CHAPTER ONE

INTRODUCTION

1.1. Global burden of salmonellosis

Salmonellosis is considered one of the predominant foodborne zoonoses of public and animal health concern across nations in the world. Infections due to Salmonellae contribute substantially to global morbidity and mortality (Eng *et al.,* 2015), with over 93.4 million cases reported globally, resulting in 155,000 deaths annually. An estimated 22 million typhoid fever cases are reported yearly out of which about 10% result in death. In addition, annual fatalities from infections caused by Non-Typhoidal *Salmonella* serotypes (NTS) are about 681,000 from an estimate of 5.4 million cases (Majowicz *et al.,* 2010).

Salmonellosis is an enteric infection of humans and livestock caused by many strains of *Salmonella*. In humans, the best described invasive *Salmonella* serovars are the host specific *Salmonella enterica* serovar Typhi and *S. enterica* serovar Paratyphi A, B, and C which cause typhoid and paratyphoid fevers respectively (Olobatoke, 2017). Clinical manifestations of typhoid and paratyphoid fevers cannot be easily distinguished; both fevers are generally referred to as 'enteric fever'. In contrast, non- typhoidal salmonellosis is usually caused by other serotypes of *Salmonella* generally referred to as NTS (Crump *et al.,* 2015). In developed countries, NTS mainly causes a self-limiting diarrhoeal illness among healthy people while invasive infection is uncommon and occur mainly in individuals with deficient immune functions and other debilitating conditions (Eng *et al.,* 2015). However, in sub-Saharan Africa, NTS are generally among the frequent causes of bacteraemia in both young and the elderly (Crump *et al.,* 2015).

1.1.1. Invasive non typhoidal salmonellosis

Invasive non- typhoidal salmonellosis (iNTS) is endemic in many sub-Saharan African countries and is among the major causes of invasive bacterial diseases in Africa overall. The global disease burden due to iNTS is estimated at 3.4 million cases with a case fatality of 20%, translating to about 681,316 deaths annually (Kariuki *et al.,* 2015). The highest number of invasive salmonellosis occur in Africa.About 2 million cases of iNTS infection were reported in Africa in 2010, which was about half of the global cases, with two-third of

this burden in children (Kariuki *et al.,* 2015). Childhood morbidity and mortality as a result of invasive salmonellosis may be higher than that from malaria in some African countries, with host risk factors playing a vital role in its epidemiology (Morpeth *et al.,* 2009).

Salmonella Typhimurium and *S.* Enteritidi*s* are mostly implicated in invasive salmonellosis across sub Saharan Africa with varying case fatalities depending on the infecting serovar. For example *S.*Typhimuriumhas a higher case fatality compared to *S*. Newport (Majowicz *et al*., 2010). The extent to which NTS strains causing invasive salmonellosis differ phenotypically and genotypically from those causing enteric infection is still unknown. Recently, a significant proportion of iNTS infections in sub-Saharan Africa were associated with a novel *S.* Typhimurium multi-locus sequence type, ST313 (Crump *et al.,* 2015). *Salmonella* infection in livestock is also of important health concern, since animal sourced foods are a major source of human outbreaks (Heredia and García 2018).

1.1.2. Bovine salmonellosis

Bovine salmonellosis is a common infection in cattle (Kemal, 2014). Bovines are usual reservoirs of *Salmonella* species with a few serotypes specifically associated with cattle, the commonest being *S.* Typhimurium and *S.* Dublin (EFSA, 2010; Kemal, 2014). Bovine *Salmonella* infections commonly occur through consumption of contaminated feed or fomites. Colonization by *Salmonella* spp. depends largely on the host's immune defense. Infections range from subclinical to clinical manifestations which include diarrhoea, dehydration, acute or chronic enteritis, septiceamia, abortion and sudden death (Adem and Bushra, 2016).

Adult cattle often appear asymptomatic while shedding *Salmonella* in their faeces while calves are more vulnerable to *Salmonella* infection. Nevertheless, symptomatic cases of salmonellosis in mature cattle have also been documented (WHO, 2016). Faecal shedding of *Salmonella* in asymptomatic cattle herds has been reported, but the relationship between faecal shedding and bovine salmonellosis outbreaks is not clearly understood (Cummings *et al.,* 2009). Salmonellosis outbreaks in cattle are of significant economic importance, they may result in reduced productivity due to treatment expenses, loss of weight, reduced lactation, reduced meat yield and eventual fatalities within the herd (Mohler and House, 2009). Bovine salmonellosis is also an important source of salmonellosis outbreaks in

humans (Heredia and García 2018). Cross contamination of dairy products with *Salmonella*e haboured by asymptomatic cattle carriers are common causes of salmonellosis in human (Heredia and García 2018). The advent of multi-drug resistant *S.enterica* Typhimurium definitive type (DT) is of public health concern; these strains have shown resistance to tetracycline, chloramphenicol, ampicillin, streptomycin, sulfonamides, trimethoprim and fluoroquinolones (Mueller-Doblis *et al.,* 2018).

The economic and medical challenges of salmonellosis are of global concern due to its shared importance in human and animal health. Concerted effort ought to be made to understand and mitigate transmission and pathogenesis of salmonellosis in cattle (Hanson *et al*., 2015). Targeted intervention strategies aimed at reducing the exposure of cattle to environmental pathogens include use of probiotics, vaccination and treatment with antimicrobial agents which minimizes or prevents pathogen colonisation and carriage (WHO, 2016). One promising alternative to antibiotics in this regard, is the use of probiotics against enteric pathogens in livestock management (Das *et al.,* 2013).

1.2. Antibiotic use in farm animal husbandry

Meat and offal from livestock are important sources of animal protein globally (FAO, 2014) andbeef is an important protein source in most Nigerian communities (Muhammad-Lawal and Balogun, 2007). There is an unprecedented increase in animal protein demand with increasing global population. In a bid to meet this enormousdemand for animal sourced protein, management of livestock routinely involves incorporation of antibiotics to animal feed for the purposes of growth enhancement, prophylaxis, metaphylaxis and therapy (Van Boeckel *et al.,* 2015). Inappropriate use of antimicrobials in livestock management practices is considered a major contributor to the emergence and dissemination of antibiotic resistance among pathogens as well as commensals of food animal origin (Adeniyi *et al.,* 2015). The rapid development of antimicrobial resistance in pathogenic bacteria is recognized as one of the main global threats to medical treatment of infectious diseases. Although inappropriate use of antibiotics in human population is thought to be the main driving factor of the current crisis of antimicrobial resistance, public health experts opined that non-judicious use of antimicrobial agents in livestock production also contributes significantly (Van Boeckel *et al.,* 2015). The contribution of agricultural antibiotics to the development of bacterial

antibiotic resistance is currently a subject of debate and research. Therapeutic use of antibiotics in livestock may be a relatively minor contributor to the problem but the non-prudent use of antimicrobial agents in apparently healthy animals is of public health concern (Van Boeckel *et al.,* 2015). European Union countries have banned antimicrobial growth promoters in livestock farming (Chattopadhyay *et al.,* 2014). There is therefore an urgent need for research targeted at providing farmers globally with natural growth promoters as possible alternatives to antibiotics.

1.3. Probiotics as natural growth promoters

The concept of "probiotics" has been defined by many researchers and at present it is viewed by different authors to mean different things. However, the generally accepted definition proposed by FAO/WHO in 2001 is “Probiotics are live microorganisms which when consumed in sufficient quantity provide the host with health benefits”. The commonest groupof microorganisms proposed for probiotic use are Lactic Acid Bacteria (LAB). The genera belonging tothis group include: *Lactobacillus*, *Lactococcus, Enterococcus,Pediococcus*, *Leuconostoc* and *Streptococcus*. *Bifidobacteria* is frequently included in probiotic preparations and the yeast *Saccharomyces boulardii* is also used. Probiotics are helpful for their role in balancing the beneficial microorganisms in the gut which have very important roles in gut health. Probiotic microorganisms have been shown to demonstrate promising novel health benefits through *in-vitro* experiments validated by *in-vivo* trials (Ewe *et al.,* 2010). There are now increasing scientific evidence that some probiotic strains are capable of providing health benefits in both humans and animals. A proposed alternative to antibiotic growth promoters in livestock is the use of probiotics; it has been demonstrated to stimulate growth promotion by improving the gut microbial balance and thus serve as a natural defense in livestock against pathogenic bacteria (Das *et al.,* 2013).

1.4. Scope of the problem

Important strategies for optimum productivity in modern livestock management involve growth promotion and disease prevention; these have encouraged the widespread incorporation of antibiotics as growth promoter in various livestock feed (Allen *et al*., 2013).

Antibiotics are believed to improve growth performance and increase feed to meat conversion, leading to increase weight gain. The mechanism of antibiotic action in growth promotion is thought to closely relate to reduction of intestinal pathogens (Das *et al.,* 2013). The incorporation of low doses of antibiotic additives to animal feed for the purpose of growth enhancement is one of the major contributors to the upsurge and dissemination of antimicrobial resistance determinants among pathogenic bacteria and commensals of animal origin (Adeniyi*et al.,* 2015). These pathogens can get into the human population through the food chain and create a huge public health challenge. One of such pathogens is *Salmonellaenterica* (Heredia and García 2018). *Salmonellaenterica* is an important but neglected zoonotic pathogen in Africa; a common cause of entero- and gastroenteritis in humans. It could also result in poor livestock productivity or mortality in cattle, particularly in calves (Adem and Bushra, 2016). Currently, vaccination and antibiotic administration are the major means of mitigating salmonellosis in livestock farming (Das *et al.,* 2013). However, both approaches have drawbacks: while the long term use of antibiotics selects for antibiotic resistant serovars, potentially resulting in dysbiosis, vaccination is often suboptimal (Hammad and Shimamoto, 2010). There have also been reports of increasing food safety concerns as regards the persistence of antibiotic residues in animal products with far reaching health implications (Van Boeckel *et al.,* 2015). The shortfalls of the above mentioned strategies coupled with the decision of the European Union to ban the use of antimicrobial growth enhancers in animal feed has necessitated the need to explore alternative intervention strategies against enterobacterialinfections in food animals. Reports from recent studies suggest that probiotics are reliable alternatives to antibiotic feed additives (Das *et al*., 2013; Adeniyi *et al*., 2015).

1.5. Hypotheses

Lactic acid bacteria with probiotic potentials have been isolated from various sources, it is therefore hypothesized that;

1. Lactic acid bacteria isolated from cattle faeces have probiotic potentials.
2. Calves fed with potential probiotic lactic acid bacteria suspension for one month will have reduced load of enterobacteria.

1.6. Research questions

i. Will LAB with probiotic potential be isolated from cattle faeces?
ii. Can the *in vitro* antimicrobial activity of LAB be achieved in *in vivo* condition?
iii. For how long will lyophilized LAB survive at room temperature?

1.7. Expected contribution of the current research to economic value

In a bid to improve livestock productivity, there is an emerging preference for Natural Growth Promoters (NGP) by livestock farmers globally. There are no known livestock probiotics in the Nigerian market and the product of this research is tailored towards meeting this need. The potential probiotic LAB strains to be characterised in this study will be lyophilized and encapsulated for further trial as feed additive which is expected to improve livestock productivity through disease prevention.

1.8. Research objectives

The general objective of this study was isolation and characterisation of LAB from cattle faeces for their anti-*Salmonella* and probiotic potential against enterobacteria in cattle.

The specific objectives were:

i. To isolate and identify *Salmonella* spp. and LAB from cattle faeces.
ii. To determine the antimicrobial activities of LAB against *Salmonella*and other enterobacteria from cattle.
iii. To determine the ability of LAB to resist bovine gastric conditions.
iv. To quantify the major organic acids produced by the LAB isolates.
v. To determine the antibiotic resistance profiles of LAB and *Salmonella*isolates.
vi. To determine the antibacterial activities of selected LAB isolates against enterobacteria *in-vivo* in calves.
vii. To determine the survival of lyophilized LAB in storage over three months.

CHAPTER TWO

LITERATURE REVIEW

2.1. Global importance of livestock farming

The subsistence of millions of people in both developed and developing nations of the world is dependent on livestock farming (World Bank, 2009; Morgavi *et al.,* 2010). It is estimated that animal farming contributes 40% to global gross domestic product (GDP) and about 30% to the economy of African countries (World Bank, 2009). These estimates underscore the importance of livestock production in economic development. The economic contribution of farm animals transcend food production and it also provides draught power (traction), organic fertilizer for crop farming; blood, milk, feathers, bones, fibres, hides and skin for the industries (Kubkomawa, 2017). Livestock farming in Nigeria and other developing countries is of great economic importance as it provides employment opportunities and household income to about 68 % of the population (Herrero *et al*., 2012), an estimate of about 1.3 billion individuals are engaged globally in various food animal product value chains (Herrero *et al*., 2009). World population is anticipated to rise from an estimate of 6.5 billion people in 2010 to over 9 billion by 2050, this parallel global population increase will consequently lead to an unprecedented increase in animal food demand which is expected to double by 2050 (Van Boeckel *et al.,* 2015). Ownership of livestock in developing countries is significant, and it is a sign of affluence and substitutable asset which can easily be sold to meet other financial obligations and may also serve as financial instruments or collateral to secure loans and other credit facilities (Herrero *et al.,* 2012). Livestock provides a steady stream of income and reduces seasonal fluctuations in the livelihood patterns of the rural dwellers, offering food security particularly at periods of crop failure (Bettencourt *et al.,* 2015). In some African cultures and traditions, livestock play a vital role in customary marriages, rituals, festivals and funerals (Tibi and Aphunu, 2010).

Livestock contribute significantly to overall global food security. Animal sourced foods are important constituents of a healthy diet as they are rich in both micro and macronutrients, since there is a link between nutrition and health. They are suitable sources of high quality protein and energy, particularly in individuals with special nutritional requirements such as children, nursing mothers and people with deficient immune functions (Herrero *et al.,* 2012).

It has been established that there is strong association between consumption of food of animal origin and improved growth, cognitive function in children and reduction in morbidity from sickness as a result of better immune response (Grace *et al.,* 2018). Food of animal source is dense in energy and a good source of a balanced diet (Herrero *et al.,* 2012). Proteins obtained from animal origin usually contain essential amino acids which are not adequately available in plant based foods. Animal proteins are also important sources of various essential micronutrients (Grace *et al.,* 2018). Absence of fibre and phytates in animal sourced food make the bioavailability of these nutrients higher than those of plant origin (Lim*et al*., 2013). Malnutrition is particularly common in economically less developed countries, partly because the major diets are deficient in macro and micronutrients as a result of limited amount of animal sourced protein. In order to combat malnutrition, it has been estimated that 20g of animal sourced protein per person per day is required (FAO, 2009). Increase in livestock production has been reported to improve productivity as well as the dietary status of individuals living in those communities (Bettencourt *et al.,* 2015).

2.2. Economic importance of cattle in Nigeria

Livestock husbandry at both subsistence and commercial levels are part of the mainstay of Nigerian's economy, and cattle contribute about 45% to meat supply in Nigeria (Kubkomawa, 2017). Nigeria is reported to have about 14.73 million cows with 13.26 million beef cattle and 1.47 million dairy cattle, thus making Nigeria one of the leading producers of cattle in Africa (Tibi and Aphunu, 2010). The contribution of cattle to agricultural GDP is approximately 12.7% and about 6% of the overall GDP in Nigeria (Kubkomawa, 2017). It is reported that cattle husbandry in Nigeria generates about 6.8 billion dollars annually with a capacity to increase to about 20 billion dollars annually (Tibi and Aphunu, 2010). Cattle production in Nigeria offers employment opportunities to a significant portion of the working population who are engaged in various value chain processes from sale, transport, butchering, processing and marketing of dairy products (FAO, 2009; Umar *et al.,* 2008). Furthermore, possession of cattle is seen in the society as a measure of an individual's wealth status, serving as mobile banks to nomadic farmers and a means of insurance against crop failure by farmers engaging in mixed farming (Glass *et*

*al.,*2014). Cattle are therefore considered an important socio-cultural asset in many Nigerian communities.

Cattle are important source of raw materials which include: hides and skin which are needed in the manufacturing leather bags, purses, belts, shoes and sandals; milk and milk products (Kubkomawa *et al.,* 2017). Fat from cattle are important materials in the production of soaps, lipsticks, lubricants and sprays (Gandhi, 2009). Bones, horns, hoofs, feathers, rumen content, and blood are also useful ingredients in compounding animal feed (Kubkomawa, 2017). Cattle are important means of draught animal and farm power in Nigeria because of its accessibility to poor farmers who may not be able to afford mechanized farm power such as tractors. Cattle are usually adapted for transportation, driving food processing equipment, water lifting and cultivation of crops (Babayemi *et al.,* 2014). Cattle dung is a good source of organic manure which is useful for the improvement of soil fertility, structure and water retention capacity (Kubkomawa, 2017).

2.3. An overview of cattle management and indigenous breeds in Nigeria

The world population of cattle is estimated to be over 1.1 billion, while Nigeria's cattle population is about 14 million (Umar, 2008), of which about 11.5 million are reared in pastoral systems and 2.4 million are kept in villages. The Fulani ethnic group, particularly the pastoralists are renowned for cattle production; they are reputed for owning about 90% of the cattle in Nigeria (Olafadehan and Adewumi, 2010). Cattle are found in every state, but are predominantly reared in northern Nigeria. About 50% of the country's total cattle population resides within the sub-humid region. Free range grazing is the commonest indigenous feeding system of cattle in Nigeria; it involves grazing animals through the nomadic pastoral system as commonly seen with the Fulani herdsmen (Umor, 2017). In the pastoral system, the herder leads the cattle herd in search of pasture and water to graze during the day. They are usually penned at night with calves kept separately in enclosures away from adults (Akpa *et al.,* 2012).

There are many cattle breeds indigenous to Nigeria which include the Zebu cattle: White Fulani, Sokoto and Adamawa Gudali cattle. The non- Zebu cattle are: Muturu, N'dama and Keteku cattle (Babayemi *et al.,* 2014). The White Fulani and the Gudali breeds are the most abundant and widespread of all indigenous cattle breeds and represents about 37% and 32%

of Nigerian national herd respectively (Alphonsus *et al.,* 2012). The White Fulani also referred to as Bunaji is known to be superior to all other indigenous breeds for their capacity to withstand diseases and survive under various environmental stress; they are also reputable for milk production, faster rate of growth, good temperament and huge body size (Olafadehan and Adewumi 2010). The major disadvantages of this cattle breed are delayed sexual maturity and short period of lactation. The Gudali breed are most popular for their milk production, they give higher milk yield than White Fulani (Alphonsus *et al.,* 2012). They are regarded as indigenous dairy breed with well-developed udder and good teats. At maturity, the male weigh about 450kg while the female weigh about 330 kg (Kubkomawa 2017).

2.4. The microbial structure of cattle's gastrointestinal tract

New born cattle are physically and functionally unique with respect to their gut system (Uyeno*et al.,* 2015). Calves are born with sterile gut; microbial colonization begins just after birth (Guzman *et al*., 2015). There begins a succession of a complex and dynamic microbiota with the emergence of a dense microbial community in the gut as the calf develops to maturity. Molecular tracking of calf's intestinal microflora suggests that the microbiota undergoes a dynamic change during the first 90 days after birth (Uyeno *et al.,* 2010). It was observed that the major bacterial groups detected in young calves at about ≤ 21 days were similar to those found in human faecal microbiota. However, the population of *Atopobium, Faecalibacterium,Lactobacillus* and *Bifidobacterium* reduces with the age of the animal (Uyeno *et al.,* 2010). This premature and fluctuating gut microbial ecosystem is challenged by an abrupt diet change which usually increases the susceptibility of young calves to onslaught of pathogens resulting in diarrhea and respiratory diseases (Li *et al.,* 2018). The normal gut flora is critical for the maintenance of animal health, and an important function of the normal flora is competitive exclusion of pathogens which prevent them from colonising the gut (Jandhyala *et al.,* 2015). The gut microflora is also particularly useful in fermentation and digestion of plant products in adult herbivores. Ruminants usually harbor a diverse microbiota consisting of anaerobic bacteria in the rumen. These consortiums of microorganisms interact with one another and digest plant polymers by anaerobic fermentation to produce source of energy to the animal host (Plaizier*et al.,* 2012).

Several factors, including diet and livestock management, can have an impact on the structural activities of the bovine microflora, sometimes resulting in reduced herd growth performance (Uyeno *et al.,* 2015). For instance, Sub-Acute Ruminal Acidosis (SARA) - an impairment that has been linked with dysbiosis in cattle gut. It was observed that the major microbial shift during SARA was a reduction in Bacteroidetes which resulted in an inflammatory response (Plaizier*et al.,* 2012).

2.5. Zoonoses

Zoonoses are naturally transmissible diseases between animals and human with or without vectors (WHO, 2015). The incidence and prevalence of zoonotic diseases is a global public health challenge (Halliday *et al.,* 2015). More than 60% of all human infections are reported to be zoonotic while about 75% of all new human diseases over the last 10 years have been associated with either pathogens of animal origin or products from animal sources, further underscoring the magnitude of this ongoing public health challenge (WHO, 2015). In Africa, and other developing countries, zoonoses contribute immensely to an already over-burdened health care system while in developed nations it is only of particular concern for risk groups such as the children below 5 years, the aged, pregnant women and individuals with debilitating immune functions (Halliday *et al.,* 2015). It is observed that the incidence and prevalence of zoonoses are higher in underdeveloped countries, partly due to inadequate control measures, health care facility deficit and insufficient public health information (Belay *et al.,* 2017). Zoonoses are transmissible from animals to human via numerous routes such as the ingestion of contaminated water and food (e.g. cryptosporidiosis, toxoplasmosis, salmonellosis), contact with diseased animals (e.g., bird flu), scratch and bites (e.g., rabies) (Metzgar *et al.,* 2010). Livestock contributes directly to the global burden of infectious diseases particularly in developing countries through food borne diseases that are transmissible from animals to humans. Some animals have been identified as reservoirs of zoonotic diseases and considered to possess the potential risk of disease transmission; ruminants, pigs, birds, rats, dogs, cats, mosquitoes and ticks (Agunos *et al.,* 2016).In some cases, livestock also act as an amplifying host for some zoonoses, for example, there is potential risk of human infection from pigs harboring and replicating the Japanese encephalitis virus after being bitten by mosquitoes (Metzgar *et al.,* 2010). Zoonotic

pathogens not only have significant impact on public health but also on the socioeconomic condition in terms of livestock productivity (McDaniel *et al.,* 2014). This results in reduced livestock productivity due to treatment cost, loss of weight, reduced milk and meat yield and sometimes mortality (Mohler and House, 2009). Due to similarities of clinical presentations between zoonotic and non-zoonotic infections, the potential for many undiagnosed cases of these zoonotic pathogens also exists (McDaniel *et al.,* 2014).

Some animal production and food consumption practices in Nigeria and other African countries that may promote zoonoses transmission include:

(1) A dense population of humans and livestock living in close proximity

(2) Operation of slaughterhouses and wet markets in unhygienic conditions

(3) Suboptimal meat inspection and inadequate cold chain meat delivery vehicles

(4) Consumption of undercooked or raw animal products

(5) Application of untreated sewage for farming purposes (Carrique-Mas and Bryant, 2013).

2.5.1. Bacterial foodborne zoonotic diseases

Foodborne zoonoses are human infections and diseases transmitted through ingestion of contaminated food and caused by pathogens with vertebrate animal species as their natural reservoir (Carrique-Mas and Bryant, 2013).The commonest food borne illnesses are microbial infection and intoxication. Intoxication occurs when pathogens produce toxin in food causing food poisoning, while infection mostly result from ingestion of food contaminated with live pathogens (Eng *et al.,* 2015).Bacteria are implicated in about 60% of foodborne diseases requiring hospitalization. The global morbidity and mortality of foodborne diseases is difficult to determine, however, it is documented that about 2.1 million children in less developed countries die annually as a result of diarrheal- related illnesses (WHO, 2015). Since the last century till date, there are four major bacterial genera that have been implicated as the main cause of foodborne infections, namely *Salmonella*, *Campylobacter, Listeria and Escherichia* (Gutić, 2015). *Salmonella* and *Campylobacter* are the most frequent bacterial contaminants found in dairy and poultry products (EFSA, 2010). Livestock are the principal reservoirs for many zoonotic pathogens. About 95% of human salmonellosis in USA and Europe are linked with the ingestion of bacterial contaminated

dairy and poultry products (EFSA, 2010). The propensity of zoonotic diseases to result in fatal outcomes is of significant "one health" importance (Heredia and Garcia 2018).

2.5.2. Common foodborne bacteria associated with bovine product

2.5.2.1. Diarrheagenic *E. coli* serotype O157:H7

Escherichia coli serotype O157:H7 is a verocytotoxigenic *E. coli* (VTEC) capable of causing potentially fatal illness in humans with symptoms including bloody diarrhoea, haemorrhage, rectal prolapse, haemolytic uraemia and anaemia. There are six major pathotypes of Diarrheagenic *E. coli;* Enteropathogenic *E. coli* (EPEC), Enterohaemorrhagic *E. coli* (EHEC), Enteroaggregative *E. coli,* Enterotoxigenic *E. coli,* Enteroinvasive *E. coli*, and diffusely adherent *E. coli* (Croxen *et al*., 2013). Enteropathogenic *E. coli* and EHEC are the major human pathogens with significant fatalities resulting from infantile diarrhea and bloody diarrhea respectively particularly in children and the elderly (Burgess and Duffy, 2011). The expression of Shiga toxin which is correlated with the development of heamorrhagic colitis distinguishes EHEC from EPEC. EPEC colonizes the small intestine while EHEC usually colonize the large intestine in human infections (Gomes*et al*., 2016).

The incidence of VTEC is relatively low when compared with other zoonotic pathogens; however, its low infectious dose and disease severity is of public health importance (EFSA, 2010). *Escherichia coli* O157:H7 is the most implicated VTEC pathotype implicated in human disease outbreaks. The pathogenicity of *E. coli* O157 is thought to be dependent on some factors which include: potential of the strain to produce verocytotoxins, adherence and colonization of the intestine and the potential of the strain to produce verocytotoxins (Burgess and Duffy, 2011). Other effectors employed in the pathogenesis of *E. coli* O157:H7 are located outside the locus of enterocyte effacement (LEE) (Karmali *et al.,* 2010). Bovine are a major reservoir of *E. coli* O157 and bovine-derived products particularly undercooked beef products, are important sources of human infection that have been linked with about 75% cases of food borne outbreaks caused by *E. coli* O157 (Callaway *et al.,* 2009).

2.5.2.2 *Listeria monocytogenes*

Listeriamonocytogenes is commonly found in nature and a serious problem in ready-made foods as a result of its ability to persist in food processing areas and its potential to grow at low temperatures including in the refrigerator. It causes listeriosis with symptoms including but not limited to influenza, meningitis, septicaemia and foetal infection or spontaneous abortion in pregnant women (Burgess and Duffy 2011). Although, the incidence is relatively low, the severity and high mortality rate (about 17.5%) are of public health concern (EFSA, 2010). Outbreaks of listeriosis with fatal outcomes are mostly associated with dairy products (Smith *et al.,* 2011).

2.5.2.3. *Campylobacter*spp.

Food borne infections caused by *Campylobacter* spp. is the commonest in both developing and developed countries with a relatively high prevalence rate in the European Union (EFSA, 2010). *Campylobacter* infection usually causes a self-limiting disease characterised by fever, nausea, bloody diarrheoa, headaches and abdominal cramps. It is also been associated with inflammatory bowel disease. The prevalence of *Campylobacter* carriage in cattle is known to be high (Chatre *et al*., 2010). Ingestion of beef not properly cooked is noted to be an important risk factor for campylobacteriosis in humans (MacDonald *et al*., 2015). The importance of cattle in *Campylobacter* zoonosis has been established with the typing of *Campylobacter* isolates from livestock and clinical sources resulting in clinical isolates clustering with isolates of livestock origin (Whiley *et al.,* 2013).

2.5.2.4 *Salmonella* spp.

Since the discovery of *Salmonella* by D.E. Salmon in 1885, it has continually been a major foodborne pathogen in livestock and human. In many countries of the world, *Salmonella* species are the commonest cause of foodborne illness and outbreaks, creating a global public health burden (Kemal, 2014).Salmonellae belong to Enterobacteriaceae, a familily of Gram-negative rods. There are about 2500 *Salmonella* serotypes identified with over 50% belonging to *Salmonella enterica*; the serotype mostly responsible for human salmonellosis (Eng *et al.,* 2015). Some serotypes of *S.enterica* are confined to a limited species of animal, while other serotypes can cause infection in a number of hosts ranging from plants to

animals (Velge *et al.,* 2012). Based on the WHO nomenclature scheme currently being used; the genus *Salmonella* consist of two species: *S. enterica* and*S. bongori* classified based on differences in 16S rRNA genes sequences. *Salmonellaenterica* also consist of six subspecies classified according to biochemical properties and genetic relatedness (Heredia and Garcia, 2018). Among the *Salmonella* subspecies, *S. enterica* subsp. enterica is the most implicated in human salmonellosis, while the other five subspecies of *S. enterica* andstrains of *S. bongori* are rarely isolated in humans (Eng *et al.,* 2015). *Salmonella* spp. can also be classified into serotype based on the agglutinating properties of their major antigenic determinants: somatic O, capsular Vi and flagellar H antigens (Eng *et al.,* 2015). More than 2600 distinct *Salmonella* serotypes have been differentiated with about 1,530 serotypes belonging to *S.*Typhimurium and *S.*Enteritidis which are responsible for more than 99% of salmonellosis in humans (Velge *et al*., 2012). Humans are the sole reservoir of *S.*Typhi and *S.* Paratyphi, while all the other serovars referred to as NTS have animals as reservoirs (Heredia and Garcia, 2018).

2.5.2.4.1. Pathogenesis of*Salmonella*

Several factors including the infecting serotype, health status, age e.t.c., determine the severity of salmonellosis in human. Usually, children less than five years old, the elderly and people with compromised immune functions or debilitating health issues are more predisposed to *Salmonella* infections than healthy individuals (WHO, 2015). Most strains of *Salmonella* are capable of invading, replicating and surviving in human host cells and are thus potential pathogens which can cause life threatening diseases (Eng *et al.,* 2015). Persistence of *Salmonella* in host cells is an important factor for pathogenesis; strains not endowed with this property are not virulent (Velge *et al.,* 2012).

Salmonellae enter the gastrointestinal tract via bacterial contaminated food products and thereafter penetrate the intestinal epithelial lining in humans. *Salmonella* demonstrate unique invasive characteristics during infection of human cells (Velge *et al.,* 2012); it induces self phagocytosis to allow access to the host cells. The gene coding for this unique invasive strategy is located in the *Salmonella* pathogenicity island (SPIs) on the chromosomal DNA (Eng *et al.,* 2015). After being engulfed into the host cell, the host cell membrane forms a vacuole which encloses the bacteria cell. Ideally, the presence of bacteria or other foreign

materials will elicit the host immune response, which results in fusion of the lysosome and secretion of digestive enzymes to digest the invading bacteria. However, *Salmonella* causes a remodeling of the vacuole by the secretion of certain effector proteins that causes structural alteration of the vacuole. The restructured vacuole prevents the fusion of lysosomes and this allows *Salmonella* to survive and replicate intracellular within host cells (Velge *et al.,* 2012; Eng *et al.,* 2015).

2.5.2.4.2. *Salmonella*carriage in cattle

Cattle are naturally susceptible to infection with non typhoidal serotypes of *Salmonella* which may eventually result in bovine salmonellosis (Elfenbein *et al.,* 2013). It is now being speculated that *Salmonella* may betransmitted from the dam *in utero* tothe foetus since faecal shedding has been reported in day-old calves (Hanson *et al.,* 2015). Cattle either respond to *Salmonella* infection by clearing the pathogen after resolution of the disease or become asymptomatic carriers and intermittently shed these organismsin their faeces. *Salmonella* carriage in cattle often leads to widespread faecal shedding of *Salmonella* resulting in environmental contamination (Cummings *et al.,* 2009). Faecal shedding of *Salmonella* within cattle herd increases the risk of bovine salmonellosis among farm cattle herd, and also a source of transmission to cattle herd on other farms thereby keeping a cycle of *Salmonella* carriage in perpetuity (Cummings *et al.,* 2009). Bovine salmonellosis is often a syndromic condition of bacteremiacharacterised by acute or chronic enteritis, and abortion may also occur in pregnant dams (Kemal, 2014). *Salmonella* Dublin and *Salmonella* Typhimurium are the commonest of the few non typhiodal serotypes of *Salmonella enterica* associated with bovine infections (Adem and Bushra, 2016). The prevalence of *Salmonella* carriage particularly by asymptomatic cattle at slaughter is a predictor of the probability of eventual carcass contamination which consequently determines the risk of human infections (Kemal, 2014). About 30% of human non- typhoidal salmonellosis have been documented to emanate from cattle (Cummings *et al.,* 2009), hence, the knowledge of the requirement for survival of Salmonellae in the guts of cattle and its transmission dynamics will give leads to new strategies of mitigating bovine colonization and in turn reduce the risk of food chain and environmental contamination of *Salmonella* spp. (Elfenbein *et al.,* 2013; Hanson *et al.,* 2015).

2.6. Cattle faeces as a source of foodborne pathogens

Cattle faeces are important sources of zoonotic pathogens. When these microorganisms are released in the faeces, they can thrive in the environment- soil and grass underlay for a long time which could be up to several months. Faeces are considered a major route of pathogen transmission among the cattle herd, food chain and the environment (Burgess and Duffy 2011). Cattle have been reported to be naturally infected by *Salmonella* spp.and the prevalence of *Salmonella* isolation in bovine faeces range from about 0 to 62% (Elfenbein *et al.,* 2013). *Escherichia coli* are members of the microbiota of the intestinal tract in cattle; it is also a predictor of the occurrence of enteropathogenic microorganisms in food and an indicator of faecal contamination (Callaway *et al.,* 2009). *Escherichia coli* O157:H7 is the commonest strain isolated from cattle faeces; it is a toxin producing pathogen also known as enteroheamorrhagic (EHEC) or verocytotoxic (VTEC)*E.coli.*This strain is highly pathogenic with low infective dose (about 10 cells) and can cause serious infections in humans while not harming the cattle host (Cummings *et al.,* 2009).The rate of occurrence of *E.coli* O157 in the faeces of calves and cattle have been estimated to range from 0 to about 60%, with some shedding at about 10^4 CFU/g (Jacob *et al.,* 2010). The prevalence of *C. jejuni* (16.5 - 94%) is comparatively higher than those of other zoonotic pathogens in cattle faeces (Chatre *et al.,* 2010). It is known to be present throughout the entire gut but mostly colonise the small intestine in bovine. *Listeria monocytogenes* causes listeriosis- a fatal invasive infection that affects both humans and livestock. The mortality rate of listeriosis in humans has been reported to be about 30% (Burgess and Duffy 2011). The few reports available on the prevalence of *L. monocytogenes* isolationin bovine faeces, showed the range to be between 4.8 and 29.4%. Beef products contaminated with bovine faecal material have been associated with major outbreaks of listeriosis outbreak worldwide (Smith *et al.,* 2011).

2.7. Vaccination in curtailing bacterial infection in livestock

Vaccination is a means of enhancing the host's immunity for the purpose of pathogen reduction through the production of antigens against particular microorganisms. Vaccination has long been used as a strategy for pathogen reduction in livestock husbandry, and some vaccines have primarily been developed against zoonotic pathogens (Amani *et al*., 2011). For example, vaccines have been developed against *Salmonella* infection in pigs and cattle

(Schwarz *et al*., 2011). Vaccination against post weaning *E.coli* edema has also been successfully used in young pigs (Schwarz *et al*., 2011). Owing to the fact that some zoonotic pathogens (e.g.*E. coli* O157) are incapable of causing disease in their host animal, it is important to vaccinate such host to mitigate human infection. Vaccines targeted at reducing the faecal shedding of *E. coli* O157:H7 in bovine have been successfully developed (Schwarz *et al*., 2011). Considering the nature of vaccination which involve the use of the native immunity of the host, vaccines could be used in synergy with other strategies aimed at pathogen reduction (Allen *et al*., 2013). It is documented that a certain *S*. Typhimurium vaccine deficient in DNA adenine methylase was able to provide multiple protection against *S.* Dublin and *S.* Newport in vaccinated calves with significant reduction in colonization and faecal shedding (Miller*et al*., 2014). On the contrary, a study involving the administration of a commercially available *S. enterica* subunit vaccine did not reduce faecal shedding of *Salmonella* in cattle. The development of a single vaccine against various serotypes of *Salmonella* and *E.coli* is challenging as a result of the difficulty in targeting the different organisms. The vaccination dose required to achieve full immunity by the animal also remains a technical challenge (Callaway *et al.,* 2013).

2.8. Antibiotic feed additives in livestock management

The incorporation of antimicrobial growth enhancers in animal feed was serendipitously observed in the 1940s, it was discovered that feeding animals with mycelia of *Streptomyces aureofaciens* containing residue of chlortetracycline usually result in growth promotion (Chattopadhyay, 2014). In 1946, the outcome of experiments revealed that low concentration of antibiotics could improve feed efficiency and stimulate growth in livestock, leading to the practice of adding several antibiotics to livestock (Chattopadhyay, 2014). The use of antibiotics as feed supplement over the counter was approved in 1951 by the FDA (Al-Khalaifah, 2018). Subsequently, this concept was exploited over the years and the use of antimicrobial growth promoters have become a global practice with the intensification of livestock production (Van Boeckel *et al.,* 2015).

A sizeable proportion of antibiotics produced worldwide are now being used in Agriculture. In the United States alone, about 24.6 million dollars' worth of antimicrobials are used in livestock production yearly, with a significant fraction of these used for purposes other than

therapeutic. About 90% of all antibiotics used in livestock management are reported to be administered at sub-inhibitory doses for prophylaxis and growth enhancement (Van Boeckel *et al.,* 2015). The principle of animal growth promotion by antibiotics is not clearly understood; it is thought that microbes compete for the absorption of nutrients, they also produce toxins which have untoward effect on the wellbeing of the animal, the growth promotion resulting from the use of antibiotic feed additive may stem from their ability to inhibit these pathogens (Das *et al.,* 2013). It is thought that keeping livestock under unhygienic conditions constantly expose them to some latent infections which usually result in cytokines production and release of certain catabolic hormones that leads to muscle wastage. Antibiotics are useful in this case to prevent the animal from producing cytocines by inhibiting the infectious organisms (Allen *et al*., 2013). The benefits of antibiotic feed additive in enhancing animal growth performance cannot be controverted. The daily growth rate of livestock provided with antibiotic feed supplements was observed to improve by 1–10% as compared with animals receiving feed without antibiotic. Pigs fed with antibiotic supplemented feed require 10–15% less feed for optimum growth performance, thus antibiotics enhances the efficiency of feed conversion to animal product (Chattopadhyay, 2014). Antibiotic fed animals usually yield better meat quality; higher protein content as well as less fat compared with meat derived from animals receiving feed without antibiotic supplementation (Park *et al.,* 2016). The addition of chlortetracycline and sulfamethazine as feed additive significantly reduced morbidity arising from bovine respiratory disease, the relapse rate and mortality of animals diagnosed with chronic respiratory disease. Tetracycline and penicillin additives in poultry feed resulted in a marked increase in hatchability and feed conversion efficiency (Chattopadhyay, 2014).

2.9. The threat of antibiotic resistance arising from antibiotic feed additives

Some advocates of antibiotic feed additives for animal growth promotion are not convinced on the propensity of this practice in aggravating the challenge of antimicrobial resistance (Wallinga and Burch, 2013). While the proliferation of antibiotic resistant bacteria strains are often associated with antibiotics usage, antimicrobial resistance has also been documented in bacteria isolated from places with relative antibiotic naivety and totally remote areas; away from human interference (Bhullar *et al*., 2012). It is also arguable that

microbial isolates of human and animal origins in most cases have been analyzed to be genetically different, thus the hypothesis on resistant gene transmissibility from farm animals to humans via the food chains is also not generally accepted (Chattopadhyay, 2014). On the contrary, the incorporation of low concentration of antibiotics as feed supplement for growth promotion is established to significantly enhance the upsurge and spread of antimicrobial resistant determinants among the normal flora and pathogenic bacteria that have livestock as reservoirs (Adeniyi*et al.,* 2015). It is also noted that the incessant exposure of bacteria to sub-therapeutic doses of some antimicrobials will in addition to enriching resistant bacteria, increase the rate of mutation and may result in evolution of multidrug-resistant strains by the facilitation of the production of reactive oxygen species which are important mutagens (Kohanski *et al.,* 2010). Sub-therapeutic concentrations of some antibiotics also enhance horizontal gene transfer which is a major means of disseminating antimicrobial resistant genes (Van Boeckel *et al.,* 2015). Antibiotic feed supplementation also enhances dissemination of antibiotic resistance by facilitating phage-mediated transfer of genetic materials (Allen *et al.,* 2013). Transfer of resistant genes from zoonotic bacteria to commensals in human has been experimented in animal models (Chang *et al.,* 2014).

The challenge of antimicrobial resistance is a burning question worldwide. Many infectious diseases with fatal outcomes are emerging as a result of increasingly difficulty in medical treatment due to antimicrobial resistance. Owing to the fact that the population of livestock greatly outnumber humans, the non-prudent use of antibiotic additives in livestock poses a huge risk to humans because of the creation of a large reservoir of resistant genes with far reaching health consequences (Van Boeckel *et al.,* 2015). Currently, the contribution of antibiotics used in livestock husbandry to the spread of antimicrobial resistance in human pathogens is a subject of debate and research (Chang *et al.,* 2014). It is imperative to note that antibiotics should be administered judiciously in livestock management.

2.10. Antibiotic resistance in *Salmonella* species

Antibiotic resistance in *Salmonella* spp. is an important public health challenge (Crump *et al.* 2015). The first documented incidence of antibiotic resistance in *Salmonella* was to chloramphenicol and it was reported in the 1960s. Thereafter, there was an upsurge in the prevalence of resistant *Salmonella* strains in both developed and developing countries (Eng

et al., 2015). Also, since the emergence of the first multi-drug resistant (MDR) *S.* Typhimurium DT104 strains in 1990, there has been a surge in the number of MDR phenotypes in many countries (Crump *et al.* 2015). Several studies have shown that serotypes having MDR phenotypes possess the ability to produce different types of hybrid plasmids. Most of the resistant determinants located on these plasmids confer resistance against sulfonamides, chloramphenicol, tetracycline, ampicillin and streptomycin (Tamamura *et al*., 2011).

Third generation cephalosporins and quinolones are the first line drugs in the treatment of MDR *Salmonella* infections, but the proliferation of *Salmonella* serotypes that are quinolone and cephalosporin resistant have created a whole new challenge (Eng *et al*., 2015). Mutation of chromosomes at *gyrA* gene-the quinolone resistance determinant region is responsible for the resistance of *Salmonella* to ciprofloxacin (Song *et al.,* 2018). Some *Salmonella* serotypes produce extended-spectrum β-lactamases and hence resistant to β-lactam antibiotics such as cephalosporin and penicillin (Crump *et al.,* 2015).

2.11. Probiotics

The concept of probiotics originated from the discovery of the Nobel Prize winner, Elie Metchnikoff around early 20th century. While working in Bulgaria, he noted that certain bacteria particularly *Lactobacillus bulgaricus* in the fermented milk consumed by some Bulgarians accounted for their extraordinary longevity. He investigated the link between these organisms and their health benefits (Reid, 2015). The term probiotics has evolved and has been referred to mean several things over the years by many researchers but the most widely accepted definition is that proposed by the FAO and WHO; "Probiotics refers to live microbes which when consumed in sufficient quantity provide the host with health benefits" (FAO/WHO, 2001; Reid, 2015). Recently, the application of probiotics in human and animal health has gained more attention as there are empirical evidences of the beneficial roles of these organisms. Lactic acid bacteria and bifidobacteria are major groups of organisms used as probiotics, although some other microorganisms including *Escherichia coli* Nissle 1917 and yeast such as *Saccharomyces boulardi* are also being employed (Reid, 2015). Lactobacilli being an integral component of the intestinal microbiota and fermented food products have earned the "Generally Regarded As Safe" status; and are the most

considered candidate for probiotic functions. Probiotic organisms are known to demonstrate various health benefits including: prevention of antibiotic related diarrhea, inhibition of cancer cells, reduction of serum cholesterol, stimulation of immune system, inhibition of resistant pathogens (Ayeni *et al.*, 2009, Ayeni *et al.,* 2011), alleviation of inflammatory bowel disease, respiratory viral infection, etc. (Fonseca *et al.,* 2017). Recently, *Lactobacillus* spp. was demonstrated to possess beneficial effects in individuals suffering psychological disorders (Shonyela *et al*., 2017). Probiotics are thought to produce health benefits through various mechanisms including: competitive exclusion of pathogens, production of antimicrobial metabolites, stimulation of immune system etc. (Mokoena, 2017). A possible alternative to antimicrobial growth enhancers in livestock is the use of probiotic organisms, which are useful in augmenting the gut microflora balance and thus creates a natural defense against pathogens (Adeniyi *et al.,* 2015; Allen *et al.,* 2013). Probiotic traits are peculiar to strains exhibiting them; such characteristics cannot be extended to strains within the same species. Probiotics are consumed live; therefore, they must be safe for consumption while producing the desired beneficial effect (Papadimitriou *et al.,* 2015).

2.12. Lactic acid bacteria

The group name LAB was recognised quite early during the 20th century. Before then, the group had been previously referred to as "lactic acid producing" and/or "milk souring" bacteria (Khalid, 2011). Lactic acid bacteria consist of a diverse group of catalase negative, aerotolerant, fastidious, non sporulating, acid tolerant, Gram positive organisms that are abundant in nature (Mokoena, 2017). Although they lack catalase, they are protected against hydrogen peroxide by peroxidases. Lactic acid bacteria are characterised by the production of organic acids (particularly lactic) as the major end product from glucose fermentation and other antimicrobial metabolites such as bacteriocins which are capable of inhibiting the proliferation of pathogens as well as bacteria implicated in food spoilage (Zacharof and Lovitt, 2012). The LAB group belongs to the phylum Firmicutes, class Bacilli, and order Lactobacillales. Lactic acid bacteria are classified into various genera based on morphology, sugar fermentation, configuration of organic acid produced, capacity to grow at various pH, temperature and salt concentrations (Khalid, 2011). They are found in diverse habitat and are known inhabitants of the human gut (Mokoena, 2017). They also occur abundantly in

meat, plants, dairy and various fermented products (Ayeni *et al.,* 2011). Lactic acid bacteria are constituents of microflora of the mouth, vagina and the guts of mammals (Mokoena, 2017). They have been used since ancient times in food preservation and their ability to ferment carbohydrates to organic acid has made them to be of industrial importance in infusing unique flavour and improving texture (Montet*et al.,* 2014). Phenotypic methods have been successfully used to identify LAB, however, the taxonomy of LAB based on 16S rRNA sequencing analysis has revealed that some taxa derived on the basis of phenotypic identification do not correspond with their phylogenetic relations (Sascha and Magdalena, 2010), hence, molecular methods such as 16S rRNA sequencing have been developed, which enable a more robust and reliable identification system for individual LAB strains (Khalid, 2011).This bacteria group are fastidious, requiring amino acids, nucleotide bases, minerals, fatty acids, vitamins and carbohydrates and grow optimally at pH 5.5–5.8 (Khalid, 2011). LAB are grouped into homofermentative and heterofermentative according to the end-product of sugar fermentation through the two main microbial fermentation pathways. Homofermentative mainlyferment sugars to form lactic acid through glycolysis, while heterofermentative LAB form alcohol or acetic acid and carbon dioxide in addition to lactic acid through the 6-phosphogluconate/phosphoketolase pathway (Mokoena *et al*., 2017). Lactic acid bacteria consist of the following genera; *Lactobacillus, Leuconostoc, Lactococcus, Tetragenococcus, Vagococcus, Aerococcus, Carnobacterium, Pediococcus Enterococcus, Oenococcus, Sporolactobacillus, Streptococcus* and *Weisselia* (Horvath *et al.,* 2009). Due to its food preservative property and probiotic potential, the genus *Bifidobacterium* is mostly listed along with LAB, although they are phylogenetically different and quite distantly related to the main lactic acid bacteria group (Turroni *et al.,* 2011).

2.12.1. ***Lactobacillus*** spp.

Lactobacillus is a vastly heterogeneous genus, comprising bacteria with a wide array of biochemical and physiological attributes. *Lactobacilli* are aerotolerant or anaerobic, non-spore forming, catalase negative, rods or coccobacilli LAB, generally characterised by a low GC content of the genome. Lactobacilli mainly form lactic acid as the primary end-product of carbohydrate fermentation. Other end-products produced include acetate, ethanol, CO_2,

formic acid and succinic acid (Hammes and Hertel, 2009). *Lactobacillus* is the largest genus of the LAB, consisting of 14 phylogenetic groups, with more than 152 species already described. The order Lactobacillales is also the largest in the Firmicutes and in the class Bacilli (Salvetti *et al*., 2012). They are fastidious, and are mostly associated with a wide array of plants and animals, they are part of the human gut and vagina microbiota, and can also be found in the gut of other mammals (Mokoena, 2017). Lactobacilli have been employed for various industrial applications particularly in the fermentation of meat, dairy and plant products (Chaillou *et al.,* 2013).

2.12.2. *Weissella*spp.

Weissella are a group of LAB, being Gram-positive, catalase-deficient and incapable of endospore formation (Björkroth *et al.*, 2014). They belong to the order *Lactobacillales* and family *Leuconostocaceae*, there are 19 known species of *Weissella* (Fusco *et al.,* 2015). They have been isolated from various habitats including plants, saliva, breast milk, human vagina, milk and faeces of animals, a wide range of fermented foods (Kamboj *et al.,* 2015). Certain *Weissella* strains are known potential probiotics useful in the management of periodontal disease (Fusco *et al.,* 2015). Some strains of *W. confusa* and *W. cibaria* are also known producers of large quantity of novel prebiotics mainly dextran which have a variety of industrial uses particularly in bakery and in the making of cereal-based fermented beverages (Fusco *et al.,* 2015). *Weissella ceti* has been implicated in "weissellosis", a bacterial disease of rainbow trouts fish. Some strains of *W.cibara,W. viridescens* and *W. confusa* have also been implicated in opportunistic infections in humans (Kamboj *et al.,* 2015). The genus *Weisella* possesses strains with both medical and technological importance (Björkroth *et al.*, 2014).

2.12.3. *Streptococcus*spp.

These are non-motile, spherical Gram-positive, catalase-negative (except *Strep. didelphis*), facultative anaerobic (some require additional CO_2 to grow) bacteria (Shewmaker *et al.,* 2017). The cells usually appear in pairs and chains when grown in broth, this is because the cell division takes place along a single axis. Streptococci are homofermentative; they are able to ferment carbohydrates to form lactic acid as the primary fermentation product.

Complex media often containing meat extract are often required as a result of their nutritional requirement. For routine microbiological analysis, streptococci can be cultivated on a variety of blood-supplemented media (Whiley and Hardie, 2009). Such media are also useful for the determination of heamolysis in *Streptococcus* species. Many streptococci species are commensals of humans and animals, while a few are pathogenic (Whiley and Hardie, 2009).

2.12.4.*Leuconostoc* spp.

Leuconostoc is a genus consisting of Gram-positive, non-motile, asporogenous and catalase-negative bacteria. They are phylogenetically related to *Lactobacillus* but morphologically composed of ovoid cocci or coccobacillary species (Khalid *et al.,* 2011). In the last ten years, novel *Leuconostoc* species have been reported to be rod-like in morphology (Kot *et al.,* 2014); however, these novel bacilli were later reclassified to another novel genus *Fructobacillus* (Endo and Okado, 2008). Presently, the genus consists only of ovoid cocci species. The species of *Leuconostoc* have optimum growth temperature between 20 and 30^{o}C and may not grow at temperatures beyond 40^{o}C. They are non-acidophilic and obligate heterofermentative in nature (Endo and Okado, 2008). Some *Leuconostoc* species grow better under aerobic conditions as a result of production of ATP by acetic acid formation. Certain species of *Leuconostoc* are employed in food biopreservation; they are known producers of bacteriocins with inhibitory activity against food-borne pathogens (Zacharof and Lovitt, 2012).

2.12.5. *Pediococcus* spp.

Pediococci are catalase-negative, non motile, oxidase-negative, Gram-positive cocci. They exist as microaerophilic or facultative aerobes. During cell division, the bacteria cells divide at right angles in two planes leading to the formation of tetrad morphology particularly when grown in broth (Haakensen *et al.,* 2009). A close relationship between *Pediococcus* and *Lactobacillus* has been revealed by rRNA and other molecular analysis and are thus phylogenetically positioned within *Lactobacillus* cluster (Zheng*et al.,* 2015). Some strains of pediococci are implicated in human infections and are regarded as opportunistic pathogens. They are capable of causing infections in people with debilitating immune functions. Some

multi-drug resistance strains with known resistance to vancomycin, teicoplanin, cephalosporins and metronidazole have been documented; this trait may give them competitive advantage and make infections caused by such strains difficult to treat (Haakensen *et al.,* 2009). A number of pediocins which is a type of bacteriocin are produced by some species of *Pediococcus*. These bacteriocins produced are useful in the prevention of meat spoilage caused by *Listeria monocytogenes* (Todorov, 2009).

2.12.6. *Enterococcus* spp.

Enterococcus species are Gram-positive, ovoid or cocci often occurring in pairs or short chains or they can also be arranged in groups, particularly when grown on agar media and are often difficult to distinguish from *Streptococci* on physical characteristics alone (Sistek *et al.,* 2011). Enterococci were initially classified as group D *Streptococcus* until they was separated on the basis of DNA-DNA and DNA-rRNA hybridization results which revealed the need for a separate genus classification. The separation of the genus *Enterococcus* from *Streptococcus* was later confirmed by 16S rRNA oligonucleotide cataloging (Byappanahalli *et al.,* 2012). They are generally catalase negative; however, some strains have pseudocatalase activity when grown on blood supplemented agar media. They are usually facultative anaerobic, homofermentative and chemo-organotrophic organisms. There are 43 species of enterococci recognized till date (Holzel *et al.,* 2010).

Species of *Enterococcus* are of both food and public health importance, but their involvement with food can be hazardous, as they can cause spoilage, or of benefit, as they are involved in ripening and giving aroma to certain locally fermented foods (Hanchi *et al.,* 2018). Enterococci have also been employed in the treatment of food borne and antibiotic-associated diarhoea, however, they have also been implicated in hospital acquired bacteraemia, endocarditis and other infections and are regarded as opportunistic pathogens (Hanchi *et al.,* 2018). *Enterococcus* spp. have not obtained the GRAS status, although some species are being used as probiotic feed additives for growth enhancement and prevention of diarrhea in livestock (Huys *et al.,* 2013). Enterococci have been isolated in a number of ecological niches ranging from soil, waste waters, manure slurry, vegetables, gut of warm blooded animals including human (Hölzel *et al.,* 2010).

2.12.7. *Vagococcus* spp.

The genus *Vagococcus* consists of facultatively anaerobic, catalase negative, Gram-positive ovoid bacteria cells. They carry out fermentative metabolism as a result of their chemo-organotrophic nutrition (Wullschleger *et al.,* 2018). The genus *Vagococcu*s and *Enterococcus* particularly share many similar traits and they have a close phylogenetic relationship, resulting in difficulties in differentiating them based on phenotypic features alone (Mattarelli *et al.,* 2014). Many conventional phenotypic identification methods have been employed to differentiate between these closely related genera. Molecular techniques employing the use of genus and species specific short nucleotide probes and checkerboard hybridization have proven to be vital in differentiating species of *Vagococcus* from other related genera (Holzapfel and Wood, 2014). Commercially available biochemical test schemes such as API Zym have been very useful in distinguishing species of *Vagococcus.Vagococcus* have been cultured from various veterinary and clinical samples (Al-Ahmad *et al.,* 2008).

2.13. Guidelines for selecting probiotic strains

Not all LAB are probiotic, there are several critical guidelines recommended by Food and Agricultural Organization on the minimum requirement for selecting probiotic strains (FAO/WHO, 2002). These are discussed below:

2.13.1. Proper identification of strain

Considering that probiotic functions are strain specific characteristics, probiotic strain designation is vital. It is imperative to associate specific health benefit(s) to a particular probiotic strain. Proposed probiotic microorganism should be identified to the strain level which must be correctly done with both phenotypic and genotypic methods (Kapitula, 2008) and the specific strain identified with an alphanumeric designation e.g.*Lactobacillus casei* DN-114. Molecular methods such as whole genome sequencing, sequencing of 16S rRNA genes and DNA-DNA hybridization have been used for strain identification. Correct identification of probiotic organisms with generally approved methods is also important for epidemiological surveillance purposes (Herbel *et al.,* 2013). It is stipulated according to FAO/WHO, that probiotic strains be registered in internationally recognized culture collections (Kapitula 2008).

2.13.2. Assessment of safety

Proposed probiotic strains should not have detrimental effects in the intended host i.e., must be nonpathogenic, non-toxic, non haemolytic etc. They must earn the Qualified Presumption of Safety (QPS) status prescribed by European Food Safety Authority (EFSA). Antibiogram, including MIC to medically important antibiotics should be determined. Potential probiotic strains are not expected to possess antibiotic resistance determinants (Gueimonde *et al.,*2013). Evaluation of toxin production should be carried out in bacterial strains belonging to species that are known producers of mammalian toxins. The safety profile of probiotic strains can also be substantiated by the inability of such strains to demonstrate infectivity in immunodeficient animal models (Papadimitriou *et al.,* 2015). Epidemiological surveillance of untoward effects of probiotic products in the host is also a crucial safety requirement (Arturo *et al.,* 2016).

2.13.3. Functional considerations

2.13.3.1. Ability to resist gastric condition

Potential probiotic LAB strains considered for oral probiotic use must be capable of surviving the gastric condition in the intended host. The gastrointestinal tract consists of a hostile environment characterised by low pH and bile salt, therefore probiotic organisms must survive in sufficient quantity capable of conferring health benefits on the host. Potential probiotic organisms of gut origin tend to have better chances of surviving the gastric conditions than those cultured from other environmental samples (Giraffa, 2012).

2.13.3.2. Adherenceand ability to colonize host's epithelial cells

Bacterial strains with probiotic potentials must possess the ability to adhere to the mucosa of the intestine and epithelial cells. This is a vital requirement for host colonization and survival of such strain. Successful colonization of the intestinal mucosa by probiotic organisms is crucial for inhibition of pathogens by competitive exclusion and immune modulation. Microorganisms with poor adherence to epithelial cells are likely to be easily washed away and prevented from colonizing the host for effective probiotic benefit (Miljkovic *et al.*, 2015).

2.13.3.3. *In vivo*validation of health benefits

Probiotics are capable of exerting health benefits through various activities in the host. *In vitro* tests alone may not be sufficient to substantiate the health benefits of probiotic organisms in the host. *In vivo* experiments are important in validating *in vitro* health benefit potentials of probiotic organisms (Vinderola *et al.,* 2017).

2.13.4. Antimicrobial activities against pathogens

Production of inhibitory metabolites against pathogens is a crucial requirement for selecting probiotic strains. Probiotic LAB are endowed with the capacity to produce inhibitory substances against pathogens. Most LAB produce antimicrobial metabolites during fermentation such as lactic, acetic, propionic acids, etc. (Ayeni *et al.,* 2011). Certain probiotic organisms also synthesize bioactive peptides and other proteinaceous inhibitory (Mokoena, 2017).

2.13.4.1. Organic acids

The end product of sugar fermentation by LAB includes organic acids: lactic, butyric, acetic and propionic acids (Ayeni *et al.,* 2011). These organic acids lead to reduction in pH of the growth medium, resulting in the inhibition of competing microorganisms. The antimicrobial activity of organic acids is largely by interfering with the integrity of the cell membrane, lowering of intracellular pH, inhibiting active transport and the various metabolic functions of the microorganisms (Ni*et al.,* 2015).

2.13.4.2. Hydrogen peroxide

Lactic acid bacteria lacking the heme group do not require the cytochrome system and therefore incapable of reducing oxygen to form water, and this results in the synthesis of hydrogen peroxide from the activities of NAD peroxides or flavoprotein oxidases. The quantity of hydrogen peroxideproduced by LAB has bacterial inhibitory potentials particularly against bacteria species lacking catalase peroxidase. Hydrogen peroxide can also serve as precusors in the formation of free radicals such as hydroxyl radical and superoxides which are capable of causing bacterial cell death as a result of oxidative damage in bacterial DNA. The production of hydrogen peroxideis also an important bacterial antagonistic mechanism in LAB (Ayeni *et al.,* 2011; Borges *et al*., 2013; Mokoena, 2017).

2.13.4.3. Bacteriocin

Lactic acid bacteria are known producers of bacteriocins; which are bioactive peptides ribosomaly synthesized during the primary phase of growth (Zacharof and Lovitt, 2012). Most of the bioactive peptides produced by LAB except nisin and pediocin possess a narrow spectrum of antimicrobial activity. Bacteriocins, particularly those produced by LAB of animal gut origin are easily degraded by proteolytic enzymes which make them safe for use in humans (Zachrof and Lovitt, 2012). They have been reported to be efficient as natural preservatives with antimicrobial activity against food spoilage pathogens; *Listeria monocytogenes, Bacillus cereus* and *Clostridium botulinum*.Bacteriocins have a wide variety of size, structure, mechanism of action, spectrum of inhibition and target cell receptors. Environmental factors such as temperature and pH of the growth medium can influence the regulation of bacteriocin production.Bacteriocins produced by LAB also tend to have greater antimicrobial activity at lower pH (Fernandez *et al.,* 2013). Bacteriocins can generally be categorized into three classes based on structure and mechanism of antimicrobial action. Nisin is an example of Class I bacteriocins; they are active against most Gram positive bacteria including pathogens and bacteria implicated in food spoilage. Nisin is also the only bacteriocin currently being employed in the food industry. Both Class II and Class III are heat stable bacteriocins, but unlike the Class II, Class III bacteriocins have relatively large molecular weghts (Zacharof and Lovitt, 2012).

Lactobacillus lactissubsp. Lactis has been reported to produce nisin, lactocin, mersacidin which are all class 1 bacteriocins containing lanthionine and methyllanthionine with molecular weight less than 5kDA (Suskovic *et al.,* 2010). Pediocin PA1, sakicin A, leucocin A are class IIa bacteriocins, and they are heat stable hydrophobic peptides known to be produced by *Leuconostoc gelidium* (Todorov, 2009; Zacharof and Lovitt, 2012). Enterocin X and Lactococcin G are typical Class IIb bacteriocins commonly produced by *Enterococcus faecium* (Perez *et al.,* 2014). *Lactobacillus acidophilus* strains are known producers of acidocin B, entereocin P and reuterin 6 (Suskovic *et al.,* 2010). Class III bacteriocins exemplified by Lysostaphin and enterolysin are produced by *Lactobacillus helveticus* (Perez *et al.,* 2014).

2.13.5. Resistance to technological conditions

An important consideration in the selection of probiotic organisms is the ability of the microorganism to be scaled up to obtain enough biomass and number of live microorganism to be included in the probiotic product. This assay can be carried out through the application of mathematical or statistical models which are important in the prediction of the behavior of the microorganisms (Govender *et al.,* 2013). It is very crucial that the probiotic strain remain viable and in amounts sufficient to produce the desired health benefit, hence, it is imperative to determine the survival of the probiotic organisms during technological procedures such as freeze drying and at different storage conditions (Ayeni *et al*., 2011). Probiotic strains can be lyophilized, spray dried and included in different products. Once in these products, they must be able to maintain the probiotic features for which they were selected which include production of organic acids and bacteriocins, adhesion or auto aggregation (Montel-Mendoza *et al.,* 2013).

2.14. Mechanism of probiotic action

The major mechanisms of action of probiotic organisms include: inhibition of pathogens by the production of antimicrobial metabolites, competitive exclusion of pathogens, formation of epithelial barrier, adherence to mucosa of the intestine, adhesion and modulation of the immune system.

2.14.1. Stimulation of immune response

Probiotics are known to exert immunomodulation in the host's immune system by their interaction with epithelial cells and other cells of the immune system. Lactic acid bacteria, like other members of the microflora are able to cross the gut mucous membrane layer and can survive in the spleen and other organs where they are capable of initiating phagocytosis (Azda*et al.,* 2018). The immune system consists of innate and adaptive systems; both can be stimulated by probiotic organisms through binding specifically to the host's immune cell receptors. These receptors initiate the synthesis of chemokines and other immune cells such as the naive and regulatory T cells which are involved in activating dendritic cells and macrophages (Wells, 2011).

The primary response to exogenous microorganisms is stimulated by pattern recognition receptors (PPRs) of which the toll-like receptors are the most studied. Probiotics are capable

of reducing inflammation of the intestine through down regulation of expression of certain toll-like receptors, and secretion of some metabolites that may prevent the entry of tumor necrotic factor into the blood mononuclear cells (Gómez-Llorente *et al.,* 2010). It is now established that probiotics can improve the immunogenicity of oral vaccines including those of rotavirus, cholera and polio (Wells, 2011).

2.14.2. Adherence to mucosa of host's intestine

The ability of probiotic strains to adhere to mucosa of the intestine is a major requirement for colonization and interaction between these strains and the host cells (Bermudez-Brito, 2012). Adherence of probiotic organisms to the mucosa of the intestine is important for inhibition of pathogens by competitive exclusion and modulation of immunity. The release of defensins- small peptides with antimicrobial activity against bacteria, viruses and fungi, can also be induced by probiotic strains from epithelial cells. Furthermore, probiotics help to stabilize the host's gut barrier functions (Wang, 2014). Lactic acid bacteria with probiotic potentials exhibit several surface determinants that are important in the interaction with the mucous membrane and epithelial cells of the intestine. The intestinal epithelial cells produce mucin which is an integral constituent of mucous, thereby inhibiting the adhesion of pathogens (Derrien *et al.,* 2010). This suggests that there is correlation between surface proteins of probiotics and their ability to competitively exclude pathogens from mucous membranes (Van-Tassell and Miller, 2011). It is now known that several *Lactobacillus* proteins are responsible for promoting mucous adhesion, which is exemplified by mucus-targeting adhesion proteins produced by *L. reuteri* (Van Tassell and Miller, 2011).

2.14.3. Competitive exclusion of pathogens

The mechanism used by probiotic strains to gain competitive advantage over other microorganisms include: creation of unconducive microecology, blocking of bacterial receptor sites, synthesis of inhibitory metabolites and depletion of available nutrients (Bermudez-Brito *et al*., 2012). The interaction between probiotic surface proteins and mucins can also inhibit adhesion and subsequent gastrointestinal colonization of pathogens. Some probiotic strains share similar carbohydrate-binding requirements with some enteric pathogens, giving the strain the opportunity to effectively compete with the pathogens for

host's receptor sites (Howarth and Wang, 2013). Inhibitory metabolites such as organic acid and bacteriocins usually lead to a detrimental modification of the environment (Adeniyi *et al.,* 2015). Organic acids particularly lactic and acetic acids possess potent inhibitory activities against Gram-negative organisms and have been adjudged the major inhibitory substances responsible for probiotic activity against pathogens (Suskovic *et al.,* 2010). The mechanism of antimicrobial activity of bacteriocin is largely by distruption of the target bacteria cell through inhibition of cell wall formation (Fernandez *et al.,* 2013). It has been established by several authors that production of bacteriocin confers a comparative survival advantage on the producers within the microecology (O'Shea *et al.,* 2012).

2.15. Current global application of probiotics in livestock management

Probiotic feed additives have been reported to be beneficial in livestock farming in; increasing the efficiency of feed conversion, increasing egg/milk production, enhancing weight gain as well as reducing lowering mortality rates (Park *et al.,* 2016). In calves, diarrheoa remains a major cause of mortality. Prevention of diarrhea is therefore important in the promotion of the growth of calves. Probiotics have been developed as effective growth promoters in improving animal health and productivity (Allen *et al.,* 2013). Gut colonization of calves early in life by LAB has been reported to prevent the colonization of the intestinal mucosa by enteric pathogens (Uyeno*et al.,* 2015). Gut microflora rich in LAB have been demonstrated to enhance weight gain and boost immune response in calves (Al-Saiady, 2010). In poultry, probiotic strains of *Lactobacillus* prevented *Salmonellaenterica* serovar Enteritidis infection (Hossain *et al*., 2012). Meat obtained from broilers fed with certain probiotic strains displayed higher content of moisture, protein and ash compared to the controls (Park*et al.,* 2016). The result showed that chicken fed with probiotics had better retention of minerals especially phosphorus, calcium and nitrogen as well as protein efficiency ratio. Higher protein efficiency ratio may subsequently help promote meat yield as observed by Hossain *et al*., (2012) where addition of probiotics increased breast weight in chicken as well as carcass quality with lesser occurrence of *Salmonella* contamination.

2.16. Probiotics in calves

A very important stage in cattle husbandry is transition from the monogastric phase of suckling calves to the herbivore condition. At this stage the pre-gastric fermentative apparatus must be active to effectively digest fibrous plant materials (Gaggìa*et al.,* 2010). In calves, at the pre-ruminant stage, probiotics are generally targeted at the lower intestine; it is an important strategy of stabilizing the gut microbial community and reducing the risk of bacterial infection. Lactic acid bacteria are well known probiotic feed additive for calves with the benefit of balancing the gut microbiota and improving animal health (Uyeno *et al.,* 2015). Antimicrobials are widely used to enhance the performance of calves and reduce diarrhea, unfortunately the risk of antibiotic resistance, release of potent antimicrobials into the environment as well as antibiotic residue in animal product associated with the use of antibiotics in such practice has necessitated the need for alternative measures (Van Boeckel *et al.,* 2015). Probiotics are considered as useful alternatives to antibiotics in the improvement of livestock productivity (Allen *et al.,* 2013). Although the efficacy of feeding probiotics to calves for the prevention of specific pathogens in the gut microbiota have been established, their interaction with the whole gut microbial community remains unclear (Gaggìa*et al.,* 2010). As earlier mentioned, the population of lactobacilli and bifidobacteria reduces with age in calves; it is important to balance the microbial ecosystem of the gut by increasing the population of these beneficial microorganisms for a successful calf rearing (Uyeno *et al.,* 2010). The addition of beneficial microorganisms to feed from birth allows the establishment of these probiotic organisms in the gut microflora of calves and helps reduce fatalities due to calfhood enteric pathogen infections. A microbiota with a stable *Lactobacillus* species load is known to enhance weight gain and immune response in calves (Al-Saiady *et al.,* 2010).When livestock are exposed to stressful conditions, the growth of the normal flora can become impaired thereby increasing the risk of infection by potential pathogens. Under stressed rearing condition, probiotic additive in calves have been shown to mitigate the risk and severity of diarrhea caused by dysbiosis (Uyeno *et al.,* 2015).

2.17. Probiotic formulation and storage

Probiotic products are expected to contain sufficient quantity of live cells capable of producing the desired health benefits up to the expiry date. For successful delivery of

probiotic preparations at the target sites, probiotic organisms must remain viable throughout the production stages, storage and shelf life (De Vos *et al.,* 2010). A major difficulty in probiotic preparation is the retention of viability of the probiotic bacteria over the shelf life. Technological processes during manufacturing and storage are major factors that can affect the viability of probiotic organisms (Gueimonde and Sanchez, 2012). Sufficient quantity of the probiotic preparation is required to be consumed in order to deliver the adequate population of live bacteria to the gut, owing to the possibility of viability loss usually encountered during gastric transit. It is suggested that a minimum of 10^7 CFU/mL viable cells must be available, therefore higher quantity have been proposed to make up for possible viability loss (Liliana and Vladimir, 2013). Several market surveys have revealed that much lower viable cells count than required for health benefits have been recorded in many probiotic products even before the expiry date. The shelf life in most probiotic product is unpredictable, such that about 200% live cells are deliberately included in probiotic products by many manufactures to make-up for possible viability loss before the product reaches the end users. This significantly increases the cost of production and makes label claims unreliable (Liliana and Vladimir, 2013).

Cultures of probiotic bacteria intended for food incorporation are usually supplied frozen or in dried form, either as spray-dried or lyophilized powders (Ayeni *et al.,* 2011). Different strains of lactobacilli and bifidobacteria have been successfully dried, however, the extreme temperature and osmotic pressure required for spray drying usually reduces the survival of most probiotic lactobacilli as a result of stress arising from temperature changes and drying which tend to damage proteins and cell membranes (Gueimonde and Sánchez, 2012). Spray-dried powder containing large amount of viable probiotic cells is a convenient way of storing and transporting probiotic cultures.Although it is a cost effective method for the large-scale production of bacterial cultures, it suffers from a setback of causing bacterial cell death due to heat and dehydration (Liliana and Vladimir, 2013). A very useful approach to circumvent these challenges is by adding thermo-protectants such as adonitol, granular starch, and gum acacia to the media before drying; these will enhance viability of probiotic bacteria cultures during drying and storage (Gueimonde and Sánchez, 2012). Incorporation of cryoprotectants during lyophilisation of lactobacilli has proven useful in circumventing

inactivation occasioned by the drying process and enhances product stability during storage (Liliana and Vladimir, 2013).

Furthermore, encapsulation of bacterial cells is a means of providing protection for the viable cells from extreme heat or moisture that may be encountered during drying and storage, this technique is increasingly gaining popularity in the probiotic industry (De Vos *et al.,* 2010). It was established that encapsulating lactobacilli in calcium-alginate beads further improved their ability to tolerate heat while encapsulation of spray-dried*Bifidobacteriumruminatium* prolonged their viability during storage (Liliana and Vladimir, 2013).

2.18. Enterobacteriaceae

Enterobacteriaceae is a diverse family of Gammaproteobacteria and also the only family within the Enterobacteriales (Potter *et al*., 2018). They are ubiquitous, found in numerous ecological niches (Jenkins *et al*., 2017). Advancement in next generation sequencing has improved the taxanomic understanding of the complexity within the family; closely related species in the same genus have been resolved and similarities between species of different genera have also been identified (Potter *et al.,* 2018). Enterobacteriaceae currently consist of more than 210 species and 53 genera with increasing number due to taxonomic changes in taxa of medical importance (Jenkins *et al*., 2017). Many strains of Enterobacteriaceae are of medical importance not only because they are pathogens but also because they serve as reservoirs for mobile genetic determinant of antibiotic resistance (Potter *et al.,* 2018). Some species are components of the microflora of animals while many are frequently implicated in intestinal and extra-intestinal infections (Leimbach *et al.,* 2013). Examples of enterobacteria implicated as opportunistic pathogens include: *Escherichia, Shigella, Enterobacter, Proteus, Morganella, Providencia, Klebsiella, Salmonella,Serratia* and *Citrobacter.*

2.18.1. *Shigella*

*Shigella*spp. are Gram-negative, non-motile, non-spore-forming pathogenic enterobacteria. They are closely related to *E. coli* but have evolved with certain traits of pathogenicity (Ud-Din and Wahid, 2014). It consists of four subgroups and several serotypes identified based on the structural arrangement of O-antigen comprising their lipopolysaccharide; *Shigella*

flexneri represented by14 serotypes, *S. boydii* comprising 20 serotypes, *S. sonnei* with only 1 serotypeand*S. dysenteriae* which consist of 15 serotypes, all of which are able to cause disease in humans (Zhang *et al.,* 2011). The global incidence of shigellosis is about 165 million cases annually, with approximately 1.1 million mortality yearly, particularly in children under 5 years old (Schroeder and Hilbi, 2008). It is an acute enteritis with clinical manifestations including mild diarrhea, inflammatory bacillary dysentery marked by violent abdominal upset, fever, mucoid and bloody stools (Marteyn *et al.,* 2012). Shigellosis is often a self-limiting disease but may become fatal in people with compromised immune functions or when adequate medical support is unavailable (Schroeder and Hilbi, 2008). Systemic complications such as septicemia, electrolyte imbalance, intestinal perforations, seizures and heamolytic uremic syndrome may occur. Shiga toxin producing *S. dysenteriae* 1 causes the most severe infections resulting in mortalitywhile endemic bacillary dysentery is mostly caused by *S. flexneri* and *S. sonnei* (Marteyn *et al.,* 2012). Multidrug resistant strains including those resistant to fluoroquinolones have been observed, which increases the risk of therapeutic failure in severe life threating cases of shigellosis (Zhang *et al.,* 2011). There are currently no vaccines available against *Shigella* spp., although there are several potential protective *Shigella* vaccines for immunization at various developmental stages and clinical trials (WHO, 2006).

2.18.2. *Klebsiella*

The genus *Klebsiella* consists of a diverse group of organisms capable of causing diseases in humans and animals while some exist in a symbiotic relationship as nitrogen fixing endophytes in plants (Hazen *et al.,* 2014). Some members formally included in the genus including *Klebsiella planticola* and *Klebsiella ornithinolytica* have now been reclassified into a new genus, *Raoultella* (Paczosa and Mecsas, 2016). The most studied species based on clinical significance is *K. pneumoniae.* The nomenclature of *Klebsiella* is somewhat complex as there are phylogenetically diverse *K. pneumoniae* isolates that are most likely to be representatives of a distinct species, for example is the recently described *Klebsiella variicola* (Hazen *et al.,* 2014).

Klebsiellapneumonia is a Gram-negative, encapsulated and non-motile enterobacterium. It is an opportunistic pathogen commonly found in environmental sources, including soil, waste waters and medical devices (Rock *et al.,* 2014). It is associated with various

community and nosocomial infections such as UTI, respiratory tract infections, bacteremia and liver abscess in humans (Paczosa and Mecsas, 2016). Initially, *K. pneumoniae* was thought to primarily cause serious infections in people with compromised immune functions, but the recent development and dissemination of hypervirulent strains have resulted in infections of healthy individuals with intact immune functions. Furthermore, the rapid upsurge in the development of multidrug resistant strains of *K. pneumonia* strains has become a global health challenge (Paczosa and Mecsas, 2016).

2.18.3. *Proteus*

Proteus spp. are Gram-negative motile rods of the family enterobacteriacea. *Proteus* consists mainly of 5 species; *Proteus mirabilis*, *P. penneri*, *P. vulgaris*, *P. myxofaciens* and *P. hauseri*. *Proteus* is related to *Morganella* and *Providencia* all being members of the tribe *Proteeae* (Giammanco *et al.,* 2011). Proteus usually colonisesthe gastrointestinal tract of humans and animals as commensals (Hamilton *et al.,* 2018). A peculiar microbiological characteristic ofspecies in this genus is their motility; they possess a few peritrichous flagella used for swarming. Swarming is seen macroscopically on solid media as a concentric ring originating from an individual colony and overtaking other species present (Liu *et al.,* 2016).

They are usual inhabitants of a variety of niches including soil, surface water and sewage (Armbruster *et al.,* 2018). *Proteus mirabilis* is not a major cause of UTI in healthy hosts; they are mostly implicated in infections of the catheterized urinary tract also referred to as catheter-associated UTI (CAUTI) (Armbruster *et al.,* 2018). *Proteus* are also reputable aetiologic agent of several infections of eye, wound and gastrointestinal tract in humans. *Proteus mirabilis* have also been recently implicated in neonatal meningoencephalitis, empyema, and osteomyelitis (Schaffer and Pearson, 2015).

2.18.4. *Citrobacter*

The genus *Citrobacter* comprises 11 species of citrate utilizing, oxidase negative, facultative anaerobic, motile, Gram-negative bacilli. Species of *Citrobacter*commonly implicated in human infections include *C. freundii, C. youngae,C. koseri*, *C. braakii* and *C. amalonaticus* (Ariza-Prota *et al.,* 2015). *Citrobacter* are commonly isolated from environmental samples such as water and soil. They are also occasional colonizers of the guts of humans and

animals, strains cultured from human guts are thought to have low virulence. This notwithstanding, they have been implicated in infections of the respiratory tract, urinary tract, wound, bone, peritoneum, endocardium, central nervous system and bloodstream. Individuals with compromised immune functions are particularly susceptible to *Citrobacter* infections, caused by *Citrobacter freundii* and *Citrobacter koseri* while *C. koseri* causes meningitis and brain abscess with high mortality in neonates (Ariza-Prota *et al.,* 2015). *Citrobacterrodentium* is a host specific pathogen restricted to mice but genetically similar to EPEC and EHEC of human origin (Petty *et al*., 2009).

2.18.5. *Enterobacter*

The genus *Enterobacter* consists of Gram-negative, non-spore-forming enterobacteria. They are saprophytic in nature, as they are found in waste water, soil and sewage (Mezzatesta *et al.,* 2012). The taxonomy of the genus *Enterobacter* has been reviewed repeatedly. Six phenotypically and genetically similar species (based on DNA relatedness to *E. cloacae*) have been identified and merged within a genetic complex referred to as "*Enterobacter cloacae* complex", i.e. *E. cloacae*, *E. asburiae*, *E. dissolvens*, *E. hormaechei*, *E. kobei*, and *E. nimipressuralis*.*Enterobacter aerogenes* and *E. cloacae* are two well-known species of clinical significance due to their emergence as opportunistic and nosocomial pathogens in patients under intensive care and those on mechanical ventilation (Mezzatesta *et al.,* 2012). *Enterobacter aerogenes* is commonly isolated from samples of blood, human respiratory, urinary, and gastrointestinal tract (Davin-Regli and Pages, 2015). There is a rapid transference of genes coding for carbapenemases in addition to extended spectrum β-lactamases (ESBL) within *E. cloacae* strains. *Enterobacter cloacae* is recently noted to be the most common enterobacteria involved in hospital acquired infections after *E. coli* and *K. pneumoniae* (Potron *et al*., 2013).

2.18.6. *Morganella*

Morganella species were initially reffered to as Morgan's bacillus and was later re-classified as *Bacillus morganii.*They are members of the tribe Proteeae also consisting of *Proteus* and *Providencia* which share some biochemical and clinical characteristics (Vanyushin, 2007).

They are motile, non-lactose fermenting Gram negative members of the*Enterobacteriaceae* with about 4,000,000 bp genome size (Olaitan *et al.,* 2014).

Like *Proteus,* they also produce urease but lack swarming ability and hydrogen sulphide production. The genus currently has only one species with two recognized subspecies, namely *M. morganii* subsp. *Morganii* and *M. morganii* subsp. *Sibonii* (Liu *et al.,* 2016).

M. morganii is widely distributed in environmental sources and in the GIT of humans and animals as constituents of the microflora (Lee *et al.,* 2009). It is considered an opportunistic pathogen known to cause both hospital-acquired and community infections. It is also been implicated in sepsis, urinary tract infections, wound infections, polymicrobial infections and rarely CNS infections in humans (Parikh *et al.,* 2011).

The urinary tract is the main port of entry of *M. morganii*. This is followed by the hepatobiliary tract, skin, soft tissue and blood. It is now being regarded as an important pathogen due to its increasing antimicrobial resistance and virulence which has led to high morbidity and mortality in human population (Liu *et al.,* 2016*). M. morganii* is equipped with virulence factors including fimbrial adhesins, LPS, IgA protease, type-III secretion system, hemolysins, ureases etc. as revealed by genome sequencing. Intrinsic resistance has been observed in *M. morganii* to almost all classes of antibiotics (Liu *et al.,* 2016*).* Furthermore*,* they have an unusual ability for extracellular biosynthesis of crystalline silver nanoparticles (Parikh *et al.,* 2011).

2.18.7. *Providencia*

The genus *Providencia* is closely related to *Morganella* and *Proteus*. It consists of urease and phenylalanine deaminase producing Gram negative bacilli (Galac and Lazzaro, 2011). The species include *Providencia stuartii*, *P. rustigianii*, *P. rettgeri*, *P. alcalifaciens* and *P. heimbachae. Providencia rettgeri*and *P. stuartii* are the commonest causes of human infections, known to cause traveler's diarrhea and urinary tract infections, but also implicated in more severe infections such as pneumonia, bacteraemia and meningitis in humans (Sipahi *et al*., 2010).

Unlike most members of the family Enterobacteriaceae, *Providencia* spp. exhibit innate resistance to colistin and tigecycline which often leads to therapeutic failure and difficulty in treatment of infections with multidrug-resistant (MDR) strains (Abdallah and

Balshi,2018).*Providencia* species are generally susceptible to meropenem, amikacin, aztreonam and cephalosporins. However, strains of*Providencia* species showing resistance tocarbapenem are increasingly being reported with the production of carbapenemase as the main mechanism of resistance to carbapenems (Abdallah and Balshi,2018). Plasmid-mediated antimicrobial resistnce mechanisms exemplified by ESBLs, among others have also been reported in strains of *Providencia* cultured from hospital-acquired infections (Oikonomou *et al.,* 2016).

2.18.8. *Serratia*

The genus *Serratia* consists of Gram-negative, facultative anaerobic bacilli, belonging to the enterobacterial group (Hadid *et al.,* 2015). They are not usual members of the microflora in human but predominantly distributed in the environment. The taxonomy of this genus is very complex, there are 14 species currently recognized; *S. marcescens, S. fonticola, S. proteamaculans, S. quinivorans, S. ficaria, S. entomophila, S. entomophila, S. glossina*and*S. nematodiphila* as examples (Mahlen, 2011). *Serratia* species were initially regarded as non-pathogens due to their low virulence in immunocompetent individuals (Kim *et al.,* 2015). *Serratia marcescens* is the main human pathogen implicatedin a number of diseases such as peritonitis, urinary tract infection, respiratory tract infection, wound infections, endocarditis and life-threatening bacteraemia (Hadid *et al.,* 2015). Septic arthritis and osteomyelitis are rare in healthy individuals but have been reported in immunocompromised hosts (Hadid *et al.,* 2015). Most strains of *Serratia* like other enterobacteria possess intrinsic resistance to β-lactam antibiotics including combination therapy exemplified by amoxicillin-clavulanate and ampicillin-sulbactam, the macrolides, clindamycin, linezolid, cephalosporins, cephamycins, cefuroxime, nitrofurantoin and rifampin (Mahlen, 2011). Most *Serratia* species are generally susceptible to the aminoglycosides while some strains of *S. marcescens* are being reported to harbor chromosomally borne ampC gene and carbapenemases with extended beta-lactam resistance potential (Mahlen, 2011).

CHAPTER THREE

MATERIALS AND METHODS

3.1. Materials

3.1.1. Major equipment, media and other materials

Microscope (Nikon, Japan), GelMax® Imager (UVP,USA), Water Bath (Grant, UK),Autoclave (Dixon, UK), Incubator (Gallenkamp, UK), Centrifuge (Eppendorf, Germany)Freeze Dryer (ALPHA, Germany), PCR Thermal Cycler (Applied Biosystem, Singapore), Weighing Scale (OHAUS, USA)HPLC System (Adept CE, UK), VITEK® 2 Compact System (Biomérieux, Germany),Microbact™24E (Oxoid, UK), VITEK®Mass Spectrometry System (Biomérieux, Germany), Fast-Prep™ Machine(BioSpec, USA)Genomic DNA Extraction Kit (Bioneer, South Korea), *Salmonella-Shigella* Agar (Oxoid, UK), Xylose Lysine Deoxychollate Agar (Oxoid, UK), Mann Rogosa Sharpe Agar and Broth (Oxoid, UK), Mueller Hinton Agar (Oxoid, UK), Tetrathionate Broth (Oxoid, UK), Tripple Sugar Iron Agar (Oxoid, UK), Epsilometer Test Strips (Biomérieux, France).

3.1.2. Bacterial strains

Salmonella enterica subsp. enterica serovar Typhimurium (ATCC 14028) was obtained from the Molecular Microbiology Laboratory of the Pharmaceutical Microbiology Department, University of Ibadan. *Staphylococcus aureus* A104, *Klebsiella spp*, *Pseudomonas aeruginosa* and an ESBL producing *Escherichia coli* T51were obtained from the culture collection of our research group.

3.1.3 Experimental animals

Eight (8 week-old) New Zealand White rabbits bred at the Rabbit Production Division and Nine calves (≤ 3 months, Sokoto Gudali) obtained from the Dairy Unit, Department of Veterinary Medicine, University of Ibadan were usedin *in-vivo* experiments.

3.2. Methods

3.2.1. Sample collection

3.2.1.1. Sample size determination for *salmonella* isolation

The sample size required for determination of the prevalence of *Salmonella* spp. on the study site was calculated as described by Daniel *et al.,* (1999).

Prevalence was determined at 10% (Umeh and Enwura, 2014).

$$n = \frac{Z^2 * (p) * (1-p)}{d^2}$$

n = Size of sample

Z = confidence level

Z value (e.g. 1.96 for 95% confidence level)

p = previous prevalence value, expressed as decimal

d = confidence interval (e.g., .0 = ±5)

n= 138

3.2.2. Ethical approval

All procedures involving handling animals were reviewed and approved by the Animal Care and Use Research Ethics Committee (ACUREC) of the University of Ibadan with the approval number UI-ACUEC/17/0011 (Appendix IV). All procedures involving animals were carried out with the supervision of an experienced veterinarian.

3.2.3 Samples for bacterial isolation

Salmonella spp. and LAB were cultured from one hundred and thirty eight and 40 different fresh bovine fecal samples respectively. Non repeated samples were collected (immediately after defecation) with disposable gloves into sterile sample collection bottles from different ear-tagged cattle (Sokoto Gudali breed), average age of 2.0 ± 0.5 years, housed at the dairy unit of the Teaching and ResearchFarm of the University of Ibadan (UI-T&RF). All the animals sampled were confirmed to be healthy by the resident veterinarian. The collected faecal samples were analysed at the Pharmaceutical Microbiology Laboratory.

3.2.4. Isolation and identification of bacteria

3.2.4.1. Isolation of *Salmonella* species

Salmonellae were isolated from bovine faeces by a modification of the method suggested by the International Standard Organisation (ISO-6579, 2000) as follows; 10 g of cattle faeces was enriched in 90 mL of buffered peptone water (Oxoid) and incubated at 37^0C for 24 hours.

One mL of the enriched sample was transferred into 10 mL of Tetrathionate-Novobiocin broth (Oxoid, UK) and incubated at 37^0C for 24 hours. A loopful of the broth culture was then inoculated on Xylose Lysine Deoxycholate agar (Oxoid, UK) and *Salmonella*-Shigella agar (Oxoid, UK) and incubated for 24 hours at 37^0C.Characteristic *Salmonella* colonies were further stabbed in Triple Sugar Iron agar with an inoculating wire and incubated for 24 hours at 37^0C.

Colonies with typical *Salmonella* characteristics were further confirmed by genus specific PCR (Hendriksen, 2002). *Salmonellaenterica*Typhimurium ATCC 14028 was used as a positive control during cultural analysis and PCR.

3.2.4.2. Identification of *Salmonella* spp.by Microbact™ 24E system

Isolates presumed to be *Salmonella* spp. based on their cultural characteristics on selective and differential media were tested with Microbact ™ 24E system (Oxoid) according to the manufacturers guide. The Microbact ™ 24E system is a simplified biochemical based identification system used for identifying Enterobacteriaceae and miscellaneous Gram-negative bacteria. Identification of microorganisms with this system is based on pH change and biochemical substrate utilization (Farmer, 1985). It utilizes 24 different biochemical reactions that produce distinct colours after an overnight incubation.

Three pure colonies of overnight culture of each presumed *Salmonella* isolate were emulsified in 5ml sterile saline and mixed thoroughly into a homogenous suspension. One hundred microliter µL of the resulting cell suspension was used to inoculate and reconstitute each well and the substrates were overlaid appropriately with mineral oil. The inoculated rows were sealed with the adhesive seal, labeled appropriately and incubated at 36±2 °C for 24 hours. An 8 digit code was generated which was read with the accompanying identification software (Oxoid Microbact) 2000 version 2.03 and interpreted based on the

manufacturer's instruction. The percentage identity obtained for each isolate represented the percentage share of the probability for that organism as part of the probabilities for all choices.

3.2.4.3. Identification of *Salmonella*isolates with genus specific primers

Three to five pure colonies of presumed *Salmonella* isolates were suspended in 50µL of molecular grade water, boiled at 100°C for 10 minutes, cooled on ice, and then centrifuged at 10,000 rpm for 10 seconds. The supernatant containing the DNA was removed and used as DNA template for PCR reaction targeting the 284 bp region of *SalmonellainvA* gene with the primers: Sal1 (5′-GTGAAATTATCGCCACGTTCGGGCAA-3') and Sal 2 (5′-TCATCGCACCGTCAAAGGAACC-3').

The Polymerase chain reaction was conducted in a 25µL reaction tube containing Ready-To-Go™ PCR master mix beads (GE Healthcare Lifescience™ illustra™ PuReTaq) with the isolate's DNA as the template. *S.enterica* serovarTyphimurium ATCC 14028 and *E. coli* T51 served as positive and negative controls respectively. The amplification was achieved in an Eppendorf Thermocycler with PCR conditions consisting of an initial incubation step at 94^{o}C for 1min, 35 cycles of 94^{o}C for 1min, followed by annealing at 64^{o}C for 30 sec and elongation at 72 ^{o}C for 30 sec, followed by 7 min at 72 ^{o}C. The amplicons were separated on agarose gels (1.5%), stained with ethidium bromide solution after electrophoresis and visualized under UV light with an expected amplified PCR product of 284bp. The molecular size marker used was a 100 bp DNA ladder.

3.2.4.4. Minimum inhibitory concentrationof *Salmonellaenterica*isolates

The Minimum Inhibitory Concentration (MIC) (µg/mL) of the 32 *Salmonella* isolates to a panel of antibiotics was determined by the automatic Vitek 2 compact system (Biomérieux, Nuertingen, Germany), with the AST-N248 cards. Bacterial suspension was prepared by emulsifying the cells in 0.45% saline to equivalent of 0.5% McFarland. The cards were filled, sealed and loaded into the Vitek 2 system for incubation and reading. The *Salmonella* isolates were classified as susceptible (S), intermediate (I) or resistant (R) by the automated machine using standard breakpoints

3.2.4.5. Isolation of lactic acid bacteria

The method described by Ayeni *et al* (2009) was employed for the isolation of lactic acid bacteria. Briefly, one gram of cattle faeces was added into 9 mL of MRS broth (Oxoid, UK) and incubated at 37^{o}C under microaerophilic condition (CampyGen^{TM} Oxoid, UK) for 24 hours. The resulting culture was serially diluted and plated out on MRS agar (Oxoid, UK) and incubated under microaerophilic condition for 48 hours. Single colonies from the MRS plate were sub-cultured and pure cultures were obtained based on colony and cell morphology. Gram's staining and catalase reaction (3% hydrogen peroxide) was carried out to select presumed LAB isolates.

3.2.4.6. Molecular identification of lactic acid bacteria isolates

Lactic acid bacteria were primarily identified by partial sequencing of the 16S rRNA genes. Extraction of the genomic DNA was done with *AccuPrep*® DNA Extraction kit (Bioneer, South Korea) based on the instruction of the manufacturer. The genomic DNA obtained was used as the PCR template targeted at the 16S rRNA gene using the primers: 27F (AGAGTTTGATCMTGGCTCAG) and 1389R (ACGGGCGGTGTGTACAAG) with the PCR condition consisting of 1 cycle of 95°C for 4 min, 25 cycles of 95°C for 1 min, followed by 55°C for 1 min, 72°C for 1 min 30s and a final extension at 72˚C for 1min (Pinoche *et al.,* 2013).

The amplicons obtained were purified and sequenced, and quality analysis (base calling and low quality trimming) was done with default parameter in CEQ™ 8000 Genetic Analysis software (Beckman Coulter). The sequences obtained were compared with others deposited in GenBank database.

3.2.4.7.Identification of lactic acid bacteriaand *Salmonella*species by MALDI-TOF MS

Matrix-Assisted Laser Desorption/Ionization Time-Of-Flight Mass Spectrometry (MALDI-TOF MS) technique according to Ayeni *et al* (2017)was employed for the identification of *Salmonella* and LAB isolates that were not identified with 16S rRNA sequencing. MALDI-TOF MS is a technique devised to identify microorganisms through the generation of highly abundant protein fingerprints, followed by correlation to reference spectra in a microorganism collection database. Bacterial extract for the mass spectrometry analysis was

prepared as follows; Thin smears of pure isolated colonies to be identified were placed on the target MALDI plate, this was overlaid with 1 µL of saturated solution of α-cyano-4-hydroxycinnamic acid in 50% acetonitrile and 2.5% trifluoroacetic acid (matrix solution), and then air dried at ambient temperature to allow co-crystallization of the matrix-sample. Measurements were taken with the aid of VITEK MS (Biomerieux, Nuertingen, Germany) and identification of the test organisms were achieved by a comparison of the mass spectra of the test isolate with reference spectra from the integrated database provided by the manufacturer. The similarity log-score thresholds of Seng *et al* (2010) were used for the identification.

3.2.5. Preservation of microrganisms

All pathogenic bacteria used were preserved in 50% glycerol/nutrient broth stock kept at -80^0C while the working cultures were maintained on Nutrient agar slant at 4^0C and sub cultured fortnightly throughout the study.

Multiple LAB stock culture batches were prepared and preserved in 50% glycerol/MRS broth at -80^0C.

3.2.6. Determination of the antibacterial activities of lactic acid bacteria

3.2.6.1. Anti-*Salmonella*activity of lactic acid bacteria

The anti-*Salmonella* activity of the Cell Free Supernatant (CFS) and viable cells of 88 isolated LAB were determined. Agar overlay method described by Ayeni *et al* (2011) was employed for the determination of the antimicrobial activity of the viable LAB cells against two bovine *Salmonella* test strains. A loopful of an overnight LAB broth culture was streaked on MRS agar as a thick line of about 20 mm in length and incubated at 37^{o}C for 24 hours. Thereafter, the MRS agar plates with well-established viable LAB streaks were overlaid with approximately 10^5CFU/mL of overnight broth culture of the two test *Salmonella*e in 10 mL Mueller Hinton soft agar (0.7% agar-agar) and incubated at 37^{o}C for 24 hours. The zones of inhibition around the LAB line of streak in the MRS agar underlay were measured and recorded.

The anti-*Salmonella* activities of the CFS of all 88 LAB isolates were also tested as follows; Lactic acid bacteria isolates were grown in MRS broth at 37^{o}C for 24 hrs and centrifuged at

12,000 rpm for 10 mins. An aliquot of 100 µL of the cell free supernatant of the LAB was placed in 6 mm well in Mueller Hinton agar seeded with approximate 0.5 McFarland standard of the test *Salmonella* using micropipette. The cell free supernatant was left to diffuse for 1 hour at room temperature before incubation at 37^{o}C for 24 hrs. Thereafter, the zones of inhibition were measured and recorded.

3.2.6.2. Antibacterial activities of lactic acid bacteria cell free supernatant

The antimicrobial activity of seven LAB isolates selected based on promising anti-*Salmonella* activity was determined by cell free supernatant assay against an array of pathogens; *S. enterica* S1, *S. enterica* S57, *S.* Typhimurium ATCC 14028, *S. aureus* A104, *Klebsiella* spp., *P. aeruginosa* and Extended Spectrum Beta-Lactam (ESBL) producing *E. coli* T51 (Balouiri *et al.,* 2016). Lactic acid bacteria isolates were grown in MRS broth at 37^{o}C for 24 hrs and centrifuged at 12,000 rpm for 10 mins. An aliquot of 100µL of the cell free supernatant of the LAB was placed in 6 mm well in Mueller Hinton agar seeded with approximate 0.5 McFarland standard of the test pathogens using micropipette. The cell free supernatant was left to diffuse for 1 hour at room temperature before incubation at 37^{o}C for 24 hrs. Thereafter, the zones of inhibition were measured and recorded.

3.2.6.3. Determination of bacteriocin-like inhibitory substances

Lactic acid bacteria with characteristic antimicrobial properties were tested for the presence or absence of bacteriocin-like inhibitory metabolites by agar-well diffusion method (Adeniyi *et al.,* 2015). Eighteen hour old cultures of LAB grown in MRS broth were centrifuged at 12,000 rpm for 10 mins to obtain the CFS (crude bacteriocin). The pH of the CFS was adjusted to 6.2 with 1.0M NaOH and the antimicrobial activity of the neutralized CFS was determined against *S. aureus* A104 in cup diffusion assay since bacteriocins are known to inhibit closely related bacteria species.

Any possible bacteriocin like inhibitory substances produced by LAB were precipitated with ammonium sulphate: briefly, 70% ammonium sulphate was added to the CFS of LAB and incubated at 4°C for 45 mins with intermittent shaking to precipitate the protein. The resulting solution was centrifuged at 12,000 rpm for 30 mins at 4°C, the supernatant was

decanted and the pellet obtained was dissolved in 1mL distill water, the peptide concentrate was then stored at -20°C for purification(Sure *et al.,* 2016).

3.2.7. Resistance of lactic acid bacteria isolates to gastrointestinal conditions

3.2.7.1. Tolerance to acidic pH

The method described by Kabore *et al* (2012) was employed to test the ability of the 88 LAB isolates to resist acidic pH levels. The LAB cells were harvested from overnight cultures of all the LAB isolates grown in MRS broth (Oxoid, UK) at 37^{o}C, centrifuged at 12,000 rpm for 5 mins. The bacterial cell pellets were washed with normal saline and resuspended in 10 mL fresh MRS broth adjusted to pH levels of 2.0, 3.0, 4.0, 5.0 and 7.0 (with 1M HCl), 100 µL from the culture was taken immediately for serial ten fold dilution for the initial count (T_0) before incubation at 37^{o}C for 3 hours under microaerophilic condition. Samples were taken after incubation for 3 hours (T_3), diluted and plated on MRS agar and incubated at 37^{o}C for 24 hours. The CFU/mL of the LAB at T_3 was compared with T_0.

3.2.7.2. Bile tolerance

The ability of the 88 isolated LAB to tolerate bile salt was determined according to the method of Kabore *et al* (2012). The LAB cells were harvested from overnight cultures of all the LAB isolates grown in MRS broth (Oxoid, UK) at 37^{o}C and centrifuged at 12,000 rpm for 5 mins. The bacterial cell pellets were washed with normal saline (0.9% NaCl) and resuspended in 10mL fresh MRS broth supplemented with bile salt (Oxoid) to obtain 0%, 0.5%, 1%, 5% and 7 % bile concentration levels, 100 µL from the cultures were taken immediately for serial ten fold dilution for the initial count (T_0) before incubation at 37^{o}C for 3 hours under microaerophilic condition. Samples were taken after incubation for 3 hours (T_3), diluted and plated on MRS agar and incubated at 37^{o}C for 24 hours. The CFU/mL of the LAB at T_3 was compared with T_0.

3.2.7.3. Consecutive acid and bile tolerance test

The ability to resist consecutive low pH and bile supplementation was tested in5 LAB strains selected for their probiotic potentials on the basis of antimicrobial activity and resistance to gastric conditions. The LAB cells were harvested from overnight cultures of selected LAB isolates grown in MRS broth (Oxoid, UK) at 37^{o}C, centrifuged at 12,000 rpm

for 10 mins. The bacterial pellets were washed with sterile saline (0.9% NaCl) and resuspended in 10ml fresh MRS broth adjusted to pH 3 (with 1M HCl), the initial viable count was noted (T_0) immediately before incubation at 37^{o}C for 3 hours under microaerophilic condition, thereafter, 100 µL from the culture was appropriately diluted and plated in MRS agar (T_3). The resultant cultures were then centrifuged and the cell pellets resuspended in 10 ml MRS broth containing 7% (w/v) bile salt, followed by incubation at 37^{o}C for 3 hours. The viability of LAB cells after exposure to consecutive low pH and bile were determined by viable colony counting of appropriate dilutions after incubation at 37^{o}C under microaerophilic condition, and comparing the viable cells with the initial count of the LAB at time 0 hour contact with bile supplemented medium.

3.2.8. Quantification of organic acids produced by lactic acid bacteria

The amount of lactic, acetic and propionic acids produced by 5 potential probiotic *Lactobacillus* strains selected on the basis of antimicrobial properties and ability to withstand consecutive low pH and bile supplementation were determined by High Performance Liquid Chromatography (HPLC) (Adept CECIL CE 4200). Filtered samples (20µL) was introduced into the HPLC system fitted with a UV absorbance detector set at 210nm, the mobile phase was degassed H_2SO_4. High Performance Liquid Chromatography grade standards of lactic, acetic and propionic acids (Sigma Adreich) were used to generate the standard curves. The quantity (mg/mL) of the tested organic acids produced by each of the strains were determined from the standard curves with linear coefficients (R^2)greater than 0.99 (Appendix VIII).

3.2.9. Determination of antibiotic susceptibility

3.2.9.1 Lactic acid bacteria susceptibility test with disk diffusion method

A major safety requirement for bacteria proposed for probiotic purpose in humans and animal is that such bacteria should be devoid of acquired antibiotic resistance determinants. The susceptibility of LAB was determined for the following antibiotics; streptomycin, ampicillin, amoxicillin, vancomycin, kanamycin, erythromycin, chloramphenicol gentamicin, clindamycin and tetracycline (Oxoid, UK). Lactic acid bacteria lawn was made with 5 x 10^7 CFU/mL (equivalent to 0.5 McFarland Standard) on Lactobacillus Susceptibility Medium (LSM) using a sterile swab (Klare *et al.,* 2007). The antibiotics disc

were placed on the seeded media and incubated at 37^{o}C for 24 hours under microaerophilic condition. The zone of bacterial inhibition was recorded, susceptibility was interpreted according to EUCAST, (2016) and the nearest species' breakpoints were used for species without clearly defined breakpoints.

3.2.9.2. Determination of minimum inhibitory concentration of lactic acid bacteria

The MIC (μg/mL) of 5 potential probiotic LAB selected on the basis of antimicrobial properties and ability to withstand consecutive low pH and bile supplementation were determined by Epsilometer test strips (E-test, bioMerieux, France) for the following antibiotics: ampicillin, tetracycline, vancomycin, kanamycin, streptomycin, erythromycin, clindamycin, gentamicin and chloramphenicol. The concentration of the test strips range from 0.016 to 256 μg/mL except for streptomycin (0.064–1024 μg/mL). The E-test strips provide an exponential gradient method of determining antibiotic resistance. This consists of gradient concentrations of antibiotics impregnated along a rectangular plastic strip. After the incubation period, a dome shaped zone of inhibition intersects the graded strip at the MIC of the antibiotic. The selected isolates were grown in MRS broth at 37^{o}C under microaerophilic condition. Sterile swab stick was used to make a lawn of the LAB with approximately 5 x 10^{7} CFU/mL (equivalent to 0.5 McFarland standard). Sterile forceps was used to place the E-test strips on the inoculated media with the graduation scale visible (facing upward), and incubated at 37^{o}C for 24 hours under microaerophilic condition. The MIC was read at the point where the ellipse intersects the scale.The MIC values for the LAB was interpreted with breakpoint suggested by EFSA (2007) for the selection of probiotic strains.

3.2.10. Haemolytic activities of lactic acid bacteria

The haemolytic potential of the 5 LAB selected on the basis of antimicrobial properties and ability to withstand consecutive low pH and bile supplementation were determined by streaking the LAB strains on 5% bovine blood agar and incubated at 37^{o}C for 24 hours (Halder *et al.,* 2017). The plates were thereafter observed for the production of green-hued zones around the colonies (alpha-hemolysis), no effect on the blood agar (Gamma-hemolysis) and those forming blood lysis around the colonies were reported as haemolyic (Beta- hemolysis).

3.2.11. Co-culturing of *Salmonella* and *Lactobacillus*

The effect of co-culturing *Salmonella* test strains with two potential probiotic strains selected based on broad spectrum antibacterial activity, ability to resist gastric conditions and production of organic acids was tested by the method of Abdel-Daim *et al* (2013). A 10mL broth containing 5 ml of MRS (double strength) and 5 ml Mueller Hinton (double strength) referred to as MRS-MH was used as the co-culture broth. The co-culture broth was inoculated with approximately 10^9CFU/mL of LAB strains and 10^8CFU/mL of the *S. enterica* spp. Experimental controls were set up with LABand *Salmonella* monocultures grown in MRS-MH broth to monitor the growth of each of the microorganism in the co-culture broth. Appropriate dilutions were plated out on SSA and MRS agar just after co-inoculation (T_0) and repeated every eight hours for twenty four hours, to achieve sampling at four time points; T_0, T_8, T_{16} and T_{24} hours. Lactobacilli monocultures were plated on MRS agar, *Salmonella* monocultures were plated on SSA agar and the co-culture were plated on both MRS agar and SSA (to check the effect of interference of both microorganisms with each other).The lactobacilli and salmonellae were incubated at 37^{o}C for 24 hours under microaerophilic and aerobic conditions respectively.The CFU/mL at every sampling time points was compared with the monoculture control.

3.2.12. Lactobacilli toxicity and translocation assay

A 7-day repeated dose toxicity and the bacterial translocation potential of two selected potential probiotic lactobacilli was tested *in-vivo* inrabbits as described by Shokryazdan *et al* (2016), in this assay, 8 New Zealand White rabbits sourced from the Rabbit Production Division, Department of Veterinary Medicine, University of Ibadan were used. The rabbits were assigned randomly into two treatment groups (n =4) as follows; the control group received 1 ml of normal saline while the test group received an approximate dose of 5.3× 10^{10} CFU/day of a mixture of the test LAB C86 and LAB C94 isolates resuspended in 1 ml of normal saline for 1 week by oral gavage. All the rabbits were fed standard rodent diet and had unrestricted access to water and the use of antibiotics was restricted throughout the experiment. Rabbitswere observed for signs of toxicity such as changes in fur and skin, diarrhoea, salivation, lethargy, changes in gait and mortality. After the feeding period, a loopful of blood was streaked in MRS agar and incubated at 37C for 48 hours under

microaerophilic condition. Spleen and liver samples were also collected aseptically, homogenized in MRS broth and incubated for 24 hours at 37C under microaerophilic condition. The resulting homogenate was then plated in MRS agar and incubated for 24 hours. The plates were then observed for microbial growth or no growth representing positive or negative results respectively for bacterial translocation.

3.2.13. *In-vivo*probiotic potential of selected lactic acid bacteria

3.2.13.1. Preparation of lactic acid bacteria feeding suspension

A modified method of Casey *et al* (2007) was employed for the preparation of bacterial suspension from two potential probiotic lactobacilli selected based on their performance in the antimicrobial and gastric resistance assays. . MRS broth (10 mL) was inoculated with 1% (vol/vol) of the appropriate culture and incubated at 37°C for 24 hours under microaerophilic condition. Thereafter, the bacteria cells for both test LAB strains were harvested by centrifugation, resuspended in 10mL of 10% reconstituted skimmed milk daily for the feeding trial. The colony forming unit per mL for each of the suspension batch was determined on MRS agar to check for consistency of the bacterial count throughout the experiment.

3.2.13.2. Calves feed trial

Nine healthy calves available at the dairy section of the UI-T&RF were used for the experiment. They were ear tagged for identification and moved to the pathogen challenge facility of the research farm. The calves were penned individually to avoid cross-contamination, and the control calves were kept away from the probiotic-treated group. All the calves had unhindered access to water and their usual daily feed ration and no antibiotic feed additive/antibiotics were administered to the animals throughout the period of the feeding trial.

The calves were administered Lactobacilli-skimmed milk suspension (LSMS) for the test group or sterile skimmed milk (SSM) in the control group for 30 days. The calves were assigned randomly to treatment groups as follows; Mixed LABsuspension group (n=6) which received a mixture of LAB C86 and LAB C94, control group (n=3) received sterile reconstituted skimmed milk. Calves receiving *Lactobacillus* culture were fed 10 mL daily

with the culture mixture, providing an approximate dose of 8.3×10^{10} CFU/day while the calves in the control group received 10 mL of sterile skimmed milk daily. Observation of faeces collected from each pen for faecal consistency, rectal temperature, lethargy, changes in gait of the calves were used as clinical scoring system.

3.2.13.3. Collection of faecal samples and DNA extraction

Faeces were collected directly using disposable gloves from the rectum of each numbered tagged calves (n= 9, weight- 50±10 kg) housed at the UI-T&RF into sterile sample bottles at two time points; before and after the 30 days feeding trial, and taken to the molecular microbiology laboratory for DNA extraction. The genomic DNA was extracted with QIAamp® DNA stool mini extraction kit based on the manufacturer's instruction with some modifications that involve bead beating steps as follows:

Cell wall lysis: 0.25 g of the faecal sample was transferred into a sterile DNAse free 2-mL screw-cap tube. 0.4 g of sterile zirconia beads consisting of 0.3 g of 0.1 mm and 0.1 g of 0.5 mm was added to the tube containing the sample. 1 mL of ASL buffer [500 mM NaCl, 50 mM Tris-HCl, pH 8.0, 50 mM EDTA, and 4% sodium dodecyl sulfate (SDS)] was added. Followed by Homogenization in FastPrep (BioSpec Products, Bartlesville, USA) at 5.5 ms for 1 min three times, keeping the samples on ice for 5 mins between each treatment. The homogenized sample mixture was then incubated at 95°C for 15 min, with gentle shaking by hand every 5 min. The samples were then centrifuged at 4°C for 5 min at 16,000× g in other to pellet the stool. The CFS was then transferred to a new sterile 2-mL Eppendorf tube referred to as lysate tube.

Nucleic acid was precipitated from the lysate with 10 M ammonium acetate and isopropanol, followed by washing with 70% ethanol. Removal of RNA, protein and purification of the genome DNA were achieved with the extraction kit according to the user's instruction.

3.2.13.4.Quantitative PCR analysis

The method of Castillo *et al* (2006) with some modifications was employed for the quantification of LAB and enterobacteria from faecal samples collected before and after thefeeding trial.Quantitative PCR was performed on a 7500 real-time PCR system (Applied

Biosystems) using optical grade 96-well plates, assays were performed in 25-µL volumes containing SYBR green I fluorophore used for the correlation of the amount of PCR product with the fluorescence signal. The primer sets used for the quantification of total lactobacilli and enterobacteria respectively are *Lactobacillus* genus-specific primer set: F-lac 5' GCAGCAGTAGGGAATCTTCCA 3' and R-lac 5' GCATTYCACCGCTACACATG3' (Walter *et al*., 2001; Castillo *et al.,* 2006) and for enterobacteria F-ent 5' ATGGCTGTCGTCAGCTCGT3' (Leser *et al*., 2002; Castillo *et al.,* 2006) and R-ent 5' CCTACTTCTTTTGCAACCCACTC3' (Sghir *et al.,* 2000; Castillo *et al.,* 2006). The reaction conditions were 50 ^{o}C for 2 mins, 95^{o}C for 10 min, 40 cycles of 95 ^{o}C for 15 s and 60 ^{0}C for 1 min.

3.2.13.4.1. Determination of standard curve for qPCR

The standard curve for qPCR was obtained according to a modified method of Castillo *et al.,* 2006 using DNA extracted from pure cultures of the target organisms. Briefly, genomic DNA was extracted from 5mL of broth culture in logarithmic growth phase with the *AccuPrep*® Genomic DNA Extraction kit (Bioneer, South Korea). The concentration (ng/µL) of the extracted DNA was determined using the PicoGreen® dsDNA Quantitation Reagent and Kits (Thermofisher, USA) with absorbance measured at 260nm. The DNA was then used to establish a standard curve. Conventional PCR was used to confirm correct amplification of the selected enterobacteriaceae and *Lactobacillus* specific primer sets with the DNA extract (Applied Biosystem Themal cycler). The amplicons were viewed in 1.8% agarose gel after electrophoresis to confirm the appropriate band sizes. The cycle threshold (CT) value was defined as the PCR cycle at which the increase in fluorescent signal was statistically significant above the background measurement. The standard curves were generated by plotting the CT values in relation to the corresponding serial double fold dilutions of the DNA extract. For the determination of the amplification specificity, analyses of the melting curves of amplicons were performed after the last cycle of every amplification. The quantities of the target DNA in the sample DNA was deduced by standard curve method. The difference in total enterobacteria between the control and treatment group was tested with paired Student's *t*- test, and $P\ value<0.05$ was taken to be significant.

3.2.14. Viability of selected lyophilized lactic acid bacteria at room temperature

The effect of freeze drying on the viability of two selected potential probiotic strainsbased on their performance in the antimicrobial and gastric resistance assays was determined with the method described by Ayeni *et al* (2011b). Lactic acid bacteria cells were harvested from an overnight broth culture by centrifugation at 3500rpm for 20 mins at 4 ^{o}C, the cells were washed once with PBS buffer and then concentrated in sterile 1mL 11% skimmed milk serving as the cryopreservant. One hundred microliter (100uL) of each sample was taken to make the initial count. The LAB cells suspended in skimmed milk were kept at -80 ^{o}C for 24 h before lyophilisation (ALPHA 1-2 LD plus). The number of viable LAB cells was determined immediately after freeze drying by plate count in MRS agar, after 2 weeks and every month for 3 months with storage in a cool dry place at room temperature. The viable count were expressed in CFU/mL and used to determine the viability of lyophilized LAB cells in storage at room temperature.

3.2.15. Data analysis

Data generated in this study were generally analysed with descriptive statistics while qPCR data were analysed withStudent's t test at $\alpha = 0.05$ using statistical software program GraphPad prism 5.0.

CHAPTER FOUR

RESULTS

4.1 Isolation and identification of *Salmonella* species

The prevalence of *Salmonella* spp. was determined in healthy cattle faeces in this study. Initially, sixty eight isolates were obtained from 138 cattle faecal samples with presumptive identification of *Salmonella* spp. Black centred colonies signifying hydrogen sulphide production on SSA and XLD, and a TSI result of pink slant (alkaline) and yellow butt (acidic) with hydrogen sulphide production were presumptively identified as *Salmonella*. Further identification was done with MALDI-TOF, Microbact 24E and molecular identification with *Salmonella* genus specific primers. Of the sixty eight isolates exhibiting typical *Salmonella* characteristics on the basis of cultural and biochemical properties, only 32 isolates were identified as *Salmonella* spp. by MALDI-TOF. These isolates were further confirmed by Microbact 24E as *Salmonella* as shown in Table 4.1. Polymerase chain reaction targeted at the amplification of *invA* gene which is specific for *Salmonella* spp. was used to validate the result of the MALDI-TOF and Microbact 24E analysis. All 32 isolates were confirmed to be *Salmonella* spp. (Fig 4.1).

4.2 Minimum inhibitory concentration of *Salmonella* isolates

The MIC of the 32 *Salmonella* isolates was determined by automated antimicrobial susceptibility testing with AST-N248 card in Vitek 2 system. All the isolates were susceptible to the entire antibiotic panel consisting of ampicillin, ampicillin-sulbactam, teteracycline, gentamicin, trimethoprim-sulfamethoxazole, cefotaxime, imipenem, meropenem, tigecycline, cefuroxime, ciprofloxacin, piperacillin/tazobactam, ertapenem, ceftazidime, moxifloxacin and cefpodoxime.

Table 4.1. Identification of *Salmonella*isolates with Microbact 24E

S/N	Sample code	Microbact ref code	Microorganisms Identity	Probability
1.	S1	77020621	*Salmonella* Sub sp 1	49.44%
2.	S2	77420661	*Salmonella* Sub sp 3b	80.28%
3	S3	77020621	*Salmonella* Sub sp 1	49.44%
4.	S4	77420621	*Salmonella* Sub sp 5	71.88%
5.	S5	77010661	*Salmonella* Sub sp 1	72.57%
6.	S10	77420621	*Salmonella* Sub sp 5	71.88%
7.	S13	77021621	*Salmonella* Sub sp 1	98.83%
8.	S15	77420621	*Salmonella* Sub sp 5	71.88%
9.	S16	77020621	*Salmonella* Sub sp 1	49.44%
10.	S19	77020621	*Salmonella* Sub sp 1	49.44%
11.	S21	77020621	*Salmonella* Sub sp 1	49.44%
12.	S25	77021621	*Salmonella* Sub sp 1	98.83%
13.	S26	77020621	*Salmonella* Sub sp 1	49.44%
15.	S31	77020621	*Salmonella* Sub sp 1	49.44%
16.	S38	67020621	*Salmonella* Sub sp 1	97.77%
17.	S41	77020621	*Salmonella* Sub sp 1	49.44%
18.	S42	77020661	*Salmonella* Sub sp 1	54.22%

Table 4.1 (cont)

S/N	Sample code	Microbact ref	Microorganisms Identity	Probability
19.	S44	77220621	*Salmonella* Sub sp 1	74.19%
20.	S47	77020621	*Salmonella* Sub sp 1	49.44%
21.	S48	77020621	*Salmonella* Sub sp 1	49.44%
22.	S49	77020621	*Salmonella* Sub sp 1	49.44%
23.	S54	77020621	*Salmonella* Sub sp 1	49.44%
24.	S56	77420621	*Salmonella* Sub sp 1	74.19%
25.	S57	77020621	*Salmonella* Sub sp 1	49.44%
26.	S58	77020621	*Salmonella* Sub sp 1	49.44%
27.	S60	77000720	*Salmonella* Sub sp 1	49.65%
28.	S62	77020621	*Salmonella* Sub sp 1	49.44%
29.	S68	77020621	*Salmonella* Sub sp 1	49.44%
30.	S70	77020621	*Salmonella* Sub sp 1	49.44%
31.	S76	77020621	*Salmonella* Sub sp 1	49.44%
32.	S77	77020621	*Salmonella* Sub sp 1	49.44%

1 2 3 4 5 M6 7 8 9 10 11 12 1314

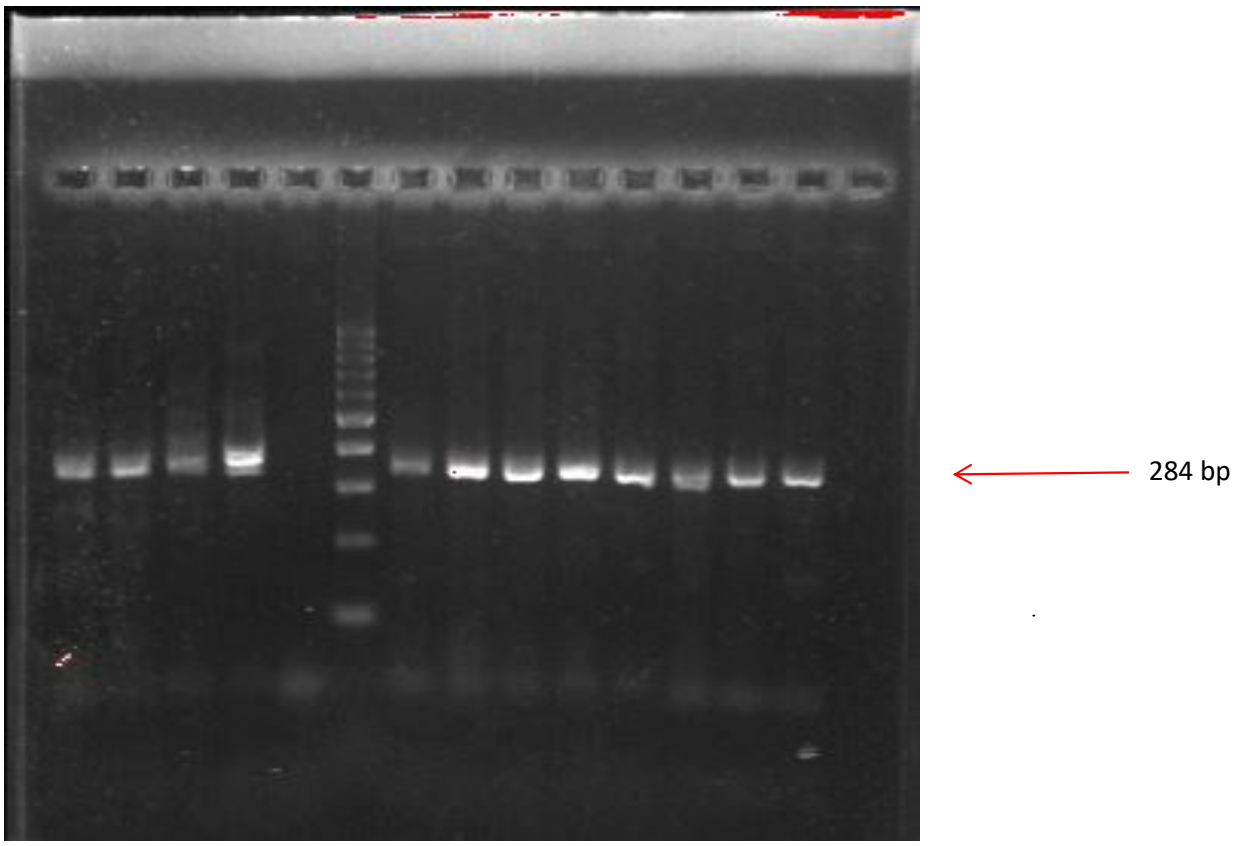

Fig 4.1.Amplification of *invA* gene (284 bp) to confirm *Salmonella* spp.

M: Molecular marker, 1-4, 6-12: Test amplicons, 13: Positive control (*S.enterica* 14028)

14: Negative control, 5- Blank control (Molecular grade water)

4.3 Isolation, identification and diversity of lactic acid bacteria

Eighty eight LAB were cultured from 40 bovine faecal samples, the isolates were presumptively identified as LAB based on their characteristic growth, morphology on MRS agar and catalase reaction. The isolates were catalase negative, with cellular morphology ranging from Gram positive short rod to long rod while some were cocci and coccobacilli (Fig 4.2- 4.3). The presumed LAB isolates were further identified based on analysis of their 16S rRNA gene sequences as *S. infantarius* (26), *E. hirae* (12), *L. mucosae* (10), *L. amylovorus* (10), *L. ingluviei* (9), *L. gasseri* (5), *L. agilis* (4), *L. taiwanensis* (3), *L. plantarum* (2), *L. salivarius* (2), *L. animalis* (1), *L. paraplantarum* (1), *L. reuteri* (1), *Streptococcus equinus* (1),and *Weissella cibaria* (1) as shown in (Table 4.2). *S. infantarius* dominated as it accounted for 30.68% of the total LAB species, *E.hirae* was the second most prominent species with 12 isolates while *L. animalis, L. paraplantarum, L. reuteri, S. equines* and *W. cibaria* all had only one strain, thus making them the least represented species cultured from the bovine faecal samples. At the genus level, *Lactobacillus* was the predominant (54.55%) with 48 isolates (Fig 4.4). The amplification of the 16S rRNA genes of eleven isolates failed and they were therefore identified by MALDI TOF as *E.hirae*, while only one *E.hirae* isolate was identified by partial sequencing of the 16S rRNA gene. The sequences obtained were deposited in the GenBank of NCBI with the accession numbers KY 810532-KY810608. The phylogenetic relationship of the various strains of LAB isolated in this study is represented in Fig 4.5.The phylogenetic relatedness of LAB isolated in this study were compared with those obtained from other bovine sources(Fig 4.6,4.7 and 4.8). It was observed that strains of LAB isolated in this study clustered in accordance with established taxonomy, alongside identical isolates from other bovine studies compared.

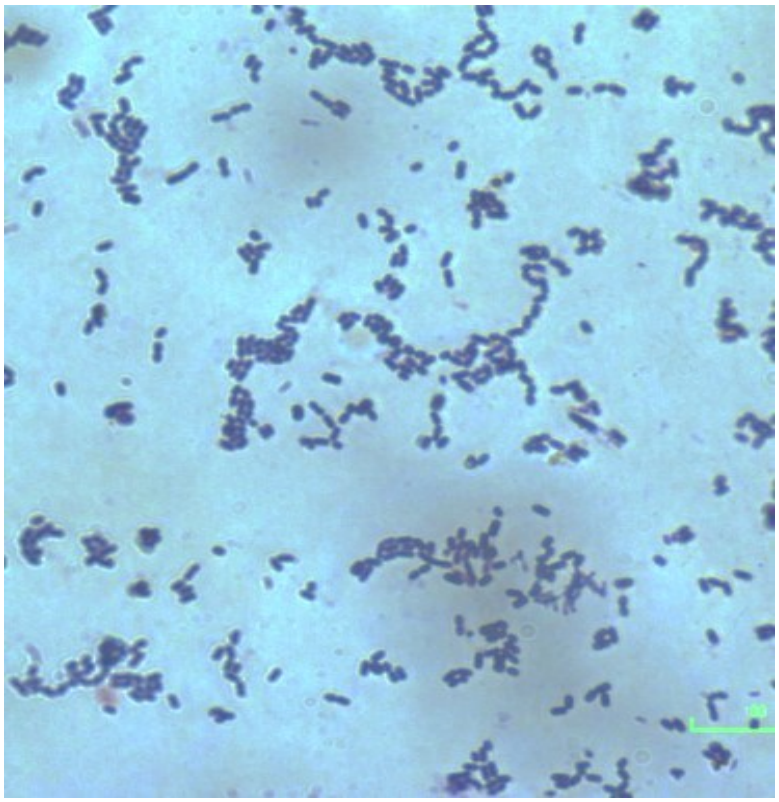

Fig 4.2. Photomicrograph of Gram's stained *Lactobacillus amylovorous* C94

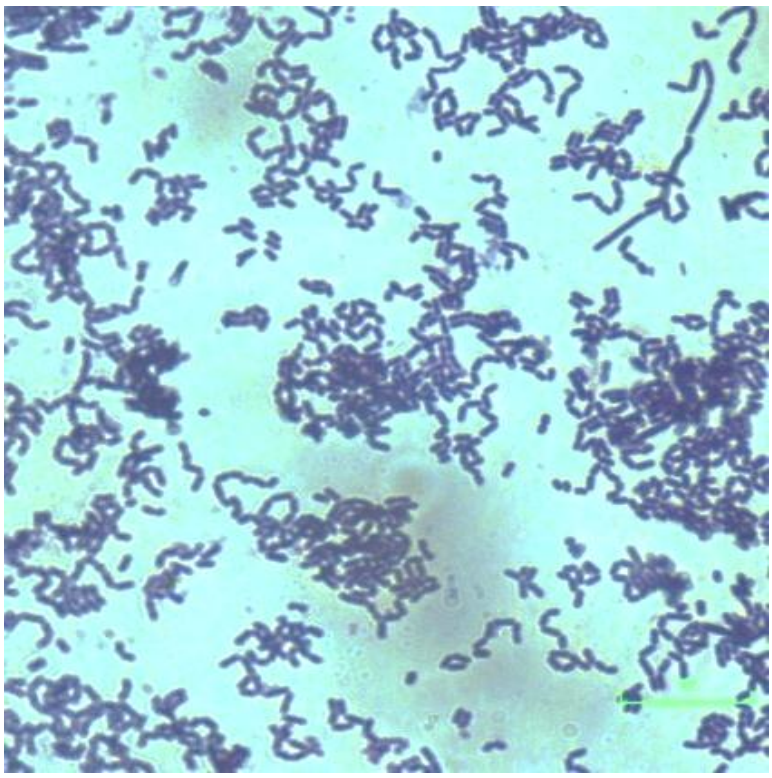

Fig 4.3.Photomicrograph of Gram's stained *Lactobacillus salivarius* C86

Table 4.2. Identification of lactic acid bacteria by partial sequencing of 16SrRNA genes

LAB Code	Isolates Identity	NCBI Ref code	% Similarity	Accession Number
C101	*Lactobacillus mucosae*	AF126738	(100)	KY810533
C103	*Lactobacillus gasseri*	AF519171	(99.6)	KY810534
C104	*Lactobacillus mucosae*	AF126738	(100)	KY810535
C105	*Lactobacillus ingluviei*	AF333975	(96.2)	KY810536
C12	*Lactobacillus agilis*	M58803	(98.9)	KY810537
C13	*Lactobacillus ingluviei*	AF333975	(96.3)	KY810538
C14	*Lactobacillus agilis*	M58803	(98.8)	KY810539
C15	*Lactobacillus amylovorus*	AY944408	(99.8)	KY810540
C16	*Lactobacillus ingluviei*	AF333975	(93.9)	KY810541
C17	*Lactobacillus ingluviei*	AF333975	(93.8)	KY810542
C19	*Lactobacillus taiwanensis*	EU487512	(97.7)	KY810543
C20	*Lactobacillus taiwanensis*	EU487512	(97.0)	KY810544
C21	*Lactobacillus mucosae*	AF126738	(100)	KY810545
C23	*Lactobacillus ingluviei*	AF333975	(93.4)	KY810546
C24	*Lactobacillus mucosae*	AF126738	(99.3)	KY810547
C25	*Lactobacillusparaplantarum*	AJ306297	(99.6)	KY810548
C26	*Lactobacillus plantarum*	AJ965482	(99.5)	KY810549

Table 4.2. Cont.

LAB Code	Isolates Identity	NCBI Ref code	% Similarity	Accession Number
C27	*Lactobacillus salivarius*	AF089108	9.8)	KY810550
C28	*Lactobacillus mucosae*	AF126738	9.8)	KY810551
C29	*Lactobacillus mucosae*	AF126738	00)	KY810552
C3	*Lactobacillus plantarum*	AJ965482	9.8)	KY810532
C31	*Lactobacillus ingluviei*	AF333975	(95.8)	KY810553
C35	*Streptococcus infantarius*	AF177729	(99.6)	KY810554
C37	*Streptococcus infantarius*	AF177729	(98.9)	KY810555
C38	*Streptococcus infantarius*	AF177729	(99.2)	KY810556
C39	*Lactobacillus mucosae*	AF126738	(98.8)	KY810557
C40	*Streptococcus equinus*	AJ301607	(99.5)	KY810558
C41	*Streptococcus infantarius*	AF177729	(99.6)	KY810559
C5	*Lactobacillus agilis*	M58803	(98.8)	KY810560
C50	*Streptococcus infantarius*	AF177729	(99.2)	KY810561
C51	*Streptococcus infantarius*	AF177729	(100)	KY810562
C53	*Streptococcus infantarius*	AF177729	(99.5)	KY810563
C54	*Streptococcus infantarius*	AF177729	(99.8)	KY810564
C55	*Streptococcus infantarius*	AF177729	(99.6)	KY810565
C56	*Streptococcus infantarius*	AF177729	(99.8)	KY810566
C57	*Streptococcus infantarius*	AF177729	(99.8)	KY810567

Table 4.2. Cont.

LAB Code	Isolates Identity	NCBI Ref code	% Similarity	Accession Number
C58	*Streptococcus infantarius*	AF177729	(99.8)	KY810568
C59	*Lactobacillus agilis*	M58803	(99.8)	KY810569
C6	*Lactobacillus taiwanensis*	EU487512	(97.5)	KY810570
C60	*Lactobacillus amylovorus*	AY944408	(99.8)	KY810571
C61	*Lactobacillus mucosae*	AF126738	(100)	KY810572
C62	*Streptococcus infantarius*	AF177729	(99.7)	KY810573
C63	*Streptococcus infantarius*	AF177729	(99.3)	KY810574
C64	*Lactobacillus gasseri*	AF519171	(99.4)	KY810575
C67	*Lactobacillus mucosae*	AF126738	(99.7)	KY810577
C68	*Streptococcus infantarius*	AF177729	(99.8)	KY810578
C69	*Streptococcus infantarius*	AF177729	(99.8)	KY810579
C70	*Streptococcus infantarius*	AF177729	(99.7)	KY810580
C71	*Streptococcus infantarius*	AF177729	(99.4)	KY810581
C72	*Lactobacillus gasseri*	AF519171	(99.6)	KY810582
C73	*Streptococcus infantarius*	AF177729	(99.8)	KY810583
C74	*Streptococcus infantarius*	AF177729	(99.8)	KY810584
C75	*Streptococcus infantarius*	AF177729	(99.3)	KY810585
C76	*Streptococcus infantarius*	AF177729	(99.7)	KY810586
C77	*Streptococcus infantarius*	AF177729	(99.8)	KY810587

Table 4.2 Cont**.**

LAB Code	Isolates Identity	NCBI Ref code	% Similarity	Accession Number
C78	*Streptococcus infantarius*	AF177729	(98.9)	KY810588
C8	*Lactobacillus mucosae*	AF126738	(97.4)	KY810589
C80	*Streptococcus infantarius*	AF177729	(99.3)	KY810590
C81	*Lactobacillus amylovorus*	AY944408	(100)	KY810591
C82	*Lactobacillus amylovorus*	AY944408	(99.6)	KY810592
C84	*Lactobacillus amylovorus*	AY944408	(100)	KY810593
C85	*Lactobacillus amylovorus*	AY944408	(99.8)	KY810594
C86	*Lactobacillus salivarius*	AY944408	(99.3)	KY810595
C87	*Lactobacillus amylovorus*	AY944408	(100)	KY810596
C88	*Streptococcus infantarius*	AF177729	(99.8)	KY810597
C89	*Lactobacillus ingluviei*	AF333975	(96.1)	KY810598
C9	*Enterococcus hirae*	Y17302	(97.7)	KY810599
C90	*Lactobacillus gasseri*	AF519171	(99.4)	KY810600
C91	*Weissella cibaria*	AJ295989	(99.7)	KY810601
C92	*Lactobacillus_ingluviei*	AF333975	(95.4)	KY810602
C93	*Lactobacillus ingluviei*	AF333975	(95.8)	KY810603
C94	*Lactobacillus salivarius*	AF089108	(99.8)	KY810604
C95	*Lactobacillus reuteri*	L23507	(96.7)	KY810605
C96	*Lactobacillus gasseri*	AF519171	(99.1)	KY810606
C98	*Lactobacillus amylovorus*	AY944408	(100)	KY810607
C99	*Lactobacillus amylovorus*	AY944408	(100)	KY810608

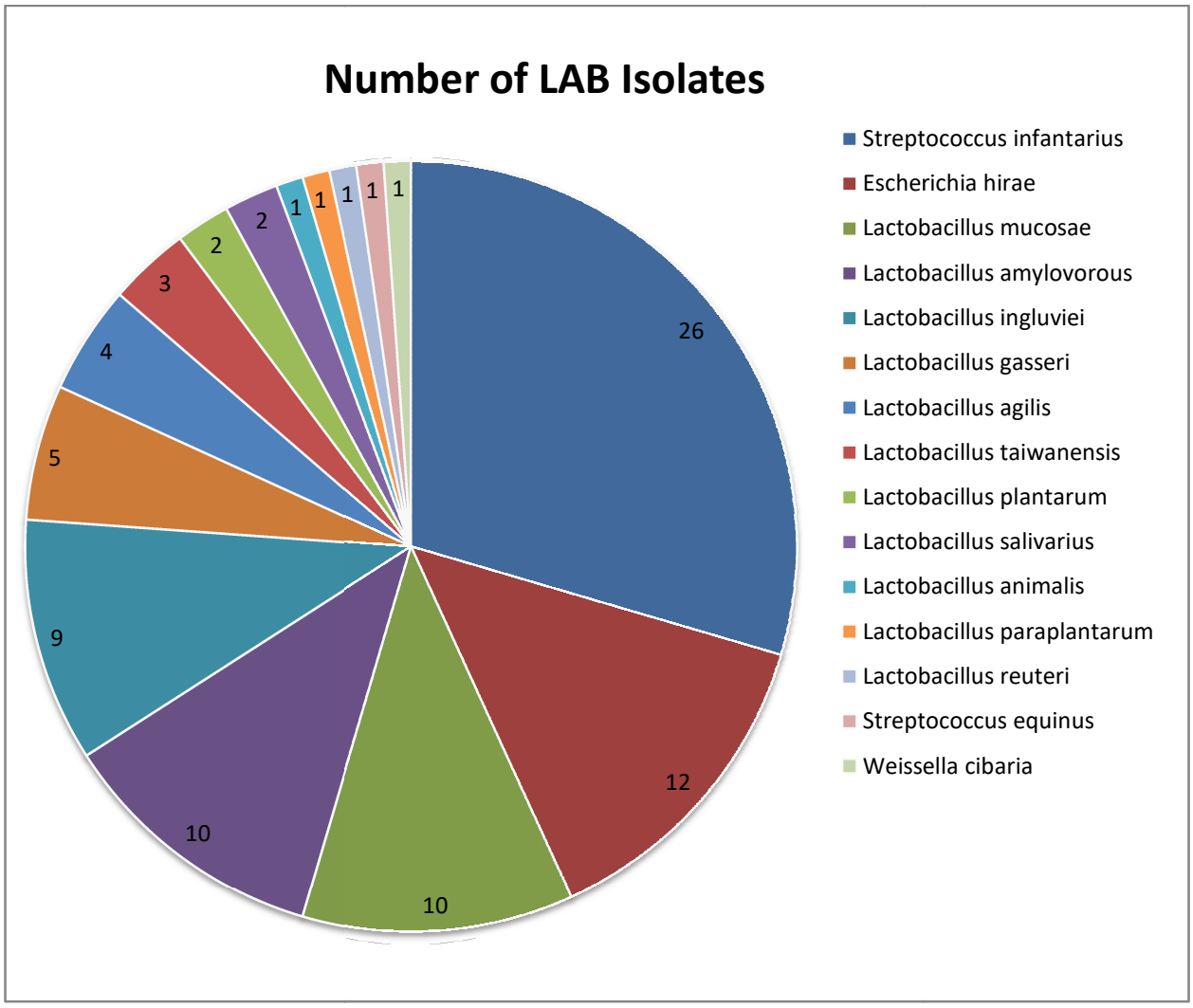

Fig 4.4.Distribution oflactic acid bacteria cultured from cattle faeces

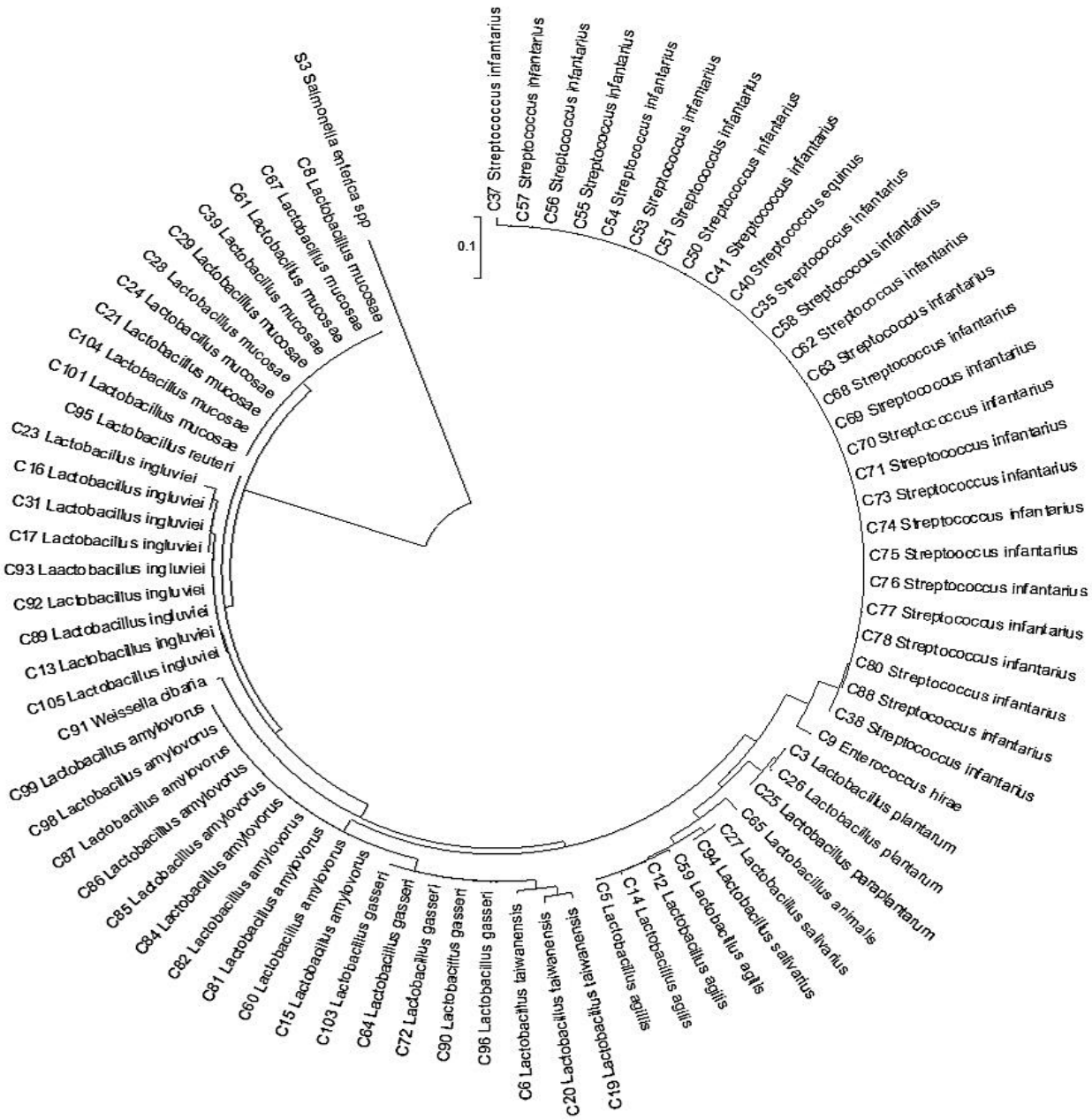

Fig 4.5. Phylogenetic tree of isolated bovine lactic acid bacteria based on 16S rRNA gene sequence alignment.

The scale bar represents 0.1-nucleotide substitutes per position.

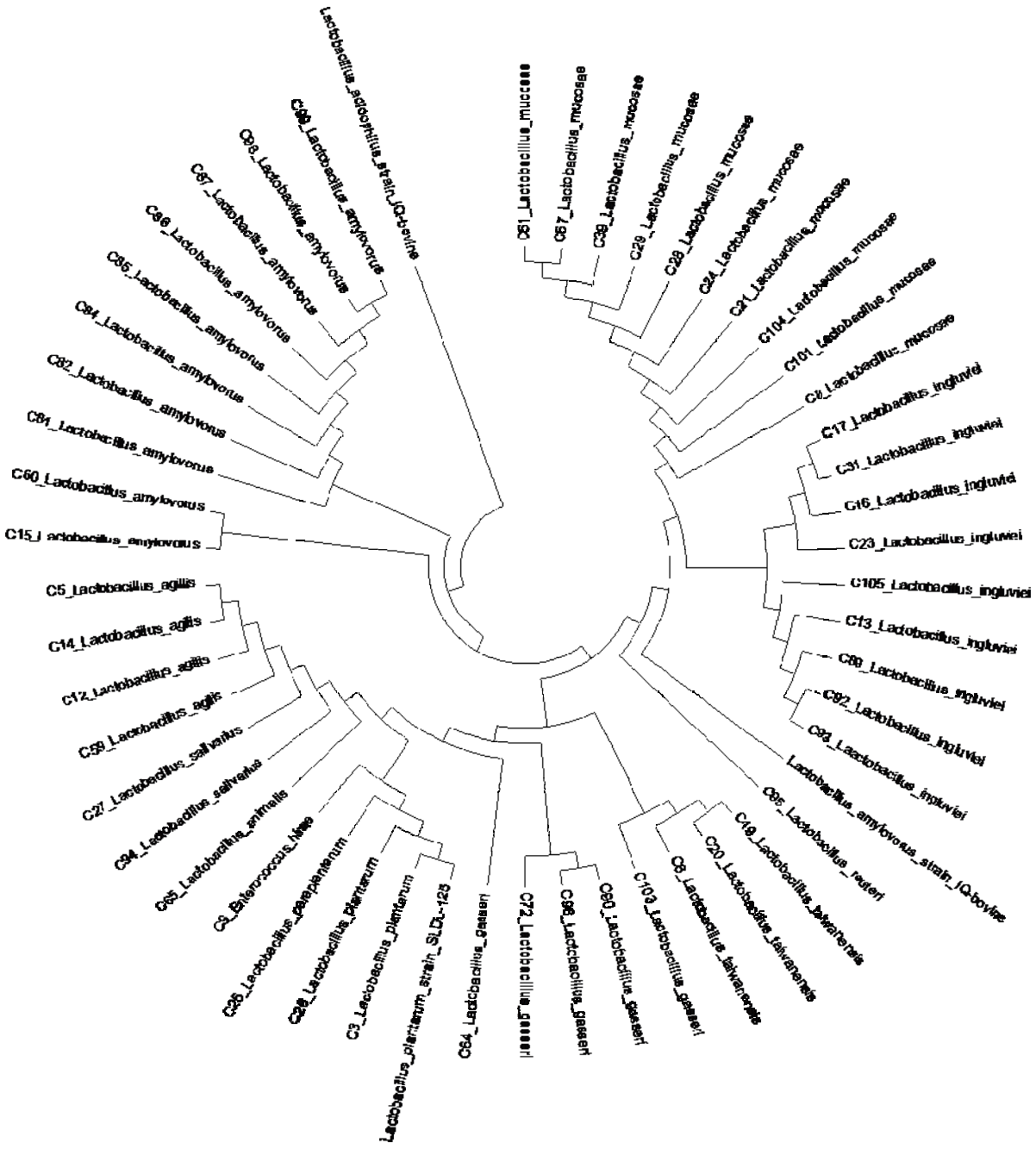

Fig 4.6. Phylogenetic tree showing the relationship between bovine *Lactobacillus* strains isolated in this study (starting with a code "C") with those from other bovine sources based on 16S rRNA gene sequence alignment**.**

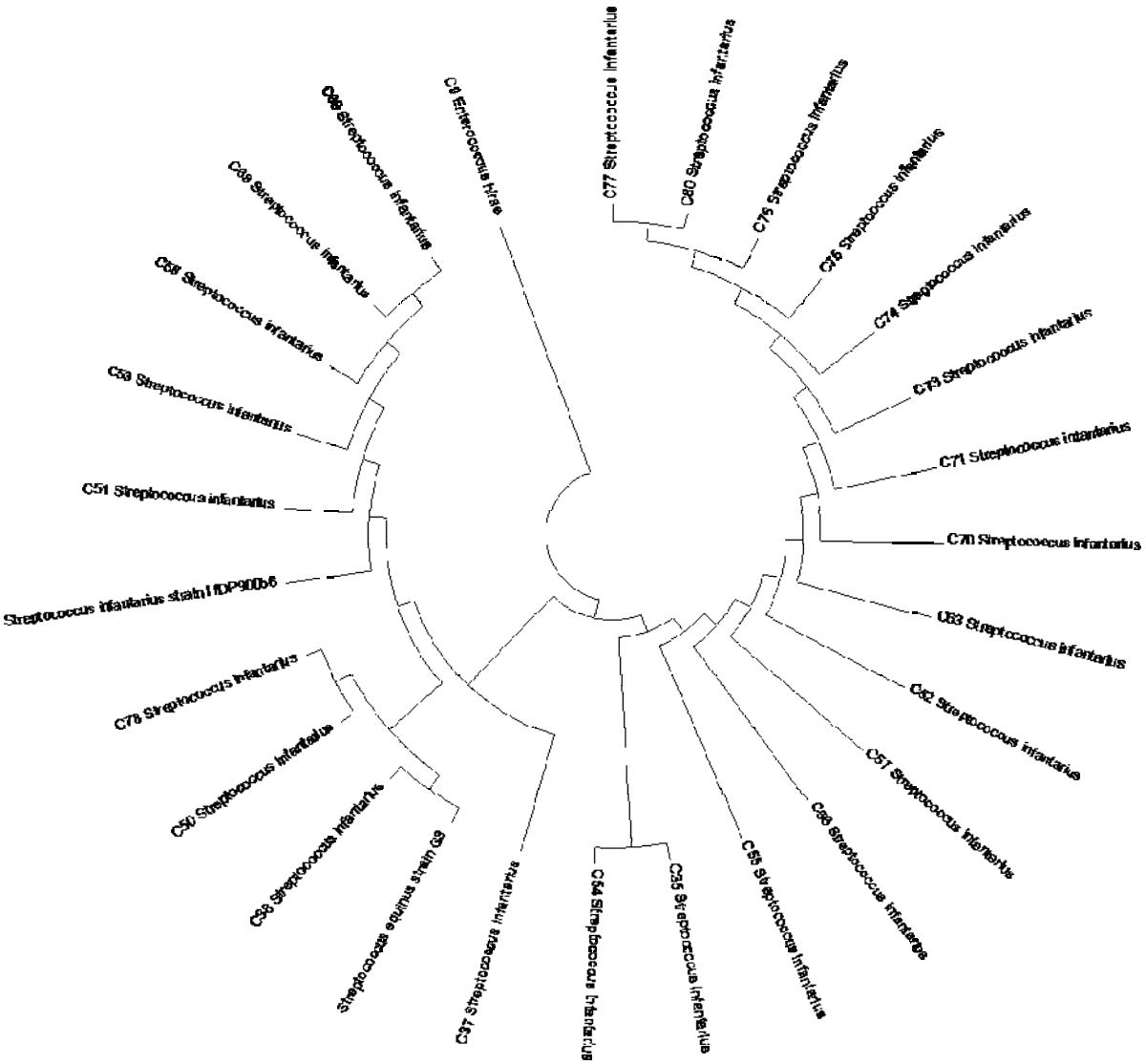

Fig 4.7. Phylogenetic tree showing the relationship between bovine Streptococci strains isolated in this study (starting with a code "C") with those from other bovine sources based on 16S rRNA gene sequence alignment.

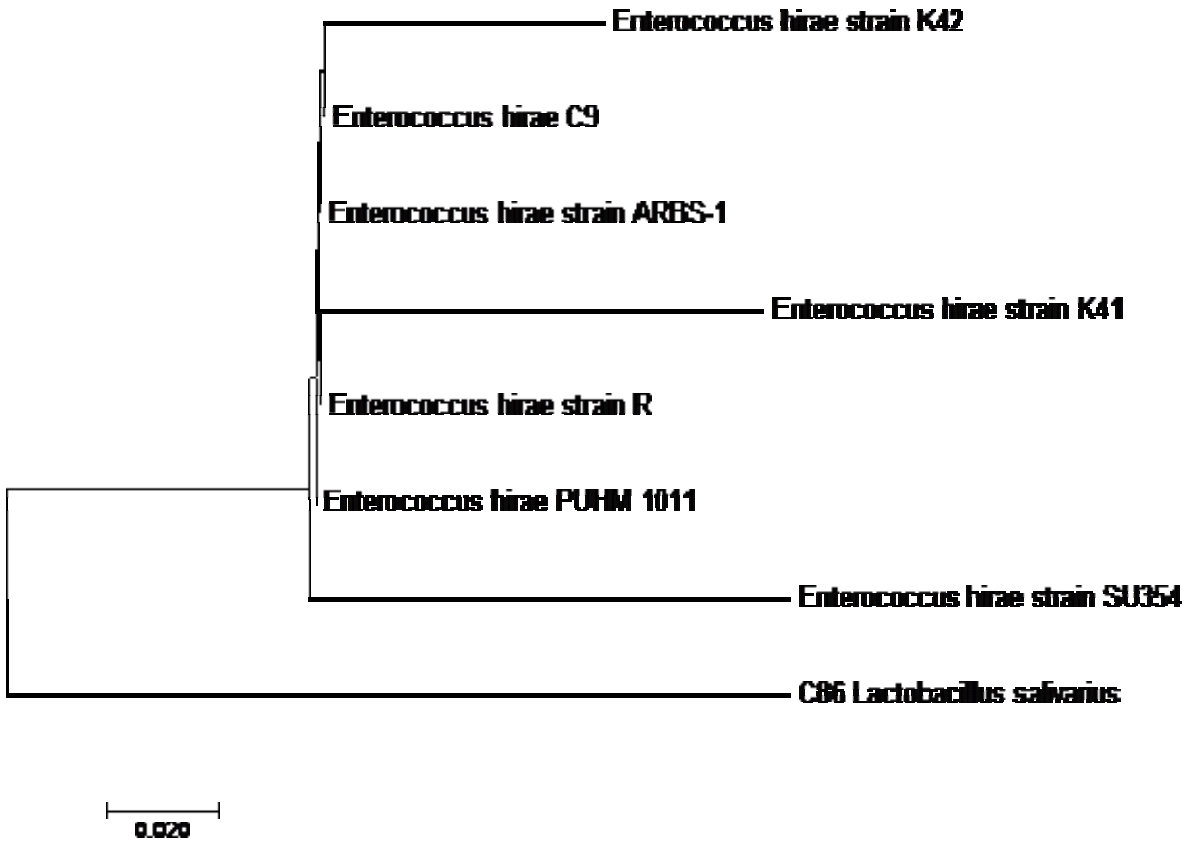

Fig 4.8. Phylogenetic tree showing the relationship between bovine *Enterococcus*strain isolated in this study (starting with a code "c") with those from other bovine sources based on 16S rRNA gene sequence alignment.

The scale bar represents 0.1-nucleotide substitutes per position

4.4 Antibacterial activity of lactic acid bacteria

The antibacterial activity of the CFS and viable cells of all 88 LAB isolates were tested against two test *Salmonella* isolates (S1 and S57) of bovine origin (Fig 4.9). In the antibacterial assays, LAB isolates exhibited varying anti-*Salmonella* activity across species with higher zones of inhibition observed with viable LAB cells in the agar overlay method. The highest anti-*Salmonella* activity against S. *enterica* S1 and S. *enterica* 57 in the agar over lay method was demonstrated by *Lactobacillus amylovorous* C94 with 21mm and 22mm respectively while some strains of *Streptococcus infantarius* (*S. infantarius* C70, *S.infantarius* C75 and *S. infantarius* C80) and *Enterococcus hirae* C9 did not show any antimicrobial activity against the test pathogens. The CFS of *L. salivarius* C86 showed the greatest anti-*Salmonella* activity with 20 mm and 22 mm diameter zones of inhibition against S. *enterica* S1 and S. *enterica* 57 respectively as shown in Table 4.3. Only the CFS of *E.hirae* C9 and *S.infantarius* C75 did not exhibit any anti-*Salmonella* activity.

Lactobacillus spp: *L. plantarum* C3, *L. amylovorus* C15,*L. ingluviei C*31,*L. mucosae* C61, *L. salivarius* C86, *L. amylovorus* C94, and *L. amylovorus* C99 selected based on their antimicrobial potentials were further tested against an array of pathogens: *Salmonella enterica* Typimurium ATCC 14028, ESBL producing *Escherichia coli* T51, *Klebsiella*spp., *Pseudomonas aeruginosa and Staphylococcus aureus* A104. All the tested potential probiotic LAB displayed appreciable antibacterial activity against the tested pathogens with *L. amylovorus* C94 and *L. salivarius* C86 both consistently exhibiting the highest antimicrobial activity on the average among the selected lactobacilli (Table 4.4). The potential of the LAB to produce inhibitory proteinaceous substance was determined by neutralizing the organic acid and protein precipitation with ammonium sulphate. Bacteriocin-like inhibitory metabolites was not detected in any of the LAB isolated in this study.

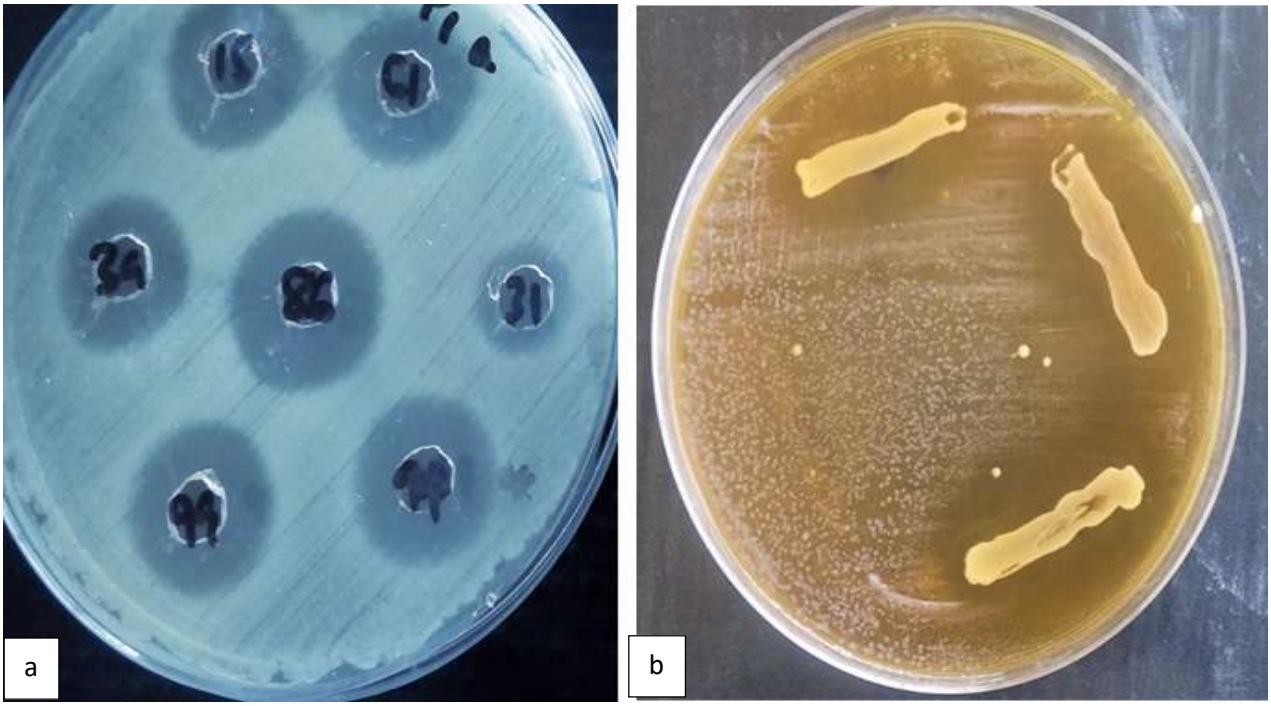

Fig 4.9.Anti-*Salmonella* activity of lactic acid bacteria isolates.Clear zones indicate antimicrobial activity

a: Anti-*Salmonella*activity of cell free supernatant of LAB isolates

b: Anti-*Salmonella* activity of viable LAB cells

Table 4.3. Antibacterial activity of lactic acid bacteria against bovine strains of *S. enterica*

LAB Species	No of Isolates (%)	Zone of inhibition (mm)							
		Salmonella enterica S1				*Salmonella enterica* S57			
		+	++	+++	++++	+	++	+++	++++
Lactobacillus agilis	4 (4.55)	(0) 0	(1) 1	(2) 3	(1) 0	(0) 0	(0) 0	(4) 4	(0) 0
Lactobacillus amylovorus	10 (11.36)	(1) 0	(1) 2	(5) 5	(3) 3	(0) 0	(1) 2	(5) 5	(4) 3
Lactobacillus animalis	1 (1.14)	(0) 0	(1) 1	(0) 0	(0) 0	(0) 0	(0) 0	(1) 1	(0) 0
Lactobacillus gasseri	5 (5.68)	(0) 0	(1) 0	(4) 5	(0) 0	(0) 0	(2) 2	(3) 3	(0) 0
Lactobacillus ingluviei	9 (10.23)	(1) 1	(1) 1	(7) 7	(0) 0	(1) 1	(1) 1	(7) 7	(0) 0
Lactobacillus mucosae	10 (11.36)	(1) 2	(2) 2	(7) 6	(0) 0	(1) 1	(4) 4	(5) 5	(0) 0
Lactobacillus paraplantarum	1 (1.14)	(0) 0	(0) 0	(1) 1	(0) 0	(0) 0	(1) 1	(0) 0	(0) 0
Lactobacillus plantarum	2 (2.27)	(0) 0	(0) 0	(1) 2	(1) 0	(0) 0	(0) 0	(2) 2	(0) 0
Lactobacillus reuteri	1 (1.14)	(0) 0	(1) 1	(0) 0	(0) 0	(0) 0	(0) 1	(1) 0	(0) 0
Lactobacillus salivarius	2 (2.27)	(0) 0	(0) 0	(1) 1	(1) 1	(0) 0	(0) 0	(1) 1	(1) 1
Lactobacillus taiwanensis	3 (3.41)	(0) 0	(1) 0	(2) 3	(0) 0	(1) 1	(0) 0	(2) 2	(0) 0
Weissella cibaria	1 (1.14)	(0) 0	(0) 0	(1) 1	(0) 0	(0) 0	(0) 0	(1) 1	(0) 0
Streptococcus equines	1 (1.14)	(0) 0	(1) 1	(0) 0	(0) 0	(1) 1	(0) 0	(0) 0	(0) 0
Enterococcus hirae	12 (13.64)	(4) 3	(3) 4	(4) 4	(1) 1	(2) 2	(8) 8	(1) 1	(1) 1
Streptococcus infantarius	26 (29.55)	(4) 5	(17) 15	(3) 5	(2) 1	(3) 3	(19) 20	(4) 3	(0) 0

Range of *Salmonella* inhibition used: 0-5 = +, >5<12= ++, 12-18 = +++, >18 = ++++. The diameter of inhibition by cell free supernatant is shown in parenthesis.

Table 4.4. Antibacterial activity of selected lactic acid bacteria against selected pathogens

Lactic Acid Bacteria	Zones of Inhibition (mm)				
	E. coli T51 (ATCC 14028)	*P. aeuroginosa*	*Klebsiella* spp.	*S. aureus* A104	*S. enterica*
Lactobacillus plantarum C3	12	18	14	28	16
Lactobacillus amylovorus C15	13	30	12	30	16
Lactobacillus ingluviei C31	12	12	11	28	18
Lactobacillus mucosae C61	12	20	15	30	15
Lactobacillus amylovorus C86	16	33	18	38	18
Lactobacillus salivarius C94	16	32	17	38	20
Lactobacillus amylovorus C99	15	30	14	32	18

4.5 Tolerance to acid and bile

The ability of all the isolated LAB to resist acidic growth condition and bile supplementation in growth medium was tested. The isolated LAB showed varying tolerance characterised by difference in the viable cell counts as compared with the initial count and control.Generally, it was observed that all the isolated LAB had the ability to survive acidic pH 3 for three hours except four *Lactobacillus* spp; *L. mucosae* C101, *L. ingluviei* C13*, L. ingluviei* C89and *L. taiwanensis* C20 which had no growth. The LAB strain that showed the highest resistance to acidic growth condition (pH 3) was *L. salivarius* C86 resulting in a log reduction from an initial cell count (T_0) of 9.3 $x10^9$ CFU/mLto a final cell count (T_3) of 5.1 $x10^8$ CFU/mL as seen in Appendix V.

LAB isolated in this study demonstrated varying capabilities to survivedifferent concentrations of bile supplementation. All the LAB survived bile supplementation at 0.1% to 1% for 3 hours with an average of about 1.5 $\log_{10}$ reduction, the viability of the LAB cells reduced with increasing bile concentration. The viability of LAB at 7% bile supplementation ranged from 9.3 $x10^8$ CFU/mL in *L. amylovorous* C94to 1.3 $x10^2$ CFU/mL in *Lactobacillus ingluviei*C13 while 6 of the isolates failed to grow at 7% bile supplementation and they include; *S. infantarius* C63, *S. infantarius* 53, *S. infantarius* C78, *L. mucosae* C104, *L. mucosae* C101 and *E. hirae* C34. Based on the outcome of the acid and bile tolerance assay and antimicrobial activity, 5 potential probiotic LAB strains were selected for further characterisation.

4.6 Growth in consecutive pH 3 and 7% bile supplementation

The ability of the 5 selected potential probiotic LAB to resist consecutive acid and bile growth medium supplementation was also determined. Two *Lactobacillus* strains: *L. amylovorus* C94 and *L. salivarius* C86 demonstrated the highest resistance to consecutive low pH of 3 and 7% bile supplementation with a final 2 $\log_{10}$ reduction in CFU/mL from 6.9x 10^{10} to 2.5 $x10^8$ CFU/mL for *L. salivarius* C86 and from 1.9 x 10^{10} to 5.7 x 10^8 CFU/mL for *L. amylovorus* C94 as shown in Table 4.5 while *L. plantarum* C3, *L. mucosae* C61 and *L. ingluvie* C31all had 3 $\log_{10}$ reduction each in viability after the consecutive low pH and bile supplementation assay.

Table 4.5. Survival of lactic acid bacteria in consecutive low pH and bile supplementation

Selected Lactobacilli	pH 3 (3 hours contact)			7% Bile (3 hours contact)			Total Log Reduction
	Initial	Final	Log reduction	Initial	Final	Log reduction	
L. plantarum C3	4.9×10^8	8.9×10^6	2 log	1.2×10^7	1.7×10^5	2 log	4 log
L. ingluvieC31	2.5×10^{10}	4.0×10^9	1 log	1.3×10^8	3.7×10^7	1 log	2 log
L. mucosae C61	3.4×10^9	5.7×10^7	2 log	8.9×10^6	1.2×10^6	nil	2 log
L.salivarius C86	6.9×10^{10}	3.2×10^9	1 log	1.0×10^9	2.5×10^8	1 log	2 log
L.amylovorous C94	1.9×10^{10}	5.7×10^9	1 log	1.2×10^9	5.7×10^8	1 log	2 log

4.7 Quantification of major organic acids produced by lactic acid bacteria

The quantity of the major acids; lactic, acetic and propionic produced by the 5 potential probiotic LAB was determined by HPLC analysis. Lactic acid bacteria in this study generally produced more quantities of lactic acid than acetic acid and propionic acid. Lactic acid accounted for 79.56% to 81.11% of all tested organic acids produced while the least produced was propionic acid (5.61% - 6.99%) except in *L. ingluvie* C31, from which we assayed more propionic acid (49.91%) and the least lactic acid (21.66%). *Lactobacillus salivarius* C86 was the highest producer of lactic acid (67.85 mg/ml; 81.11%), followed closely by *L. amylovorous* C94 which produced 54.91 mg/ml (80.93%) while *L. ingluivie* C31 produced the smallest concentration (8.88 mg/ml; 21.66%) as shown in (Fig 4.10) and Appendix VI.

4.8 Antibiotic susceptibility of lactic acid bacteria

The antibiotic susceptibility of all the LAB isolates was tested. The LAB showed general susceptibility to chloramphenicol, ampicillin, amoxicillin-clavunalic acid and erythromycin while there was 98.8% susceptibility to tetracycline. There was complete resistance to kanamycin, vancomycin and aminoglycosides; gentamicin and clindamycin (Fig 4.11).

The MIC of nine antibiotics of human and veterinary importance was determined with E-test strips. The LAB tested at this stage were five selected potential probiotic lactobacilli: *L. amylovorus* C94, *L. salivarius* C86, *L. ingluvie* C31, *L. mucosae* C61 and *L. plantarum* C3. All the selected LAB isolates were susceptible to the panel of antibiotics tested for the selection of probiotic organisms (Table 4.6).

4.9 Anti-*Salmonella*activity of lactobacilli in co-culture

The two selected lactobacilli: *L. salivarius* C86 and *L. amylovorus* C94 showing the most promising probiotic potentials in terms of overall antimicrobial activity, production of inhibitory organic acids and tolerance to consecutive low pH and bile supplementation were tested for anti-*Salmonella* activity in a 24-hour kill rate co-culture assay (Fig 4.12). A rapid decline in the viability of *Salmonella* was observed from 8 $\log_{10}$ to no bacterial growth between 8 hours and 16 hours contact time of both selected lactobacilli strains with the two test Salmonellae in co-culture. The bacterial cell count for *S. enterica* S1 and *S. enterica* S57 was 3.9×10^8 and 5.7×10^8 respectively in the control *Salmonella* monoculture at T_{16} while there was

no*Salmonella* growth from the co-culture inoculum at T_{16} in SSA. The growth of both lactobacilli in theLAB-*Salmonella* mix and *Lactobacillus* monoculture controls were similar as seen in Appendix VII.

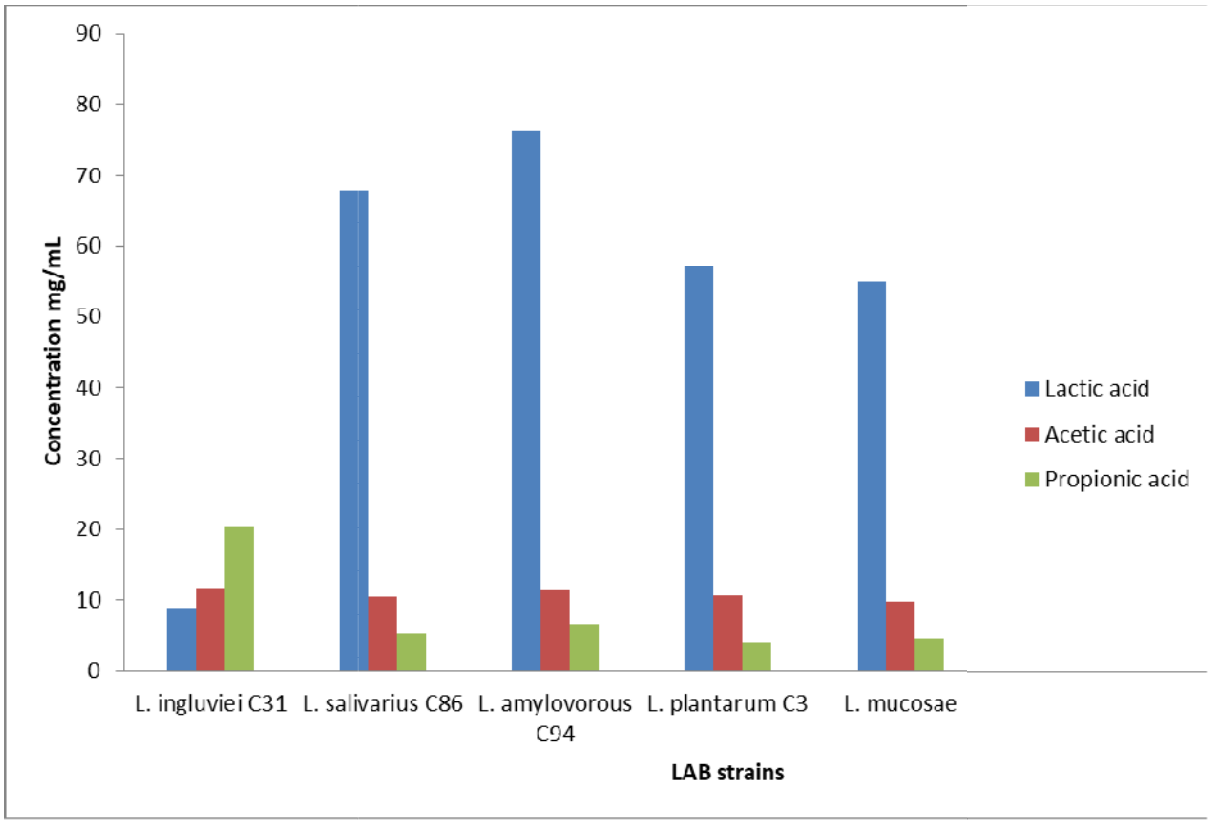

Fig 4.10. Quantity (mg/mL) of selected organic acid produced by potential bovine probiotic lactobacilli

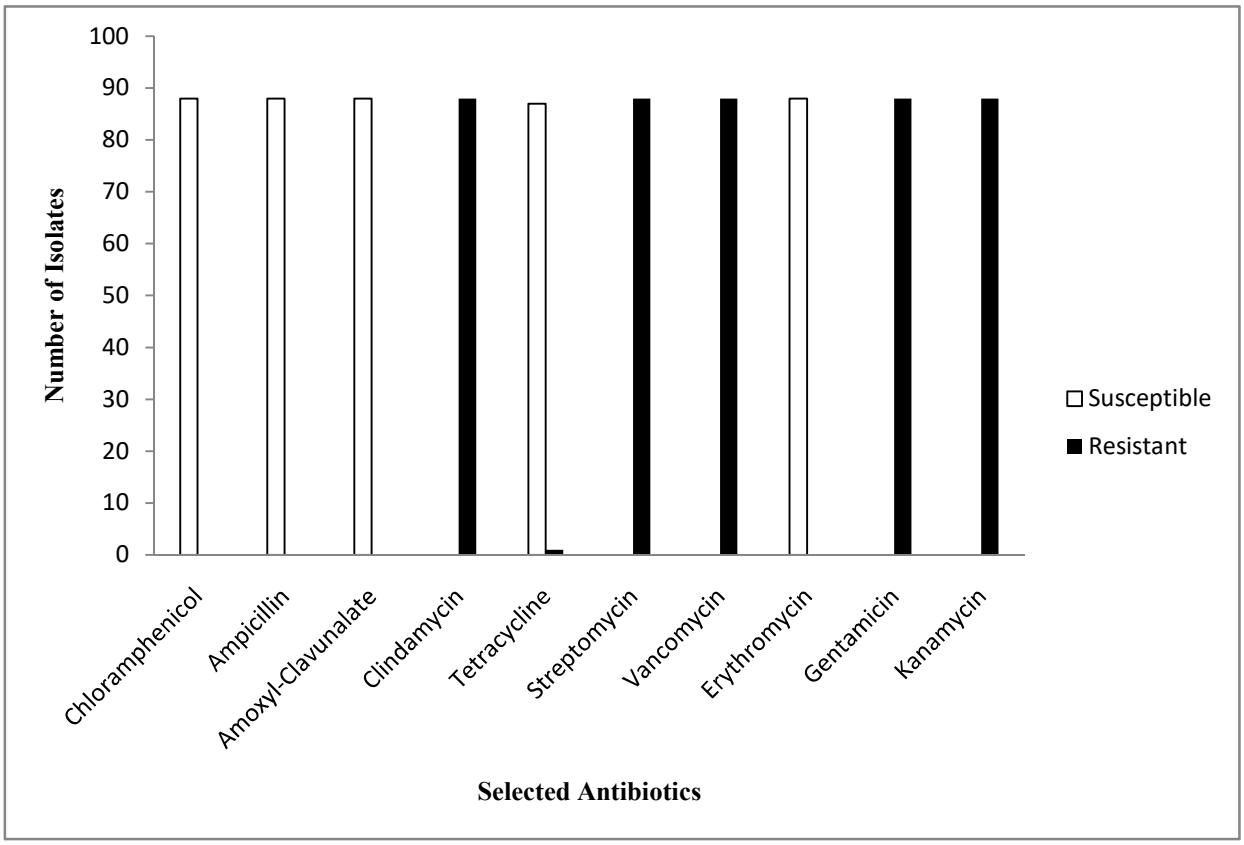

Fig 4.11. Antibiotic susceptibility of bovinelactic acid bacteria isolates

Table 4.6. Minimum inhibitory concentration of potential probiotic LABto selected antibiotics

	ampicillin (bp = 4)	gentamicin (bp =16)	kanamycin (bp = 64)	streptomycin (bp =64)	erythromycin (bp =1)	clindamycin(bp =1)	tetracycline (bp =8)	chloramphenicol (bp=4)
MIC (EFSA cut –off values) [mg/L]								
Lactobacillus plantarum C3	1.0	2.0	5.0	8.0	0.2	0.6	4.0	2.0
Lactobacillus ingluvie C31	0.5	5.0	15	12	0.5	0.6	6.0	2.0
Lactobacillus mucosae C61								1.5
	2.5	10	16	0.5	0.5	4.0	3.0	
Lactobacillus salivarius C86	1.0	1.5	4.0	8.0	0.3			
	0.5	2.0	3.0					
Lactobacillus amylovorous C94				0.5	1.0	8.0	10	0.1
	0. 5	1.0	2.0					

Note- bp = breakpoint as recommended by European Food Safety Authourity

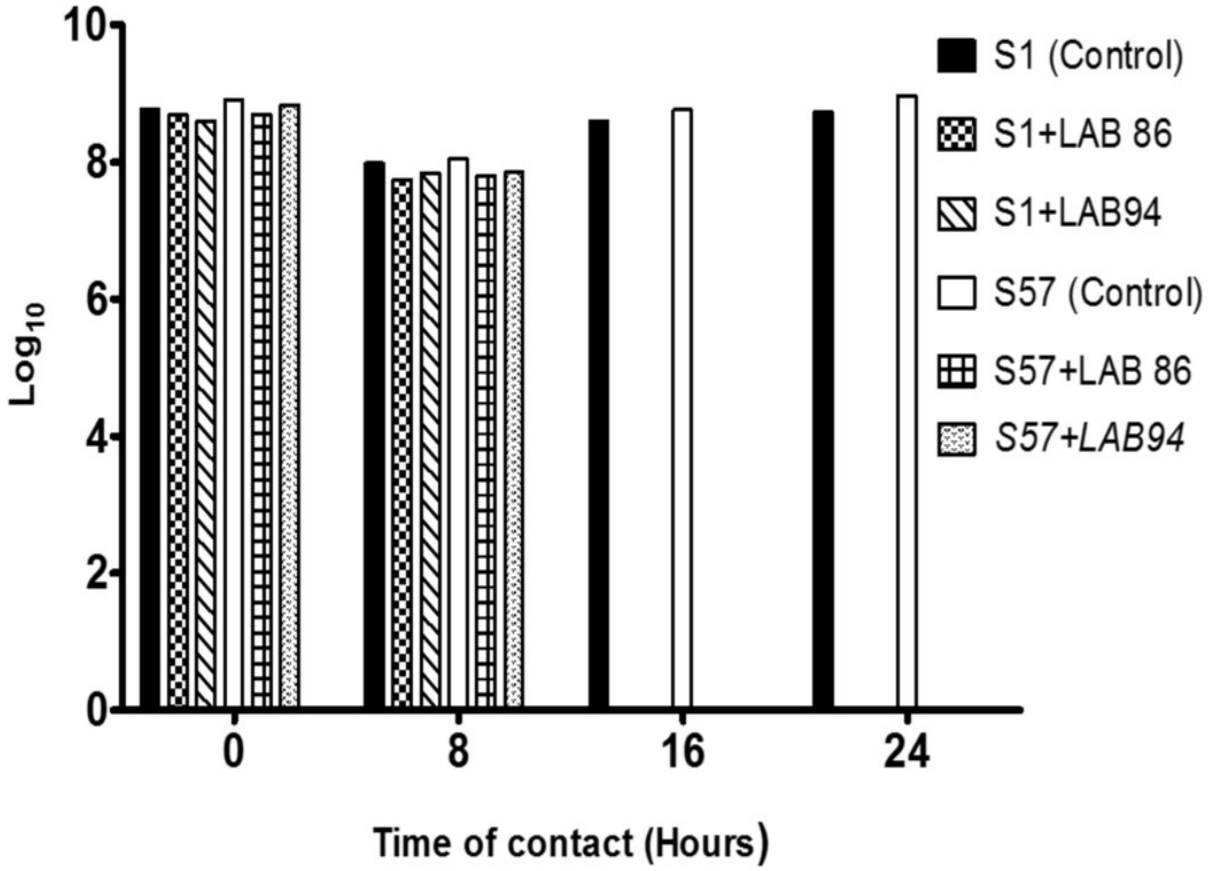

Fig 4.12. Growth of *Salmonella* and lactobacilli in co-culture

S1: *S. enterica* S1; S57: *S. enterica* 57;LAB 86: *L. salivarius* C86; LAB 94: *L. amylovorous* C94

4.10. *In-vivo*safety assesment of isolated lactic acid bacteria

The ability of the 5 selected LAB to lyse red blood cells was also determined as part of the safety profile of the isolates with probiotic potential.None of the LAB tested exhibited haemolytic effects as observed with the absence of lysis on bovine blood agar.

Bacterial translocation is an indication of potential pathogenicity. As part of the safety consideration, the ability of two potential probiotic LAB strains; *L. salivarius* C86 and *L. amylovorus* C94 to migrate to extra-intestinal sites was tested in rabbits. No viable Lactobacilli was detected in the blood cultured plate of all the rabbits in both control and test group. There was also no growth in the tissue homogenates for both the test and control samples plated in MRS agar after incubation for 24 hours. The outcome of the toxicity study suggests that oral dosage of about 5.3×10^{10} CFU/day of a mixture of *L. salivarius* C86 and *L. amylovorus* C94 for 7 days did not result in treatment related sign of toxicity or death in any of the rabbits. There were no untoward changes in appearance or behavior and there was no difference in faecal consistency between the treatment and control group, hence, no evidence of toxicity in the studied animal as a result of the administered potential probiotic lactobacilli.

The antibacterial activity of*L. salivarius* C86 and *L. amylovorus* C94 was also tested *in-vivo*in calves. Generally, clinical signs of disease such as diarrhea, fever, loss of appetite and behavioral changes were not observed in all the experimental animals administered with the LAB in the probiotic intervention experiment.

4.11. Quantification of enterobacteria and lactobacilli in cattle faeces

The specificity of the primer sets used for qPCR was determined by end-point PCR and the PCR products were checked with gel electrophoresis. The amplicons were confirmed to correspond to the expected size for the species of interest and no amplicon for non target species as shown with only one specific PCR product for each set of primers as illustrated by only one peak in the melting curve analysis (Fig 4.13).

Quantitative PCR was employed to determine the relative increase/decrease in number of members of the genus *Lactobacillus* and the family Enterobacteriaceae after probiotic feeding intervention period of 30 days. A total of 9 calves [6 (treatment), 3 (control)] completed the feeding trial. The DNA concentration interpolated from the Ct values of the qPCR analysis for these microorganisms is directly proportional to the bacterial concentration in the samples. At baseline, before the probiotic intervention, the concentration of lactobacilli in all subject's

faecal DNA ranged from 0.7ng/uL to 3.7ng/uL with a mean of 2.1ng/uL while the concentration of Enterobacteriacea was between 2.77ng/uL and 3.2ng/uL with a mean of 3.0 ng/uL. After one month of probiotic intervention, there was a significant (p= 0.01) increase in the population of lactobacilli in all the calves fed with the probiotic suspension when compared with the initial baseline concentration. One of the three calves in the control group had a marginal increase in the lactobacilli concentration at the end of the feeding trial while there was a marked reduction in the lactobacilli concentration in the other control as seen in Fig 4.14 and AppendixVIII. An independent *t*-test showed a significant reduction in the concentration of enterobacteria (p= 0.01) following the probiotic intervention in the treatment group in contrast to the calves in control group which had higher concentration of enterobacteria after the feeding period than the baseline concentration (Fig 4.15).

4.12. Survival of lactobacilli during lyophilisation and storage

The ability of *Lactobacillus amylovorous* C94 and *Lactobacillus salivarius* C86 to retain viability after freeze drying and during storage at 25±2^{o}C was determined. Both strains survived the lyophilisation process with about one logarithm reduction in nuber of viable colony forming unit/ml from 3.9x 10^{10} to 8.7 x10^{9} CFU/mL for *L. salivarius* C86 and from 8.2 x 10^{10} to 1.0 x10^{10} CFU/mL for *L. amylovorous* C94 . A 4 log reduction in viability from 3.9x 10^{10} to 1.8 x10^{6} CFU/mL for *L. salivarius* C86 and from 8.2 x 10^{10} to 1.0 x 10^{6} CFU/mL for *L. amylovorous* C94 was observed in both lactobacilli over a storage period of three monthswith an average monthly reduction of one logarithm as shown in Table 4.9.

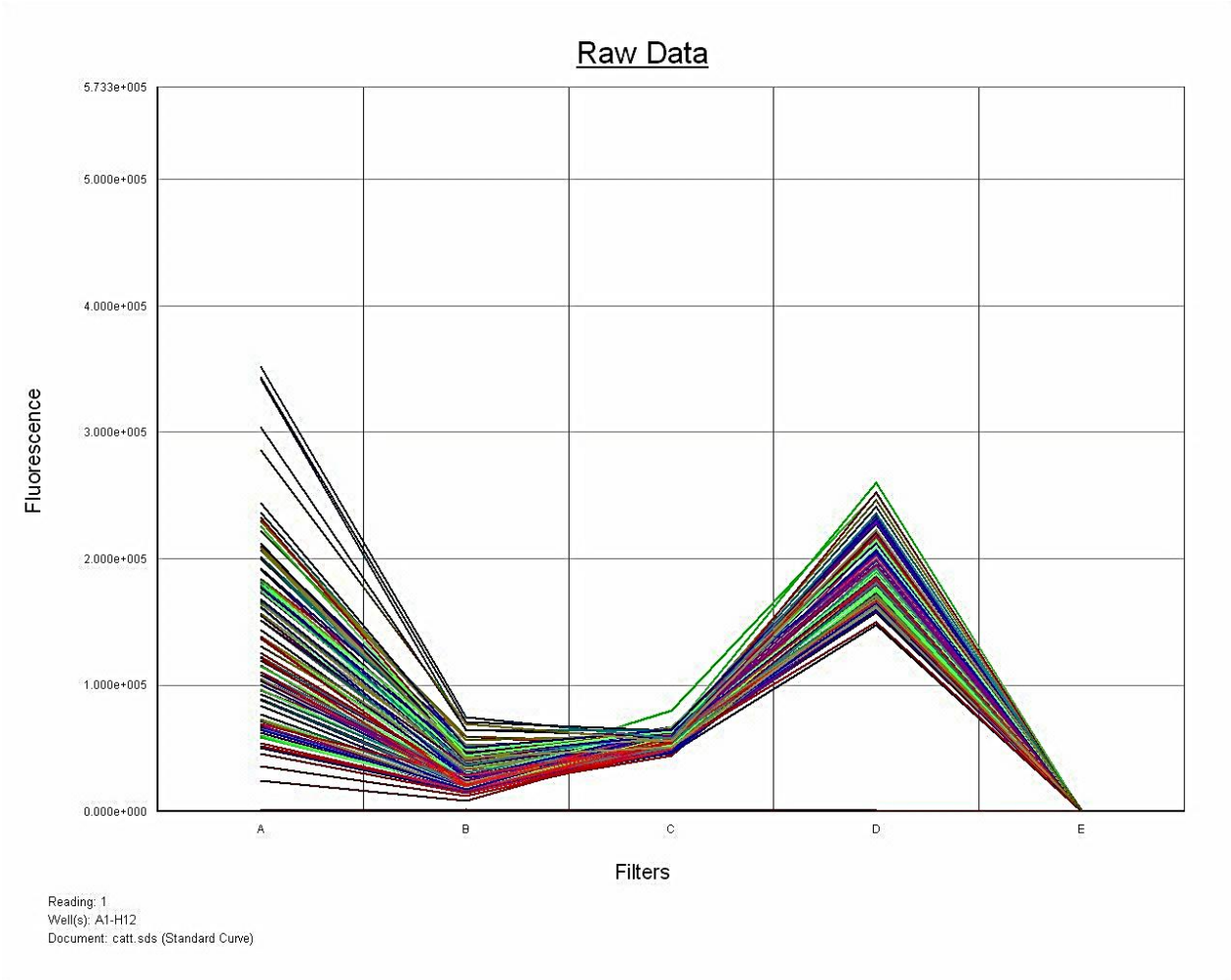

Fig 4.13.Melting curve analysis showing a single specific PCR product for primers used in qPCR.

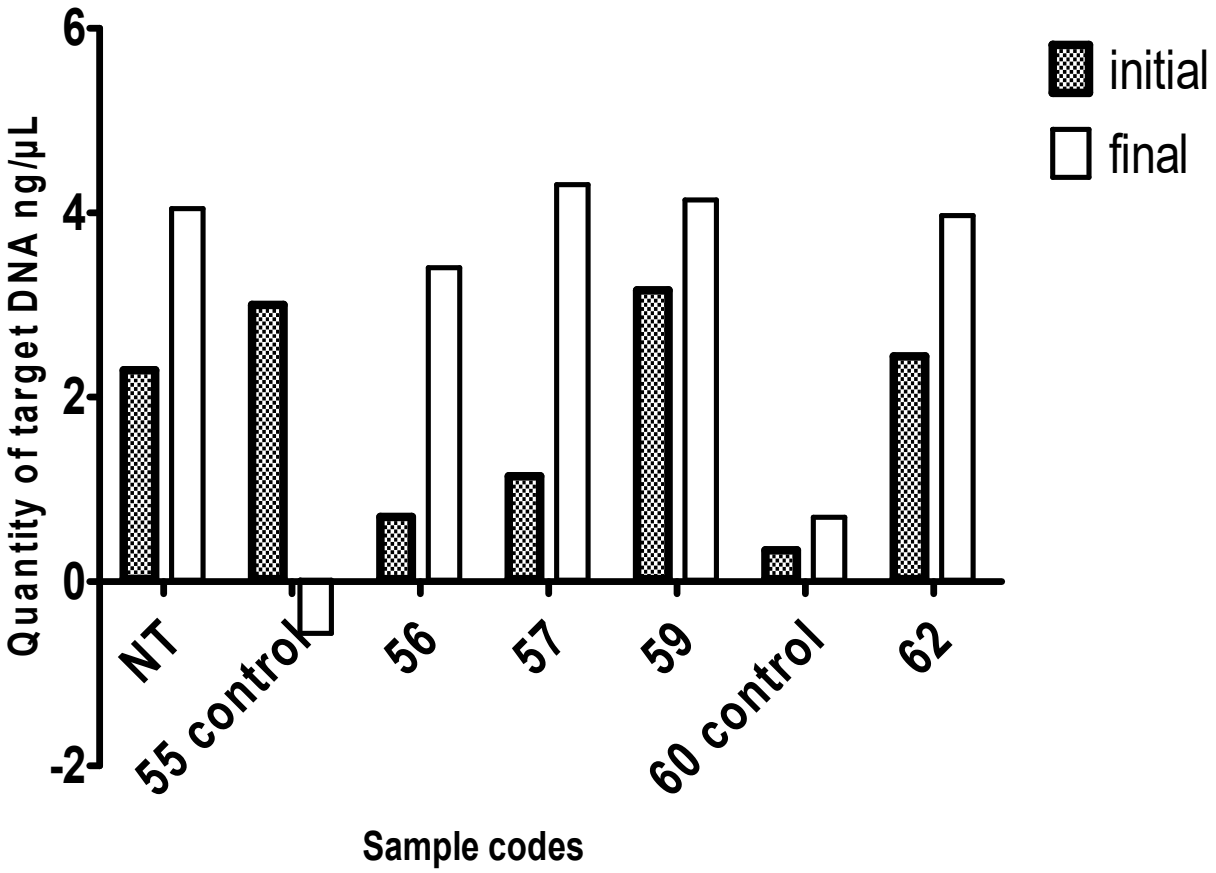

Fig 4.14. Quantification of total lactobacilli in cattle faecal samples after the feeding intervention

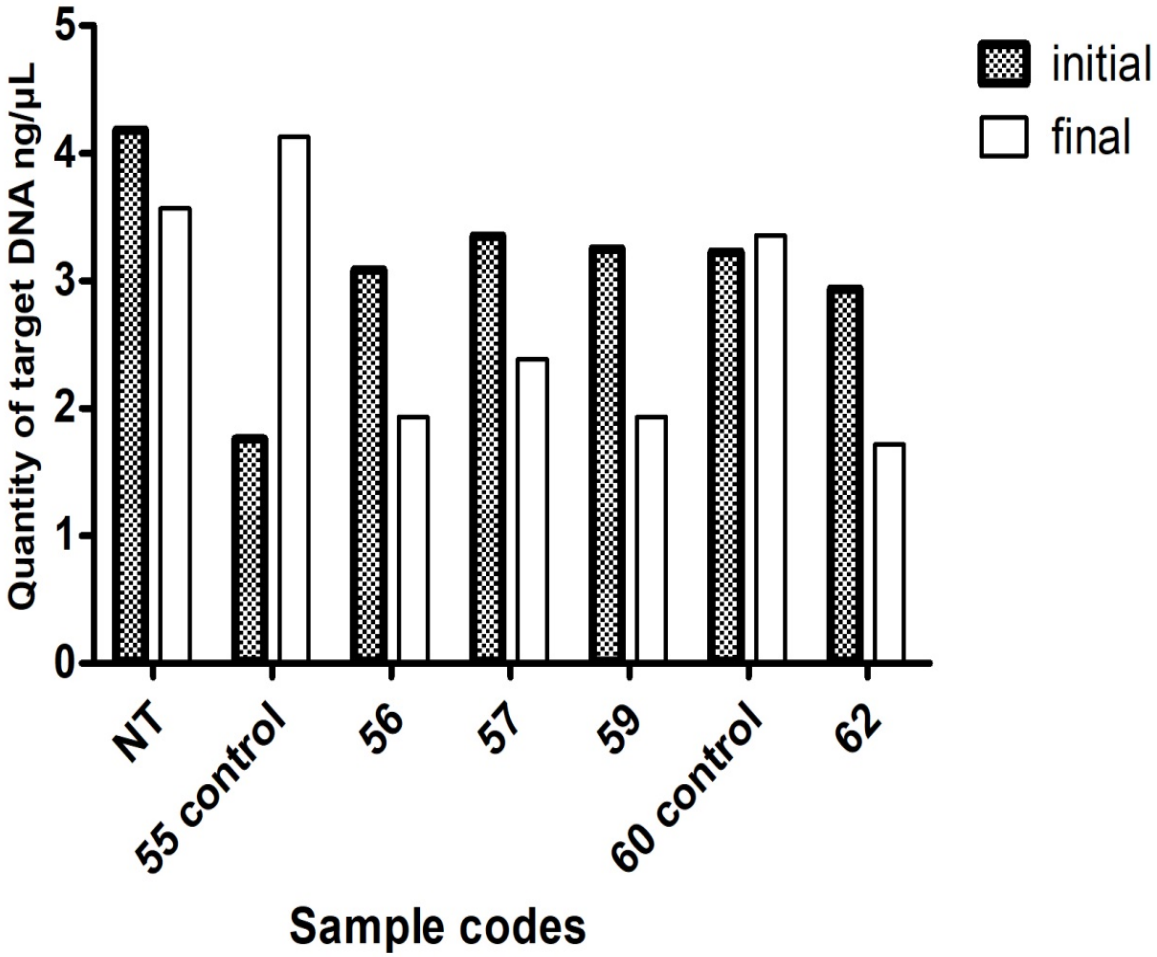

Fig 4.15. Quantification of Enterobacteriaceae in cattle faecal samples after the feeding intervention.

Table 4.7. Viability of lactic acid bacteria during freeze drying and storage

	Before freeze drying	After Freeze drying	2 Weeks	4 Weeks	8 Weeks	12 weeks
L. salivarius C86	3.9×10^{10}	8.7×10^{9}	2.3×10^{9}	3.6×10^{8}	8.1×10^{7}	1.8×10^{6}
*L. amylovorous*C96	8.2×10^{10}	1.0×10^{10}	7.0×10^{9}	1.0×10^{8}	4.8×10^{7}	3.0×10^{6}

CHAPTER FIVE

DISCUSSION

5.1 Identification of *Salmonella*species

Accurate identification of bacterial pathogens is critical in many aspects of public and animal health including disease diagnosis, epidemiologic surveillance, food safety and environmental monitoring. Phenotypic and biochemical methods alone are largely insufficient to correctly identify microorganisms, often leading to misidentification of bacteria (Ayeni and Odumosu, 2016). *Salmonella*e in this study were identified by growth on selective and differential media, biochemical identification system (Microbact 24E), MALDI-TOF and amplification of generic *invA* gene. A number of studies have compared conventional methods of bacterial identification such as growth in differential media and biochemical reaction with MALDI-TOF and molecular identification methods (Jesumirhewe *et al*., 2016; Ayeni *et al.,* 2017). In this study, only 32 out of the 68 (47.1%) isolates presumed to be *Salmonella* based on cultural methods were identified by Microbact 24E, PCR amplification of *invA* gene and MALDI-TOF as *Salmonella* spp. This is in agreement with the outcome of Ayeni *et al.,*(2015) where all *S.aureus* identified by MALDI-TOF were also positive for *spa* geneamplification but in discordance with biochemical identification by slide agglutination. Misidentification of Enterobacteriaceae isolates from clinical samples by conventional methods has also been reported by Jesumirhewe *et al*., (2016) in comparison with MALDI-TOF.Polymerase chain reaction as been reported by many authors to be more sensitive and less labourintensive than conventional cultural method of *Salmonella* identification, possibly because it relies on the presence of genetic sequences of interest for detection and identification rather than serial enrichments and growth on selective-differential media (Langkabel *et al*., 2014; Jinu *et al.,* 2014; Bell *et al.,* 2016).

The results of Microbact 24E, PCR and MALDI-TOF were in accordance in the identification of *Salmonella* spp. in this study, these methods have been credited as reliable for bacterial identification (Ayeni *et al*., 2015; Jesumirhewe *et al*., 2016).

5.2. Prevalence of *Salmonella*spp. in cattle faeces

Salmonella spp. have been reported in healthy cattle at slaughterand the consumption of beef and other dairy products have been linked with food-borne disease outbreaks (Elfenbein *et al.,* 2013). The prevalence of *Salmonella* carriage in beef cattle at slaughter is a predictor of the chances of eventual carcass contamination which in turn determines the risk of human *Salmonella* infections (Kemal *et al.,* 2014). There are only a few reports available with quantitative data on *Salmonella* present in faeces of healthy cattle (Cummings *et al.,* 2010). Hence, the prevelance of *Salmonella* carriage in healthy cattle in the Teaching and Research Farm of the University of Ibadan was studied. In a similar prevalence study of *Salmonella* in pigs, it was observed that the prevalence determined was influenced by the size of the samples, such that the larger the amount of faecal sample used, the higher the chances of *Salmonella* detection. In another study, the prevalence of *Salmonella* was examined in 80 porcine faecal samples using 1, 10 and 25 g and the resulting prevalence was 11, 22 and 24% respectively (Funk *et al.,* 2000). Large sample weights are not mostly used by researchers, possibly for convenience and/or economic reasons; hence, the ten gram (10g) of cattle faeces that was screened for *Salmonella* spp. in this study was a compromise between sensitivity of the method and economy. The single time point sampling protocol employed in this study is likely to underestimate the actual prevalence of intermittent shedding of *Salmonella*, the method mirrors that previously reported in many other *Salmonella* surveys (Sorensen *et al.,* 2003).

The result of this study revealed that the prevalence of *Salmonella* spp. in cattle faeces on UI-T&RF is 23.2%. The present finding is considerably higher than that of a previous study in northern Nigeria where the prevalence of *Salmonella* was 10% (Umeh and Enwura, 2014). Relatively, lower prevelence of *Salmonella* spp. in slaughter cattle have also been reported in many African countries and in other western climes.The prevalence of *Salmonella* isolated from cattle faeces in Egypt is 0.0% (El-Gamal and El-Bahi, 2016), 0.5% in Namibia (Renatus *et al.,* 2015), in Ethiopia 11.3% (Takele *et al.,* 2018). Hah *et al.,*(2011) revealed a 1.2% to 2.0% prevalence of *Salmonella* in faeces of ready-for–slaughter cattle in Korea. *Salmonella*prevalence in beef cattle is about 0.5% in Japan and 3.0% in the United Kingdom (Ishihara *et al.,* 2009). Interestingly, *Salmonella* prevalence higher than that obtained in this study (38%) had been previously reported in feedlot cattle in the United States about 2 decades ago (Fedorka-Cray *et al.,* 1998). The differences in

Salmonella prevalence observed in this study and those from other countries could be due the husbandry management practices, geographical distribution, sampling techniques and sample size.

It has been established that the prevalence of *Salmonella* carriage in slaughter cattle directly correlate with the probability of *Salmonella* contamination in the carcass and in turn the risk of human salmonellosis. Molecular methods have provided clues of a clonal relationship between antimicrobial resistant *Salmonella*e from livestock and human sources (Tamang *et al.,* 2011).

Although, several intervention strategies have been initiated in the meat processing chain to limit carcasse contamination with potential pathogens harbored by food animals and thus mitigate the risk of food borne infections (Economou and Gousia, 2015), the dearth of modern beef processing facilities in Nigeria has made faecal contamination of meat in the process of slaughtering almost inevitable. Asides hygienic management practices, vaccination and use of antibiotics are two strategies often used to combat *Salmonella* carriage in cattle (Das *et al.,* 2013). However, both interventions have shortcomings: while vaccination does not provide complete protection, persistent use of antibiotics can result in selection of antibiotic resistant *Salmonella* strains with potential public health risks (Hammad and Shimamoto, 2010). Probiotic lactic acid bacteria with anti-*Salmonella* capability have been demonstrated as promising alternative strategies against *Salmonella* carriage in livestock farming (Puphan *et al.,* 2015). Thus, this work explored the anti-*Salmonella* and probiotic potential of LAB cultured from cattle faecal microbiota.

5.3. Antibiotic susceptibility of isolated *Salmonella* species

All *Salmonella* isolates from this survey were susceptible to the panel of antibiotics tested. The high susceptibility of LAB to antibiotics recorded in this study is in tandem with the report of Dargatz *et al* (2015) where almost all the *Salmonella*e isolated were sensitive to all the antibiotics tested. The pan susceptibility reported in this study is however in disagreement with that of Sorensen *et al* (2003) in which none of the *Salmonella* isolates tested was sensitive to the panel of tested antibiotics. Kim *et al,* (2014) reported that nearly all the *Salmonella* spp. isolated from cattle in their study were resistant to all the antibiotics tested, although the particular antibiotics employed are different from those used in this study. More than 99% of *Salmonella* isolates from livestock in China were reported to be

resistant to at least one antibiotic, with about 41.5 % resistant rate to ciprofloxacin (Lai *et al.,* 2014).

Our findings on the susceptibility of *Salmonella* in this study to ciprofloxacin, trimethoprim-sulfamethoxazole and tetracycline were similar to that of Adzitey *et al,* (2015) but differ in susceptibility to gentamicin. General resistance of bovine *Salmonella* to macrolides, aminoglycosides and tetracycline have been published by many authors (Hah *et al*., 2011; Umeh and Enwuru, 2014; Kim *et al.,* 2014), but those isolated in this study showed high susceptibility to these classes of antibiotics. *Salmonella* strains from other livestock have been reported to be commonly resistant to tetracycline (EFSA, 2014). This is linked to the indiscriminate use of tetracycline and oxytetracyline in food producing animals. The general susceptibility of *Salmonella enterica* isolated from cattle faeces, as observed in this study, suggests a positive association between judicious use of antibiotics in farm animals and antibiotic susceptibility in farm animal-borne microorganisms. Meta-data obtained from the resident farm veterinarians showed that antibiotics are not used on the UI-T&R farm other than for therapeutic purposes. This is likely tobe a major determinant of high antimicrobial susceptibility observed on isolates from the farm.

5.4 Isolation, identification and diversity of lactic acid bacteria isolated in this study

Lactic acid bacteria are ubiquitous in nature and have been cultured from several environmental niches and gastrointestinal tracts of animals and humans. In this study, LAB were isolated from cattle faeces for the characterisation of their probiotic potential. The source of isolation of probiotic strain is germane to its survival and efficiency at the intended site of beneficial action, for optimum probiotic activity. The strain must survive, proliferate and colonize the specific site of presumed action (De Vos *et al.,* 2010). There are also emerging evidence that probiotic strains are host specific (Mills *et al.,* 2011), suggesting that LAB intended for oral administration in animals are better isolated from the gut of the intended host other than environmental sources. The microbial community of the gut in humans and animals consist of more than one thousand different species of microorganisms **(**Mokoena, 2017). Bacteroidetes and Firmicutes are the two dominant phyla of the mammalian bacterial community. Gut microflora are diverse and unique depending on the animal species (Karlsson *et al.,* 2011). About 90% of the dominant bacterial groups in bovine gut are recognized as defined groups, however, some members

of the cattle gut flora are yet to be identified as a result of incomplete knowledge of the gastrointestinal bacterial ecosystem implied from the many 16S rRNA genes obtained from cattle faecal samples that have not been previously reported in the gut microflora (Uyeno *et al.,* 2010). Lactic acid bacteria represent a significant group in the Firmicutes group resident in the bovine intestine.

Since probiotic features are strain specific attributes which cannot be extended to other strains within the same species, it is expedient that microorganisms to be considered must be correctly identified to the species level with internationally recognized techniques such as sequencing of 16S rRNA genes or DNA-DNA hybridization, as phenotypic methods alone are not sufficient for thorough bacterial identification. Correct identification of probiotic strains is also important for linking specific health benefits to a particular strain (Kapitula, 2008). As a result, the main method of identification for the LAB isolated in this study was through sequencing of the 16S rRNA genes, and the sequences were deposited in Genbank of NCBI.Phylogenetic information of probiotic strains are important for epidemiological surveillance (Herbel *et al.,* 2013). Strains of LAB isolated in this study clustered closely with one another as per species, along with similar species obtained from other studies which suggest common ancestral lineage; this comform with the established taxonomy. Analysis of diversity of culturable LAB isolated in this study showed that eighty eight isolates identified belong to 15 species of lactic acid bacteria distributed within the genera: *Lactobacillus, Weissella, Streptococcus* and *Enterococcus***.** Ayeni *et al.,* (2009) have also previously isolated *Weissella, Enterococcus and Lactobacillus* species from bovine intestine in Nigeria. *Lactobacillus* (11) and *S. infantarius* (26) were the dominant genus and species isolated respectively. This contrasts the findings of Adeniyi *et al* (2015) in which *Enterococcus hirae* was the most abundant species of LAB isolated from cattle faecal samples collected in the same geographical location, and no *Lactobacillus* spp. was isolated. This vast variation could be as a result of differences in methodology such as bacterial isolation procedure and species identification since Ayeni *et al.,* (2009) were able to isolate *Lactobacillus* spp. in a similar study. All the *Enterococcus* spp. isolated in this study were observed to belong to *E. hirae*, agreeing with the report of Anderson *et al.,* (2008) which found *E.hirae* as the predominant enterococciin cattle.

It is worthy of note that a number of LAB strains isolated in the current study, exemplified by *Lactobacillus taiwanens* are not known residents of the bovine gut microflora. *Lactobacillus taiwanensis* was first reported by Wang *et al.,* (2009) in Taiwan, where it

was isolated from silage in a cattle ranch and thus named after the geographical location where the sample was collected. *S. infantarius* which was dominant in this research is a prominent LAB found in various processed dairy products but rarely in fresh cattle milk (Wullschleger *et al.,* 2013). *Lactobacillus mucosae* is a novel porcine gastrointestinal LAB species first reported by Roos *et al*., (2000) while *S. equinus* is mainly a species of horse origin related to *S. bovis* which is predominantly found in cattle faeces and are sometimes referred to as the *S. bovis/ S. equinus* complex (Clarke *et al*., 2016).

16S rRNA targeted PCR was unsuccessful for 10 out of the 88 LAB isolates, probably due to primer incompatibility, necessitating the use of another identification method. MALDI TOF MS technique was therefore employed, and all 10 isolates were identified as *E. hirae.*

5.5 Antimicrobial activity of lactic acid bacteria

The gastrointestinal tract of cattle consists of a complex array of microorganisms constantly competing with one another for limited resources in the same ecological niche. The possession of antimicrobial property is a survival "strategy" by some gut microbial residents to outcompete other species. A vital consideration in the selection of LAB for probiotic use is their antimicrobial activity against pathogens (WHO, 2006). Lactic acid bacteria identified in this study displayed significant antimicrobial activity against *Salmonella* spp. of bovine origin. Seven LAB demonstrating promising antibacterial potential were tested against an array of pathogens; *S.enterica* Typhimurium ATCC 14028, *P.aeruginosa,Klebsiella* spp., *S.aureus* and ESBL producing *E.coli. Lactobacillus salivarius* C86and *L. amylovorous* C94 consistentlyshowed the greatest and broadest range of antimicrobial activity against *Salmonella*e and all the pathogens tested. The antagonistic activity of LAB of intestinal origin against enteropathogens in this study is in agreement with the reports of Adeniyi *et al.,* (2015) and Sirichokchatchawan *et al.,* (2018) where LAB isolated from cattle faeces demonstrated remarkable antibacterial activity against some enteric pathogens. Lactic acid bacteria with antibacterial activity against *Salmonella* and other enteropathogens have being isolated from various sources such as fermented food, breast milk, vegetables, animal faeces and a host of other environmental sources (Casey *et al*., 2007; Ayeni *et al.,* 2011b; Adeniyi *et al*., 2015; Sirichokchatchawan, 2018).

The observed antibacterial activities of LAB could be as a result of certain products of metabolism with antimicrobial effects exemplified by hydrogen peroxide, certain organic acids and bacteriocins(Ayeni *et al*., 2009; Adeniyi *et al*., 2015). Bacteriocins and other

proteinaceous inhibitory substances were not detected in this study, although the production of bacteriocins has been reported in several strains of *Lactobacillus* spp. (Todorov, 2009), *Weisella* spp. (Srionnual *et al.,* 2007), *Enterococcus* spp. (Perez *et al.,* 2014) and *Streptococcus* spp. (Mokoena, 2017). The main mechanism of antimicrobial activities observed in LAB studied in this research is thought to be production of lactic acid, as all but one of the five LAB selected for that assay produced higher quantities of lactic acid than other organic acids tested. This observation is in agreement with the report of Ayeni *et al.,* (2011) where LAB isolated from cattle intestine produced larger quantities of lactic acid than acetic acid. Lactic acid production is one of the main characteristics of LAB as suggested by their name; lactic acidis known to inhibit a broad spectrum of pathogens. Other short-chain organic acids including acetic and propionic acids were also detected, further confirming the heterolactic nature of the tested strains.

The remarkable antibacterial activity of both *L. salivarius* C86 and *L. amylovorus* C94 correspond with copious production of lactic acid when compared with the antibacterial potential of other lactobacilli strains tested, which produced lower concentrations of lactic acid. This is in concordance with several reports that have attributed the antibacterial properties of various *Lactobacillus* spp. to the production of acids which in turn result in lower pH (Ouwehand and Vesterlund, 2004). The CFS of *Lactobacillus casei* cultured from fermented milk demonstrated potent inhibition of multi-drug resistant *Shigella sonnei* and *S .flexneri* (Mirnejad *et al* 2013). A strain of *Lactobacillus fermentum* reported by Ilayajara *et al.,* (2011) displayed broad spectrum antibacterial activity against enterobacteria including *Proteus* spp., *E. coli*, *Enterococcus* spp., *P aeruginosa* and *K. pneumonia.* The antimicrobial activity of strains of *Lactobacillus delbrueckii* and *L. casei* against *E.coli* O157:H7 correlates with the production of lactic acid (Poppi *et al.,* 2015). A positive correlation has also been reported between decrease in pH, quantity of lactic acid produced and degree of antimicrobial activity observed in some *Lactobacillus* strains against *Shigella sonnei*. It was observed that upon adjustment of pH of CFS to 6.5, no antibacterial activity was recorded, suggesting that the antimicrobial activity was as a result of production of organic acids (Zhang *et al*., 2011). It is worthy of note that *L. salivarius* C86 and *L. amylovorus* C94 completely inhibited the growth of *Salmonella enterica* spp. in less than 16 hours after co-culturing such that no single viable colony of *Salmonella* spp. was recovered upon subculturing on solid growth medium. This

corroborates reports from several authors who have also reported the anti-*Salmonella* ability of LAB in co-culture (Abdel-Daim *et al.,* 2013; Szala *et al.,* 2012).

5.6 Survival in gastrointestinal conditions

An important selection criterion for probiotic strains proposed for oral administration is the ability to withstand adverse condition of the gastrointestinal tract characterised by bile toxicity and low gastric pH (Hawaz, 2014). The capacity to survive these harsh conditions is a critical factor to be considered in *in vitro* selection of probiotic strains (Ayeni *et al.,* 2011).

Most of the isolated LAB were able to withstand varying degrees of low pH and bile salt supplementation. This is not very surprising as similar results have been obtained in a number of studies of LAB isolated from animal gut (Puphan *et al*., 2015), while LAB from food products and other environmental sources have been observed to have reduced potential of surviving the gastric condition (Hassanzadazar *et al.,* 2012). All selected 5 LAB were able to resist consecutive low pH and bile supplementation to various extent with *L. amylovorous* C94 and *L.salivarius* C86 demonstrating excellent ability to survive this condition similar of bovine gut while it receives food (Puphan *et al*., 2015), pH 3.0 is regarded as the standard for acid tolerance screening (Sahadeva *et al.,* 2011). The viable counts of both LAB isolates after the gastric challenge assays were 2.5 X 10^8 and5.7 X 10^8 CFU/mL respectively. These values lie in the range of live bacteria considered sufficient to confer probiotic functions in the gut. It has been demonstrated that ingestion of about 1.0x10^6 to1.0 x10^{10} viable cells daily is required for probiotic effect, which qualifies this isolates as potential probiotic strains (Puphan *et al*., 2015). The ability of LAB strains in this study to survive gastric simulation in contrast to reports on LAB from other sources (Hassanzadazar *et al.,* 2012) was not very surprising since isolation of the studied LAB was from gut of cattle. The ability of *Lactobacillus* species of intestinal origin to resist low pH and bile salt is considered a strategy of evolution to aid survival and migration across the intestine. *bsh*-1 and *bsh*-2 are two bile salt hydrolyzing genes, whose gene products have been reported to confer acid and bile tolerance on *L. salivarius* strainUCC118 (Neville and O'Toole, 2010).

5.7. Antibiotic susceptibilityprofile of lactic acid bacteria

Several antibiotic susceptibility assay methods have been reported for LAB including disc diffusion method, broth dilution, agar dilution and E-test (Abdul-sattar *et al.,* 2011). Of these methods, the disk diffusion susceptibility test also referred to as Kirby-Baur method is the most widely used owing to its high levels of antibiotic concentration standardization and relative ease of use (Huys*et al.,* 2002). Considering the fastidious nature of LAB species; requiring special growth medium and conditions, the convectional media recommended by CLSI for susceptibility testing which are Mueller-Hinton and Iso-Sensitest (IST) agar are unsuitable for such assay in LAB (Klare *et al.,* 2007). The antibiotic susceptibility of LAB and MIC of selected potential probiotic strains in this study were determined by disk diffusion method and the MIC of selected potential probiotic strains was determined by Kirby-Baur method and E-test respectively on *Lactobacillus* Susceptibility Media (LSM) as suggested by ISO/International Dairy Federation (IDF) (ISO and IDF 2010).

Considering the taxonomical complexity of LAB and there rare association with clinical infections, there are still no known generally accepted susceptibility breakpoints for most antibiotics. The focus of the breakpoints suggested by Clinical Laboratory Standards Institute (CLSI) is mainly on clinical isolates while LAB species are not typically associated with clinical infections (Gueinmonde *et al.,* 2013). Also, the antibiotic breakpoints for *Lactobacillus* spp. are not stated in the EUCAST guidelines, thus making the determination of antibiotic susceptibility difficult. For instance, Charteris *et al* (2001) in their study on antibiotic susceptibility of lactobacilli used the breakpoint values recommended for testing clinical isolates. Such comparison is not ideal since the antibiotic breakpoint standardization among clinical and non-clinical isolates is unlikely to be achieved. Some authors have also proposed a range of values for the interpretation of LAB susceptibility as follows; Sensitive (S = 21 mm); Intermediate (I, 16 to 20 mm) and Resistant (R = 15 mm) (Vlkova *et al.,* 2006; Puphan *et al.,* 2015). However, this generalization of antibiotic breakpoint for LAB may not be true for all species of the lactic acid bacteria because the minimum inhibitory concentration breakpoints values have been demonstrated to be species specific and thus cannot be generalized (Danielsen and Wind, 2003). Therefore, the breakpoint used for Lactobacilli in this study was assumed from that of *Streptococcus* spp., a member of the LAB group with defined breakpoint in CLSI

guidelines to give an idea of the susceptibility of the LAB as a quantitative parameter with further determination of the MIC; being the standard recommended by FEEDAP for probiotic additives (EFSA, 2012).

Bacteria are known to acquire or develop resistance to antimicrobial agents with resultant grave public health consequences (Van Boeckel *et al.,* 2015). The safety of bacterial strains to be considered as potential probiotic is of utmost importance, because of the increasing risk of resistant genes dessiminating to other microorganisms. The antibiotic resistance profile of probiotic LAB strains is an importantsafety consideration to forestall the likelihood of horizontal transfer of genes coding for resistance amongthe microflora (Gueimonde *et al.,* 2013). A major means of differentiating between intrinsic and acquired antimicrobial resistance is by comparing the antibiotic susceptibility patterns across different representative strains for each species (Gueimonde *et al.,* 2013).

In this study, the susceptibility of 88 LAB to 11 antibiotics of medical importance was tested. The result of the antibiotic susceptibility of LAB in this study revealed a 100% susceptibility to ampicillin, amoxicillin-clavunalic acid, chloramphenicol, erythromycin, tetracycline and complete resistance to clindamycin, streptomycin, vancomycin, gentamicin and kanamycin among all species of *Lactobacillus, Enterococcus, Streptococcus* and *Weissella*. This general pattern of phenotypic resistance and susceptibility seen across all LAB strains tested is indicative of intrinsic resistance. Antibiotic resistance in probiotic strains is not a problem *per se,* in fact, intrinsic antibiotic resistance could be of advantage in situations where co-administration of probiotics with antibiotics is desired (Gueimonde *et al.,* 2013). This trait is desirable in probiotic LAB administered for preventing antibiotic-related diarrheoa due to dysbiosis and also in replenishing the gut microbiota after an antibiotic treatment course (Adagbada *et al.,* 2012; Gueimonde *et al.,* 2013). Antibiotic resistance becomes a safety issue when the risk of antibiotic resistant genes transfer is present which could have some therapeutic consequences.

Antibiotic resistance genes borne on mobile genetic elements are most probably capable of being transferred horizontally (Chang *et al.,* 2014). Lactobacilli are well known for their innate ability to resist a plectora of antibiotics. These resistant genotypes are typically not transferrable and therefore not of safety concern. Therefore, only *Lactobacillus* species were considered as potential probiotics in this study. The antibiotic susceptibility results obtained in this work is similar to those reported by Maldonado and Nader-Macías (2015)

who isolated erythromycin, ampicillin and kanamycin susceptible LAB from faecal samples of calves.

Lactobacillus spp. exhibit a high natural resistance to streptomycin, gentamicin and kanamycin (Erginkaya *et al.,* 2018), which agreed with the results obtained in this study. The report of Gueimonde *et al* (2013) reiterates that lactobacilli are generally sensitive to penicillin and other betalactamase antibiotics such as ampicillin and amoxicillin while Flórez *et al* (2007) isolated LAB exhibiting innate resistance to erythromycin and other macrolides due to reduced affinity of the antibiotics to the ribosomes as a result of point mutation in the gene coding for 23S rRNA. A high *Lactobacillus* spp. resistance to tetracycline was observed in the study of Hoque *et al* (2010), as tetracycline resistant genes are the commonest resistance determinants found in lactobacilli of animal sources. This observation may be as a result of the widespread use of tetracycline in livestock management for prophylaxis and growth promotion. Interestingly, there was 100% susceptibility of *Lactobacillus* spp. isolated in this study to tetracycline. Conversely, there was high resistance to vancomycin by the lactobacilli isolates. Several authors have reported the exhibition of intrinsic resistance to aminoglycoside antibiotics by lactobacilli (Sornplang and Leelavatcharamas, 2010; Gueimonde *et al.,* 2013).

Vancomycin resistance in *Lactobacillus* species has been reported as the best characterised intrinsic resistance in LAB (Gueimonde *et al.,* 2013), which is likely to be as a result of the inactivation of vancomycin by substitution of the last residue of D-alanine with either D-lactic acid or D- serine in the pentapeptide chain muramyl preventing vancomycin from binding (Gueimonde *et al.,* 2013; Erginkaya*et al.,*2018). Vancomycin resistant phenotypes in *Lactobacillus* spp. are not of safety concern, and the MIC determination is not required in probiotic strains according to the requirement stipulated by FEEDAP (EFSA, 2012). Genes conferring resistance to many medically important antibiotics such as chloramphenicol, erythromycin, streptomycin and tetracycline are borne on plasmids or transposons which are highly mobile genetic elements have already been characterised in *Lactobacillus* spp. (Devirgiliis *et al.,* 2013).

The susceptibility of *E. hirae* to ampicillin in this study agrees with previous reports for *Enterococcus* spp. isolated from cattle (Adeniyi *et al.,* 2015) but in contrast to the work of Bouymajane *et al.,* (2018) where all enterococci of bovine origin were resistant to ampicillin. While no resistance was observed in the *Enterococcus* spp. to tetracycline in this work, tetracycline resistance was the greatest resistance phenotype observed in cattle

in a similar study (Anderson *et al.,* 2008). Detection of gentamicin resistance in *Enterococcus* spp. in this work is corroborated by the report of Torres *et al.,*(2018)where *Enterococcus* spp. of animal origin showed high gentamicin resistance. In contrast, susceptility of enterococci of cattle origin to gentamicin and vancomycin has been reported (Jackson *et al.,* 2010). Resistance to tetracycline and erythromycin was not observed in this study. However, Anderson *et al* (2008) detected substantial levels of resistance to tetracycline and erythromycin in enterococci isolated from cattle and opined that it was likely to be due to selective pressure as a result of antibiotics growth promoters. Also the result of the antibiotic susceptibility of *Enterococcus hirae* reported by Jackson *et al* (2010) is partly in agreement with the results of this study, where high resistance to kanamycin and streptomycin was seen in enterococci of bovine origin but differ in that of erythromycin and tetracycline resistance which is contrary to the findings of this present study.

All the *Enterococuss hirae* strains identified in this work are vancomycin resistant. Vancomycin resistance in *Enterococcus* spp. poses an increasing healthcare problem worldwide. There is an increased frequency in the report of bacteremia and infective endocarditis caused by vancomycin resistant *Enterococcus* spp.which are also implicated in the infection of the urinary tract, pelvis and intra-abdomen (Hanchi *et al.,* 2018) and bovine mastitis in cattle (Gomes *et al.,* 2016). *Enterococcus hirae* isolates of animal origin have been reported to contain *vanA* transposons, being a highly mobile genetic element coding for high level vancomycin resistance in *Enterococcus* spp. (Beukers*et al.,* 2017). *Enterococcus* spp.are being suggested as indicator organisms for the development of antibiotics resistance. The safety of antibiotic-resistant *Enterococcus* spp. intended for probiotic purpose must be proven with molecular techniques and the risk of pathogenicity of *Enterococcuss* spp. in causing infections should be investigated thoroughly (Hanchi *et al.,* 2018). The upsurge of antimicrobial-resistant strains of *Enterococcus* with an increasing prevalence of antimicrobial resistant determinants has emerged a global public health concern (Gueimonde *et al.,* 2013). These demerits coupled with the infectivity potentials of *Enterococcus* spp. excluded *E. hirae* isolated in this study from further consideration as good probiotic candidates.

It is required that the MIC of antibiotics be determined to differentiate between susceptible and resistant strains, since probiotic strains must not possess antimicrobial resistant determinants. Absence of mobile antimicrobial resistance determinants is an important

requirement for selection of potential probiotic strain (EFSA, 2012), hence in addition to the determination of the susceptibility patterns of all the LAB by disk diffusion, the MICs of nine antibiotics of medical importance was determined for 5 selected potential probiotic Lactobacilli with E-test. Epsilometer-test has been described by many authors as a simple quantitative method commonly employed for determination of the antibiogram of various microorganisms; hence, it was chosen for the MIC assay in this study with little modifications of the original protocol to suit lactobacilli (Huys*et al*., 2010). Two major categories of antibiotics recommended by EFSA were tested: cell-wall synthesis inhibitors (ampicillin) and protein synthesis inhibitors (chloramphenicol, gentamicin, streptomycin, kanamycin, tetracycline, erythromycin and clindamycin). The five tested lactobacilli strains were sensitive to all the antibiotics tested with MICs lower than the breakpoints proposed by the FEEDAP Panel for selection of probiotic feed additive (EFSA, 2012).

The MIC obtained from this study for LAB is in agreement with the report of Georgieva *et al* (2015) where most of the lactobacilli intended for use as probiotics and starter cultures had high susceptibility to ampicillin, gentamicin, erythromycin, tetracycline, kanamycin, clindamycin, streptomycin and chloramphenicol in the MIC assay. Minimum inhibitory concentration higher than the established breakpoint for at least one antibiotic would require molecular investigation to distinguish between acquired and natural resistance (EFSA, 2012). The detection of MIC values above the cut-off values suggested by the FEEDAP Panel for the antibiotics tested requires further investigation, so that the nature and probable mechanism of resistance can be ascertained. According to the result of this study, acquired antimicrobial resistance is not present in any of the potential probiotic lactobacilli strains based on the MIC determined and therefore molecular characterisation of antibiotic resistance was not required.

5.8. Pathogenicity of potential probiotic bacteria

Another important safety consideration for selection of probiotic organisms is the absence of pathogenicity and infectivity. Lactic acid bacteria over the years have been regarded generally as safe, but the frequency of isolation of these organisms from clinical infections recently raised some doubts over the safety of these organisms and the ability of this group of bacteria to cause infection is now being investigated (Papadimitriou *et al.,* 2015). The infectivity of LAB cannot be generalized as the isolation of LAB from infective lesions is mostly as a result of opportunistic infections. Endogenous infection resulting from

translocation of gut microflora is one of the causes of opportunistic infection in hosts with impaired immune functions (Liu *et al*., 2016).

The infectivity and pathogenic potential of selected potential probiotic LAB strains were tested by the determination of their ability to lyse red blood cells, migrate into internal organs and cause infection especially as lactic acid bacteria have been implicated in some pathological conditions such as bacteraemia and endocarditis (Encarnacion *et al.,* 2016). None of the tested LAB demonstrated pathogenic potentials, further ascertaining their safety profile as probiotic candidates; they were non-hemolytic and did not translocate to the blood, spleen or liver.

Experimental animals did not exhibit any sign of bacterial infection after repeated feeding with high doses of selected LAB. This agreed with the reportof Cheng-Chih et al. (2014) where suspension of about 9×10^9 CFU/kg/d and 4.5×10^{10} CFU/kg/d of *Lactobacillus plantarum* HK006, and *Pediococcus pentosaceus* PP31administered to rats for 28 days did not result in translocation of these organisms to extra-intestinal sites. Similarly, a high dose of *L. acidophilus* or *L. paracasei* administered to mice did not result in translocation of these bacteria to the spleen, liver, or blood. Translocation of *Lactobacillus* spp. to liver, spleen, and blood was not observed in mice fed either *L. acidophilus* or *L. paracasei* or in the control mice (Paturi and Kasipathy, 2008). Asahara *et al.,* (2003) also reported that there was no colonization of peripheral blood and also no evidence of histopathological changes as a result of infection due to the administration of *L. casei* strain Shirota, *L. acidophilus* ATCC 4356, and *L. gasseri* DSM 20243 in rabbits. On the contrary, Rodriguez *et al.,* (2001) reported the detection of viable bacteria in the liver and spleen of healthy mice after oral administration of *L. rhamnosus* suspension.

A clear zone around colonies of LAB in blood agar signifies no haemolysis; this is considered a safety prerequisite in the selection of probiotic strains. None of the potential probiotic LAB assayed in this study exhibited hemolysis. Conversely, a green zone around LAB colonies representing alpha-hemolysis have been reported in *L. coagulans* and *L. rhamnosus* (Hawaz, 2014). While *Lactobacillus* spp. are "Generally Regarded as Safe", *Enterococcus* spp. in recent times have been identified as one of the main causes of bacteriamia and hospital-acquired infections (Hanchi *et al.,* 2018).

5.9. Survival and antimicrobial activity of selected lactic acid bacteria *in-vivo*

A crucial selection criterion for probiotic LAB strains is their capability to survive the prevailing conditions at the site of application (De Vos *et al.,* 2010). The probiotic potential of microorganisms *in-vitro* may not directly translate to similar benefits *in-vivo* because both conditions differ in reality. However, *in vitro* selection criteria gives insight to the selection of potential probiotic candidates since activity in the gut and effects on the gut microflora can only be adequately substantiated *in vivo* (Papadimitriou *et al.,* 2015). To this end, the two most promising lactobacilli: *L. amylovorous* C94 and *L.salivarius* C86 characterised in this study with putative probiotic property *in-vitro* were further tested *in-vivo* in calves to validate their probiotic activity against enteric pathogens. Although rodents are the best studied models for studying interactions between gut microbes and the host, *Salmonella* infection in rodents have not been established without immunosuppression. Many researchers have also reported that most rodents are naturally resistant to *Salmonella* infections, and studies involving rodent model of salmonellosis usually involve pretreatment with antibiotics to disrupt the gut microflora before being challenged with *Salmonella* (Mathur *et al.,* 2012).*Salmonella enterica* serovar Typhimurium are capable of inducing enterocolitis in humans and cattle resulting in intestinal inflammation and diarrhea (Adem and Bushra, 2016). Conversely, mice possess intrinsic resistance to *Salmonella* infection. Mathur *et al* (2012) in their study discovered that Toll-like receptor 11 (TLR11) in the intestine of mice recognises flagellin and helps prevent *Salmonella* spp. infection via the oral route and subsequent dissemination. It was noted that absence of TLR11 renders mice susceptible to *Salmonella* infection with increased lethality. Mouse strains deficient in genes coding SLC11A1 have also been demonstrated to be susceptible to *Salmonella* infections with symptoms similar to typhoid disease in humans (de Jong *et al.,* 2012). Many rodent model studies only achieved *Salmonella* infection with immunocompromised neonatal mice, pre-treatment with streptomycin, colonization of germ free animal or non-physiological routes of administration (Mathur *et al.,* 2012). Some other studies employed infection of ligated murine, rabbit and bovine ileal loops model (Schulte and Hensel, 2016), tissue culture and *ex vivo* culture of intestinal organs (Wildenberg and van den Brink, 2012).

While these models have proven very useful in various studies, they have significant limitations in the current study since our interest is to achieve natural *Salmonella* infection

through the oral route in an immunocompetent animal model. The shortcomings of the small animal models necessitated the use of calves; which are ideal in-vivo model for cattle probiotic feeding trial since the study aimed at preventing *Salmonella* onslaught in cattle.

Evaluation of the effects of probiotic interventions on gut microflora is limited, owing to the unculturable nature of the vast majority of intestinal species (Uyeno *et al.,* 2010). However, we determined total faecal Enterobacteriaceae;which are intestinal pathogen indicators and total lactobacilliin a bid to assess any major effects of the administration of potential probiotic strains on these representative intestinal species. Cattle are known healthy carriers of *Salmonella* spp.; the work of Hanson *et al* (2015) provided compelling evidences of vertical transmission of *Salmonella* from dam to her foetus such that new borne calves are already infected and do not require faecal-oral exposure for transmission. This assertion is supported by the results obtained from *Salmonella* screening of all the experimental calves recruited in this study. All the nine calves available on the farm were confirmed to be shedding *Salmonella* in their faeces prior to the lactobacilli intervention.

The probiotic lactobacilli feeding intervention in this study resulted in marked reduction in the number of enteric pathogens as expressed in the qPCR analysis data with the control calves exhibiting significantly higher load of enteric pathogens than the probiotic group. A number of studies have reported probiotic-mediated reduction of enteric pathogens in livestock; cattle fed a standard finishing diet with *L. acidophilus* NP51 as feed additive for 168 days had better resistance to *E. coli* O157:H7 colonisation and faecal shedding than controls (Menconi*et al.,* 2011). Our result also agreedwith the report of Casey *et al* (2007) where administration of a combination of probiotic lactobacilli strains resulted in reduction of faecal *Salmonella*, even though their study was on pigs and most probable number (MPN) technique was used for quantification of *Salmonella*. In another intervention study involving the administration of *L. acidophilus* (LA51) and *Propionibacterium freudenreichii* (PF 24), there was no marked reduction in *Salmonella* observed but a significant reduction in faecal shedding of *E. coli* O157 in naturally infected feedlot cattle was seen (Tabe *et al.,* 2008) while Stephens *et al*., (2007) showed marked decrease in carriage of *E. coli* O157 and *Salmonella* in cattle as a result of *L. acidophilus* feed supplementation. Conversely, the use of direct fed microbial culture of *Bacillus subtilis* have been shown to cause no significant reduction in prevalance and faecal shedding of *E. coli* O157 in cattle (Arthur *et al.,* 2010).

The current study detected significant increase in cumulative lactobacilli count in the test calves after the feeding period, compared to the not-fed control group, suggesting that the administered lactobacilli were able to colonize and survive in the gut of cattle with resultant reduction in population of enterobacteria. Similar finding was reported by Chiang *et al*., (2015) where *L. johnsonii* x-1d-2 and *L. mucosae* x-4w-1 fed weaned piglets had significant rise in intestinal lactobacilli population and marked reduction in *E. coli* count in comparison with controls after 21 days of intervention. Unlike the remarkable increase in total lactobacilli observed in this study, Casey *et al* (2007) were unable to establish a significant difference in total fecal lactobacillicount between any of the treatment and control groups after a five-strain *Lactobacillus* probiotic combination was administered for 30 days. The ability of *Lactobacillus* strains selected in this study to survive cattle gut conditions and resultantly increase the total lactobacilli population with a consequent reduction in the enterobacteria further corroborates the potentials of the selected strains as good probiotic candidates.

6.0. Stability of lactic acid bacteria during processing and storage

There is need to preserve bacterial cells from losing viability during technological manipulations and storage. Freezing is a method commonly employed for probiotic bacteria preservation but poor transportation and storage temperatures are the major demerits of frozen starter cultures (Liliana and Vladimir, 2013). Frozen direct-to-vat probiotic cultures require low temperature for storage and a cold chain distribution which may be a limitation in most developing countries where power supply is epileptic. Lyophilisation on the other hand is a technology more convenient, given that it does not require freezing conditions for storage and distribution (Fonseca *et al.,* 2015). It's a means of bacterial preservation which involves reducing the water activity values below 0.2, thus allowing for long term storage with minimal loss in functionality and viability (Liliana and Vladimir, 2013). In order to ensure that the probiotic strains are capable of surviving storage and shipment and would have sufficient quantity of viable cells when administered to the animals, the two selected lactobacilli were lyophilized with skimmed milk as cryopreservant and evaluated for stability during storage at 25 ±2^{o}Cover a period of 3 months. Both *L. amylovorous* C94 and *L. salivarius* C86 maintained viability during lyophilisation procedure and over a storage period of 3 months. A log reduction in colony forming unit/ml was observed after the lyophilisation process cumulating to 3 log

reduction in both *Lactobacillus* strains after 3 months of storage at room temperature with an average of about one logarithmic unit reduction in survival per month. This result is in agreement with the report of Ayeni *et al* (2011b) where lyophilisation of *W. confusa* and *L. paracasei* strainsresulted in a percentage reduction in survival of less than 0.5 log, further suggesting that freeze drying is a suitable method of preserving probiotic LAB. The viable lactobacilli cells remaining after storage for 3 months were still within the quantity considered adequate for beneficial probiotic effects (Liliana and Vladimir, 2013; Purphan *et al*., 2015).

CONCLUSION

The outcomes of this study revealed a significantly high prevalence of *Salmonella* carriage in healthy ready-to-slaughter cattle on the teaching and research farm of the University of Ibadan with an associated risk of these zoonotic pathogens finding their way to the human population through the food chain if preventive strategies aimed at reducing the carriage of these enteric pathogens in slaughter cattle is not instituted on the farm. The high susceptibility of the *Salmonella* isolates is also worthy of note, reiterating the importance of antibiotic stewardship in livestock management as exemplified in the studied farm.

Lactobacillus amylovorus C94 and *Lactobacillus salivarius* C86 of bovine origin were able to survive the austere physico-chemical environment of the cattle gut with a resultant improvement in the lactobacilli microflora. Both strains are considered safe, possessing enormous potential as probiotic strains; being non pathogenic, devoid of antimicrobial resistant determinants and demonstrating significant activity against enterobacteria in calves. These potential probiotic strains can be vehiculated in skimmed milk and lyophilized as feed additive; they are able to survive lyophilisation in skimmed milk cryopreservant with a marginal viability loss while maintaining their viability in storage at room temperature for 3 months. An average of $1\log_{10}$ reduction per month in viability observed during storage is crucial for the determination of the quantity of the starting bacteria cultures required in the development of lyophilized cultures of these potential probiotic strains. Although, the viable lyophilized cells of *Lactobacillus amylovorus* C94 and *Lactobacillus salivarius* C86 after a 3 months storage were still within the recommended quantity considered adequate to exert the desired health benefits, however, scaling up of the starting quantities of the probiotic preparation of *Lactobacillus amylovorous* C94 and *Lactobacillus salivarius* C86 is recommended to extend the shelf life of the product and make up for any possible viability loss in transit to the site of action.

A mixture of *Lactobacillus amylovorous* C94 and *Lactobacillus salivarius* C86 culture suspension can be safely administered in cattle for reduction of enterobacteria and are potential natural control strategy for zoonotic pathogens of global “One Health” importance.

RECOMMENDATIONS

It is recommended that the Nigerian government should implement the National Antimicrobial Resistance Action Plan (NARAP) to regulate the use of antimicrobials in livestock farming, considering the untoward effect of antimicrobial resistant pathogens on human and animals alike, which could sometimes result in fatal outcomes. Use of natural growth promoters such as probiotics should be encouraged by all stakeholders in the Agro-allied sector.

Tertiary institutions and research institutes in Nigeria should collaborate with indigenous pharmaceutical companies for research in the area of probiotics development and eventual production such as this current study.This will ultimately provide farmers with safe and cost effective alternatives to antibiotic growth promoters and ensure the transition of research outputs in the various institutions into products of invaluable benefits to humanity.

CONTRIBUTIONS TO KNOWLEDGE

1. To the best of my knowledge, as at the time of writing this thesis, this is the first study to determine the diversity of culturable lactic acid bacteria in cattle faeces in Nigeria.
2. Adetoye *et al*, (2018) was the first to report the isolation of *Lactobacillus taiwanensis* from cattle faeces in Nigeria.
3. This study corroborates a positive correlation between antibiotic use in livestock and the development of antibiotic resistance in bacteria of farm animal origin.
4. The outcome of this research provides insight into the possible use of beneficial gut microflora to combat enteropathogens in livestock. *Lactobacillus amylovorous* C94 and *Lactobacillus salivarius* C86 are prospective probiotic organisms and possible alternatives to antibiotic feed additives capable of reducing the carriage of enterobacteria in cattle management, and consequently mitigating enterobacterial zoonoses in human population.
5. This study demonstrated lyophilisation as an effective preservation method for probiotic bacteria strains

FUTURE DIRECTIONS

Clinical trials aimed at determining the mechanism of probiotic action and effect of *Lactobacillus amylovorous* C94 and *Lactobacillus salivarius* C86 on weight gain with larger cattle sample size should be considered.

Whole genome sequencing is imperative for the two prospective probiotic strains; the sequence data will further provide information necessary for genomic understanding of the probiotic traits and possible new biotechnological application of these promising strains.

Whole genome sequencing of the *Salmonella* spp. isolated in this study is equally important to elucidate the genomic basis of their antimicrobial susceptibility.

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APPENDIX I

LIST OF AWARDS AND PUBLISHED ARTICLE FROM THIS THESIS

Awards

International Conference Travel Grant awarded by Society for Applied Microbiology (*Sfam*). 2016.

University of Ibadan Postgraduate School 2017/2018 session award for publication of articles from Ph.D thesis.

Publication

Adetoye, A., Pinloche, E., Adeniyi, B. A., and Ayeni, F. A. 2018. Characterisation and anti-*Salmonella* activities of lactic acid bacteria isolated from cattle faeces. *BMC microbiology*, *18*(1), 96.doi:10.1186/s12866-018-1248-y.

Identification, Prevalence of *Salmonella* and *in-vivo* antibacterial activity of potential probiotic Lactobacilli against Enterobacteria in cattle. –Manuscript in preparation.

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RESEARCH ARTICLE Open Access

Characterization and anti-salmonella activities of lactic acid bacteria isolated from cattle faeces

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Abstract

Background: Non typhoidal salmonellosis is one of the neglected zoonoses in most African countries. The use of sub-therapeutic doses of antibiotics as animal growth promoter enhances the emergence and dissemination of antimicrobial resistance in bacteria with food animal reservoirs and may also results in antibiotics residue in animal products. One promising alternative to antibiotics in animal feed is Lactic Acid Bacteria (LAB) as probiotics. This study was carried out to determine the anti-salmonella activities and suitability of LAB isolated from cattle faeces in Nigeria as potential probiotics in cattle feed.

Method: The test *Salmonella enterica* spp strains and LAB were isolated from cattle faeces and identified by MALDI-TOF MS and partial sequencing of 16S rRNA genes respectively. The anti-salmonella activities of the isolated LAB in co-culture, cell-free supernatant, inhibition of growth by viable LAB cells and quantification of organic acids were determined by standard techniques. The ability of the LAB strains to withstand gastric conditions, antibiotic susceptibility and their haemolytic ability on blood agar were also determined.

Results: A total of 88 LAB belonging to 15 species were isolated and identified from cattle faeces. The most abundant species were *Streptococcus infantarius* (26), *Enterococcus hirae* (12), *Lactobacillus amylovorus* (10), *Lactobacillus mucosae* (10) and *Lactobacillus ingluviei* (9). Most of the LAB strains showed good anti-salmonella activities against the test *Salmonella enterica* spp. with 2 *Lactobacillus* strains; *Lactobacillus amylovorus* C94 and *Lactobacillus salivarius* C86 exhibiting remarkable anti-salmonella activities with total inhibition of *Salmonella* spp after 18 hours of co-incubation. The selected strains were able to survive simultaneous growth at pH 3 and 7% bile concentration and are non hemolytic.

Conclusion: This study reports the vast diversity of culturable LAB in cattle faeces from Nigeria and their putative *in-vitro* antibacterial activity against *Salmonella enterica* spp isolated from cattle. *Lactobacillus amylovorus* C94 and *Lactobacillus salivarius* C86 demonstrated promising probiotic potentials *in-vitro* and will be further tested *in-vivo* in animal field trial.

Keywords: Lactic acid bacteria, Cattle, Faeces, *Salmonella*, Probiotics

Background

Antibiotics resistance is a global health challenge and the causes are multifactorial with human activities being a major culprit. Antibiotics misuse and overuse in humans and livestock are major contributory factors to the emergence and transmission of antibiotics resistant organisms, the contribution of farm animals in this public health challenge is noteworthy. Growth promotion and disease prevention are important strategies in modern livestock farming; hence, there has been widespread use of antibiotics as animal feed additives [1]. The addition of such antibiotics feed additive at sub therapeutic doses for growth enhancement is a major contributing factor to the emergence and spread of antimicrobial resistant determinants among bacterial pathogens and commensals in animal reservoirs [2]. *Salmonella* is an important zoonotic pathogen [3]. *Salmonella enterica* is one of the major food borne pathogens resulting in infections ranging from acute gastroenteritis to systemic infections like typhoid fever [4]. There are about 93.8 million cases of

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salmonellosis in humans worldwide resulting in about 155,000 deaths annually [5]. In Africa, non-typhoidal *Salmonella* is a major cause of bacteremia particularly among children and people with impaired immune functions [6, 7] and invasive infections.

Bovine salmonellosis is also of enormous economic importance, leading to a reduction in productivity as a result of cost of treatment, weight loss, reduced meat and milk yield and mortality within the cattle herd [8]. The use of antibiotics and vaccination are some of the strategies currently being employed to combat salmonellosis [4]. However, both strategies have shortfalls while vaccination is suboptimal. The prolong use of antibiotics have a resultant effect of selecting for resistant *Salmonella* serovars and may also alter the intestinal microflora [9]. There is therefore a need for an alternative intervention against *Salmonella* infection in livestock management.

Probiotics are now being considered a promising alternative to antibiotics against enteropathogens infections [10–14]. It has been demonstrated that probiotics are useful substitutes to conventional antibiotics growth promoters especially in newly born animals [15]. Probiotics are added as feed additives to promote animal health and productivity [16]. A stable microflora of lactobacilli has been demonstrated to improve overall health performance in calves [17]. However, there is limited information on the diversity and probiotic potentials of LAB in the gut of cattle. Therefore, this study describes the diversity of culturable LAB in cattle faeces and their anti-salmonella probiotic potential *in vitro*.

Methodology

Samples Collection

Fresh fecal samples were collected on the ground (immediately after defecation) from 40 different cattle (Sokoto Gudali breed) , aged 2.0 ± 0.5 years at University of Ibadan Teaching and Research Dairy Farm for the isolation of LAB within a period of three months (May to July, 2015). All the cattle were certified healthy by the resident farm veterinarian. Samples collected were taken to the Pharmaceutical Microbiology laboratory for microbiological analysis within one hour of collection.

Bacterial Isolation and Identification

Test Pathogens

Two *Salmonella enterica* spp designated *Salmonella enterica* S1 and *Salmonella enterica* S57 previously isolated from cattle faeces according to standard procedure [18, 19] were selected as test *Salmonella* pathogens. *Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aureus* and *Klebsiella* spp from our research culture collections were also used as general test pathogens

Lactic Acid Bacteria

1g of cattle fecal samples were added into 9 ml of MRS broth and incubated at 37°C under microaerophilic condition (CampyGen™ Oxoid, UK) for 24 hours, the culture were appropriately plated out on MRS agar (Oxoid, UK) and viable cells were counted. Distinct morphologically different colonies were picked from each plates and sub-cultured to obtain pure cultures. Gram positive and catalase negative isolates were preserved in 50% glycerol stock at -80°C.

Identification of the Lactic Acid Bacteria Isolates.

Identification of lactic acid bacteria in this study was done primarily by partial sequencing of 16S rRNA genes. The genomic DNA of the LAB were extracted by *Accu-Prep®* Genomic DNA Extraction kit (Bioneer, South Korea) according to the manufacturer`s instruction. The extracted DNA was used as template in PCR targeted at 16S rRNA gene using the primers: 27F (AGAGTTTGA TCMTGGCTCAG) and 1389R (ACGGGCGGTGTGTA CAAG) with the following PCR conditions: 1 cycle of 95°C for 4 min followed with 25 cycles of 95°C for 1 min, 55°C for 1 min and 72°C for 1 min 30s and finally 1 cycle of 7 min at 72°C [20].

The PCR products obtained for 77 LAB strains were purified and sequenced. The sequences were compared with GenBank database using the basic local alignment search tool (BLAST) program for the identification of the isolates. Eleven strains whose DNA did not amplify with 16S primers were subsequently identified by MALDI-TOF MS according to standard procedure [21].

Determination of Antimicrobial Activities of Lactic Acid Bacteria.

The anti-salmonella activities of 88 isolated viable LAB cells were carried out using a modified agar overlay method [22]. A loopful of LAB grown in MRS broth was inoculated on MRS agar plate as a line of about 2 cm long and incubated under microaerophilic condition at 37°C for 24 h. After incubation, the MRS agar plates were overlaid with approximately 10^5 cfu/ml of an overnight broth culture of the two *Salmonella* test pathogens inoculated in 10 ml of Mueller Hinton (MH) soft agar (0.7% agar-agar). The overlay was allowed to set and incubated at 37°C under aerobic condition for 24 h and the zones of inhibition were measured.

The cell free supernatants (CFS) of all the 88 LAB isolates were further tested for antibacterial activities. The LAB were grown overnight in MRS broth and centrifuged at 12,000 rpm for 10 mins. One hundred μl of the CFS of the LAB strains were placed in wells (6 mm) bored into Mueller Hinton agar pre-seeded with approximately 10^5 cfu/ml of the test *Salmonella* spp. The supernatant was allowed to diffuse for one hour before incubation at 37°C

for 24 hrs. The plates were examined and clear zones of inhibition were measured. The antibacterial activities of seven selected LAB isolates with promising anti-salmonella activity were further determined against *Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aureus* and *Klebsiella spp* in a cell free supernatant assay as described above.

Lactic acid bacteria showing promising antagonistic properties were assayed to determine the presence or absence of bacteriocin-like inhibitory substances using the agar-well diffusion method [23]. The LAB were grown in MRS broth for 18 hours and centrifuged at 12,000 rpm for 10 mins. The pellets were discarded and the pH of the cell free supernatant was adjusted to 6.2 using 1.0M NaOH. The antibacterial activities of unneutralized and neutralized CFS was tested against *Staphylococcus aureus* A104 by putting 100 μl of the CFS of the LAB strains in wells (6 mm) bored into Mueller Hinton agar pre-seeded with approximately 10^5 cfu/ml of the test *Staphylococcus aureus*. The supernatant was allowed to diffuse for one hour before incubation at 37°C for 24 hrs. The plates were examined and clear zones of inhibition were measured.

Resistance to Gastrointestinal Conditions

Tolerance to acidic pH

All the 88 LAB isolates were grown overnight in MRS broth under microaerophilic condition. The overnight culture was centrifuged at 12,000 rpm for 5 mins for the collection of bacterial cells. The bacterial cells were washed with sterile saline and resuspended in 10 ml fresh MRS broth and 100 μl from the culture was then inoculated into 10 ml of MRS broth which has been adjusted to pH 3.0, 4.0, 5.0 and 7.0 (with 1M HCl) and incubated at 37°C for 3 hours under microaerophilic condition. The initial count was done (T_o) before incubation at 37°C for 3 hours under microaerophilic condition Thereafter, appropriate dilutions of the resultant culture was plated on MRS agar and incubated at 37°C for 24 hours under microaerophilic condition. The LAB viable count after 3 hours of contact with the modified medium was compared with the initial count.

Bile Tolerance

An overnight culture of all the isolated 88 LAB in MRS broth were grown at 37°C under microaerophilic condition and centrifuged at 12,000 rpm for 5 mins for the collection of bacterial cells. The bacterial cells were washed with sterile saline and resuspended in 10 ml fresh MRS broth. 100 μl from the culture was then inoculated into 10 ml of MRS broth supplemented with bile salt (Oxoid) to achieve 0% bile salt (control), 0.1%, 0.5%, 1%, 5% and 7 % bile concentration levels respectively. The initial count was done (T_o) before incubation at 37°C for 3 hours under microaerophilic condition and incubated at 37°C for 3 hours under microaerophilic condition. Thereafter, appropriate dilution of the resultant culture were plated in MRS agar and incubated at 37°C for 24 hours under microaerophilic condition. The LAB viable count after 3 hours contact time was compared with the initial count at time 0 hour.

Continuous Acid and Bile Tolerance Test

Five LAB strains belonging to different *Lactobacillus* species were selected based on their antibacterial activities and resistance to gastric conditions to determine their survival in continuous acid and bile simulation. Selected LAB strains which were able to resist bile and acid separately were tested for their resistance to low pH and then bile. An overnight broth culture of LAB grown in MRS broth was centrifuged at 12,000 rpm for 10 mins for the collection of bacterial cells. The bacterial cells were washed with sterile saline and resuspended in fresh MRS broth, 100 μl from the culture was then inoculated into 10 ml of MRS broth which has been adjusted to pH 3 (with 1M HCl). The initial viable count was taken and the mixture incubated at 37°C for 3 hours under microaerophilic condition, after which 100 μl from the mixture were then inoculated into 10 ml of MRS broth containing 7% (w/v) bile salt and also incubated at 37°C for 3 hours under microaerophilic condition. The survival of the LAB were determined by plating appropriate dilution and incubating at 37°C under microaerophilic condition. The log reduction in the final viable LAB count in comparison with the initial count was evaluated.

Determination of the Antibiotic Susceptibility of Lactic Acid Bacteria Isolates

As part of the European Food Safety Authority (EFSA) requirements for safety assessment of bacteria intended for probiotic purpose, such organism should not possess acquired resistance determinants to antibiotics of medical importance.

The antibiotics ampicillin, amoxicillin-clavunanic acid, vancomycin, gentamicin, kanamycin, streptomycin, erythromycin, clindamycin, tetracycline and chloramphenicol (Oxoid, UK) were tested for all the 88 isolated LAB with the disk diffusion method. A lawn of the lactic acid bacteria were made with approximately 5 x 10^7 cfu/ml (equivalent to 0.5 McFarland standard) on *Lactobacillus* Susceptibility Test Media (LSTM). The antibiotics disc was placed on the inoculated media and incubated under microaerophilic condition at 37°C for 24 hours. The plates were then examined and the zones of inhibition were measured. The results were interpreted with European Committee on Antimicrobial Susceptibility Testing (EUCAST) 2016 breakpoint and the nearest species breakpoints were used for species without clearly defined breakpoints.

Determination of Organic Acids Production by LAB

Five LAB strains belonging to different *Lactobacillus* species were selected based on their antibacterial activities and resistance to gastric conditions to determine the level of acids produced. The concentration of lactic, acetic and propionic acids produced by the selected LAB were determined using High Performance Liquid Performance Chromatography (HPLC). The HPLC system (Adept CECIL CE 4200) consisted of an HICHROM NUCLEOSIL 120-10C18 column (25cm X 4.6mm id), the column was maintained at room temperature and an aliquot (20ul) of the filtered samples was injected into the HPLC system equipped with a UV absorbance detector set at 210nm, degassed H_2SO_4 was used as the mobile phase. The standard curves were generated with HPLC grade lactic acid, acetic acid and propionic acid (Sigma Adreich) standards, the peak areas (mAS) were plotted against standard concentration (mg/L) to produce a standard calibration graph.

Hemolytic Activities of LAB

Five LAB strains belonging to different *Lactobacillus* species were selected based on their antibacterial activities and resistance to gastric conditions to determine their hemolytic potential. The LAB strains were streaked on blood agar and incubated at 37°C for 24 hours [24]. The LAB strains that produce green-hued zones around the colonies (alpha-hemolysis) or those that do not produce any effect on the blood agar (Gamma- hemolysis) were considered non hemolytic. Those producing zones of blood lyses around the colonies are classified as hemolyic (Beta- hemolysis).

Salmonella and Lactobacillus Co culture Experiment

Two lactic acid bacterial strains, *Lactobacillus salivarius* C86 and *Lactobacillus amylovorus* C94 were selected for *Salmonella* co culture experiments due to above average results in all the screening methods employed above. The rate of inhibition of growth of the two test *Salmonella enterica* strains by the two LAB strains were determined by a modified method of Drago *et al.* [25] in a kinetic study. A broth culture medium containing 5 ml of double strength MRS broth and 5 ml of double strength Mueller Hinton broth (MRS-MH), prepared to support the growth of both *Salmonella* and *Lactobacillus* was employed in the experiment. For the co-culture, the MRS-MH broth was inoculated with approximately 10^9cfu/ml of LAB and 10^8cfu/ml of the test *Salmonella enterica* spp. Two experimental controls were set up which consist of 10^9cfu/ml of LAB as monoculture and also 10^8cfu/ml of *Salmonella enterica* spp as monoculture. Serial dilution was carried out immediately after inoculation and appropriate dilutions of the co culture mixture were plated for time T_0 on both MRS agar and SSA (to determine the initial counts of both organisms) at the condition of growth for each organism. LAB and *Salmonella* monoculture were plated out on MRS and SSA agar respectively This procedure was repeated every 8 hours for 24 hours, such that the cultures were serially diluted and plated out at times T_0, T_8, T_{16} and T_{24} hours and the viable count (cfu/ml) at each time were compared with the control grown in monoculture.

Results

Diversity of LAB in Bovine Faeces.

Eighty eight lactic acid bacteria were identified, belonging to 4 Genera and 15 species; *Enterococcus hirae* (12), *Lactobacillus agilis* (4), *Lactobacillus amylovorus* (10), *Lactobacillus animalis* (1), *Lactobacillus gasseri* (5), *Lactobacillus ingluviei* (9), *Lactobacillus mucosae* (10), *Lactobacillus paraplantarum* (1), *Lactobacillus plantarum* (2), *Lactobacillus reuteri* (1), *Lactobacillus salivarius* (2), *Lactobacillus taiwanensis* (3), *Streptococcus equinus* (1), *Streptococcus infantarius* (26) and *Weissella cibaria* (1) (Table 1, Fig. 1). *Streptococcus infantarius* was the most isolated species accounting for 30.68% of all the isolated LAB while *Lactobacillus animalis, Lactobacillus paraplantarum, Lactobacillus reuteri, Streptococcus equines* and *Weissella cibaria* were the least isolated with only one strain each. *Lactobacillus* (54.55%) was the most frequent genera isolated in this study. The phylogenetic relationship of the isolated lactic acid bacteria is represented in Fig. 1 showing the diversity relatedness of the different isolated species.

Anti Microbial Activities

The anti-salmonella activities of the cell free supernatant and viable cells of the 88 isolated LAB were determined against the two test *Salmonella* strains of bovine origin. The difference between the diameters of the zones of inhibition in both assay averaged about ± 4mm with greater activities observed with the viable LAB in the agar overlay method. In both assays, the LAB isolates showed varying zones of *Salmonella* inhibition across species. Some strains of *Enterococcus hirae* and *Streptococcus infantarius* showed no activity against the test pathogens, however *Lactobacillus salivarius* C86 showed a remarkable 20 mm and 22 mm zones of inhibition, *Enterococcus hirae* 1F produced an appreciable 18mm and 20mm while *Lactobacillus amylovorus* C94 showed 21mm and 20mm zones of inhibition against *Salmonella enterica* S1 and *Salmonella enterica* S57 respectively as seen in Table 1. Based on the anti-salmonella activities, 7 LAB isolates were further tested against an array of pathogens as shown in Table 2. All the selected lactobacilli showed varying antimicrobial activities against *E. coli, S. aureus, Klebsiella* spp and *Pseudomonas aeuroginosa. Lactobacillus amylovorus* C94 and *Lactobacillus salivarius* C86 consistently exhibited the best antibacterial activities

Table 1 Distribution of lactic acid bacteria isolates and their anti-salmonella activity

LAB Species	No of Isolates (%)	Zone of inhibition (mm)							
		Salmonella enterica S1				*Salmonella enterica* S57			
		+	++	+++	++++	+	++	+++	++++
Lactobacillus agilis	4 (4.55)	(0) 0	(1) 1	(2) 3	(1) 0	(0) 0	(0) 0	(4) 4	(0) 0
Lactobacillus amylovorus	10 (11.36)	(1) 0	(1) 2	(5) 5	(3) 3	(0) 0	(1) 2	(5) 5	(4) 3
Lactobacillus animalis	1 (1.14)	(0) 0	(1) 1	(0) 0	(0) 0	(0) 0	(0) 0	(1) 1	(0) 0
Lactobacillus gasseri	5 (5.68)	(0) 0	(1) 0	(4) 5	(0) 0	(0) 0	(2) 2	(3) 3	(0) 0
Lactobacillus ingluviei	9 (10.23)	(1) 1	(1) 1	(7) 7	(0) 0	(1) 1	(1) 1	(7) 7	(0) 0
Lactobacillus mucosae	10 (11.36)	(1) 2	(2) 2	(7) 6	(0) 0	(1) 1	(4) 4	(5) 5	(0) 0
Lactobacillus paraplantarum	1 (1.14)	(0) 0	(0) 0	(1) 1	(0) 0	(0) 0	(1) 1	(0) 0	(0) 0
Lactobacillus plantarum	2 (2.27)	(0) 0	(0) 0	(1) 2	(1) 0	(0) 0	(0) 0	(2) 2	(0) 0
Lactobacillus reuteri	1 (1.14)	(0) 0	(1) 1	(0) 0	(0) 0	(0) 0	(0) 1	(1) 0	(0) 0
Lactobacillus salivarius	2 (2.27)	(0) 0	(0) 0	(1) 1	(1) 1	(0) 0	(0) 0	(1) 1	(1) 1
Lactobacillus taiwanensis	3 (3.41)	(0) 0	(1) 0	(2) 3	(0) 0	(1) 1	(0) 0	(2) 2	(0) 0
Weissella cibaria	1 (1.14)	(0) 0	(0) 0	(1) 1	(0) 0	(0) 0	(0) 0	(1) 1	(0) 0
Streptococcus equines	1 (1.14)	(0) 0	(1) 1	(0) 0	(0) 0	(1) 1	(0) 0	(0) 0	(0) 0
Enterococcus hirae	12 (13.64)	(4) 3	(3) 4	(4) 4	(1) 1	(2) 2	(8) 8	(1) 1	(1) 1
Streptococcus infantarius	26 (29.55)	(4) 5	(17) 15	(3) 5	(2) 1	(3) 3	(19) 20	(4) 3	(0) 0

Diameter of zone of inhibition: 0-5 = +, >5<12= ++, 12-18 = +++, >18 = ++++. The results of cell free supernatant assay are shown in parenthesis

against all tested pathogens. None of the isolates tested produced bacteriocin-like inhibitory substances.

Acid and Bile Tolerance

All the tested LAB isolates were able to survive growth at the varying pH levels including the acidic pH of 3 except four *Lactobacillus* strains; *Lactobacillus* mucosae C101, *Lactobacillus ingluviei* C13, *Lactobacillus ingluviei* C89 and *Lactobacillus taiwanensis* C20 which showed no growth. The tested LAB survived the varying bile salt levels up to 5% concentration, while only six of the isolates failed to grow at 7% bile supplementation and they include; *S. infantarius* C63, *S. infantarius* 53, *S. infantarius* C78, *L. mucosae* C104, *L. mucosae* C101 and *Enterococcus hirae* C34 (results not shown). These organisms were not considered for further tests.

Both *Lactobacillus amylovorus* C94 and *Lactobacillus salivarius* C86 further demonstrated the best probiotic potentials among the selected LAB by showing considerable resistance to continuous acid and bile challenge. They were able to withstand both low pH level of 3 and simultaneous 7% bile supplementation with a 2 $\log_{10}$ reduction in cfu/ml cell count from 6.9 x 10^{10} to 7.5 x 10^{8} for *Lactobacillus salivarius* C86 and 1.9 x 10^{10} to 1.7 x 10^{8} for *Lactobacillus amylovorus* C94 as seen in Table 3.

Antibiotics Susceptibility of Lactic Acid Bacteria

All the 88 LAB isolates were generally susceptible to chloramphenicol, ampicillin, amoxicillin-clavunalic acid and erythromycin as represented in Fig 2, there was 98.8% susceptibility to tetracycline with only one organism showing resistance, while on the other hand, there was total resistance to kanamycin, vancomycin gentamicin and clindamycin.

Quantification of Organic Acids

Generally, the concentration of lactic acid produced by all the tested strains was more than acetic acid, accounting for about 79.56% to 81.11% of all organic acid tested while propionic acid was the least produced (5.61% - 6.99%) except for *Lactobacillus ingluvie* C31 which produced mostly propionic acid (49.91%) and lactic acid was the least (21.66%) produced organic acid by this strain. *Lactobacillus salivarius* C86 produced the highest concentration of lactic acid 67.85 mg/ml (81.11%), followed by *Lactobacillus amylovorus* C94 which produced 54.91 mg/ml (80.93%) while *Lactobacillus ingluivie* C31 produced the least 8.88 mg/ml (21.66%) (Fig 3).

Hemolytic activity of the LAB

The tested LAB did not exhibit any haemolytic effect on the blood agar

Co-Culture kinetic study

The two selected *Lactobacillus* strains; *Lactobacillus salivarius* C86 and *Lactobacillus amylovorus* C94 for co culture showed that both *Lactobacillus salivarius* C86 and *Lactobacillus amylovorus* C94 possess potent anti-salmonella activities *in vitro*. There was a drastic reduction in value from 8 $\log_{10}$ to no viable *Salmonella* cell count

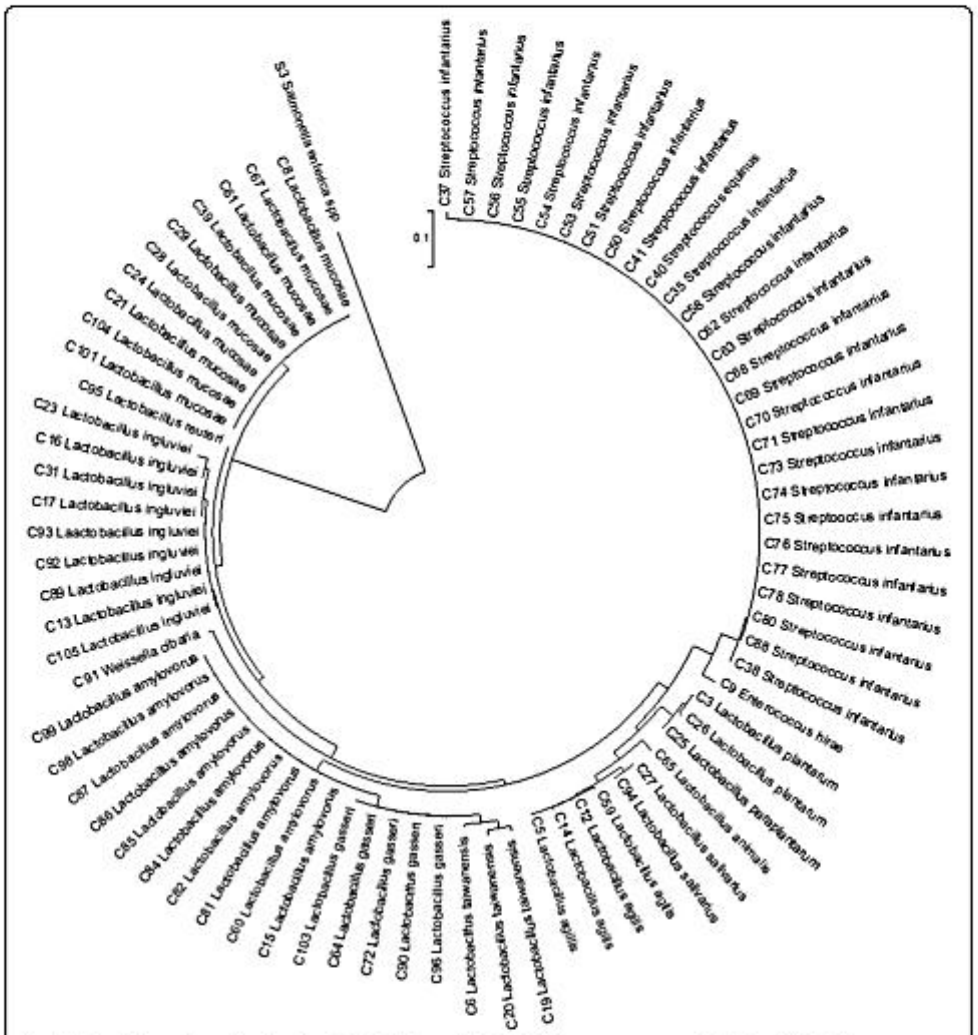

Fig. 1 Circular phylogenetic tree based on the neighbor-joining method of 16S rRNA genes sequences of the isolated LAB and the outgroup was Salmonella enterica spp constructed using MEGA version 6. The scale bar represents 0.1-nucleotide substitutes per position

between 8 hours and 16 hours contact time with the two LAB strains. However, *Salmonella enterica* S1 and *Salmonella enterica* S57 groew at 3.9 x10^8 and 5.7 x 10^8 respectively in the *Salmonella* monoculture control at T_{16}. There was no difference in the *Lactobacillus* count in the LAB-Salmonella mix for both strains as compared with the *Lactobacillus* monoculture controls (Fig 4).

Discussion

Lactic acid bacteria are usually part of the normal flora of animals and humans. The diversity of the culturable LAB in bovine faeces isolated in MRS media in this study reveals eighty eight lactic acid bacteria belonging to 15 species and 4 genera; *Lactobacillus, Weissella, Streptococcus* and *Enterococcus*. *Lactobacillus* was identified as the most

Table 2 Antimicrobial Activity of Selected LAB against other Pathogens

Lactic Acid Bacteria	Zones of Inhibition (mm)			
	E. coli	*Pseudomonas Aeuroginosa*	*Klebsiella spp*	*S. aureus*
Lactobacillus plantarum C3	12	18	14	28
Lactobacillus amylovorus C15	13	30	12	30
Lactobacillus ingluviei C31	12	12	11	28
Lactobacillus mucosae C61	12	20	15	30
Lactobacillus amylovorus C86	16	33	18	38
Lactobacillus salivarius C94	16	32	17	38
Lactobacillus amylovorus C99	15	30	14	32

frequent genera while *Streptococcus infantarius* was the most abundant species isolated in this study, followed by *Enterococcus hirae*. This is contrary to the report of Adeniyi *et al.* [23] where 94.12% of the isolated LAB from cattle faeces were *Enterococcus* spp, and no *Lactobacillus* spp was isolated. Although LAB are usual residents of the bovine gut, it is noteworthy that some LAB not commonly reported in cattle faeces were identified in this study. *L. taiwanensis* is a novel *Lactobacillus* species first isolated from cattle silage in Taiwan and named after the geographical location of sample collection [26], *Streptococcus infantarius* which was the most isolated species in our study is a predominant LAB species in African fermented dairy product of animal origin but not usually isolated from fresh milk [22, 27, 28]. *L. mucosae* is a novel pig intestinal *Lactobacillus* species first described in 2000 [29] while *Streptococcus equinus* which is predominantly of horse origin and are related to *Streptococcus bovis* commonly found in cattle faeces are often grouped together as the *S. bovis/ S. equinus* complex [30].

Lactobacillus salivarius C86, Lactobacillus salivarius C94 and Enterococcus 1F all demonstrated significant antibacterial activity against the two test *Salmonella enterica* S1 and *Salmonella enterica* S57 isolated from cattle faeces. However, only *Lactobacillus salivarius* C86 and *Lactobacillus amylovorus* C94 were selected for further characterization. While *Lactobacillus* strains have earned the "Generally Regarded as Safe" status, *Enterococcus* spp have recently emerged as one of the leading causes of nosocomial infections and bloodstream infections [31, 32]. The spread of antibiotic-resistant enterococci has also become a major public health concern worldwide [33, 34] based on the aforementioned reasons, *Enterococcus hirae* 1F was excluded from further work.

An important attribute of LAB intended for oral route of administration is the ability to survive the resistance of the gastrointestinal tract including the presence of bile salt and acidity of the gastric content [35]. The ability to withstand bile salt is an important factor for the *in vitro* selection of probiotic bacteria [22, 36]. Both *Lactobacillus salivarius* C86 and *Lactobacillus amylovorus* C94 were able to survive simultaneous low pH and bile simulation at the pH of the stomach of cattle while it receives food [37]. The survived viable LAB cells in this study are within the range of viable organisms regarded adequate to exert probiotic functions in the gut, as it has been established by various authors that the consumption of about 1.0 x 10^6 to 1.0 x10^{10} viable cells per day is required for beneficial probiotic effects [38, 39]. The ability of these two strains to withstand gastric conditions is not very surprising considering that they were isolated from the gut of cattle and thus will have better resistance than LAB isolated from other sources. Acid tolerance and bile resistant traits of intestinal *Lactobacillus* species are thought to be evolutionary means of withstanding the host defenses and surviving transit through the gastrointestinal tract. The possession of *bsh*-1 and *bsh*-2 genes which are bile salt hydrolyze genes were found to be responsible for acid and bile tolerance in *L. salivarius* UCC118 [40].

Lactobacillus spp can serve as microbial barrier against intestinal pathogen through competitive exclusion of pathogen binding, modulation of host's immune system, production of antimicrobial compounds such as organic acids (e.g., lactic acid, acetic acid, propionic acid) and proteinaceous compounds such as bacteriocins [41, 42]. One of the mechanisms of anti-salmonella activities of LAB in this study is the production of organic acids since no bacteriocin-like inhibitory substance was detected. The high antibacterial activity of *Lactobacillus salivarius* C86 and *Lactobacillus amylovorus* C94 against *Salmonella* spp and other pathogens in this study correspond with the high production of lactic acid as

Table 3 Viability of selected LAB after exposure to continuous acid and bile conditions

LAB ISOLATES	Viable count at pH 3 (after 3 hours contact)		Viable count in Bile (after 3 hours contact)	
	initial	final	Initial	final
Lactobacillus plantarum C3	4.9 X 10^8	8.9 X 10^6	1.2 X 10^7	1.7 X 10^5
Lactobacillus ingluvie C31	2.5 X 10^{10}	4.0 X 10^9	1.3 X 10^8	3.7 X 10^7
Lactobacillus mucosae C61	3.4 X 10^9	5.7 X 10^7	8.9 X 10^6	1.2 X 10^6
Lactobacillus salivarius C86	6.9 X 10^{10}	3.2 X 10^9	1.0 X 10^9	7.5 X 10^8
Lactobacillus amylovorous C94	1.9 X 10^{10}	5.7 X 10^9	1.2 X 10^9	1.7 X 10^8

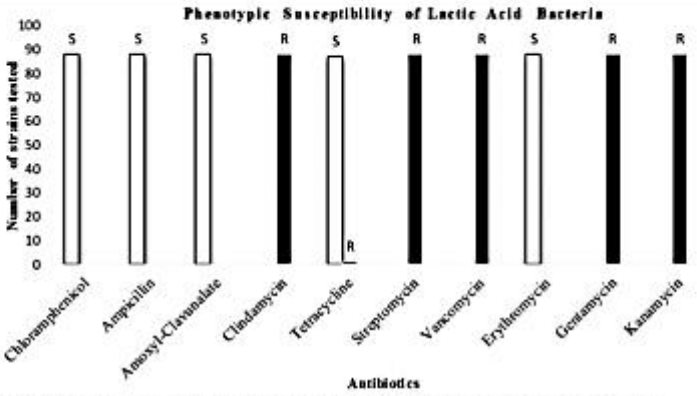

Fig. 2 Antibiotic susceptibility pattern of the isolated lactic acid bacteria. S represents susceptible. R represents resistance

compared with the antimicrobial activity of other LAB strains tested. It was observed that *Lactobacillus ingluivie* C31 produced the least quantity of lactic acid and consequently had the least activity against the tested pathogens, this is in tandem with the report of many researchers who have attributed the antimicrobial activity of *Lactobacillus* spp in their various studies to the production of lactic acid which usually results in low pH [43, 44]. De-Keersmaecker *et al.*, [45] reported that the anti-salmonella activity of *Lactobacillus rhamnosus* was due to accumulation of lactic acid. H'utt *et al.*, [46] also reported a correlation between the pH decreases, amount of lactic acid produced, and the degree of antibacterial activity of probiotic LAB strains.

Interestingly *Lactobacillus salivarius* C86 and *Lactobacillus amylovorus* C94 in this study were able to inhibit the growth of both test *Salmonella enterica* spp completely between 8 and 16 hours of co-incubation such that no *Salmonella* spp was recoverable in the growth media. Several authors have also reported strong

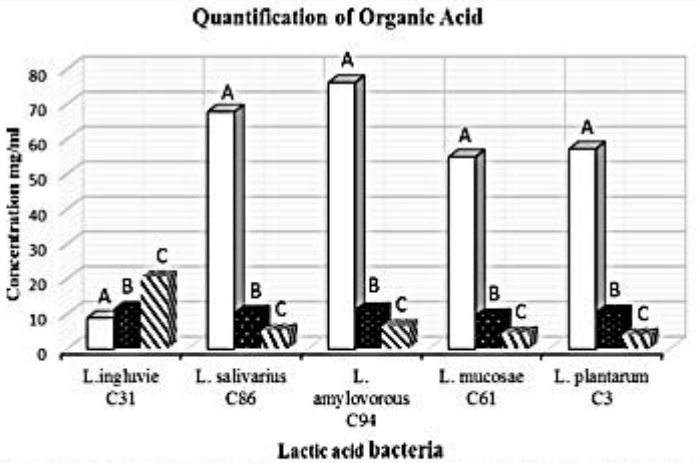

Fig. 3 Concentration (mg/ml) of organic acid produced by selected LAB strains isolated from cattle faeces. **a** indicates yield of lactic acid. **b** indicates yield of acetic acid. **c** indicates yield of propionic acid

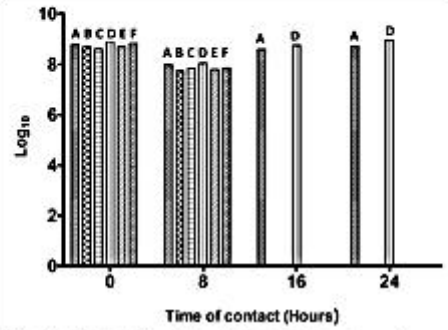

Fig. 4 Kill Kinetics of two Salmonella enterica strains in co-culture with Lactobacillus salivarius C86 and Lactobacillus amylovorus C94. **a** represents Salmonella enterica strain S1 (control). **b** represents Salmonella enterica S1 and Lactobacillus salivarius C86. **c** represents Salmonella enterica S1 and Lactobacillus amylovorus C94. **d** represents Salmonella enterica strain S57 (control). **e** represents Salmonella enterica S57 and Lactobacillus salivarius C86. **f** represents Salmonella enterica S57 and Lactobacillus amylovorus C94

inhibition of *Salmonella* activities by LAB in co-culture [47–49].

The safety of LAB to be used as probiotics is also of utmost importance as the risk of dissemination of resistant genes to other microorganisms is increasing. Potential probiotic strains should not possess transferrable antibiotic resistant determinants. A major consideration is to distinguish between intrinsic and acquired resistance in probiotic organisms and this can be suggested by the comparison of antibiotic susceptibility patterns of different representative strains from each species [50]. A general susceptibility and resistant pattern was observed among species of all the isolates tested which suggest intrinsic resistance. Lactobacilli are known to exhibit a wide range of antibiotic resistance naturally, which are not transmissible and do not form a safety concern [51]. The result of our antimicrobial susceptibility testing is corroborated with the report of Maldonado and Nader-Macias [52] where the entire LAB isolated from calves faeces were all susceptible to erythromycin, ampicillin and chloramphenicol and all but one isolate was resistant to the aminoglycoside kanamycin. Intrinsic resistance to aminoglycoside antibiotics in *Lactobacillus* spp has been reported by several authors [53–57]. The resistance of *Lactobacillus* species to vancomycin has also been described as intrinsic) [50, 58]. Hoque *et al.* [59] reported a high *Lactobacillus* spp resistance to tetracycline but all *Lactobacillus* spp isolated in our study were susceptible to tetracycline. The selected strains were non haemolytic, further qualifying them as potential probiotic candidates.

Conclusion

This study demonstrated the *in vitro* anti-salmonella ability of cattle intestinal lactic acid bacteria and their potentials to function as probiotic feed additive in livestock especially to act against salmonellosis in cattle. The two selected *Lactobacillus* strains demonstrated promising potential probiotic property *in vitro*. The strains will be further tested *in vivo* for the reduction of salmonella carriage in cattle.

Abbreviations

16SrRNA: 16S ribosomal ribonucleic acid; CFS: Cell free supernatant; CFU/ml: Colony forming unit per millilitre; DNA: Deoxyribonucleic; Fig.: Figure; HPLC: High performance liquid chromatography; *L.*: *Lactobacillus*; LAB: Lactic acid bacteria; MALDI : Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry; MH: Mueller Hinton; MRS: De Man, rogosa and sharpe; PCR: Polymerase chain reaction; *S.*: *Streptococcus*; spp.: Species; SSA: Salmonella-shigella agar

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Ethical approval and consent to participate

Not applicable

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Availability of data and materials

The 16S sequences were deposited in NCBI with the accession numbers KY 810532-KY810608.

Authors' contributions

AA carried out the isolation, antimicrobial assay and drafted the manuscript. EP is involved in acquisition of some data and revising the manuscript. BAA participated in the design of the study and read the manuscript. FAA participated in the study design, acquisition of some data, coordination and revision of the manuscript. All authors read and approved the final manuscript.

Consent for publication

Not applicable

Competing interests

The authors declare no competing interest on this study.

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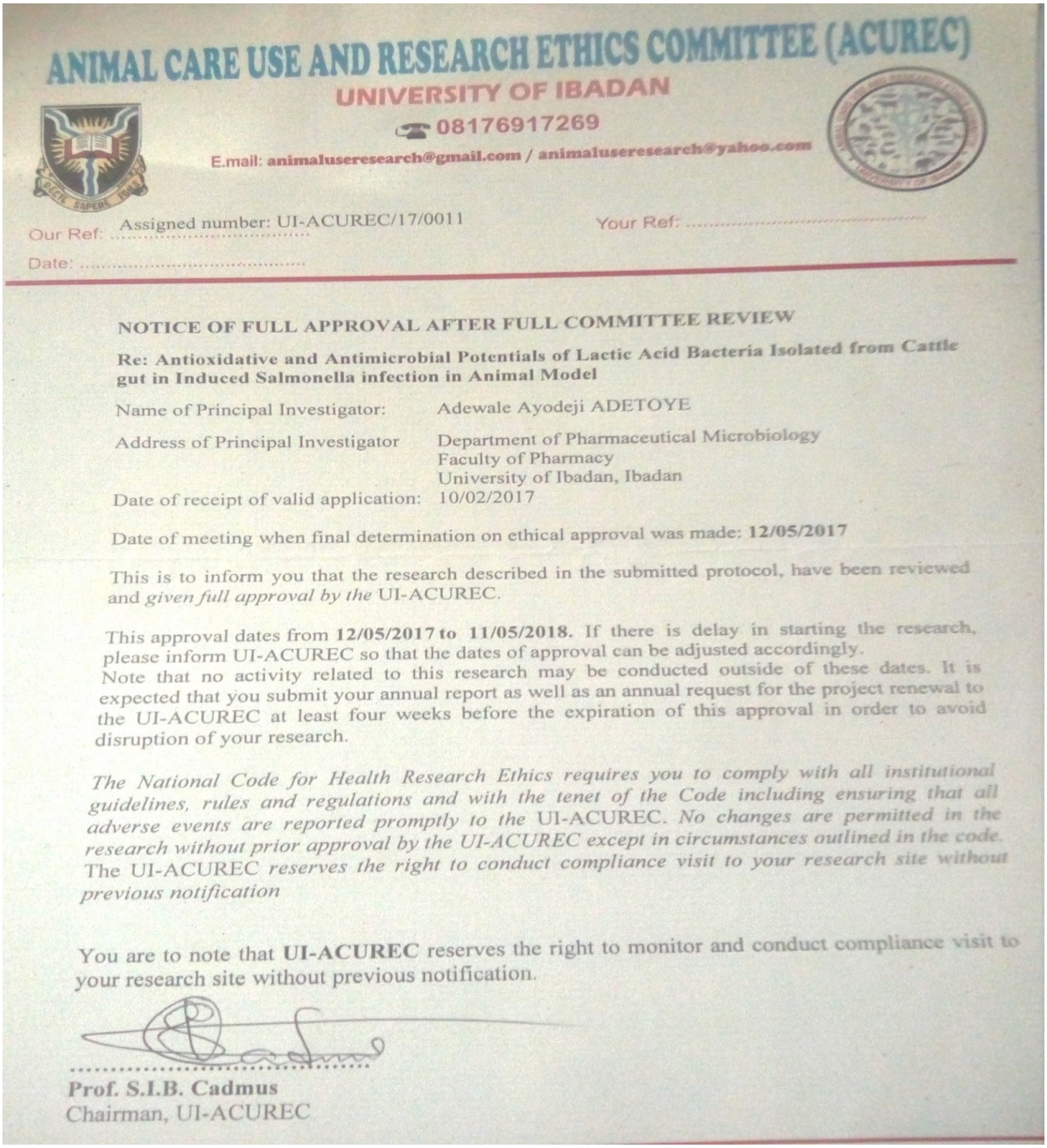
APPENDIX II

ACUREC Ethical Approval



ANIMAL CARE USE AND RESEARCH ETHICS COMMITTEE (ACUREC)

UNIVERSITY OF IBADAN

08176917269

E.mail: animaluseresearch@gmail.com / animaluseresearch@yahoo.com

Our Ref: Assigned number: UI-ACUREC/17/0011 Your Ref: .....

Date: .....

NOTICE OF FULL APPROVAL AFTER FULL COMMITTEE REVIEW

Re: Antioxidative and Antimicrobial Potentials of Lactic Acid Bacteria Isolated from Cattle gut in Induced Salmonella infection in Animal Model

Name of Principal Investigator: Adewale Ayodeji ADETOYE

Address of Principal Investigator: Department of Pharmaceutical Microbiology, Faculty of Pharmacy, University of Ibadan, Ibadan

Date of receipt of valid application: 10/02/2017

Date of meeting when final determination on ethical approval was made: **12/05/2017**

This is to inform you that the research described in the submitted protocol, have been reviewed and *given full approval by the* UI-ACUREC.

This approval dates from **12/05/2017 to 11/05/2018.** If there is delay in starting the research, please inform UI-ACUREC so that the dates of approval can be adjusted accordingly. Note that no activity related to this research may be conducted outside of these dates. It is expected that you submit your annual report as well as an annual request for the project renewal to the UI-ACUREC at least four weeks before the expiration of this approval in order to avoid disruption of your research.

The National Code for Health Research Ethics requires you to comply with all institutional guidelines, rules and regulations and with the tenet of the Code including ensuring that all adverse events are reported promptly to the UI-ACUREC. *No changes are permitted in the research without prior approval by the UI-ACUREC except in circumstances outlined in the code.* The UI-ACUREC *reserves the right to conduct compliance visit to your research site without previous notification*

You are to note that **UI-ACUREC** reserves the right to monitor and conduct compliance visit to your research site without previous notification.

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Prof. S.I.B. Cadmus

Chairman, UI-ACUREC

APPENDIX III

Minimum Inhibitory Concentration of *Salmonella* Isolate

	Amp	Amp-Sul	Tet	Gen	TMP-SMX	Cefpo	Cefuro	Cip	Mofix	Pipe-Taz	Cefo	Cefta	Imi	Meso	Erta	Tig
S1	2 I	2 I	1 S	1 S	20 S	0.25 S	4 S	0.25 S	0.25 S	4 S	1 S	1 S	0.25 S	0.25 S	0.5 S	0.5 S
S2	2 I	2 I	1 S	1 S	20 S	0.25 S	4 S	0.25 S	0.25 S	4 S	1 S	1 S	0.25 S	0.25 S	0.5 S	0.5 S
S3	2 I	2 I	1 S	1 S	20 S	0.25 S	4 S	0.25 S	0.25 S	4 S	1 S	1 S	0.25 S	0.25 S	0.5 S	0.5 S
S4	2 I	2 I	1 S	1 S	20 S	0.25 S	4 S	0.25 S	0.25 S	4 S	1 S	1 S	0.25 S	0.25 S	0.5 S	0.5 S
S5	2 I	2 I	1 S	1 S	20 S	0.25 S	4 S	0.25 S	0.25 S	4 S	1 S	1 S	0.25 S	0.25 S	0.5 S	0.5 S
S10	2 I	2 I	1 S	1 S	20 S	0.25 S	4S	0.25 S	0.25 S	4 S	1 S	1 S	0.25 S	0.25 S	0.5 S	2.0 I
S13	2 I	2 I	1 S	1 S	20 S	0.25 S	4 I	0.25 S	0.25 S	4 S	1 S	1 S	0.25 S	0.25 S	0.5 S	0.5 S
S15	2 I	2 I	1 S	1 S	20 S	0.5 S	8 S	0,5 S	0.2, S	4 S	1 S	1 S	0.25 S	0.25 S	0.5 S	0.5 S
S16	2 I	2 I	1 S	1 S	20 S	0.25 S	4 S	0.25 S	0.25 S	4 S	1 S	1 S	0.25 S	0.25 S	0.5 S	0.5 S
S19	2 I	2 I	1 S	1 S	20 S	0.25 S	4 S	0.25 S	0.25 S	4 S	2 S	1 S	0.25 S	0.25 S	0.25 S	0.5 S
S21	2 I	2 I	1 S	1 S	20 S	0.25 S	4 S	0.25 S	0.25 S	4 S	2 S	1 S	0.25 S	0.25 S	0.25 S	0.5 S
S25	2 I	2 I	1 S	1 S	20 S	0.25 S	4 S	0.25 S	0.25 S	4 S	2S	1 S	0.25 S	0.25 S	0.25 S	0.5 S
S26	2 I	2 I	1 S	1 S	20 S	0.25 S	4 S	0.25 S	0.25 S	4 S	1 S	1 S	0.25 S	0.25 S	0.25 S	0.5 S
S31	2 I	2 I	1 S	1 S	20 S	0.25 S	4 S	0.25 S	0.25 S	4 S	1 S	1 S	0.25 S	0.25 S	0.5 S	0.5 S
S38	2 I	2 I	1 S	1 S	20 S	0.25 S	4 S	0.25 S	0.25 S	4 S	2 S	1 S	0.25 S	0.25 S	0.25 S	0.5 S
S41	2 I	2 I	1 S	1 S	20 S	0.25 S	4 S	0.25 S	0.25 S	4 S	2 S	1 S	0.25 S	0.25 S	0.25 S	0.5 S
S42	2 I	2 I	1 S	1 S	20 S	0.25 S	4 S	0.25 S	0.25 S	4 S	2S	1 S	0.25 S	0.25 S	0.25 S	0.5 S
S44	2 I	2 I	1 S	1 S	20 S	0.25 S	4 S	0.25 S	0.25 S	4 S	1 S	1 S	0.25 S	0.25 S	0.5 S	0.5 S
S47	2 I	2 I	1 S	1 S	20 S	0.25 S	4 S	0.25 S	0.25 S	4 S	1 S	1 S	0.25 S	0.25 S	0.25 S	0.5 S
S48	2 I	2 I	1 S	1 S	20 S	0.25 S	4 S	0.25 S	0.25 S	4 S	2 S	1 S	0.25 S	0.25 S	0.25 S	0.5 S
S49	2 I	2 I	1 S	1 S	20 S	0.25 S	4 S	0.25 S	0.25 S	4 S	2 S	1 S	0.25 S	0.25 S	0.25 S	0.5 S
S54	2 I	2 I	1 S	1 S	20 S	0.25 S	4 S	0.25 S	0.25 S	4 S	2 S	1 S	0.25 S	0.25 S	0.25 S	0.5 S
S56	2 I	2 I	1 S	1 S	20 S	0.25 S	4 S	0.25 S	0.25 S	4 S	2 S	1 S	0.25 S	0.25 S	0.25 S	0.5 S
S57	2 I	2 I	1 S	1 S	20 S	0.25 S	4 S	0.25 S	0.25 S	4 S	2 S	1 S	0.25 S	0.25 S	0.25 S	0.5 S
S58	2 I	2 I	1 S	1 S	20 S	0.25 S	4S	0.25 S	0.25 S	4 S	1 S	1 S	0.25 S	0.25 S	0.25 S	0.5 S
S60	2 I	2 I	1 S	1 S	20 S	0.25 S	4S	0.25 S	0.25 S	4 S	1 S	1 S	0.25 S	0.25 S	0.25 S	0.5 S
S62	2 I	2 I	1 S	1 S	20 S	0.25 S	4S	0.25 S	0.25 S	4 S	1 S	1 S	0.25 S	0.25 S	0.25 S	0.5 S
S68	2 I	2 I	1 S	1 S	20 S	0.25 S	4S	0.25 S	0.25 S	4 S	1 S	1 S	0.25 S	0.25 S	0.25 S	0.5 S
S70	2 I	2 I	1 S	1 S	20 S	0.25 S	4S	0.25 S	0.25 S	4 S	1 S	1 S	0.25 S	0.25 S	0.25 S	0.5 S
S76	2 I	2 I	1 S	1 S	20 S	0.25 S	4S	0.25 S	0.25 S	4 S	1 S	1 S	0.25 S	0.25 S	0.25 S	0.5 S
S77	2 I	2 I	1 S	1 S	20 S	0.25 S	4S	0.25 S	0.25 S	4 S	1 S	1 S	0.25 S	0.25 S	0.25 S	0.5 S

APPENDIX IV a

Lactic Acid Bacteria 16S rRNA Sequences Obtained in this Study.

Lactobacillus plantarum C3

GCGT GCCT AATA CATG CAAGT CAGA ACGA ACTC TGGT ATAT GATT GGTG CTTGC ATCAT GATTT ACATT TAGTG AG TGGG AACT GGTG AGTA ACAC GTGG GAAA CCTG CCCA GAAG CGGG GGAT AACA CCTGG AA AC AG AT GCTA ATACC GCAT AACA ACTTG GACCG CATGG TCCGA GTTTG AAAG ATGG CTTCG GCTAT CA CTTT TG GATGG TCCC CGGC GTATT AGCTA GATGGTG GGGTA ACGGCT CACCA TGGC AATGA TACG TAGC CGAC CTGA GAGG GTAA TCGG CCAC ATTG GGAC TGAG ACAC GGCC CAAA CTCC TACG GGAG GCAG CAGT AGGG AATC TT CCACA ATGGA CGAAA GTCTG ATGGA GCAAC GACCG CGTGA GTGAA GAAG GGTT TC GGCT CGTAA AACTC TGTTG TTAAA GAAG AACA TATCT GAGA GTAAC TGTTC AGGT ATTGA CGGTA TTGAA CCAG AAAG CCAC GG

Lactobacillus agillis C5

GTGC CTAA TACAT GCAA GTCGA ACGC TTTT ATTC AATC ATCGTA GCTT GCTAC ACCG ATTGA AAAT TGAGT GGCGA ACGG GTGA GTAAC ACGTG GGTA ACCTG CCCAA AAGAG GGGG ATAAC ACTTG GAAAC AGGTG CTAAT ACCGC ATAAC CATGAT GACC GCAT GGTCA TTATG TAAAA GATGG TTTCG GCTAT CACTT TTGGA TGGAC CCGCG GCGTA TTAA CTTG TTGGT GGGGT AACGG CCTAC CAAGG TGAT GATA CGTAG CCGAA CTGA GAGG TTGA TCGG CCAC ATTG GGAC TGAG ACAC GGCC CAAA CTCC TACG GGAG GCAG CAGT AGGG AATC TTCC ACAA TGGG CGCA AGCC TGAT GGAG CAAC GCCG CGTGA GTGA AGGAA GGTC TTCG GATCG TAAA ACTC TGTT GTTAG AGAA GAAC ATGCA GGAGA GTAA CTGT TCTT GTAT TGAC TGTA TCTAA CCAG AAAG CCAC GGCT AACT ACGA TGCC AGCTG CCG CGGT CATAC GTACG TGGC

Lactobacillus *taiwanensis C6*

GGCG GCGTG CCTAA TACA TGCA AGTCA GAGCG AGCT TGCCT AGAT GATT TTAGTG CTATGC ACTAAA TGAAA CTAGAT ACAAGC GAGCG GCGGAC GAGGTG AGTAA CACGT GGGTAA CCTGCC CAAGA GACTG GGAT AACAC CTGGA AACAG ATGCT AATACC GGATAA CAGAC ACTAG ACGC ATGT CTAGA GTTT GAAAG ATGGT TCTG CTATC ACTCTTG GATGGAC CTGCG GTGCAT TAGCT AGTTAGG TAAGG TAACG GCTTAC GCATA GGCAAT GATGC ATAGAC CGAGT TGAGA GACTG ATCGAG CCACAT CGGGA CATGAG ACAC GGCCC AAACT CCTACG GGTA GGCAGA CAGTA AGGAA TCTTCC ACAAT GGACG AAAGTG CTGAT GGAGC AACGC CGCGT GTAGT GAAG AATGG GTTT CGGC TCGTA CGATA GCTAAT ACCG CATAA CAGCA TTTAA CACA TGTTAG ATGCT TGAAA GGAGC AATTG CTTC ACTAG TAGATG GACCT GCGTTG TATT AGCTA GTTG GTGAG GTAAC GGCT CACCAA GGCGAC GATACA TAGCCG ACCTG AGAGG GTGATC GGCCA CACTG GGAC TGAGA CACGG CCCAG ACTCCTA CGGG AGGCA GCAGT AGGGAA TCTTC GGCAAT GGGGGC AACCC TGACC GAGCA ACGCCG CGTGA GTGAA GAAGG TTTTCG GATCGT AAAG CTCTG TTGTA AGAGA AGAAC GTGTGT GAGAG TGGAA AGTTC ACACA GTGAC GGTA ACTTAC CAGAA AGGG ACGGC TAACT ACGT GCCA GCAG CCGCG GTAAT ACGT AGGTC CCGAG CGTT GTCCG GATTT ATTG

Lactobacillus mucosae C8

TGTGCC TAATAC ATGCAA GTCGAA CGCGTT GGCCCA ACTGATT GAACGT GCTTGCA CGGACT TGACGTT GGTTTA CCAGCG AGTGGC GGACGG GTGAG TAACAC GTAAGT AACCTG CCCCAA AGCGGG GGATAA CATTT GGAAA CAGAT GCTAA TACCGC ATAGA CAATTT AGAATC GCATGA TTCAAA TTTAAA AGATG GCTTC GGCTAT CACTTT GGGAT GGACCT GCGGC GCATTA GCTTG TTGGTA GGGTAA CGGCC TACCA AGGCT GTGATG CGTAG CCGAGT TGAGA GACTG ATCGGC CACAA TGGAAC TGAGA CACGG TCCATA CTCCTA CGGGA GGCAG CAGTA GGGAAT CTTCCA CAATGG GCGCAA GCCTG ATGGA GCAACA CCGCG TGAGTG AAGAA GGGTT TCGGC TCGTAT AAGCT CTGTTG TTAGA GAAGA ACGTG CGTGA GAGCA ACTAGT TCACGC AGTGAC GGTAT CTAACC AGAGAG GCACGG CTAACT ACGTGCC AGCAGC CGCGGT AGACG TAGGTG GCAAG CGTCATC CGGATC TATTGG GCGTA CAGCG AGCGC AGGCG GATCTG ATAC GTCTG ATGT GACAG

Lactobacillus mucosae C101

TGCCT AATAC ATGCA AGTCG AACGC GTTGGC CCAAC TGATT GAACG TGCTT GCACG GACTT GACGT TGGTT TACCA GCGAG TGGCG GACGG GTGAG TAACA CGTAG GTAAC CTGCCC CAAAG CGGGG GATAA CATTTG GAAAC AGATG CTAAT ACCGC ATAAC AATTT GAATCG CATGA TTCAA ATTTA AAAG ATGGT TTCGG CTAT CACT TTGGG ATGGA CCTGC GGCG CATTA GCTTG TTGGT AGGGT AACGG CCTAC CAAGG CTGTG ATGCG TAGCC GAGT TG AGAGA CTGAT CGGC CACAA TGGAA CTGA GACAC GGTCCA TACTC CTACG GGAG GC AGC AGTAG GGAAT CTTCC ACAAT GGGC GCAAGC CTGATG GAGCA ACACC GCGT GAGTG AAGAA GGGT TTCG GCTCG TAAA GCTC TGTTG TTAGA GAAGA ACGTG CGTGA GAGCA ACTG TTCAC GCAG TGAC GGTA TCTAA CCAGA AAGT CACG GCTA ACTA CGTG CCAG CAGC CGCG GTAA TACGT AGGTG GCAAG CGTTA TCCG GATTT ATTGG GCGTA AAGCG AGCGC AGGCG GTTTGA TAAGTC TGATGT GAAA GC CTTTGG CTTAA CCAA NGAA GTGC ATCG GAAA CTGTC

Lactobacillus gasseri C103

GTGC CTAAT ACATG CAAGT CGAG CGAGC TTGCC TAGA TGATT TTAGTG CTTGC ACTAA ATGAA ACTAG ATACA AGCGA GCGG CGGAC GGGTG AGTA ACACG TGGGT AACCT GCCCA AGAGA CTGGG ATAAC ACCTG GAAAC AGATG CTAATA CCGGA TAACA ACACT AGACG CATGT CTAGA GTTTG AAAGA TGGTT CTGCT ATCA CTCTT GGAT GGACC TGCGG TGCAT TAGC TAGTT GGTAA GGTA ACGGC TTACC AAGG CAATG ATGCATAGC CGAG TTGAG AGACT GATCG GCCAC ATTGG GACTG AGACA CGGCC CAAAC TCCTA CGGG AGGC AGCAG TAGGG AATCTT CCACA ATGGA CGAAA GTCTG ATGGA GCAA CGCC GCGTG AGTGA AGAA GGGT TTCGG CTCG TAAA GCTC TG TTGGT AGTGA AGAAA GATA GAGG TAGTA ACTGG CCTTT ATTTG ACGG TAAT TA CTTA GAAA GTCA CG GCTAA CTACG TGCCA GCAG CCGCG GTAA TACGTA GGTG GCAA GCGTTG TC CGGA TTTATT GGGCG TAAAG CGAG TGCAG GCGG TTCAAT AAGTCT GATGT GAAAG CCTTCG GCTCAA CCGGA GAATT GCATCA GAAA CTGTT GAACT TGAGT GCAGA AGAG GAG AGTG

Lactobacillus mucosae C104

TGCCT AATAC ATGCAA GTCGA ACGCG TTGGC CCAAC TGATT GAACG TGCTTG CACGGA CTTGA CGTTG GT TTA CCAG CGAGT GGCG GACG GGTGA GT AACACG TAGGT AACCT GCCC CAAAG CGGG GGATA ACATTT GGAA ACAG ATGCT AATA CCGC ATAA CAA TTTG AATCG

CATGA TTCAA ATTTA AAAGA TGGT TTCG GCTA TC ACTTT GG GATG GACCTG CGGCG CATT AGCT TGTT GGTAG GGTAA CGGCC TACCA AGGCT GTG ATGCG TA GCCG AGTTGA GAGA CTGA TCGG CCAC AATGG AACTGA GACAC GGTCC ATACT CCTAC GGGA GGCAG CA GTAGG GAATC TTCCA CAATG GGCG CAAG CCTG ATGGA GCAA CACC GCGTG AGTGA AGAAG GGTTT CGGCT CGTA AAGCT CTGTTG TTAGA GAAG AACGT GCGTG AGAG CAACT GTTCA CGCA GTGAC GGTATC TAACC AGAA AGTCA CGGCTA ACTACG TGCCA GCAG CCGCG GTAAT ACGTA GGTG GCAA GCGTT ATCCGG AT TTAT TGGG CGTA AAGC GAGCG CAGG CGGT TTGA TAAG TCTGA TGTGAA

Lactobacillus ingluviei C105

GTGTG CCTAA TACATG CAAGTC GAACG CGTTG GCCCA ATTGA TTGATG GTGCTT GCAC CTGAT TGATTT TGGT CGCC AACG AGTG GCGG ACGG GTGA GTAACAC GTAGGT AACCTG CCCA GAAG CGGG GGAC AA CATT TGG AAACAG ATGCTAA TACCGCA TAACAAC GTTGTTC GCATGA ACAACG CTTAAA AGATGG CTTCT CG CTAT CACTTC TGGATG GACCTG CGGTG CATTAG CTTGTT GGTGG GGTAAC GGCCT ACCAA GGC GATGA TGCA TAGCC GAGTT GAGAG ACTGAT CGGCCA CAATGG GACTG AGACA CGGC CCAT ACTC CTACG GGAG GCAG CAGT AGGG AATCTT CCAC AATG GGCG CAAG CCTGA TGGA GCAA CACC GCGT GAGT GAAG AAGG GTTTC GGCTC GTAAA GCTCT GTTG TTAAA GAAG AACA CGTA TGAG AGTAA CTGTT CATA CGTT GACG GTAT TTAAC CAGAA AGTCA CGGCT AACTA CGTGC CAGCA GCCGC GGTAA TACGT AGGTG GCAA GCGTT ATCCG GATTT ATTG GGCG TAAA GAGA GTGC AGGC GGTT TTCT AAGT CTGA TGTGA

Lactobacillus agilis C12

GTGCC TAAT ACATG CAAG TCGAA CGCTT TTTTC AATC ATCGT AGCTT GCTAC ACCGAT TGAA AATTG AGTGG CGAAC GGGTG AGTAA CACGT GGGTA ACCTG CCCAA AAGA GGGG GATA ACAC TTGG AAACAG GTGCT AATAC CGCA TAACC ATGAT GACCG CATG GTCA TTAT GTAA AAGA TGGT TTCG GCTA TCAC TTTT GGATG GACC CGCG GCGT ATTAA CTTG TTGGT GGGGT AACGG CCTAC CAAGG TGATG ATACG TAGCC GAAC TGAG AGGTT GATCG GCC ACAT TGGGA CTGAG ACACGG CCCAA ACTCC TACGG GAGG CAGCA GTAGG GAATC TTCCA CAATG GGCG CAAG CCTGA TGGAG CAACG CCGCG TGAGT GAAGA AGGTC TTCGG ATCGT AAAA CTCTG TTGTT AGAG AAGAA CATGC AAGA GAGT AACTG TTCTTG TATTG ACGG TATCT AACC AGAA AGCC ACGG CTAA CTACG TGCCA GCAG CCGC GGTA ATACG TAGGT GGCAA GCGT TGTC CGGA TTTAT TGGG CGTA AAGG GAAC GC AG GCGG TCCTT TAAGTC TGATGT GAAA GCCT TCGG CTTA ACCG AAGAA TTGC ATTGG AAACT GGAG GACT TGAG TGCA GAAG AGGA GAGG TGGA

Lactobacillus ingluviei C13

TGTG CCTA ATAC ATGC AAGT CGAA CGCG TTGG CCCA ATTG ATTGAT GGTG CTTGC ACCTG ATTGA TTTT GGTCG CCAA CGAG TGGC GGACG GGTG AGTAA CACG TAGG TAAC CTGC CCAG AAGC GGGG GACAA CATT TGGA AACA GATGC TAATA CCGC ATAAC AACG TTGTT CGCAT GAACA ACGCT TAAAA GATGGC TTCT CGTA TCAC TTCTGG ATGGA CCTGC GGTG CATT AGCT TGTT GGTG GGGT AACG GCCT ACCA AGGC GATGA TGCA TAGC CGAG TTGAG AGAC TGAT CGGC CACAA TGGGA CTGA GACA CGGC CCAT ACTC CTAC GGGA GGCA GCAG TAGG GAAT CTTCC ACAA TGGG CGCA AGCC TGAT GGAG CAACA CCGCGT GAGTG AAG AAGG GTT TCGG

CTCG TAAAG CTCT GTTG TTAA AGAAG AACAC GTAT GAGAG TAAC TGTTC ATAC GTTG ACGGT ATTTA ACCAG AAAG TCACG GCTAA CTAC GTGC CAGCA GCCGC GGTA ATAC GTAG GTGGC AAGC GTTA TCCGG ATTT ATTG GGCG TAAA GAGA GTGC AGGCG GTTT TCTA AGTC TGAT GTGA AAGC CTTCG GCTTA ACCGG AGAA GTGCA TCGG AAAC TGGA TAA CTT GAGTG CAGA AGAGG GTAGTG GAACT CCATG TGTA GCGG TGGA ATGCG

Lactobacillus agilis C14

GTGC CTAA TACA TGCA AGTC GAACG CTTTT ATTCA ATCAT CGTAG CTTGC TACAC CGATT GAAAA TTGA GTGG CGAA CGGG TGAGT AACA CGTG GGTAA CCTGC CCAA AAGAG GGGG ATAA CACT TGGAA ACAG GTGC TAAT ACCG CATAA CCATG ATGAC CGCAT GGTC ATTAT GTAA AAGAT GGTTT CGGC TATCA CTTTT GGATG GACC CGCG GCGTA TTAA CTTG TTGG TGGG GTAA CGGC CTACC AAGG TGAT GATAC GTAG CCGA ACTG AGAG GTTGA TCGG CCAC ATTG GGAC TGAG ACAC GGCC CAAA CTCC TACGG GAGG CAGC AGTAC GGAA TCTT CCAC AATG GGCG CAAG CCTGA TGGAG CAACG CCGC GTGA GTGAA GAAGG TCTTC GGAT CGTAA AACTC TGTTG TTAGA GAAGAA CATGC AGGAG AGTA ACTGT TCTTGT ATTGA CTGTA TCTAA CCAGA AAGCCA CGGCTA ACTA CGAT GCCA GCTGC CGCGG TAAT ACGT ACGTG GCG AGCG

Lactobacillus amylovorus C15

GTGCC TAAT ACAT GCAA GTCG AGCG AGCGG AACC AACA GATT TACTT CGGTA ATGAC GTTGG GAAA GCGA GCGG CGGA TGGG TGAG TAAC ACGT GGGG AACCT GCCT CTAAG TCTGG GATA CCATT TGGA AACA GGTG CTAA TACCG GATA ATAAA GCAGA TCGCA TGAT CAGC TTTT GAAA GGCG GCGT AAGC TG TCGC TAAG GGAT GGCC CCGC GGTG CATT AGCT AGTTG GTAA GGTAA CGGC TTACC AAGG CGAC GATG CATA GCCG AGTT GAGA GACT GATC GGCC ACATT GGGA CTGA GACAC GGCC CAAA CTCC TACG GGAG GCAG CAGTA GGGA ATCTT CCAC AATGG ACGC AAGT CTGAT GGAG CAACG CCGC GTGA GTGAA GAAGG TTTT CGGAT CGTAA AGCTC TGTT GTTGG TGAAG AAGGA TAGA GGTA GTAA CTGG CCT TTA TTTG ACGG TAATC AACCA GAAAGT CACGG CTAAC TACGT GCCA GCAG CCGC GGTA ATAC GTAGGT

Lactobacillus ingluviei C16

TGCCT AATA CATG CAAGT CGAAC GCGTT GGC CCAA TTGA TTGA TGGT GCTT GCAC CTGA TTGAT TTTA GGTC GCCA ACGAG TGGC GGAC GGGT GAGT AACAC GTAG AGTA ACCTG CCCA GAAGC GGGG GACA ACAT TTGG AAAC AGATG CTAAT ACCG CATAA CAAC GTTA GTTA CGCAT GAAC AACG CTTA AAAG ATGGC TTCT CGCT ATCA CTTC TGGA TGGA CCTG CGGT GCAT TAGC TTGTTG GTGG GGTAA TGGC CTAC CAACG GCGA TGATG CATA GCCG AGTT GAGA GACT GATC GGCC ACAA TGGG ACTG AGAC ACGG CCCA TACTC CTAC GGGA GGCA GCAG TACG GAAT CTTC CACA ATGG GCGC AAGC CTGA TGGA GCAA CACC GCGT GAGT GAAG AATG GTTT CGGC TCGT ATAG CTCT GTTG TTGA AAGA AGAA CACGT ATGA GAGT AACT AGTT CATA CGTT GACG GTAT ACAA CCAG AGAG TCAC TGCT AACT ACGT GCCA GCAG CCGC GGTA GACG TANGT GGCC AGCG TCAT CCGG ATCT ATTG GGCG TACAT GAGA GTGC AGGC

Lactobacillus ingluviei C17

TGTG CCTA ATAC ATGC AAGT CGAA CGCGT TGGC CCAA TTGA TTGAT GGTG CTTG CACC TGAT TGAT TTTG GTCG CCAA CGAG TGGC GGAC GGGT GAGT AACA CGTA GGTA ACCT GCCC AGAA

GCGG GGG ACAA CATTT GGAA ACAG ATGC TAAT ACCG CATA ACAA CGTT GTTCG CATG AACA ACGC TTAA AAGA TGGC TTCT CGCTA TCAC TTCT GGAT GGAC CTGC GGTG CATT AGCT TGTT GGTG GGGT AATG GCCT ACCA AGGC GATG ATGCA TAGC CGAG TTGA GAGA CTGA TCGG CCAC AATG GGAC TGAG ACAC GGCC CATA CTCC TACG GGAG GCAG CAGT AGGG AATCT TCCA CAATG GGCGC AAGC CTGAT GGAG CAAC AC CGCGTG AGTGA AGAA GGGT TTCG GCTC GTAA AGCT CTGT TGTTA AAGA AGA ACAC GTAT GAGA GTAA CTGT TCAT ACGT

Lactobacillus taiwanensis C19

GCAC TAAA TGAA ACTA GATA CAAG CGAG CGGC GGAC GAGG TGAG TAAC ACGT GGGT AGCC TGCC CAAG AGAC TGGG ATAA CACC TGGA AACA TGAT GCTAA TACCG GATAA CAGAC ACTAG ACGC ATGT CTAG AGTT TGAA AGAT GGTT CTGCT ATCA CTCT TGGA TGGA CCTG CGGTG CATT AGCTA GTTA GGTA AGGT AACG GCTT ACGC GATA GGCA CATG ATGC ATAG CCGAG TTGA GAGA CTGA TCGA GCCA CATCG GGAC ATGAG ACACG GCCCA ACACG TCCTAC GGGAG GCAG ACAGT AAAGG AATCT TCCAC AATG GACGA CAAGT GCTGA TGGA GCAA CGCC GCGTG CTTC ACTAG TAGATG TGGGA TAACA CCTG GAAA CAGA TGCT AATA CCGG ATAA CAGA CACTAG ACGCA TGTCT AGAG TTTG AAAG ATGG TTCTG CTAT CACTC TTGG ATGG ACCT GCGG TGCA TTAG CTAG TTAG GTAAG GTAAC GGCT TACG CGAT AGGC AATGA TGCA TAGCC GAGT TGAG AGAC TGAT CGAG CCAC ATCG GGAC ATGA GACA CGGC CCAA ACTC CTAC GGGA GGCA GACA GGTA AGGA ATCT TCCA CAATG GACGA CAAGT GCTGA TGGAGC AACGCC GCGTG TAGTG AAGCAA TGGTTT CGGCT CGTA

Lactobacillus taiwanensis C20

GCAC TAAA TGAA ACTA GATA CAAG CGAG CGGC GGACG AGGT GAGT AACA CGTG GGTA AGCC TGCC CAAG AGAC TGAG ACACG GCCC AGACTCC TACG GGAG GCAG CAGTA GGGAA TCTTC GGCTT TGGGG GCAAC CCTGA CCGAGC AACGC CGCGTG AGTGA AGAA GGTT TTCG GATC GTAA A G C T C C TGTTG TAAGA GAAGA ACGT GTGT GAGA GTGG AAAG TTCAC ACAG CGATA GCTAAT ACCG CATAA CAGCA TTTAA CACA TGTTAG ATGCT TGAAA GGAGC AATTG CTTC ACTAG TAGATG TGGGA TAACA CCTG GAAA CAGA TGCT AATA CCGG ATAA CAGA CACTAG ACGCA TGTCT AGAG TTTG AAAG ATGG TTCTG CTAT CACTC TTGG ATGG ACCT GCGG TGCA TTAG CTAG TTAG GTAAG GTAAC GGCT TACG CGAT AGGC AATGA TGCA TAGCC GAGT TGAG AGAC TGAT CGAG CCAC ATCG GGAC ATGA GACA CGGC CCAA ACTC CTAC GGGA GGCA GACA GGTA AGGA ATCT TCCA CAATG GACGA CAAGT GCTGA TGGAGC AACGCC GCGTG TAGTG AAGCAA TGGTTT CGGCT CGTA

Lactobacillus mucosae C21

TGCC TAATA CATG CAAG TCGA ACGC GTTG GCCC AACTG ATTG AACG TGCT TGCAC GGAC TTGA CGTT GGTTT ACCA GCGA GTGG CGGA CGGGT GAGT AACA CGTA GGTA ACCT GCCC CAAA GCGG GGGAT AACA TTTGG AAAC AGAT GCTA ATACC GCATA ACAA TTTG AATCG CATG ATTC AAAT TTAA AAGA TGGC TTCG GCTAT CACTT TGGG ATGG ACCT GCGG CGCA TTAG CTTG TTGG TAGGG TAAC GGCCT ACCA AGGCT GTGAT GCGTA GCCG AGTT GAGA GACT GATC GGCC ACAA TGGA ACTGA GACA CGGTC CATAC TCCT ACGGG AGGCA GCAG TAGG GAAT CTTC CACA ATGG GCGCA AGCCT GATG GAGC AACA CCGC GTGAG TGAA GAAG GGTTT CGGCT CGTAA AGCT

CTGTT GTTAG AGAA GAAC GTGC GTGA GAGT AACT GTTCA CGCA GTGA CGGTA TCTA ACCAG AAAG TCAC GGCT AACT ACGTG CCAG CAGC CGCG GTAA TACGT AGGT GGCA AGCG TTAT CCGG ATTT ATTG GGCG TAAA GCGAG CGCAG GCGG TTTGA TAAG TCTG ATGT GAAA GCCT TTGGCT TAACCA AAGAA GTGCA TCGG AAACT GTCAG ACTTG AGTG CAGAA GAGG ACAGT

Lactobacillus ingluviei C23

GGTG GGGT AATG GCCT ACCA ACGG CGAT GATG CACT AGCC GAGT TGAG AGACT GATCG GCCAC AATGG GACTG AGAC ACGG CCCAT ACTC CTACG GGAGG CAGCA GTACG GAATC TTCC AGCGA TATGG GCGCA TGAG ACACG GCCC AGACTCC TACG GGAG GCAG CAGTA GGGAA TCTTC GGCTT TGGGG GCAAC CCTGA CCGAGC AACGC CGCGTG AGTGA AGAA GGTT TTCG GATC GTAA A G C T C C TGTTG TAAGA GAAGA ACGT GTGT GAGA GTGG AAAG TTCAC ACAG CGATA GCTAAT ACCG CATAA CAGCA TTTAA CACA TGTTAG ATGCT TGAAA GGAGC AATTG CTTC ACTAG TAGATG AGCC TGAT AGAG CAAC ACCG GCGT GAGTG AAGA ATGGT TTCG GCTC GTATA GCTCT GTTG TTATA GCAAG AACAC GTAT GAGA GTAA CAGT TCA TAC GTTGA CGGT ATAT AACCA GAGAG TCACT GCTA ACTAC GTGC CAGCA GC

Lactobacillus mucosae C24

TGCC TAATACA TGCAA GTCG AACG CTGT TGGCC CAAC TGATT GAAC GTGC TTGCA CGGAC TTGAC GTTG GTTTA CCAGC GAGTG GCGGA CGGG TGAG TAAC ACGT AGGTA ACCT GCCC CAAAG CGGG GG ATAAC ATTTG GAAA CAGAT GCTA ATAC CGCA TAACA ATTTG AATCG CATG ATTCA AATT TAAAA GATG GTTTC GGCTA TCACT TTGGGA TGGA CCTGC GGCGC ATTA GCTTG TTGGT AGGG TAAC GGCC TACCA AGGC TGTG ATGC GTAG CCGA GTTGA GAGA CTGA TCGG CCACA ATGGA ACTGAG ACACG GTCCA TACTC CTACG GGAG GCAGC AGTAG GGAA TCTTC CACAA TGGGC GCAAG CCTG ATGGA GCAA CACCG CGTG AGTG AAGA AGGG TTTCG GCTC GTAA AGCT CTGTT GTTAG AGAAG AACG TGCG TGAGA GCAAC TGTTC ACGCA GTGACG GTAT CTAA CCAG AA AGTC ACGG CTAA CTAC GTGC CAGC AGCC GCGG TAAT ACGT AGGT GGCA AGCG TTAT CCGG ATTTAT TGGG CGTA AAGC GAGC GCAG GCGGT TTGAT AAGT CTGAT GTGA AAGCC TTGGG CTTAA CCAAA GAAG TGCAT CGGA AACTG TCAGAC TTGAGT GCAGA AGAGG ACAGT GGAAC CTCAT GTGTA CCGGT GGAA TGCG

Lactobacillus paraplantarum C25

GTGC CTAA TACA TGCA AGTC GAAC GAACT CTGGT ATTG ATTGGT GCTTG CATCA TGATT TACAT TTGAG TGAGT GGCGA ACTG GTGAG TAACA CGTG GGAA ACCT GCCC AGAA GCGG GGG ATAAC ACCT GGAA ACAGA TGCT AATA CCGC ATAAC AACTT GGACC GCATG GTCCG AGTC TTGA AAGA TGGC TTCG G CTAT CACT TCTG GATG GTCC CGCG GCGT ATTA GCTA GATG GTGA GGTA A CGGCT CACCAT GGCAA TGAT ACGTA GCCG ACCT GAGA G GGTA ATCG GCCAC ATTG GGACT GAGA CACGG CCCA AACTC CTAC GGGA GGCA GCAG TAGG GAAT CTTC CACA ATGG ACGA AAGT CTGA TGGAG CA AC GC CG CGT G AGT GAAG AAGG GTTT CGGC TCGT AAAA CTCT GTTG TTA AAGA AGAA CATA TCTG AGAGT AACT GTTCA GGTA TTGA CGGT ATTT AACC AGAA AGCC ACGGC TAACT ACGTG CCAGC AGCCG CG G TAAT A CGTA GGTG GCAA GCGT TGTC CGGA TTTA TTGG GC GTAA AGCG AGCGCA

Lactobacillus plantarum C26

GGCG GCGT GCCT AATA CATG CAAG TCAG AACG AACTC TGGT ATAT GATTG GTGCT TGCAT CATGA TTTAC ATTTG AGTG AGTGG CGAAC TGGTG AGTA ACACG TGGGA AACCT GCCC AGAAG CGGGG GATAA CACC TGGAA ACAGA TGCT AATA CCGCA TAACA ACTTG GACCG CATGG TCCGA GTTTG AAAG ATGG CTTCG GCTA TCAC TTTT GGAT GGTCC CGCG GCGTA TTAGC TAGA TGGT GGGG TAAC GGCT CACCA TGGCA ATGAT ACGTA GCCG ACCTG AGAG GGTAA TCGG CCAC ATTG AGGA CTGAG ACACG GCCCA AACTC CTACG TGGAG GCAGC AGTAG GGAA TCTTC CACA ATGG ACGA TAAG TCTGA TGGA GCAA CGACC GCGTG AGTGA AGAA CGGT TTCG GCTCG TAAAA CTCTG TTGT TAAAG AAGA AGCA TATC TGAGA GTAAC TGTT CTGGT ATTG ACAG TATT TAAC CAGAA AGCC ACGG

Lactobacillus salivarius C27

GTGCC TAATA CATGC AAGT CGAAC GAAA CTTTC TTACA CCGAA TGCTT GCATT CACCG TAAGA AGTTG AGTGGC GGACG GGTGA GTAAC ACGTG GGTA ACCTG CCTAA AAGAA GGGGA TAACA CTTGG AAACAG GTGCTA ATACC GTATA TCTCT AAGGAT CGCAT GATCC TTAGAT GAAAGA TGGTTC TGCTA TCGCT TTTAG ATGGA CCCGC GGCG TATT AACTA GTTGG TGGGG TAACG GCCTA CCAAG GTGAT GATA CGTAG CCGA ACTGA GAGG TTGAT CGGC CACA TTGGG ACTG AGACA CGGC CCAA ACTC CTAC GGGAG GCAG CAGT AGGG AATC TTCC ACAA TGGAC GCAAG TCTGA TGGAG CAACG CCGCGTG AGTG AAGA AGGT CTTCG GATC GTAAA ACTC TGTTG TTAG AGAAG AACAC GAGTG AGAGT AACT GTTCA TTCGA TGAC GGTA TCTA ACCA GCAA GTCA CGG CTAA CTAC GTGC CAGC AGCC GCGG TAAT ACGT AGGT GGCA AGCG TTGT CCGG ATTT ATTG GGCG TAAA GGGA ACGC AGGC GGTC TTTT AAGT CTGA TGTGAA

Lactobacillus mucosae C28

TGTG CCTAA TACA TGCAA GTCG AACGC GTTGG CCCAA CTGA TTGAA CGTG CTTGC ACGGA CTTGA CGTTG GTTTA CCAG CGAG TGGCG GACG GGTG AGTA ACACG TAGG TAAC CTGC CCCAA AGCG GGGG ATAA CATT TGGA AACA GATG CTAA TACCG CATA ACAAT TTGAA TCGCA TGAT TCAA ATTTA AAAG ATGGT TTCGG CTATC ACTT TGGGA TGGAC CTGCG GCGC ATTA GCTT GTTG GTAGG GTAA CGGC CTAC CAAGG CTGTG ATGCG TAGCC GAGT TGAGA GACT GATCG GCCA CAATG GAACT GAGA CACG GTCC ATACT CCTA CGGG AGGC AGCA GTAGG GAAT CTTCC ACAAT GGGCG CAAGC CTGAT GGAG CAAC ACCGC GTGAG TGAAGA AGGGT TTCGGCT CGTAA AGCTC TGTTG TTAGA GAAGA ACGTGC GTGAGA GCAAC TGTTCA CGCAGT GACGG TATCTA ACCAG AAAGT CACGG CTAAC TACGT GCCA GCAG CCGC GGTA ATAC GTAG GTGG CAAG CGTT ATCC GGATT TATTG GGCGT AAAGCG AGCGC AGGCG GTTGG ATAAG TCTGAT GTGA AAGC

Lactobacillus mucosae C29

GTGT GCCT AATA CATG CAAG TCGAA CGCG TTGGC CCAAC TGAT TGAA CGTGC TTGCA CGGA CTTGA CGTT GGTTT ACCAG CGAGT GGCG GACGG GTGAG TAACA CGTAG GTAAC CTGCC CCAAA GCGGG GGAT AACAT TTGGA AACAG ATGC TAATA CCGC ATAA CAAT TTGAA TCGC ATGAT TCAA ATTT AAAA GATG GCTT CGGC TATC ACTT TGGG ATGG ACCT GCGG CGCA TTAG CTTG TTGG TAGG GTAA CGGC CTAC CAAG GCTG TGAT GCGT AGCC GAGT TGAG AGAC TGAT CGGC CACA ATGG AACT GAGA CACG GTCC ATAC TCCT ACGG GAGG CAGCAG TAGG GAAT CTTC CACAA TGGG CGC AAGC CTGA TGGA GCAA CACC GCGTG AGTG AAGA AGGGT TTCG

GCTC GTAA AGCTC TGTT GTTA GAGA AGAA CGTG CGTG AGAG CAACT GTTCA CGCAG TGACGG TATCT AACCA GAAA GTCAC GGCTA ACTAC GTGCC AGCAG CCGCG GTAAT ACGTA GGTGG CAAG CGTT ATCC GGAT TTAT TGGG CGTA AAGCG AGCGC AGGC GGTT TGAT AAGT CTGA TGTG

Lactobacillus ingluviei C31

GTGT GCCT AATA CATGC AAGT CGAAC GCGT TGGC CCAAT TGATT GATG GTGCTTG CACC TGATT GATT TTGG TCGC CAACGA GTGGC GGACG GGTGA GTAAC ACGTA GGTA ACCTG CCCAG AAGCG GGGG ACAAC ATTTG GAAA CAGA TGCTA ATAC CGCA TAACA ACG TTGT TCGCA TGAA CAAC GCTT AAAAG ATGG CTTCT CGCTA TCAC TTCT GGATG GACCT GCGG TGCAT TAGCT TGTT GGTGG GGTAA TGGCC TACCA AGGCG ATGAT GCAT AGCCG AGTT GAGAG ACTGA TCGGC CACA ATGGGA CTGA GACA CGGCC CATAC TCCT ACGG GAGGC AGCA GTAG GGAA TCTT CCAC AATG GGCG CAAG CCTG ATGG AGCA ACAC CGCG TGAG TGAA GAAG GGTT TCGG CTCGT AAAG CTCT GTTGT TAAAG AAGAA CACGT ATGAG AGTA ACTGT TCATA CGTTG ACGGT ATTTA ACCAG AAAG TCACG GCTAA CTACG TGCC AGCA GCCGC GGTAA TACGT AGGTG GCAA GCGT TATCCG GATT TATT GGGC GTAA AGAG AGTGC

Streptococcus infantarius C35

GTGC CTAA TACA TGCA AGTA GAACG CTGAA GACT TTAGCT TGCTA AAGTT GGAA GAGTT GCGAA CGGGT GAGT AACGC GTAG GTAAC CTGC CTACT AGCGG GGGA TAACT ATTG GAAA CGA TAGCT AATA CCGC ATAA CAGC ATTT AACA CATG TTAGA TGCT TGAAA GGAG CAATT GCTT CACTA GTAGA TGGA CCTGC GTTGT ATTA GCTA GTTG GTGAG GTAAC GGCT CACC AAGGC GACG ATAC ATAG CCGA CCTG AGAG GGTG ATCG GCCA CACTG GGAC TGAGAC A CGGC CCAG ACTC CTAC GGGAGGC AGCA GTAG GGAATCTT CGGC AATG GG GGCAA CCCT GACCGA GCAACG CCGCGT GAGTGA AGAAGGT TTTCGGA TCGTA AAGC TCTGT TGTA AGAG AAGA ACGT GTGT GAGA GTGG AAAGT TCAC ACAGT GACGG TAAC TTACC AGAA AGGG ACGGC TAAC TACGT GCCA GCAG CCGC GGTA ATAC GTANG TCCCC GAGC GTTGT CCGG ATTTA TTGGGC GTAAA GCGAG CGCAG GCGG TTTAA TAAG TNTGA AGTAA AAGG CAGTGG

Streptococcus infantarius C37

GCGG CGTG CACTA ATACAT GCAA GTAGA ACGCT GAAGA CTTTA GCTTGC TAAAG TTGGAAG AGTT GCGAA CGGG TGAG TAACG CGTAG GTAAC CTGCC TACTA GCGGG GGATA ACTAT TGGA AACG ATAGCT AATACC GCAT AACAG CATT TAACA CATGT TAGA TGCTT GAAAG GAGCA ATTGC TTCAC TGAG ACACG GCCC AGACTCC TACG GGAG GCAG CAGTA GGGAA TCTTC GGCTT TGGGG GCAAC CCTGA CCGAGC AACGC CGCGTG AGTGA AGAA GGTT TTCG GATC GTAA A G C T C C TGTTG TAAGA GAAGA ACGT GTGT GAGA GTGG AAAG TTCAC ACAG CGATA GCTAAT ACCG CATAA CAGCA TTTAA CACA TGTTAG ATGCT TGAAA GGAGC AATTG CTTC ACTAG TAGATG TAGTA GATG GACCT GCGTT GTATT AGCTA GTTG GTGA GGTA ACGG CTCA CCAAG GCGA CGATA CATA GCCGA CCTG AGAG GGTG ATCG GCCA CACTG GGAC TGAG ACACG GCCC AGACTCC TACG GGAG GCAG CAGTA GGGAA TCTTC GGCTT TGGGG GCAAC CCTGA CCGAGC AACGC CGCGTG AGTGA AGAA GGTT TTCG GATC GTAAA GCTC C TGTTG TAAGA GAAGA ACGT GTGT GAGA GTGG AAAG TTCAC ACAG

Streptococcus infantarius C38

GCGG CGTGCC TAATA CATG CAAGT AGAAC GCTGA AGACT TCCA GTCTTG CTAAA GTTGGA AGAG TTGC GAAC GGGT GAGT AACGC GTAG GTAA CCTG CCTA CTAG CGGG GGAT AACT ATTG GAAA CGATA GCTAA TACC GCAT AACA GCAT TTAA CTCA TGTT AGAT GCTT GAAA GGAG CAAT TGCT TCAC TAGT AGATG GACCT GCGTT GTATT AGCTAG TTGGT GAGGT AACGG CTCAC CAAGG CGACG ATACA TAGC CGACC TGAG AGGGT GATCG GCCA CACTGG GACTG AGACA CGGCC CAGA CTCCT ACG GGAGGC AGCAG TAGGG AATCT TCGGC AATG GTGG GCAAC CCTGA CCGA GCAAC CGCCG CGTGA GTGAA GAAGG TTTTC GGATC GTAAA GCTCC TGTTG TAAGAG AAGAA CGTGT GTGAG AGTGGAA TGAG ACACG GCCC AGACTCC TACG GGAG GCAG CAGTA GGGAA TCTTC GGCTT TGGGG GCAAC CCTGA CCGAGC AACGC CGCGTG AGTGA AGAA GGTT TTCG GATC GTAA A G C T C C TGTTG TAAGA GAAGA ACGT GTGT GAGA GTGG AAAG TTCAC ACAG CGATA GCTAAT ACCG CATAA CAGCA TTTAA CACA TGTTAG ATGCT TGAAA GGAGC AATTG CTTC ACTAG TAGATG

Lactobacillus mucosae C39

GCGG TGTG CCTA ATAC ATGC AAGT CGAA CGCT TTGG CCCA ACTG ATTG AACG TGCT TGCA CGGA CTTG ACGT TGGT TTAC CAGC GAGT GGCG GACG GGTG AGTA ACAC GTAGG TAACC TGCCC CAAAG CGGG GG AT AACA TTTG GAAA CAGA TGCT AATA CCGC ATAA CAAT TTGA ATCG CATG A TTCA AATT TAAA AGAT GGCTT CGGCT ATCAC TTTG GGAT GGAC CTGC GGCG CATT AGCT TGTT GGTAG GGTAA CGGC CTAC CAAG GCTG TGATG CGTAG CCGA GTTGA GAGAC TGAT CGGC CACAA TGGAA CTGA GACAC GGTCC ATACT CCTA CGGG AGGC AGCA GTAG GGAA TCTT CCAC AATG GGCG CAAG CCTG ATGG AGCA ACAC CGCG TGAG TGAA GAAG GGTT TCGG CTCGT AAAG CTCTG TTGTT AGAG AAGAA CGTG CGTG AGAG CAAC TGTT CACG CAGT GACG GTATC TAAC CAGAA AGTCA CGGCT AACT ACGTG CCAGC AGCC GCGGT AATAC GTAG GTGGCA AGCG TTAT CCGGA TTTA TTGG GCGT AAAG CGAG CGCAG GCGGT TTTGA TAAGT CTGAT GGTGA AAGCC

Streptococcus equinus C40

GACG ATCG CCGG CGGC GTGC CTAA TACA TGCA AGTA GAACG CTGAA GACTT TAGCT TGCT AAAG TTGG AAGAG TTGC GAAC GGG TGAG TAAC GCGT AGGT AACC TGCC TACTA GCGG GGGA TAAC TATT GGAA ACGA TAGC TAATA CCGCA TAACA GCATT TAAC ACATG TTAGA TGCTT GAAA GGAGC AATTG CTTCA CTAGT AGAT GGAC CTGC GTTGT ATTA GCTA GTTGG TGAG GTAA CGGCT CACC AAGGC GACG ATACA TAGCC GACC TGAG AGGG TGAT CGGC CACA CTGG GACT GAGA CACG GCCC AGAC TCCT ACGGG AGGC AGCA GTAG GGAA TCTT CGGC AATG GGGG CAAC CCTG ACCG AGCAA CGCC GCGT GAGTG AAGA AGGTT TTCGG ATCGT AAAGC TCTGT TGTAA GAGAA GAAC GTGTG TGAGA GTGGA AAGTT CACAC AGTGA CGGT AACTT ACCAG AAAG GGACG GCTA ACTACG TGCCA GCAGC CGCGG TAAT ACGT AGGT CCCG AGCG TTGT CCGG ATTTA TTGGG CGTA AAGCG AGCG CAGG CGGT TTAA TAAG TCTG AAGT TAAAG GCAG TGGC TTAAC CATTG TTCGC TTTGGA AACTG TTAG ACTT GAGTG CAGA AGGG GAGAG TGGAA TTCCA TGTGT AGCG GTGA AATG

Streptococcus infantarius C41

TGCC TAAT ACAT GCAA GTAGA ACGCT GAAGA CTTTA GNCTT GCTAA AGTTG GAAGA GTTGC GAACG GGTGAG TAACG CGTA GGTA ACCT GCCT ACTAG CGGGG GATA ACTAT TGGAA ACGAT AGCTA ATACC GCAT AACA GCATT TAACA CATGT TAGAT GCTT GAAA GGAG CAATT GCTT CACTA GTAGA TGGAC CTGCG TTGTA TTAGC TAGT TGGT GAGG TAACG GCTCA CCAAG GCGAC CCAAG GCGA CGATA CATA GCCGA CCTG AGAG GGTG ATCG GCCA CACTG GGAC TGAG ACACG GCCC AGACTCC TACG GGAG GCAG CAGTA GGGAA TCTTC GGCTT TGGGG GCAAC CCTGA CCGAGC AACGC CGCGTG AGTGA AGAA GGTT TTCG GATC GTAA A G C T C C TGTTG TAAGA GAAGA ACGT GTGT GAGA GTGG AAAG TTCAC ACAG

Streptococcus infantarius C50

TGCT AAAG TTAG GAAG AGTT GCGA ACGG GTGA GTAA CGCG TAGG TAAC CTGC CTAC TAGC GGGG GATA ACTA TTGG AAAC GATA GCTAA TACC GCAT AACA GCATT TAACT CATG TTAG ATGC TTGA AAGG AGCA ATTG CTTC ACTA GTAG ATGG ACCT GCGT TGTA TTAG CTAG TTGG TGAG GTAAC GGCT CACCA AGGC GACG ATAC ATAG CCGA CCTG AGAG GGTG ATCGG CCACA CTGG GACT GAGA CACGG CCCAG ACTC CTAC GGGA GGCA GCAG TAGG GAAT CTTC GGCA ATGG GGGC AACC CTGAC CGAG CAAC GCCG CGTG AGTG AAGA AGGT TTTC GGAT CGTA AAGCT CTGTT GTAAG AGAA GAAC GTGTG TGAG AGTG GAAA GTTC ACAC AGTG ACGG TAAC TTAC CAGA AAGG GACG GCTA ACTA CGTG CCAG CAGCC GCGG TAAT ACGT AGGT CCCG AGCG TTGT CCGG ATTT ATTGG GCGTA AAGCGA GCGC AGGC GGTT TAATA AGTC CTGA AGTT AAAGG CAGT GGCT TAAC CATT GTTCG CCTT TGGA AACT G TTAGA

Streptococcus infantarius C51

GTGC CTAA TACA TGCA AGTA GAAC GCTG AAGA CTTT AGCT TGCT AAAG TTGG AAGA GTTG CGAA CGGG TGAGT AACG CGTA GGTAA CCTGC C TACTA GCGGG GGATA ACTA TTGGA AACGA TAGCT AATACC GCAT AACAG CATT TAACCC ATGTT AGATG CTTGA AAGGA GCAATT GCTTC ACTAGT AGAT GGACC TGCGT TGTA TTAGC TAGT TGGTG AGGTA ACGG CTCA CCAAG GCGAC GATA CATAG CCGAC CTGAG AGGGT GATCG GCCAC ACTG GGACT GAGA CACGG CCCAG ACTCCT ACGG GAGGC AGCAG TAGGG AATCT TCGGC AATGG G G GCAA CCCT GACC GAGC AACG CCGC GTGA GTGA AGAA GGT TTTC GGAT CGTA AAGCT CTGTT GTAA GAGA AGAAC GTGT GTGA GAGT GGAA AGTTC ACACA GTGAC GGTAA CTTAC CAGAA AGGGA CGGC TAACT ACGT GCCAG CAGC CGCGG TAATA CGTAG GTCC CGAGC GTTGT CCGGAT TTATT GGGC GTAA AGCG AGCG CAGGC GGTT TAATA AGTCT GAAG TTAAA GGCAG TGGCT TAAC CATTG TTCGC TTTGG AAACT GTTA GACT TGAG TGCA

Streptococcus infantarius C53

CGAT CGCC GGCG GCGTG CCTA ATACA TGCAA GTAGA ACGCT GAAGA CTTTA GCTTG CTAA AGTT GGAA GAGT TGCG AACGG GTGA GTAAC GCGTA GGTAA CCTGC CTACT AGCGG GGGA TAACT ATTG GAAA CGAT AGCTA ATACC GCATA ACAGC ATTTA ACCC ATGTT AGATG CTTGAA AGGAG CAAT TGCTT CACTA GTAGA TGGAC CTGCG TTGTA TTAG CTAG TTGGT GAGG TAACG GCTC ACCAA GGCGA CGATA CATA GCCG ACCTG AGAGG GTGA TCGG CCACA CTGG GACTG

AGACA CGGC CCAGA CTCCT ACGGG AGGCA GCAGT AGGGA ATCTT CGGC AATGG GGGCA ACCCT GACC GAGCA ACGCC GCGT GAGTG AAGAA GGTT TTCGG ATCGT AAAG CTCT GTTG TAAG AGAA GAAC GTGT GTGA GAGT GGAA AGTT CACA CAGT GACG GTAA CTTA CCAG AAAGG GACG GCTA ACTAC GTGC CAGCA GCCGC GGTA ATAC GTAG GTCCC GAGC GTTGT CCGG ATTTA TTGG GCGTA AAGC GAGCG CAG GCGGT TTAAT AAGTC TGAA GTTA AAGG CAGT GGCT TAAC CATT GTTC GCTT TGGA AACTG TTAG ACTT GAGT GCAGA AGGGG AGAGT GGAAT TCCA TGTGT AGCG GTGA AATGCG

Streptococcus infantarius C54

GTGC CTAA TACA TGCA AGTA GAAC GCTG AAGAC TTTA GCTT GCTA AAGT TGGAA GAGTT GCGAA CGGGT GAGT AACG CGTA GGTA ACCT GCCTA CTAGCG GGGG ATAA CTATT GGAA ACGAT AGCT AATAC CGCA TAAC AGCA TTTA ACAC ATGT TAGA TGCT TGAA AGGA GCAA TTGCT TCAC TAGTA GATG GACC TGCG TTGT ATT AGCT AGTT GGTG AGGT AACG GCTCA CCAA GGCG ACGAT ACAT AGCCG ACCT GAGA GGGT GATC GGCC ACACT GGGA CTGAG ACACG GCCC AGAC TCCTA CGGGA GGCAG CAGTA GGGAA TCTT CGGCA ATGGG GGCAA CCCT GACC GAGCA ACGCC GCGTG AGTG AAGA AGGT TTTC GGAT CGTA AAGC TCTG TTGT AAGA GAAG AACG TGTG TGAG AGTG GAAA GTTC ACAC AGTG ACGG TAAC TTAC CAGA AAGG GACG GCTA ACTA CGTG CCAG CAGC CGCG GTAA TACG TAGG TCCC GAGC GTTG TCCG GA TT TATT GGGC GTAA AGCG AGCGC AGGCG GTTT AATAA GTCT GAAGT TAA AGGC AGTGG CTTA ACCAT TGTTC GCTT TGG AAAC T GTTA GACT TGAG TGCA GAAGGG GAGA GTGG AATT CCATG TGTA GCGGTGA

Streptococcus infantarius C55

GTGC CTAA TACA TGCA AGTA GAAC GCTG AAGA CTTT AGCT TGCT AAAG TTGG AAGA GTTG CGAAC GGGT GAGT AACG CGTA GGTAA CCTGC CTAC TAGC GGGGG ATAAC TATTG GAAAC GATAG CTAAT ACCG CATAA CAGCA TTTAA CACA TGTT AGAT GCTTG AAAG GAGC AATT GCTT CACT AGTA GATGG ACCT GCGT TGTA TTAGC TAGT TGGT GAGG TAAC GGCT CACCA AGGC GACGA TACAT AGCCG ACCTG AGAGG GTGA TCGGC CACA CTGG GACT GAGA CACG GCCC AGAC TCCT ACGG GAGG CAGC AGTAG GGAAT CTTC GGCA ATGG GGGC AACC CTGA CCGA GCAA CGCC GCGT GAGT GAAGA AGGT TTTC GGAT CGTA AAGC TCTGT TGTA AGAGA AGAA CGT GTGT GAGAG TGGA AAGTT CACACA GTGAC GGTAA CTTAC CAGAAA GGGA CGGCT AACT ACGT GCCA GCAGC CGCG GTAAT ACG TAGG TCCCG AGCGT TGTCC GGATT TATTG GGCGT AAAG CGA GCGCAG GCGGT TTAA TAAGT CTGAA GTTAA AGGCA GTGG CTTAA CCATG GTTCG CTTTGG AAACT GTTAG ACTTGA GTGCAG AAGG GGAG AGTG

Streptococcus infantarius C56

GTGC CTAAT ACATG CAAGT AGAA CGCTG AAGA CTTTA GCTTG CTAAA GTTGG AAGAG TTGCG AACGG GTGA GTAAC GCGTA GGTAA CCTGCC TACT AGCGGG GGATA ACTATT GGAAA CGATA GCTAAT ACCG CATAA CAGCA TTTAA CACA TGTTAG ATGCT TGAAA GGAGC AATTG CTTC ACTAG TAGATG GACCT GCGTTG TATT AGCTA GTTG GTGAG GTAAC GGCT CACCAA GGCGAC GATACA TAGCCG ACCTG AGAGG GTGATC GGCCA CACTG GGAC TGAGA CACGG CCCAG ACTCCTA CGGG AGGCA GCAGT AGGGAA TCTTC GGCAAT GGGGGC AACCC TGACC GAGCA ACGCCG CGTGA GTGAA GAAGG TTTTCG GATCGT AAAG CTCTG TTGTA AGAGA AGAAC GTGTGT

GAGAG TGGAA AGTTC ACACA GTGAC GGTA ACTTAC CAGAA AGGG ACGGC TAACT ACGT GCCA GCAG CCGCG GTAAT ACGT AGGTC CCGAG CGTT GTCCG GATTT ATTG GGCGT AAAGC GAGCG CAGG CGGT TTAA TAAGT CTGA AGTT AAAG GCAG TGGC TTAAC CATT GTTCG CTTTG GAAAC TGTT AGACT TGAG TGCAG AAGG GGAG AGTGG AATCC ATGT GTAG CGGT GAAATGC

Streptococcus infantarius C57

GTGC CTAA TACA TGCA AGTA GAAC GCTG AAGAC TTTAG CTTG CTAAA GTTGG AAGA GTTG CGAAC GGGT GAGTA ACGC GTAG GTAAC CTGCC TACTA GCGGG GGATA ACTA TTGGA AACGA TAGCT AATA CCGCA TAAC AGCA TTTAA CACAT GTTAGAT GCTT GAAAG GAGCAA TTGCT TCACT AGTAG ATGGA CCTG CGTTG TATT AGCT AGTT GGTG AGGTA ACGGC TCAC CAAG GCGA CGAT ACAT AGCCG ACCT GAGAG GGTGA TCGGC CACAC TGGG ACTG AGACA CGGC CCAGA CTCCT ACGGG AGGCA GCAG TAGGG AATCTT CGGC AATGG GGGC AACCC TGACC GAGCA ACGC CGCG TGAGT GAAG AAGG TTTT CGGAT CGTA AAGCT CTGTT GTAAG AGAA GAACG TGTGT GAGAG TGGAA AGTT CACAC AGTGA CGGT AACTT ACCA GAAAG GGAC GGCTA ACTA CGTG CCAGC AGCC GCGGT AATA CGTAGG TCCCG AGCGT TGTCC GGATT TATTG GGCGT AAAG CGAG CGCA GGCG GTTTA ATAAG TCTGAA GTTAAA GGCAG TGGCT TAAC CATT GTTC GCTTT GGAAA CTGT TAGAC TTGAG TGCA GAAG GGGA GAGTG GAATT CCAT GTGTA GCGGT GAAATG CGTA GATAT ATGGA GGAA CACCGG

Streptococcus infantarius C58

GTGC CTAAT ACATG CAAG TAGAA CGCTG AAGAC TTTAG NCTTGC TAAAG TTGG AAGA GTTG CGAAC GGGTG AGTA ACGCG TAGGT AACC TGCCTA CTAGCG GGGGAT AACT ATTGG AAACG ATAG CTAATA CCGCA TAAC AGCAT TTAACC CATGTT AGATG CTTGA AAGGA GCAATT GCTTCAC TAGTAG ATGGA CCTGC GTTG TATTA GCTAG TTGGT GAGGT AACGG CTCAC CAAGGCG ACGATA CATAG CCGAC CTGAGAG GGTGATC GGCCA CACTGG GACTG AGACAC GGCCC AGACT CCTACG GGAG GCAGC AGTAG GGAAT CTTCG GCAATGGG GGCAA CCCT GACCG AGCA ACGCC GCGTGAG TGAAG AAGGT TTTCG GATCG TAAAG CTCTG TTGTAA GAGAA GAAC GTGTGT GAGAG TGGAA AGTT CACAC AGTGAC GGTAA CTTAC CAGAAAG GGACG GCTA ACTA CGTGCC AGCAG CCGCGGT AATACG TAGGT CCCG AGCGT TGTCCG GATTTA TTGGGC GTAA AGCGA GCGCAG GCGG TTTAAT AAGTC TGAA GTTAA AGGCA GTGGC TTAACC ATTGT TCGCTT TGGAA ACTGT TAGA CTTGAG TGCAG AAGGG GAGAG TGGAAT CCCATG TGTAGC GGTGA AATGCG

Lactobacillus agilis C59

CGTG CCTA ATACAT GCAAG TCGAA CGCTT TTTTC AATCA TCGTA GCTTGC TACAC CGAT TGAAAA TTGAG TGGCG AACG GGTGA GTAAC ACGTG GGTA ACCTG CCCAAA AGAGG GGGAT AACAC TTGGAA ACAGG TGCTA ATAC CGCATA ACCAT GATGA CCGCAT GGTCAT TATGT AAAAG ATGGT TTCGG CTATC ACTTT TGGAT GGAC CCGC GGCGT ATTAA CTTGT TGGTG GGGTA ACGGC CTACCA AGGTA ATGATA CGTAG CCGAA CTGAG AGGTT GATCG GCCAC ATTGGG ACTGA GACAC GGCCC AAACT CCTA CGGGA GGCAG CAGTA GGGA ATCTTC CACAA TGGGC GCAAG CCTGA TGGAG CAACG CCGCG TGAGT GAAGA AGGTC TTCGG ATCGT AAAAC TCTGT TGTTA GAGAA GAACA TGCGA GAGAG TAACT GTTCT TGTAT TGACG GTATCT AACCA GAAA GCCAC GGCT

AACTA CGTGC CAGCA GCCGC GGTAA TACGT AGGT GGCAA GCGTT GTCC GGATT TATTGG GCGTA AAGG GAACG CAGGC GGTCC TTTAA GTCTG ATGTG AAAG CCTTC

Lactobacillus amylovorus C60

GTGC CTAAT ACAT GCAAG TCGAG CGAGC GGAAC CAACA GATTTA CTTCGG TAATGA CGTTGGG AAAGCG AGCGGC GGATG GGTGA GTAA CACG TGGG GAAC CTGC CCCT AAGT CTGG GATA CCAT TTGG AAACA G GTGC TAAT ACCG GATAA TAAA GCAG ATCG CATG A TCAG CTTT TGAA AGGC GGCG TAAG CTGT CGCT AAGG GATG GCCC CGCG GTGCAT TAGC TAGTT GGTAA GGTAAC GGCTTA CCAAGGCG ACGATG CATAG CCGA GT TGAGA GACTGAT CGGCCAC ATTGGGA CTGAGAC ACGGC CCAAA CTCCT ACGGG AGGC AGCA GTAG GGAA TC TTCCACAATGG ACGCAA GTCTG ATGGA GCAAC GCCGC GTGAG TGAAG AAGG TTTT CGGAT CGTA AAGC TCTG TTGT TGGT GAAG A AGGA TAGA GGTA GTAA CTGG CCTT TATT TGAC GGTA ATCA ACCA GAAA GTCA CGGCTAA CTACGT GCCAG CAGC CGCG GTAATA CGTAG GTGGC AAGCGT TGTCCG GATTT ATTGG GCGTA AAGCG AGCGC AGGCG GAAA AATAA GTCTA ATGTG AAAGC CCTC GGCTT AACC GAGG AACT GCAT CGGA AACT GTTT TTCT TGAG TGCA GAAG AGGA GAGT GGAA CTCC ATGT GTAT CGGT GGAA TGCG

Lactobacillus mucosae C61

TGTG CCTA ATAC ATGC AAGT CGAA CGCG TTGG CCCA ACTGA TTGAA CGTGC TTGCA CGGACT TGACGT TGGTT TACCA GCGAGT GGCGG ACGGG TGAG TAACA CGTAG GTAAC CTGCC CCAAA GCGGG GGATAA CATTT GGAAA CAGAT GCTAA TACCGCA TAACAA TTTGA ATCGC ATGAT TCAAA TTTA AAAGA TGGCT TCGGC TATC ACTT TGGGA TGGACC TGCGGC GCATT AGCTTG TTGGT AGGGT AACGGC CTACCA AGGC TGTGA TGCGT AGCC GAGTT GAGAG ACTGA TCGG CCACA ATGGA ACTG AGAC ACGG TCCAT ACTC CTAC GGGAG GCAGC AGTA GGGAAT CTTCC ACAAT GGGCGC AAGCC TGAT GGAG CAACA CCGC GTGAG TGAA GAAGG GTTT CGGC TCGT AAAG CTCTG TTGT TAGA GAAG AACG TGCG TGAG AGCA ACTGT TCACG CAGTG ACGGT ATCTA ACCA GAAAG TC AC GGCTAA CTACG TGCC AGCAG CCGCG GTAAT ACGTAG GTGGC AAGCG TNATC CGGATT TATTG GGCGT AAAG CGAGC GCAG GCGG TTTGA TAAGT CTGA TGTG AAAGC

Streptococcus infantarius C62

GTGCC TAATA CATGC AAGTA GAACG CTGAAG ACTTT AGCTTG CTAAA GTTGGA AGAGT TGCGAA CGGGT GAGTAA CGCGT AGGTA ACCTGC CTACT AGCGG GGGAT AACT ATTGG AAAC GATA GCTAA TAC CGCAT AACA GCATT TAAC ACATG TTAGA TGCT TGAAA GGAGC AATT GCTT CACTA GTAG ATGG ACCTG CGTTG TATTA GCTA GTTGG TGAGG TAACG GCTC ACCA AGGCG ACGA TACA TAGCC GACCT GAGAG GGTGA TCGGC CACAC TGGGA CTGAG ACACG GCCCA GACT CCTAC GGGAG GCAGC AGTAG GGAAT CTTC GGCAA TGGG GGCAA CCCT GACC GAGC AACG CCGCG TGA GTGAAG AAGGT TTTC GGATCG TAAAG CTCTG TTGTA AGAGA AGAAC GTGTG TGAGA GTGGA AAGTT CACAC AGTGA CGGTA ACTTA CCAGA AAGGG ACGGC TAACT ACGT GCCAG CAGCC GCGGT AATAC GTAGG TCCC GAGCG TTGTC CGGAT TTATT GGGCG TAAAG CGAG CGC AGGCG GTTT AATAAG TCTG AAGTT AAAGG CAGTG GCTTA ACCAT TGTTC GCTTTG GAAA CTGTT AGAC TTGAG TGCA GAAG GGGA GAGT GGAAT TCCAT GTGTA GCGGT GAAAT GCGTA AATA TATGG AGGA

Streptococcus infantarius C63

GTGC CTAAT ACATG CAAGT AGAAC GCTGA AGAC TTTA GACTT GCTAA AGTTG GAAGA GTTGC GAACG GGTGA GTAAC GCGTA GGTA ACCT GCCT ACTA GCGG GGGA TAACT ATTG GAAAC GATA GCTAA TACCGC ATAA CAGC ATTTA ACACA TGTTA GATGC TTGAA AGGAG CAATT GCTTC ACTAGT AGAT GGACC TGCGT TGTAT TAGC TAGTTG GTGA GGTA ACGG CTCAC CAAGG CGACG ATACA TAGCC GACCTG AGAGG GTGAT CGGCC ACACT GGGAC TGA GACA CGGCC CAGAC TCCTA CGGGA GGCAG CAGTA GGGAA TCTTC GGCAA TGGGG GCAA CCCTG ACCGA GCAAC GCCGC GTGA GTGAA GAAGG TTTTCG GATCG TAAAG CTCTG TTGTAA GAGAAG AACGTG TGTGAG AGTGGA AAGTTC ACACA GTGAC GGTAA CTTAC CAGAA AGGGA CGGCT AACTA CGTGC CAGCA GCCGC GGTAA TACGT AGGTC CCGA GCGT TGTC CGGAT TTAT TGGG CGTAA AGCGA GCGCA GGCGG TTTAA TAAGT CTGAG GTTAA AGGCA GTGG CTTAAC ATTGTT CGCTT TGGAA ACTGTT AGACT TGAGTG CAGAAGG GGAGAG TGGAAT CCCATG TGTAN CGGGT GAAA TGCGT AAATA TATGGA

Lactobacillus gasseri C64

CGTG CCTA ATACA TGCAA GTCG AGCGA GCTT GCCT AGAT GATT TTAG TGCT TGCA CTAA ATGA AACTA GATA CAAG CGAGC GGCG GACGG GTGA GT AACACG TGGGT AACCT GCCCAA GAGACT GGGAT AACACC TGGAAA CAGATG CTAATA CCGGAT AACAA CACTA GACGC ATGTC TAGAG TTTGAA AGATGG TTCTGC TATCAC TCTTGGA TGGACCT GCGGTGC ATTAGCT AGTTGG TAAGGT AACGG CTTA CCAA GGCA ATGAT GCATA GCCG AGTTG AGAGA CTGAT CGGCC ACATT GGGACT GAGA CACGG CCCAA ACTCC TACGG GAGGC AGCA GTAGG GAATC TTCCA CAATG GACGA AAGTC TGA TGGAG CAAC GCCG CGTGA GTGAA GAAGG GTTTC GGCTC GTAAA GCTCT GTTG GTAGT GAAGA AAGAT AGAGG TAGT AACT GGCC TTTA TTTG ACGG TAAT TACT TAGA AAGT CACG GCTA ACTA CGTG CCAG CAGC CGCG GTAA TACG TAGG TGGC A AG CGTTG TCCGG ATTAA TTGGGC GTAAAG CGA GTGCA GGCGG TTCA ATAAGT CTGAT GTGAA AGCCT TCGGC TCAAC CGGAG AATTG CATC

Lactobacillus animalis C65

CGTG CCTA ATAC ATGCA AGTCG AACGA AACTT CTTCA TCAC CGAGT GCTTG CACTCA CCGAT GAAGAG TTGAG TGGCG AACGG GTGAG TAACA CGTGG GCAAC CTGCC CGAAA GAGGG GGATA ACACT TGGAA ACAG GTGCTA ATAC CGCATA ACCAT GAACA CCGCA TGGTG TTTATG TGAAA GGTGGT TTCG GCTA CCGCT TTCGG ATGG GCCC GCGGC GCATT AGCT AGTT GGTGG GGTA AAGG CTT ACCAA GGCAA TGATG CGTAG CCGAAC TGAGAG GTTGA TCGGC CACAT ATGGG CGAAA GCCTG ATGGAG CAACG CCGCG TGGGT GAAGAA GGTCT TCGGAT CGTAA AACCC TGTTG TCAGA GAAGAA AGTGC ATGAG AGTAA CTGTT CATGT TTCGAC GGTAT CTGACC AGAAA GCCAC GGCTA ACTAC GTGCC AGCAG CCGCGG TAATA CGTAG GTGGC AAGCG TTATC CGGATT TATTG GGCGT AAAGG GAACG CAGGC GGTCT TTTAA GTCTG ATGTG AAAGC CTTCG GCTTA ACCGG AGTAT TGCAT TGGA AACTG GTGC CTAA TACATGC AAGT CGAGC GAGC GGAA CCAA CAGA TTTACT TCGGTA ATGA CGTT GGGA AAGC GAGC GGCGG ATGGG TGAG TAACA CGTG GGGA ACCT GCCTC TAAGTC TGGG ATAC CATT TGGA AACAG GTGC TAATA CCGGAT AATAAAG CAGA TCGC ATGAT CAGCTT

Lactobacillus mucosae C67

TGTG CCTA ATAC ATGC AAGTC GAAC GCGT TGGCC CAACT GATT GAACG TGCT TGCAC GGACT TGAC GTTG GTTTA CCAGC GAGT GGCG GACGG GTGA GTAA CACG TAGG TAACC TGCC CCAA AGCGG GGGA TAACA TTTGG AAAC AGATG CTAAT ACCGC ATAAC AATTT GAAT CGCAT GATTC AAATT TAAAA GATG GTTT CGGCT ATCAC TTTGG GATGG ACCTG CGGCG CATTA GCTTG TTGGTA GGGTAACGGC CTACC AAGG CTGTG ATGCG TAGCC GAGTT GAGAG ACTGA TCGGCCACAA TGGAA CTGAG ACAC GGTCC ATACT CCTAC GGGAG GCAGC AGTAG GGAAT CTTCC ACAAT GGGCG CAAGC CTGAT GGAGC AACAC CGCGT GAGTG AAGAA GGGTT TCGGC TCGTA AAGCT CTGTT GTTA GAGAA GAAC GTGCG TGAGA GCAAC TGTTC ACGCA GTGACG GTATC TAACC AGAAA GTCAC GGCTA ACTAC GTGCC AGCAG CCGCG GTAAT ACGT AGGTG GCAAG CGTTAT CCGGA TTTAT TGGGC GTAAA GCGAG CGCAG GCGGT TTGATA AGTCT GATGT GAAAG CCTTT GGCTT AACCA AAGAAG TGCAT CGGAA ACTGTC AGACT GGAG TGCA GAGG AGGA CAGT GGAAC TCCA TGTG

Streptococcus infantarius C68

GTGC CTAA TACAT GCAAGT AGAAC GCTGA AGACT TTAGC TTGCT AAAGT TGGAA GAGTT GCGAA CGGGT GAGTA ACGCG TAGGT AACCT GCCTAC TAGCGG GGGAT AACTA TTGGAA ACGAT AGCTA ATACC GCATA ACAGCA TTTAAC CCATGT TAGATG CTTGAA AGGAGCA ATTGCTT CACTA GTAG ATGG ACCT GCGT TGTA TTAG CTAGTT GGTGA GGTAAC GGCTCA CCAA GGCGA CGATA CATAG CCGAC CTGAG AGGG TGAT CGGC CACAC TGGGAC TGAGAC ACGGCC CAGAC TCCTAC GGGAGG CAGCA GTAGGG AATCTT CGGCAA TGGG GG CAAC CCTGAC CGAGC AACGCC GCGT GAGT GAAGA AGGTT TTCGGA TCGTAA AGCTCT GTTG TAAGA GA AGAAC GTGTG TGAGAG TGGAAA GTTCAC ACAGT GACGG TAACT TACC AGAAA GGGA CGGC TAAC TACGT GCCA GCAGC CGCG GTAAT ACGTA GGTC CCGAGC GTTGT CCGGA TTTA TTGGG CGTA AAGC GA GCG CAGGC GGTT TAATA AGTCT GAAGT TAAAG GCAGT GGCTT AACCA TGGTTC GCTTT GGAAA CTGTT A GACT TGAGT GCAG AAGGG GAGAG TGGAA TCCA TGTGT AGCGG TGAAA TGCGT.

Streptococcus infantarius C69

GTGC CTAA TACAT GCAAG TAGAA CGCTG AAGAC TTTAG CTTGCT AAAGT TGGAA GAGTT GCGA ACGGG TGAGT AACGC GTAG GTAA CCTGC CTACT AGCGG GGGA TAAC TATTG GAAA CGATA GCTA ATACC GCATA ACAG CATT TAACC CATGT TAGAT GCTTG AAAGG AGCAA TTGCT TCACT AGTA GATGG ACCT GCGTT GTATT AGCT AGTT GGTG AGGTA ACGGC TCAC CAAGG CGAC GATAC ATAGC CGAC CTGA GAGGG TGAT CGGC CACA CTGGG ACTGA GACAC GGCCC AGAC TCCTA CGGGA GGCAG CAGTAG GGAA TCTTC GGCAA TGGGG GCAA CCCTG ACCGA GCAAC GCCGC GTGAG TGAAG AAGGT TTTCG GATCG TAAA GCTCT GTTGT AAGAG A AGAA CGTG TGTG AGAG TGGA AAGT TCAC ACAG TGAC GGTA ACTT ACCA GAAA GGGA CGGC TAAC TACGT GCCAG CAGCC GCGGT AATAC GTAGG TCCCG AGCGT TGTC CGGAT TTATT GGGCG TAAAG CGAGC GCAGGCG GTTTA ATAA GTCTGA AGTTA AAGGCA GTGGC TTAAC CATNG TTCG CTTT GGAA ACTG TTAG ACTT GAGT GCAG AAGG GGAG AGTG GAATC CATGT GTACC GGTGAA ATGC GTAGA

Streptococcus infantarius C70

GTGC CTAAT ACATG CAAGT AGAA CGCTG AAGA CTTTA GCTTG CTAA AGTTGG AAGA GTTGC GAACG GGTGA GTAAC GCGTA GGTAA CCTGC CTACT AGCGG GGGAT AACTA TTGG AAACGAT AGCTA ATACC GCATA ACAGC ATTTA ACACA TGTTAG ATGCT TGAAAG GAGC AATTG CTTCA CTAGT AGATGG ACCTGC GTTGTA TTAGCT AGTTG GTGAG GTAACG GCTCAC CAAGGC GACGA TACAT AGCCG ACCTG AGAG GGTG ATCG GCCA CACT GGGA CTGA GACAC GGCCC AGAC TCCTA CGGGA GGCA GCAGT AGGG AATC TTCGG CAATG GGGGC AACC CTGAC CGAGC AACGC CGCG TGAGT GAAG AAGGTT TTCGG ATCGTA AAGCTC TGTTG TAAGA GAAGA ACGTGT GTGAG AGTGG AAAGTT CACAC AGTGAC GGTAA CTTAC CAGA AAGG GACG GCTA ACTA CGTG CCAG CAGCC GCGGT AATA CGTA GGTCC CGAG CGTT GTCC GGAT TTAT TGGG CGTA AAGCG AGCGC AGGCGG TTTAAT AAGTCT GAAGTT AAAGG CAGT GGCT TAACC ATTGTT CGCTT TGGA AACT GTTA GACT TGAG TGCA GAAG GGGA GAGT GGAA TCCATGTG TAGC CGTGAAAT GCG

Streptococcus infantarius C71

TTGCTAA AGTTAG GAAGAG TTGCG AACGG GTGAG TAACGC GTAGGT AACCTG CCTAC TAGCG GGGGA TAAC TATT GGAAAC GATAGC TAATAC CGCAT AACA GCATTT AACAC ATGTT AGATG CTTGAA AGGAG CAATT GCTTCA CTAGT AGATGG ACCTG CGTTG TATTAG CTAGT TGGTGA GGTAA CGGCT CACCA AGGCG ACGA TACAT AGCC GACCT GAGAG GGTGA TCGGC CACAC TGGGA CTGAG ACACG GCCCA GACT CCTAC GGGA GGCAG CCAGT AGGGA ATCTT CGGCA ATGGG GGCAA CCCTG ACCGA GCAAC GCCGCG TGAGT GAAGA AGGTT TTCGG ATCGT AAAGCT CTGTT GTAAG AGAAGA ACGTGT GTGAGA GTGGAA AGTTCAC ACAGTGAC GGTAAC TTACCA GAAAGGG ACGGCTA ACTACG TGCCAG CAGCCG CGGTA ATACGT AGGTNC CCGAG CGTTG TCCGG ATTTA TTGGG GCGT AAAG CGAGC GCAAG GCGGT TTAAT AAGTT TGAAGT

Lactobacillus gasseri C72

TGCCT AATAC ATGC AAGT CGAGCG AGCTT GCCTAG ATGAT TTTAG TGCTT GCACT AAATG AAACT AGATA CAAGC GAGCG GCGGA CGGGT GAGTA ACACG TGGGT AACCT GCCCA AGAGA CTGGG ATAAC ACCTG GAAACA GATGC TAATA CCGGAT AACAA CACTAG ACGCA TGTCT AGAGT TTGAAA GATGG TTCTG CTATC ACTCT TGGAT GGACC TGCG GTGCA TTAGC TAGTT GGTAA GGTAA CGGCT TACCA AGGCA ATGAT GCATA GCCGAG TTGA GAGAC TGAT CGGC CACAT TGGGA CTGAG ACAC GGCC CAAA CTCC TACG GGAG GCAG CAGT AGGGAA TCTTC CACA ATGG ACGAA AGTCT GATG GAGCA ACGCC GCGTG AGTGAA GAAGG GTTTC GGCT CGTAA AGCTCTG TTGGTAG TGAAGAA AGATAG AGGTAGT AACTG GCCTT TATTTG ACGGT AATT ACTT AGAA AGTCA CGGCT AACTA CGTGC CAGCA GCCGC GGTAA TACGT AGGTG GCAAG CGTTG TCCGG ATTTAT TGGGCG TAAAGC GAGTG CAGG CGGTTC AATAAG TCTGAT GTGAA AGCCT TCGGC TCAACC GGAGAAT TGCATC AGAAA CTGTTG AACTT GAGTGC AGAAG AGGAG AGTGGA

Streptococcus infantarius C73

GTGCC TAATA CATGCA AGTAGA ACGCT GAAGACT TTAGCT TGCTAA AGTTG GAAGA GTTGCG AACGGG TGAGTA ACGCGT AGGTA ACCTG CCTAC TAGCG GGGGA TAACT ATTGG AAACGA T AGCT AATA CCGCA TAACAG CATT TAACAC ATGTTA GATGCT TGAAA GGAG CAATT GCTTCA CTAGT AGATGG ACCTG CGTTGT ATTAGC TAGTT GGTGA GGTAAC GGCTC ACCAAG GCGAC

GATAC ATAGCC GACCTG AGAGG GTGATC GGCCAC ACTGG GACTG AGACA CGGC CCAGA CTCCT ACGGGA GGCAG CAGTA GGGAA TCTTCG GCAAT GGGGG CAACC CTGAC CGAGC AACGC CGCGT GAGTG AAGAAG GTTTTC GGATCG TAAAGC TCTGT TGTAAG AGAAG AACGT GTGTG AGAGT GGAAA GTTCA CACAGT GACGG TAACT TACCA GAAAGG GACGG CTAAC TACGTG CCAGC AGCCGC GGTAA TACGTA GGTCCC GAGCGT NGTCCG GATTT ATTGGG CGTAAA GCGAG CGCAGGC GGTT TAAT AAGTCT GAAG TTAA AGGCA GTGGCTT AACCA TTGTT CGCTTT GGAAA CTGTT AGAC TTGA GTGCAG AAGGGG AGAGTG GAATT CCATG TGTA

Streptococcus infantarius C74

GTGCC TAATA CATG CAAGT AGAACG CTGAAG ACTTTA GCTTGC TAAAGTT GGAA GAGT TGCGA ACGGGTG AGTAACG CGTAGGT AACCTG CCTACT AGCGG GGGATA ACTATT GGAAA CGATA GCTAATA CCGCATA ACAGCA TTTAA CACAT GTTAGA TGCTTGAA AGGAG CAATT GCTTCA CTAGTA GATGGA CCTGC GTTGT ATTAGC TAGT TGGTGA GGTAAC GGCTCA CCAAG GCGAC GATAC ATAGC CGACC TGAGAG GGTGA TCGGC CACAC TGGGA CTGAG ACACG GCCCA GACTCCT ACGGGA GGCAG CAGTA GGGAA TCTTC GG CA ATGG GGGCAACC CTGAC CGAGC AACGC CGCGTG AGTGAA GAAGGT TTTCGG ATCGTA AAGCT CTGTTG TAAGA GAAGA ACGTG TGTGAG A GTGGAA AGTTC ACACA GTGAC GGTA ACTT ACCA GAAAG GGACG GCTA A CTACGTG CCAGC AGCCG CGGTA ATACG TAGGT CCCGA GCGTTG TCCGG ATTTA TTGGG CGTAA AGCGA GCGCA GGCGG TTTAA TAAGT CTGAAG TTAAA GGCAG TGGCT TAACC ATTGT TCGCT TTGGA AACTGT TAGAC TTGAGT GCAGA AGGG GAGAG TGGAA TTCAT GTGTA

Streptooccus infantarius C75

GTGCC TAATA CATGC AAGTAG AACGCT GAAGAC TTTAG CTTGCT AAAGT TGGAA GAGTT GCGAA CGGGTG AGTAAC GCGTAG GTAAC CTGCCT ACTAG CGGGGG ATAAC TATTGG AAACG ATAGCT AATACC GCATA ACAGCA TTTAA CACAT GTTAG ATGCTT GAAAGG AGCAA TTGCTT CACTAG TAGATG GACCT GCGTTG TATT AGCT AGTT GGTG AGGTAAC GGCTCA CCAAG GCGACG ATACA TAGCC GACCT GAGAG GGTGA TCGGCC ACACT GGGAC TGAG ACACG GCCCA GACTC CTACG GGAGGCA GCAGT AGGG AATCT TCGGC AATG GGGG CAAC CCTGA CCGAG CAACG CCGCGT GAGTG AAGAA GGTTT TCGGAT CGTAA AGCT CTGTTG TAAGA GAAGA ACGTG TGTG AGAGTG GAAAGT TCACA CAGTG ACGGT AACTT ACCAG AAAGG GACG GCTA ACTACG TGCCA GCAGC CGCG GTAATA CGTA GGTCCC GAGCG TTGT CCGGA TTTAT AGGGC GTAAA GCGAG CGCAG GCGGTT TAATA AGTCT GAAGT TAAAG GCAGT GGGC TTAAC CATTG TTCGC TTTT GGAAA CTGTT AGAC TTGAG TGCAGA

Streptococcus infantarius C76

TGCCTAA TACAT GCAAGT AGAAC GCTGA AGACTT TAGNCT TGCTAA AGTTG GAAG AGTT GCGA ACGG GTGA GTAA CGCG TAGGT AACCTG CCTA CTAG CGGG GGATA ACTA TTGG AAAC GATAGC TAAT ACCG CATA ACAG CATTT AACA CATG TTAG ATGC TTGA AAGG AGCA ATTG CTTCAC TAGT AGATG GACC TGCG TTGT ATTAG CTAG TTGGT GAGG TAACGG CTCACCA AGGCG ACGA TACATAGCC GACCT GAGAGG GTGA TCGGCC ACACT GGGA CTGA GACAC GGCC CAGAC TCCT ACGG GAGG CAGCA GTAG GGAAT CTTCGG CAAT GGGG GCAACC CTGA CCGA GCAA CGCC GCGT GAGTG AAGAA GGTT TTCG GATC GTAA AGCT CTGT TGTAA GAGA AGAACGT GTGT

GAGAGT GGAA AGTTCA CACAG TGACG GTAACT TACCAG AAAGGG ACGGCT AACTAC GTGCC AGCAG CCGCGG TAATACG TAGG TCCC GAGC GT TGTCCGG AT TTATT GGGC GTAAAG CGAGCG CAGG CGG TTTA ATAA GTCT GAAGTT AAAG GCAG TGGC TTAAC CATTG TTCG CTTTG GAAA CTGTT AGAC TTGA GTGC AGAA GGGG AGAGT GGAAT TCCATGT GTAGC GGTGA AATGCG TAAATA TATGGA GGAA CACC GGGT GGCG AAAG CGGC TCTC TGGG TCTG TAAC TGAC

Streptococcus infantarius C77

GTGCC TAAT ACAT GCAA GTAG AACG CTGAA GACTT TAGC TTGCTAA AGTTG GAAG AGTTGCG AACGG GTGAG TAACGCG TAGG TAAC CTGCC TACT AGCG GGGGA TAAC TATTG GAAA CGAT AGCT AATA CCGC ATAA CAGC ATTT AACA CATGT TAGAT GCTTG AAAG GAGC AATT GCTT CACTA GTAG ATGG ACCT GCGTT GTAT TAGC TAGTTGG TGAGG TAACG GCTCA CCAA GGCG ACGA TACA TAGCC GACC TGAG AGGG TGAT CGGC CACA CTGG GACT GAGACA CGGCCCA GACTCC TACG GGAG GCAG CAGTA GGGAA TCTTC GGCAA TGGGGGC AACCCTG ACCGAG CAACGCCG CGTGAGT GAAG AAGG TTTTC GGATC GTAA AGCT CTGTT GTAAGA GAAGAA CGTGT GTGA GAGT GGAA AGTT CACA CAGT GACG GTAA CTTAC CAGAA AGGGA CGGC TAACTAC GTGCCAG CAGC CGCG GTAAT ACGT AGGTC CCGA GCGT TGTC CGGA TTTA TTGGGCG TAAA GCGA GCGC AGGC GGTT TAAT AAGTCT GAAGT TAAA GGCA GTGG CTTAA CCAT TGTT CGCTT TGGA AACTG TTAGA CTTGA GTGCAAACGC CGCGTG AGTGAA GAAGGT TTTCGG ATCGTA AAGCT CTGTTG TAAGA GAAGA ACGTG TGTGAG A GTGGAA AGTTC ACACA GTGAC GGTA ACTT ACCA GAAAG GGACG GCTA A CTACGTG CCAGC AGCCG CGGTA ATACG TAGGT CCCGA GCGTTG TCCGG ATTTA TTGGG CGTAA AGCGA GCGCA GGCGG TTTAA TAAGT CTGAAG TTAAA GGCAG TGGCT TAACC ATTGT TCGCT TTGGA AACTGT TAGAC TTGAGT GCAGA AGGG GAGAG TGGAA TTCAT GTGTA

Streptooccus infantarius C75

GTGCC TAATA CATGC AAGTAG AACGCT GAAGAC TTTAG CTTGCT AAAGT TGGAA GAGTT GCGAA CGGGTG AGTAAC GCGTAG GTAAC CTGCCT ACTAG CGGGGG ATAAC TATTGG AAACG ATAGCT AATACC GCATA ACAGCA TTTAA CACAT GTTAG ATGCTT GAAAGG AGCAA TTGCTT CACTAG TAGATG GACCT GCGTTG TATT AGCT AGTT GGTG AGGTAAC GGCTCA CCAAG GCGACG ATACA TAGCC GACCT GAGAG GGTGA TCGGCC ACACT GGGAC TGAG ACACG GCCCA GACTC CTACG GGAGGCA GCAGT AGGG AATCT TCGGC AATG GGGG CAAC CCTGA CCGAG CAACG CCGCGT GAGTG AAGAA GGTTT TCGGAT CGTAA AGCT CTGTTG TAAGA GAAGA ACGTG TGTG AGAGTG GAAAGT TCACA CAGTG ACGGT AACTT ACCAG AAAGG GACG GCTA ACTACG TGCCA GCAGC CGCG GTAATA CGTA GGTCCC GAGCG TTGT CCGGA TTTAT AGGGC GTAAA GCGAG CGCAG GCGGTT TAATA AGTCT GAAGT TAAAG GCAGT GGGC TTAAC CATTG TTCGC TTTT GGAAA CTGTT AGAC TTGAG TGCAGA

Streptococcus infantarius C76

TGCCTAA TACAT GCAAGT AGAAC GCTGA AGACTT TAGNCT TGCTAA AGTTG GAAG AGTT GCGA ACGG GTGA GTAA CGCG TAGGT AACCTG CCTA CTAG CGGG GGATA ACTA TTGG AAAC GATAGC TAAT ACCG CATA ACAG CATTT AACA CATG TTAG ATGC TTGA AAGG AGCA ATTG CTTCAC

TAGT AGATG GACC TGCG TTGT ATTAG CTAG TTGGT GAGG TAACGG CTCACCA AGGCG ACGA TACATAGCC GACCT GAGAGG GTGA TCGGCC ACACT GGGA CTGA GACAC GGCC CAGAC TCCT ACGG GAGG CAGCA GTAG GGAAT CTTCGG CAAT GGGG GCAACC CTGA CCGA GCAA CGCC GCGT GAGTG AAGAA GGTT TTCG GATC GTAA AGCT CTGT TGTAA GAGA AGAACGT GTGT GAGAGT GGAA AGTTCA CACAG TGACG GTAACT TACCAG AAAGGG ACGGCT AACTAC GTGCC AGCAG CCGCGG TAATACG TAGG TCCC GAGC GT TGTCCGG AT TTATT GGGC GTAAAG CGAGCG CAGG CGG TTTA ATAA GTCT GAAGTT AAAG GCAG TGGC TTAAC CATTG TTCG CTTTG GAAA CTGTT AGAC TTGA GTGC AGAA GGGG AGAGT GGAAT TCCATGT GTAGC GGTGA AATGCG TAAATA TATGGA GGAA CACC GGGT GGCG AAAG CGGC TCTC TGGG TCTG TAAC TGAC

Streptococcus infantarius C77

GTGCC TAAT ACAT GCAA GTAG AACG CTGAA GACTT TAGC TTGCTAA AGTTG GAAG AGTTGCG AACGG GTGAG TAACGCG TAGG TAAC CTGCC TACT AGCG GGGGA TAAC TATTG GAAA CGAT AGCT AATA CCGC ATAA CAGC ATTT AACA CATGT TAGAT GCTTG AAAG GAGC AATT GCTT CACTA GTAG ATGG ACCT GCGTT GTAT TAGC TAGTTGG TGAGG TAACG GCTCA CCAA GGCG ACGA TACA TAGCC GACC TGAG AGGG TGAT CGGC CACA CTGG GACT GAGACA CGGCCCA GACTCC TACG GGAG GCAG CAGTA GGGAA TCTTC GGCAA TGGGGGC AACCCTG ACCGAG CAACGCCG CGTGAGT GAAG AAGG TTTTC GGATC GTAA AGCT CTGTT GTAAGA GAAGAA CGTGT GTGA GAGT GGAA AGTT CACA CAGT GACG GTAA CTTAC CAGAA AGGGA CGGC TAACTAC GTGCCAG CAGC CGCG GTAAT ACGT AGGTC CCGA GCGT TGTC CGGA TTTA TTGGGCG TAAA GCGA GCGC AGGC GGTT TAAT AAGTCT GAAGT TAAA GGCA GTGG CTTAA CCAT TGTT CGCTT TGGA AACTG TTAGA CTTGA GTGCA

Streptococcus infantarius C78

GTGC CTAAT ACATG CAAGTA GAACG CTGAA GACT TTAG CTTG CTAA AGTT GGAA GAGTTGC GAAC GGGT GAGT AACG CGTA GGTA ACCTG CCTACT AGCGG GGGA TAAC TATT GGAA ACGAT AGCTA ATACCGCA TAACA GCA TTTAA CTCAT GTTAGA TGCTTGA AAGGAGC AATTGCTT CACTA GTAGATG GACCTG CGTT GTAT TAGC TAGT TGGT GAGGTAA CGGC TCAC CAAG GCGAC GATACA TAGCCGA CCTGAG AGGGTG ATCGGC CACA CTGGG ACTGA GACA CGGC CCAGACT CCTACG GGAG GCAG CAGT AGGGA ATCTT CGGCA ATGGG GGCAA CCCT GACCG AGCAA CGCCG CGTGA GTGAAG AAGGT TTTCG GATCGT AAAGC TCTGT TGTAA GAGAA GAACG TGTGT GAGAG TGGAA AGTTCA CACAG TGACG GTAAC TTACC AGAAA GGGAC GGCTA ACTAC GTGCC AGCAG CCGCGG TAATA CGTAGG TCCCTA GCGTN GTCCGG ATTAAT TGGGCG TAAAG CGAGC GCAGG CGGTTT AATAA GTCTGA AGTTAA AGGC AGTT GGCT TAACCA TGGTT CGCTT TGGA AACT GTAA GACT

Lactobacillus mucosae C8

TGTGCC TAATAC ATGCAA GTCGAA CGCGTT GGCCCA ACTGATT GAACGT GCTTGCA CGGACT TGACGTT GGTTTA CCAGCG AGTGGC GGACGG GTGAG TAACAC GTAAGT AACCTG CCCCAA AGCGGG GGATAA CATTT GGAAA CAGAT GCTAA TACCGC ATAGA CAATTT AGAATC GCATGA TTCAAA TTTAAA AGATG GCTTC GGCTAT CACTTT GGGAT GGACCT GCGGC GCATTA GCTTG

TTGGTA GGGTAA CGGCC TACCA AGGCT GTGATG CGTAG CCGAGT TGAGA GACTG ATCGGC CACAA TGGAAC TGAGA CACGG TCCATA CTCCTA CGGGA GGCAG CAGTA GGGAAT CTTCCA CAATGG GCGCAA GCCTG ATGGA GCAACA CCGCG TGAGTG AAGAA GGGTT TCGGC TCGTAT AAGCT CTGTTG TTAGA GAAGA ACGTG CGTGA GAGCA ACTAGT TCACGC AGTGAC GGTAT CTAACC AGAGAG GCACGG CTAACT ACGTGCC AGCAGC CGCGGT AGACG TAGGTG GCAAG CGTCATC CGGATC TATTGG GCGTA CAGCG AGCGC AGGCG GATCTG ATAC GTCTG ATGT GACAG

Streptococcus infantarius C80

GGCG GCGTG CCTAA TACAT GCAAG TAGAA CGCTG AAGACT TTAGCT TGCTA AAGTTG GAAGAG TTGCG AACGGG TGAGT AACGC GTAGGT AACCT GCCTAC TAGCG GGGGAT AACTAT TGGAA ACGATA GCTAA TACCG CATAA CAGCA TTTAA CACAT GTTAG ATGCT TGAAAG GAGCA ATTGC TTCAC TAGTA GATG GACCT GCGTT GTATT AGCTA GTTGG TGAGGT AACGG CTCAC CAAGG CGACGA TACA TAGC CGACC TGAGA GGGTG ATCGGC CACAC TGGGA CTGAG ACACGG CCCA GACTC CTACG GGAGG CAGCA GTAGG GAATC TTCGG CAAT GGGGG CAAC CCTGA CCGAG CAACG CCGCG TGAGT GAAGA AGGTT TTCGG ATCGT AAAGC TCTGT TGTAA GAGAA GAACG TGTGT GAGAGT GGAA AGTTC ACACA GTGAC GGTAA CTTAC CAGAAA GGGAC GGCTA ACTAC GTGCCA GCAGCC GCGGT AATACG TAGGT CCCGA GCGTT GTCCG GATTT ATTGG GCGTA AAGC GAGCG CAGG CGGTT TAAT AAGTC TGAAG TTAAA GGCAG TGGCTT ACCCA TTGTT CGCTT TGGAA ACTGT TAGAC TTGAG TGCAG AAGGG GAGAG TGGAA TCCAT GTGTA CCCGT GAAATGC

Lactobacillus amylovorus C81

TGCCT AATA CATGC AAGTC GAGCG AGCGG AACCA ACAGA TTTA CTTC GGTAA TGAC GTTGGG AAAGC GAGCG GCGGA TGGGT GAGTA ACACGT GGGGA ACCTG CCTCTA AGTCT GGGAT ACCATT TGGA AACAG GTGCT AATAC CGGAT AATAA AGCAG ATCGCAT GATC AGCT TTTG AAAGG CGGCGTA AGCT GTCG CTAAGG GATG GCCC CGCG GTGC ATTA GCTA GTTG GTAA GGTA ACGGCT TACCAA GGCGAC GATG CA TAGC CGAGTT GAGAG ACTGA TCGGC CACATT GGGAC TGAGA CACGGC CCAAA CTCCT ACGGG AGGCA GCAGT AGGGA ATCTT CCACA ATGGA CGCAA GTCTG ATGGA GCAAC GCCGC GTGAG TGAAG AAGG TTTT CGGAT CGTAA AGCTC TGTTG TTGGT GAAGA AGGA TAGA GGTAG TAACT GGCCT TTATT TGACG GTAATC AACCAG AAAGT CACGG CTAAC TACGT GCCAG CAGCCG CGGTAA TACGT AGGTG GCAAG CGTTG TCCGG ATTTA TTGGG CGTAA AGCGA GCGCAG GCGGA AAAAT AAGTC TAATGT GAAA GCCCT GTGC CTAA TACATGC AAGT CGAGC GAGC GGAA CCAA CAGA TTTACT TCGGTA ATGA CGTT GGGA AAGC GAGC GGCGG ATGGG TGAG TAACA CGTG GGGA ACCT GCCTC TAAGTC TGGG ATAC CATT TGGA AACAG GTGC TAATA CCGGAT AATAAAG CAGA TCGC ATGAT CAGCTT

Lactobacillus amylovorus C82

GTGC CTAA TACATGC AAGT CGAGC GAGC GGAA CCAA CAGA TTTACT TCGGTA ATGA CGTT GGGA AAGC GAGC GGCGG ATGGG TGAG TAACA CGTG GGGA ACCT GCCTC TAAGTC TGGG ATAC CATT TGGA AACAG GTGC TAATA CCGGAT AATAAAG CAGA TCGC ATGAT CAGCTT TTGAAA GGCGGC GTAAGC TGTCGCT AAGG GATGG CCCC GCGG TGCATTA GCTAGT TGGTAA GGTAA CGGC TTACC AAGGC GACGA TGCAT AGCCG AGTTGA GAGAC TGATC GGCCA CATTG GGACT GAGAC ACGGCC CAAA CTCCTA CGGGA GGCAG CAGTAG GGAAT CTTCC ACAATG

GACGC AAGTC TGATG GAGCAA CGCCG CGTGA GTGAA GAAGG TTTTC GGAT CGTA AAGCT CTGTT GTTGG TGAA GAAG GATAG AGGTA GTAA CTGGC CTTAA TTTGA CGGTA ATCAA CCAG AAAGT CACGG CTAAC TACGT GCCAG CAGCCG CGGT AATAC GTAGG TGGCA AGCGT TGTCCG GATTA ATTGG GCGTA AAGCG AGCGC AGGCG GAAAA ATAAG TCTAAT GTGA AAGC GTGC CTAA TACATGC AAGT CGAGC GAGC GGAA CCAA CAGA TTTACT TCGGTA ATGA CGTT GGGA AAGC GAGC GGCGG ATGGG TGAG TAACA CGTG GGGA ACCT GCCTC TAAGTC TGGG ATAC CATT TGGA AACAG GTGC TAATA CCGGAT AATAAAG CAGA TCGC ATGAT CAGCTT

Lactobacillus amylovorus C84

GTGCC TAATA CATGCAA GTCGAG CGAGC GGAAC CAACAG ATTT ACTT CGGTA ATGAC GTTGG GAAAG CGAGCG GCGGA TGGGT GAGTA ACACG TGGGGA ACCTGC CCCTA AGTCT GGGAT ACCAT TTGGAA ACAGGT GCTAAT ACCGG ATAATA AAGCA GATCG CATGAT CAGCTT TTGAAA GGCGG CGTAA GCTGTC GCTAA GGGAT GGCCC CGCGG TGCA TTAGC TAGTT GGTAA GGTAA CGGCT TACCA AGGCG ACGAT GCATA GCCGAGT TGAG AGAC TGAT CGGC CACA TTGGG ACTGA GACAC GGCCC AAACT CCTA CGGGA GGCA GCAG TAGGG AATC TTCCA CAATG GACGC AAGTC TGATG GAGCA ACGCC GCGTG AGTGA AGAAG GTTT TCGG ATCGT AAAGC TCTGT TGTTG GTGAAG AAGGA TAGAG GTAGT AACTG GCCT TTAT TTGACGG TAATC AACCA GAAAG TCACG GCTA ACTA CGTG CCAG CAGCC GCGGT AATA CGTAG GTGGC AAGCG TTGTC CGGAT TTATT GGGCG TAAAG CGAGC GCAGG CGGA AAAAT AAGTC TAATG TGAAAG CCCTC GGCTT AACC GAGGA ACTGCA TCGGA AACTGT TTTTC TTGAGT GCAG AAGA GGAG AGTG GAAC TCCA TGTG TAGC GGTG GAAT GCGT AGAT ATAT GGA

Lactobacillus amylovorus C85

TGCCT AATA CATGC AAGTC GAGCG AGCGG AACCA ACAGA TTTA CTTC GGTAA TGAC GTTGGG AAAGC GAGCG GCGGA TGGGT GAGTA ACACGT GGGGA ACCTG CCTCTA AGTCT GGGAT ACCATT TGGA AACAG GTGCT AATAC CGGAT AATAA AGCAG ATCGCAT GATC AGCT TTTG AAAGG CGGCGTA AGCT GTCG CTAAGG GATG GCCC CGCG GTGC ATTA GCTA GTTG GTAA GGTA ACGGCT TACCAA GGCGAC GATG CA TAGC CGAGTT GAGAG ACTGA TCGGC CACATT GGGAC TGAGA CACGGC CCAAA CTCCT ACGGG AGGCA GCAGT AGGGA ATCTT CCACA ATGGA CGCAA GTCTG ATGGA GCAAC GCCGC GTGAG TGAAG AAGG TTTT CGGAT CGTAA AGCTC TGTTG TTGGT GAAGA AGGA TAGA GGTAG TAACT GGCCT TTATT TGACG GTAATC AACCAG AAAGT CACGG CTAAC TACGT GCCAG CAGCCG CGGTAA TACGT AGGTG GCAAG CGTTG TCCGG ATTTA TTGGG CGTAA AGCGA GCGCAG GCGGA AAAAT AAGTC TAATGT GAAA GCCCT GTGC CTAA TACATGC AAGT CGAGC GAGC GGAA CCAA CAGA TTTACT TCGGTA ATGA CGTT GGGA AAGC GAGC GGCGG ATGGG TGAG TAACA CGTG GGGA ACCT GCCTC TAAGTC TGGG ATAC CATT TGGA AACAG GTGC TAATA CCGGAT AATAAAG CAGA TCGC ATGAT CAGCTT

Lactobacillus amylovorus C86

CGTG CCTAAT ACATGC AAGTC GAGCGA GCGGAA CCAAC AGATTTA CTTCG GTAATG ACGTTG GAAAA GCGA GCGG CGGA TGGG TGAG TAAC ACGTGG GGAACC TGTC CCTA AGTC TGGG ATAC CATTTGG AAACA GGTGCT AATAC CGGATA ATAAA GCAGAT CGCATGA TCAGC TTTTGA AAGGCG GCGTA AGCTG TCGCTA AGGGAT GGCCCC GCGGT GCATT AGCTAG TTGGTA AGGTA

ACGG CTTA CCAAG GCGAC GATGC ATAGC CGAGT TGAGA GACTG ATCGGC CACAT TGGGA CTGAGA CACGG CCCAAA CTCCT ACGGGA GGCAG CAGTA GGGAAT CTTCCA CAATGGA CGCAAG TCTGA TGGAGC AACGC CGCGT GAGTG AAGAA GGTT TTCGG ATCGTA AAGC TCTGT TGTTGG TGAAG AAGGA TAGAG GTAGT AACT GGCCT TTATT TGACG GTAAT CAACC AGAAA GTCAC GGCTA ACTAC GTGCC AGCAG CCGCG GTAAT ACGTA GGTGG CAAGC GTTGT CCGGA TTTAT TGGGC GTAAA GCGAG CGCAG GCGGA AAAAT AAGTC TAATG TGAAA GCCCT CGGCT TAACC GAGGA ACTGC ATCGG AAACT GTTTT CCTTG AGTGC AGAA GAGGA GAG TGGACCT CATGTG

Lactobacillus amylovorus C87

CGTGC CTAAT ACATG CAAGT CGAGC GAGCG GAAC CAACAG ATTTA CTTCGGT AATGA CGTTGG GAAAGC GAGC GGCGG ATGGG TGAGT AACA CGTG GGGA ACCT GCCCC TAAGT CTGGG ATACC ATTTG GAAAC AGGTG CTAATA CCGG ATAAT AAAGCA GATCG CATGAT CAGCT TTTGA AAGGC GGCGT AAGCTG TCGC T AAGG GATG GCCC CGCGG TGCA TTAG CTAG TTGG TAAG GTAA CGGC TTACC AAGG CGAC GATG CATA GCCG AGT TGAG AGAC TGAT CGGC CACAT TGGG ACTG AGAC ACGG CCCA AACT CCTA CG GGAGG CAGCA GTAG GGAAT CTTCC ACAAT GGACG CAAGT CTGAT GGAGC AACG CCGCG TGAGT GAAG AAGGT TTTC GGAT CGTA AAGC TCT GTTG TTGG TGAAG AAGGA TAGAG GTAGT AACTG GCCTT TATTT GACG GTAATC AACCA GAAAG TCAC GGCTA ACTAC GTGCC AGCAG CCGCG GTAATA CGTAG GTGGCA AGCGTT GTCCG GATTT ATTGG GCGTA AAGCG AGCGC AGGCG GAAAA ATAAG TCTA ATGTG AAAGC GTGC CTAA TACATGC AAGT CGAGC GAGC GGAA CCAA CAGA TTTACT TCGGTA ATGA CGTT GGGA AAGC GAGC GGCGG ATGGG TGAG TAACA CGTG GGGA ACCT GCCTC TAAGTC TGGG ATAC CATT TGGA AACAG GTGC TAATA CCGGAT AATAAAG CAGA TCGC ATGAT CAGCTT

Streptococcus infantarius C88

GTGCCT AATACAT GCAAGT AGAA CGCT GAAG ACTT TAGCTT GCTA AAGT TGGA AGAG TTGCGA ACGG GTGAG TAAC GCGT AGGT AACC TGCCT ACTA GCGGG GGATA ACTA TTGG AAAC GATA GCTA ATAC CGCA TAAC AGCA TTTA ACAC ATGT TAGAT GCTTGA AAGGA GCAAT TGCT TCAC TAGTA GATGGA CCTGCG TTGTAT TAGCT AGTTGG TGAG GTAAC GGCTCA CCAAGGC GACGATA CATAGCC GACC TGAGA GGGTG ATCGG CCAC ACTG GGACT GAGACA CGGCCC AGACTCC TACGGGA GGCAG CAGT AGGGA ATCTT CGGC AATG GGGG CAAC CCTGAC CGAGCAA CGCC GCGT GAGT GAAG AAGGTT TTCGGAT CGTAAAG CTCTG TTGT AAGAG AAGA ACGTGTG TGAG AGTG GAAA GTTC ACAC AGTG ACGG TAAC TTAC CAGA AAGG GACG GCTA ACTA CGTGC CAGC AGCCG CGGTA ATACGTA GGTC CCGA GCGTT GTCCG GATTT ATTG GGCG TAAA GCGA GCGCAGG CGGTTT AATAA GTCTGAA GTTAAA GGCAG TGGCTT AACCA TTGTT CGCTTT GGAAA CTGTT AGACT TGA GTGCA GAAG GGG AGAGT GGAA TCCAT GTGT AGCG GTGAA

Lactobacillus ingluviei C89

TGTG CCTA ATACAT GCAAG TCGA ACGC GTTGG CCCA ATTG ATTG ATGG TGCT TGCA CCTG ATTG ATTTTGG TCGC CAAC GAGT GGCG GACG GGTG AGTA ACACG TAGGT AACCTGC CCAG AAGC GGGG GACAA CATTTGG AAAC AGAT GCTA ATAC CGCA TAAC AGCG TTGT TCGCATG AACA ACGC TTAA AAGA TGGC TTCT CGCTAT CACTT CTGG ATGG ACCT GCGGTG CATTAG CTTG TTGG TGGG GTAA CGGCCT ACCA AGGC GATG ATGC ATAG CCGA GTTGA GAGA CTGAT CGGC

CACAAT GGGA CTGAG ACACG GCCCA TACTC CTACGG GAGGC AGC AGTA GGGA ATCTTC CACAAT GGGC GCAA GCCTG ATGGA GCAA CACC GCGTGAG TGAA GAAG GGTTTCG GCTCG TA AAGC TCTGT TGTTA AAGAAGA ACACGTA TGAGA GTAA CTGT TCAT ACGT TGACGG TATTT AACC AGAA AGTCAC GGCT AACT ACGTGCC AGCA GCCG CGGTA ATAC GTAG GTGGCA AGCG TTAT CCGG ATTT ATTG GGCG T AAAG AGAG TGCAGGC GGTTT TCTA AGTCT GATGT GAAA GCCT TCGGC TTAACC GGAG AAGTGC ATCG GAAA CTGG ATAA CTTGAG TGCAG AAGA

Enterococcus hirae C9

GCGGC GTGCCT AATACAT GCAAGT CGAA CGTCTTC TTTT TCCA CCGG AGCT TGCTC CACC GGAA AAAG AGGA GTGG CG AACG GGT GAGT AACA CGTGG GTAA CCTGC CCAT CAGA AGGGG ATAACA CTTG GAAA CAGGTG CTAATA CCGTATA ACAATC GAAA CCGCA TGGTT TTGAT TTGAA AGGC GCTT TCGG GTGTCGC TGATGG ATGG ACCC GCGGTG CATTAGCT AGTTG GTGAGG TAAC GGCT CACCA AGGCG ACGA TGCAT AGCC GACC TGAG AGGG TGAT CGGCC ACATTG GGACT GAGA CACGG CCCA AACC TCCT ACGG GAGGC AGCAG TAGG GAAT CTTC GGCA ATGG ACGA AAGT CTGAC CGAG CAAC CGCC GCCGT GAGT GAAG AAGG TTTT CGGA TCGT AAAA CTCT GTTGG TT AGAGA AGAA CCAG GATG AGAGT ACTG

Lactobacillus gasseri C90

GTGC CTAAT ACATGC AAGT CGAGC GAGC TTGC CTAG ATGA TTTTA GTGCT TGCA CTAA ATG AAAC TAGA TACA AGCGA GCGGCG GACGG GTGAG TAAC ACGT GGGT AACC TGCC CAA GAGA CTGG GATA ACAC CTGG AAA CAG ATGC TAATA CCGG ATAA CAAC ACT A GACGC ATGTCTA GAGTTT GAAA GATG GTTC TGCTA TCAC TCTTGGA TGGACCT GCGGTGC ATTA GCTAG TTGGT AAGG TAAC GGCT TACCA AGGCA ATGAT GCAT AGCC GAGTT GAGA GACT GATCG GCCAC ATTG GGACT GAGA CACG GCCC AAA CTCCT ACGG GAG GCAG CAGT AGGGA AT CTTC CACAA TGGAC GAAAG T CTGATG GAGCAAC GCCG CGTGAGT GAAGAA GGGTT TCGG CTCG TAAA GCTCTG TTGG TAGT GAAGA AAGAT AGAG GTAG TAAC TGGC CTTTA TTTGAC GGTAATT ACTTAG AAAG TCACG GCTA ACTAC GTGCC AGCA GCCG CGGTAA TACGT AGGTG GCAA GCGTT GTCC GGATT TATTGG GCGTA AAGCG AGTGC AGGC GGTT CAAT AAGT CTGA TGTG AAAG CCTT CCGG CTCA

Weissella cibaria C91

GTGCCT AATACA TGCAAGT CGAAC GCTT TGTGG TTCAA CTGAT TTGA AGAG CTTG CTCA GATA TGACG ATGGA CATTG CAAAG AGTGG CGAAC GGGT GAGTAA CACGT GGGAA ACCTA CCTC TTAGCA GGGG ATAAC ATTTG GAAA CAGA TGCT AATA CCGT ATAACA ATAGCAA CCGCATG GTTG CTAC TTAAAAG ATGG TTCTG CTATCA CTAAG AGATGGTCC CGCG GTGC ATTAG TTAGTTG GTGA GGTA ATGG CTCA CCAA GACGAT GATGC ATAGCC GAGT TGAG AGACT GATCG GCCA CAATG GGAC TGAG ACACG GCCC ATAC TCCTA CGGGAGG CAGCA GTAGG GAATC TTCCACAA TGGGCGA AAGC CTGA TGGAGCA ACGC CGCG TGTG TGAT GAAG GGTT TCGGCTC GTAAAAC ACTG TTGT AAGA GAAG AATGA CATT GAGAG TAACT GTTCA ATGTG TGAC GGTA TCTTAC CAGAA AGGA ACGGC TAAA TACGT GCCA GCAG CCGCGGT AATAC GTATGT TCCAA GCGT TATC CGGA TTTA TGGG GCGTA AAGC GAGC GCAG ACGG TTATT TAAG TCTGA AGTGAA AGCCC TCAGCT CAACT GAGGAA TTGC TTTG GAAA CTGGA TGACTT GAGT GCAG TATA GGAA AGTG GAACTC

Lactobacillus ingluviei C92

TGTGCC TAATACA TGCAAGTCG AACGC GTTG GCCCA ATTG ATTG ATGGT GCTTG CACCT GATTG ATTTT GGTCG CCAA CGAGT GGCG GACGG GTGAG TAACA CGTA GGTAA CCTGCC CAGA AGCGG GGGAC AACA TTTGG AAACA GATGCT AATACCG CATAAC AGCG TTGTT CGCAT GAACA ACGC TTAA AAGA TGGCTT CTCG CTAT CACTTCT GGATG GACC TGCG GTGCATT AGCT TGTT GGTG GGGTA ACGG CCTA CCAA GGCG ATGA TGCA TAGCC GAGTTG AGAG ACTG ATCG GCCACAA TGGG ACTG AGACA CGGCC CATACT CCTAC GGGAGG CAGCA GTAGGG AATC TTCC ACAAT GGGC GCAA GCCT GATG GAGC AACA CCGC GTGA GTGA AGAAG GTGT CTGCG GCTCGTA AAGC TCTG TTGTTA AAGAAG AACACGT ATGA GAGT AACTGTT CATACG TTGA CGGT ATTTAAC CAGAAA GTCA CGGC TAACT ACGT GCCAG CAGC CGCGG TAAT ACGT AGGT GGCA AGCG TTAT CCGG ATTTA TTGGG CGTAA AGAGA GTGCA GGCG GTTT TCTAA GTCT GATGTGA

Laactobacillus ingluviei C93

GTGTG CCTAAT ACATG CAAGT CGAA CGCG TTGG CCCAA TTGA TTGAT GGTGC TTGC ACC TGATT GATTT TGGTC GCCA ACGAG TGGCG GACG GGTG AGTA ACACG TAGG TAACC TGCCC AGAA GCGG GGGA CAAC ATTT GGAA ACAGA TGCTA ATAC CGCA TAACA GCGTT GTTCG CATG AACA ACGCT TAAAA GATGG CTTCT CGCTA TCACT TCTGG ATGG ACCTG CGGTG CATTA GCTTG TTGGTG GGGTA ACGGCC TACCA AGGCG ATGAT GCATA GCCG AGTTG AGAGA CTGA TCGGC CACAA TGGGA CTGA GACAC GGCC CATAC TCCTA CGGGA GGCAG CAGTA GGGA ATCTTC CACAA TGGGC GCAAG CCTGA TGGAG CAACA CCGC GTGAGT GAAG AAGGG TTTCG GCTC GTAA AGCTC TGTTGT TAAAG AAGAA CACG TATGA GAGTA ACTGT TCATA CGTTG ACGGT ATTT AACCA GAAAG TCACG GCTA ACTAC GTGCC AGCA GCCG CGGT AATA CGTA GGTGG CAAGC GTTATC CGGA TTTATG GGGC GTAAA GAGA GTGCA GGCGG TTTTC TAAG TCTGA TGTGA

Lactobacillus salivarius C94

GTGCCT AATACAT GCAAG TCGAA CGAA ACTTT CTTAC ACCGAA TGCTT GCATT CACCG TAAGA AGTTG AGTGG CGGAC GGGTG AGTAA CACGT GGGTA ACCTG CCTAA AAGAA GGGG ATAAC ACTTG GAAAC AGGTG CTAAT ACCGTA TATCT CTAAG GATCG CATGAT CCTTA GATGA AAGAT GGTTC TGCTA TCGCTT TTAGA TGGAC CCGC GGCGT ATTAA CTAGT TGGTG GGGT AACGG CCTAC CAAG GTGA TGATA CGTAG CCGAA CTGAG AGGTT GATCG GCCAC ATTGGG ACTG AGACA CGGC CCAAA CTCCT ACGGG AGGCA GCAGT AGGGA ATCTTC CACA ATGGAC GCAA GTCTG ATGGA GCAA CGCCGC GTGAG TGAAG AAGGT CTTCGG ATCGT AAAA CTCT GTTGTT AGAGAAG AACAC GAGTGA GAGT AACTG TTCATT CGATG ACGGT ATCTAA CCAGC AAGTC ACGGC TAACT ACGT GCCAG CAGCC GCGGT AATAC GTAGG TGGCA AGCGT TGTCC GGATT TATTG GGCGT AAAGG GAACG CAGGC GGTCT TTTAA GTCTG ATGTG AAAG CCTTC GGCTT AACCGG AGTA GTGC ATTGG AAACTG GAAGAC TTGAGT GCAGA AGAGG AGAG TGGA

Lactobacillus reuteri C95

TGCCTAA TACAT GCAAGT CGTACG CACTG GCCCA ACTGAT TGATG GTGCTT GCACCT GATTGA CGATGG ATCACC AGTGAG TGGCG GACGG GTGAGT AACACG TAGGTA ACCTG CCCCG GAGCG GGGGAT AACATT TGGAA ACAGAT GCTAA TACCG CATAAC AACAAA AGCCGC ATGGCT TTTGTT TGAAAG ATGGCT TTGGC TATCA CTCTG GGATG GACCTG CGGTGCA TTAGCTA GTTGGTA

AGGTAACGG CTTACC AAGGCG ATGAT GCATA GCCGAG TTGA GAGA CTGATC GGCCAC AATGGAA CTGAGAC ACGGTC CATACT CCTACG GGAGG CAGCA GTAGGG AATCTT CCACA ATGGGC GCAAGCC TGATGG AGCAACA CCGCGT GAGTGA AGAAGG GTTTCG GCTCG TAAAGCT CTGT TGTT GGAGAAG AACGTGC GTGAGA GTAACT GTTCAC GCAGTG ACGGTA TCCAA CCAGA AAGTC ACGG CTAAC TACG TGCC AGCA GCCG CGGT AATA CGTA GGTG GCAA GCGT TATC CGGA TTTA TTGG GCGT AAAG CGAG CGCA GGCG GTTG CTTA GGTC TGAT GTGA AAGC CTTC GGCT TAAC CGAA GAAG TGCA TCGG AAAC CGGG CGAC TTGA GTGCA GAAG AGGACA GTGG AACTC ATGTG

Lactobacillus gasseri C96

GTGCCT AATACA TGCAAG TCGAGCG AGCTTG CCTAGA TGATTT TAGTGC TTGCAC TAAATG AAACT AGATACA AGCGAG CGGCGG ACGGGT GAGTAA CACGTG GGTAAC CTGCC CAAGA GACTG GGATA ACACCT GGAA ACAG ATGCT AATAC CGGAT AACAA CACTAG ACGCAT GTCTA GAGTT TGAAA GATGG TTCTG CTATCA CTCTT GGATG GACCT GCGGT GCATT AGCTA GTTGG TAAGGT AACGG CTTAC CAAGG CAATG ATGCA TAGCC GAGTT GAGAG ACTGA TCGGC CACAT TGGG ACTGA GACA CGGCC CAAAC TCCTA CGGGA GGCAGC AGTAGG GAATC TTCCA CAATGG ACGAA AGTCTG ATGGA GCAAC GCCGCG TGAGT GAAGA AGGGT TTCGG CTCGTA AAGCT CTGT TGGTA GTGAA GAAAG ATAGA GGTAG TAACT GGCCT TTATT TGACG GTAAT TACTT AGAAA GTCAC GGCTA ACTAC GTGCC AGCAG CCGCG GTAAT ACGTA GGTGGC AAGCG TTGTC CGGATT TATTG GGCGTA AAGCGA GTGCAG GCGG TTCAA TAAGT CTGATG TGAAAG CCTTC GGCTCA ACCGGA GAATTG CATCAG AAACTG TGGAAC TTGAGT GCCGAA AAGGA GAGTG

Lactobacillus amylovorus C98

GGCGTG CCTAA TACATG CAAGTC GAGCG AGCGG AACCAA CAGATT TACTT CGGTA ATGACG TTGGGA AAGCG AGCGG CGGAT GGGTGA GTAAC ACGTGG GGAACC TGCCC CTAAG TCTGG GATAC CATTT GGAAA CAGGT GCTAA TACCG GATAAT AAAGC AGATC GCATGA TCAGCT TTTGA AAGGCG GCGTA AGCTG TCGCT AAGGG ATGGCC CCGCGG TGCATT AGCTAG TTGGT AAGGT AACGG CTTAC CAAGGC GACGA TGCA TAGCC GAGTT GAGAG ACTGA TCGGC CACAT TGGGA CTGAG ACACG GCCCA AACTC CTACGG GAGGCA GCAGTA GGGAA TCTTCC ACAATG GACGC AAGTCT GATGGA GCAAC GCCGCG TGAGTG AAGAAG GTTTT CGGATCG TAAAG CTCTG TTGTTG GTGAA GAAGG ATAGAGG TAGTAA CTGGCC TTTATT TGACGG TAATCA ACCAGA AAGTCAC GGCTA ACTACG TGCCAG CAGCCG CGGTAA TACGTA GGTGG CAAGCG TTGTCC GGATTT ATTGG GCGTAA AGCGA GCGCAG GCGG AAAAA TAAGT CTAATG TGAAAG CCCTCG GCTTAA CCGAG GAACT GCATC GGAAA CTGTTT TTCT TGAG TGCA GAAG AGGA GAGT GGAA CTCC ATGT GTAGC GGTGGA ATGCG

Lactobacillus amylovorus C99

GTGCCT AATACAT GCAAGT CGAGC GAGCGG AACCAA CAGAT TTACT TCGGTA ATGACG TTGGGA AAGCGA GCGGCG GATGG GTGAG TAACA CGTGG GGAACC TGCCC CTAAGT CTGGGA TACCA TTTGG AAACA GGTGC TAATA CCGGA TAATA AAGCA GATCGC ATGA TCAGC TTTTGA AAGG CGGCG TAAGCT GTCGCTA AGGGA TGGCC CCGCG GTGCAT TAGCT AGTTG GTAAGG TAACGG CTTAC CAAGGC GACGA TGCA TAGC CGAGT TGAGAG ACTGA TCGGC CACATT GGGAC

TGAGA CACGG CCCAA ACTCC TACGGG AGGCA GCAGT AGGGA ATCTT CCACAA TGGA CGCAA GTCTGA TGGAG CAACG CCGCGT GAGTG AAGAAG GTTTTC GGATCG TAAAGC TCTGT TGTTG GTGAA GAAGGA TAGAGG TAGTA ACTGG CCTTT ATTTG ACGGT AATCAA CCAGAAA GTCACGG CTAAC TACGTG CCAGCA GCCGCG GTAAT ACGTA GGTGG CAAGCG TTGTC CGGAT TTATT GGGCG TAAAG CGAGC GCAGGC GTGC CTAA TACATGC AAGT CGAGC GAGC GGAA CCAA CAGA TTTACT TCGGTA ATGA CGTT GGGA AAGC GAGC GGCGG ATGGG TGAG TAACA CGTG GGGA ACCT GCCTC TAAGTC TGGG ATAC CATT TGGA AACAG GTGC TAATA CCGGAT AATAAAG CAGA TCGC ATGAT CAGCTT

APPENDIX IVb

Bovine LAB Sequences Obtained from NCBI Genebank used for Phylogenetic Analysis

Lactobacillus amylovorus strain IQ-bovine

CAGG TCTT GACA TCTA GTGC AATC TGTA GAGA TACG GAGT TCCC TTCGG GGAC GCTA AGAC AGGTG GTGC ATGG CTGT CGTCA GCTCG TGTCG TGAGA TGTT GGGT TAAG TCCC GCAAC GAGC GCAA CCC TTGTT ATTAG TTGC CAGC AT TAA GTTG GGCA CTCTA ATGA GACT GCCG GTGA CAAA CCGG AGGA AGGT GGGG ATGA CGTC AAGT CATC ATGCC CCTT ATGA CCTGG GCTA CACAC GTGC TACA ATGG GCAG TACAA CGAG AAGC AAGC CTGC GAAG GCAAG CGAAT CTCT GAAA GCTG TTCT CAGT TCGG ACTG CAGT CTGC AACT CGACT GCAC GAAG CTGG AATC GCTA GTAA TCGC GGAT CAGC ACGC CGCG GTGAA TACGT TCCC GGGC CTTG TACA CACC GCCC GTCA CACC ATGG GAGT CTGC AATG CCCA AAG CCGGTG CAGG TCTT GACA TCTA GTGC AATC TGTA GAGA TACG GAGT TCCC TTCGG GGAC GCTA AGAC AGGTG GTGC ATGG CTGT CGTCA GCTCG TGTCG TGAGA TGTT GGGT TAAG TCCC GCAAC GAGC GCAA CCC TTGTT ATTAG TTGC CAGC AT TAA GTTG GGCA CTCTA ATGA GACT GCCG GTGA CAAA CCGG AGGA AGGT GGGG ATGA CGTC AAGT CATC ATGCC CCTT ATGA CCTGG GCTA CACAC GTGC TACA ATGG GCAG TACAA CGAG AAGC AAGC CTGC GAAG GCAAG CGAAT CTCT GAAA GCTG TTCT CAGT TCGG ACTG CAGT CTGC AACT CGACT GCAC GAAG CTGG AATC GCTA GTAA TCGC GGAT CAGC ACGC CGCG GTGAA TACGT TCCC GGGC CTTG TACA CACC GCCC GTCA CACC ATGG GAGT CTGC AATG CCCA AAG CCGGTG

Lactobacillus plantarum strain SLDL-125

ACGAA CGCTG GCGG CGTGC CTAA TACAT GCAAG TCGAA CGAACT CTGGTA TTGAT TGGTG CTTGC ATCAT GATTT ACATT TGAGT GAGTG GCGAA CTGGT GAGTA ACACG TGGGA AACCT GCCCA GAAGC GGGGGA TAACA CCTGG AAACA GATGC TAATA CCGCA TAAC AACTT GGACC GCATG GTCCG AGCTT GAAAGA TGGCT TCGGC TATCAC TTTTGG ATGGT CCCGC GGCGT ATTAG CTAGAT GGTGG GGTAA CGGCTC ACCATG GCAATGA TACGTA GCCGAC CTGAG AGGGT AATCG GCCAC ATTGGG ACTGAG ACACG GCCCA AACTC CTACGG GAGGC AGCAG TAGGG AATCT TCCAC AATGG ACGAA AGTCT GATGG AGCAA CGCCG CGTGA GTGAA GAAGG GTTTC GGCTC GTAAA ACTCT GTTGT TAAAG AAGAAC ATATC TGAGA GTAAC TGTTC AGGTA TTGA CGGTA TTTAA CCAG AAGC CACG GCTA ACTA CGTG CCAG CAGC CGCG GTAA TACG TAGG TGGC AGCG TTGT CCGG ATTT ATTG GGCG TAAA GCGA GCGC AGGC GGTT TTTT AAGT CTGAT GTGA AAGC CTTC GGCT CAAC CGAA GAAG TGCA TCGG AAAC TGGG AAAC TTGA GTGC AGAA GAGG ACAG TGGA ACTC CAT G TGTA GCGG TGAA ATGC GTAG ATAT ATGG AAGA ACAC CAGT GGCG AAGG CGGC TGTCTG GTCT GTAA CTGA CGCT GAGG CTCG AAAG TATG GGTA GCAA ACAG GATT AGAT ACCC TGGTA GTCCA TACCG TAAAC GATGA ATGCT AAGTG TTGGA GGGTT TCCGC CCTTC AGTGC TGCAG CTAAC GCATT AAGCAT TCC CCTG GGGA GTACG GCCGC AAGGC TGAAAC TCAAA GGAAT TGAC GGGG GCCC GCAC AAGC GGTG GAGC ATGT GGTT TAAT TCGA AGCT ACGC GAAGA ACCTT ACCAGG TCTTGA CATAC TATGC AAATC TAAGA GATTAGA CGTTCC

CTTCGG GGACAT GGATAC AGGTGG TGCAT GGTTG TCGTC AGCTCG TGTCGT GAGA TGTT GGGT TAAG TCCC GCAA CGAG CGCAA CCCTT ATTA TCAG TTGC CAGC ATTA AGTT GGGC ACTC TGGT GAGA CTGC CGGTG ACAAA CCGGA GGAAGG TGGGG ATGACGT CAAA TCATC ATGC CCCTT ATGACC TGGGC TACAC ACGTGC TACAAT GGATG GTACAA CGAGTT GCGAA CTCGC GAGAG TAAGC TAATC TCTTA AAGCC ATTCT CAGTT CGGAT TGTA GGCT GCAA CTCGC CTACA TGAAG TCGGA ATCGC TAGTA ATCGC GGATC AGCATG CCGCGG TGAATAC GTTCC CGGGC CTTGT ACACA CCGCC CGTCA CACCA TGAGA GTTTGT AACAC CCAAA GTCGG TGGGG TAACCT TTTAGG AACCAG CCGCCT AAGGT GGGAC AGATG ATTAG GGTG AAGTC

Lactobacillus acidophilus strain IQ-bovine

GAGC GCAGG CGGAA GAATA AGTCT GATGT GAAAGC CCTCG GCTTA ACCGAG GAACT GCATC GGAAA CTGTT TTTCT TGAGT GCAGA AGAGGA GAGT GGAAC TCCAT GTGTA GCGGT GGAAT GCGTAG ATATAT GGAAGA ACACCA GTGGC GAAG GCGG CTCTC TGGT CTGCA ACTGAC GCTG AGGCT CGAA AGCAT GGGTAG CGAAC AGGAT TAGAT ACCCT GGTAG TCCAT GCCGT AAACGA TGAGT GCTAA GTGTT GGGAG GTTTC CGCCT CTCAG TGCTG CAGCT AACGC ATTAA GCACT CCGCC TGGGG AGTAC GACCG CAAGG TTGAA ACTCAA AGGAA TTGACG GGGG CCCGC ACAAG CGGTG GAGCA TGTGG TTTAA TTCGA AGCAA CGCGAA GAACCT TACCA GGTCT TGACA TCTAG TGCAA TCCGT AGAGA TACGG AGTTCC CTTCGG GGACA CTAAG ACAGG TGGTG CATG GCTGT CGTCA GAGC GCAGG CGGAA GAATA AGTCT GATGT GAAAGC CCTCG GCTTA ACCGAG GAACT GCATC GGAAA CTGTT TTTCT TGAGT GCAGA AGAGGA GAGT GGAAC TCCAT GTGTA GCGGT GGAAT GCGTAG ATATAT GGAAGA ACACCA GTGGC GAAG GCGG CTCTC TGGT CTGCA ACTGAC GCTG AGGCT CGAA AGCAT GGGTAG CGAAC AGGAT TAGAT ACCCT GGTAG TCCAT GCCGT AAACGA TGAGT GCTAA GTGTT GGGAG GTTTC CGCCT CTCAG TGCTG CAGCT AACGC ATTAA GCACT CCGCC TGGGG AGTAC GACCG CAAGG TTGAA ACTCAA AGGAA TTGACG GGGG CCCGC ACAAG CGGTG GAGCA TGTGG TTTAA TTCGA AGCAA CGCGAA GAACCT TACCA GGTCT TGACA TCTAG TGCAA TCCGT AGAGA TACGG AGTTCC CTTCGG GGACA CTAAG ACAGG TGGTG CATG GCTGT CGTCA.

Lactobacillus crispatus strain IQ-bovine

GCAC AAGC GGTG GAGC ATGT GGTT TAAT TCGA AGCA ACGC GAAG AACC TTAC CAGG TCTT GACA TCTA GTGC CATT TGTA GAGA TACA AAGT TCCC TTCG GGGA CGCT AAGA CAGG TGGT GCAT GGCT GTCG TCAG CTCGTGTC GTGA GATGT TGGGT TAAGT CCCG CAACG AGCG CAAC CCTTG TTAT TAGTT GCCA GCATT AAGT TGGGC ACTC TAAT GAGA CTGC CGGT GACA AACC GGAGG AAGGT GGGGA TGACG TCAAG TCATC ATGC CCCT TATG ACCT GGGC TACA CACG TGCT ACAA TGGG CAGT ACAA CGAG AAGC GAGC CTGC GAAG GCAA GCGA ATCTCTGA AAGC TGTT CTCA GTTC GGAC TGCA GTCT GCAA CTCG ACTG CACG AAGC TGGA ATCG CTAG TAAT CGCG GATC AGCA CGCC GCGG TGAA TACG TTCC CGGG CCTT GTAC ACAC CGCC CGTC ACAC CATG GGAG TCTG GCAC AAGC GGTG GAGC ATGT GGTT TAAT TCGA AGCA ACGC GAAG AACC TTAC CAGG TCTT GACA TCTA GTGC CATT TGTA GAGA TACA AAGT TCCC TTCG GGGA CGCT AAGA

CAGG TGGT GCAT GGCT GTCG TCAG CTCGTGTC GTGA GATGT TGGGT TAAGT CCCG CAACG AGCG CAAC CCTTG TTAT TAGTT GCCA GCATT AAGT TGGGC ACTC TAAT GAGA CTGC CGGT GACA AACC GGAGG AAGGT GGGGA TGACG TCAAG TCATC ATGC CCCT TATG ACCT GGGC TACA CACG TGCT ACAA TGGG CAGT ACAA CGAG AAGC GAGC CTGC GAAG GCAA GCGA ATCTCTGA AAGC TGTT CTCA GTTC GGAC TGCA GTCT GCAA CTCG ACTG CACG AAGC TGGA ATCG CTAG TAAT CGCG GATC AGCA CGCC GCGG TGAA TACG TTCC CGGG CCTT GTAC ACAC CGCC CGTC ACAC CATG GGAG TCTG

Lactobacillus acidophilus strain U234 bovine

CAAGTA GAACG CTGAA GACT TTAG CTTG CTAA AGTT GGAA GAGTTGC GAAC GGGT GAGT AACG CGTA GGTA ACCTG CCTACT AGCGG GGGA TAAC TATT GGAA ACGAT AGCTA ATACCGCA TAACA GCA TTTAA CTCAT GTTAGA TGCTTGA AAGGAGC AATTGCTT CACTA GTAGATG GACCTG CGTT GTAT TAGC TAGT TGGT GAGGTAA CGGC TCAC CAAG GCGAC GATACA TAGCCGA CCTGAG AGGGTG ATCGGC CACA CTGGG ACTGA GACA CGGC CCAGACT CCTACG GGAG GCAG CAGT AGGGA ATCTT CGGCA ATGGG GGCAA CCCT GACCG AGCAA CGCCG CGTGA GTGAAG AAGGT TTTCG GATCGT AAAGC TCTGT TGTAA GAGAA GAACG TGTGT GAGAG TGGAA AGTTCA CACAG TGACG GTAAC TTACC AGAAA GGGAC GGCTA ACTAC GTGCC AGCAG CCGCGG TAATA CGTAGG TCCCTA GCGTN GTCCGG ATTAAT TGGGCG TAAAG CGAGC GCAGG CGGTTT AATAA GTCTGA AGTTAA AGGC AGTT GGCT TAACCA TGGTT CGCTT TGGA AACT GTAA GACTTGTGCC TAATAC ATGCAA GTCGAA CGCGTT GGCCCA ACTGATT GAACGT GCTTGCA CGGACT TGACGTT GGTTTA CCAGCG AGTGGC GGACGG GTGAG TAACAC GTAAGT AACCTG CCCCAA AGCGGG GGATAA CATTT GGAAA CAGAT GCTAA TACCGC ATAGA CAATTT AGAATC GCATGA TTCAAA TTTAAA AGATG GCTTC GGCTAT CACTTT GGGAT GGACCT GCGGC GCATTA GCTTG TTGGTA GGGTAA CGGCC TACCA AGGCT GTGATG CGTAG CCGAGT TGAGA GACTG ATCGGC CACAA TGGAAC TGAGA CACGG TCCATA CTCCTA CGGGA GGCAG CAGTA GGGAAT CTTCCA CAATGG GCGCAA GCCTG ATGGA GCAACA CCGCG TGAGTG AAGAA GGGTT TCGGC TCGTAT AAGCT CTGTTG TTAGA GAAGA ACGTG CGTGA GAGCA ACTAGT TCACGC AGTGAC GGTAT CTAACC AGAGAG GCACGG CTAACT ACGTGCC AGCAGC CGCGGT AGACG TAGGTG GCAAG CGTCATC CGGATC TATTGG GCGTA CAGCG AGCGC AGGCG GATCTG ATAC GTCTG ATGT GACAG GTGC CTAAT ACATG

Lactobacillus amylovorus strain IQ-bovine milk no.2

CAGG TCTT GACA TCTA GTGC AATC TGTA GAGA TACG GAGT TCCC TTCG GGGA CGCT AAGA CAGG TGGT GCAT GGCT GTCG TCAG CTCG TGTC GTGA GATG TTGG GTTA AGTC CCGC AACG AGCG CAAC CCTT GTTA TTAG TTGC CAGC ATTA AGTT GGGC ACTC TAAT GAGA CTGC CGGT GACA AACC GGAG GAAG GTGG GGAT GACG TCAA GTCA TCAT GCCC CTTA TGAC CTGG GCTA CACA CGTG CTAC AATG GGCA GTAC AACG AGAA GCAA GCCT GCGA AGGC AAGC GAAT CTCT GAAA GCTG TTCT CAGT TCGG ACTG CAGT CTGC AACT CGAC TGCA CGAA GCTG GAAT CGCT AGTA ATCG CGGA TCAG CACG CCGC GGTG AATA CGTT CCCG GGCC TTGT ACAC ACCG CCCG TCAC ACCA TGGG AGTC TGCA ATGC CCAA AGCC GGTG CAGG TCTT GACA TCTA GTGC AATC TGTA GAGA TACG GAGT TCCC TTCG GGGA CGCT AAGA CAGG TGGT GCAT GGCT GTCG TCAG

CTCG TGTC GTGA GATG TTGG GTTA AGTC CCGC AACG AGCG CAAC CCTT GTTA TTAG TTGC CAGC ATTA AGTT GGGC ACTC TAAT GAGA CTGC CGGT GACA AACC GGAG GAAG GTGG GGAT GACG TCAA GTCA TCAT GCCC CTTA TGAC CTGG GCTA CACA CGTG CTAC AATG GGCA GTAC AACG AGAA GCAA GCCT GCGA AGGC AAGC GAAT CTCT GAAA GCTG TTCT CAGT TCGG ACTG CAGT CTGC AACT CGAC TGCA CGAA GCTG GAAT CGCT AGTA ATCG CGGA TCAG CACG CCGC GGTG AATA CGTT CCCG GGCC TTGT ACAC ACCG CCCG TCAC ACCA TGGG AGTC TGCA ATGC CCAA AGCC GGTG

Streptococcus equinus strain G3

ATACA TGCAAG TAGAAC GCTGAA GACTTT AGCTTG CTAAAG TTGGAA GAGTTG CGAAC GGGTG AGTAA CGCGT AGGTAA CCTGCC TACTA GCGGG GGAT AACT ATTGG AAACG ATAGC TAATA CCGCA TAACA GCATT TAACT CATGT TAGA TGCT TGAA AGGA GCAA TTGC TTCA CTAG TAGAT GGACC TGCGT TGTAT TAGCT AGTTGG TGAG GTAAC GGCTC ACCAA GGCGA CGATA CATAG CCGAC CTGAG AGGGT GATCGG CCACA CTGGG ACTGAG ACACG GCCCA GACTC CTACGGG AGGCA GCAGT AGGGA ATCTT CGGCA ATGGG GGCAA CCCTGA CCGAGC AACGC CGCGT GAGTG AAGAA NGGTTT TCGGA TCGGT AAAGC TCTGT TGTAA GAGAA GAAC GTGT GTGA GAGT GGA AAGT TCACA CAGTG ACGGTA ACTTA CCAGA AAGGG ACGGC TAACT ACGTG CCAGC AGCCG CGGTAAT ACGTAG GTCCCG AGCGT TGTC CGGA TTTA TTGG GCGT AAAG CGAGCG CAGGC GGTTTAA TAAGT CTGAAG TTAA AGGC AGTGGC TTAACC ATTGTT CGCTT TGGAAA CTGTTA GACT TGAG TGCA GAAG GGGA GAGT GGAA TTCC ATGT GTAG CGGT GAAAT GCGT AGATA TATGG AGGAAC ACCG GTGG CGAA AGCGGC TCTCTG GTCTGT AACTG ACGCTG AGGCTC GAAAG CGTGG GGAGC AAACA GGATT AGATA CCCTGG TAGTC CACGCC GTAAA CGATG AGTGCT AGGTG TTAGGC CCTTTC CGGGGC TTAGTG CCGCAG CTAACG CATTAA GCACTC CGCCT GGGG AG TACGAC CGCAAG GTTGAA ACTCAA AGGAA TTGACG GGGGC CCGCA CAAGC GGTGG AGCAT GTGGT TTAATT CGAAGC AACGCG AAGAAC CTTAC CAGGTC TTGAC ATCC CGAT GCTA TTCCTA GAGATAG GAAGT TTCTTC GGAAC ATCGG TGACAG GTGGTG CATGGT TGTCGT CAGCTC GTGTCC TGAGAT GTTGG GTTAAA TCCCG CAACGA GCGCA ACCC CTAT TGTT AGTTGC CATC ATTA AGTG GGGC ACTC TAAC GAGA CTGC CGGT AATA AACC GGAA GAAA GGTG GGGA TAAC GTCAA ATCA TCATGC CCCTT ATGACC TGGGC TACACA CCGTGC TACA GGTT GGGAC AACC AAGT CCCGA ATCCGT GGAACG GCAAGC AAATC TCTT AAAG CCAA TCTC AGTT CGGA TTGTAGG CTGCAA CTCGCC TACATG AAGT CGGA ATCGC TAGT AATC GCGGA TCAG CACG CCGC GGTGAA TACGT TCCCGG GCCTTG TACACA CCGCCC GTCACA CCACGA GAGTTT GTAACA CCCGAA GTCG GTGA GGTAA CCTTT TAGGA GCCAG CCGC

Streptococcus infantarius strain HDP90056

CTAAT ACAT GCAAG TAGAA CGCT GAAA ACTTT AGCTT GCTAA AGTTT GAAG AGTT GCGAA CGGG TGAGT AACGC GTAG GTAA CCTG CCTA CTAG CGGG GGATA ACTA TTGG AAAC GATA GCTA ATACC GCAT AACA GCAT TTAA CCCAT GTTAG ATGC TTGAA AGG AGCA ATTG CTTCA CTAGT AGAT GGAC CTGC GTTG TATT AGCT AGTT GGTG AGGT AACG GCTC ACCA AGGC GACG ATAC ATAG CCGA CCTG AGAG GGTG ATCG GCCA CACT GGGA CTGA GACA CGGC CCAG ACTCC TACG GGAG GCAG CAGT AGGG AATC TTCG GCAAT GGGGG CAACC CTGA CCG AGCA ACGC

CGCG TGAG TGAA GAAG GTTT TCGG ATCG TAAA GCTC TGTTG TAAGA GAAG AATG TGTG TGAG AGTG GAAAG TTCA CACA GTGA CGGT AACT TACCA GAAA GGGA CGGC TAAC TACG TGCCA GCAG CCGC GGTA ATAC GTAG GTCC CGAG CGTT GTCC GGAT TTAT TGGG CGTA AAGC GAGC GCAG GCGGT TTAA TAAG TCTG AAGTT AAAG GCAGT GGCTT AACC ATTGT TCGCT TTGGAA ACTG TTAGA CTTGA GTGC AGAA GGGG AGAG TGGAA TTCC ATGTG TAGCG GTGAA ATGC GTAGA TATAT GGAGG AACAC CGGT GGCG AAAG CGGC TCTC TGGTC TGTAA CTGAC GCTGA GGCT CGAAA GCGTG GGGAG CAAA CAGGA TTAGA TACCC TGGTA GTCC ACGCC GTAA ACGAT GAGT GCTA GGTGT TAGG CCCT TTCCG GGGC TTAGT GCCG CAGC TAAC GCATT AAGC ACTC CGCC TGGG GAGT ACGAC CGCAA GGTT GAAA CTCA AAGGA ATTG ACGGG GGCC CGCA CAAG CGGTG GAGC ATGT GGTTT AATT CGAA GCAA CGCG AAGA ACCT TACC AGGTC TTGAC ATCGA TGCT ATTC CTAGA GATA GGAA GTTTC TTCG GAAC ATCG GTGA CAGG TGGT GCAT GGTT GTCGT CAGCT CGTG TCGT GAGAT GTTG GGTT AAGT CCCGC AACG AGCGC AACC CCTA TTGT TAGTT GCCA TCAT TAAGT TGGG CACT CTAGC GAGA CTGC CGGTA ATAA ACCG GAGG AAGG TGGGG ATGA CGTC AAAT CATC ATGC CCCT TATGA CCTGG GCTA CACAC GTGC TACA ATGG TTGGT ACAA CGAG TCGCG AGTC GGTG ACGG CAAG CAAA TCTC TTAA AGCC AATCT CAGT TCGGA TTGT AGGC TGCA ACTCG CCTA CATG AAGT CGGA ATCGC TAGTA ATCG CGGA TCAG CACG CCGC GGTG AATAC GTTC CCGG GCCT TGTA CACA CCGC CCGT CACA CCACG AGA GTTT GTAAC

Streptococcus bovis isolate LP339

GACG AACG CTGG CGGC GTGC CTAA TACA TGCAA GTAG AACG CTGA AGAC TTTA GCTT GCTA AAG TTGG AAGA GTTG CGAA CGGG TGAG TAAC GCGT AGGT AACC TGCC TACT AGCG GGGG ATAA CTAT TGGA AACG ATAG CTAA TACC GCAT AACA GCAT TTAA CCCA TGTT AGAT GCTT GAAA GGAG CAAT TGCT TCAC TAGT AGAT GGAC CTGC GTTG TATT AGCTA GTTG GTGA GGTA ACGG CTCA CCAAG GCGA CGAT ACAT AGCC GACC TGAG AGGG TGAT CGGC CACA CTGG GACT GAGA CACG GCCC AGAC TCCT ACGG GAGG CAGC AGTA GGGAATCT TCGG CAAT GGGG GCAA CCCT GACC GAGC AACG CCGC GTGA GTGA AGAAG GTTT TCGG ATCG TAAA GCTC TGTTG TAAG AGAA GAACG TGTG TGAG AGTGG AAAG TTCAC ACAGT GACGG TAAC TTAC CAGA AAGG GACG GCTAA CTAC GTGC CAGC AGCC GCGG TAAT ACGT AGGT CCCG AGCG TTGT CCGG ATTT ATTGG GCGT AAAG CGAG CGCA GGCG GTTT AATA AGTC TGAA GTTA AAGG CAGT GGCT TAAC CATT GTTC GCTT TGGA AACT GTT AGAC TTGA GTGC AGAA GGGG AGAG TGGA ATTCC ATGT GTAG CGGT GAAA TGCG TAGA TATA TGGA GGAA CACC GGTG GCGA AAGC GGCT CTCT GGTC TGTA ACTG ACGC TGAG GCTCG AAAG CGTG GGGAG CAAA CAGG ATTA GATA CCCT GGTA GTCC ACGC CGTAA ACGAT GAGTG CTAGG TGTTA GGCCC TTTC CGGGG CTTAG TGCCG CAGCT AACG CATT AAGC ACTC CGCC TGGG

Streptococcus bovis isolate LP2990

GACGA ACGC TGGC GGCG TGCC TAAT ACAT GCAA GTAG AACG CTGA AGAC TTTAG CTTG CTAA AGTT GGAA GAGT TGCG AACG GGTG AGTAA CGCGT AGGT AACC TGCCT ACTA GCGG GGGA TAAC TATT GGAA ACGA TAGC TAAT ACCG CATA ACAG CATTT AACC CATGT TAGA TGCT TGAA AGGAG CAAT TGCT TCACT AGTA GATG GACCT GCGT TGTA TTAG CTAG TTGG TGAG GTAA

CGGC TCAC CAAG GCGA CGAT ACAT AGCC GACC TGAG AGGG TGAT CGGC CACA CTGG GACT GAGA CACG GCCC AGAC TCCT ACGG GAGG CAGC AGTA GGGA ATCT TCGG CAAT GGGG GCAA CCCT GACC GAGC AACG CCGC GTGA GTGA AGAA GGTT TTCG GATC GTAA AGCT CTGT TGTA AGAG AAGA ACGT GTGT GAGA GTGG AAAG TTCA CACA GTGA CGGT AACT TACC AGAA AGGG ACGG CTAA CTAC GTGC CAGC AGCC GCGG TAAT ACGT AGGT CCCG AGCG TTGT CCGG ATTT ATTG GGCG TAAA GCGA GCGC AGGC GGTT TAAT AAGT CTGA AGTT AAAG GCAG TGGC TTAA CCAT TGTT CGCT TTGG AAAC TGTT AGAC TTGA GTGC AGAA GGGG AGAG TGGA ATTC CATG TGTA GCGG TGAA ATGC GTAG ATAT ATGG AGGA ACAC CGGTG GCGA AAGC GGCTC TCTG GTCT GTAA CTGAC GCTG AGGC TCGA AAGC GTGGG GAGCA AACA GGAT TAGA TACC CTGG TAGT CCACG CCGT AAAC GATG AGTG CTAG GTGT TAGG CCCT TTCC GGGG CTTA GTGC CGCA GCTA ACGC ATTA AGCG TCCG CTTG CACG ACGC CGGG GGTTAT

Streptococcus bovis strainLP278 16S ribosomal RNA gene, partial sequence

GACG AACG CTGG CGGC GTGC CTAA TACA TGCA AGTA GAAC GCTG AAGA CTTT AGCT TGCT AAAG TTGG AAGA GTTG CGAA CGGG TGAG TAAC GCGT AGGT AACC TGCC TACT AGCG GGGG ATAA CTAT TGGA AACG ATAG CTAA TACC GCAT AACA GCAT TTAA CCCA TGTT AGAT GCTT GAAA GGAG CAAT TGCT TCAC TAGT AGAT GGAC CTGC GTTG TATT AGCT AGTT GGTG AGGT AACG GCTC ACCAA GGCG ACGA TACAT AGCC GACC TGAG AGGG TGAT CGGC CACA CTGG GACT GAGA CACG GCCC AGAC TCCT ACGG GAGG CAGC AGTA GGGA ATCT TCGG CAAT GGGG GCAA CCCT GACC GAGCA ACGC CGCG TGAG TGAA GAAG GTTT TCGG ATCG TAAA GCTC TGTT GTAA GAGA AGAA CGTG TGTGA GAGT GGAA AGTT CACA CAGT GACG GTAA CTTA CCAG AAAG GGAC GGCT AACT ACGT GCCA GCAG CCGC GGTA ATAC GTAG GTCC CGAG CGTT GTCC GGAT TTATT GGG CGTA AAGC GAGC GCAG GCGG TTTA ATAA GTCT GAAG TTAA AGGC AGTG GCTT AACC ATTGT GACG AACG CTGG CGGC GTGC CTAA TACA TGCA AGTA GAAC GCTG AAGA CTTT AGCT TGCT AAAG TTGG AAGA GTTG CGAA CGGG TGAG TAAC GCGT AGGT AACC TGCC TACT AGCG GGGG ATAA CTAT TGGA AACG ATAG CTAA TACC GCAT AACA GCAT TTAA CCCA TGTT AGAT GCTT GAAA GGAG CAAT TGCT TCAC TAGT AGAT GGAC CTGC GTTG TATT AGCT AGTT GGTG AGGT AACG GCTC ACCAA GGCG ACGA TACAT AGCC GACC TGAG AGGG TGAT CGGC CACA CTGG GACT GAGA CACG GCCC AGAC TCCT ACGG GAGG CAGC AGTA GGGA ATCT TCGG CAAT GGGG GCAA CCCT GACC GAGCA ACGC CGCG TGAG TGAA GAAG GTTT TCGG ATCG TAAA GCTC TGTT GTAA GAGA AGAA CGTG TGTGA GAGT GGAA AGTT CACA CAGT GACG GTAA CTTA CCAG AAAG GGAC GGCT AACT ACGT GCCA GCAG CCGC GGTA ATAC GTAG GTCC CGAG CGTT GTCC GGAT TTATT GGG CGTA AAGC GAGC GCAG GCGG TTTA ATAA GTCT GAAG TTAA AGGC AGTG GCTT AACC ATTGT

Streptococcus bovis strain LP

GACGA ACGC TGGC GGCG TGCC TAAT ACAT GCAA GTAG AACG CTGA AGAC TTTAG CTTG CTAA AGTT GGAA GAGT TGCG AACG GGTG AGTAA CGCGT AGGT AACC TGCCT ACTA GCGG GGGA TAAC TATT GGAA ACGA TAGC TAAT ACCG CATA ACAG CATTT AACC CATGT TAGA TGCT TGAA AGGAG CAAT TGCT TCACT AGTA GATG GACCT GCGT TGTA TTAG CTAG TTGG TGAG GTAA CGGC TCAC CAAG GCGA CGAT ACAT AGCC GACC TGAG AGGG TGAT CGGC CACA CTGG GACT

GAGA CACG GCCC AGAC TCCT ACGG GAGG CAGC AGTA GGGA ATCT TCGG CAAT GGGG GCAA CCCT GACC GAGC AACG CCGC GTGA GTGA AGAA GGTT TTCG GATC GTAA AGCT CTGT TGTA AGAG AAGA ACGT GTGT GAGA GTGG AAAG TTCA CACA GTGA CGGT AACT TACC AGAA AGGG ACGG CTAA CTAC GTGC CAGC AGCC GCGG TAAT ACGT AGGT CCCG AGCG TTGT CCGG ATTT ATTG GGCG TAAA GCGA GCGC AGGC GGTT TAAT AAGT CTGA AGTT AAAG GCAG TGGC TTAA CCAT TGTT CGCT TTGG AAAC TGTT AGAC TTGA GTGC AGAA GGGG AGAG TGGA ATTC CATG TGTA GCGG TGAA ATGC GTAG ATAT ATGG AGGA ACAC CGGTG GCGA AAGC GGCTC TCTG GTCT GTAA CTGAC GCTG AGGC TCGA AAGC GTGGG GAGCA AACA GGAT TAGA TACC CTGG TAGT CCACG CCGT AAAC GATG AGTG CTAG GTGT TAGG CCCT TTCC GGGG CTTA GTGC CGCA GCTA ACGC ATTA AGCG TCCG CTTG CACG ACGC CGGG GGTTAT

Streptococcus bovis Tu

TGGT GCTA TCCT TGTA GTAG CTTC TACA GATG GTCC AATG CCAC AAAC ACGT GAAC ACAT CCTT CTTT CACG TCAA GTTG GTGT TAAA CACC TTAT CGTC TTCA TGAA CAAA GTTG ACCT TGTT GATG ACGA AGAA TTGC TTGA ATTG GTTG AAAT GGAA ATCC GTGA CCTT CTTT CAGA ATAT GATT TCCC AGG TGAT GAAA TCCC TGTA ATCC AAGG TTCAG CTCT TAAA GCCC TTGA AGGT GACA CTCA CTAC GAAG ACAT CATC ATGG AATT GATG AACA CTGT AGAT GAAT ACAT TCCA GAAC CAAA ACGT GATA CTGA CAAA CCAT TGCT TCTT CCAG TCGA AGAC GTAT TCTC AATC ACTG GTCG TGGT ACTG TAGC ATCA GGAC GTAT CGAC CGTG GTAC TGTT AAAG TCAA CGAC GAAG TTGA AATC GTTG GTAT CCGT GACG ACAT CCAA AAAG CTGT TGTT ACTG GTGTT GAAA TGTT CCGT AAAC AACT TGAT GAAG GTAT CGCA GGGGATA ACGT TGGT GTTC TTCT TCGT GGTA TCCA ACGT GATG AAAT CGAA CGTG GTCA AGTT CTTG CTAA ACCA GGTT CAAT CCAC CCAC ACAC TAAA TTCA AAGG TGAA GTTT ACAT CCTT ACTA AAGA AGAA GGTG GACG TCAC ACTC CATT CTTC AACA ACTA CCGT CCTC AATT CTAC TTCC GTAC AACT GACG TTAC AGGT TCAA TCGA ACTT CCAG CAGG TACT GAAA TGGT AATG CCTG GTGA TAAC GTTA CTAT CGAC GTTG AATT GATT CACC CAAT CGCC GTTG AACA AGGT ACTACAT

Enterococcus hirae strain K41

TAGA AAAA GGGG GTCC TAAA AATG CAAG TCGA GCGC TTCT TTTT CCTC CGGA ACTTG CTCC ACCG CGAA AAAG AGGA GTGG CGAA CGGG TGAG TAAC ACTT GGGT GACC TGCC CATC TTAA GGGG GTAA AACT TGGA CCAG GTGC TAAT ACCT TATAA CAAT CGAA ACCG CATG GTTT TGAT TTGA AAGG CGCT TTCG GGTG TCAC TGAT GGAT GGAC CCGC GGTG CATT AGCT AGTT GGCG AGGT AACG GCTC ACCA AGGC GACG ATGC CAGC CGAG CTGA GAGG GTGA TCGG CCAC CTTG GAACT GAGA CACG GCCC AGACT CCTA CGGGA GGGA GCAA TAGG GAGT CTTC GGCA ATGG ACGA AAGT CTGA CCGA CCAA CGCC GCGG GAGT GATC ATTG TTTT CGGA TCGA AAAA CTCT GTTG GTAG AGAA GAAC AAGG ATGA TAGT AACTG TTCAT CCCTT GACG GTAT CTAA CCAG AAAG CCAC GGCT AACT AGTG GCCA GCAT CCGC GGTAA TACA TAGG TGGC AAGC TTTG TCCG GATT TATT GGGC GTAA AGCG ACGC AAGC ATTTC TTAA GTCT GATG TGAA ATCCC CCGGC TCATC CTGG GGAG GTGC TTTG GAAC TGGGA

Enterococcus hirae strain SU

CCCG NTAA CAAT CGAAA CCGCA AGGTT TCGNA TTGAA AGGGC CTTT CGGGG TCCGC TGATG GATG GACC CCCG GTGC ATTA GCTAG TGGT GAGG TAAC GGCT CCCC AAGG CGAC GATG CATA GCCG ACCT GAG AGGG TGAT CGGC CACA TTGG GACTG AGACA CGGC CCAA ATTC CTAC GGGA GGCAG CAGT AGGG AATC TTCG GCAA TGGA CGAA AGTC TGAC CGAG CAAC GCCG CGTGA GTGA AGAA GGTT TTCG GATC GTAA AACT CTGT TGTT AGAG AAGA ACAA GGAT GAGA GTAA CTGT TCAT CCCT TGACG GTAT CTAAC CAGA AAGC CACG GCTA ACTA CGTG CCAG CAGCC GCGG TAATA CGTA GGTGG CAAG CGTT GTCCG GATT TATT GGGC GTAA AGCG AGCG CAGGC GGTT TCTT AAGT CTGA TGTG AAAG CCCC CGGC TCAA CCGG GGAG GGTCA TTGGA AACT GGGA GACT TGAG TGCA GAAG AGGA GAGT GGAA TTCCA TGTG TAGC GGTGA AATG CGTA GATA TATG GAGG AACA CCAG TGGC GAAG GCGG CTCT CTGG TCTG TAACT GACGC TGAG GCTCG AAAGC GTGGG GAGCA AACAG GATT AGAT ACCC TGGT AGTC CACG CCGT AAACG ATGA GTGC TAAGT GTTG GAGG GTTT CCGC CCTT CAGTG CTGC AGCT AACGC ATTA AGCA CTCC GCCT GGGG AGTAC GACCG CAAGG TTGA AACTC AAAG GAAT TGACG GGGG CCCG CACA AGCG GTGG AGCA TGTG GTTT AATT CGAA GCAAC GCGA AGAA CCTT ACCA GGTC TTGA CATC CTTT GACCA CTCT AGAG ATAG AGCT TCCC CTTCG GGGG CAAA GTGA CAGG TGGT GCAT GGTT GTCG TCAG CTCG TGTCG TGAG ATGT TGGG TTAA GTCC CGCAA CGAG CGCA ACCCT TATT GTTA GTTG CCAT CATT TAGT TGGG CACT CTAG CAAG ACTG CCGG TGACA AACC GGAG GAAG GTGG GGAT GACG TCAAA TCAT CATG CCCC TTAT GACC TGGG CTAC ACAC GTGC TACA ATGG GAAG TACAA CGAGT CGCA AAGT CGCGA GGCT AAGC TAAT CTCT TAAA GCTT CTCT CAGT TCGG ATTG TAGG CTGC AACT CGCC TACA TGAAG CCGG AATC GCTA GTAA TCGC GGAT CAGC ACGC CGCG GTGA ATAC GTTCC CGGGC CTTG TACA CACC GCCC GTCA CACCA CGAG AGTT TGTA ACCC CGAA GTCG GTGA GGTA ACCT TTTG GAGC CAGC CGCC TAAA

Enterococcus hirae strain ARBS-1

TGGC GGCG TGCC TAAT ACAT GCAA GTCG AACG CTTCT TTTT CCACC GGAG CTTG CTCC ACCG GAAA AAGA GGAG TGGC GAAC GGGT GAGT AACA CGTG GGTA ACCT GCCC ATCA GAAG GGGA TAAC ACTT GGAA ACAG GTGC TAAT ACCG TATA ACAA TCGA AACC GCAT GGTT TTGA TTTG AAAG GCGC TTTC GGGT GTCG CTGA TGGA CAAG GATG AGAG TAAC TGTT CATC CCTT GACG GTAT CTAA CCAG AAAG CCAC GGCT AACT ACGT GCCA GCAG CCGC GGTA ATAC GTAG GTGG CAAG CGTT GTCC GGAT TTAT TGGG CGTA AAGC GAGC GCAG GCGG TTTC TTAA GTCT GATG TGAAA GCCC CCGG CTCA ACCG GGGA GGGT CATT GGAA ACTG GGAG ACTT GAGT GCAG AAGA GGAG AGTG GAAT TCCA TGTG TAGC GGTG AAAT GCGT AGAT ATAT GGAG GAACA CCAG TGGC GAAG GCGG CTCT CTGG TCTGT AACT GACG CTGA GGCT CGAA AGCG TGGG GAGC AAAC AGGA TTAG ATAC CCTGG TAGTC CACG CCGT AAAC GATG AGTG CTAA GTGT TGGA GGGT TTCC GCCCT TCAG TGCT GCAG CTAA CGCA TTAA GCAC TCCG CCTG GGGA GTAC GACC GCAA GGTT GAAA CTCA AAGG AATT GACG GGGG CCCG CACA AGCG GTGG AGCA TGTG GTTT AATT CGAA GCAA CGCG AAGA ACCT TACC AGGT CTTG ACAT CCTT TGAC CACT CTAG AGAT AGAG CTTC CCCTT CGGG GGCA AAGT GACA GGTG GTGC ATGG TTGT CGTC AGCT CGTG TCGT GAGA TGTT GGGT TAAG TCCC GCAA CGAG CGCA ACCC TTAT TGTT AGTT GCCA TCAT TTAG TTGG GCAC

TCTA GCAA GACT GCCG GTGA CAAA CCGG AGGA AGGT GGGG ATGA CGTC AAAT CATC ATGCC CCTT ATG ACCT GGGC TACA CACG TGCTA CAAT GGGA AGTA CAAC GAGT CGCA AAGT CGCG AGGC TAAG CTAA TCTC TTAA AGCT TCTC TCAG TTCG GATT GTAG GCTG CAAC TCGC CTAC ATGA AGCCGGA

Enterococcus hirae strain R

CCTG GCTC AGGA CGAA CGCT GGCGG CGTG CCTA ATAC ATGC AAGT CGAA CGCT TCTT TTTC CACC GGAG CTTG CTCC ACCG GAAA AAGAG GAGTG GCGA ACGG GTGAG TAAC ACGTG GGTAA CCTG CCCA TCAG AAGG GGAT AACA CTTG GAAA CAGG TGCT AATA CCGT ATAA CAAT CGAA ACCG CATG GTTT TGAT TTGA AAGG CGCT TTCG GGTG TCGC TGAT GGAT GGAC CCGC GGTG CATTA GCTA GTTG GTGA GGT AACG GCTC ACCA AGGCG ACGA TGCA TAGC CGAC CTGA GAGGG TGAT CGGC CACA TTGG GACT GAGA CACG GCCC AAAC TCCT ACGG GAGG CAGC AGTA GGGA ATCT TCGG CAAT GGAC GAAA GTCT GACC GAGC AACG CCGC GTGAG TGAA GAAG GTTT TCGG ATCG TAAA ACTC TGTT GTTA GAG AGAA CAAG GATG AGAG TAACT GTTC ATCC CTTG ACGG TATC TAAC CAGA AAGC CACG GCTA ACTA CGTG CCAG CAGC CGCG GTAA TACG TAGGT GGCA AGCGT TGTC CGGA TTTA TTGG GCGT AAAG CGAG CGCA GGCG GTTT CTTA AGTC TGAT GTGA AAGC CCCC GGC TCAA CCGG GGAG GGTC ATTG GAAA CTGG GAGA CTTG AGTG CAGA AGAG GAGA GTGG AATT CCAT GTGT AGCG GTGA AATG CGTA GATA TATG GAGG AACA CCAG TGGC GAAG GCGG CTCT CTGG TCTG TAAC TGAC GCTG AGGC TCGA AAGC GTGG GGAG CAAA CAGG ATTA GATA CCCT GGTA GTCC ACGC CGTA AACG ATGA GTGC TAAGT GTTG GAGG GTTT CCGC CCTT CAGT GCTG CAGCT AACG CATT AAGC ACTC CGCC TGGG GAGT ACGA CCGC AAGG TTGAA ACTC AAAG GAAT TGAC GGGG GCC CGCA CAAG CGGT GGAG CATG TGGT TTAA TTCG AAGC AACG CGAA GAAC CTTAC CAGG TCTT GACAT CCTTT GACC ACTC TAGA GATA GAGC TTCC CCT TCGG GGGC AAAG TGAC AGGT GGTG CATG GTTG TCGT CAGC TCGTG TCGT GAGA TGTT GGGT TAAG TCCC GCAA CGAG CGCA ACCC TTAT TGTTA GTTG CCAT CATT TAGT TGGG CACT CTAG CAAG ACTG CCGG TGAC AAAC CGGA GGAA GGTGG GGAT GACG TCAA ATCA TCAT GCCC CTTA TGAC CTGG GCTA CACA CGTG CTAC AATG GGAA GTAC AACG AGTC GCAA AGTC GCGA GGCT AAGC TAAT CTCT TAAA GCTT CTCT CAGT TCGG ATTG TAGG CTGC AACT CGCC TACA TGAA GCCG GAAT CGCT AGTA ATCG CGGA TCAG CACG CCGC GGTG AATA CGTT CCCG GGCC TTGT ACAC ACCG CCCG TCAC ACCA CGAG AGTT GTAA CACC CGAA GTCG GTGA GGTA ACCT TTTG GAGC CAGC CGCC TAAG GTGG GATA GATG ATTG GGGT GAAG TCGT AACA AGGT AGCC GTAT CGGA AGGT GCGG CTGG ATCA

Enterococcus hirae strain K42

AAGC AATG CGGG TACT ATAA TGCA GTCG AACG CTTC TTTT TCAC CGGA GCTT GCTC CACC GGAA AAAG AGGA GTGG CGAA CGGG TGAG TAAC ACGT GGGT AACC TGCC CATC AGAA GGGG ATAA CACT TGGA AACA GGTG CTAA TACC GTAT AACA ATCG AAAC CGCA TGGT TTTG ATTT GAAA GGCG CTTTC CGGTG TCGC TGAT GGAT GGAC CCGC GGTG CATT AGCT AGTT GGTG AGGG AACGG CTCA CCAA GGGG ACGA TGCA TACT CGAC CTGA TAGG GTGA TCGG TCCA ATGG GAC GAGGG TGAT CGGC CACA TTGG GACT GAGA CACG GCCC AAAC TCCT ACGG GAGG CAGC AGTA GGGA ATCT TCGG CAAT GGAC GAAA GTCT GACC GAGC AACG CCGC GTGAG TGAA GAAG GTTT

TCGG ATCG TAAA ACTC TGTT GTTA GAG AGAA CAAG GATG AGAG TAACT GTTC ATCC CTTG ACGG TATC TAAC CAGA AAGC CACG GCTA ACTA CGTG CCAG CAGC CGCG GTAA TACG TAGGT GGCA AGCGT TGTC CGGA TTTA TTGG GCGT AAAG CGAG CGCA GGCG GTTT CTTA AGTC TGAT GTGA AAGC CCCC GGC TCAA CCGG GGAG GGTC ATTG GAAA CTGG GAGA CTTG AGTG CAGA AGAG GAGA GTGG AATT CCAT GTGT AGCG GTGA AATG CGTA GATA TATG GAGG AACA CCAG TGGC GAAG GCGG CTCT CTGG TCTG TAAC TGAC GCTG AGGC TCGA AAGC GTGG GGAG CAAA CAGG ATTA GATA CCCT GGTA GTCC ACGC CGTA AACG ATGA GTGC TAAGT GTTG GAGG GTTT CCGC CCTT CAGT GCTG CAGCT AACG CATT AAGC ACTC CGCC TGGG GAGT ACGA CCGC AAGG TTGAA ACTC AAAG GAAT TGAC GGGG GCC CGCA CAAG CGGT GGAG CATG TGGT TTAA TTCG AAGC AACG CGAA GAAC CTTAC CAGG TCTT GACAT CCTTT GACC ACTC TAGA GATA GAGC

Enterococcus hirae PUHM 1011

GCTC CACC GGAA AAAG AGGA GTGG CGAA CGGG TGAG TAAC ACGT GGGTA ACCT GCCC ATCA GAAG GGGA TAAC ACTT GGAA ACAG GTGC TAAT ACCG TATA ACAA TCGA AACC GCAT GGTT TTGA TTTG AAAG GCGC TTTC GGGT GTCG CTGA TGGAT GGAC CCGC GGTG CATT AGCT AGTT GGTG AGGT AACG GCTC ACCA AGGC GACG ATGC ATAG CCGA CCTG AGAG GGTG ATCG GCCA CATT GGGA CTGAG ACAC GGCC CAAA CTCC TACG GGAG GCAG CAGT AGGG AATC TTCG GCAA TGGA CGAA AGTC TGAC CGAG CAAC GCCG CGTG AGTG AAGA AGGT TTTC GGAT CGTA AAAC CTGT TCAT CCCT TGACG GTAT CTAAC CAGA AAGC CACG GCTA ACTA CGTG CCAG CAGCC GCGG TAATA CGTA GGTGG CAAG CGTT GTCCG GATT TATT GGGC GTAA AGCG AGCG CAGGC GGTT TCTT AAGT CTGA TGTG AAAG CCCC CGGC TCAA CCGG GGAG GGTCA TTGGA AACT GGGA GACT TGAG TGCA GAAG AGGA GAGT GGAA TTCCA TGTG TAGC GGTGA AATG CGTA GATA TATG GAGG AACA CCAG TGGC GAAG GCGG CTCT CTGG TCTG TAACT GACGC TGAG GCTCG AAAGC GTGGG GAGCA AACAG GATT AGAT ACCC TGGT AGTC CACG CCGT AAACG ATGA GTGC TAAGT GTTG GAGG GTTT CCGC CCTT CAGTG CTGC AGCT AACGC ATTA AGCA CTCC GCCT GGGG AGTAC GACCG CAAGG TTGA AACTC AAAG GAAT TGACG GGGG CCCG CACA AGCG GTGG AGCA TGTG GTTT AATT CGAA GCAAC GCGA AGAA CCTT ACCA GGTC TTGA CATC CTTT GACCA CTCT AGAG ATAG AGCT TCCC CTTCG GGGG CAAA GTGA CAGG TGGT GCAT GGTT GTCG TCAG CTCG TGTCG TGAG ATGT TGGG TTAA GTCC CGCAA CGAG CGCA ACCCT TATT GTTA GTTG CCAT CATT TAGT TGGG CACT CTAG CAAG ACTG CCGG TGACA AACC GGAG GAAG GTGG GGAT GACG TCAAA TCAT CATG CCCC TTAT GACC TGGG CTAC ACAC GTGC TACA ATGG GAAG TACAA CGAGT CGCA AAGT CGCGA GGCT AAGC TAAT CTCT TAAA GCTT CTCT CAGT TCTG TTGT TAGA GAAG AACA AGGA TGAG AGTA ACTG TTCA TCCC TTGA CGGT ATCT AACC AGAA AGCC ACGG CTAA CTAC GTGC CAGC AGCC GCGG TAAT ACGT AGGT GGCA AGCG TTGT CCGG ATTT ATTG GGCG TAAA GCGA GCGC AGGC GGTT TCTT AAGT CTGA TGTG AAAG CCCC CGGC TCAA CCGG GGAG GGTC ATTG GAAA CTGG GAGA CTTG AGTG CAGA AGAG GAGA GTGG

Enterococcus hirae strain SU354

CCCG NTAA CAAT CGAAA CCGCA AGGTT TCGNA TTGAA AGGGC CTTT CGGGG TCCGC TGATG GATG GACC CCCG GTGC ATTA GCTAG TGGT GAGG TAAC GGCT CCCC AAGG CGAC GATG CATA GCCG ACCT GAG AGGG TGAT CGGC CACA TTGG GACTG AGACA CGGC CCAA ATTC CTAC GGGA

GGCAG CAGT AGGG AATC TTCG GCAA TGGA CGAA AGTC TGAC CGAG CAAC GCCG CGTGA GTGA AGAA GGTT TTCG GATC GTAA AACT CTGT TGTT AGAG AAGA ACAA GGAT GAGA GTAA CTGT TCAT CCCT TGACG GTAT CTAA CCAG AAAG C CACG GCTA ACTA CGTG CCAG CAGCC GCGG TAATA CGTA GGTGG CAAG CGTT GTCCG GATT TATT GGGC GTAA AGCG AGCG CAGGC GGTT TCTT AAGT CTGA TGTG AAAG CCCC CGGC TCAA CCGG GGAG GGTCA TTGGA AACT GGGA GACT TGAG TGCA GAAG AGGA GAGT GGAA TTCCA TGTG TAGC GGTGA AATG CGTA GATA TATG GAGG AACA CCAG TGGC GAAG GCGG CTCT CTGG TCTG TAACT GACGC TGAG GCTCG AAAGC GTGGG GAGCA AACAG GATT AGAT ACCC TGGT AGTC CACG CCGT AAACG ATGA GTGC TAAGT GTTG GAGG GTTT CCGC CCTT CAGTG CTGC AGCT AACGC ATTA AGCA CTCC GCCT GGGG AGTAC GACCG CAAGG TTGA AACTC AAAG GAAT TGACG GGGG CCCG CACA AGCG GTGG AGCA TGTG GTTT AATT CGAA GCAAC GCGA AGAA CCTT ACCA GGTC TTGA CATC CTTT GACCA CTCT AGAG ATAG AGCT TCCC CTTCG GGGG CAAA GTGA CAGG TGGT GCAT GGTT GTCG TCAG CTCG TGTCG TGAG ATGT TGGG TTAA GTCC CGCAA CGAG CGCA ACCCT TATT GTTA GTTG CCAT CATT TAGT TGGG CACT CTAG CAAG ACTG CCGG TGACA AACC GGAG GAAG GTGG GGAT GACG TCAAA TCAT CATG CCCC TTAT GACC TGGG CTAC ACAC GTGC TACA ATGG GAAG TACAA CGAGT CGCA AAGT CGCGA GGCT AAGC TAAT CTCT TAAA GCTT CTCT CAGT TCGG ATTG TAGG CTGC AACT CGCC TACA TGAAG CCGG AATC GCTA GTAA TCGC GGAT CAGC ACGC CGCG GTGA ATAC GTTCC CGGGC CTTG TACA CACC GCCC GTCA CACCA CGAG AGTT TGTA ACCC CGAA GTCG GTGA GGTA ACCT TTTG GAGC CAGC CGCC TAAA AATT CCAT GTGT AGCG GTGA AATG CGTA GATA TATG GAGG AACA CCAG TGGC GAAG GCGG CTCT CTGG TCTG TAAC TGAC GCTG AGGC TCGA AAGCG

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APPENDIX V

Viability of LAB in Varying pH Conditions

LAB ISOLATES	pH	3		4		6		7	
		Initial	*Final*	*Initial*	*Final*	*Initial*	*Final*	*Initial*	*Final*
Lactobacillus mucosae C101		2.3×10^8	5.0×10^6	2.5×10^8	9.9×10^7	3.0×10^8	2.1×10^8	7.5×10^8	1.0×10^9
Lactobacillus gasseri C103		1.7×10^9	2.0×10^8	2.2×10^8	1.5×10^8	2.6×10^8	2.0×10^8	2.0×10^9	4.5×10^9
Lactobacillus_mucosae C104		6.0×10^7	2.6×10^5	3.9×10^7	4.0×10^6	6.9×10^7	2.3×10^7	5.0×10^7	7.0×10^7
Lactobacillus_ingluviei C105		6.4×10^{10}	5.1×10^8	6.1×10^{10}	8.8×10^9	7.0×10^{10}	7.9×10^{10}	5.5×10^{10}	3.0×10^{10}
Enterococcus hirae C106		7.0×10^8	9.9×10^6	3.4×10^8	2.1×10^7	5.2×10^8	5.6×10^8	6.1×10^8	8.5×10^8
Lactobacillus_agilis C12		4.3×10^8	1.0×10^7	5.0×10^8	6.6×10^8	4.0×10^8	7.0×10^8	5.7×10^8	8.9×10^8
Lactobacillus_ingluviei C13		7.0×10^8	9.5×10^6	5.9×10^8	7.0×10^8	1.0×10^9	8.0×10^8	6.0×10^8	2.3×10^9
Lactobacillus agilis C14		7.3×10^9	3.0×10^8	8.0×10^9	1.5×10^9	9.7×10^8	1.0×10^9	7.0×10^8	8.7×10^8
Lactobacillus amylovorus C15		5.3×10^8	2.9×10^7	4.3×10^8	2.6×10^8	4.0×10^8	7.0×10^8	5.3×10^8	8.1×10^8
Lactobacillus_ingluviei C16		7.4×10^8	9.9×10^6	3.0×10^8	2.1×10^7	5.9×10^8	5.2×10^8	6.1×10^8	8.5×10^8
Lactobacillus ingluviei C17		7.0×10^8	8.6×10^6	1.4×10^8	4.2×10^7	5.0×10^8	6.5×10^8	6.1×10^8	6.5×10^8
Lactobacillus_taiwanensis C19		2.5×10^8	6.9×10^6	4.5×10^8	9.1×10^7	3.3×10^8	2.6×10^8	8.5×10^8	1.0×10^9
Lactobacillus taiwanensis C20		1.3×10^8	9.9×10^7	4.4×10^8	2.1×10^7	5.2×10^8	5.7×10^8	7.3×10^8	8.7×10^8
Lactobacillus mucosae C21		9.9×10^7	3.0×10^8	2.1×10^8	7.5×10^8	8.0×10^9	1.5×10^9	9.7×10^8	1.0×10^9
Lactobacillus ingluviei C23		6.3×10^{10}	5.7×10^8	6.1×10^{10}	8.8×10^9	7.0×10^{10}	7.9×10^{10}	5.5×10^{10}	3.4×10^{10}
Lactobacillus mucosae C24		8.3×10^9	4.4×10^7	6.7×10^9	4.1×10^8	8.6×10^9	7.3×10^9	4.9×10^{10}	5.5×10^{10}
Lactobacillus paraplantarum C25		5.3×10^9	4.0×10^8	7.0×10^9	4.6×10^9	8.7×10^9	7.3×10^{10}	7.9×10^{10}	5.5×10^{10}
Lactobacillus_plantarum C26		2.1×10^8	5.9×10^7	5.2×10^8	6.3×10^8	8.5×10^8	1.7×10^9	2.0×10^8	9.8×10^8
Lactobacillus salivarius C27		7.4×10^9	3.2×10^8	8.3×10^9	2.5×10^9	9.7×10^8	1.2×10^9	6.0×10^8	8.7×10^8
Lactobacillus mucosae C28		5.5×10^7	4.6×10^5	3.9×10^7	3.7×10^7	4.0×10^6	6.9×10^7	2.3×10^7	1.0×10^8
Lactobacillus mucosae C29		3.9×10^8	8.9×10^6	2.6×10^8	3.1×10^7	6.1×10^7	9.6×10^8	7.1×10^8	9.5×10^8
Lactobacillus plantarum C3		5.4×10^9	2.6×10^7	5.8×10^8	4.7×10^8	4.7×10^8	6.0×10^8	5.7×10^8	1.9×10^9
Lactobacillus ingluviei C31		8.3×10^8	1.0×10^7	5.0×10^8	6.6×10^8	4.0×10^8	7.0×10^8	5.7×10^8	8.9×10^8
Enterococcus hirae C33		2.8×10^9	5.0×10^8	2.2×10^8	1.6×10^8	2.3×10^8	3.0×10^8	2.3×10^9	4.1×10^9
Streptococcus infantarius C35		2.6×10^9	5.5×10^7	7.2×10^8	1.9×10^8	4.5×10^8	2.4×10^8	1.3×10^9	5.4×10^9
Enterococcus hirae C36		3.2×10^8	2.0×10^7	5.3×10^8	7.6×10^8	4.0×10^8	6.2×10^8	8.7×10^8	9.9×10^8
Streptococcus infantarius C37		7.2×10^8	2.6×10^7	6.0×10^7	8.6×10^8	9.0×10^8	7.0×10^8	5.7×10^8	7.7×10^8
Streptococcus_infantarius C38		4.4×10^9	3.4×10^7	5.3×10^9	2.6×10^9	8.8×10^8	1.3×10^9	6.2×10^8	8.5×10^8
Lactobacillus mucosae C39		3.3×10^8	6.0×10^7	5.5×10^8	6.6×10^8	4.2×10^8	7.3×10^8	5.1×10^8	8.9×10^8
Streptococcus equinus C40		1.9×10^9	1.0×10^7	2.3×10^8	7.5×10^8	6.0×10^9	1.8×10^9	5.7×10^8	1.0×10^9
Streptococcus infantarius C41		2.5×10^8	5.4×10^6	3.7×10^8	8.4×10^7	5.1×10^8	6.3×10^8	7.5×10^8	1.0×10^9
Lactobacillus agilis C5		7.3×10^8	4.3×10^6	2.5×10^8	9.3×10^7	4.6×10^8	4.1×10^8	5.6×10^8	1.0×10^9
Streptococcus infantarius C50		3.1×10^8	4.2×10^7	6.6×10^8	5.1×10^8	6.5×10^8	2.5×10^9	4.0×10^8	8.1×10^8
Streptococcus infantarius C51		8.8×10^9	6.1×10^8	9.2×10^8	6.4×10^8	5.3×10^8	7.0×10^8	2.3×10^9	8.1×10^9
Enterococcus hirae C52		5.3×10^{10}	2.7×10^8	4.1×10^{10}	8.1×10^9	5.0×10^{10}	7.9×10^9	1.5×10^{10}	7.4×10^{10}
Streptococcus infantarius C53		3.3×10^9	7.7×10^8	6.3×10^9	7.7×10^9	7.0×10^9	7.5×10^9	5.3×10^9	3.4×10^9

Streptococcus infantarius C54	2.9×10^{8}	5.7×10^{7}	2.2×10^{8}	5.1×10^{7}	6.7×10^{7}	8.6×10^{8}	7.1×10^{8}	8.7×10^{8}
Streptococcus infantarius C55	4.1×10^{9}	6.4×10^{7}	4.3×10^{9}	7.6×10^{9}	3.8×10^{8}	7.3×10^{9}	5.4×10^{8}	9.5×10^{8}
Streptococcus infantarius C56	2.3×10^{8}	6.3×10^{6}	3.5×10^{8}	7.1×10^{7}	4.6×10^{8}	9.1×10^{8}	5.2×10^{8}	2.0×10^{9}
Streptococcus infantarius C 57	2.3×10^{8}	1.0×10^{7}	3.0×10^{8}	8.6×10^{8}	2.0×10^{8}	7.7×10^{8}	5.7×10^{8}	8.8×10^{8}
Streptococcus infantarius C58	4.3×10^{8}	6.0×10^{6}	5.0×10^{8}	6.6×10^{8}	4.0×10^{8}	6.0×10^{8}	5.7×10^{8}	8.0×10^{8}
Lactobacillus agilis C59	1.9×10^{8}	5.7×10^{6}	2.0×10^{8}	6.1×10^{7}	6.7×10^{7}	8.6×10^{8}	4.1×10^{8}	8.3×10^{8}
Lactobacillus taiwanensis C6	3.2×10^{9}	3.8×10^{7}	2.2×10^{8}	7.1×10^{7}	6.2×10^{8}	7.6×10^{8}	7.3×10^{8}	9.7×10^{8}
Lactobacillus amylovorus C60	9.3×10^{8}	8.4×10^{6}	3.4×10^{8}	8.5×10^{8}	5.5×10^{8}	1.0×10^{9}	5.4×10^{8}	3.2×10^{9}
Lactobacillus mucosae C61	3.9×10^{9}	5.0×10^{7}	4.3×10^{8}	7.1×10^{8}	6.0×10^{9}	1.8×10^{9}	2.7×10^{8}	1.4×10^{9}
Streptococcus infantarius C62	1.4×10^{9}	1.0×10^{8}	2.3×10^{8}	5.4×10^{8}	6.0×10^{9}	2.5×10^{9}	5.4×10^{8}	6.2×10^{9}
Streptococcus infantarius C63	6.1×10^{10}	7.5×10^{8}	3.4×10^{10}	8.1×10^{9}	6.3×10^{10}	8.7×10^{10}	6.4×10^{10}	8.4×10^{10}
Lactobacillus gasseri C64	2.9×10^{9}	5.0×10^{7}	4.3×10^{8}	3.5×10^{8}	6.0×10^{9}	2.6×10^{9}	6.5×10^{8}	1.8×10^{9}
Lactobacillus animalis C65	8.0×10^{8}	6.6×10^{6}	1.4×10^{8}	4.3×10^{7}	5.0×10^{8}	5.5×10^{8}	4.1×10^{8}	6.5×10^{8}
Enterococcus hirae C66	2.3×10^{9}	7.4×10^{7}	7.7×10^{9}	2.1×10^{8}	8.6×10^{9}	7.3×10^{9}	4.0×10^{9}	2.5×10^{10}
Lactobacillus mucosae C67	9.3×10^{9}	6.5×10^{7}	3.7×10^{9}	5.4×10^{8}	7.4×10^{9}	2.3×10^{9}	5.9×10^{10}	8.5×10^{10}
Streptococcus infantarius C68	4.4×10^{10}	2.1×10^{9}	5.1×10^{10}	8.8×10^{9}	6.3×10^{10}	2.9×10^{10}	1.5×10^{10}	4.0×10^{10}
Streptococcus infantarius C69	6.4×10^{9}	5.1×10^{7}	1.1×10^{9}	5.8×10^{8}	3.0×10^{9}	4.9×10^{9}	4.5×10^{9}	7.0×10^{9}
Streptococcus infantarius C70	5.3×10^{8}	4.7×10^{6}	1.5×10^{8}	7.3×10^{7}	4.6×10^{8}	2.2×10^{8}	4.6×10^{8}	1.0×10^{9}
Streptococcus infantarius C71	2.2×10^{8}	7.9×10^{7}	4.2×10^{8}	6.5×10^{8}	8.3×10^{8}	1.8×10^{9}	2.0×10^{8}	7.6×10^{8}
Lactobacillus gasseri C72	2.1×10^{8}	7.9×10^{6}	5.0×10^{8}	6.1×10^{8}	8.5×10^{8}	2.7×10^{9}	2.4×10^{8}	7.8×10^{8}
Streptococcus infantarius C73	5.0×10^{7}	7.6×10^{5}	3.9×10^{7}	3.0×10^{6}	6.6×10^{7}	2.9×10^{7}	2.0×10^{7}	7.0×10^{7}
Streptococcus infantarius C74	6.3×10^{9}	7.5×10^{7}	9.4×10^{8}	1.1×10^{8}	6.3×10^{10}	8.7×10^{10}	6.4×10^{10}	8.4×10^{10}
Streptococcus infantarius C75	6.1×10^{10}	7.5×10^{8}	3.4×10^{9}	8.1×10^{9}	6.7×10^{9}	8.9×10^{9}	4.4×10^{9}	7.6×10^{9}
Streptococcus infantarius C76	5.3×10^{9}	2.4×10^{7}	4.5×10^{9}	4.4×10^{8}	5.6×10^{9}	9.3×10^{9}	3.9×10^{9}	7.5×10^{9}
Streptococcus infantarius C77	2.8×10^{9}	4.1×10^{7}	2.2×10^{8}	5.4×10^{8}	5.3×10^{8}	7.1×10^{8}	2.7×10^{9}	8.4×10^{9}
Streptococcus infantarius C78	5.5×10^{8}	7.9×10^{6}	3.5×10^{8}	4.1×10^{7}	3.3×10^{8}	2.6×10^{8}	8.0×10^{8}	7.6×10^{9}
Lactobacillus mucosae C8	6.3×10^{8}	1.0×10^{7}	3.0×10^{8}	5.7×10^{8}	3.1×10^{8}	7.0×10^{8}	5.7×10^{8}	8.9×10^{8}
Streptococcus infantarius C80	1.9×10^{8}	8.2×10^{6}	3.3×10^{8}	3.5×10^{7}	7.1×10^{7}	8.4×10^{8}	4.1×10^{8}	9.5×10^{8}
Lactobacillus amylovorus C81	5.1×10^{8}	5.9×10^{7}	3.2×10^{8}	1.3×10^{8}	9.5×10^{8}	2.7×10^{9}	2.0×10^{8}	9.8×10^{8}
Lactobacillus amylovorus C82	1.3×10^{8}	9.9×10^{7}	4.4×10^{8}	2.1×10^{7}	5.2×10^{8}	5.7×10^{8}	7.3×10^{8}	8.7×10^{8}
Lactobacillus amylovorus C84	1.1×10^{9}	5.9×10^{7}	3.0×10^{8}	6.1×10^{8}	9.5×10^{8}	3.7×10^{9}	2.6×10^{8}	7.8×10^{8}
Lactobacillus amylovorus C85	4.3×10^{9}	4.4×10^{6}	6.7×10^{9}	4.4×10^{7}	6.6×10^{9}	8.2×10^{9}	3.3×10^{9}	2.5×10^{10}
Lactobacillus amylovorus C86	1.9×10^{10}	5.7×10^{9}	2.0×10^{9}	6.1×10^{9}	6.7×10^{9}	8.6×10^{9}	9.9×10^{9}	4.4×10^{10}
Lactobacillus amylovorus C87	3.0×10^{8}	5.0×10^{6}	5.3×10^{8}	7.6×10^{8}	5.2×10^{8}	8.3×10^{8}	4.1×10^{8}	8.8×10^{8}
Streptococcus infantarius C88	5.3×10^{9}	3.0×10^{8}	5.0×10^{9}	1.5×10^{9}	7.7×10^{8}	1.1×10^{9}	6.3×10^{8}	8.5×10^{8}
Lactobacillus ingluvie C89	3.3×10^{10}	5.4×10^{8}	6.6×10^{9}	2.1×10^{8}	8.3×10^{9}	5.3×10^{9}	4.0×10^{9}	2.1×10^{10}
Enterococcus hirae C9	3.3×10^{8}	4.1×10^{7}	5.3×10^{8}	7.4×10^{8}	4.3×10^{8}	7.3×10^{8}	5.4×10^{8}	7.7×10^{8}
Lactobacillus gasseri C90	6.4×10^{10}	2.2×10^{8}	6.3×10^{9}	8.5×10^{8}	5.7×10^{8}	1.2×10^{8}	3.0×10^{8}	8.7×10^{8}
Weissella cibaria C91	1.6×10^{8}	3.5×10^{7}	4.2×10^{8}	1.2×10^{8}	4.5×10^{8}	2.3×10^{8}	1.5×10^{9}	7.5×10^{9}
Lactobacillus ingluviei C92	3.0×10^{9}	7.4×10^{8}	4.3×10^{9}	7.7×10^{8}	5.4×10^{9}	7.5×10^{9}	5.3×10^{9}	5.5×10^{9}

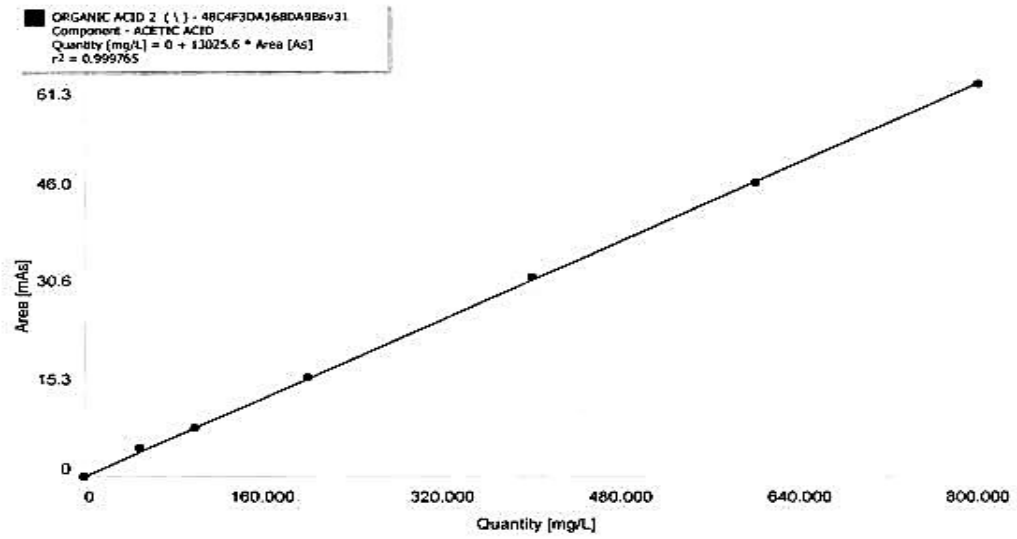
Lactobacillus ingluviei C93	4.4×10^{8}	1.6×10^{7}	4.1×10^{8}	6.6×10^{8}	4.0×10^{8}	7.0×10^{8}	5.7×10^{8}	8.9×10^{8}
Lactobacillus salivarius C94	2.9×10^{10}	5.0×10^{9}	4.3×10^{9}	7.1×10^{9}	6.0×10^{10}	4.8×10^{10}	2.7×10^{10}	2.4×10^{10}
Lactobacillus reuteri C95	2.4×10^{9}	2.0×10^{8}	2.6×10^{8}	5.4×10^{8}	6.0×10^{9}	2.5×10^{9}	5.4×10^{8}	6.2×10^{9}
Lactobacillus gasseri C96	9.8×10^{8}	8.2×10^{6}	3.4×10^{8}	8.5×10^{8}	5.5×10^{8}	1.0×10^{9}	5.1×10^{8}	3.6×10^{9}
Lactobacillus amylovorus C98	6.5×10^{8}	7.9×10^{6}	3.5×10^{8}	4.3×10^{7}	3.0×10^{8}	2.6×10^{8}	4.0×10^{8}	7.6×10^{9}
Lactobacillus amylovorus C99	5.3×10^{9}	4.0×10^{8}	5.2×10^{9}	2.5×10^{9}	7.7×10^{8}	1.1×10^{9}	6.1×10^{8}	9.2×10^{8}

APPENDIX VI

Standard Curves for Quantification of Organic Acid Produced by LAB

Component Calibration Report
ORGANIC ACID 2 (\ ) - 48C4F3DA168DA9B6v31

Component:	ACETIC ACID	**Retention Time:**	02:40.5	**Window %:**	8.000
Identify By:	Largest	**Quantify By:**	Area	**Curve Type:**	Linear
Force Origin:	Yes				



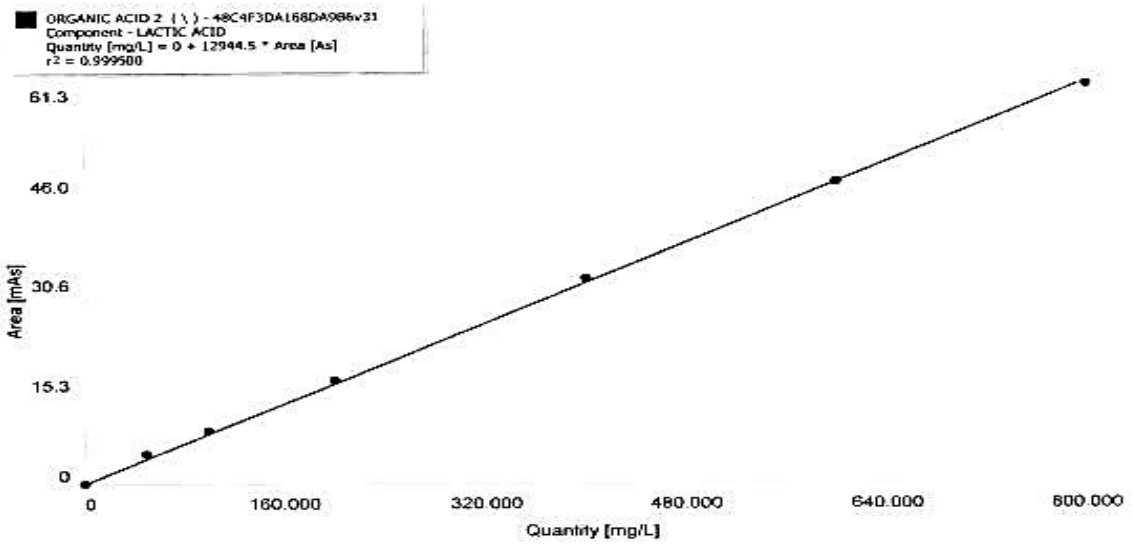
STANDARDS

Std. No	Active	Area [mAs]	Height [mA]	Quantity [mg/L]	Comment / Cgm File ID
01	Y	4.5	0.9	50.000	
...\STANDARD 1 - 50PPM001-1		4.3	1.0		47C4A39AED7850C8v2
...\STANDARD 1 - 50PPM001-1		4.6	0.9		2628608EF4B39FE0v2
02	Y	7.7	1.5	100.000	
...\STANDARD 2 - 100PPM-1		8.2	1.6		EDA687D2F3E365D1v3
...\STANDARD 2 - 100PPM001-		7.1	1.4		98C3420554C96852v2
03	Y	15.6	3.0	200.000	
...\STANDARD 3 - 200PPM001-		15.9	3.2		18C903420BB3A676v2
...\STANDARD 3 - 200PPM001-		15.2	2.8		A990B73D717ED765v2
04	Y	31.2	5.7	400.000	
...\STANDARD 4 - 400PPM001-		30.2	5.4		07E23B6042979AABv2
...\STANDARD 4 - 400PPM001-		32.1	6.0		9E6E4942745EE787v2
05	Y	45.8	8.7	600.000	
...\STANDARD 5 - 600PPM001-		44.5	8.5		EAF2C404FBE5462Ev2
...\STANDARD 5 - 600PPM001-		47.1	8.9		A76FF5E8C5FD9DE5v1
06	Y	61.3	11.3	800.000	
...\STANDARD 6 - 800PPM001-		63.8	11.7		E1274F36739BAB60v2
...\STANDARD 6 - 800PPM001-		58.8	10.9		0DD4F711854D68D6v2

Component Calibration Report

ORGANIC ACID 2 (\ ) - 48C4F3DA168DA9B6v31

Component:	LACTIC ACID	**Retention Time:**	02:30.4	**Window %:**	8.000
Identify By:	Largest	**Quantify By:**	Area	**Curve Type:**	Linear
Force Origin:	Yes				



STANDARDS

Std. No	Active	Area [mAs]	Height [mA]	Quantity [mg/L]	Comment / Cgm File ID
01	Y	4.6	0.9	50.000	
...\STANDARD 1 - 50PPM001-1		4.5	0.9		47C4A39AED7850C8v2
...\STANDARD 1 - 50PPM001-1		4.6	0.9		2628608EF4B39FE0v2
02	Y	8.2	1.6	100.000	
...\STANDARD 2 - 100PPM-1		8.2	1.6		EDA687D2F3E365D1v3
...\STANDARD 2 - 100PPM-2		9.2	1.9		BC87E82455905B41v3
...\STANDARD 2 - 100PPM001-		7.1	1.4		96C3420554C96852v2
03	Y	16.0	3.0	200.000	
...\STANDARD 3 - 200PPM001-		16.8	3.1		E58837829AC6572Fv2
...\STANDARD 3 - 200PPM001-		15.9	3.2		18C903420BB3A676v2
...\STANDARD 3 - 200PPM001-		15.2	2.8		A990B73D717ED765v2
04	Y	31.5	5.9	400.000	
...\STANDARD 4 - 400PPM001-		32.2	6.2		F2AE37A2DD095524v2
...\STANDARD 4 - 400PPM001-		30.2	5.4		07E23B6042979AABv2
...\STANDARD 4 - 400PPM001-		32.1	6.0		9E6E4942745EE787v2
05	Y	46.3	8.8	600.000	
...\STANDARD 5 - 600PPM001-		44.5	8.5		EAF2C404FBE5462Ev2
...\STANDARD 5 - 600PPM001-		47.1	8.9		A76FF5E8C5FD9DE5v1
...\STANDARD 5 - 600PPM001-		47.3	8.9		374DF048B3B97F44v2

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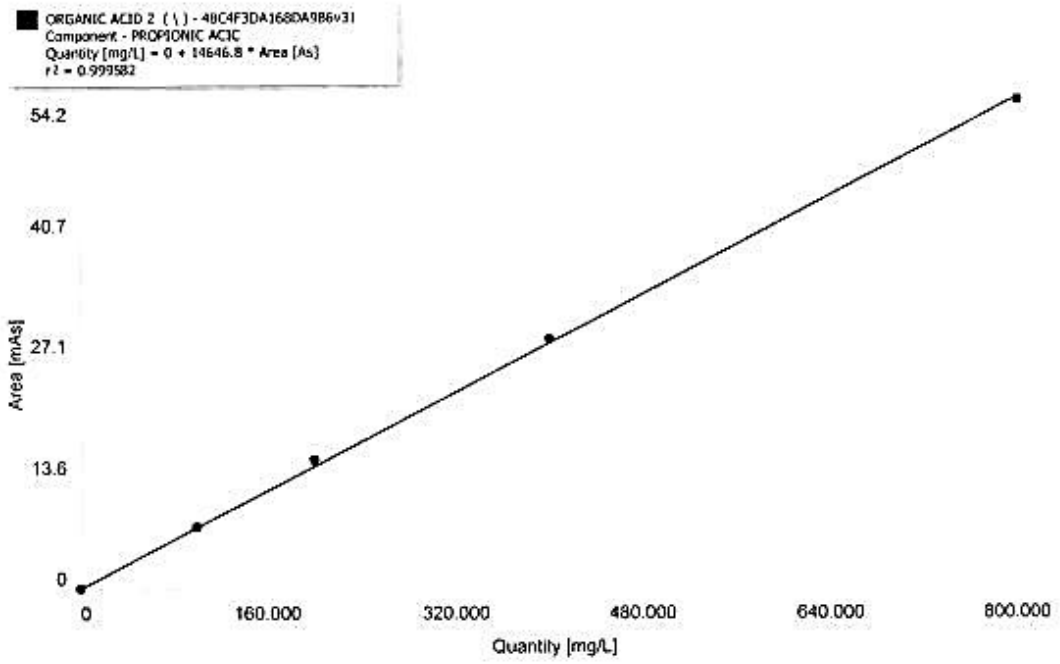
Component Calibration Report

ORGANIC ACID 2 (\ ) - 48C4F3DA168DA9B6v31

Component:	PROPIONIC ACIC	**Retention Time:**	06:04.5	**Window %:**	8.000
Identify By:	Largest	**Quantify By:**	Area	**Curve Type:**	Linear
Force Origin:	Yes				



STANDARDS

Std. No	Active	Area [mAs]	Height [mA]	Quantity [mg/L]	Comment / Cgm File ID
02	Y	6.9	0.9	100.000	
...\STANDARD 2 - 100PPM-1		6.9	0.9		EDA667D2F3E365D1v3
03	Y	14.3	1.5	200.000	
...\STANDARD 3 - 200PPM001-		14.3	1.5		A990B73D717ED765v2
04	Y	27.7	2.8	400.000	
...\STANDARD 4 - 400PPM001-		27.7	2.8		07E23B6042979AABv2
06	Y	54.2	5.6	800.000	
...\STANDARD 6 - 800PPM001-		55.1	5.6		E1274F36739BAB60v2
...\STANDARD 6 - 800PPM001-		53.4	5.9		EC5F429E16F83E8Fv2

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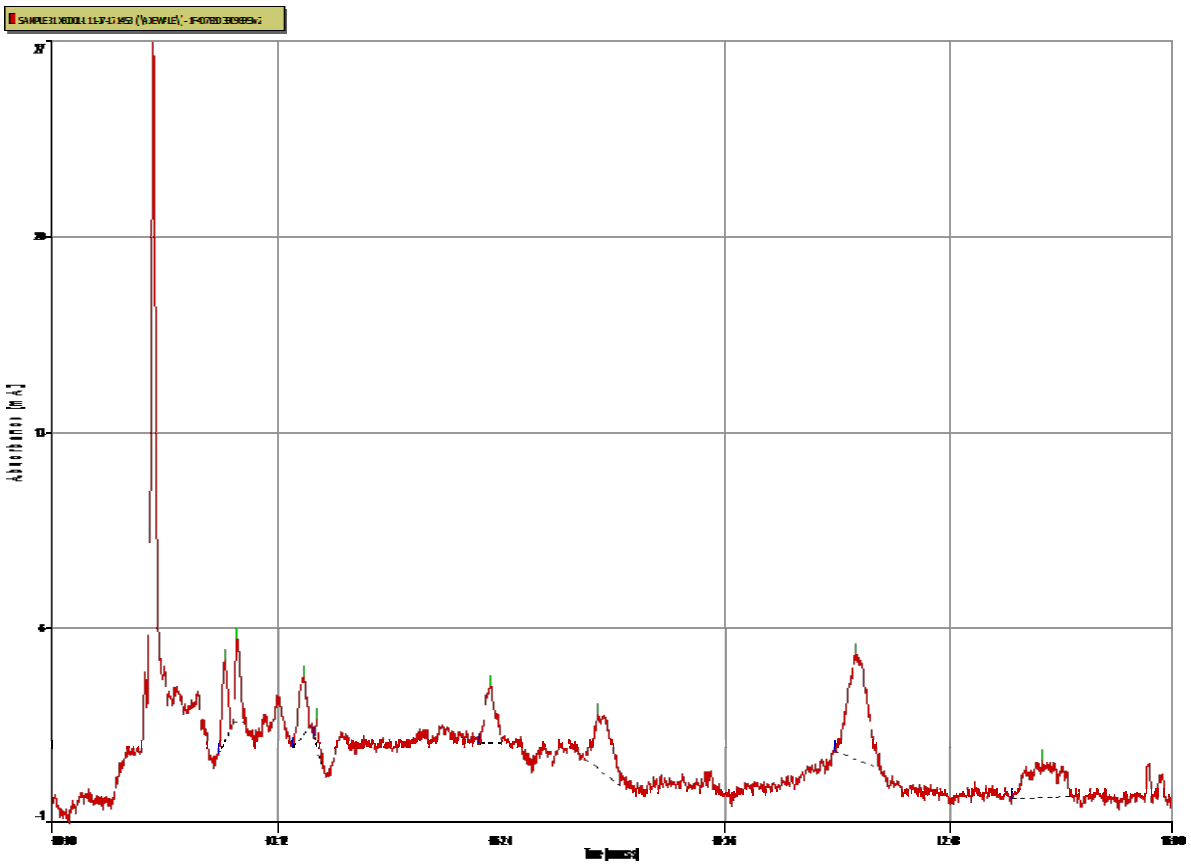
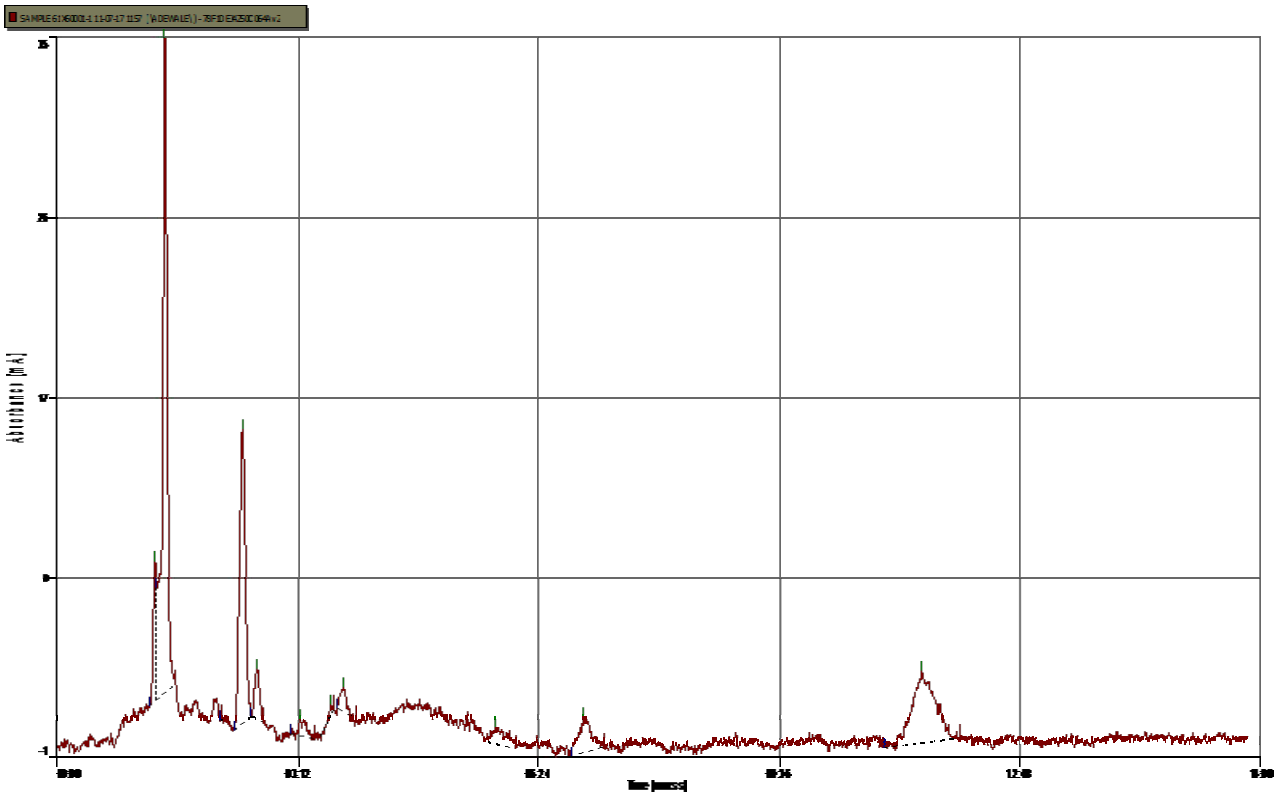
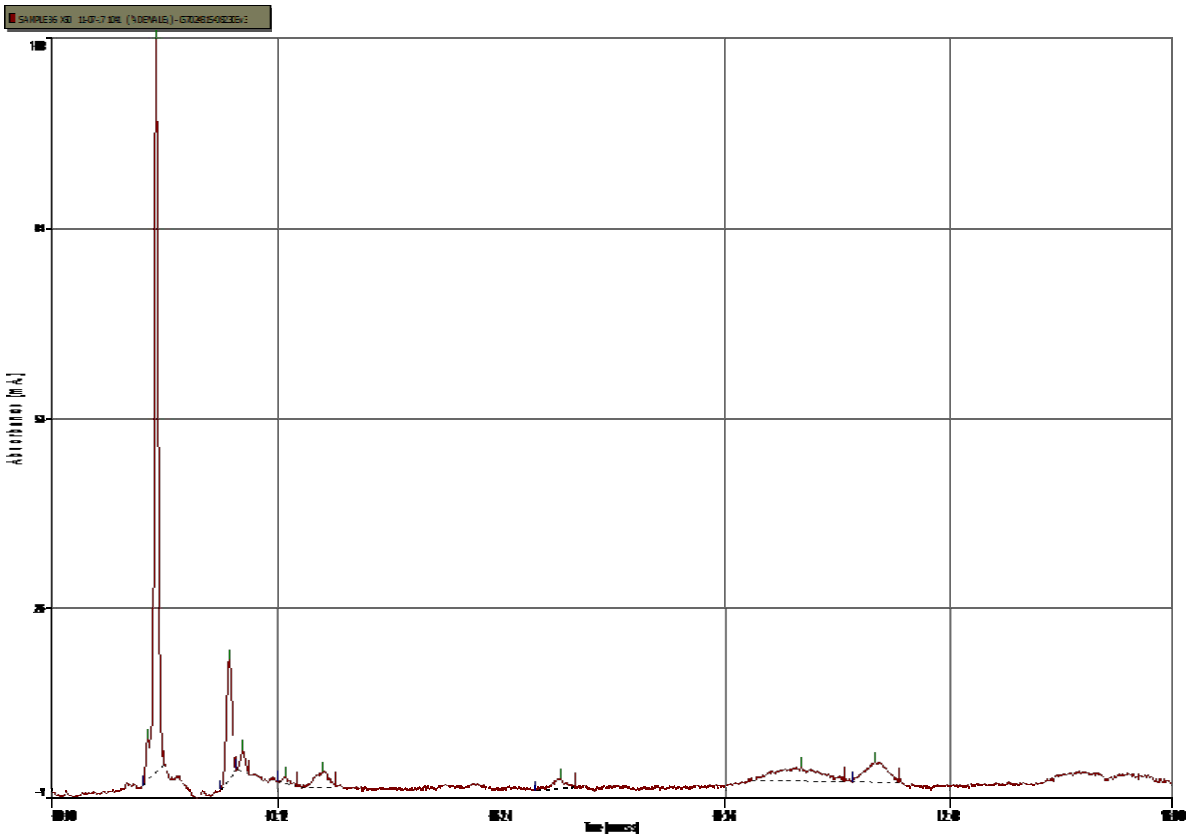
C31 X60



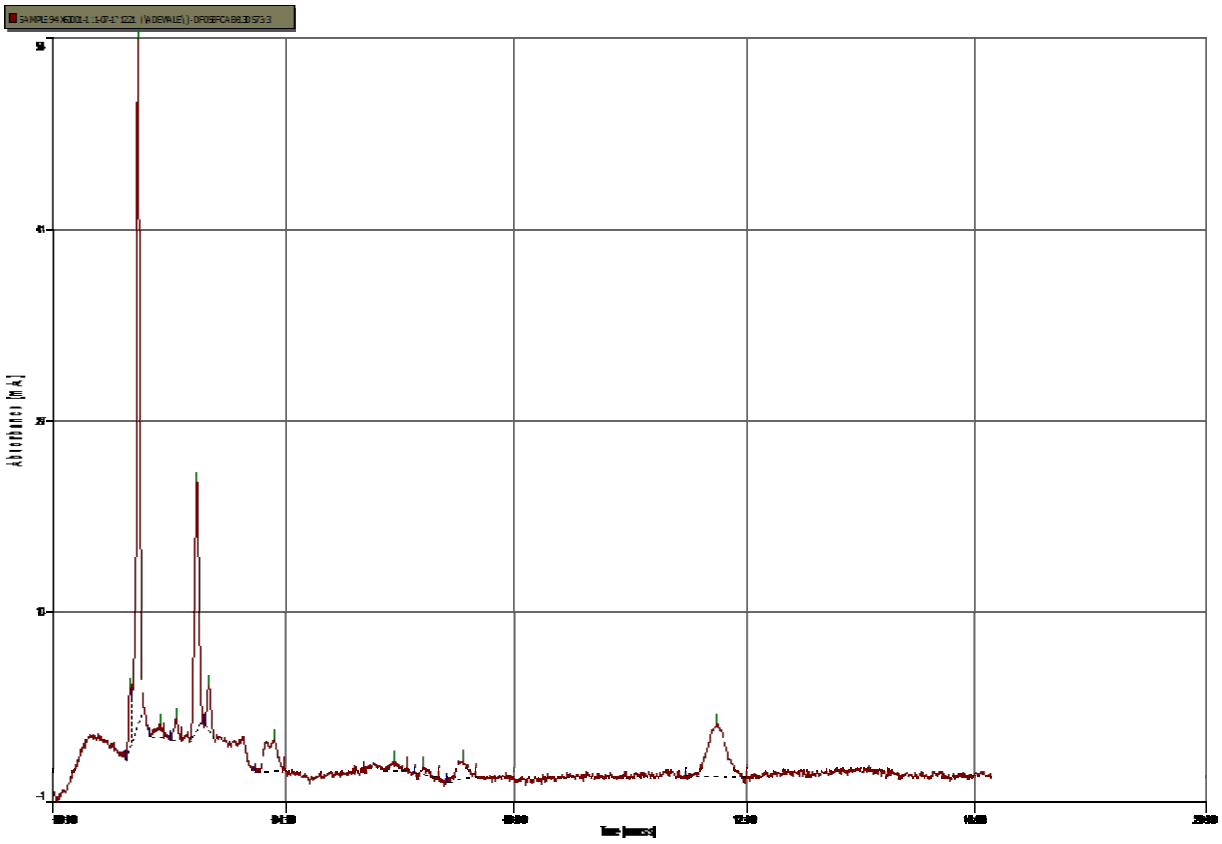
C61 X60



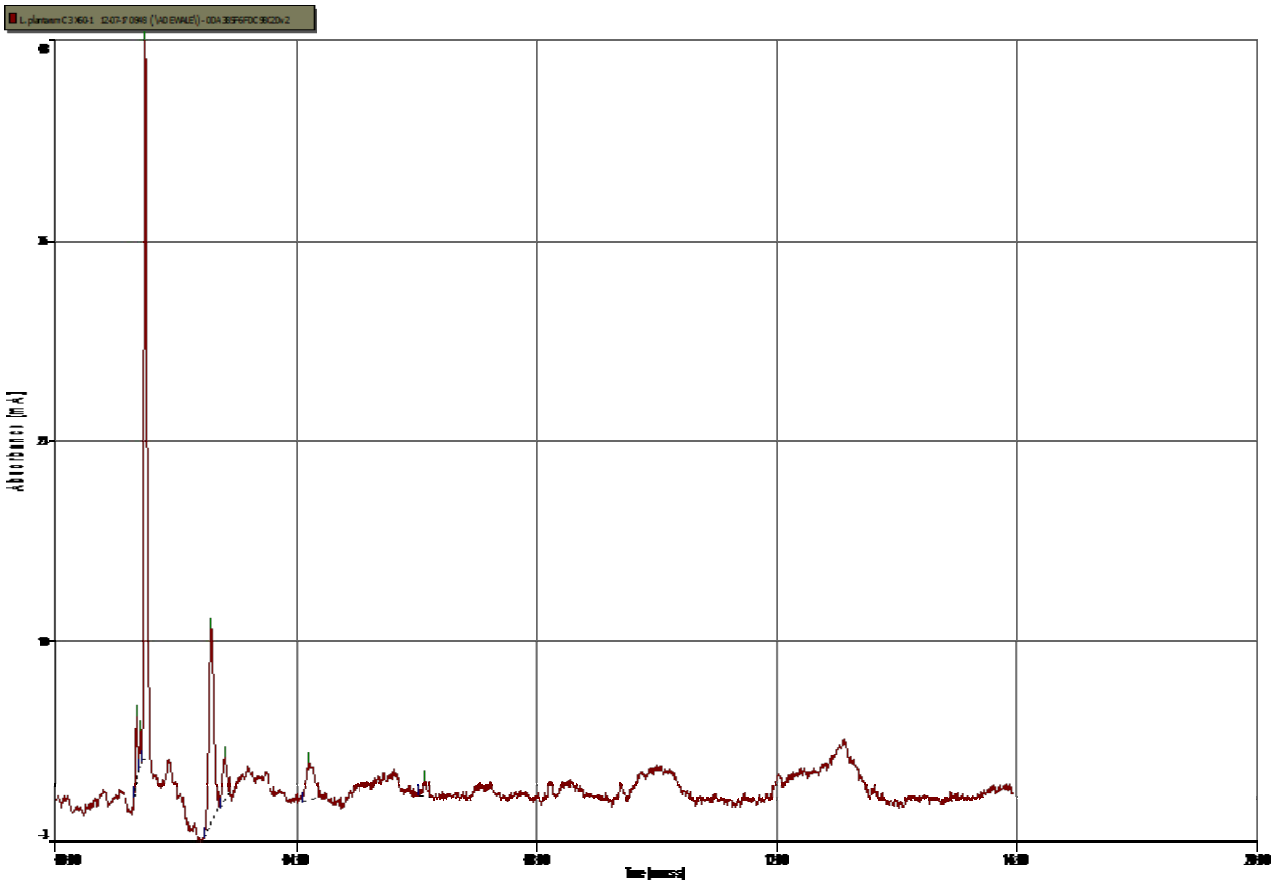
C86 X60



C94 X60



C3X60



APPENDIX VII

Antimicrobial Activity of *Lactobacillus* spp. in Co-culture with *Salmonella*

Organisms	Incubation Time (Hours)							
	0		8		16		24	
	CFU/mL	Log_{10}	CFU/mL	Log_{10}	CFU/mL	Log_{10}l	CFU/mL	Log_{10}
S1[C]	6.0 $X10^8$	8.778	9.5 $X10^7$	7.978	3.9 $X10^8$	8.591	5.3 $X10^8$	8.724
S1 + LAB 86	4.9 $X10^8$	8.690	5.5 $X10^7$	7.740	NG	-	NG	-
S1 + LAB 94	4.0 $X10^8$	8.602	6.9 $X10^7$	7.839	NG	-	NG	-
S57[C]	8.0 $X10^8$	8.903	1.1 $X10^8$	8.041	5.7 $X10^8$	8.756	9.2 $X10^8$	8.963
S57+ LAB 86	5.4 $X10^8$	8.699	6.3 $X10^7$	7.799	NG	-	NG	-
S57+ LAB 94	6.7 $X10^8$	8.826	7.1 $X10^8$	8.851	NG	-	NG	-

Growth of Selected Lactobacilli in Co-culture with *Salmonella*

Organisms	Incubation Time (Hours)							
	0		8		16		24	
	CFU/mL	Log_{10}	CFU/mL	Log_{10}	CFU/mL	Log_{10}	CFU/mL	Log_{10}
LAB 94[C]	$8.4 X10^{10}$	10.929	$1.0 X10^{9}$	9.004	$8.0 X10^{9}$	9.903	$3.0 X10^{10}$	10.477
S1 + LAB 86	$6.7 X10^{10}$	10.826	$8.3 X10^{8}$	8.919	$6.2 X10^{9}$	9.792	$4.0 X10^{10}$	10.602
S1 + LAB 94	$6.0 X10^{10}$	10.770	$4.9 X10^{8}$	8.690	$2.1 X10^{9}$	9.324	$9.4 X10^{9}$	9.973
LAB 86[C]	$7.4 X10^{10}$	10.867	$9.0 X10^{8}$	8.954	$4.1 X10^{9}$	9.613	$1.2 X10^{10}$	10.079
S57+ LAB 86	$4.0 X10^{10}$	10.602	$5.0 X10^{8}$	8.699	$3.4 X10^{9}$	9.531	$3.5 X10^{10}$	10.544
S57+ LAB 94	$3.7 X10^{10}$	10.568	$4.0 X10^{8}$	8.602	$1.1 X10^{9}$	9.045	$5.1 X10^{10}$	10.708

APPENDIX VIII

Quantification of Lactobacilli and Enterobacteria from qPCR Data

Enterobacteria			Lactobacilli		
Sample Code	CT Value	Quantity	Sample Code	CTValue	Quantity
Stock ENT	7.44283	7.844	Stock LAB	3.55659	13.67
1;4	11.2407	3.989	1;4	9.75004	5.47
1;8	15.0953	1.435	1;8	12.0243	1.9
1;10	10.8629	1.382	1;10	11.8636	1.9
NT initial	5.007	3.205457	NT initial	25.1832	2.291381
NT final	13.6482	3.095865	NT final	16.0923	4.046709
55 (control)initial	39.0561	2.773628	55 (control)initial	21.5128	3.000085
55 final	5.788	3.195552	55 final	39.96	-0.56181
56 initial	20.4467	3.009643	56 initial	33.4241	0.700178
56 final	36.6722	2.803862	56 final	19.4123	3.405662
57 initial	16.69	3.057287	57 initial	31.135	1.142171
57 final	30.2719	2.885034	57 final	14.75	4.305888
58 initial	20.0337	3.014881	58 initial	17.6131	3.753063
58 final	N.D	N.D	58 final	N.D	N.D
59 initial	18.0984	3.039425	59 initial	20.6845	3.160018
59 final	36.672	2.803864	59 final	15.612	4.139448
60 (control) initial	18.4824	3.034555	60 (control) initial	35.2965	0.338643
60 final	16.6639	3.057618	60 final	33.4454	0.696065
62 initial	22.5561	2.98289	62 initial	24.4055	2.441545
62 final	39.6804	2.76571	62 final	16.4904	3.969841
$r^2 = 0.9973$			$r^2 = 0.9986$		