CHARACTERISATION OF LACTIC ACID BACTERIA ISOLATED FROM HUMAN BREAST MILK AND INFANT FAECES AS POTENTIAL PROBIOTICS

BY

ABIOLA OLUWATOSIN, OBISESAN

142880

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ABSTRACT

Infant mortality, particularly in developing countries; remains a concern and one of its major causes is diarrhoea. Human breast milk and faeces contain lactic acid bacteria (LAB), which could have probiotic potential. However, there is a paucity of information on the effect of LAB from faeces of healthy infants and mothers' breast milk on disease caused by diarrhoeagenic *E. coli*. This study was undertaken to evaluate the potential of LAB from human breast milk and infant faeces to contribute to the management of infantile diarrhoea.

Lactic acid bacteria were isolated from the breast milk of 16 mothers and faeces of 13 infants (healthy volunteers) from Ado-Ekiti, Ekiti State. The LAB strains were identified by partial sequencing of the 16S rRNA gene. The antimicrobial activities of LAB viable cells and Cell-Free Supernatant (CFS) against enterohaemorrhagic, enterotoxigenic, Shiga-toxin producing, enteroinvasive and enteropathogenic *E. coli* were tested by agar overlay and diffusion methods, respectively. Production of bacteriocin-like substances was evaluated by ammonium sulphate precipitation of CFS. The kill rate of LAB was determined by co-culture with diarrhoeagenic *E.coli* pathotypes. Organic acid production by selected LAB was quantified by HPLC, while the MICs wer/e determined by VITEK®2. The ability of selected strains to withstand simulated gastric conditions (consecutive pH 3 and 0.3% bile) was determined. Autoaggregation, co-aggregation and hydrophobicity abilities of selected strains in *n*-hexadecane and xylene were assessed by UV spectrophotometry, while anti-biofilm effects of1:1, 1:9 and 1:99 dilutions of selected LAB CFS were evaluated by crystal violet assay. The level of IL-6 and IL-10 cytokines in groups of immunosuppressed mice (n=5, 22±4 g) treated with LAB and levamisole hydrochloride (positive control) groups were evaluated in blood and spleen by ELISA as recommended by FAO guidelines. Data were analysed by one-way ANOVA at $\alpha_{0.05}$.

Ninety-three LAB belonging to five genera and 15 species were identified. *Lactobacillus* (44) was the predominant genus, while *L. plantarum* (27) was the most abundant species. Inhibition zones of CFS against the tested pathogens ranged from 8.0 to 25.0±1 mm, while viable cell inhibition zones were 12.0 to 20.0±3 mm. Eight LAB strains produced bacteriocin-like substances. Complete inhibition of *E.coli* strains were observed between

16 and 24h. The highest concentration (76.8 mg/mL) of the most prominent organic acid (lactic) was produced by *L. rhamnosus* A012 (from faeces). *L. rhamnosus* A012 was susceptible to all tested antibiotics while *L. plantarum* A011 (from faeces) was resistant to only one of the antibiotics. *L. rhamnosus* A012 and *L. plantarum* A011 had the highest resistance to gastric conditions with one $\log_{10}$ reduction in CFU/mL. *L. rhamnosus* A012 had hydrophobicity of 25.0% in *n*-hexadecane, 15.0% in xylene, autoaggregation of 32.0% at 5^{th} h and co-aggregation of 6.8%. All dilutions of *L. plantarum* A011 CFS showed antibiofilm activity. There was a significant decrease in IL-6 and an increase in IL-10 levels in immunosuppressed mice treated with LAB compared to control groups.

L. rhamnosus A012 demonstrated antimicrobial activity against diarrhoeagenic *E. coli* strains and immunomodulatory properties. It is, therefore, a potential probiotic candidate.

Keywords: *Lactobacillus rhamnosus*, *Lactobacillus plantarum*, Probiotics, Diarrhoeagenic *E.coli*.

Word count: 495

DEDICATION

I dedicate the project work to the ALPHA and OMEGA, the Beginning and the End and Christ Jesus, my Wisdom, Righteousness, and Justification. To YOU alone the glory.

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I give all glory, honour and adoration to Most High God, the Author and the Finisher of my faith, for the success of this work. Thank you, Abba Father.

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I show my appreciation to everyone who has rendered help to the success of this research, thank you all.

CERTIFICATION

This is to certify that this project was carried out by ABIOLA OLUWATOSIN, OBISESAN under my supervision in the Department of Pharmaceutical Microbiology, Faculty of Pharmacy,
University of Ibadan, Nigeria.

…….

Supervisor
Funmilola A. Ayeni
B.Tech (FUTA), M.Sc., Ph.D. (Ibadan)
Senior Lecturer, Department of Pharmaceutical Microbiology,
University of Ibadan, Nigeria.

LIST OF MAIN ABBREVIANS

Abbreviation	Full Meaning
BLAST	Basic Local Alignment Search Tool
CFCS	Cell Free Culture Supernatant
CTX	Cyclophosphamide
DNA	Deoxyribonucleic acid
EDTA	Ethylnediamine tetra acetic Acid
FAO	Food and Agricultural Organization
FEEPAP	European Food Safety Authority Panel on Additives and Products
GG	Code for probiotic *L. rhamnosus*
GRAS	Generally Regarded As Safe
HPLC	High Performance Liquid Chromatography
IL	Interleukins
LAB	Lactic Acid Bacteria
MDG	Millenium Development Goal
MIC	Minimum Inhibitory Concentration
MRS	Mann Rogosa Sharpe
PBS	Phosphate Buffer Solution
PCR	Polymerase Chain Reaction
rRNA	ribosomal Ribonuceic Acid
WHO	World Health Organization

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CHAPTER ONE

INTRODUCTION

1.1. The universal threat of diarrhoea

Diarrhoea is a gastrointestinal infection caused by viral, bacterial and parasitic microorganism and the risk factors are unhygienic environment, contaminated food or unclean water. According to World Health Organization (WHO), it is defined as 'the passage of three or more loose or liquid stools per day (WHO, 2017). It can last a few days, sometimes longer or shorter, resulting in loss of fluid and electrolyte leading to dehydration. Globally, diarrhoea disease accounted for high cases among the children under the age of five between 1990 and 2016, with millions of deaths recorded in 2016 (Global Burden of Disease Study, 2016). This death threat among children led to a discussion at the general meeting of the United Nations on international development to reduce the high mortality rate by at least two thirds at the end of the year 2015 (UNICEF, 2019). Their goal was to discover the measure to minimize the rate at which children are dying of diarrhoea. Besides, 2.5 billion cases of diarrhoea are reported yearly, causing around 525,000 deaths among the infants that are not up to five years (WHO, 2017).

In Africa, diarrhoea disease is a major challenge. According to the WHO (2017), the incidence of diarrhoea is generally high in the developing countries, till date diarrhoea disease is still a threat with a little reduction in mortality rate (UNICEF, 2019). Children are exposed to diarrhoeal infections because of improper disposal of waste, improper sanitation, lack of appropriate access to pure water and unclean environments.

In Nigeria, diarrhoea is one of the principal reasons for childhood death. Over 315,000 deaths are recorded annually among preschool age, and a major cause of these children's death is diarrhoeal disease (Akinnibosun, *et al*., 2015). Diarrhoea disease are

prevalent in North West and North Central. According to Dairo *et al*., (2017), Jos (Northcentral) had a 27% prevalence; Kaduna (Northwest region) had 21.1% prevalence and Ondo in South Western Nigeria had a prevalence of 8.1% of diarrhoea disease. This is a problem common to most region in Nigeria, as the prevalence of diarrhoea among the growing children were reported (UNICEF, 2019). Therefore, there is need for proper management of diarrhoea in developing and developed countries (UNICEF, 2019).

1.2. Diarrheagenic *Escherichia coli*

Diarrhoeagenic *E. coli,* according to the Global Burden of Disease Study (2016) is the main causative agent for diarrhoea infection among children apart from rotavirus. It affects children under five years. The mortality rate is high as well as the infection rate. The report from WHO (2017) also showed an estimate of 80% diarrhoea-related deaths among children under two years, sub-Saharan Africans have the highest mortality rate (WHO, 2015). The frequent prevalence of diarrhoea among African children have been reported (Dairo *et al*., 2017; Omole *et al*., 2019) with evidence of children having two or three episodes of diarrhoea per year.

Reports from different geographical regions have shown the high rate of diarrhoeagenic *E. coli* resistance among children below five years in Africa, and Nigeria is one of the major countries affected (Akinnibosun *et al.*, 2015; WHO, 2015; Saka *et al*., 2019). In a study, 20% of the implicated microorganisms observed in faecal specimens of children with diarrhoea was diarrheagenic *E. coli* while other implicated microorganisms were rotavirus *Salmonella*, and *Clostridium* (Charyeva *et al*., 2015). A report from Mozambique in 2015 recorded 41.8% of surveyed children with diarrheagenic *E. coli* implicated in their diarrhoea (WHO, 2015). Furthermore, diarrheagenic *E. coli* infections increased in the region with low social-economy status, absence of potable water supply, and unhygienic environment, most especially among the children resident in IDP camp (Saka *et al*., 2019).

Diarrhoeagenic *E. coli* strains are divided into six pathotypes groups according to their clinical syndrome and specific virulence factors. These six pathotypes are; enteropathogenic *E. coli* (EPEC), diffusely adherent *E. coli* (DAEC), enteroaggregative *E. coli* (EAggEC), shiga toxin producing *E. coli* enterohaemorrhagic *E. coli*

(STEC/EHEC), enteroinvasive *E. coli* (EIEC), and enterotoxigenic *E. coli* (ETEC) (Jafari *et al*., 2012).

1.3. Human gut

The human gut has millions of bacteria living in a symbiotic relationship in the gastrointestinal tract (GIT), they are referred to as gut microbiota (Thursby and Juge, 2017). They help in the digestion of food and protecting the gastrointestinal tract from invasion. The gastrointestinal tract supplies the bacterial communities with nutrients and good condition for their growth. Certain drugs (antibiotics) or diet can result in microbiota imbalance (Dudek-Wicher *et al*., 2018) especially when they have an influence on GIT motility in addition to their original effect on gut microbiota. For example, excessive consumption of alcoholic beverages has a negative influence on gut microbiota, (Collado *et al.*, 2016), and thus results in imbalance. Excessive consumption of drugs with purgative effect could also result in excessive motility of GIT that can result in gut microbiota imbalance and could lead to diarrhoea. However, there is a continuous battle between the gut microflora and infectious microorganisms, the pathogens creep in, colonize the gut and initiate infection upon successful entry. When these pathogens successfully colonise the host, they alter the microbial balance (homeostasis) by breaking the protective barrier created by the normal flora. This alteration leads to disruption of the physiological and absorptive functions, thus resulting in chronic diarrhoea, enlargement of the colon or shock. Some of these pathogens are *E. coli*, *Salmonella*, *Clostridium*, *Pseudomonas,* e.t.c. (Kotloff *et al*., 2019).

The microflora of the gut plays a significant function in preventing infectious microorganisms from invading the gut. They thereby create a healthy gut, as well as, promoting a healthy environment. The healthy gut is important for disease prevention; because when the pathogens invade the organ, it becomes infected with symptoms or could be asymptomatic. The infected gut might be leaking, resulting in diarrhoea or affect intellectual development in children and may lead to death (Pinkerton *et al.* 2016). Pathogenic microorganisms compete for space and attachment sites with gut microbiota; their entrance into the gut prompts an attack within the niche and reduces the population of gut flora. Diarrhoea-causing microbes throw the normal gut flora off balance. However, probiotic microorganisms have the potential to reverse the effect of infection

due to diarrhoeagenic pathogens. Not all diarrhoea cases should be treated with antibiotics. As the initial major concern is often rehydration and replacement of electrolyte imbalance due to the passage of loose stool. Currently, acute infectious diarrhoea is responsive to therapy by oral rehydration (Ali *et al*., 2014). However, the use of antimicrobial therapy should be for severely ill patients (Ochoa *et al.,* 2009; Bezatu *et al.,* 2013). Also, prescribing antibiotics before the proper susceptibility test could lead to inappropriate use of antibiotics. The inappropriate prescription of antibiotics in the management of persistent diarrhoea might render treatment ineffective because of the differences in pathogenicity of diarrhoea, which can also lead to antimicrobial resistance.

Antimicrobial resistance (AMR) is a major public health issue as agents that are more infectious are becoming resistant to antibiotic treatments. Previous studies identified the alarming rates of AMR in Africa. *E. coli* that was often susceptible to gentamicin and ciprofloxacin now exhibit a range of resistance to the same antibiotics (Tadesse *et al.,* 2017). Pathogenic microbes are resistant to nearly all the old generation antibiotics, although, efforts are put in place to discover new drugs (Tadesse *et al.,* 2017). Also, inappropriate use of antibacterial drugs against diarrhoea infections disrupts gut microbes. Antibiotics have both bacteriostatic and bactericidal action against pathogenic microorganisms, and can be detrimental to the growth of gut flora, hence, this affects its physiology, causing pathology of the gastrointestinal tract, and suppressing the immune system. Antimicrobial resistance has caused a nuisance to universal health; therefore, a major concern for healthcare workers (Charyeva *et al*., 2015; WHO 2016; UNICEF, 2016). There should be appropriate management of persistent diarrhoea among children; apart from treating the infection with the use of antibiotics, probiotics might be a good therapeutic option.

1.4. Probiotics

There is an urgent need to improve children's health; therefore, there should be more effort to reduce infant mortality rate, at least to a minimal level. The diverse mechanisms of interaction amongst microbial populations within the host and body immunity has brought about an interesting part of microorganisms, which influence man's health. The

World Health Organization defines probiotics as ‘live microorganisms which when administered in adequate amounts confer a health benefit on the host’ (FAO/WHO, 2002). The efficacy of probiotics was previously in the context of alternative medicine, but now incorporated into conventional medicine (Islam, 2016). The impact of probiotics as gut and immune modulators in restoring the gut microbial balance and expression of immune cells were analyzed by Probiotic Association of India (PAi) and the evidence revealed that probiotics not only improve human health but also restore and maintain the gut microbial niche (Grover *et al*., 2012).

The Food and Agriculture Organization (FAO) panel established the guidelines in 2002 on how to evaluate potential probiotic strains for therapeutic purposes (FAO/WHO, 2002). These are used for the selection of probiotic strains to date. In 2006, probiotics were generally accepted as 'life microorganisms that release health benefit into the host when administred in the right proportions’ (FAO 2006). The probiotic strains release some metabolites as their by-product, which produced antagonistic actions against pathogens. The inhibitory compounds are; organic acids, bacteriocins, H_2O_2 and diacetyl (Ayeni *et al.,* 2009; Fernandes *et al*., 2019). Probiotic strains possess the ability to restore and maintain human health and replenish the lost microflora in the gastrointestinal tract. They are toxic to the infectious microbes but not to the host cell and thereby preventing the pathogenic microorganisms from evading the mucosa cell wall. Probiotics bring about a microbial balance of the intestines and one of the major groups of bacteria used is the Lactic Acid Bacteria (LAB), also bifidobacteria, *E. coli* Nissle 1917 and *Saccharomyces boulardii* (Ukena *et al*., 2007).

Lactic acid bacteria are used therapeutically in the treatment of intestinal diarrhoea (gastroenteritis infection) and in the prevention of travellers’ diarrhoea (Nditange *et al*., 2013; Denkova *et al*., 2013). The commonly used strains are the heterogeneous LAB such as lactobacilli species, enterococci species, *Leuconostoc, Pediococcus* e.t.c. These genera are Gram-positive, non-spore formers, facultative or obligate anaerobes, rod or cocci shapes. They produce organic acids during fermentation as the finished products; hence, they are important in fermentation industries. The lactic acid bacteria strains are ubiquitous; these strains can be isolated in soil or vegetables, fermented milk, honey, animals and man. They can exist as normal flora of the GIT and women vagina (Ayeni and Adeniyi, 2013). They occur naturally in breast milk, faeces of animals and man.

Some foods such as sauerkraut, cheese, milk, meat, pickles, locust beans, cucumber, sausage etc contain the bacteria. Examples include *L. bulgaricus* found in yoghurt; *L. casei* found in cheeses and accepted in Canada as probiotics (Health Canada, 2009). Other natural sources are; fermented foods; human and animal milk; human and bovine intestines (Afolayan *et al*., 2017; Adetoye *et al*., 2018).

1.5. Beneficial microbes in human breast milk

Breast milk is the best food given to an infant to encourage healthy and rapid growth. According to Moussa *et al*. (2013), breastmilk is the best nutritional meal for neonates; it protects them from various diseases such as gastroenteritis infections, asthma and allergy. According to WHO (2013) report, in African, only 38 per cent of infants were exclusively breastfed while the larger percentage of infants were formula food fed. Meanwhile, mother's breast milk contains important constituents that include essential minerals, natural anti-aging substances, hormones, enzymes and antibodies for proper child. s growth. According to WHO (2013), when a child is exclusively breastfed for at least the first six months of life, they do not often contract infections, they are protected and shielded from any outbreak of infections or any form of illnesses compared to the formula-fed children (Martin *et al*., 2016; Krol and Grossmann, 2018). The risk of contracting infectious diarrhoea is high among neonates who were inadequately breastfed (WHO, 2013).

According to Moles *et al*. (2013), breast milk contributes to the colonization of lactobacilli and bifidobacteria in the GIT of a newborn baby. This is demonstrated by the colonization of LAB and *Bifidobacterium* spp in the gut of a newborn baby after they are introduced to the breast milk within few days of birth, with the isolation of these bacteria in their faeces (Milani *et al*., 2017). A healthy gut is influenced by the diet. Report from Milani *et al*. (2017) showed that a child who takes mother breast milk has richer gut microbiota than the formula-fed' infants. According to Timmerman *et al*. (2017), the first colonizers in breast-fed infants are facultative anaerobes such as enterobacteria, streptococci, enterococci, staphylococci, or lactobacilli, with strictly anaerobic strains of *Lactobacillus* and *Bifidobacteriium*. They enhance the intestinal barrier and protect the gut from pathogenic microorganisms (Chukwu, *et al.*, 2014). The gut colonizers can function as probiotics, especially the LAB and bifidobacteria species.

Application of probiotic bacteria boosts the body protection against pathogens and function as prophylaxis against infections.

Probiotic bacteria contribute positively to gut microflora, it restores depleted microbial content and decreases the microbial toxic activity (Zhang, *et al*., 2015). Potential probiotic isolates from human breast milk can be use therapeutically. Other functional properties of probiotics are hypocholesterolemic activity through lowering of plasma cholesterol (Kumar *et al*, 2012; Shehata *et al*., 2019), an anti-infectious barrier to pathogens (Ayeni, *et al.,* 2011), preventing diarrhoea and modulate the immune system (Khaneghah *et al*., 2020; Rocha-Ramírez *et al*., 2020).

1.6. Statement of the problem

Diarrhoea is one of the major health challenges among growing children. When the stooling persists for more than twenty-four hours, there could be development of severe symptom in which children lose the ability to eat, and also have continuous vomiting and possibly develop a fever. When this happens, the child is dehydrated and the dehydration can be severe leading to seizure, brain damage, and even lead to death (Collado *et al*., 2009).

Diarrhoea can also affect the beneficial microbes of the gut that serve as protection in the GIT. The pathogens can evade the mucosa area of intestines and cause a harmful effect. Furthermore, the presence of infection in the GIT causes alteration of the gut microbes leading to deregulation and dysfunction of the immune system, resulting in inflammation (Collado *et al*., 2016). It could also result in severe and persistent gastroenteritis that are treated with antibiotics. Frequent use of antibiotics affects gut flora, it reduces the high population of the microbes in the gut (Dudek-Wicher *et al*., 2018). Microorganisms can become resistant to antibiotics when these drugs are inappropriately used (Alshara, 2011; Akingbade, *et al*., 2013).

1.7. Rationale for the study

Human beings, irrespective of gender and age are regularly predisposed to numerous microorganisms; both beneficial and pathogenic via diet; this supports the adage that

says ‘you are what you eat’. The colonization of microorganisms in the gut starts from birth, the diversity of species increases and reach a stable climax in adult life. The types of colonized bacteria in the child depend on many factors such as; diet; breastmilk or formula, the mode of delivery; natural or caesarean, the mothers’ diet, regular use of antibiotic and early exposure to the environment (WHO, 2013). The perception of protective role by beneficial bacteria is that the gut microbes are in contact with the epithelial cells and intestinal mucosa forming a mechanical barrier against invading pathogenic microorganisms. For example, *L. rhamnosus* strain GG and *L. plantarum* strain 299v inhibit *E. coli* from adhering to epithelial cells (La Fata *et al*., 2018).

The rationale for using probiotic strains in the management of diarrhoeal infections arises from the ability of the strains to inhibit the growth of enteric pathogens, interact with the host natural defense system and protect the epithelial cell from invasion. Probiotic strains also supplement the lost gut microflora during diarrheal infection. Potential probiotic strains isolated from fermented food, cow intestine and wine possess antimicrobial activity against various gastroenteritis microorganisms causing diarrhoeal infections. (Ayeni *et al*., 2009; Jensen, *et al*. 2012; Adeniyi *et al*., 2015; Afolayan and Ayeni 2017; Adetoye *et al*., 2018; Kwasi *et al*. 2019; Sunmola *et al*. 2019). However, there is a scarcity of information on the management of infantile diarrhoea caused by diarrheagenic *E. coli* using probiotic strains in Nigeria. Therefore, this research is evaluating LAB isolates from mothers’ breast milk and/or their babies’ faeces and antagonist effect against diarrheagenic *E. coli*. Prophylactic and therapeutic use of strains isolates from mothers’ breast milk and their babies’ faeces will be evaluated as advocated by the WHO on the implementations of an alternative disease control strategy.

1.8. Research hypothesis

- Anti diarrhoeagenic *E. coli* lactic acid bacterial strains are present in mothers’ breastmilk and faeces of their healthy children.
- Potential probiotic strains are safe for oral use.
- Lactic acid bacteria possess aggregation and cell adherence properties to attach to the host cell.
- Potential probiotic strains produce general antimicrobial compounds.
- Potential probiotic strains modulate the immune response.

1.9. Research question:

- Can anti diarrhoeagenic *E. coli* lactic acid bacteria strains be isolated from mothers' breast milk and faeces of healthy children in Nigeria?
- Are these strains safe for oral consumption?
- Do the strains possess aggregation and cell adherence properties?
- Do these strains produce antimicrobial compounds?
- Do the isolated strains have immunomodulatory properties?

1.10. Aim and objectives

This research aims to characterize the LAB isolated from mothers' breast milk and the faeces of their healthy children as potential probiotics in the management of infantile diarrhoea.

1.10.1. Specific objectives

The specific objectives of this research are:

- To isolate and characterize anti diarrhoeagenic *E. coli* lactic acid bacteria from mothers' breast milk and faecal samples of their healthy children.
- To evaluate the safety properties of LAB strains for oral probiotic application.
- To evaluate the adhesion properties of LAB strains.
- To evaluate the antimicrobial compounds produced by selected potential probiotics strains.
- To determine the immunomodulatory property of selected strains of LAB *in vivo*.

CHAPTER TWO

LITERATURE REVIEW

2.1. The gut microbiota

Microbiota, microflora or normal flora is used synonymously for the group of microbes that live together in coexistence with their hosts in the gastrointestinal tract. Joshua Lederberg was the first person to introduce the concept of the human microbiome to the world of science (Ursell *et al*., 2012). He defined it as 'the ecological community of the different microorganisms as commensal, symbiotic, or even pathogenic, which we factually share our body space with but have been disregarded to some level as the main factors of health and ailment' (Lederberg and McCray, 2001).

According to some researchers, the gut microflora of healthy individuals produces numerous health benefits which include; protection from pathogens, nourishment, host metabolism and modulation of host' immune system (Sekirov *et al*., 2010). However, some factors, which include old age, food, use of antibiotics and the environment, can modify the microbial habitat. Once the ecological equilibrium of the gut microbiota is disrupted, the physiological status of the habitat is affected, the pH of the stomach is altered, and the immune response is compromised, leading to free passage of the invaders to the epithelium wall.

Previously, it has been reported that newborn baby intestines are supposed to be sterile and free from microbes or contain a small number of microorganisms (Fouhy *et al*., 2012), conversely, the gastrointestinal tract of the infants have been found to compose microorganisms that colonized the organs after delivery. The colonization of the gut depends on the method of delivery, the type of food, use of antibiotics, prebiotic supplements and the growing environment (Fouhy *et al*., 2012). Infants obtain their gut microbes from the vagina, faeces, milk, mouth and skin of their mothers during the gestation period (Fouhy *et al*., 2012).

Neonates' gut comprises of the most common strains of microorganisms isolated from the intestine generally called 'early colonizers'. These early colonizers in neonates are; majorly Gram-positive anaerobic microorganisms like bifidobacteria, *Lactobacillus*, clostridia, enterococci, streptococci, and others such as *Bacteroides* spp. *Enterobacter* (Houghteling *et al*., 2015). Moreover, a new population of microorganisms continues to evolve until a child is aged two when the gut's community resembles the adult's gut (Houghteling *et al*., 2015). However, the stabled and abundance gut microbes' exert an outstanding function in the management of child health by breaking down food substances and making the nutrients available to the host. The gut microbes produce some proteinous and organic acid substance. However, metabolites produced empowered the gut, help in maintaining a homeostasis environment, protecting the gut, fighting against the invaders, and boost the immune system to ward off invading pathogens (Milani *et al.*, 2017).

Many factors influence the diversity of gut microbiota. The diverse composition of the infants' gut microbes can arise from variations in diet, environment or the use of antibiotics. Antibiotics use can affect the gut microbes resulting in dysbiosis condition such as gastroenteritis majorly infantile diarrhoea, immune system and metabolic disorders (Lazar *et al*., 2018).

2.2. Diarrhoea

Diarrhoea is defined 'as the passage of three or more loose or liquid stools in 24 hours, or frequent stooling than the normal for an individual (WHO, 2017). Depending on the types of diarrhea, it can last for 2 weeks, 4 weeks or more. Diarrhoeal disease leads to alteration in bowel motility. Different aetiological agents such as virus, parasites and bacteria are implicated in the disease. The disease may be because of poorly absorbed solutes in the intestinal lumen, an increase in toxins secretion into the intestinal lumen by bacterial agents such as, *Vibrio cholera*, enterotoxigenic *E. coli*, and *Clostridium perfringes*, which cause uncontrollable outpour of fluid (Tejan *et al*., 2018). Diarrhoea disease may also be due to inflammatory or cytotoxicity action of some bacterial agents such as *Shigella* sp, *Campylobacter* sp, *Vibrio parahemolyticus*, non-typhoidal *Salmonella* sp, which distrupt the mucosa and cause damage to colon and ileum resulting in dysentery (Tejan *et al*., 2018). The diarrhoeal disease occurs in both adult and children

but the morbidity and mortality rate is higher in children while the adult rate is controllable. The fatality in diarrhoeal diasease among children is therefore a global concern.

According to Global Burden of Disease in Pediatrics (2016), diarrhoea was estimated to cause 1 in 9 child deaths worldwide; therefore, diarrhoea was ranked second amongst the diseases that cause death among children before their fifth birthday (Mokomane *et al*., 2018). Based on their findings, about 80% of the deaths were recorded to occur before the children reach the age of two. Moreover, 50% of these sudden deaths take place majorly in sub-Saharan Africa (Mokomane *et al*., 2018). Although, implementation of ORT and antibiotic therapy for the treatment of severe and persistent diarrhoea is operational, however, enteric infections (diarrhoea) are still on the increase, and the cause of a global increase in death rate (Fischer Walker *et al*., 2012). Also , diarrhoea infections affect children growth and thereby causing low cognitive development. Diarrhoea disease can also become an economic burden as large amount of money are used for the treatment of patients with infantile diarrhoea. The causative agents of diarrhoea infections include; rotavirus, *Shigella*, *Campylobacter jejuni,* cryptosporidia, *Vibrio cholerae*, *Salmonella* spp, parasitic microorganisms and *E. coli* of different pathotypes group such as; enteroinvasive *E. coli*, adherence *E. coli,* enteropathogenic *E. coli* and enterotoxigenic *E. coli*.

2.2.1. Childhood diarrhoea

Diarrhoea is a serious health challenge among infants that are not up to 5 years. Acute and persistent cases of diarrhoea are common among children that are not up to five years,. Rotavirus accounts for 40 per cent in all cases of diarrhoea reported (Troeger *et al*., 2018). Additionally, WHO (2016) also reported that rotavirus is one of the major causative agents of diarrhoea infection. However, some bacteria are also implicated in diarrhoeal disease e.g. *Campylobacter, E. coli, Shigella, Salmonella, E. histolytica, Vibro cholera* (Tian *et al*., 2016). The infection is contracted through food or water that is already contaminated by these agents. The epidemiological data showed that above 80% of the diarrhoea-causing infections are prevalence in developing and under-developed countries (WHO 2017; UNICEF 2019).

India has the highest numbers of children with cases of diarrhoea followed by Nigeria with the second largest percentage (UNICEF, 2016). Okeke (2009) also reported that the clinical condition of a child with at least two episodes of diarrhoea every year is common in the developing world. Clinically, diarrhoeal disease occurs in three main forms viz:

2.2.1.1. The watery acute diarrhoea

Acute watery diarrhoea is usually a prolonged illness for some numbers of hours or days. The symptoms are connected with significant loss of electrolytes and quick dehydration of body fluids in infected patients. *V. cholerae, E. coli* and rotavirus are usually the causative agents (Afroza *et al.*, 2013).

2.2.1.2. Bloody diarrhoea

Bloody diarrhoea is often referred to as dysentery. It is manifested by the presence of blood in an infected stool. It normally causes damage in the gastrointestinal tract and loss of essential nutrients. *S. dysentreae, Salmonella, Campylobacter* and *Aeromonas* sp are implicated in bloody diarrhoea (Akinnibosun and Nwafor, 2015).

2.2.1.3. Persistent diarrhoea

Persistent diarrhoea is the kind of stooling accompanied by the presence or absence of blood and lasts not less than 14 days. Children who are not fed properly, the undernourished and the immunocompromised children with a severe illness, as HIV/AIDS are prone to this type of diarrhoeal infection (WHO, 2011).

2.2.2. Aetiology of Diarrhoea

In both developed and developing countries, the implicated microorganisms in diarrhoea amongst young children are virus (rotavirus), parasites, *E. coli, S. dysentrae, C. jejuni, Cryptosporidium* spp, and *V. cholera.* (Mokomane *et al*., 2018).

2.2.2.1. Rotavirus

Rotavirus according to Afroza, *et al*. (2013) is the major cause of diarrhoeal disease. The viral diarrhoea is severe and life threatening. Globally, it affects children not up to two years old. The viral infection is observed in children before their second birthday. The frequent occurrence of the viral diarrhoea infection is dangerous and can lead to significant fatal illness. According to the same author, at least two episodes of rotavirus diarrhoea are experienced among 30% of children under the age of two. The virus usually attacks the bowels of the children to cause inflammation and eventually leads to gastroenteritis infection and it can be contracted from one person to another.

The viral diarrhoea alone causes more than 20% of death among infants globally. It accounts for 500,000 deaths of children that are younger than three years every year (Crawford *et al*., 2017). Rotavirus affects urban and rural areas alike both in developed and in developing countries (Latipov *et al*., 2011). The rotaviral diarrhoea' symptoms are liquid stools, dehydration and vomiting; the rotaviral diarrhoea can occur at any time of the year (Afroza *et al.*, 2013).

Rotavirus replicates in the host cell within the epithelium layer of the intestine. It causes the erratic destruction of epithelial cell and shrinking of the villous (Tomislav, 2018). The absorptive villous later disappeared and are replaced by immature secretory crypt cells. The replacement of the lost villous by immature crypt cells triggers the intestines, secrets saltwater, and electrolytes. The lost villous cells lead to the loss of enzymes that produce disaccharides and this reduces the availability of disaccharide for absorption, most especially lactose sugar (Tomislav, 2018. The whole process can be recovered when there are reproduction and maturation of villous epithelia with stimulation of the enzymes to produce lactose.

2.2.2.2. *Escherichia coli*

Escherichia coli is a Gram-negative microorganism, Theodore Escherich discovered it in 1885. *Escherichia coli* strains are facultative anaerobic organism that resides in the lower part of the gastrointestinal tract of both human beings and animals. After a child is weaned, the organism inhabits the colon as a commensal and continues to exist as

normal flora of the gut within the epithelial layer of the mucus and large intestine, most especially the lumen (Donaldson *et al*., 2016). However, infection set in when there is ingestion of any faecal contaminated food or water (Donaldson, *et al*., 2016).

The microorganisms can also become pathogenic when they acquire virulence factors like toxins, effector proteins secreted and adhesions. They can as well acquire plasmids, phages and pathogenic islands (PAI) which modulate the response of the host. (Longstreth *et al.*, 2006; Mwambete *et.al*. 2010). The pathogenicity of *E. coli* is because of combined virulence factors leading to severe diarrhoea. The infectious *E. coli* strains are grouped as pathotypes, these include enterohemorrhagic *E. coli* (EHEC), adherent-invasive *E. coli* (AIEC), sepsis/meningitis causing *E. coli* (MNEC), enteropathogenic *E. coli* (EPEC), diffusely adherent *E. coli* (DAEC), atypical enteropathogenic *E. coli* (ATEC), enterotoxigenic *E. coli* (ETEC), enteroinvasive *E. coli* (EIEC), uropathogenic *E. coli* (UPEC) and enteroaggregative *E. coli* (EAEC) (Croxen *et al*., 2013). Within each of the pathotype, *E. coli* strains are categorized further according to the antigenic variants, which include; K-antigen (capsular), types O-antigen (lipopolysaccharide), and H-antigen (flagellar) (DebRoy *et al*., 2016).

2.2.2.3. *Shigella*

Shigella species are also implicated in gastroenteritis infection. They are the main source of dysentery. According to Ifeanyi *et al*. (2009), in severe cases of dysentery, 60% of the microorganisms implicated are *S. dysenteriae*. The symptoms are mostly watery diarrhoea. *Shigella* species are divided into four serogroups viz: *S. sonnei, S. boydii, S. flexner,* and *S. dysenteriae.* The most common is *Shigella flexneri,* and is mostly found in developing countries. *S. flexneri* is implicated in provincial epidemics place where it causes asterndysentery disease (Ifeanyi *et al.,* 2009). *Shigella dysenteriae* type 1 produces Shiga toxin, which destroys tissue to release a large amount of watery diarrhoea. The transmission is mostly person-person contact. *Shigella spp* are susceptible to antibiotics such as co-trimoxazole and nalidixic acid, however, the use of ampicillin has been recorded to be effective in a few areas while resistance to some antibiotics has been reported (Karimi-Yazdi *et al*., 2020).

2.2.2.4. *Campylobacter jejuni*

Another gastroenteritis causing microorganisms is *C. jejuni*. It is implicated mostly in infantile diarrhoea in developing countries (Lehtopolku *et al.,* 2010). *C. jejuni* also affects animals of different species such as dogs and chicken. The microorganism is spread either by having direct contact with infected animal faeces or by the consumption of water, milk or food that have been contaminated.

C. jejuni causes two-third of watery diarrhoea episodes but one-third of the episodes of dysentery. Its symptoms might be fever and a mild severe disease that last for 2 to 5 days. Erythromycin antibiotic can be administered in the early stage of the symptoms to reduce its replication, but it is not always recommended, because *C. jejuni* infections are difficult to be clinically distinguished from other infectious-causing agents that also exhibit the same symptoms (Lehtopolku *et.al*., 2010).

2.2.2.5. *Vibrio cholerae*

According to Akingbade *et al*. (2013), there are two biotypes and two serotypes of *V. cholerae*, the classical and El Tor. *Vibrio cholerae* is a non-invasive microorganism that produced cholera toxin, which results in a prolific secretion of electrolytes and fluids in the intestine. *V.cholerae* causes severe diarrhoea, which leads to dehydration, and if the patients are not treated with the replacement of fluid and electrolytes in a short time, it can lead to death. *V.cholerae* infection occurs in children and sometimes adult in both endemic and non-endemic areas. Administration of antibiotics like tetracycline, erythromycin, metronidazole, chloramphenicol, furazolidone, or co-trimoxazole can be used to manage the infection; however, resistance to these antibiotics has been recorded (Akingbade *et al.,* 2013).

2.2.2.6. *Salmonella*

Salmonella spp is a foodborne pathogen that causes serious form of acute gastroenteritis infections like typhoid fever (Das *et al.,* 2013). Globally, over 93 million cases of *Salmonella* infections are on records resulting in about 155,000 deaths annually (Garedew *et al.,* 2018). Besides, *Salmonella* species are implicated in most of the infections of animals or contaminated animal products. *Salmonella* spp normally causes

typhoid with the usual symptoms of diarrhoea in most developing countries and in an area where processed foods for commercial purpose are common.

2.2.3. Behaviours that increase the risk of diarrhoea

The statistics in the world for the last 20 years have shown that unsafe drinking water, insufficient hygiene and inadequate sanitation cause 88% of diarrhoea-associated deaths in infants (WHO, 2016). The Global Burden of Disease Pediatrics Collaboration (2016) evaluated a major percentage of diarrhoea-associated deaths to be attributable to the behaviours that are listed below;

1. The inability of a child to be exclusively fed with breast milk for at least six months. If a child is not well breastfed or breastfed at all, the risk of developing severe diarrhoea is very high. This is because breast milk contains essential components thatt are transported from mothers to their infants, this helps in building the natural early colonizers microflora in infants' gut.
2. Failure to continue with breast-feeding of the infant after the first six months of exclusively breastfeeding. The continuous breastfeeding of a child for the first 6 months or more can reduce the chance of the child contacting certain infections or diseases like cholera, dysentery and diarrhoea.
3. Use of feeding bottles: Feeding bottle can be contaminated easily with faecal microbes if not handled properly. Using the same bottle to prepare the infants formula can transfer the contaminants into the body and it can lead to diarrhoea infections.
4. Method of storage: The methods used in storing the infant food for a later used can also encourage the growth of microbes. The food that is already cooked and saved for a later used, when exposed, can grow mould or have bacteria contaminants when it is not refrigerated. It is advisable to feed the infants with freshly prepared food as keeping the food for a long time can encourage microbial growth.
5. Drinking of water with faecal bacterial contaminants: If water is not gotten from hygienic source, it can be contaminated. Besides, failure to cover the water during storage at home can make the water not suitable for drinking. Moreover, dipping dirty hands inside drinking water can introduce microbes into the water in the container.

6. Inappropriate disposal of infant faeces: Although, infant faeces are believed to be innocuous and contain beneficial microbes (Watkins *et al*., 2017), however, they contain microorganisms like fungi, viruses, bacteria and protozoans and become infectious when exposed to contaminants.

2.2.4. Pathogenesis of diarrhoea

A minimum of eight litres of fluid are secreted into the intestinal lumen (Leiper, 2015). In a normal host, there is a balance between secretion of fluids in the crypt and absorption of fluids in the villi. However, malfunction of the intestine disrupts the function of the intestinal lumen and causes a prolonged opening of the chloride channels which leads to uncontrolled secretion of water and wrong absorption of water, ions and solutes, thereby, the secretion of fluids exceeds absorption, resulting in diarrhoea.

The pathogenesis of diarrhoea disease occurs through various mechanisms, some of which are discussed below:

- Alteration of the gut: Upon the entering of the diarrheagenic *E. coli* into the gut, they strictly adhere to the mucosa wall of the small intestine to avoid being swept away before proliferation. Bacteria that possess fimbriae or pili cause adhesion of the bacteria to the small intestine. The pili bind to the receptors at the peripheral layer of the intestine. The enterotoxin producers most especially *V. cholera* and *E. coli* make use of the mechanism. The adherence of bacteria to the mucosa wall effects a change in the gut; this reduces the capacity of the epithelial cells to absorb and secretes free-flowing fluids. This mechanism applies to infection with enteropathogenic or enteroadherent *E. coli*. Below are the other mechanisms used by different diarrhoeagenic *E. coli* (Mirhoseini *et al*., 2018).

- **Toxins that cause secretions**. Some microorganisms produce toxins thereby modifying the cells and cause intestinal secretions that alter the functions of epithelial cells. The toxins released incapacitate the villi by reducing its uptake of sodium and increase the production of chloride in the crypts. Examples of such diarrhoea-causing microorganisms are Enterotoxigenic *E. coli, V. cholerae* and possibly *Salmonella.* Total recovery of the gut can occur after the

replacement of the intoxicated cells with healthy ones after two to four days (Qiangde *et al*., 2019).

- **Mucosal invasion**. The pathogenic microorganisms gain access to the host cells; they invade and destroy the epithelial cells of the colon and distal part of the ileum. These common microorganisms are *Shigella* spp, *C. jejuni* and enteroinvasive *E. coli*. They perforate the tissues and form superficial ulcers resulting in bloody diarrhoea with the evidence of red and white blood cells in the stool. They also produced toxins, damage the tissues and cause secretion of excess saltwater (Thiagarajah *et al*., 2015)

2.3. Diarrhoegenic *Escherichia coli*

Diarrheagenic *E. coli* (DEC) is the source of acute, moderate and severe diarrhoea (Platts-Mills *et al*., 2015; Jain *et al*., 2019). It is well observed that enteropathogenic serotypes harboured virulence genes, and other diarrhagenic *E. coli* produce toxins, which are not observed in normal flora *E. coli* or non-pathogens (Okeke, 2009; Platts-Mills *et al*., 2015; Seidman *et al*., 2016). Diarrheagenic *E. coli* are divided into five categories discussed below:

2.3.1. Enteropathogenic *Escherichia coli*

Enteropathogenic *E. coli* (EPEC) is defined as the type of diarrheagenic *E. coli* possessing the Loci for Enterocyte Effacement (LEE), but lack Shiga-toxins genes found in phage-borne of other strain (Gomes *et al*., 2016). Enteropathogenic *E. coli* strains produce attaching and effacing (A/E) lesions mechanism on the surface of enterocytes, and those strains carry plasmid with virulence factors (Ochoa *et al*., 2009). Within their genes are encoding regulator and some putative virulence genes responsible for diarrheagenic *E. coli*.

The classical EPEC serotypes were reported to be the principal source of diarrhoea disease in Africa (Mandomando *et al.*, 2007; Okeke, 2009). The cases are severe among children that are below one year of age, and spread from the contact with the faeces of an infected person to another healthy person. The disease can also spread through

drinking of unclean water, eating spoilt food, or being in contact with non-sterile objects. Enteropathogenic *E. coli* symptom is associated with watery diarrhoea, which could be severe and fatal.

2.3.2. Enterotoxigenic *Escherichia coli*

Enterotoxigenic *E. coli* (ETEC) are known to agglutinate the heat-stable or heat-labile enterotoxin using immunologic reagents. Enterotoxigenic *E. coli* are the common causative agent of infantile diarrhoea in developing countries. Children below five years suffer more from the diarrhoea caused by ETEC. The diarrhoea can easily produce major fatal dehydration in young children including persistent diarrhoea that claims life among the children that are malnourished (Okeke, 2009).

The small intestine can be colonized by ETEC strains and cause watery diarrhoea but with less abdominal pain and vomiting. Enterotoxigenic *E. coli* strains produce heat labile (LT) or/and heat stable (ST) enterotoxins (Mirhoseini *et al*., 2018). Heat labile enterotoxin have a high percentage of similarity with the structures and mode of action of cholera toxins. It binds to GM-1 ganglioside irreversibly on the surface of intestinal epithelial cells and activates adenylate cyclase resulting in the expression of cyclic AMP, which stimulates the secretion of excess chloride in the crypt cells and inhibits the absorption of neutral sodium chloride in the villi (Mirhoseini *et al*., 2018). Heat stable enterotoxin on the other hand, binds to guanylate cyclase C (GC-C) on the intestinal epithelia surface and stimulate the production of cyclic GMP (Fleckenstein and Kuhlmann, 2019). The two enterotoxins cause the alteration of cystic fibrosis transmembrane regulator (CFTR) chloride channel and inhibit Na+/H+ exchanger NHE3 producing excess salt and water in the intestinal lumen which leads to diarrhoea (Mirhoseini *et al*., 2018; Fleckenstein and Kuhlmann, 2019). However, ETEC strains are resistant to various antibiotics, which is a huge concern in global health (Medina *et al*., 2015).

2.3.3. Enteroinvasive *Escherichia coli*

Enteroinvasive *E. coli (*EIEC*)* are closely related to *Shigella* sp; its infections are attributed to virulence mechanisms like that of *Shigella* (Gomes *et al*., 2016). Its plasmid

encodes for Mxi-Spa type III secretion system and an antigen (Ipa) encoding for the T3SS effectors (Gomes *et al*., 2016). The plasmid gives EIEC the ability to attack the multicellular cells. It also encodes for IcsA that gives the bacteria a full potential to travel intracellularly within a host cell to invade the immune system (Mattock and Blocker, 2017). Its infection is established when the bacteria (EIEC) enter the M-cells in payer's patches, phagocytosed by resident macrophage and cause the death of macrophage (Pasqua *et al*., 2017). The escaped bacteria invade the enterocytes via the basolateral membrane. Through the acquisition of F-type plasmid (pINV), EIEC are able to invade the enterocytes, survive, diffuse and cause intracellular replication resulting in inflammation and severe damage to the intestina mucosa leading to dysentery (Pasqua *et al*., 2017). The symptoms include watery and mucoid diarrhoea, abdominal cramps, fever and tenesmus. *Shigella dysenteriae,* on the other hand, possesses Shiga toxin genes, which has a link with epidemic dysentery. *Shigella* is also an important cause of infantile diarrhoea

2.3.4. Enteroaggressive *Escherichia col*

Enteroaggregative *E. coli* (EAEC) are grouped under heterogeneous strains of *E. coli* with pathogenesis that results in non-bloody type of diarrhoea. The EAEC are defined by their ability to adhere to human epithelial cell, such as, HEp-2 cells in a 'stacked-bricked' pattern. This specie is implicated in acute and persistent diarrhea.

Enteroaggregative *E. coli* infection is established when EAEC aggregatively adhere to microvilli via aggregative adherence fimbriae resulting in the excessive production of mucus and formation of biofilm; the salient features of pathogenicity and resistance of EAEC (Ellis *et al*., 2020). The inflammatory response is induced leading to crypt dilation, microvillous vesiculation and production of toxins to cause epithelial cell extrusion (Wanda and Reygaert, 2017). The symptoms of EAEC infections are watery diarrhea, mucoid diarrhea, low-grade fever, nausea e.t.c.

2.3.5. Enterohemorrhagic *Escherichia coli*

Enterohemorrhagic *E. coli* (EHEC) infection results in bloody diarrhoea and hemolytic uremic syndrome (HUS). The infections can also lead to kidney disease such as kidney

failure (Marejková *et al.,* 2013). Enterohemorrhagic *E. coli* survive the harsh condition of GI tract and release a potent Shiga toxin with irreversibly cytotoxic effect leading to severe EIEC infections. Enterohemorrhagic *E. coli* bacteria break the acidic barrier of digestive sytem, after the passage, they attach themselves to the host epithelial cell forming attaching and effacing (A/E) lesions on the mucosa epithelium cells and also producing Shiga toxins which cause the destruction of microvilli (Cordonnier *et al*., 2017). The Shiga toxin causes bloody diarrhoea that is present in just about 5-10% of cases of HUS (Marejková *et al.*, 2013).

2.4. The resistance of diarrheagenic *Escherichia coli* to antibiotics

Some decades ago, antibiotic was employed as a direct approach to fight against infections. Unfortunately, the actions of antibiotics do not only destroy pathogenic microorganisms but also disrupt gastrointestinal microflora. Also, the introduction of antibiotics and the resistance of pathogenic bacteria have become a major health concern in clinical practice. Therefore, opportunistic microorganisms are becoming pathogenic and resistant to most antibacterial drugs (Seidman *et al*., 2016).

Resistance of enteric pathogens to antibiotics pose a risk to the health sector in Africa; several researches are on going to overcome this threat (Seidman *et al*., 2016). The resistance of diarrheagenic *E. coli* to antibiotics varies between the locations of samples collections, but generally, most pathogenic microorganisms are developing resistance to old and new generations of antibiotics such as penicillin, β- lactams, trimethoprim-sulfamethoxazole and cephalosporins (Seidman *et al*., 2016). Besides, the infectious microorganisms carry the transmissible genetic elements encoding for resistance genes, which are resistance to first- line and older antibiotics (Alanazi *et al*., 2018).

2.5. Prevention of diarrhoea

Infantile diarrhoea can be prevented. Although, diarrhoeagenic *E. coli* possess some factors, which impaired host defences and caused severe diarrhoea, however, numerous numbers of measures have been put in place to improve the host defences and decrease the global threat of infantile diarrhoea. Among these measures is the provision of

vaccines against rotavirus; exclusive breastfeeding and an improved nutritional status of food given to weaned children.

In addition, therapeutic intake of probiotics such as *L. reuteri, L. acidophilus* LB1, and *B. lactis* was reported to shorten the duration of diarrhoea (Denkova *et al.*, 2013). The probiotics used should have potentials of restoring and replacing lost gut flora. For instance, *S. boulardii*s has therapeutic effects in the treatment of C. *difficile* (Fijan, 2014).

2.6. Human breast milk

Breast milk is described as human fluid comprising several components and basic nutrition that is necessary for a growing child (Jara *et al.*, 2011). Breastfeeding starts immediately after birth; this is to make the child have all the nutrition required for growth. Furthermore, breast milk also protects the newborn against infectious microorganisms. This is made possible by the production of several components e.g. immunoglobulins, immunocompetent cells and antimicrobial compounds that are beneficial for the improvement of immune systems and protection against diseases (Martin *et al*., 2012a). These components play different roles such as anti-infectious, immunomodulatory and metabolic (Martin *et al*., 2012a). Human breastmilk contains proteinaceous molecules, nutrition of different classes and, beneficial bacteria. It produces bioactive molecules like immunocompetent cells, polyamines, fatty acids, lysozyme, oligosaccharides, lactoferrin, and different immunoglobulins like IgG; IgA; and IgE.

Breast milk is one of the products involved in colonization and development of neonatal gut microbes (Albesharat *et al*., 2011; Jara *et al.*, 2011). Different strains of *Weisella, Lactococcus, Lactobacillus, Bifidobacterium, Streptococcus, Micrococcus, Enterococcus* and *Leuconostoc*, have been isolated from breast milk (Perez-Caro *et al.*, 2010; Albesharat *et al*., 2011; Jara, *et al.*, 2011). Breast milk protects the neonates against asthma and allergy (Boix-Amorós *et al*., 2019).

2.7. Microbial component of faeces

In general, the neonates' faeces are made up of millions of microorganisms, which serve as normal flora of the gastrointestinal tract. There are variations in the components of human faeces; this variance to some extent depends on the kind of neonates' diet. Children that are breastfed exclusively have a higher content of water in their faeces (Boix-Amorós *et al*., 2019). Also, 25% of faeces contain organic solids with small proportion of inorganic material (Rose *et al*., 2015). These organic solids consist of 25-54% of microorganisms like bacteria, parasites, viruses and protozoans of both viable and non-viable cells (Rose *et al*., 2015).

The types of bacteria present in faeces depend solely on the consumption of the diet. For example; *Prevotella* bacteria are most common in hard faeces due to high fibre consumption content; while *Ruminococcaceae* bacteria are present in soften stools, they assist in the breakdown of complex carbohydrates, and soften the harder faeces (Poeker *et al.,* 2018).

2.8. Biodiversity of the gut microbiota

Gut flora consists of a diverse population of microbes. The ecosystem has various microorganisms that live as commensals with the host; e.g. bacteria, archaea, fungi, viruses and bacteriophages (Hillman *et al*., 2017). Among the microbial commensals residing in the gut, bacteria are the most abundant and they are diversified. The combined data generated from the Human Microbiome Project (HMP) showed an abundance of microbial communities with thousands of strains associated with humans (Hillman *et al*., 2017). From the results, 2172 species were isolated from humans and were classified into 12 different phyla. Phyla Bacteroidetes and Firmicutes account for about 93.5% of the total community. Out of all the species identified, 386 were found to be strictly anaerobic and these reside in the mucosal regions of the intestines (Hillman *et al*., 2017). Microorganisms that reside in the gut can become stable and live permanently in the gut, depending on the food intake, water and interactions with environmental components (Tasnim *et al*., 2017).

Microbial colonization is needed to maintain and establish the microbes in the gut to make them function effectively and provide Microbe Associated Molecular Patterns (MAMPs) signal. The MAMPs contribute to the growth of Gut Associated Lymphoid Tissue (GALT) and maturation of immune cells (Arrazuria *et al.*, 2018). Besides, the metabolic pathway assists the gut flora in making important components of indigestible food available for the host (Sekirov *et al*., 2010; Fouhy *et al.*, 2012).

Moreover, the diversity of gut microbiota is subjective to the diet, cultural practisess, geographical location, genetics and metabolism (Fouhy *et al.*, 2012; Tasnim *et al*., 2017; Ayeni *et al*., 2018; Afolayan *et al.*, 2019). The populations of gut flora can also be diversified base on geographical regions (Tasnim *et al*., 2017). In other studies, some conditions such as exposure to medications, type of diet, lifestyle, and health largely influence the diversity of microbes in the gut (Flandroy *et al*., 2018; Afolayan *et al*., 2019; Afolayan *et al.*, 2020).

2.9. Beneficial microorganisms of the gut

Gastrointestinal tract is a large organ with different section, starting from the mouth and ends in the rectum. Gastrointestinal tract has a luminal surface of 400 m^2; it is referred to as the largest organ (Sekirov *et al*., 2010). Each section of gastrointestinal tract performs various physiological functions reflected by oxygen level, pH values, availability of the enzymes and nutrients presents. For instance, the predominant bacterial groups in the large intestine are *Faecalibacterium* spp, *Ruminococcus* spp, *Eubacterium* spp, *Bifidobacterium* spp. *Peptococcus* spp, the genera *Enterobacteriaceae*, *Bacteroides* spp, *Lactobacillus* spp, *Streptococcus* spp and *Clostridium* spp, (Hillman *et al*., 2017).

2.9.1. The genus *Bifidobacterium*

A significant member of the human flora of the gut is *Bifidobacterium*. A scientist named Tissier in the year 1899 first discovers them. The first member of the genus isolated is *Bacillus bifidus communi*. This genus was isolated from faeces of healthy breastfed infants' gut. In 1924, Orla-Jensen reclassified the strain as *Bifidobacterium* genus but it took another 50 years before this reclassification could gain acceptance (Lee and

O'Sullivan, 2010). *Bifidobacterium* species are Gram-positive bacteria and strict anaerobes. Their names were derived from Y-shaped Bifidus, which means cleft division. They grow without oxygen but some of the species in the genus can tolerate low oxygen concentrations. They are non-spore formers with irregular rod-shaped. The genera are classified within Actinobacteria phylum; the largest unit of bacteria taxonomy (Lee and O'Sullivan, 2010). There are 36 different *Bifidobacterium* species with various sub-species. *Bifidobacterium* species form a total percentage of 91% microflora of the faeces in breastfed infants while they form 75% of the microflora of formula-fed infants' faeces (Lewis and Mills, 2017). Bifidobacteria are beneficial bacteria, they are used as probiotics (Stavropoulou and Bezirtzoglou, 2020).

2.9.2. The genus *Faecalibacterium*

Faecalibacterium is a member of Firmicutes phylum with single specie known as *Faecalibacterium prausnitzii* (Francesca *et al*., 2020). *Faecalibacterium prausnitzii* is a nonmotile, non-sporing, mesophilic, anaerobic and gram-positive microorganism. It is an abundance commensal bacterium of the gut microbiota, which constitutes 5 % of human faeces. It is extremely oxygen sensitive bacterium, therefore, difficult to cultivate (Francesca *et al*., 2020). Through fermentation of dietary fibre, *Faecalibacterium prausnitzii* produces short-chain fatty acid and synthesis butyrate and peptides as their metabolites (Francesca *et al*., 2020). Alteration in the relative abundance of *Faecalibacterium prausnitzii* has been associated with obesity and inflammatory disease most especially Crohn's disease (Francesca *et al*., 2020).

2.9.3. The genus *Ruminicoccus*

Ruminococcus is an important member of phylum Firmucutes, which belongs to a class of Clostridia. *Ruminococcus* spp are gram-positive anaerobic bacteria within the colonic microbial communities of which the significant numbers are found in the human gut microbiota (La Reau and Shen, 2018). They degrade complex polysaccharides and convert it to simply nutrients for their host use (La Reau and Shen, 2018). *Ruminococcus bromii* and *Ruminococcus gnavus* are prevalent species of the human gut and are detected in human fecal samples by metagenomic sequencing (Crost *et al*., 2018). *Ruminococcus bromii* is non-digestible dietary polysaccharide and it is the keystone

species in degrading resistant starch (RS) to produce formate, acetate, and lactate as their by-products (Crost *et al*., 2018). *Ruminoccocus gnavus* generates toxic metabolites due to the presence of beta- glucuronidase. *Ruminoccocus gnavus* has recently reclassified into genus *Blautia* a member of the family Lachnospiraceae by 16S rRNA gene sequencing, but still maintains its original name (Gren *et al*., 2019).

2.9.4. The genus *Clostridium*

The genus *Clostridium* bacteria are commensal bacteria that live in the intestine. The *Clostridium* species are spore forming, rod-shaped and gram-positive anaerobes. They are widely distributed in water, soil and intestinal tract of both human and animals (Guo *et al*., 2020). *Clostridium* species possess ability to utilize complex and large amount of of nutrients, which are indigestible to their host and produce short chain fatty acid that plays a major role in intestinal homeostasis (Guo *et al*., 2020). Clostridia are early colonizers of infants' gut and these are detectable some days after birth (Guo *et al*., 2020).

2.9.5. Lactic acid bacteria

The lactic acid bacteria (LAB) are Gram positive, facultative or obligate anaerobes, cocci or bacilli in shapes, non-sporulating, catalase-negative, with DNA < 55 mol percent G+C content. The members of the group are *Carynobacterium, Pediococcus, Enterococcus, Melissococcus, Weissella, Lactococcus* spp, *Lactobacillus* spp, *Lactosphaera, Streptococcus* spp, *Oenococcus, Carnobacterium, Vagococcus, Leuconostoc*. Some members of the group such as *Lactobacillus* species have "Generally Regarded as Safe" (GRAS) status, but some other genera like *Enterococcus* and *Streptococcus* can become infectious and pathogenic. Lactic acid bacteria are found in various environments e.g. gastrointestinal tract of different endothermic animals, plant products, fermented food and milk products (Ayeni *et al*., 2009; Ayeni *et al*., 2011; Afolayan and Ayeni, 2017). Lactic acid bacteria are asporogenous bacteria and fastidious microorganisms; therefore, they occupy environments highly rich in nutrients.

The LAB strains exhibit resistance to low pH. They survive in acidic environments, survive bile concetration, and release organic acids as by product; therefore, they can

display antimicrobial effect against pathogenic microorganisms. They are found in naturally fermented food products and are used in the production of yoghurt (Mathur *et al*., 2020). Some of the LAB strains also produce proteinaceous compound, polypeptides and bacteriocin like substances as their metabolites (Mathur *et al*., 2020). The acid produced by LAB helps in inhibiting the food from spoilage and assist in the fermentation of food products (Mathur *et al*., 2020).

2.9.5.1. *Enterococcus*

Enterococcus species are cocci and they appear in pairs, that is, diplococci; or short chains. Most times, they are not easily distinguished from *Streptococci* when using morphological characteristics solely but can be identified using molecular identification (Kok, 1991). *Enterococcus* species consist of 58 members found in both humans and animals. Some of the most common species in man are *E. faecalis*, *E. lactis*, *E. pseudoavium*, *E. faecium*, *E. durans*, *E. hirae* etc (Dolka *et al*., 2020). *Enterococcus* species are very resilient, so they can survive in hot temperature of 60^{o}C, high salt concentration, or acidic environments in a short time. They are normal flora of the intestines but when found in an unwanted site it can become pathogenic causing serious infections. Vancomycin-resistant enterococci (VRE) are identified as threat to global health (Dolka *et al*., 2020).

2.9.5.2. *Lactococcus*

Lactococcus species are homo-fermenters, their role as homo-fermenters is altered when pH conditions and glucose concentrations needed for their growth are adjusted or the availability of nutrients is limited. They were once characterized as a member of the genus *Streptococcus* Group N1 (Sekirov *et al*., 2010). *Lactococcus* spp has a coccus shape. The shapes can occur singly, in pairs, or occur together as chains.

Lactococcus species are very important in dairy industries and are used as starter cultures in the preparation of fermented products and the manufacture of cheese in dairy industries. Besides, the strains are also used as mixed strains cultures with *Lactobacillus* and *Streptococcus* spp. The commonly used strains of *Lactococcus* are *L.*

lactis subsp. *Cremoris* and *L. lactis* subsp. *Lactis*. Their metabolites are used as flavour in fermented food products (Fouhy *et al.*, 2012).

2.9.5.3. *Leuconostoc*

Leuconostoc belongs to Leuconostocaceae family (Nieminen *et al.*, 2014). *Leuconostoc* spp are Gram-positive bacteria, cocci in chain forms. They are intrinsically resistant to vancomycin, unlike staphylococci that are susceptible to vancomycin antibiotics (Fouhy *et al.*, 2012). *Leuconostoc* are sometimes resistant to clindamycin and commonly susceptible to gentamycin. They are homo-fermenters and they are used in the metabolism of carbohydrate. They are also used in the production of dextran from glucose. They form slime and usually cause 'stink' in a sourdough starter. However, some strains of *Leuconostoc* cause infections in human (Fouhy *et al.*, 2012).

2.9.5.4. *Lactobacillus*

Lactobacillus genus is a normal flora of the gut, they are fastidious microorganisms and they survive in acidic and bile environment. They ferment hexose sugars and convert the lactose to produce lactic acid. Their metabolism can be both homo-fermenters and hetero-fermenters as they produce either lactic acid alone or with alcohol from sugar. They are unable to synthesized purines, fatty acids, vitamins and amino acid but adopt different strategies to adapt to their ecological niche including the transport of amino acid. The genus can co-aggregate with pathogenic strains and still replicate to protect the mucous layer of the intestines (Monteagudo-Mera *et al*., 2019).

2.9.5.5. *Pediococcus*

Pediococcus species are coccus shaped, non-motile, non-spore forming and facultative anaerobes, most times, their appearance is in tetrads form and sometimes in pairs. *Pediococcus* resembles *Aerococcus* and *Tetragenococcus* as their division is symmetrically down to two planes. *Pediococcus* metabolised using homo-fermentative process (Fouhy *et al.*, 2012). They produce pediocin, a bacteriocin, which exerts a bactericidal action against listeria; hence, pediocin is commonly referred to as 'antilisterial' (Porto *et al*., 2017). *Pediococcus* species are used as starter culture as they

ferment lactose, glucose and mannose without gas production (Diguta *et al*., 2020). The successful strains use for probiotics in the food industry are *P. pentosaceus* and *P. acidilactici*.

2.9.5.6. *Oenococcus*

Oenococcus is a family member of Leuconostocaceae, the genus *Oenococcus* species are acidophilic lactic acid bacteria, which grow best at pH 3.5 or lower (Lorentzen *et al.*, 2019). *Oenococcus* species are gram-positive, microaerophilic and obligatory heterofermentative bacteria. *Oenococcus oeni* was first classified as *Leuconostoc oeni* base on phenotypic and morphological similarities with *Leuconostoc* species until 1995 when it was re-classified to *O. oeni* (Lorentzen *et al.*, 2019). However, in 2006, another two species were isolated *O. kitaharae* from Japanese Shochu and *O. alcoholitolerans* from bioethanol plants and Branzilian Cachaca (Lorentzen *et al.*, 2019; Lorentzen and Lucas, 2020). *O. oeni* is very important in Oenology field, it is primarily involved in malolactic fermentation where it converts L-malate to L-lactate and CO_2 in wine production to improve the quality of wine and gain a soften taste (Lorentzen *et al.*, 2019; Lorentzen and Lucas, 2020).

2.9.5.7. *Weissella*

The genus *Weissella* belongs to the family Leuconostocaceae, facultative anaerobes, Gram-positive, catalase-negative and can either be spherical or irregular shapes in rod form. *Weissella* genus was formally grouped as *L. paramesenteroides*. Some of the species in the genus are *W. cibaria* and *W. confusa*. *Wiessella* species are distributed in a wide range of habitat such as raw milk, meat, fermented cereals, fish, vegetables, soil, oral cavity, faeces, gastrointestinal tract of animals and humans and urogenital of humans. The only species isolated from humans are *W. confusa*, *W. cibaria* and *W. viridenscens* (Abriouel *et al*., 2015). Several strains of *W. confusa* and *W. cibaria* are used as probiotics respectively; however, some of these strains are found to be opportunistic pathogens as they are involved in human infection (Fusko *et al*., 2015). They are implicated in infective endocarditis, bacteremia, prosthetic joint infection and abscssses (Kamboj *et al*., 2015), the alteration of these species in the gut flora due to surgery or use of antimicrobial facilitate their translocation into unwanted environment.

2.9.5.8. *Streptococcus*

The genus *Streptococcus* bacteria are cocci, non-motile, non-spore forming, catalase-negative, gram-positive, facultatively anerobes, and homofermentative lactic acid bacteria. During fermentation of glucose, they produce lactic acid as major metabolites (Hatti-Kaul *et al*., 2018). They reside in gastrointestinal tract of animals and raw milk. Out of the ninety-three classified species, only *Streptococcus thermophilus* have GRAS status as probiotic strains, and plays important role in biopreservation of food (Hatti-Kaul *et al*., 2018).

2.10. Probiotics

The awareness on the health benefit of consuming functional food has increased drastically. These health benefits include the prevention of GIT diseases. Moreover, some functional food contains living microorganisms that are used as probiotics. They have antioxidant, anti-inflammatory and immune potential properties. The word 'probiotic' was derived from the Greek language 'pro bios' that meant 'for life'. Russian researcher, ‘Elite Metchnikoff introduced probiotics in 1908 (Fuller, 1992). According to him, the Bulgarians were known to be healthy and usually live very long. He observed that their longevity was because of the consumption of fermented milk products such as yoghurt. He carried out studies on the fermented food products and observed that they contained *Lactobacillus* spp (Fuller, 1992). Probiotics was termed in 1965 by Lilly and Stillwell, they described that the microorganisms secreted substances, which stimulate the growth of other microorganisms (Fuller, 1992). Probiotic is used in various ways based on its mechanism of action, effects and functions on human well being. However, in 1974, Parker worked on probiotic strains and later redefined it as ‘live microorganisms which contribute to the formation and growth of intestinal microbial world’ (Guarner *et al*., 2008). Nonetheless, in 1989, Fuller improved the meaning of probiotic. Therefore, probiotic’according to Fuller is defined as a ‘live microbial supplement that when ingested contributes positively to the individual’s health, by assisting in the building of microbial population in the intestine’ (Fuller, 1992). But in 2001, a meeting held in October by the Expert Consultation of International scientist working on behalf of Food and Agriculture Organization of the United Nations and the World Health Organization accepted probiotic as alternative medicine and re-defined probiotic definition as ‘living

microorganisms, which when administered in adequate amounts confer health benefits on the host' (FAO/WHO, 2001). Since this period, this definition has been adopted, and it is used worldwide. This means that certain microbes can be beneficial, and when ingested, could provide health benefits for humans.

Probiotic products are marketed, and consumed worldwide (Alison *et al*., 2013). The products are often used as starter cultures, dietary supplements or living and functional foods. The most common strains in the market are *Bifidobacterium* and *Lactobacillus* spp of different strains; these have GRAS status and mostly found in gastrointestinal tracts (Alison *et al*, 2013). Besides, Macpherson and Uhr (2004), reported that *Bifidobacterium* spp and genus *Lactobacillus* are regularly used probiotic in functional foods and supplements, and consumed by both humans and animals.

2.10.1. Criteria for the selection of probiotics

For any strain to be accepted as probiotic, it must follow the developed guidelines for the evaluation of probiotic in food (FAO/WHO, 2002). The guidelines are listed below:

- Strain Identification by phenotypic and genotypic methods
- Characterirization of the strain using *invitro* tests
- Demonstration of functional properties of the strain
- Safety considerations of the strain
- Technological aspects of probiotics
- Evidence of therapeutics potential of Probiotics

2.10.1.1. Strain identification

According to (FAO/WHO, 2002), any potential probiotic isolate must be taxonomically identified. The validity and reproducibly of the strains must be carried out using genotypic methods. This is very important, as it is the first criterion used to select potential probiotic species (FAO/WHO, 2006). The effects of probiotics are strain-specific; therefore, the correct name of the strain must be confirmed. It is recommended that DNA-DNA hybridization should be a reference method of identification. Sequencing of the 16S rRNA gene is also an acceptable method.

2.10.1.2. Characterisation of the strain

The potential probiotic strain must successfully survive the recommended *invitro* test use to characterize it as probiotic. According to the FAO/WHO guidelines (2002), the characterization of probiotic candidate should be demonstrated by *invitro* assays These *invitro* tests include; antimicrobial activity against pathogenic bacteria, bile acid resistance, resistance to gastric acidity, adherence to mucus and/or human epithelial cells and cell lines, bile salt hydrolase activity, ability to reduce pathogen adhesion to surfaces, if the strain is used on the vagina, then resistance to spermicides. Potential probiotic strains must maintain their viability after the final product (FAO/WHO, 2002).

2.10.1.3. Safety considerations

According to FAO/WHO, (2002), a potential probiotic strain must be safe when consume and must not be infectious. It must be have the Generally Regarded As Safe (GRAS) status, some of the genera in these categories are *Lactobacillus*, *Bifidobacterium*, *Pediococcus* and some strains of *Streptococcus*, these are already being used as supplements, The WHO and FAO recommend series of tests to characterise probiotic strains, these include absence of transferrable antibiotic resistance genes determinant, evaluate if the strain haemolyse blood or belong to any species that cause haemolysis, absence of virulence factors, not producing toxins and absence of adverse effect during clinical trials.

2.10.1.4. Invivo studies using experimental animals and human

Another criterion from the guidelines is the *invivo* study using an animal model (FAO/WHO, 2002). This is required to validate *invitro* effects and to evaluate the mechanism of probiotic effect. The assessment using the animal model before the use of human for clinical trials is encouraged. Oral probiotic strains should be able to adhere to the intestinal epithelial cells and interfere with colonization of pathogens. The strains should also stimulate immune responses positively.

2.10.1.5. Technological properties

The ability of the strain to scale up and retain its viability to produce the desired characteristics is an important way of screening potential probiotics strains, as this is critical to their exploitation (Alison *et al*., 2013). The viability and the stability of probiotic product are essential for the production companies to overcome the marketing and technological challenge. A probiotic strain must survive the processing and maintain an appropriate level of viable cells during storage. Likewise, when incorporated into a food product, good probiotic bacteria should be able to utilize the substrates of the food products, improve the taste and texture of the products (Diguta *et al*., 2020). The viability of a selected strain in probiotic functional product must be between 10^6 and 10^9 cfu/g, and little loss until it reaches the site of action of the host regarding the lyophilization procedure (Diguta *et al*., 2020).

2.10.2. Evidence of therapeutic potential of probiotics

The therapeutic evidence of probiotics in diseases such as urogenital infection, *Helicobacter pylori* and gastric ulcer (Khoder *et al*., 2016), gastroenteritis infections (Kumar *et al*., 2016), cancer, immune disorder and liver disease (Nabila *et al*., 2019) have been reported. Furthermore, *invivo* studies of probiotic have indicated that they are used therapeutically to reduce the high level of cholesterol in serum (Ettinger *et al*., 2015), in the treatment of candidiasis and in slowing down bacteria translocation in HIV patient (Nwosu *et al*., 2014). The intake of probiotics for 6 months results in the 60.0% clearance of cytological lesions in patients with human papillomavirus (HPV) (Verhoeven *et al*., 2013). Probiotic strains also produce therapeutic effects against enteritis, necrotizing and enterocolitis, and inflammatory bowel disease (Kumar *et al*., 2016). However, each probiotic strain is unique and specific; therefore, the potentiality of every strain must not be generalized. Certain probiotic strains produced antagonistic effect against enteric pathogens such as; *Salmonella* spp (Adetoye *et al*., 2018), *Shigella* (Trikha *et al*., 2017), *Y. enterolytica*, enterotoxigenic *E. coli* (Karimi *et al*., 2018; Kwasi *et al*., 2019), *Campylobacter, V. cholera, Pleisiomonas,* enteropathogenic *E. coli,* and *Aeromonas* (Gao *et al*., 2017; Kwasi *et al*., 2019). Antimicrobial effects of probiotic strains are made possible by the inhibitory substances they release as metabolites; these include organic acids, hydrogen peroxide, and bacteriocins (Maldonado-Barragán *et al*.,

2016; Adetoye *et al*., 2018; Dahiya *et al*., 2019). Also, probiotic strains should possess the ability to competitively exclude pathogens on epithelial cells (La Fata *et al*., 2018).

2.10.3. Probiotic mechanisms of action

The mechanism of action of probiotics includes: improving the barrier mechanism of epithelial cells; improving adhesion facility of intestinal mucosa, blocking the pathogens from adhering to intestines, exclusion of pathogenic microbes from the intestine, production of various antimicrobial compounds and immune system modulations (Figure 2.1).

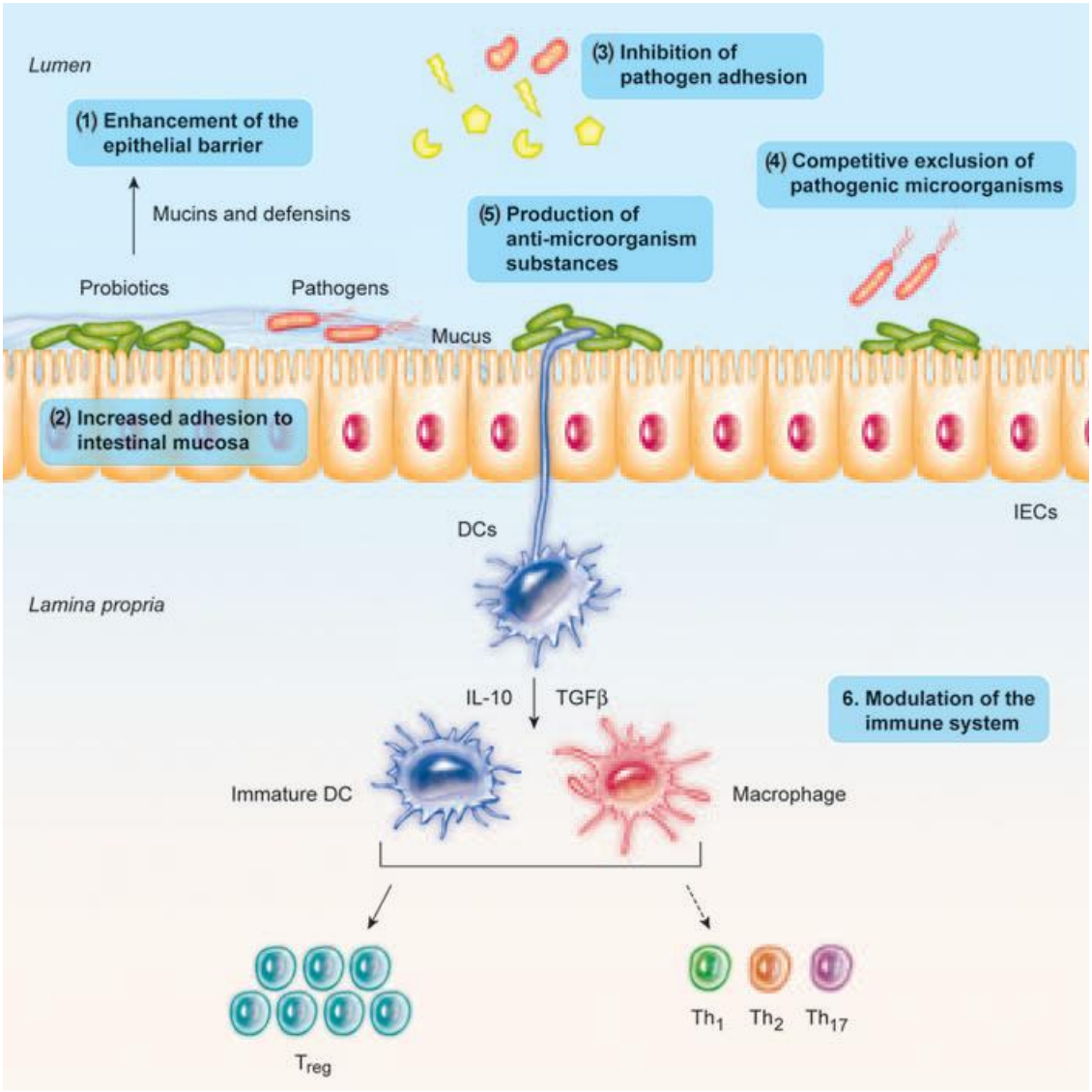

Figure 2.1: Major mechanisms of action of probiotics

Schema extracted from www.karger.com©2012 S. Karger AG, Basel

2.10.3.1. Improvement of the epithelial barrier

The intestinal epithelium is an important tissue that protects the host from invaders. It is closely connected to luminal contents, surrounded by enteric microflora and forms a barrier around to protect the epithelial cell. Intestinal epithelial cells are known to be immune sentinels (Liu *et al*., 2020). They play a major role in maintaining the integrity of the intestinal mucosa of the host. The monolayer of the intestinal epithelium acts as a barrier limiting interaction between luminal content and underlying immune system and serve as defense mechanim against inflammation and infection (Liu *et al*., 2020). Probiotics produce some metabolites such as organic acids, extracellular proteins, bacteriocins, nitric oxide, hydrogen peroxide etc; the metabolites help to maintain the integrity of the intestinal epithelium barrier (Liu *et al*., 2020). Probiotics also accelerates the production of antimicrobial peptides, mucous layer, epithelial adhesion complex and the secretion of IgA; thereby strengthen the intestinal barrier (Zhang *et al*., 2016). However, any defect in intestinal barrier mechanism can disrupt the function of epithelial cells and permit the passage of bacteria and antigen which finds its way into the submucosa to trigger inflammatory responses, resulting in dysbiosis such as IBD (Mendes *et al*., 2019). Probiotics assist in maintaining the intestinal barrier functions by protecting the intestine (Bron *et al*., 2017). Probiotic strains can possess flagella that propel it into the epithelial cells through the mucus membrane. For instance, *E. coli* Nissle (EcN) is a probiotic strain, it has a potential to inhibit the adherence and invasion of *Y. enterolitica*, *L. monocytogens* and *S. typhirium* to epithelial cell lines because of its flagellated and motility ability (Bron *et al*., 2017).

2.10.3.2. Improvement of adhesion to intestinal mucosa

The mucus layer contains glycoproteins and glycolipids that covers the intestinal epithelium (Monteagudo-Mera *et al*., 2019). The viscosity of mucus layer serves as a physical barrier that protects the intestinal cells from mechanical damage and microbial invasion (Garcia-Gonzalez *et al*., 2018). Probiotic strains such as *L. plantarum* possess an ability to adhere to the host mucus layer and persist long in gastrointestinal tract through the production of hydrolases and trans-glycosylases enzymes (Garcia-Gonzalez *et al*., 2018); this plays a fundamental role in microbial adherence mechanism. Probiotic bacteria colonize mucosa layer and prevent colonisation by enteric pathogens thereby,

protecting the host from invasion. They enhance the secretion of mucus, which regularly replenishes the mucosa layer, the physical barrier against pathogens (Liu *et al*., 2020). Also, *Bifidobacterium* and *Lactobacillus* contain surface molecules like surface layer associated proteins (SLAPs), lipoteichoic acid (LTA) and mucin binding proteins (Mubs) that are involved with mucus component of the intestinal epithelia cells (Monteagudo-Mera *et al*., 2019).

2.10.3.3. Competitive exclusion of pathogenic microorganisms

There is competition for existence among the microorganisms in the gastrointestinal tract, hence, the survival of the fittest among these microorganisms. Probiotic strains possess the ability to displace the pathogens from adhering to available space. Probiotic strains could possess a blocking mechanism that aids in excluding the pathogens from their desire sites (La Fata *et al*., 2018). Fibronectin, an adhesive glycoprotein is an essential constituent of extracellular matrix of intestinal epithelial cells (Hymes *et al*., 2016). Fibronectin binding protein molecules have been identified in Gram-positive and Gram-negative bacteria either pathogenic or commensal bacteria. Fibronectin binding proteins characterized in probiotic bacteria bind specifically to fibronectin (Hymes *et al*., 2016); these molecules allow the bacteria to interact directly with the gastrointestinal tract of the host. Probiotic bacteria colonize the intestinal layer of the host and competitively prevent subsequent attachment of the pathogenic bacteria to the intestine, hence, competitive exclusion (Hymes *et al*., 2016)

The mechanisms of exclusion adopted by probiotic strains include the formation of aggressive microecology, alteration of the receptor sites, depletion of vital nutrients and secretion of antagonistic metabolites (Papadimitriou *et al*., 2015). The study conducted by Denkova *et al*. (2013) reported that tested lactobacilli and bifidobacteria are binding to the same receptor with enteropathogens, therefore, lactobacilli and bifidobacteria strains successfully compete with pathogenic bacteria for specific receptor sites.

2.10.3.4. Antimicrobial substances production

Lactic acid bacteria produce inhibitory compounds like organic acids as a metabolite. The organic acids; butyric, acetic, citric, propionic, and lactic acid, in particular, produce

high antagonistic action against infectious microbes. Different studies reported various strains of *Leuconostoc, Weisella, Lactobacillus, Streptococcus, Bifidobacterium* and *Enterococcus* to secrete organic acids with antagonistic activities against pathogens (Ayeni *et al*., 2011; Fijan, 2014; Adetoye *et al*., 2018). Also, probiotic strains produce proteinaceous compounds like bacteriocins from their metabolic activities and other bioactive compounds such as enzymes, amino acids, polypeptides, vitamins, carbohydrate, short-chain fatty acid and oligosaccharides to maintain the health of the host (Indira *et al*., 2019).

2.10.3.4.1. Organic acid productions

Organic acids are of different types, which include butyric, lactic, propionic, piruvic, lactic, formic and acetic acids. Probiotic strains release organic acids as metabolites during the fermentation process (Kralik *et al*., 2018). These possess antagonistic properties against different pathogenic bacterial strains. Their antimicrobial activity is through penetration into the pathogenic bacteria cells to disrupt the cells (Bermúdez-Brito *et al*., 2012). Also, they avert the metabolic activity of the cell and hinder active transport (Kralik *et al*., 2018). Organic acid enters intact into the cell of infectious microorganisms, dissociates within the cytoplasm, lowering the pH of cytoplasm, and causes the death of the pathogens (Bermúdez-Brito *et al*., 2012).

2.10.3.4.2. Bacteriocins

Bacteriocins are ribosomal proteins; they exert inhibitory action against strains that are closely related (Indira *et al*., 2019). Bacteriocins are narrow spectrum and its mechanism of action is by targeting the invading cells, disrupt the cells by forming pores within the cells and inhibit cell wall synthesis (Meade *et al*., 2020). There are several reports on production and purification of bacteriocins from *Lactobacillus spp*. For instance, Gassericin E from *L. gasseri*, (Maldonado-Barragán*et al*., 2016), and Plantarin from *L. plantarum* (Meade *et al*., 2020) respectively. Some other bacteriocins are; Nisin, Cytolysin and Acidophilucin produced from *E. faecalis, L. lactis* and *L. acidophilus* (Maldonado-Barragán *et al*., 2016; Meade *et al*, 2020).

2.10.4. Immune system'modulation

Probiotics have immunomodulating potential, as they possess the ability to interact with the dendritic cells such as Th1, Th2, macrophages, monocytes and lymphocytes of the host (Alison *et al.*, 2013; Meng *et al*., 2018). Some species of *Bifidobacterium* and *Lactobacillus* play essential roles in non-specifific immunity, for example natural killer cells (innate immune cells) as they increase the cytotoxity of these innate immune cells (Azad *et al*., 2018). Probiotic bacteria possess the ability to adhere to the intestinal epithelial cells. They interact with the epithelial cells by binding to Toll-like receptors and facilitate the modulation, stimulation, regulation of immune responses. The uptake of the probiotic bacteria cell or the fragment are internalized and interact with antigen presenting cell, lymphocytes, macrophages and dendritic cells associated with the lamina propria of the gut. The interaction of epithelial cell with probiotic bacteria induces production of various cytokines mediated by antigen presenting cell (Meng *et al*., 2018). The production of cytokines can either up-regulate or down regulate the immune response. The whole cell or their fragments are transported to the lymphocytes, activating the antigen-presenting cell where they induce B and T cells to exert adaptive immune responses. In addition, dendritic cells and macrophages phagocytose probiotic bacteria and are induced to produce pro-inflammatory cytokines such as interferon gamma and tumor necrotic factor, stimulating an increase of epithelial cell and initiate cross talk among all the associated immune cells (Azad *et al*., 2018). Integration of the whole cell or fragments of probiotic bacteria can also stimulate mast cells present in payer's patches through transepithelia vesicular transport mechanism to produce cytokines such as IL-10, IL-4 and IL-6 to enhance cytokine-networking signal. TNF-α is a pro-inflammatory cytokine which is produced in the macrophage and dendritic cells in inflammatory condition (Mendes *et al*., 2019). TNF-α responds by triggering the molecules of the immune system and inducing the neutrophil activation, which is a defining step in the inflammatory response (Mendes *et al*., 2019), therefore, the amount is increased. On the other hand, IL-10 is an anti-inflammatory cytokine that maintains the balance in the immune system. The presence of an antigen increases IL-10 production, it initiates the signal transduction activity using Jak-STAT pathway, controlling the proliferation and differentiation of macrophages (Mendes *et al*., 2019). The stimulation of IL-6 triggers when aggravated by acute intestinal inflammation similar to other pro-inflammatory immune response (Kittana *et al*., 2018).

Probiotic strains can cross the intestinal layer of the gut membrane and bind to the Toll-Like receptors to produce the therapeutic effect (Azad *et al*., 2018). The receptors increase the productions of chemokines and regulatory T cells to activate dendritic cells and macrophages to produce an immune response (Alison *et al.*, 2013). Probiotic strains affect the immune system by stimulation of macrophages, increasing the secretion of IgA antibody and concentrations of cytokines produced (Nditange *et al*., 2013; Meng *et al*., 2018). Probiotics strengthen the immune system and prevent the evasion of the pathogenic organism (Lazar *et al*., 2018).

2.11. Resistance to gastrointestinal condition

A good oral probiotic strain should withstand the gastrointestinal tract conditions. The oral probiotic strains should pass through the lysozyme enzyme in the oral cavity, survive the low pH in the stomach and enter the intestines that contain deconjugated bile without losing their viability greatly (Markowiak and Śliżewska, 2017) Ability to resist acidic environment is an important criterion for probiotic bacteria. Probiotic bacteria produce organic acid, which can dissociate to form hydrogen proton, this reduces the strength of cytoplasmic membrane and the bacteria able to cross the cell membrane. Some probiotic bacteria possess bshA gene, therefore, they can express bile salt hydrolase. Probiotics lack catalase enzymes and superoxide dismutase, these make them to detoxify and decompose reactive oxygen specie, thereby, surviving microaerophilic environment (Markowiak and Śliżewska, 2017).

CHAPTER THREE

MATERIALS AND METHODS

3.1. Materials

3.1.1. Equipment, media, reagents, enzymes, chemicals and other materials.

Equipment, materials e.g. culture media, antibiotics, AST- GP75 kit, DNA extraction kit, IL 10, IL 6, TNF-α, enzymes buffers, chemicals, and instructions for preparation of different media are stated in appendix III-V. The sterilization of all buffer and media was at 121^{o}C for 15 minutes.

3.1.2. Bacterial strains

Five pathotypes of diarrheagenic *E. coli*: enterotoxigenic *E. coli* (ETEC H40B), enteropathogenic *E. coli* (EPEC H62E), enteroinvasive *E. coli* (EIEC H68D), Shiga toxin-producing *E. coli* (STEC H77E), enteroaggregative *E. coli* (EAEC H40C), enteroaggregative *E. coli* (EAEC D49B) and enteroaggregative *E. coli* (EAEC D47C) were obtained from Molecular and Genetics Laboratory, Department of Pharmaceutical Microbiology, Faculty of Pharmacy, University of Ibadan.

3.2. Methods

3.2.1. Ethical considerations

The clearance certificate of ethical approval for the collections of human samples were approved by the Ethics and Research Committee of Ekiti State University Teaching Hospital Ado Ekiti, with the approval number EKSUTH/A67/2015/03/008. All procedures involving the use and proper handling of animals for research purpose was approved by Afe-Babalola University, Ado-Ekiti Ethical Committee with the reference number AB/EC/19/06/047.

3.2.2. Isolation of lactic acid bacteria

Breast milk and faecal samples were isolated from sixteen mothers and thirteen children. The collection of the samples was at the Community Medicine Department of Ekiti State University Teaching Hospital, Ado Ekiti and Afe Babalola University Ado-Ekiti, Ekiti State. The breast milk and infant faeces were collected from healthy participants as follows; the volunteer mothers manually expressed 5 ml of breast milk samples into a sterile tube and faeces of their infants were collected from their children using a sterile swab stick, the collection of the samples were carried out in a sterile condition. After the collection, all the samples were stored at 4°C and transported to the Microbiology laboratory, College of Sciences, Afe-Babalola University within an hour of collection. The isolation of the samples were carried out immediately.

The method of Medjaoui *et al*. (2016) was employed for the isolation of lactic acid bacteria from the samples. This is briefly described as follows; 1 ml of breast milk sample and 1 g of faecal samples were added into 9 ml of MRS-cysteine broth (Oxoid, U.K) respectively, homogenized by vortex mixing and incubated at 37^{o}C under anaerobisc condition (Anaerogen Gen^{TM} Oxoid, UK) for 24 h. The resulting cultures were serially diluted and plated on MRS agar (Oxoid, UK) supplemented with L-cysteine HCl (0.05mg/100ml) for 48 h. Colonies were picked at random and purified by streaking onto MRS-cysteine agar. Single colonies from the MRS- cysteine agar plate were sub-cultured to obtain pure cultures.

3.2.3. Presumptive identification of lactic acid bacteria

The pure isolates selected based on colonial and cell morphology were presumably characterized using Gram's staining, KOH test and catalase reaction (3% Hydrogen peroxide). The isolates presumably identified as LAB were stored in the freezer in 40% MRS/glycerol.

3.2.3.1. Gram's staining

The isolated organisms were Gram stained according to standard procedure as summarized below: crystal violet was applied to a heat-fixed smear, followed by the addition of a mordant (Gram's Iodine), rapid decolorization with alcohol and lastly,

counterstained with safranin. The Gram positive organisms were desribed base on their characteristic purple colour when viewed under the microscope and they were kept for further studies.

3.2.3.2. Potassium hydroxide test

The Potassium hydroxide (KOH) test was used to determine the Gram reaction of LAB isolates. Lactic acid bacteria cultures were grown on MRS-cys agar at 37°C for 24 h under anaerobic conditions. A drop of 3% aqueous KOH was placed on a clean slide. Using a sterile loop, visible cells from fresh cultures were transferred to the drop of 3% KOH. The cells and KOH were mixed thoroughly on the slide and stirred constantly over an area of 1-2 cm^2. The isolates, which did not give a viscid product, were selected since lactic acid bacteria (LAB) are known as Gram-positive cells.

3.2.3.3. Catalase test

Lactic acid bacteria isolates were grown on MRS-cys agar at 37°C for 24 h under anaerobic conditions. The catalase test was conducted by dripping two drops of hydrogen peroxide (3%) on 24 h-old cultures on a glass slide. The catalase test showed positive reaction characterized by the formation of oxygen bubbles that indicate the production of catalase enzyme by the test bacterium. Therefore, the isolates, which did not give gas bubbles, were selected for subsequent activities sice LAB are known to be catalase negative.

The isolates presumptive identified as LAB were used for molecular identification and further research studies.

3.2.4. Molecular identification of lactic acid bacteria strains

3.2.4.1. DNA extraction of bacterial strains.

Lactic acid bacteria were molecularly identified using partial sequencing of the 16S rRNA genes. The extraction of DNA was carried out on each of the isolate presumptively identified as LAB.

The LAB cells were inoculated into MRS broth for 24 hours. The DNA was extracted by Accu® Prep Genomic DNA extraction kit (Bioneer, USA) according to the manufacturer`s instructions. In summary, Fresh 10^8 cultured cells of 200 µl were pipette and added to the eppendorf tube that contains proteinase K (20 µl) and 200 µl of binding buffer (GC) was pipette to the mixture and vortex immediately with care. The lysate was incubated in a water bath at 60 ℃ for 10 min and 100 µl of isopropanol was added and mixed by pipetting. Then, the lysate was spun briefly in a centrifuge; transferred into the binding column tube (upper reservoir), without allowing the rim to wet. The binding column tube was centrifuged at 8,000 rpm for 1 min. The column tube was transferred to a new 2 ml tube for washing and filtration. Then, 500 µl of first washing buffer was added, and centrifugation was done at 8,000 rpm for 1 min. The solution in the ependorf tube was transfered from the 2 ml tube into a disposal bottle. The washing was repeated with the second washing buffer, and centrifuge for 1 min at 8,000 rpm. The centrifugation was repeated for the same minute but this time at 12,000 rpm to remove all the ethanol completely. The solution in binding column tube was transferred to another new sterile 1.5 ml tube for elution using 200 µl of Elution buffer (EL). After 5 min at room temperature, complete absorption of EL was centrifuge at 8,000 rpm for 1 min. The supernatant, which is the pure DNA was collected in a 2 ml sterile ependorf tube and stored in -20^{o}C for further use.

3.2.4.2. Polymerace chain reaction and 16S rRNA gene sequencing

The genomic DNA templates obtained was used in a Polymerace chain reaction (PCR) reaction for amplification of 16S rRNA gene using 27F (AGAGTTTGATCMTGGCTCAG) and 1389R (ACGGGCGGTGTGTACAAG) primers. Polymerace chain reaction was performed using a thermocycler (Applied Biosystems, USA) with the following condition: 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. (Pinloche *et al*., 2013). The PCR products were separated by agarose gel electrophoresis (1.5% w/v) and visualize by staining with EZ vision. The procedure was repeated for the second batch of samples collected. The first and second batch of PCR products of strains obtained were sequenced by Beckman Coulter, Germany and Inqaba Biotech, South Africa. The 16S rDNA sequences were compared with known sequences in GeneBank using the basic local alignment search tool

(BLAST) and phylogenetic tree was constructed for relatedness. The obtained 16S rRNA sequences were deposited at European Molecular Biology Laboratory (EMBL) under accession number PRJNA628165 (https://www.ncbi.nlm.nih.gov/sra/PRJNA628165).

3.2.4.2.1. Determination of antibacterial spectrum of cell free supernatant obtained from lactic acid bacteria

The antimicrobial activities of culture free supernatant (CFS) of 93 LAB strains against seven strains of diarrheagenic *E. coli* were carried out using the agar well diffusion method. The 93 strains were cultured for 72 h in MRS-cysteine broth under anaerobic condition (Gaspak Jar, BBL, USA) and then centrifuge at 12,000 rpm for 10 min to obtain CFS. An aliquot of 100 µl of each CFS of the LAB was placed in 6 mm well bored in Mueller Hinton agar (Becton, Dickson and Co, Spark, MD, USA) pre-seeded with 100 µl of 10^6 cfu/ml of: ETEC H40B, EPEC H62E, EIEC H68D, STEC H77E, EAEC H40C EAEC D49B and EAEC D47C respectively. The CFS was allowed to diffuse for 1 h at room temperature before incubating aerobically at 37ºC for 24 h. The diameter of clear zones of inhibitions around each well were recorded.

3.2.4.3. Antibacterial activity of lactic acid bacteria viable cells

The agar overlay method described by Ayeni *et al*. (2009) was employed for the determination of the activity of the viable LAB cells against ETEC H40B, EPEC H62E, EIEC H68D, STEC H77E, EAEC H40C, EAEC D49B and EAEC D47C respectively. A loopful of each overnight LAB broth culture was streaked on MRS-cysteine agar (Oxoid, UK) as a straight line of about 20 mm in length, then incubated anaerobically at 37ºC for 48 h. Thereafter, the plates were overlaid with 10^6 cfu/ml of a fresh broth culture of each diarrheagenic *E. coli* vehiculated in 10 ml Mueller Hinton soft agar (0.7%). The overlay was allowed to set and aerobically incubated at 37ºC for 24 h. The diameter of zones of inhibition around the LAB line of streak were recorded.

3.2.4.4. Determination of bacteriocin-like inhibitory substances

The antimicrobial properties of bacteriocin-like substances produced were determined by agar cup diffusion method (Malheiros *et al*., 2015). Seventy-two hours old cultures of LAB grown in MRS-cysteine broth were centrifuged at 12,000 rpm for 10 min to obtain the cell-free supernatant. The pH of the CFS was adjusted with 1.0 M NaOH to 6.5 followed by filtration of the supernatant through a 0.4 µm pore-size cellulose membrane filter. An aliquot of 100 µl of each CFS of the LAB was placed in 6 mm well bored in Mueller Hinton agar (Becton, Dickson and Co, Spark, MD, USA) seeded with 100 µl of 10^6 cfu/ml of *S. aureus* ATCC 29213.

For all strains with observed inhibition against *S. aureus*, the proteinaceous nature of the CFS was determined by partial purification with ammonium sulphate precipitation (Sure *et al*., 2016). Briefly, solid 70% ammonium sulphate (Mallinckrodth Chemical, Inc., USA) was added to the adjusted CFS, the mixtures were stirred for 2 h at 4ºC and later centrifuged at 12,000 rpm for 30 min (4ºC). The precipitate were collected after centrifuging the mixture and resuspended in phosphate buffer. The pellet that still retain its precipitation in phosphate buffer was recorded as being proteinaceous and having bacteriocin like substance.

3.2.4.5. Determination of rate of growth inhibition with time by co-culture assay

A 10 ml of Mann Rogosa Sharpe-Mueller Hinton (MRS-MH) broth (Oxoid, UK-Becton, Dickson USA) containing 5 ml of MRS-cysteine (double strength) and 5 ml of Mueller Hinton (Oxoid, UK- Becton, Dickson USA) (double strength) was prepared. The co-culture broth was a mixture of 10^8 cfu/ml of each 15 *Lactobacillus* strains and 10^6 cfu/ml of each of ETEC H40B, EPEC H62E, EIEC H68D, STEC H77E, EAEC H40C pathogens respectively. Each strain of *Lactobacillus* and *E. coli* was also monocultured as experimental control to monitor the growth of the srains that were co-cultured. The co-culture broths were mixed thoroughly and appropriate dilution were plated out on Eosin Methyline Blue (Sigma-Aldrich, USA) and MRS-cys agar (Oxoid, UK) immediately after co-inoculation (T_0), 8 h after co-inoculation (T_8), 16 h after co-inoculation (T_{16}) and 24 h after co-inoculation (T_{24}). All the experimental set ups were

incubated at 37 ºC. Each strain of *Lactobacillus* was plated on MRS-cys agar and anaerobically monocultured at 37ºC, and each *E. coli* strain was plated out on EMB agar at appropriate dilutions and aerobically monocultured at 37ºC just immediately after inoculation (T_0), 8 h of incubation (T_8), 16 h of incubation (T_{16}) and 24 h of incubation (T_{24}). (Alebiosu *et. al.,* 2017). The monoculture was used as control. Viable cells of LAB and *E. coli* determined are used to estimate microbial cells that survived.

3.2.5. Quantification of organic acids produced by lactic acid bacteria

The quantification of propionic, acetic, lactic and butyric acids produced by selected five *Lactobacillus* strains was carried out using High-Performance Liquid Chromatography (HPLC) (Adept CECIL CE 4200) with UV detection using standard procedure. The assay was carried out thus: 20 µL of the supernatant of overnight cultureof *Lactobacillus* strain which was introduced into the HPLC system that is fitted to a UV absorbance detector set at the wavelength of 210 nm. The mobile phase used was H_2SO_4 at 55ºC. The standard curves of HPLC were plotted using the response factor and the retention time data of the organic acids. The quantity (mg/ml), the area (mAs) and Height (mA) of the tested organic acids produced by each of the selected strain were determined from the standard curves with linear coefficients (R^2) greater than 0.99.

3.2.6. Determination of the safety of lactic acid bacteria

3.2.6.1. Determination of antibiotic susceptibility pattern of selected lactic acid bacteria.

Minimum inhibitory concentration of 15 selected LAB isolates was determined by using Vitek 2 (Biomerieux diagnostics, France) using AST-GP75 test cards. The 15 LAB isolates were grown anaerobically for 24 hours at 37ºC in MRS-cysteine agar. The inoculum suspensions of each of the 15 LAB to be used for susceptibility test were prepared in sterile saline at a turbidity of 0.5 McFarland standard, as measured using a Densi Chek instrument (bioMérieux). The individual test cards were automatically filled with the prepared culture suspension, sealed, and incubated by the Vitek 2 instrument. The system determined the susceptibility of the LAB strains according to manufacturer s procedures. The results obtained from the Vitek 2 system were compared with the

standard reference breakpoints of antibiotics outlined by EFSA (2018) for lactic acid bacteria.

3.2.6.2. Haemolytic activities of lactic acid bacteria

The haemolytic potential of 93 LAB strains was assessed. Each strain of LAB was cultured aerobically on human blood agar (5%), for 24 hours at 37ºC (Halder *et al*., 2017). After 24 h, the production of clear zones around the colonies was observed. The formation of clear zones around cell colonies was reported as blood lysis.

3.2.7. The resistance of lactic acid bacteria to gastrointestinal conditions

3.2.7.1. Resistance to low pH

The method employed for this assay was modified from Hassanzadazar *et al*., (2012). Ninety-three LAB strains were assessed for their abilities to survive different pH level. One ml of 24 h old culture was inoculated into 9 ml of MRS-cysteine broth; incubated anaerobically (Gaspak Jar, BBL, USA) at 37°C for 24 h. The resulting culture was centrifuged for 10 min at 12,000 rpm. The cell pellets were rinsed two times with phosphate buffer saline (PBS) of pH 7.2 and resuspended in 10 ml sterile MRS-cysteine broth already adjusted to pH 2.0, and 3.0 respectively. For LAB suspension in pH 3 tubes, the viability count of the culture was done at time 0 h (immediately after re suspension in acidified broth) by plating out serially diluted culture and incubating for 24 h at 37ºC). The original tubes was incubated for 3 h at 37°C, and the plating out process was repeated to get viability counts after 3 h. The same procedure was repeated for LAB suspensions in pH 2.0 tubes, the viability counts of the cultures were done at 0 h and 3 h. The results were expressed as cfu/ml. The LAB strains grown without any adjustment of pH was used as control. The viable cells at (T_0) was recorded as initial count and viable count at (T_3) was recorded as final count in cfu /ml.

3.2.7.2. Tolerance to bile salt

The method employed for this assay was modified from Hassanzadazar *et al*., (2012). The 93 LAB strains were assessed for their ability to survive different concentration of bile. One ml of 24 h old LAB culture was inoculated into 10 ml of MRS-cys broth;

incubated anaerobically at 37°C for 24 h and centrifuged for 10 mins at 12,000 rpm. The cell pellets were rinsed two times in phosphate buffer saline (pH 7.2), and resuspended in 10 ml sterile MRS-cys broth supplemented with 0.3% (w/v) of bile salt followed by incubation for 4 h at 37°C under anaerobic condition (Gaspak Jar, BBL, USA) with inserted AnaeroGen^{TM} 3.5 L. At 0 h (T_0), Immediately after resuspension of the pellet, 0.1 ml was serially diluted and plated out onto on MRS-cys agar for 24 h at 37°C under anaerobic condition (Gaspak Jar, BBL, USA) with inserted AnaeroGen^{TM} 3.5 L (Thermo Scientific, Oxoid, Japan). The viability counts of the cultures done at 0 h that is T_0, was recorded as intial reading. The original tube was incubated at 37°C for 4 h (T_4), a 0.1 ml was serially diluted from T4; the diluent was cultured on MRS-cys agar and anaerobically incubated at 37^{o}C for 24 h. The viability counts of the cultures done at 4 h that is T_4, was recorded as final reading.The viable counts at T_0 and T_4 were recorded, and the results were expressed as cfu/ml. The strains grown without bile salt supplementation was used as control.

3.2.7.3. Consecutive acid and bile tolerance test

The ability to survive consecutive low pH and bile supplementation was performed for twenty LAB strains using a modified method of Hassanzadazar *et al*., (2012). The LAB cells grown in MRS-cys broth (Oxoid, UK) were harvested after incubation at 24 h for 37^{o}C, the cell was centrifuged for 10 min at 12,000 rpm. The pellets were washed with buffer saline (0.9% NaCl), resuspended in 10 ml MRS-cysteine broth that has been adjusted to pH 3. The culture was incubated aerobically for 3 h. Before the incubation, 0.1 ml of culture was serially diluted (T_0) and incubated anaerobically for 24 h at 37^{o}C to evaluate the initial viable counts of LAB. After 3 h incubation of the original tube, 0.1 ml (T_3) sample was diluted appropriately, plated onto MRS-cys agar, and incubated anaerobically at 37^{o}C for 24 h. Then the whole tube that has been incubated for 3 h was centrifuged and the pellet was resuspended in 10 ml MRS-cysteine broth containing 0.3% bile, then incubated at 37°C for 4 h. A 0.1 ml of the suspension was taken out before the incubation and diluted appropriately plated onto MRS-cys agar, and incubated anaerobically at 37^{o}C for 24 h to get the viable count. The remaining suspension in the tube was incubated for 4 h at 37^{o}C. After the incubation, 0.1 ml was pippete out, appropriately diluted and plated out onto MRS-cys agar plate, incubated at 37^{o}C for 24 h under anaerobic condition. The survived viable cells were enumerated using a manual

colony counter (Scan 100, Intersciencelab, USA). The strains grown without acid and bile salt supplementation were used as control.

3.2.7.4. Cell surface hydrophobicity

Cell surface hydrophobicity is considered one of the important properties improving the first contact between bacteria and host cells (Krausova *et al*., 2019). Therefore, the ability of 93 LAB strains to hydrocarbon solvents was evaluated by the cell surface hydrophobicity using the microbial adhesion to hydrocarbon MATH) method described by Rosenberg *et al*. (1980). The solvents used were n-hexadecane and xylene. The LAB isolates were sub-cultured in MRS-cys broth (Oxoid, UK), incubated at 37^{o} C for 24 h, under anaerobic condition (Gaspak Jar, BBL, USA) with inserted AnaeroGen^{TM} 3.5 L (Thermo Scientific, Oxoid, Japan). The cultured cells were centrifuged at 5,000 rpm for 15 minutes; pellets were washed twice with Phosphate Buffer Solution (PBS) at 7.0 pH and resuspended in 3 ml of sterile PBS. The optical densities (OD) of the bacteria in cell suspension; (initial absorbance) was measured using UV spectrophotometer (Unico, Flinn Scientific, Canada) after adjusting the absorbance to approximately 0.00 at 600 nm wavelength (A_{600}) with water. Thereafter, 1 ml of xylene solvent (Sigma, USA) was added into a test-tube containing the cell suspension and vortexed vigorously for 30 s, the cell suspension was incubated for 1 h for the separation of phases. Final absorbance of the cell suspension was taken by gently pippeting out an aliquot of 1 ml from the aqueous phase and its optical density was read at 600 nm. In addition, the same procedure was carried out for hydrophobicity of *n*-hexadecane solvent (Sigma, USA) as a 3 ml of the LAB cell suspension was prepared and vortexed vigorously for 30 s. Then, 1 ml of the suspension was taken for absorbance, optical density was read at 600 nm on a UV spectrophotometer and this was recorded as the initial absorbance. The cell suspension was incubated at 37^{0}C for 1 h to allow them to separate into two phases. After separation of the phases, OD at 600 nm of the aqueous phase was measured using UV spectrophotometer (Unico, Flinn Scientific, Canada) by pippeting out an aliquot of 1 ml from the surface, the optical density result was recorded as final absorbance. Bacterial cell surface hydrophobicity of the two solvents were expressed as a percentage of the cell population that had passed into the hydrophobic phase of the solvent. Hydrophobicity result was calculated from the percentage of decrease between initial suspension and final suspension using the equation below:

$$H_{\%} = \frac{\text{Optical Density initial absorbance (600 nm) – O.D.final absorbance)}}{\text{Optical Density initial absorbance (600 nm)}} \times 100$$

3.2.7.5. Autoaggregation assay

Auto-aggregation of LAB strain is essential and used as a pre-test to determine the adhesion property of the strain to epithelial cells as well as mucosal surfaces and consequently to colonize the gastrointestinal tract, this assay helps to determine the ability of a strain to survive and be viable in the intestine (Krausova *et al*., 2019). Therefore, in this study, auto-aggregation of 15 LAB strains was evaluated using Tuo *et al*. (2013) method. Each of the 15 LAB strains were incubated under anaerobic condition (Gaspak Jar, BBL, USA) with inserted AnaeroGen™ 3.5 L (Thermo Scientific, Oxoid, Japan) overnight at 37^0C in MRS-cysteine broth, each of the strain was centrifuged using Spectrafuge™ 6C Compact Centrifuge (Biocompare, USA) at 5,000rpm for 15 min. The cells pellets were washed with 2 ml of 0.9% normal saline twice, resuspended in 10 ml of PBS. The suspension was vortexed vigorously for 30 s and the initial absorbance was measured at 600 nm by taking 1 ml of the suspension. The suspension was vortexed again and then incubated at 37^0C for 5 hours. At every hour, (t1, t2, t3, t4 and t5) an aliquot (upper suspension) of 1 ml was transferred into the cuvet and absorbance at 600 nm was checked using a visible spectrophotometer (Chongqing Gold, China). Autoaggregation's result was calculated using the equation below:

Autoaggregation % = [(Ao-At)/Ao) x 100]

Where A_t represents the absorbance values at time t1, t2, t3, t4, t5 and A_0 is the initial absorbance; at the onset of the incubation.

3.2.7.6. Co-aggregation assay

The co-aggregation ability of LAB allows it to prevent the attachment and colonization of the pathogenic microorganisms in the intestine. Therefore, the co-aggregation potential of 11 selected LAB with each strain of *E. coli* ETEC H40B, EPEC H62E, EIEC H68D, STEC H77E, EAEC H40C were evaluated using Collado *et al*. (2009) and Tuo *et al*., (2013) methods respectively. Each of the LAB strain was innoculated in MRS-cysteine broth (Oxoid, USA), incubated at 37^o C under anaerobic condition (Gaspak Jar,

BBL, USA) with inserted AnaeroGen^{TM} 3.5 L (Thermo Scientific, Oxoid, Japan) for 24 h. Each of ETEC H40B, EPEC H62E, EIEC H68D, STEC H77E, and EAEC H40C strain was prepared overnight in Mueller Hinton broth (Oxoid, USA) and incubated aerobically at 37^0C. A 10 ml of fresh cultures of LAB and *E. coli* strains were centrifuged separately at 5,000 rpm for 15 minutes using Spectrafuge^{TM} 6C Compact. Centrifuge (Biocompare, USA). Lactic acid bacteria cells and *E. coli* cells were washed two times separately with 2 ml of 0.9% normal saline; each was resuspended in 10 ml of PBS. An equal volume each of 1 ml suspension of LAB and *E. coli* were mixed, the mixture was vortex for 10 s and incubated aerobically at 37ºC for 5 h. Mono cultured LAB and *E. coli* strains were performed under the conditions described above and served as control. At the end of incubation period, the absorbance of co-incubated suspension and mono-incubated suspension was determined using UV spectrophotometer (Chongqing Gold, China) at 600 nm wavelength. The co-aggregation was calculated in percentage using the formula below:

$$\text{Coaggregation\%} = [(Ax + Ay)/2 - A(x+y)]/(Ax + Ay)]$$

Where Ax and Ay represents addition of the absorbance of each mono-cultured suspension of LAB and *E. coli* strains (control) and A(x+y) is the absorbance of co-cultured suspension of LAB and diarrheagenic *E. coli* strains.

3.2.8. Evaluation of antibiofilm potential

Inoculum preparation

The enteroaggregative *E. coli* are associated with biofilm formation that is mostly difficult to treat because of their resistance to antibiotics. This assay is carried out to determine if the 15 LAB with bacteriocidal effect against enteroaggregative *E. coli* (EAEC) also possess antibiofilm potential. Antibiofilm assay was carried out on the 15 LAB strains against enteroaggregative *E. coli* strain 042 using the modified method of Jadhav *et al*. (2013). Enteroaggregative *E. coli* 042 was inoculated into 10 ml of Mueller Hinton (MH) broth, (Oxoid, UK), incubated aerobically at 37ºC for 16 h to harvest the bacterial cells at exponential phase. Each of the fifteen LAB strains was inoculated in MRS-cysteine broth, (Oxoid, USA), incubated at 37º C for 24 h under anaerobic condition (Gaspak Jar, BBL, USA) with inserted AnaeroGen^{TM} 3.5 L (Thermo

Scientific, Oxoid, Japan). Thereafter, they were centrifuged using Spectrafuge™ 6C Compact Centrifuge (Biocompare, USA**)**, the supernatant was prepared in three dilutions; 1:1, 1:10 and 1:100 and each was filtered using membrane filter 0.45 µm pore size (Sigma Aldrich, St. Louis, Missouri, USA). Anti- biofilm assay of three dilutions of each 15 LAB was carried out as prescribed below. A 180 µl of high glucose Dulbecco′s Modified Eagle′s Medium (DMEM) broth (Sigma Aldrrich, USA), was aliquoted into 96-well polystyrene flat-bottom microtitre plates; 15 µl of CFS of each LAB strain was added, 5 µl of EAEC 042 cultures were also added into the wells (total 200 µl). Sterile high glucose DMEM broth without EAEC 042 served as control. Microtitre plates were sealed and incubated aerobically at 37^0C for 18 h. OD of the suspended culture at 595 nm was initially measured. The suspended culture was discarded and the plate washed with sterile distilled water to remove any cells that fail to adhere. The plates were air-dried, and also put in the oven at 60^oC for 35 min to dry, then stained with 150 µl crystal violet (0.1%) and fixed with 75 % ethanol, then incubated at room temperature for 20 min. The stained biofilm was washed with deionized water to remove unbound dye. The wells were rinsed twice with sterile distilled water to eliminate unabsorbed stain. Then, the crystal violet bound was eluted using 100% ethanol and quantified by measuring OD at 545 nm using microplate absorbance reader (BioRad, Richmond, CA, USA). The quantity of the stain absorbed showed the quantity of biofilm formed.

To calculate biofilm inhibition density, the percentage inhibition was calculated using the formula below:

$$\text{Percentage inhibition} = \frac{[\text{O.D of control (595 nm)} - \text{O.D.of inhibition of biofilm)}]}{\text{O. D of control (595 nm)}} \times 100$$

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

3.2.9.1. Experimental animals

Five weeks old, male swiss mice weighing 22±4 g were obtained from Ekiti State University Animal Breeding Experimental Center (Ado-Ekiti). All the mice were housed

in cages that were covered with wires at 22-24^{o}C and humidity of 50%. They were fed with standard laboratory mice pellets with water *ad libitum*.

3.2.9.2. Preparation of bacterial strains

Lactobacillus plantarum A011 and *L. rhamnosus* A012 were cultured in MRS- cysteine medium and incubated at 37^{o} C under anaerobic condition (Gaspak Jar, BBL, USA) with inserted AnaeroGen^{TM} 3.5 L (Thermo Scientific, Oxoid, Japan) for 24 h. The two lactobacilli strains had an approximate counts of 1.0 x 10^{8} cfu/mL. The cells were harvested and centrifuged using Compact Centrifuge (Biocompare, USA) at 4,000 rpm for 10 min, after which they were washed twice and re-suspended in 10 ml of sterile PBS.

3.2.9.3. Experimental design

All mice used for the experiment were divided randomly into seven groups of five mice in a group (n=5). A negative control group was treated with 20 mg/kg of cyclophosphamide (i.p.) and PBS (vehicle). The positive control (PC) group, was treated with cyclophosphamide and standard drug (levamisole HCl 40 mg/kg). The *L. rhamnosus* treated group was treated with cyclophosphamide and *L. rhamnosus* A012 (1.0 x 10^{8} cfu/mL) and *L. plantarum* treated group was treated with cyclophosphamide and *L. plantarum* A011 (1.0 x 10^{8} cfu/mL). The *L. rhamnosus* group was given *L. rhamnosus* A012 (1.0 x 10^{8} cfu/mL) without cyclophosphamide and *L. plantarum* group was given *L. plantarum* A011 (1.0 x 10^{8} cfu/mL) without cyclophosphamide and a normal control (NC) group, was given PBS without cyclophosphamide. (Kwom *et al*., 2018). They were administered with appropriate drugs using intended route of drug administration for treatment, at the specific number of days as shown in Table 3.1.

Table 3.1: Experimental design

Groups	Identity	Treatment	Route of Administration
1	Negative control	3 days CTX 20 mg/kg + 15 days PBS	I.P + oral
2	Positive control	3 days CTX 20 mg/kg + 15 days Levamisole HCl 40 mg/kg	I.P + oral
3	*L.rhamnosus* treated group	3 days CTX 20 mg/kg + 15 days of 1.0 x 10^8 cfu/mL *L. rhamnosus* (oral)	
4	*L. plantarum* treated group	3 days CTX (i.p.) + 15 days of 1.0 x 10^8 cfu/mL *L. plantarm* (oral)	
5	*L. rhamnosus* alone	15 days of 1.0 x 10^8 cfu/mL *L. rhamnosus* (oral)	
6	*L. plantarum* alone	15 days of 1.0 x 10^8 cfu/mL *L. plantarum* (oral)	Oral
7	Healthy Control group	15 days PBS (oral)	

Key

CTX - cyclophosphamide

I.P – intraperitoneal

Note: 20 mg/kg of cyclophosphamide and 40 mg/kg of levamisole HCl was administered to the appropriate groups.

3.2.9.4. Body weight analysis

The experimental mice'weights were properly monitored using a table weighing balance (USA) every four days of the experiment until the last day of the experiment. The weight of the mice in each of these days was recorded.

3.2.9.5. Analysis of immune organ index

The experiments were done in each group as described above for 15 days, the mice were euthanized using standard procedures with phentermine hydrochloride at the dose of 0.1 ml or 0.2 ml depending on the body weight of the animal, and then sacrificed through cervical dislocation. The blood was collected, and dispensed in EDTA bottle. The spleen and intestine of each mouse were surgically excised immediately and their weights were recorded. The harvested spleen of the experimental mice was used to calculate the spleen index as follows:

$$\text{Spleen or thymus indices (mg/g)} = \frac{\text{spleen or thymus weight (mg)}}{\text{Body weight (g)}}$$

3.2.9.6. Quantification of white blood cells

The whole blood was collected from each animal and diluted 1 in 19 in White Blood Cells (WBC) diluting fluids that contain gentamicin violet. The diluting fluid was used to haemolyze the red blood cells. The WBC were counted microscopically using a haemocytometer. The enumeration ofeach WBC was determined in blood per litre.

3.2.9.7. Cytokine quantitation

The level of IL-10, IL-6, and TNF-alpha produced from each mouse were measured and quantified by mouse TNF-$\boldsymbol{\alpha}$ ELISA kit with pre-coated plates Cat No: 430907, mouse IL-10 ELISA kit Cat No: 431417 and IL-6 ELISA kit Cat No: (LEGEND MAX^{TM}, BioLegend, U.K). The blood from the experimental animals was collected in a sterile eppendorf bottle at sacrifice and allowed to stand for 1 h at room temperature, then placed accordingly into the centrifuge and spun at 3,000 rpm for 10 min to obtain serum. The serum was kept at −20°C until further use. The spleen of each mouse was harvested

and homogenized. The homogenate was obtained by centrifugation at 5000 rpm for 30 min after which it was kept at−20°C until further use. The level of cytokines (IL-10, IL-6, and TNF-alpha) produced in serum and spleen homogenate were quantified using BioLegend ELISA assay kits with ELISA absorbance reader and ELISA washer following instructions of the manufacturer. The procedure for the ELISA is described thus: A 1,000 µL of the top standard at a concentration of 500 pg/mL is prepared from Assay Diluent stock. Then, a six two-fold serial dilutions of the 500 pg/mL top standard with Assay Diluent is prepared in separate tubes. The dilutions are used to prepare mouse IL-6, IL-10 and TNF alpha standard concentrations at 500 pg/mL, 250 pg/mL, 125 pg/mL, 62.5 pg/mL, 31.25 pg/mL, 15.6 pg/mL, and 7.8 pg/mL, respectively. The assay diluent serves as the zero standard (0 pg/mL). A 50 µL Assay Buffer is dispensed into each well, then 50 µL diluted standards or samples are added into the wells. The plate is incubated for 2 hrs at room temperature with shaking. After the incubation, the plate was washed 4 times, and 100 µL of Detection Antibody solution was added to each well and incubated at room temperature for 1 hour with shaking. The plate was washed 4 times and 100 µL of diluted Avidin-HRP solution was added to each well and incubated at room temperature for 30 minutes with shaking. The plate was washed 5 times with Wash Buffer. A 100 µL of TMB Substrate Solution was added into the well and incubated in the dark for 15-30 minutes until the colour of the plate turned blue. After which a 100 µL of Stop Solution was added to each well to stop the reaction. The absorbance was read at 450 nm and 570 nm. The results were obtained by calculating the quantity of the cytokines produced using the line of regression obtained from the standard curve range in pg/ml of mouse serum and homogenate to obtain the values of cytokine produced in each group.

3.2.9.8. Histopathological examination

The tissue specimens of the small intestine were examined for histologically using Xie *et al*. (2016) method. Jejunum section, which is four µm thick, was mounted on slides after embedding in molten paraffin. After deparaffinization, (putting the waxed slides in the water bath to dewax them), the sample on the glass slide was stained with hematoxylin and eosin (HE), mounted in neutral balsam and covered with a coverslip. The histological variation of the group was observed under a biological microscope (Olympus, Japan, 100x magnification), the images were acquired with a digital camera.

An Image-Pro Plus 6.0 software (Media Cybernetics, MD, United States) was used to measure the intestinal villus length and the crypt depth.

3.2.9.9. Statistical analysis

Graph pad prism 10.0 statistical software program was used to analyse the results. The statistical significance of every data generated was determined with one-way ANOVA and the p values that were significantly different were recorded.

CHAPTER FOUR

RESULTS

4.1. Diversity of lactic acid bacteria in human breast milk and infant faeces

Ninety-three LAB strains were identified from mothers' breast milk and their neonates'faeces based on their growth on MRS-cys agar, morphological characteristics, Gram positive, catalase negative and partial sequencing of 16S rRNA gene. The 16S rRNA gene band are shown (Figure 4.1). Fifty five strains (59.0%) from mothers' breast milk and 38 (41.0%) from neonates' faeces were isolated respectively. These were identified from five genera: *Lactobacillus*, *Enterococcus*, *Weisella*, *Leuconostoc* and *Pediococcus*. The sequences from 93 LAB isolates showed high degree of similarity with the nucleotides present in the standard strains in the BLAST program ranging from 97.8% to 100% (Appendix IV).

Lactobacillus spp. were the most predominant genus with 43 isolates (46.24%) while the least predominant genus was *Pediococcus* spp. with 1 isolate (1.08%) (Figure 4.2). The genera identified consist of 15 different species namely; *L. fermentum* (4), *L. plantarum* (27), *E. faecium* (12), *L. pentosus* (8), *L. rhamnosus* (2), *L. paracasei* (1), *L. xianqfrangensis* (1), *W. cibaria* (5), *W. confusa* (1), *P. pentosaceus* (1), *L. pseudomesenteroides* (9), *E. durans* (9), *E. faecalis* (7), *E. lactis* (4), and *E. thailadicus* (2) as shown in Figure: 4.3. The most prevalent species is *L. plantarum* (29.03%), followed by *E. faecium* (12.90%). The least prevalent species are *L. paracasei, L. xianqfrangensis, W. confusa,* and *P. pentosaceus,* each of the strain has 1.08% (Figure 4.3). In breast milk samples, *L. plantarum* was the most prevalent 20 (36.36%) and the least prevalent strains were *L. paracasei, L. rhamnosus, L. xianqfrangensis, P. pentosaceus* and *W. confusa,* each strain had 1 (2.0%) occurrence, meanwhile, *L. fermentum, E. thailandicus* and *E. faecalis* were absent but these strains were present in faecal samples (Figure 4.4). However, *L. paracasei, L. xianqfrangensis, P. pentosaceus, W. cibaria* and *W. confusa* were all absent from neonates' faeces but were present in

human breast milk. The most prevalent strain in human breast milk was *L. plantarum* with 7 (18.42%) prevalence while the least prevalent strain was *L. rhamnosus*, which had 1 (2.63 %) prevalence (Figure 4.5).

The circular phylogenetic tree in figure 4.6 shows the diversity and relatedness of different species among the lactic acid bacteria strains isolated. However, phylogenetic tree of *Lactobacillus* strains isolated in this study was enerated from the 16S rRNA gene sequence alignment (Fig 4.7). Based on the findings, the LAB strains isolated from mothers' breast milk and neonates' faeces were aligned with Multiple Sequence Alignment using the Neighbour Joining Algorithm.

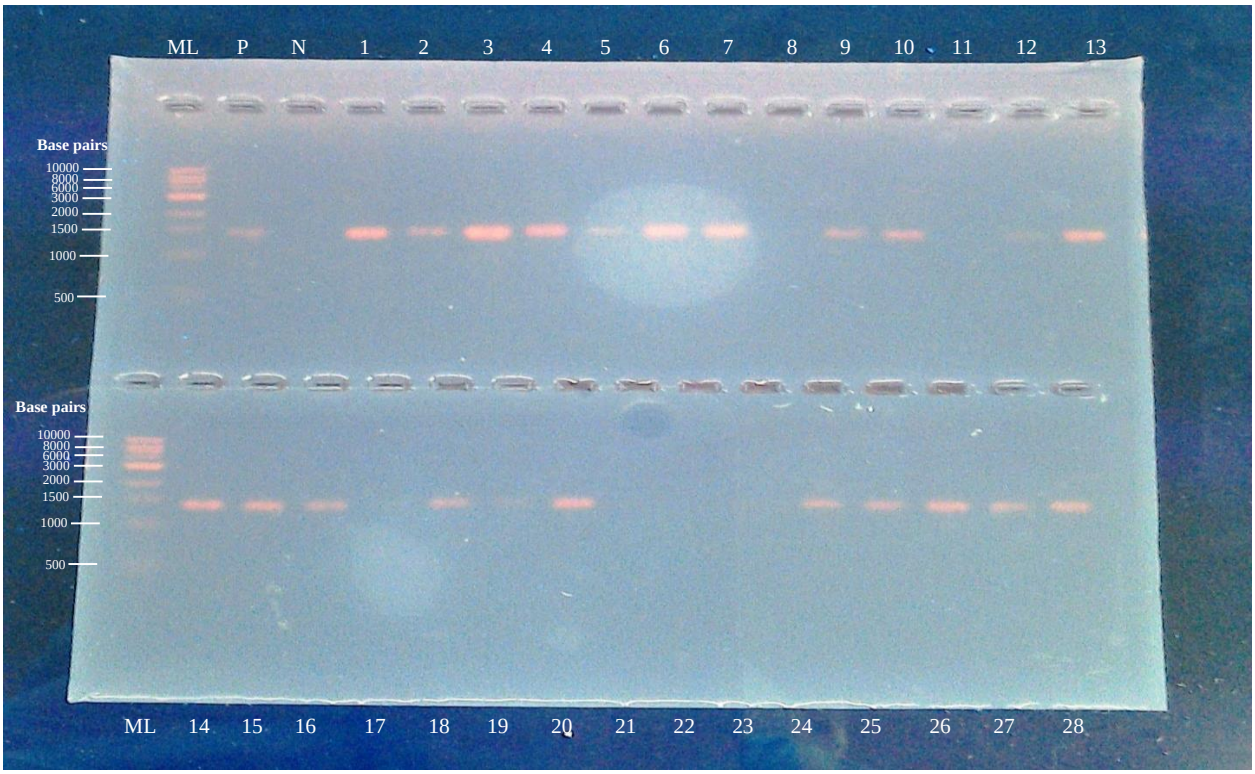

Figure 4.1. Amplification of 16S rRNA gene

Key:

P - Positive;

N- Negative;

ML- Molecular ladder;

1-28 reps: the amplicons of LAB DNA.

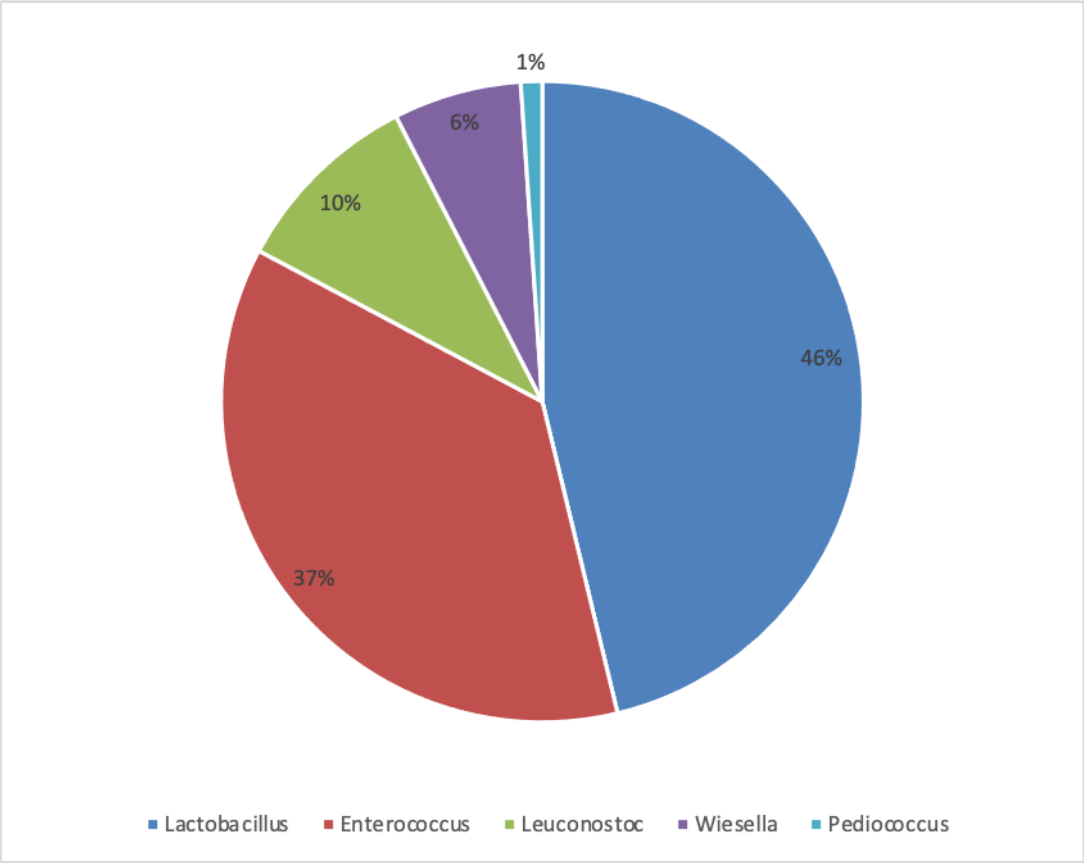

Figure 4.2. Distribution of lactic acid bacteria from mothers' breast milk and their neonates' faeces at the genus level.

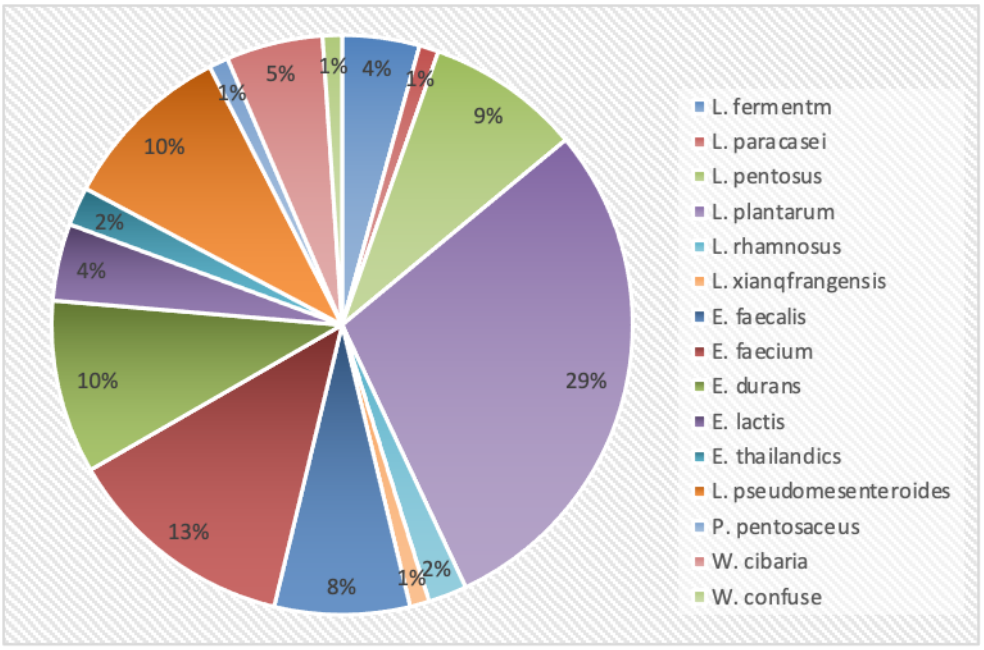

Figure 4.3. Percentage occurrences of lactic acid bacteria from both mothers' breast milk and their neonate faecal samples

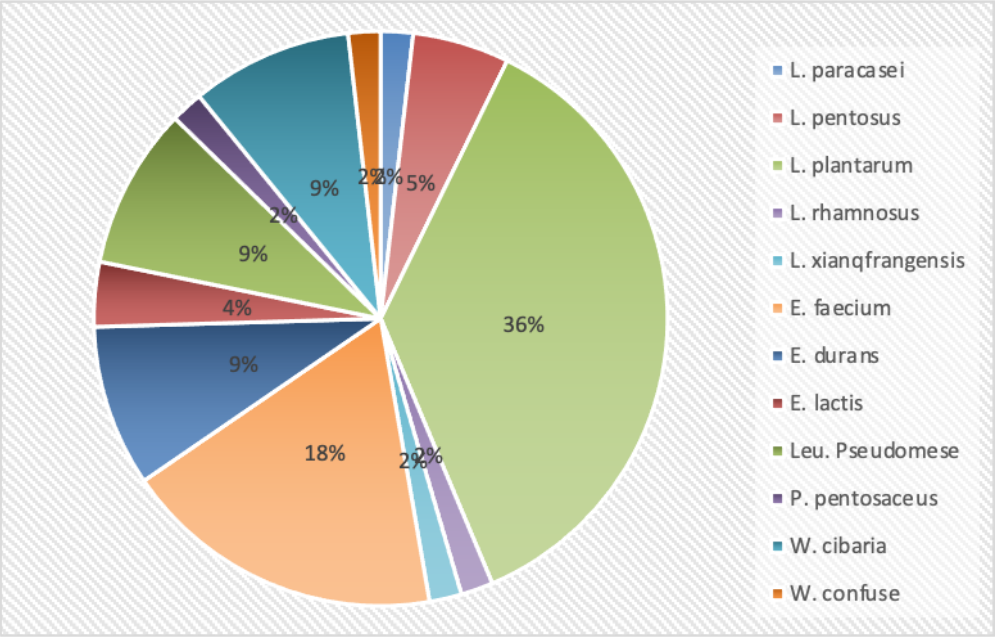

Figure 4.4. Percentage occurrence of lactic acid bacteria from mothers' breast milk

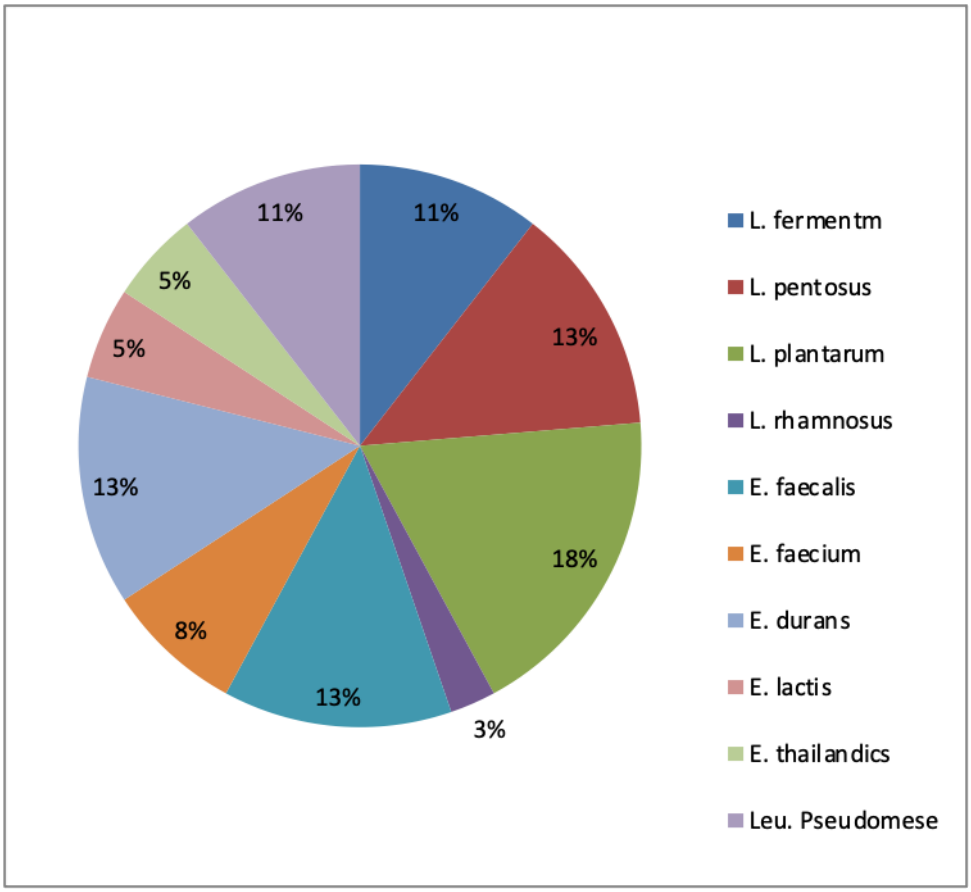

Figure 4.5. Percentage occurrence of lactic acid bacteria from neonates faeces

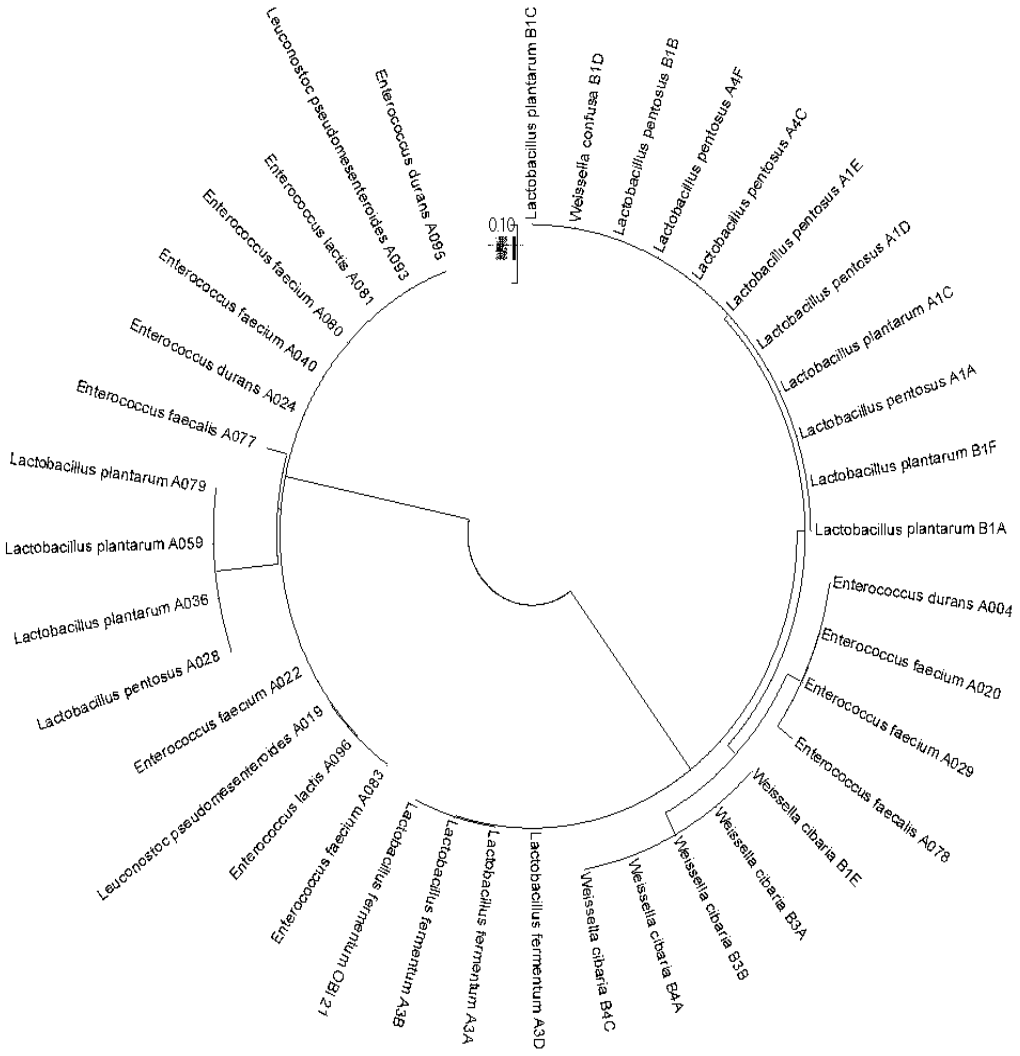

Figure 4.6. Phylogenetic tree relationship among lactic acid bacteria isolated

Note: The scale bar of nucleotide/position is 0.1

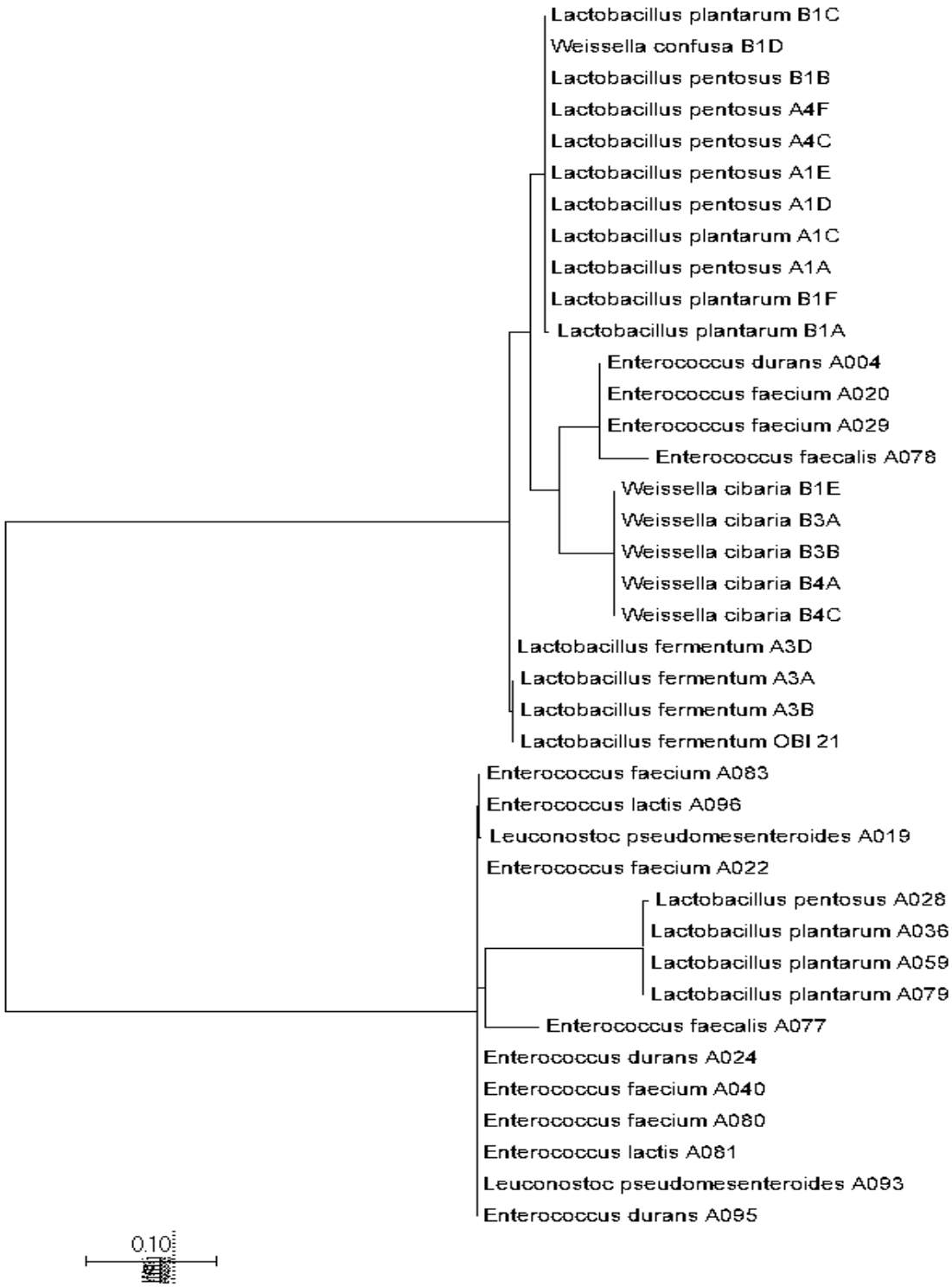

Figure 4.7. Phylogenetic tree relationship between lactic acid bacteria isolated from human breast milk and neonates faeces.

4.2. Antibacterial activities of isolated lactic acid bacteria

The anti-diarrhoeagenic activity of CFS of 93 LAB isolates against 5 pathotypes of diarrhoeagenic *E. coli* was reported. Most of the LAB exhibited appreciable zones of inhibition against the test *E. coli* strains (Table 4.1). The antimicrobial activity of LAB against ETEC H40B (showed that *L. pseudomesenteroides* A093 produced the highest zone of inhibition (26.0 mm) while the least zones of inhibition (8.1 mm) were produced by *L. pentosus* A1a and *W. confuse* B1d. Eighty-five LAB produced antimicrobial activity against ETEC H40B with the following zone of inhibition range; ≥20.1 mm (15 LAB); 16.1 – 20.0 mm (17 LAB), 12.1 – 16.0 mm (33 LAB), 8.1 – 12.0 mm (15 LAB) and five LAB produced between 7.0 – 8.0 mm (Table 4.1). However, eight LAB did not produce any antimicrobial activity against ETEC H40B. Antimicrobial activity of LAB against EPEC H62E) was also observed; *E. faecium* A039 possessed the highest zone of inhibition (18.0 mm while *L. pseudomesenteroides* A010 produced the least value (7.0 mm). Only 65 LAB had antimicrobial activity against EPEC H62E with the range of 16.1 – 20.0 mm (10 LAB), 12.1 – 16.0 mm (29 LAB), 8.1 – 12.0 mm (20 LAB) and 7.0 – 8.0 mm (6 LAB).

Furthermore, 28 LAB did not produce antimicrobial activity against EPEC H62E (Table 4.1). Also, 56 LAB produced antagonistic effect against EIEC (H68D) with inhibition zone of ≥20.1 mm (1 LAB). 16.1 – 20.0 mm (3 LAB), 12.1 – 16.0 mm (15 LAB), 8.1 – 12.0 mm (30 LAB) and 7.0 – 8.0 mm (7 LAB) while 37 LAB CFS did not have any inhibition effect on the test pathogen. *L. pentosus* B1b from breast milk has the highest zone of inhibition with 24.0 mm against EIEC while the least zone of inhibition (8.0 mm), was produced by *L. pseudomesenteroides* A082 and *L. fermentum* A3b respectively. Eighty-two LAB produced antimicrobial effect against STEC H77E at the range of ≥20.1 mm (13 LAB). 16.1 – 20.0 mm (27 LAB), 12.1 – 16.0 mm (32 LAB), 8.1 – 12.0 mm (6 LAB) and 7.0 – 8.0 mm (4 LAB), while 11 LAB isolates had no antimicrobial effect against STEC H77E. The highest zone of inhibition against STEC was observed in *L. pentosus* B1b and *P. pentosaceus* A074 with 26.0 mm and 22.1 mm respectively, while *E. faecalis* A4d and *L. fermentum* A3b produced the least zone of inhibition of 7.0 mm (Table 4.1).

On the other hand, 87 LAB produced antimicrobial activity against EAEC H40C with the zone of inhibition range ≥20.1 mm (11 LAB). 16.1 – 20.0 mm (25 LAB), 12.1 – 16.0 mm (32 LAB), 8.1 – 12,0 mm (13 LAB) and 7.0 – 8.0 mm (6 LAB) while 6 LAB strains did not produce any antimicrobial activity against EAEC H40C, the highest inhibition zone was produced by *P. pentosaceus* A074 (24.0 mm) while the least value (7.0 mm) was produced by *L. plantarum* B1f. In addition, 71 LAB produced antimicrobial activity against EAEC D49B with the zone of inhibition range at ≥20.1 mm (2 LAB), 16.1 – 20.0 mm (2 LAB), 12.1 – 16.0 mm (26 LAB), 8.1 – 12,0 mm (25 LAB) and 7.0 – 8.0 mm (16 LAB) while 22 LAB had no antimicrobial activity. *L. plantarum* A059 produced the highest zone of inhibition (22.0 mm) while *L. plantarum* B1f, *L. fermentum* A3d and *E. durans* A004 produced the least zone of inhibition (7.0 mm) respectively.

Furthermore, 76 LAB produced antimicrobial activity against EAEC D47C, the zone of inhibition range between 16.1 – 20.0 mm (6 LAB), 12.1 – 16.0 mm (26 LAB), 8.1 – 12,0 mm (26 LAB) and 7.0 – 8.0 mm (18 LAB) while 17 LAB had no antimicrobial effect. *L. plantarum* B1a2 have the highest zone of inhibition (20.0 mm) while the least inhibition was produced by *L. plantarum* A035, *L. plantarum* A037, *E. faecium* A040, *E. lactis* A060, *E. durans* A004 and *E. faecalis* A063 with 7.0 mm each (Table 4.1 and Appendix V).

In summary, each LAB isolates possess an antagonistic effect on at least three pathotypes of *E. coli* strains, in addition, *L. plantarum* AO11, *L. plantarum* A1c, *L. rhamnosus* A012, *P. pentosaceus* A074, *L. rhamnosus* A072, *L. pentosus* A028, *L. pentosus* B1b, *L. pentosus* A4c, *E. faecium* A080 and *E.s faecium* A087 exhibited varying zones of *E. coli* inhibition across the pathotypes (Table 4.2).

The antimicrobial activities of viable cells of the isolated LAB were observed on 5 pathotypes of diarrhoeagenic *E. coli*. The LAB isolates showed appreciable antimicrobial activity against the tested pathogens (Table 4.3). Eighty-one LAB produced antimicrobial activity against EPEC H62E. The highest inhibition zone against EPEC H62E was produced by *E. faecalis* A077 (32 mm) while the lowest inhibition zone was produced by *E. faecalis* A033 (Table 4.3). Twelve of the LAB viable cells were unable to produce zone of inhibition against the pathogen (Table 4.3).

In addition, the antimicrobial activity of LAB against ETEC H40B showed that 89 isolates have antimicrobial effect against ETEC H40B. *E. durans* produced the highest inhibition zone (33 mm) while the least zone of inhibition was produced by *L. pentosus* A1a (10 mm), *L. fermentum* A3d (10 mm), *L. pentosus* A1e (10 mm), *W. confusa* B1d (10 mm) and *W. cibaria* B4a (10 mm). However, 4 isolates did not produce antimicrobial activity against ETEC (H40B). The antimicrobial activity of the viable cells of LAB against EAEC H40C showed that 71 LAB isolates produced antagonistic effect against the pathogens while 22 isolates did not inhibit the growth of the pathogens (Table 4.3). *E. durans* produced the highest inhibition (33 mm) while the least zone of inhibition was produced by *L. pentosus* A1a (10 mm), *W. cibaria* B4a (10 mm), *L. plantarum* A075 (10 mm) *E. faecium* A039 (10 mm) and *L. pentosus* A016 (10 mm) respectively(Table 4.3). Furthermore, 68 LAB viable cells produced antimicrobial activity against EIEC (H68D) while 25 LAB viable cells have no effect against the pathogen. It was observed that *L. pseudomesenteroides* A082 produced the highest zone of inhibition (28 mm) while *L. pseudomesenteroides* A030 produced the least inhibition (10 mm). On the other hand, 87 LAB viable cells produced antagonistic effect against STEC (H77E) while 6 LAB had no antagonistic effect against the pathogen, therefore, *L. pseudomesenteroides* A019 produced the highest inhibition zone and *L. pentosus* A4f produced the least inhibition zone (10 mm) against the pathogen (Table 4.3). The viable cells of 11 LAB strains produced appreciable zones of inhibition across the test *E. coli* pathogens using agar overlay method (Table 4.3). These strains include: *L. rhamnosus* A012 which produced antimicrobial activity against; EPEC H62E (24 mm), ETEC H40B (18 mm), EAEC H40C (18 mm), EIEC H68D (18 mm) and STEC H77E (18 mm). *L. rhamnosus* A072 produced antimicrobial against EPEC H62E (25 mm), ETEC H40B (18 mm), EAEC H40C (14 mm), EIEC H68D (18 mm) and STEC H77E (20 mm). *P. pentosaceus* A074 produced antimicrobial activity against; EPEC H62E (24 mm), ETEC H40B (22 mm), EAEC H40C (17 mm), EIEC H68D (18 mm) and STEC H77E (18 mm), *L. plantarum* A011; produced antimicrobial activity against; EPEC H62E (21 mm), ETEC H40B (18 mm), EAEC H40C (15 mm), EIEC H68D (14 mm) and STEC H77E (16 mm). *L. pentosus* A4c produced antimicrobial activity against; EPEC H62E (22 mm), ETEC H40B (18 mm), EAEC H40C (16 mm), EIEC H68D (14 mm) and STEC H77E (16 mm). *L. plantarum* A023 produced antimicrobial activity against; EPEC H62E (23 mm), ETEC H40B (28 mm), EAEC H40C (24 mm), EIEC H68D (18 mm) and STEC H77E (25 mm).

Table 4.1. Antibacterial activities of supernatant of lactic acid bacteria on pathotypes of *E. coli*.

Name of Isolates	Source of the Isolates	ETEC (H40B)	EPEC (H62E)	EIEC (H68D)	STEC (H77E)	EAEC (D49C)	EAEC (D49B)	EAEC (D47C)
L. plantarum A002	Breast Milk	++++	0	++	+++	++	+	+
E. faecium A003	Breast Milk	+++	++	++	+++	+++	++	++
E. faecium A008	Breast Milk	+++	+	0	+++	+++	++	++
L. pseudomenseteroides A010	Breast milk	+++	+	++	++++	+++	++	+
L. plantarum A011	Faeces	+++	+++	++	+++++	++++	++	++
L. rhamnosus A012	Faeces	+++++	++	+++	++++	++++	+++	+++
E. durans A013	Faeces	+++++	+++	++	++++	++++	++	+
L. plantarum A014	Faeces	+++	+++	+++	+++	++++	+++	+++
L. pentosus A016	Breast Milk	+++	++	0	++++	+++	0	0
L. paracasei A017	Breast Milk	++++	0	0	+++.	+++	++	++
E. faecium A018	Breast Milk	++	+++	++	++++	++++	+++	++
L. pseudomenseteroides A019	Breast Milk	+++	0	0	+++++	++++	0	+
E. faecium A022	Breast Milk	+++	0	++	+++	+++	+++++	+++
L. plantarum A023	Feaces	+++++	+++	+++	++++	++	++++	+++
E. durans A024	Feaces	++++	+++	+++	++++	+++++	0	++++
L. pseudomenseteroides A026	Faeces	+++	+++	+++	+++	++++	0	0
E. .thailandicus A027	Faeces	++	+++	++	+++	+++++	++	+++
L. pentosus A028	Breast Milk	++++	++++	++	++++	+++	++	++
L. pseudomenseteroides A030	Feaces	+++	0	0	+++	+++	0	++
E. thailandicus A031	Faeces	++	0	0	+++	+++	0	+
L. plantarum A033	Breast Milk	++	0	0	+++++	++++	++	+
L. plantarum A034	Breast Milk	+++	++	++	++++	++++	++	++
L. plantarum A035	Breast Milk	+++	++++	++++	++++	++++	0	+
L. plantarium A036	Breast Milk	+++	++	0	++++	+++	+++	+++
L. plantarium A037	Breast Milk	+++	0	0	++++	+++	+	+
E. durans A038	Breast Milk	++	+++	0	+++	+++	0	0
E. faecium A039	Breast Milk	+++++	++++	++	+++++	++++	0	++
E. faeciuum A040	Breast Milk	++++	+++	+++	+++	++++	+	+
L. plantarium A041	Breast Milk	++++	0	0	++++	++++	+++	++
E. durans A043	Breast Milk	+++	0	0	+++	++	+	0
L. pseudomenseteroides A044	Breast Milk	++++	+++	+++	++++	++++	+++	+++
L. plantarium A046	Breast Milk	+++++	++++	++	+++	++++	+++	++
L. plantarium A047	Breast Milk	++++	++++	0	+++	++	+	+++
L. plantarium A048	Breast Milk	+++	+++	0	++	+++	+	++
E. faecium A049	Breast Milk	+++	0	0	++++	+++	+	0
E. durans A050	Breast Milk	++++	+++	+++	++++	++++	++	0

Table 4.1. cont.

Name of Isolates	Source of the Isolates	ETEC (H40B)	EPEC (H62D)	EIEC (H68D)	STEC (H77E)	EAEC (H40C)	EAEC (D49B)	EAEC (D47C)
L. plantarium A051	Breast Milk	++++	+++	++	+++++	++++	++	++
E. lactis A052	Breast Milk	++	+++	0	++++	+++++	+++	+++
E. faecalis A058	Faeces	+++	0	0	0	+++++	0	++
L. plantarium A059	Faeces	+++++	++	++	+++++	+++++	+	++
E. lactis A060	Faeces	+++	0	0	++	+++	0	+
L. pseudomenseteroides A064	Faeces	+++++	++	++	++++	+++++	+++	++++
E. faecium A066	Faeces	+++++	++	+++	+++++	+++	++	+
L. plantarum A071	Breast Milk	+++	+++	0	++	+++	+	+
L. rhamnosus A072	Breast Milk	+++++	++++	++	++++	++++	++	+
P. pentosaceus A074	Breast Milk	+++++	+++	++	+++++	+++++	++	++
L. plantarum A075	Breast Milk	+++	++++	++	++++	+++	+	0
E. faecalis A077	Faeces	+++++	+++	++	+++++	++++	+	0
L. plantarum A079	Faeces	+++	++	0	++++	++++	+++	+
E. faecium A080	Breast Milk	++++	+++	+	+++++	+++++	+++	+++
E. lactis A081	Breast Milk	+++++	+++	0	+++++	+++	+++	+++
L. pseudomenseteroides A082	Breast Milk	++++	+++	++	+++	++	++	++
E. faecium A083	Breast Milk	+++++	+++	++	++++	++++	0	+
L. plantarium A084	Breast Milk	+++	++	++	++++	++	+	+++
E. faecium A087	Faeces	++++	+++	++	++++	++++	++	++
L. pseudomenseteroides A089	Faeces	+++	++	+++	+++	++++	+++	+++
E. faecium A090	Faeces	++++	++	0	+++++	+++++	0	0
L. pseudomenseteroides A093	Breast Milk	+++++	+++	+	++	++	++	++
L. plantarium A094	Faeces	++	+++	0	+++	++	+	++
E. durans A095	Faeces	+++++	++++	0	+++	+++++	+++	+++
E. lactis A096	Faeces	+++	0	0	+++	+++	+	0
E. durans A097	Faeces	++++	++	0	++++	++++	+++	+++
E. durans A098	Faeces	++	++++	++	++++	+++	+++	+++
E. faecalis A4D	Faeces	+++	++	++	+	+++	+++	+++
W. cibaria B3b	Breast Milk	++	++	0	0	++	+++	+++
L. pentosus A1d	Faeces	+	+	0	0	0	0	0
W. cibaria B3a	Breast Milk	++	++	+++	+++	++	+++	+++
L. fermentum A3b	Faeces	0	0	+	+	0	++	++
L. plantarum B3c	Breast Milk	+++	+	+++	+++	++	+++	+++
L..plantarum A4b	Faeces	+++	++	+	++	++	+++	++++
L. plantarum B1b2	Breast Milk	+++	0	++++	0	++++	++++	++++
L. plantarum A1c	Faeces	+++	++	+++	+++	+	+++	+++

Table 4.1. cont.

Name of Isolates	Source of the Isolates	ETEC (H40B)	EPEC (H62D)	EIEC (H68D)	STEC (H77E)	EAEC (H40C)	EAEC (D49B)	EAEC (D47C)
W. cibaria B4a	Breast Milk	+++	0	++	0	0	+	++
L. pentosus A4f	Faeces	++	0	++	+++	+++	+++	++
W. cibaria B1e	Breast Milk	++	0	0	0	+	++	0
L. fermentum A3a	Faeces	+	0	+++	+++	++	+++	++
W. cibaria B4c	Breast Milk	+	0	0	0	0	0	0
L. pentosus A4c	Faeces	+++	+++	+++	+++	+++	++	++
W. confusa B1d	Breast Milk	+	0	0	0	0	0	0
L. pentosus A1a	Faeces	+	+	++	+++	+++	0	+++
L. plantarum B1f	Breast Milk	0	0	+	+	+	0	0
L. fermentum A3d	Faeces	0	0	+	0	+	++	+++
L. plantarum B1c	Breast Milk	++	++	+++	+	+++	+++	++++
L. fermentum A3c	Faeces	0	+++	++	++	+	++	+++
L. pentosus B1b	Breast Milk	++	++++	+++++	+++++	+++	++	+++
L. pentosus A1e	Faeces	++	0	++++	+++	+++	+	0
L. plantarum B1a	Breast Milk	+++	0	++	+++	+++	++	+++
L. Xianqfangensis B1a2	Breast Milk	0	+++	++	0	++	+++++	++++
E. durans A004	Breast Milk	0	+	0	+++	+++	0	+
*E. faecalis*A020	Breast Milk	++++	++	++	+++	+++	0	++
E. faecalis A029	Breast Milk	++++	+++	+	++++	+++	+++	+
E. faecalis A063	Faeces	0	0	0	+++	0	0	+
E. faecalis A078	Faeces	++++	0	0	0	+++++	0	0

Key:

+ indicates 7.0 mm – 8.0 mm zone of inhibition
++ indicates 8.1 mm -12.0 mm zone of inhibition
+++ indicates 12.1 mm – 16.0 mm zone of inhibition
++++ indicates 16.1 mm – 20.0 mm zone of inhibition
+++++ indicates 20.1mm zone of inhibition and above

Table 4.2. Antibacterial activity of lactic acid bacteria against pathotypes of *E. coli* strains

LAB species	No of isolates (%)	Zones of inhibition																				
		ETEC (H40B)			EPEC (H62D)			EIEC (H68D)			STEC (H77E)			EAEC (H40C)			EAEC (D 49B)			EAEC (D 47B)		
		+	++	+++	+	++	+++	+	++	+++	+	++	+++	+	++	+++	+	++	+++	+	++	+++
L. fermentum	4(4%)	1	0	0	0	1	0	3	1	0	2	2	0	2	0	0	3	1	0	2	2	0
L. paracasei	1(1%)	0	0	1	0	0	0	0	0	0	0	1	0	0	1	0	1	0	0	1	0	0
L. pentosus	8 (10%)	4	2	1	3	2	1	3	1	2	1	2	3	0	6	0	4	1	0	3	2	0
L. plantarum	27 (29%)	2	17	7	9	8	3	11	5	1	5	8	13	8	8	11	14	8	2	11	8	3
L. rhamnosus	2(2%)	0	0	2	1	0	1	1	1	0	0	0	2	0	0	2	2	0	0	1	1	0
L. xianqfrang.	1(1%)	0	0	0	1	0	0	1	0	0	0	0	0	1	0	0	0	0	1	0	0	1
E. faecalis	5 (4%)	0	2	2	1	1	0	2	1	0	1	1	1	0	1	3	1	1	0	1	1	0
E. faecium	13(14%)	1	4	7	4	5	1	7	1	0	0	4	8	0	5	7	6	2	1	7	2	0
E. durans	10 (10%)	2	1	7	4	4	2	5	1	0	0	5	5	1	5	4	3	4	0	3	3	0
E. lactis	4(4%)	1	2	1	0	2	0	0	0	0	0	2	2	0	3	1	1	2	0	0	2	0
E. thailadicus	2 (3%)	2	0	0	0	1	0	1	0	0	0	2	0	0	1	1	1	0	0	1	1	0
Le.pseudomes	9 (10%)	0	6	3	2	4	0	3	3	0	1	4	3	2	2	5	3	3	0	5	2	1
Ped. Pentosac	1(1%)	0	0	1	0	1	0	1	0	0	0	0	1	0	0	1	1	0	0	1	0	0
W. cibaria	5(6%)	3	1	0	2	0	0	1	1	0	0	1	0	3	0	0	1	2	0	2	2	0
W. confse	1(1%)	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

Key:

The diameter of zone of inhibition (mm) of LAB against the test microorganisms:
From 8mm to 12mm = +
From 12.1mm to 16mm = ++, From 16.1mm and above = +++

Table 4.3. Inhibition of pathotypes of *E. coli* by viable lactic acid bacterial cells

LAB STRAINS	EPEC (H62E)	ETEC (H40B)	EAEC (H40C)	EIEC (H68D)	STEC (H77E)
L. plantarum A002	16.0	20.0	12.0	12.0	25.0
E. faecium A003	12.0	18.0	0	0	18.0
E. faecium A008	20.0	28.0	18.0	0	32.0
L. pseudomenseteroides A010	15.0	22.0	13.0	15.0	0
L. plantarum A011	21.0	18.0	15.0	14.0	16.0
L. rhamnosus A012	24.0	18.0	18.0	18.0	18.0
E. durans A013	20.0	28.0	28.0	0	32.0
L. plantarum A014	20.0	20.0	15.0	11.0	22.0
L. pentosus A016	18.0	20.0	10.0	0	24.0
L. paracasei A017	21.0	28.0	0	11.0	28.0
E. faecium A018	24.0	20.0	18.0	20.0	22.0
L. pseudomenseteroides A019	26.0	28.0	0	20.0	34.0
E. faecium A022	22.0	26.0	16.0	0	28.0
L. plantarum A023	23.0	28.0	24.0	18.0	25.0
E. durans A024	25.0	20.0	21.0	18.0	28.0
L. pseudomenseteroides A026	15.0	16.0	15.0	10.0	20.0
E. .thailandicus A027	15.0	24.0	18.0	12.0	28.0
L. pentosus A028	19.0	16.0	0	16.0	15.0
L. pseudomenseteroides A030	10.0	17.0	0	10.0	13.0
E. thailandicus A031	20.0	28.0	17.0	0	25.0
L. plantarum A033	22.0	24.0	20	0	0
L. plantarum A034	19.0	14.0	0	15.0	18.0
L. plantarum A035	0	20.0	0	0	24.0
L. plantarium A036	19.0	24.0	18.0	15.0	18.0

Table 4.3. cont.

LAB STRAINS	EPEC (H62E)	ETEC (H40B)	EAEC (H40C)	EIEC (H68D)	STEC (H77E)
L. plantarium A037	26.0	32.0	19.0	11.0	32.0
E. durans A038	0	20.0	18.0	10.0	17.0
E. faecium A039	17.0	23.0	10.0	0	0
E. faeciuum A040	0	21.0	0	0	15.0
L. plantarium A041	24.0	22.0	14.0	15.0	10.0
E. durans A043	0	0	10.0	0	10.0
L. pseudomenseteroides A044	21.0	16.0	16.0	14.0	16.0
L. plantarium A046	23.0	19.0	15.0	0	15.0
L. plantarium A047	24.0	26.0	24.0	18.0	26.0
L. plantarium A048	21.0	24.0	18.0	14.0	18.0
E. faecium A049	0	20.0	0	0	22.0
E. durans A050	16.0	16.0	0	0	20.0
L. plantarum A051	19.0	17.0	20.0	20.0	28.0
E. lactis A052	18.0	20.0	16.0	16.0	24.0
E. faecalis A058	16.0	17.0	18.0	12.0	20.0
L. plantarium A059	18.0	24.0	17.0	16.0	28.0
E. lactis A060	15.0	18.0	12.0	0	24.0
L. pseudomenseteroides A064	0	0	16.0	16.0	16.0
E. faecium A066	20.0	26.0	18.0	19.0	26.0
L. plantarum A071	16.0	15.0	0	17.0	20.0
L. rhamnosus A072	25.0	18.0	14.0	18.0	20.0
P. pentosaceus A074	24.0	22.0	17.0	18.0	18.0
L. plantarum A075	26.0	24.0	10.0	0	22.0
E. faecalis A077	32.0	29.0	14.0	26.0	28.0

Table 4.3. cont.

LAB STRAINS	EPEC (H62E)	ETEC (H40B)	EAEC (H40C)	EIEC (H68D)	STEC (H77E)
L. plantarum A079	20.0	15.0	20.0	12.0	25.0
E. faecium A080	18.0	16.0	12.0	10.0	14.0
E. lactis A081	18.0	22.0	0	14.0	22.0
L. pseudomenseteroides A082	24.0	32.0	23.0	28.0	28.0
E. faecium A083	10.0	12.0	0	0	10.0
L. plantarium A084	20.0	20.0	18.0	16.0	24.0
E. faecium A087	25.0	18.0	20.0	14.0	28.0
L. pseudomenseteroides A089	22.0	32.0	18.0	18.0	25.0
E. faecium A090	20.0	23.0	0	0	32.0
L. pseudomenseteroides A093	17.0	22.0	0	0	20.0
L. plantarium A094	24.0	24.0	14.0	10.0	28.0
E. durans A095	18.0	28.0	14.0	0	25.0
E. lactis A096	26.0	20.0	18.0	24.0	32.0
E. durans A097	0	20.0	10.0	16.0	24.0
E. durans A098	20.0	15.0	26.0	20.0	28.0
E. faecalis A4D	18.0	30.0	23.0	20.0	32.0
W. cibaria B3b	15.0	20.0	18.0	18.0	25.0
L. pentosus A1d	0	10.0	11.0	17.0	0
W. cibaria B3a	21.0	18.0	16.0	18.0	14.0
L. fermentum A3b	10.0	0	0	10.0	0
L. plantarum B3c	15.0	20.0	0	0	24.0
L .plantarum A4b	10.0	25.0	17.0	15.0	28.0
L. plantarum B1b2	26.0	24.0	20.0	24.0	32.0
L. plantarum A1c	10.0	22.0	18.0	16.0	20.0
W. cibaria B4a	10.0	10.0	10.0	10.0	12.0

Table 4.3. cont.

LAB STRAINS	EPEC (H62E)	ETEC (H40B)	EAEC (H40C)	EIEC (H68D)	STEC (H77E)
L. pentosus A4f	10.0	10.0	0	13.0	10.0
W. cibaria B1e	14.0	11.0	20.0	15.0	11.0
L. fermentum A3a	19.0	16.0	15.0	0	20.0
W. cibaria B4c	18.0	0	0	15.0	16.0
L. pentosus A4c	22.0	18.0	16.0	14.0	16.0
W. confusa B1d	0	10.0	0	12.0	12.0
L. pentosus A1a	0	15.0	10.0	0	19.0
L. plantarum B1f	14.0	12.0	0	0	20.0
L. fermentum A3d	18.0	20.0	0	10.0	16.0
L. plantarum B1c	16.0	15.0	15.0	14.0	22.0
L. fermentum A3c	17.0	10.0	14.0	10.0	18.0
L. pentosus B1b	21.0	20.0	16.0	18.0	18.0
L. pentosus A1e	0.0	10.0	11.0	0	0
L. plantarum B1a	20.0	17.0	24.0	22.0	30.0
L. Xianqfangensis B1a2	25.0	28.0	20.0	24.0	30.0
E. durans A004	23.0	33.0	16.0	14.0	27.0
*E. faecalis*A020	0	20.0	14.0	0	25.0
E. faecalis A029	25.0	22.0	18.0	24.0	32.0
E. faecalis A063	18.0	22.0	15.0	15.0	28.0
E. faecalis A078	15.0	25.0	12.0	10.0	22.0

Note: zone of inhibition results are in diameter (mm)

The inhibitory potential of non-neutralized and neutralized CFS of 93 LAB strains against *S. aureus*, was evaluated. Forty non-neutralized LAB strains produced a zone of inhibition against *S. aureus* ATCC 29213. *E. thailandicus* A027 and *L. plantarum* B3C produced the largest zone of inhibition (20.0 mm) followed by *E. lactis* A060 and *L. pseudomesenteroides* A010 with 18.0 mm and 17.0 mm inhibition zones respectively. Meanwhile, *P. pentosaceus* A074, *L. plantarum* A011, *L. plantarum* A1c, *L. plantarum* B1a2 and *E. lactis* A052 have inhibition zones of 16.0 mm each. The least zone of inhibition was produced in *E. durans* A013 with 8.0 mm (Table 4.4.)

Out of 40 strains that shown inhibitory activity against *S. aureus*, only 8 strains produced antimicrobial activity against *S. aureus* after neutralising their metabolites. The neutralized LAB with inhibition zones produced are: *L. plantarum* A011 (12.0 mm), *L. plantarum* A1c (11.0 mm), *L. plantarum* A084 (8.0 mm), *W. cibaria B1e* (12.0 mm) *L. plantarum* A036 (10.0mm) *L. plantarum* B1a2 (11.0 mm), *L. pseudomesenteroides* A044 (9.0 mm) and A089 (11.0 mm) (Table 4.44). *L. plantarum* A011, *L. plantarum* A1c (11.0 mm), *L. plantarum* A084, *W. cibaria B1e*, *L. plantarum* A036, *L. plantarum* B1a2, *L. pseudomesenteroides* A044 and *L. pseudomesenteroides* A089 produced protein precipitation with Ammonium Sulphate.

Seventeen LAB were tested for anti-diarrheagenic *E. coli* activity against EAEC H40C, EPEC H62E, EIEC H68D, ETEC H40B, and STEC H77E strains in co-culture assay (Table 4.5). The longitudinal inhibition of 17 selected LAB against ETEC H40B was demonstrated, (Figure 4.8). It was observed that, at 24 h of contact; 13 of the LAB strains (*L. plantarum* A011, *L. plantarum* A046, *L. plantarum* A084, *L. plantarum* A1c and *L. plantarum* B1c, *L. pentosus* A028, *L. pentosus* B1b), *L. rhamnosus*A012 and *L. rhamnosus* A072, *W. cibaria* B3a and *L. pseudomesenteroides* A044 and *L. pseudomesenteroides* A064 completely inhibited the growth of ETEC H40B. However, at 16 h of contact, 8 LAB strains; *L. plantarum* (A011, A034) *L. pentosus* B1b, *L. rhamnosus* strains (A012 and A072), *W. cibaria* B3a, *P. pentosaceus* A074 and *L. pseudomesenteroides* A044, completely inhibited the pathogen (Figure 4.8). The growth of EPEC (H62E) was completely inhibited at 24 h of contact with 13 LAB, however, at 16 h of co-culturing the selected LAB with EPEC H62E;

five of the strains (*L. rhamnosus* A012 and *L. rhamnosus* A072, *L. plantarum* A011, *W. cibaria* B3a, *L. pseudomesenteroides* A044, completely inhibited the growth of the pathogen (Figure 4.9). Furthermore, 11 strains out of the selected LAB inhibited EIEC (H68D) at 24 h while only 5 of the strains (*L. rhamnosus* A012, *L. rhamnosus* A072, *L. plantarum* A011, *L. pentosus* B1b and *P. pentosaceus* A074) completely inhibited the growth of EIEC H68D (Figure 4.10).

It was also observed that at 24 h of contact of selected LAB with STEC H77E, most of the selected LAB inhibited the growth of the pathogen except *L. plantarum* B1c. At 16 h of contact of selected LAB with STEC H77E, *L. rhamnosus* A012, *L. rhamnosus* A072, *L. plantarum* A011, *L. pseudomesenteroides* A044, *L. pentosus* A4c, *P. pentosaceus* A074, *L. plantarum* B3c and *L. plantarum* A034 completely inhibited the growth of the pathogen, (Figure 4.11). Ten strains of LAB out of the 17 selected for the study inhibited the growth of EAEC H40C at 24 h of contact, however, only 6 of these strains; (*L. rhamnosus* A012, *L. rhamnosus* A072, *L. plantarum* A011, *L. pentosus* B1b, *L. pentosus* A4c, *W. cibaria* B3a), inhibited the growth of the pathogen at 16 h of contact (Figure 4.12). It was observed that *L. rhamnosus* A012, *L. rhamnosus* A072 and *L. plantarum* A011 exert bacteriocidal effect against the diarrhoegenic *E. coli* at 16 h of contact with the pathogens.

All the 15 LAB strains used in the study possess growth inhibition against enteroaggregative *E. coli* but only few possess biofilm inhibition. At 1 in 1 dilution, *L. plantarum* A011 was the only strain with biofilm inhibition, other strains showed growth inhibition against the pathogen. At 1 in 9 dilution, *L. plantarum* A1c and *L. plantarum* A011 showed biofilm inhibition while *L. plantarum* A011 and *L. plantarum* A014 showed biofilm inhibition at 1 in 99 dilutions (Table 4.6). It was observed that, *L. plantarum* A011 had biofilm inhibition at every concentration used (Table 4.6).

The organic acid (lactic and acetic) produced by five selected LAB was quantified by HPLC (Figure 4.13). Lactic acid produced was 72.52% while acetic acid accounted for 27.45%. *L. rhamnosus* A012 produced the highest quantity of lactic acid (76.8 mg/ml; 73.71%) and *L. pentosus* A028 produced the least quantity (23.12 mg/ml; 9.14%). The highest quantity

of acetic acid was produced by *L. rhamnosus* A012 (27.39 mg/ml; 28.59%) while the least quantity was produced by *L. pentosus* A028 (9.13 mg/ml; 9.53%) (Figure 4.13).

Table 4.4. Antibacterial activity of non-neutralized and neutralized cell free supernatant of lactic acid bacteria on *S. aureus*

LAB ISOLATES	pH of CFS (Non-neutralised)	Zone of Inhibition(mm)	pH of CFS (Neutralised)	Zone of Inhibition (mm)
L. platarum A002	4.23	0. 0	6.56	0. 0
E. faecium A003	4.26	0. 0	6.70	0. 0
E. faecium A008	4.18	0. 0	6.58	0. 0
L. pseudomenseteroides A010	3.52	17. 0	6.55	9. 0
L. plantarium A011	3.44	16. 0	6.50	12. 0
L. rhamnosus A012	3.57	10. 0	6.53	0. 0
E. durans A013	4.38	8. 0	6.42	0. 0
L. plantarum A014	3.44	15. 0	6.55	0. 0
L. pentosus A016	4.29	0. 0	6.76	0. 0
L. paracasei A017	3.43	13. 0	6.53	0. 0
E. faecium A018	4.53	0. 0	6.64	0. 0
L. pseudomenseteroides A019	3.69	0. 0	6.58	0. 0
E. faecium A022	3.78	13. 0	6.55	0. 0
L. plantarium A023	3.56	14. 0	6.63	0. 0
E. durans A024	3.72	12. 0	6.56	0. 0
L. pseudomenseteroides A026	3.46	9. 0	6.58	0. 0
E. thailandicus A027	3.58	20. 0	6.65	0. 0
L. pentosus A028	3.61	0. 0	6.54	0. 0
L. pseudomenseteroides A030	4.24	0. 0	6.58	0. 0
E. thailandicus A031	4.11	0. 0	6.51	0. 0
L. plantarium A033	3.89	10. 0	6.54	0. 0
L. plantarium A034	3.44	9. 0	6.56	0. 0
L. plantarium A035	4.08	0. 0	6.60	0. 0
L. plantarium A036	3.45	15. 0	6.62	10.0
L. plantarium A037	3.60	0. 0	6.70	0. 0
E. durans A038	4.26	0. 0	6.55	0. 0
E. faecium A039	3.95	0. 0	6.45	0. 0
E. faecium A040	4.10	0. 0	6.57	0. 0
L. plantarium A041	3.50	0. 0	6.53	0. 0
E. durans A043	4.36	0. 0	6.57	0. 0
L. pseudomenseteroides A044	3.51	14. 0	6.50	9. 0
L. plantarium A046	3.58	0. 0	6.50	0. 0
L. plantarium A047	3.39	15. 0	6.53	0. 0
L. plantarium A048	3.59	0. 0	6.56	0. 0
E. faecium A049	4.38	0. 0	6.56	0. 0

Table 4.4. Cont.

LAB ISOLATES	pH of CFS (Non-neutralised)	Zone of Inhibition(mm)	pH of CFS (Neutralised)	Zone of Inhibition (mm)
E. durans A050	4.12	0. 0	6.54	0. 0
L. plantarium A051	3.84	0. 0	6.56	0. 0
E. lactis A052	4.28	16. 0	6.74	0. 0
E. faecalis A058	3.56	0. 0	6.50	0. 0
L. plantarium A059	3.55	12. 0	6.59	0. 0
E. lactis A060	3.52	18. 0	6.54	0. 0
L. pseudomenseteroides A064	3.43	11. 0	6.75	0. 0
E. faecium A066	3.55	0. 0	6.50	0. 0
L. plantarum A071	3.60	0. 0	6.50	0. 0
L. rhamnosus A072	3.58	0. 0	6.78	0. 0
P. pentosaceus A074	3.59	16. 0	6.57	0. 0
L. plantarium A075	4.29	0. 0	6.53	0. 0
E. faecalis A077	4.52	0. 0	6.53	0. 0
L. plantarium A079	3.56	0. 0	6.54	0. 0
E faecium A080	4.09	0. 0	6.51	0. 0
E lactis A081	4.02	0. 0	6.52	0. 0
L pseudomenseteroides A082	3.78	0. 0	6.56	0. 0
E faecium A083	4.12	0. 0	6.50	0. 0
L. plantarium A084	3.44	10. 0	6.52	8. 0
E. faecium A087	4.22	0. 0	6.54	0. 0
L. pseudomenseteroides 089	3.42	12. 0	7.20	11. 0
E. faecium 090	4.25	0. 0	6.80	0. 0
L. pseudomenseteroides A093	4.10	0. 0	6.58	0. 0
L. plantarium A094	4.28	0. 0	6.52	0. 0
E. durans A095	4.22	0. 0	6.50	0. 0
E. lactis A096	4.28	0. 0	6.50	0. 0
E. durans A097	3.80	0. 0	6.58	0. 0
E. durans A098	4.06	0. 0	6.50	0. 0
E. faecalis A4D	3.47	10. 0	6.56	0. 0
W. cibaria B3b	3.57	10. 0	7.69	0. 0
L. pentosus A1d	3.72	0. 0	6.56	0. 0
W. cibaria B3a	3.46	0. 0	6.56	0. 0
L. fermentum A3b	4.26	0. 0	6.51	0. 0
L.. plantarum B3c	3.41	20. 0	7.11	0. 0
L.. plantarum A4b	3.63	14. 0	6.52	0. 0
L.. plantarum B1b2	3.46	14. 0	6.50	0. 0

Table 4.4. cont.

LAB ISOLATES	pH of CFS (Non-neutralised)	Zone of Inhibition(mm)	pH of CFS (Neutralised)	Zone of Inhibition (mm)
L. plantarum A1c	3.45	16. 0	6.52	11. 0
W. cibaria B4a	3.70	14. 0	6.64	0. 0
L. pentosus A4f	3.56	10. 0	6.55	0. 0
W. cibaria B1e	4.04	14. 0	6.56	12. 0
L. fermentum A3a	4.23	0. 0	6.70	0. 0
W. cibaria B4c	3.88	0. 0	6.60	0. 0
L. pentosus A4c	3.70	0. 0	7.41	0. 0
W. confusa B1d	3.64	0. 0	6.50	0. 0
W. cibaria A1a	3.49	15. 0	6.52	0. 0
L. plantarum B1f	3.72	0. 0	6.52	0. 0
L. fermentum A3d	4.20	0. 0	6.50	0. 0
L. plantarum B1c	3.50	12. 0	7.11	0. 0
L. fermentum A3c	4.12	0. 0	6.51	0. 0
L. pentosus B1b	3.40	14. 0	6.56	0. 0
L. pentosus A1e	3.38	0. 0	6.52	0. 0
L. plantarum B1a	3.48	14. 0	6.51	0. 0
L. xanqfangensis B1a2	3.58	16. 0	6. 76	11. 0
E. durans A004	3.80	0. 0	6.51	0. 0
E. faecalis A020	3.74	0. 0	6.50	0. 0
E. faecalis A029	3.63	12. 0	6.59	0. 0
E. faecalis A063	3.55	14. 0	6.50	0. 0
E.f aecalis A078	3.56	13. 0	6.50	0. 0

Table 4.5. Killing rate of lactic acid bacteria in co-culture with diarrhoeagenic *E. coli*

	Incubation Time (Hour)			
	0	**8**	**16**	**24**
Organisms	**CFU/mL**	**CFU/mL**	**CFU/mL**	**CFU/mL**
Enteroinvasive *E. coli* H68D	$8.6x10^6$	$5.6 x10^6$	$8.9 x10^6$	$1.3 x10^7$
L. pentosus A028 + enteroinvasive *E. coli* H68D	$3.9 x10^5$	$2.5 x10^4$	$7.2 x10^3$	NG
L. plantarum A046 + enteroinvasive *E. coli* H68D	8.3×10^5	1.89×10^4	1.20×10^4	6.0×10^2
L. plantarum A084 + enteroinvasive *E. coli* H68D	4.2×10^5	8.1×10^3	3.2×10^3	NG
L. pentosus B1b + enteroinvasive *E. coli* H68D	7.1×10^5	1.52×10^4	NG	NG
L. plantarum A034 + enteroinvasive *E. coli* H68D	7.4×10^5	9.1×10^3	1.8×10^3	5.0×10^2
L. pentosus A4c + enteroinvasive *E. coli* H68D	7.7×10^5	7.5×10^3	1.8×10^3	NG
L. rhamnosus A072 + enteroinvasive *E. coli* H68D	7.0×10^5	1.0×10^4	NG	NG
L. rhamnosus A012 + enteroinvasive *E. coli* H68D	2.6×10^6	6.4×10^3	NG	NG
P. pentosaceus A074+ enteroinvasive *E. coli* H68D	$6.5 x10^5$	6.1×10^3	NG	NG
W. cibaria B3a + enteroinvasive *E. coli* H68D	2.11×10^6	1.17×10^4	4.0×10^2	NG
L. pseudomesenteroides A044 + enteroinvasive *E. coli* H68D	1.13×10^6	1.22×10^4	7.8×10^3	$6.5 \text{ X } 10^3$
L. pseudomesenteroides A064 + enteroinvasive *E. coli* H68D	2.0×10^5	1.51×10^4	3.0×10^3	NG
L. plantarum B1c + enteroinvasive *E. coli* H68D	3.6×10^5	2.3×10^4	5.8×10^3	7.0×10^2
L. plantarum A1c + enteroinvasive *E. coli* H68D	4.1×10^5	2.4×10^4	7.0×10^2	NG
L. plantarum B3c + enteroinvasive *E. coli* H68D	$4.7 \text{ X } 10^5$	1.7×10^4	3.6×10^3	7.0×10^2
L. plantarum A011+ enteroinvasive *E. coli* H68D	5.6×10^5	2.6×10^3	NG	NG

Note: NG means no growth

Table 4.5. cont.

	Incubation Time (Hour)			
	0	**8**	**16**	**24**
Organisms	**CFU/mL**	**CFU/mL**	**CFU/mL**	**CFU/mL**
Enterotoxigenic *E. coli* H40B	6.0 x10^6	7.0 x10^7	8.3 x10^6	6.7 x10^6
L. pentosus A028 + enterotoxigenic *E. coli* H40B	3.2 x 10^5	1.92 x 10^4	4.1 x 10^3	NG
L. plantarum A046 + enterotoxigenic *E. coli* H40B	3.3 x 10^5	1.65 x 10^4	1.0 x 10^3	NG
L. plantarum A084 + enterotoxigenic *E. coli* H40B	6.6 x 10^5	8.1 x 10^3	1.7 x 10^3	NG
L. pentosus B1b + enterotoxigenic *E. coli* H40B	3.7 x 10^5	9.7 x 10^3	NG	NG
L. plantarum A034 + enterotoxigenic *E. coli* H40B	5.6 x 10^5	7.6 x 10^3	NG	NG
L. pentosus A4c + enterotoxigenic *E. coli* H40B	8.1 x 10^5	9.6 x 10^3	1.0 x 10^3	NG
L. rhamnosus A072 + enterotoxigenic *E. coli* H40B	3.1 x 10^5	1.16 x 10^4	NG	NG
L. rhamnosus A012 + enterotoxigenic *E. coli* H40B	3.2 x 10^6	9.1 x 10^3	NG	NG
P. pentosaceus A074+ enterotoxigenic *E. coli* H40B	6.2 x 10^6	9.7 x 10^3	NG	NG
W. cibaria B3a + enterotoxigenic *E. coli* H40B	2.5 x 10^5	1.02 x 10^4	NG	NG
L. pseudomesenteroides A044 + enterotoxigenic *E. coli* H40B	5.6 x 10^5	3.2 x 10^3	NG	NG
L. pseudomesenteroides A064 + enterotoxigenic *E. coli* H40B	3.1 x 10^5	1.55 x 10^4	4.1 x 10^3	1.0 x 10^2
L. plantarum B1c + enterotoxigenic *E. coli* H40B	1.1 x 10^6	3.01 x 10^4	8.0 x 10^2	NG
L. plantarum A1c+ enterotoxigenic *E. coli* H40B	1.3 x 10^5	1.2 x 10^4	2.2 x 10^3	NG
L. plantarum B3c+ enterotoxigenic *E. coli* H40B	1.7 x 10^6	1.9 x 10^4	2.0 x 10^3	5.0 x 10^2
L. plantarum A011+ enterotoxigenic *E. coli* H40B	3.1 x 10^5	8.2 x 10^3	NG	NG

Note: NG means no growth

Table 4.5. Cont.

	Incubation Time (Hour)			
	0	**8**	**16**	**24**
Organisms	**CFU/mL**	**CFU/mL**	**CFU/mL**	**CFU/mL**
Enteropathogenic *E. coli* H62E	3.2 x10^6	4.1 x10^6	6.1 x10^6	2.2 x10^7
L. pentosus A028 + enteropathogenic *E. coli* H62E	2.2 x 10^5	1.75 x 10^4	3.9 x 10^3	NG
L. plantarum A046 + enteropathogenic *E. coli* H62E	5.2 x 10^5	1.50 x 10^4	4.6 x 10^3	NG
L. plantarum A084 + enteropathogenic *E. coli* H62E	3.4 x 10^5	9.3 x 10^3	2.5 x 10^3	NG
L. pentosus B1b + enteropathogenic *E. coli* H62E	2.8 x 10^5	1.01 x 10^4	1.6 x 10^3	NG
L. plantarum A034 + enteropathogenic *E. coli* H62E	8.9 x 10^5	1.12 x 10^4	7.1 x 10^3	1.2 x 10^3
L. pentosus A4c + enteropathogenic *E. coli* H62E	6.7 x 10^5	1.02 x 10^4	1.2 x 10^3	NG
L. rhamnosus A072 + enteropathogenic *E. coli* H62E	1.0 x 10^6	9.8 x 10^3	NG	NG
L. rhamnosus A012 + enteropathogenic *E. coli* H62E	2.3 x 10^6	1.0 x 10^3	NG	NG
P. pentosaceus A074 + enteropathogenic *E. coli* H62E	1.7 x 10^6	1.0 x 10^4	1.0 x 10^3	NG
W. cibaria B3a + enteropathogenic *E. coli* H62E	1.8 x 10^6	1.34 x 10^4	2.0 x 10^2	NG
L. pseudomesenteroides A044 + enteropathogenic *E. coli* H62E	1.2 x 10^6	4.6 x 10^3	NG	NG
L. pseudomesenteroides A064 + enteropathogenic *E. coli* H62E	1.9 x 10^5	1.10 x 10^4	2.3 x 10^3	NG
L. plantarum B1c + enteropathogenic *E. coli* H62E	2.5 x 10^5	1.6 x 10^4	3.0 x 10^3	4.0 x 10^2
L. plantarum A1c+ enteropathogenic *E. coli* H62E	3.8 x 10^5	1.9 x 10^5	2.4 x 10^4	1.6 x 10^3
L. plantarum B3c + enteropathogenic *E. coli* H62E	4.0 x 10^5	2.1 x 10^4	NG	NG
L. plantarum A011+ enteropathogenic *E. coli* H62E	3.9 x 10^5	7.2 x 10^3	NG	NG
Shiga-toxin *E. coli* H77E	6.2 x10^6	9.1 x10^6	1.1 x10^7	8.6 x10^6
L. pentosus A028 + Shiga-toxin *E. coli* H77E	4.2 x 10^5	6.70 x 10^4	5.2 x 10^3	NG
L. plantarum A046+ Shiga-toxin *E. coli* H77E	6.2 x 10^5	1.10 x 10^4	4.2 x 10^3	NG

Note: NG means no growth

Table 4.5. cont.

	Incubation Time (Hour)			
	0	**8**	**16**	**24**
Organisms	**CFU/mL**	**CFU/mL**	**CFU/mL**	**CFU/mL**
L. plantarum A084 + Shiga-toxin *E. coli* H77E	4.4×10^5	3.5×10^3	2.2×10^3	NG
L. pentosus B1b + Shiga-toxin *E. coli* H77E	2.8×10^5	1.4×10^4	1.3×10^3	NG
L. plantarum A034 + Shiga-toxin *E. coli* H77E	2.6×10^5	1.5×10^3	NG	NG
L. pentosus A4c + Shiga-toxin *E. coli* H77E	4.1×10^5	4.4×10^3	NG	NG
L. rhamnosus A072 + Shiga-toxin *E. coli* H77E	2.0×10^6	1.22×10^4	NG	NG
L. rhamnosus A012+ Shiga-toxin *E. coli* H77E	3.2×10^6	1.62×10^4	NG	NG
P. pentosaceus A074 + Shiga-toxin *E. coli* H77E	6.0×10^6	7.1×10^3	NG	NG
W. cibaria B3a + Shiga-toxin *E. coli* H77E	2.6×10^5	1.12×10^4	5.0×10^3	NG
L. pseudomesenteroides A044 + Shiga-toxin *E. coli* H77E	7.6×10^5	5.2×10^3	NG	NG
L. pseudomesenteroides A064 + Shiga-toxin *E. coli* H77E	6.5×10^5	2.51×10^4	2.0×10^3	NG
L. plantarum B1c + Shiga-toxin *E. coli* H77E	6.6×10^5	3.2×10^4	5.1×10^3	7.0×10^2
L. plantarum A1c + Shiga-toxin *E. coli* H77E	1.4×10^6	1.1×10^4	2.2×10^3	NG
L. plantarum B3c+ Shiga-toxin *E. coli* H77E	2.01×10^6	2.2×10^4	NG	NG
L. plantarum 11+ Shiga-toxin *E. coli* H77E	2.1×10^6	8.6×10^3	NG	NG
Enteroaggregative *E. coli* H40C	5.6×10^6	6.9×10^6	1.3×10^6	2.1×10^7
L. pentosus A028 + enteroaggregative *E. coli* H40C	3.0×10^5	1.91×10^4	3.1×10^3	NG
L. plantarum A046 + enteroaggregative *E. coli* H40C	1.3×10^5	1.05×10^4	1.0×10^3	1.5×10^2
L. plantarum A084+ enteroaggregative *E. coli* H40C	4.6×10^5	5.2×10^3	2.3×10^3	NG

Note

NG means no growth

Table 4.5. cont.

	Incubation Time (Hour)			
	0	**8**	**16**	**24**
Organisms	**CFU/mL**	**CFU/mL**	**CFU/mL**	**CFU/mL**
L. pentosus B1b + enteroaggregative *E. coli* H40C	5.1×10^5	1.50×10^4	NG	NG
L. plantarum A034 + enteroaggregative *E. coli* H40C	6.5×10^5	1.26×10^4	5.1×10^3	1.2×10^3
L. pentosus A4c + nteroaggregative *E. coli* H40C	5.7×10^5	2.2×10^4	NG	NG
L. rhamnosus A072 + enteroaggregative *E. coli* H40C	5.6×10^5	1.50×10^4	NG	NG
L. rhamnosus A012 + enteroaggregative *E. coli* H40C	5.1×10^6	1.22×10^4	NG	NG
P. pentosaceus A074 + enteroaggregative *E. coli* H40C	6.5×10^5	4.7×10^3	1.0×10^3	NG
W. cibaria B3a + enteroaggregative *E. coli* H40C	2.0×10^6	1.02×10^4	NG	NG
L. pseudomesenteroides A044 + enteroaggregative *E. coli* H40C	1.5×10^6	4.1×10^3	NG	NG
L. pseudomesenteroides A064 + enteroaggregative *E. coli* H40C	7.1×10^5	1.25×10^4	6.2×10^3	2.0×10^2
L. plantarum B1c + enteroaggregative *E. coli* H40C	3.5×10^6	1.8×10^4	6.5×10^3	2.6×10^3
L. plantarum A1c + enteroaggregative *E. coli* H40C	2.1×10^6	2.1×10^4	1.7×10^3	NG
L. plantarum B3c + enteroaggregative *E. coli* H40C	1.51×10^6	1.16×10^4	2.6×10^3	6.0×10^2
L. plantarum 11 + enteroaggregative *E. coli* H40C	1.12×10^5	7.5×10^3	NG	NG

Note

NG means no growth

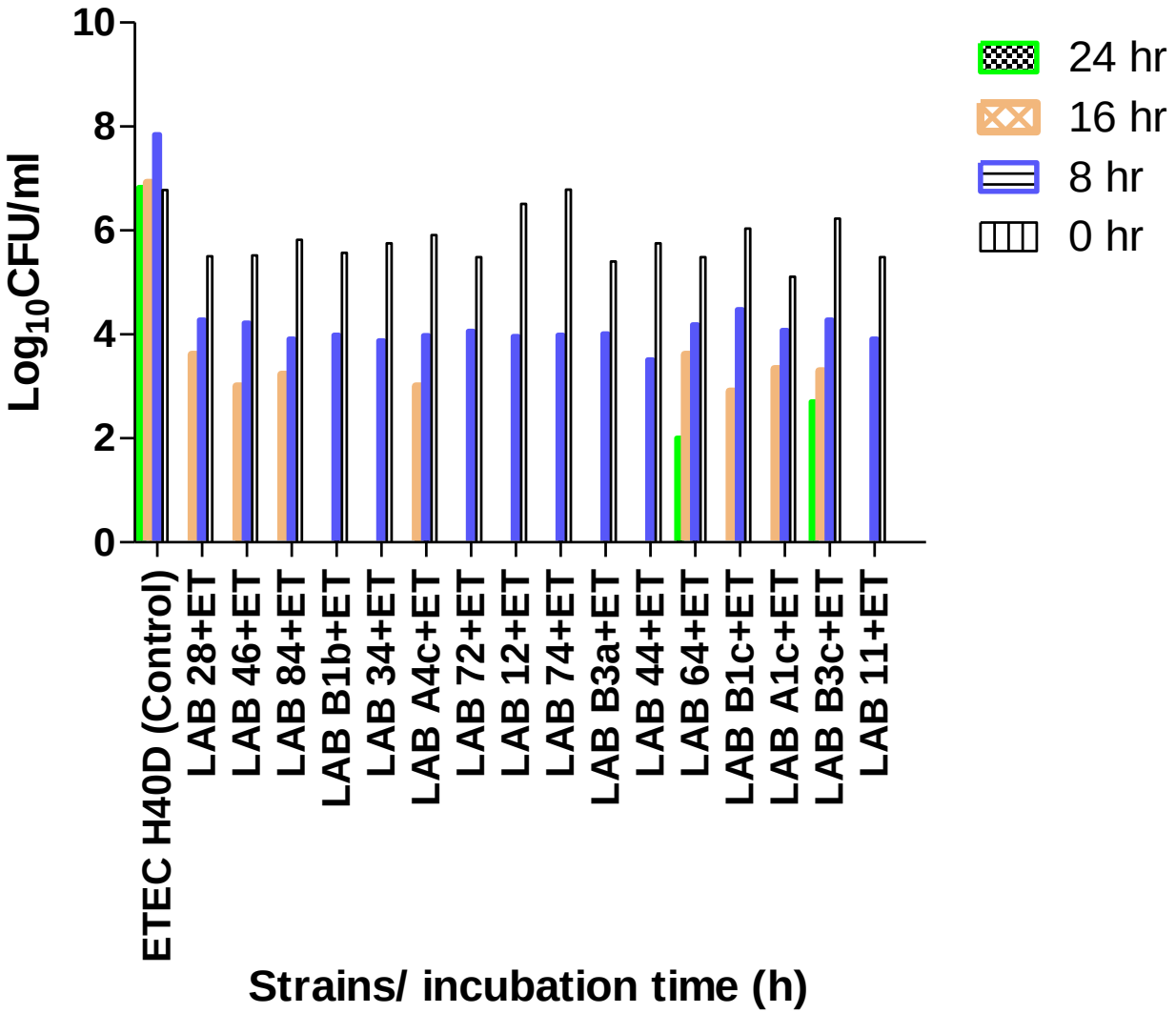

Figure 4.8. Longitudinal inhibition of enterotoxigenic *E. coli* H40D in a co culture with lactic acid bacteria strains.

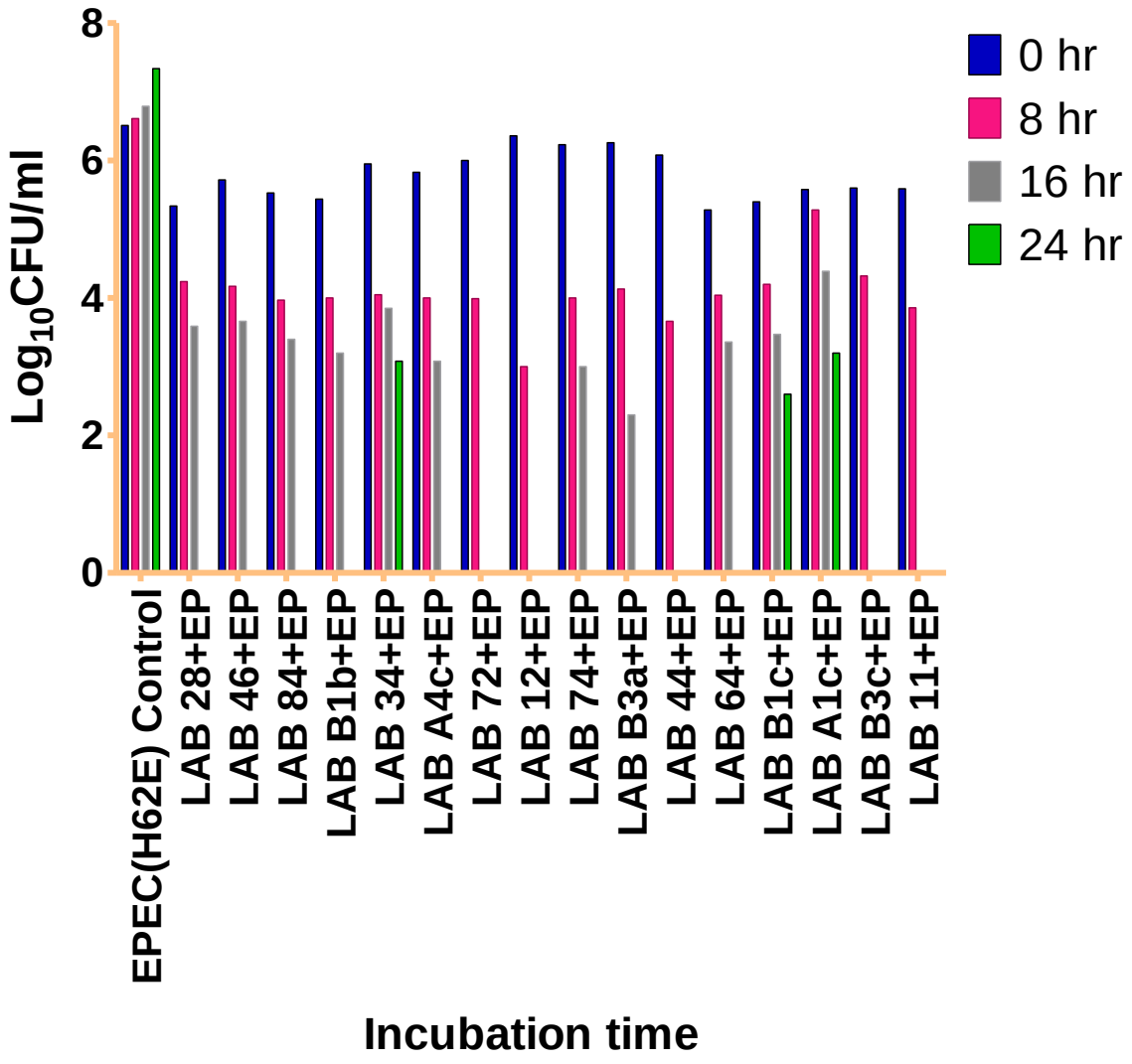

Figure 4.9. Longitudinal inhibition of enteropathogenic *E. coli* H62E in a co culture with lactic acid bacteria.

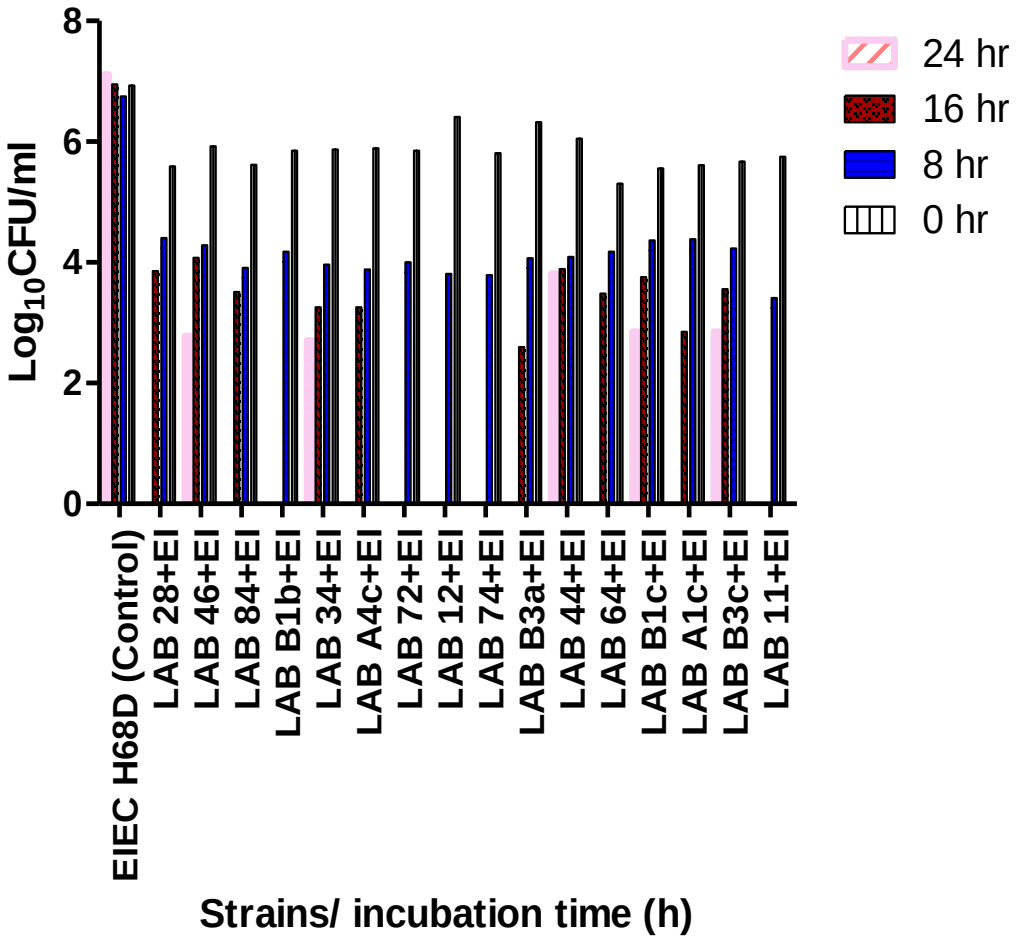

Figure 4.10. Longitudinal inhibition of enteroinvasive *E. coli* H68D in a co culture with lactic acid bacteria.

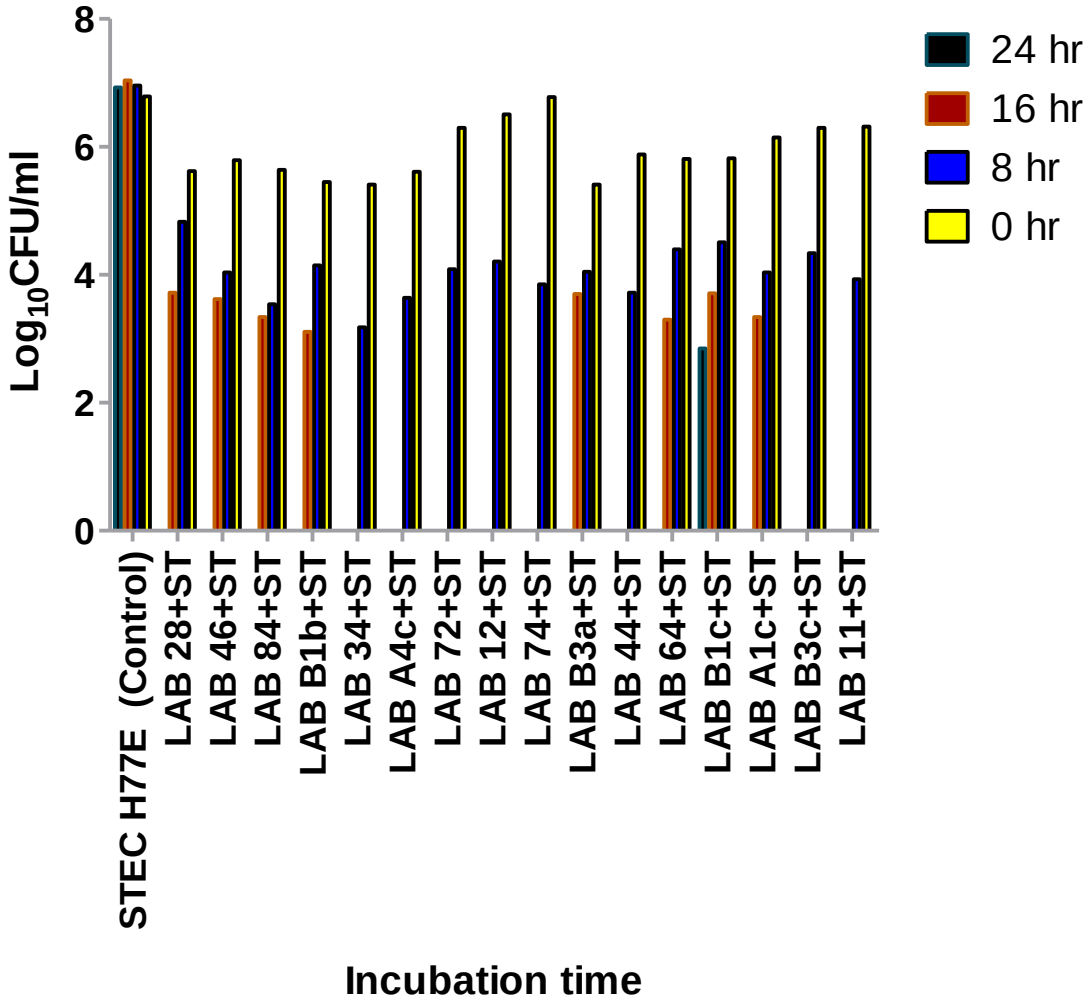

Figure 4.11. Longitudinal inhibition of shiga toxin *E. coli* strains in a co culture with lactic acid bacteria.

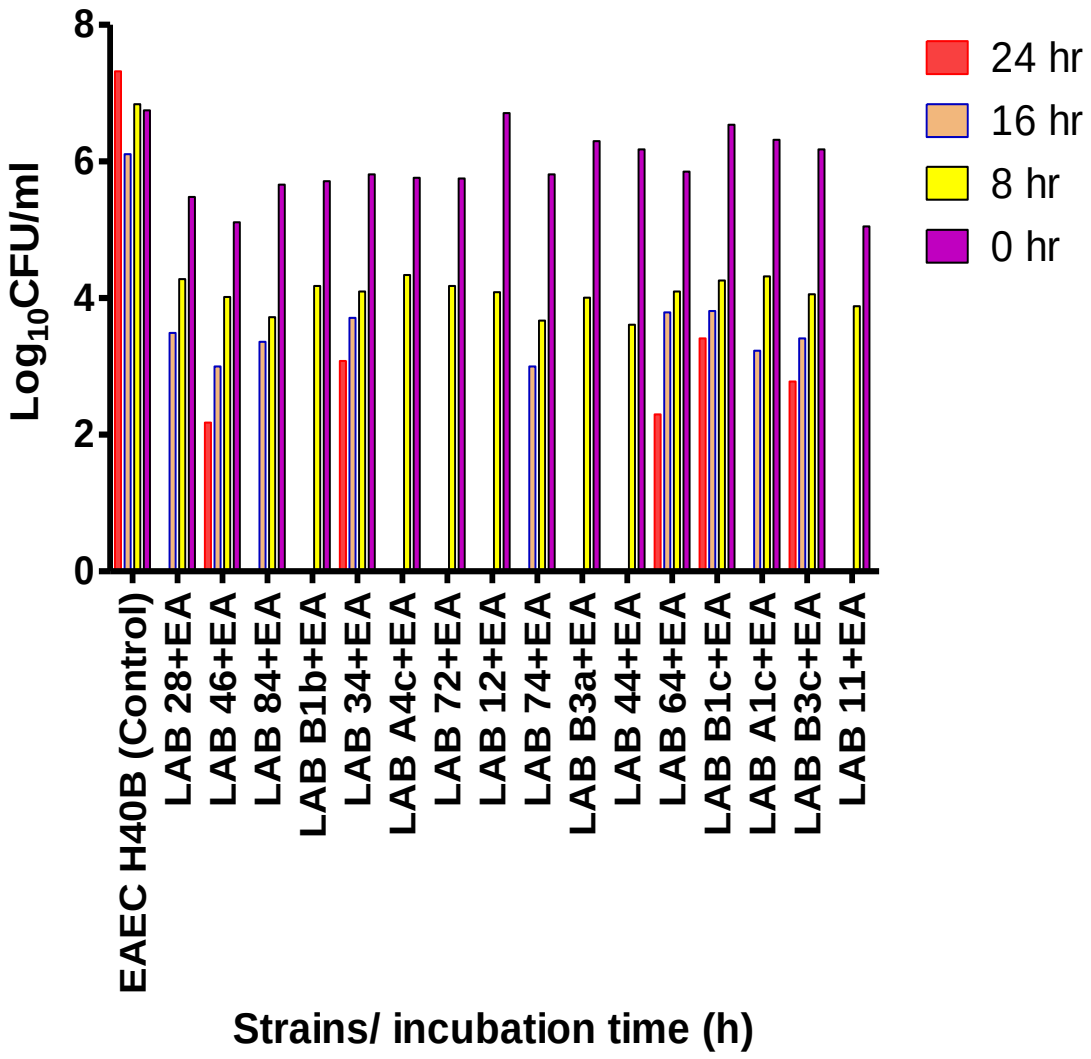

Figure 4.12. Longitudinal inhibition of enteroaggregative *E. coli* H40C strains in a co culture with lactic acid bacteria.

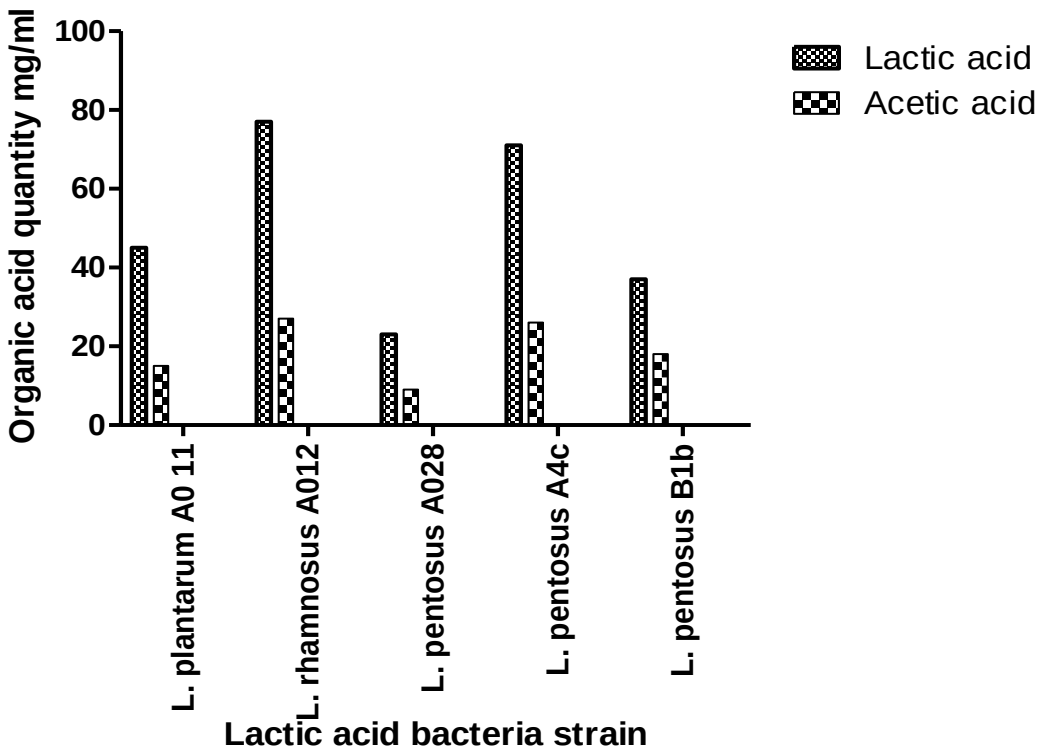

Figure 4.13. Quantity of organic acid produced by potential probiotic lactic acid bacteria

Table 4.6. Antibiofilm properties of lactic acid bacteria against enteroaggregative *E. coli* 042

Strains of LAB	**Percentage of biofilm inhibition (** 1 in 1 dilution)	**Percentage of biofilm inhibition** (1 in 9 dilution)	**Percentage of biofilm inhibition** (1 in 99 dilution)	**Percentage of growth inhibition**
L. pentosus B1b	-2.77	-1.11	87.95	21.31
L. plantarum A1c	-0.29	99.91	85.00	22.15
L. pentosus A4c	99.28	-2.22	78.59	22.57
W. cibaria B3a	-1.19	-5.55	75.64	22.29
L. plantarum A011	93.98	99.86	78.76	17.51
L. rhamnosus A012	99.46	-1.76	86.03	21.17
L. plantarum A014	-0.72	-2.73	78.42	17.79
L. pentosus A028	99.46	-1.99	68.63	23.91
L. plantarum A034	99.50	-1.20	89.87	28.13
L. pseudomesenteroides A044	-0.18	-0.97	83.50	24.75
L. plantarum A046	99.86	-0.83	78.46	24.40
L. pseudomesenteroides A064	98.95	-1.29	88.29	27.00
L. rhamnosus A072	-0.14	-2.08	79.96	24.82
P. pentosaceus A074	99.39	-2.40	76.32	24.89
L. plantarum A084	99.03	-2.59	86.32	26.86

4.3. Safety evaluation of isolated lactic acid bacteria

All the ninety-three strains of LAB identified were evaluated for their ability to haemolyse human red blood cells. None of the LAB strains lysed the red blood cells, as there was no evidence of any clear zone around the colonies. The MIC of 15 selected LAB against selected antibiotics was carried out. According to EFSA (2018) breakpoint, 80 % of the LAB tested were resistant to ampicillin while only 20 % were susceptible to the antibiotics; the susceptible strains include *L. rhamnosus* A012, *L. pentosus* A028 and *P. pentosaceus*. The entire LAB tested were susceptible to gentamicin (100%), however, 73.3 % of the LAB strains were susceptible to tetracycline while the remaining 26.7 % (*P. pentosaceus* A074, *L. pseudomesenteroides* A064, *L. pseudomesenteroides* A044, and *W. cibaria* B3a) were resistant to the antibiotics. On the other hand, 33.3 % of the selected LAB (*L. plantarum* A046, *L. plantarum* A034, *L. plantarum* A1c, *W. cibaria* B3a, and *L. rhamnosus* A072) were resistant to erythromycin while 66.7 % were susceptible to the antibiotics (Table 4.7). *L. rhamnosus* A072 (256 µg/ml), *L. plantarum* A034 (128 µg/ml), and *L. pentosus* A028 (128 µg/ml) were resistant to nitrofurantoin antibiotic. Rifampicin were sensitive at 1 µg/ml, therefore, *P. pentosaceus* A074 (1 µg/ml) was sensitive and *L. rhamnosus* A012 was intermediate at 2 µg/ml while the remaining LAB were resistant to rifampicin antibiotic base on Vitek 2 system interpretation of AST result. Other antibiotics used in the study have no recommended breakpoint because according to EFSA (2018) they are not required (n.r). Tested lactic acid bacteria strains were also susceptible to cefoxitin inducible clindamycin resistance except *W. cibaria* AB3a and *L. pseudomesenteroides* A044. Meanwhile, *L. rhamnosus* A012 was susceptible to all the 21 antibiotics tested (Table 4.7).

Table 4.7. Antibiotic Susceptibility of potential probiotic lactic acid bacteria

Lactic aci bacteria strains	Cef oxit in Scr een	Am picill in (bp= 2 &4)	Ox acil lin	Genta micin High Level (syner gy)	Strepto mycin High Level (syner gy)	Gent amic in (bp= 16)	Cipr oflo xaci n (n.r)	Levo floxa cin	Moxi floxa cin	Induc ible Clind amyc in Resis tance	Eryth romy cin (bp= 1)	Clind amyc in (bp= 1 & 4)	Line zoli d	Dapt omyc in	Vanc omyc in (n.r)	Doxy cycli ne	Tetra cycli ne (bp= 8 & 32	Tige cycli ne	Nutrof uranto in	Rifa mpic in	Trimeth oprim/ Sulfam ethoxaz ole
L. plantarum A011	posi tive	16	S	S	S	≤ 0.5	≥8	≥8	2	NEG	≤0.2 5	<=0. 12		≥8	≥32	4	>=16	0.25	<=16	16	<=10
L. plantarum A034	posi tive	≥32	≥4	S	S	≤ 0.5	≥8	4	2	NEG	4	≥4		2	≥32	2	>=16		128	8	80
L. plantarum A046	posi tive	≥16	≥4	S	S	≤ 0.5	≥8	4	2	NEG	≥8	≥4		8	≥32	8	>=16	0.5	32	16	<=10
L. plantarum A084	posi tive	8	≥4	S	S	≤ 0.5	≥8	≥8	4	NEG	0.5	0.5		≥8	≥32	4	>=16	0.12	32	8	<=10
L. plantarum A1c	posi tive	≥32	≥4	S	S	≤ 0.5	≥8	≥8	4	NEG	≥8	0.5		≥8	≥32	4	>=16		≤ 16	≥32	80
L.xianqfan gensis B1a2	posi tive	8	≥4	R	S	≤ 0.5	≥8	≥8	2	NEG	≤0.2 5	≥4	2	≥8	≥32	2	>=16		>=16		
L. rhamnosus A012	posi tive	4	TR M	S	S	≤ 0.5	2	4	2	NEG	≤0.2 5	≤0.1 2	2	TR M	≥32	TR M	2	TR M	126	2	20
L. rhamnosus A072	posi tive	≥32	≥4	S	S	≤ 0.5	4	4	2	NEG	≥8	≥4		>8	≥32	TR M	TRM	0.5	256	≥32	320

Keys: S- sensitive, R- resistance

Table 4.7. cont'd

Lactic aci bacteria strains	Cef oxit in Scr een	Am picill in (bp= 2 &4)	Ox acil lin	Genta micin High Level (syner gy)	Strepto mycin High Level (syner gy)	Gent amic in (bp= 16)	Cipr oflo xaci n (n.r)	Levo floxa cin	Moxi floxa cin	Induc ible Clind amyc in Resis tance	Eryth romy cin (bp= 1)	Clind amyc in (bp= 1 & 4)	Line zoli d	Dapt omyc in	Vanc omyc in (n.r)	Doxy cycli ne	Tetra cycli ne (bp= 8 & 32	Tige cycli ne	Nutrof uranto in	Rifa mpic in	Trimeth oprim/ Sulfam ethoxaz ole
L. pentosus A028	posi tive	≤2	≥4	S	S	1	1	4	2	NEG	0.5	≥4	2	0.25	≤0.5	≤0.5	≤1	≤0.1 2	128	≥32	<=10
L. pentosus A4c	posi tive	8	TR M	S	S	≤ 0.5	≥8	≥8	2	NEG	≤0.2 5	≤0.1 2	4	TRM	≥32	TR M	>=16	TR M	≤16	16	<=10
L. pentosus B1b	posi tive	8	TR M		S	≤ 0.5	≥8	≥8	2	NEG	≤0.2 5	≤0.1 2	4	TRM	≥32	TR M	>=16	TR M	≤16	8	<=10
L.pseudom ensenteroi des A044	posi tive	16	4	S	S	≤ 0.5	≥8	≥8	2	POS	≤0.2 5	0.5		≥8	≥32	≥8	>=16		32	8	≤10
L.pseudom ensenteroi des A064	posi tive	16	TR M	S	S	≤ 0.5	≥8	≥8	2	NEG	≤0.2 5	≤0.1 2		TRM	≥32	TR M	>=16	TR M	32	8	≤10
P.pentosac eus A074	posi tive	≤2	TR M	SYN-S	S	≤ 0.5	4	4	2	NEG	≤0.2 5	≤0.1 2	4	TRM	≥32	TR M	>=16	TR M	64	1	≤10
W.cibaria B3a	posi tive	8	≥4	S	S	≤ 0.5	≥8	4	2	POS	≥8	≤0.1 2	4	≥8	≥32	2	>=16	≤ 0.12	≤16	8	<=10

Key: S – sensitive, R – resistance, (n.r) – not required.

Note: The breakpoint was adapted from the European Food Safety Authority (EFSA). 2018. 16.3: 5206. Any MIC that is greater than the breakpoint is considered to be resistant. According to EFSA, (2018), the cut off values of lactic acid bacteria to antibiotics are varried, the cut-off values for each LAB specie is therefore used to check their susceptibility or resistance to different antibiotics in the table.

4.4. Survival of lactic acid bacterial strains in simulated gastrointestinal condition

The ability of 93 LAB strains to resist acidic growth condition of pH 3.0 and 2.0 was assessed. The isolated LAB showed varying resistance. At pH 3, all the LAB strains isolated survived the condition, 54.54% of the strains survived with 1 $\log_{10}$ reduction while 45.46% of the strains survived the acidic condition with no $\log_{10}$ reduction respectively. At pH 2, 53 LAB strains (57.0%) survived the acidic condition while the remaining 40 LAB strains (43.01%) were totally killed . Out of the 57.0% LAB that survived; only 4.3% survived at 1 $\log_{10}$ reduction, while 19.35% survived at 2 $\log_{10}$ reduction, 26.88% survived at 3 $\log_{10}$ reduction and 6.45% survived at 4 $\log_{10}$ reduction (Table 4.8). At 0.3% of bile concentration, 88.17% of LAB strains survived the condition while 11.83% of LAB strains were unable to survive the condition respectively. Out of these 88.17% of LAB that survived; 53.7% survived with no $\log_{10}$ reduction, 31.18% of LAB strains survived with 1 $\log_{10}$ reduction and 3.22% of the LAB strains survived with 2 $\log_{10}$ reduction (Table 4.8). The exceptions were *L. plantarum* A023, *E. thailandicus* A027, *L. plantarum* A051, *E. durans* A098, *E. durans* A095, *E. faecium* A066, *E. faecium* A087 and *L. pseudomesenteroides* A089 which were unable to survive the 0.3% bile condition (Table 4.8). However, *L. plantarum* A011, *L. rhamnosus* A012, *L. rhamnosus* A072 and *P. pentosaces* A074 were among the strains that survived the high condition of acid and bile environment (Table 4.8).

The ability of 20 LAB to resist consecutive acid and bile conditions were reported. *L. plantarum* A011, *L. rhamnosus* A072, *L. plantarum* A046, *L. pentosus* A028, *P. pentosaceus* A074, *L. pseudomesenteroides* A084, *W. cibaria* B3a demonstrated the highest resistance to consecutive low pH of 3 and 0.3% bile supplementation with no $\log_{10}$ reduction in cfu/ml (Table 4.9). However, the viability of *L. rhamnosus* A012, *L. plantarum* A1c, *L. pentosus* A4c, *L. pseudomesenteroides* A044 and *L. pseudomesenteroides* A064 at consecutive pH 3 and 0.3% bile reduced with only 1 $\log_{10}$.. The viability of *L. rhamnosus* A012 reduced from 1.22 x 10^8 to 9.7 x 10^7 CFU/ml showing one $\log_{10}$ reduction, *L. plantarum* A014 viable cells reduced from 9.8 x 10^8 CFU/ml to 1.3 x 10^7 CFU/ml; 1 log reduction, *L. plantarum* A034 viable cells reduced from 1.94 x 10^8 to 7.4 x 10^7 CFU/ml. However, *E. faecium* A080 viable cells reduced from 1.21 x 10^8 CFU/ml to 5.0 x 10^6 CFU/ml and *L. pseudomesenteroides* A082 viable

cells reduced from 2.65 x 10^8 CFU/ml to 6.0 x 10^6 CFU/ml, showing the two LAB strains had 2 $\log_{10}$ reductions in their viable cells (Table 4.9).

Table 4.8. Survival of lactic acid bacteria in acidic medium and in bile condition

LAB Isolate	pH 3.0 (CFU/ml)			pH 2 (CFU/ml)			0.3% Bile (CFU/ml)		
	Control	Initial	Final	Control	Initial	Final	Control	Initial	Final
*L. platarum*A002	2.4×10^8	2.08×10^8	9.7×10^7	2.7×10^8	7.8×10^7	8.0×10^4	2.66×10^8	2.6×10^8	2.74×10^8
E. faecium A003	2.1×10^8	1.5×10^8	2.0×10^7	2.0×10^8	1.04×10^8	1.2×10^5	1.5×10^8	1.3×10^8	2.5×10^7
E. faecium A008	3.0×10^8	1.09×10^8	1.9×10^7	2.3×10^8	1.12×10^8	1.8×10^5	1.09×10^8	1.0×10^8	1.2×10^7
L. pseudomenseteroides A010	1.9×10^9	8.1×10^8	5.0×10^8	1.6×10^9	2.12×10^8	1.01×10^6	2.19×10^8	2.1×10^8	1.60×10^8
*L. plantarum*A011	3.3×10^8	2.57×10^8	1.94×10^8	2.3×10^8	1.15×10^8	1.8×10^5	2.08×10^8	1.1×10^8	1.6×10^8
L. rhamnosus A012	1.9×10^9	8.4×10^8	5.5×10^7	1.9×10^9	1.75×10^8	8.0×10^4	3.06×10^8	2.2×10^8	2.99×10^8
*E. durans*A013	3.2×10^8	5.8×10^7	1.4×10^7	2.2×10^8	2.0×10^8	0	1.52×10^8	1.3×10^8	6.7×10^7
L. plantarum A014	1.3×10^9	2.48×10^8	2.45×10^8	1.1×10^9	1.82×10^8	4.1×10^5	2.77×10^8	2.5×10^8	2.61×10^8
L. pentosus A016	5.4×10^8	3.12×10^8	2.93×10^8	1.4×10^8	1.01×10^8	1.01×10^6	1.65×10^8	1.6×10^8	2.22×10^8
L. paracasei A017	2.8×10^8	2.55×10^8	2.16×10^8	1.8×10^8	9.8×10^7	1.0×10^5	2.86×10^8	2.7×10^8	2.16×10^8
E. faecium A018	1.5×10^9	2.5×10^8	1.7×10^7	1.5×10^8	1.04×10^8	3.5×10^5	1.13×10^8	1.1×10^8	8.5×10^7
L. pseudomenseteroides A019	2.6×10^8	1.41×10^8	9.7×10^7	1.6×10^8	1.09×10^8	1.0×10^5	8.6×10^7	3.5×10^7	1.0×10^7
E. faecium A022	1.6×10^9	4.4×10^8	1.07×10^8	1.2×10^9	1.51×10^8	0	2.03×10^8	2.0×10^8	1.02×10^8
L. plantarum A023	2.1×10^8	1.51×10^8	4.5×10^8	4.1×10^8	1.28×10^8	5.0×10^4	1.51×10^8	1.1×10^8	0
E. durans A024	2.0×10^9	4.0×10^8	2.33×10^8	1.0×10^9	1.1×10^8	0	1.44×10^8	1.5×10^8	7.7×10^7
L. pseudomenseteroides A026	3.2×10^8	2.38×10^8	1.52×10^8	1.2×10^9	8.8×10^8	0	2.38×10^8	2.2×10^8	1.12×10^8
E. thailandicus A027	2.4×10^8	1.06×10^8	6.5×10^7	1.4×10^8	1.2×10^7	3.8×10^5	1.06×10^8	9.7×10^7	0
L. pentosus A028	1.7×10^9	2.06×10^8	1.78×10^8	1.3×10^9	1.62×10^8	1.19×10^6	3.01×10^8	3.0×10^8	2.60×10^8
L. pseudomenseteroides A030	7.3×10^8	1.66×10^8	1.4×10^7	2.8×10^8	2.31×10^8	7.0×10^4	1.42×10^8	1.3×10^8	1.02×10^8
E. thailandicus A031	1.9×10^9	1.52×10^8	6.5×10^7	1.7×10^8	1.13×10^8	0	1.52×10^8	1.2×10^8	8.4×10^8
L. plantarum A033	5.4×10^8	1.52×10^8	3.2×10^7	7.3×10^8	1.55×10^8	0	1.52×10^8	1.1×10^8	5.0×10^6
L. plantarum A034	3.7×10^8	1.89×10^8	1.0×10^7	2.5×10^8	1.89×10^8	3.0×10^4	2.73×10^8	1.9×10^8	1.85×10^8
L. plantarum A035	3.3×10^8	2.34×10^8	1.51×10^8	3.1×10^8	1.22×10^8	0	3.02×10^8	2.9×10^8	1.86×10^8
L. plantarum A036	4.0×10^8	8.2×10^8	6.9×10^8	2.4×10^8	1.21×10^8	6.0×10^5	2.28×10^8	1.8×10^8	2.21×10^8
L. plantarum A037	1.6×10^9	2.05×10^8	1.98×10^8	2.6×10^8	9.8×10^7	2.3×10^5	3.28×10^8	2.1×10^8	2.31×10^8

Table 4.8. cont.

LAB Isolate	pH 3.0 (CFU/ml)			pH 2 (CFU/ml)			0.3% Bile (CFU/ml)		
	Control	Initial	Final	Control	Initial	Final	Control	Initial	Final
E durans A038	5.0×10^8	3.62×10^8	2.27×10^8	3.0×10^8	1.4×10^8	0	1.62×10^8	1.1×10^8	2.59×10^8
E. faecium A039	2.7×10^8	1.38×10^8	6.2×10^7	1.5×10^8	6.9×10^7	8.0×10^4	2.18×10^8	2.2×10^8	3.02×10^8
E. faecium A040	1.3×10^9	1.05×10^8	0	1.3×10^9	3.1×10^7	0	1.03×10^8	1.0×10^8	7.8×10^7
L. plantarum A041	6.1×10^8	3.7×10^8	2.03×10^8	6.1×10^8	8.1×10^7	0	2.88×10^8	1.0×10^8	2.05×10^8
E. durans A043	2.1×10^8	1.05×10^8	1.0×10^7	1.1×10^9	2.06×10^8	0	1.05×10^8	1.0×10^8	6.5×10^7
L. pseudomenseteroides A044	1.6×10^8	1.55×10^8	1.8×10^7	1.8×10^8	1.36×10^8	3.0×10^4	2.20×10^8	2.1×10^8	2.47×10^8
L. plantarum A046	2.3×10^8	1.83×10^8	1.25×10^8	1.7×10^8	1.51×10^8	1.5×10^5	2.18×10^8	2.0×10^8	1.11×10^8
L. plantarum A047	3.4×10^8	2.89×10^8	2.37×10^8	1.4×10^8	1.07×10^7	2.2×10^5	2.77×10^8	2.6×10^8	2.57×10^8
L. plantarum A048	4.2×10^8	1.92×10^8	1.56×10^8	4.9×10^8	3.09×10^8	3.9×10^5	1.87×10^8	1.0×10^8	1.05×10^8
E. faecium A049	5.1×10^8	1.81×10^8	1.05×10^8	6.2×10^8	1.2` $\times 10^8$	0	1.81×10^8	1.3×10^8	0
E. durans A050	2.2×10^8	4.8×10^8	1.6×10^7	1.8×10^8	1.11×10^8	0	1.15×10^8	1.0×10^8	1.3×10^7
L. plantarum A051	2.7×10^8	9.8×10^8	1.4X $\times 10^7$	1.7×10^8	1.1×10^8	0	9.8×10^7	3.1×10^7	0
E. lactis A052	1.2×10^9	2.7×10^8	1.74×10^8	4.2×10^8	1.21×10^8	2.2×10^5	1.22×10^8	1.4×10^8	8.3×10^7
E. faecalis A058	1.8×10^8	1.05×10^8	2.5×10^7	1.6×10^9	2.9×10^8	0	1.88×10^8	1.4×10^8	1.01×10^8
L. plantarum A059	1.9×10^9	1.92×10^8	1.55×10^8	3.4×10^8	1.91×10^8	0	1.92×10^8	2.0×10^8	1.05×10^8
E. lactis A060	1.1×10^9	1.72×10^8	8.5×10^7	1.0×10^9	2.06×10^8	0	1.25×10^8	1.1×10^8	3.2×10^7
L. pseudomenseteroides A064	2.9×10^9	5.4×10^8	3.2×10^8	3.0×10^8	2.58×10^8	3.2×10^5	2.48×10^8	2.2×10^8	2.46×10^8
E. faecium A066	2.4×10^8	1.85×10^8	1.5×10^7	2.9×10^8	2.1×10^8	2.0×10^5	1.85×10^8	1.9×10^8	2.8×10^7
L. plantarum A071	3.4×10^8	1.68×10^8	1.21×10^8	2.2×10^8	9.4×10^7	0	1.68×10^8	1.5×10^8	8.5×10^7
L. rhamnosus A072	3.5×10^8	3.22×10^8	2.76×10^8	1.6×10^8	1.42×10^8	1.96×10^6	2.93×10^8	2.6×10^8	2.53×10^8
P. pentosaceus A074	1.6×10^8	2.84×10^8	2.52×10^8	1.3×10^8	2.5×10^7	1.3×10^5	3.08×10^8	3.1×10^8	2.98×10^8
L. plantarum A075	1.5×10^8	1.36×10^8	2.3×10^7	2.1×10^8	1.9×10^8	3.0×10^4	1.36×10^8	1.4×10^8	1.21×10^8
E. faecalis A077	1.1×10^9	2.55×10^8	2.2×10^7	3.3×10^9	1.62×10^8	0	1.16×10^9	1.5×10^8	8.1×10^8
L. plantarum A079	2.3×10^8	1.88×10^8	1.08×10^8	3.3×10^8	2.99×10^8	7.5×10^5	3.05×10^8	2.4×10^8	3.9×10^7
E. faecium A080	1.2×10^9	3.2×10^8	1.11×10^8	2.6×10^9	1.0×10^8	2.1×10^5	1.01×10^8	1.3×10^8	2.3×10^7
E. lactis A081	4.4×10^8	3.22×10^8	9.5×10^7	2.4×10^8	1.78×10^8	0	1.33×10^8	1.2×10^8	8.1×10^7

Table 4.8. cont.

LAB Isolate	pH 3.0 (CFU/ml)			pH 2 (CFU/ml)			0.3% Bile (CFU/ml)		
	Control	Initial	Final	Control	Initial	Final	Control	Initial	Final
L. pseudomenseteroides A082	3.5×10^8	2.33×10^8	4.1×10^7	2.7×10^8	1.81×10^8	2.2×10^5	9.7×10^7	3.9×10^7	3.3×10^7
E. faecium A083	2.1×10^8	1.81×10^8	6.1×10^7	1.1×10^8	8.9×10^7	8.0×10^4	1.55×10^8	1.5×10^8	9.1×10^7
L. plantarum A084	1.1×10^9	6.2×10^8	4.0×10^8	3.1×10^8	2.78×10^8	4.4×10^5	2.25×10^8	1.0×10^8	6.1×10^7
E. faecium A087	3.3×10^8	9.5×10^7	1.4×10^7	3.3×10^8	1.5×10^8	0	1.02×10^8	1.0×10^8	4.1×10^7
L pseudomenseteroides A093	3.9×10^8	2.10×10^8	1.22×10^8	2.7×10^8	9.8×10^7	3.8×10^5	2.03×10^8	1.2×10^8	2.0×10^7
E. faecium A090	2.6×10^8	1.08×10^8	1.2×10^7	2.1×10^8	6.3×10^7	0	1.08×10^8	2.6×10^7	0
L. pseudomenseteroides A093	2.01×10^8	1.75×10^8	1.4×10^7	3.0×10^8	7.9×10^7	0	7.9×10^7	1.2×10^7	5.3×10^7
L. plantarum A094	2.2×10^8	1.52×10^8	1.0×10^7	2.4×10^8	1.91×10^8	2.1×10^5	1.52×10^8	1.4×10^8	8.0×10^6
E. durans A095	2.5×10^8	1.04×10^8	1.01×10^8	2.2×10^8	2.11×10^8	2.1×10^5	3.07×10^8	2.9×10^8	1.78×10^8
E. lactis A096	1.7×10^8	1.55×10^8	8.5×10^7	3.1×10^8	1.22×10^8	0	1.55×10^8	1.4×10^8	1.4×10^7
E. durans A097	1.1×10^9	5.0×10^8	3.5×10^7	2.0×10^8	1.2×10^8	3.1×10^5	1.71×10^8	1.6×10^8	1.04×10^8
E. durans A098	2.6×10^8	1.03×10^8	1.3×10^7	2.9×10^8	2.5×10^8	0	1.06×10^8	2.8×10^7	0
E. faecium A4D	1.9×10^8	1.15×10^8	1.01×10^8	3.7×10^8	0	0	1.15×10^8	1.0×10^8	1.02×10^8
W. cibaria B3b	1.3×10^9	2.9×10^8	1.2×10^8	2.8×10^9	2.3×10^8	0	2.90×10^8	1.5×10^8	3.6×10^7
L. pentosus A1d	3.5×10^8	1.83×10^8	7.6×10^7	2.5×10^8	1.8×10^8	2.1×10^5	1.83×10^8	1.7×10^8	1.05×10^8
W. cibaria B3a	1.6×10^8	1.31×10^8	1.02×10^8	4.6×10^8	1.91×10^8	2.6×10^5	1.31×10^8	1.3×10^8	1.26×10^8
L. fermentum A3b	1.5×10^8	1.05×10^8	6.2×10^7	1.1×10^8	0	0	1.25×10^8	1.1×10^7	0
L. plantarum B3c	3.1×10^8	1.5×10^8	4.8×10^7	2.6×10^8	1.85×10^8	0	1.5×10^8	1.4×10^8	1.42×10^8
L. plantarum A4b	3.9×10^8	2.65×10^8	6.4×10^7	1.7×10^8	8.6×10^7	1.1×10^5	2.65×10^8	2.3×10^8	1.81×10^8
L. plantarum B1b2	4.2×10^8	2.32×10^8	1.8×10^7	6.1×10^8	3.1×10^8	1.7×10^5	2.32×10^8	2.2×10^8	1.86×10^8
L. plantarum A1c	3.8×10^8	9.5×10^7	3.5×10^7	3.4×10^9	2.9×10^9	1.0×10^6	1.15×10^8	1.0×10^8	9.8×10^7
W. cibaria B4a	1.2×10^9	1.87×10^8	1.72×10^8	2.5×10^9	1.54×10^8	2.2×10^5	1.87×10^8	1.4×10^8	0
L. pentosus A4f	3.7×10^8	1.15×10^8	4.2×10^7	1.6×10^8	1.18×10^8	0	1.15×10^8	1.1×10^8	1.06×10^8
W. cibaria B1e	3.2×10^8	1.65×10^8	1.5×10^7	5.5×10^8	1.7×10^8	0	1.65×10^8	1.5×10^8	0
L. fermentum A3a	1.9×10^8	1.17×10^8	1.07×10^8	5.1×10^8	1.1×10^8	0	1.17×10^8	9.9×10^7	0
W. cibaria B4c	1.5×10^8	1.01×10^8	6.5×10^7	2.7×10^8	1.81×10^8	0	1.01×10^8	1.1×10^8	1.2×10^7

Table 4.8. cont.

LAB Isolate	pH 3.0 (CFU/ml)			pH 2 (CFU/ml)			0.3% Bile (CFU/ml)		
	Control	Initial	Final	Control	Initial	Final	Control	Initial	Final
L. pentosus A4c	2.8×10^8	1.2×10^8	1.16×10^8	3.1×10^8	1.52×10^8	7.1×10^5	1.21×10^8	1.1×10^8	1.03×10^8
W. confuse B1d	3.6×10^8	1.65×10^8	1.2×10^7	2.7×10^8	2.3×10^8	0	1.65×10^8	1.3×10^8	3.8×10^7
L. pentosus A1a	4.2×10^8	2.26×10^8	3.2×10^7	1.1×10^9	1.42×10^8	3.0×10^5	2.26×10^8	2.1×10^8	1.85×10^8
L. plantarum B1f	3.1×10^8	2.32×10^8	1.18×10^8	2.8×10^8	1.62×10^8	0	2.32×10^8	2.0×10^8	1.06×10^8
L. fermentum A3d	2.5×10^8	1.66×10^8	5.2×10^7	1.9×10^8	1.1×10^8	3.3×10^5	1.66×10^8	1.4×10^8	0
L. plantarum B1c	2.9×10^8	2.48×10^8	2.46×10^8	3.1×10^8	1.1×10^8	0	2.48×10^8	2.3×10^8	2.03×10^8
L. fermentum A3c	3.4×10^8	1.61×10^8	1.42×10^8	1.5×10^8	1.31×10^8	6.1×10^5	1.61×10^8	315×10^8	9.5×10^7
L. pentosus B1b	4.1×10^8	2.52×10^8	2.34×10^8	3.0×10^8	2.0×10^8	7.7×10^5	2.52×10^8	2.6×10^8	2.08×10^8
L. pentosus A1e	3.8×10^8	2.08×10^8	4.5×10^7	1.3×10^8	$5. \times 10^7$	0	2.06×10^8	1.9×10^8	1.81×10^8
L. plantarum B1a	3.5×10^8	1.88×10^8	1.76×10^8	1.2×10^9	1.11×10^8	5.5×10^5	1.88×10^8	1.5×10^8	1.69×10^8
L. xianqfangensis B1a2	5.2×10^8	2.69×10^8	2.27×10^8	1.5×10^9	2.91×10^8	2.3×10^5	2.69×10^8	2.6×10^8	2.6×10^8
E. durans A004	3.0×10^8	1.78×10^8	1.05×10^8	1.1×10^8	0	0	1.78×10^8	1.7×10^8	8.2×10^7
E. faecalis A020	3.1×10^8	2.35×10^8	1.08×10^8	1.7×10^8	1.66×10^8	9.0×10^4	2.35×10^8	1.6×10^8	8.5×10^7
E. faecalis A029	2.5×10^8	1.05×10^8	1.21×10^8	1.2×10^8	3.5×10^7	0	1.05×10^8	1.2×10^8	9.8×10^7
E. faecalis A063	3.1×10^8	1.05×10^8	1.6×10^7	2.0×10^8	1.5×10^8	0	1.05×10^8	1.0×10^8	8.0×10^6
E. faecalisA078	5.2×10^8	3.12×10^8	2.65×10^8	3.3×10^8	2.8×10^7	0	2.63×10^8	2.1×10^8	5.0×10^6

Table 4.9. Survival of lactic acid bacteria in consecutive low pH and bile supplementation

Lactic acid bacteria strains	pH (3 hours contact)			0.3% (3 hours contact)		
	Initial	Final	Log reduction	Initial	Final	Log reduction
L. plantarum A011	2.05 x10^8	1.8 x10^8	Nil	1.6 x10^8	1.13x10^8	nil
L. rhamnosus A012	1.22 x10^8	9.2 x10^7	1 log	9.9 x10^8	9.7 x10^7	nil
L. plantarum A014	9.8 x10^8	4.2 x10^8	Nil	1.1 x10^8	1.3 x10^7	1 log
L. pentosus A028	2.83 x10^8	1.9 x10^8	Nil	1.91 x10^8	1.42x10^8	nil
E. faecalis A029	2.57 x10^8	1.1 x10^8	Nil	1.6 x10^8	1.5 x10^7	nil
L. plantarum A034	1.94 x10^8	9.1 x10^7	1 log	9.5 x10^7	7.4 x10^7	nil
L. pseudomenseteroides A044	3.16 x10^8	5.4 x10^7	1 log	9.7 x10^7	8.8 x10^7	nil
L. plantarum A046	2.58 x10^8	2.0 x10^8	Nil	2.1 x10^8	2.19 x10^8	nil
E. lactis A052	2.06 x10^8	1.6 x10^8	Nil	1.4 x10^8	2.3 x10^7	nil
L. plantarum A059	1.81 x10^8	1.3 x10^8	Nil	1.2 x10^8	4.2 x10^7	nil
L. pseudomenseteroides A064	1.87 x10^8	1.3 x10^8	Nil	1.4 x10^8	6.6 x10^7	1 log
L. rhamnosus A072	2.91 x10^8	2.1 x10^8	Nil	2.3 x10^8	1.44 x10^8	nil
P. pentosaceus A074	1.55 x10^8	1.3 x10^8	Nil	1.2 x10^8	1.14 x10^8	nil
E .faecium A080	1.21 x10^8	1.02 x10^8	Nil	1.0 x10^8	5.0 x10^6	2 log
L. pseudomenseteroides A082	2.65 x10^8	1.96 x10^7	1 log	2.0 x10^7	6.0 x10^6	2 log
L. plantarum A084	1.91 x10^8	1.4 x10^8	Nil	1.5 x10^8	1.2 x10^8	nil
L. plantarum A1c	1.97 x10^8	9.8 x10^7	1 log	9.6 x10^7	3.3 x10^7	nil
L. plantarum B1c	1.75 x10^8	8.5 x10^7	1 log	7.2 x10^7	4.0 x10^7	nil
W. cibaria B3a	1.84 x10^8	1.5 x10^8	Nil	1.6 x10^8	1.13 x10^8	nil
L. plantarum B3c	2.61 x10^8	1.0 x10^8	Nil	1.2 x10^8	7.9 x10^7	nil

The cell surface hydrophobicity is most frequently determined by evaluating the affinity of LAB to hydrocarbon (*n*-hexadecane, xylene and toluene). The hydrophobicity of 93 LAB strains obtained showed that 67.74 % of the bacterial strains had affinity towards *n*-hexadecane solvent while 56 99 % of the bacterial strains had affinity towards xylene solvents respectively. The hydrophobicity percentages obtained for all the 93 LAB strains ranged from 0.29 % to 90.73% in *n*-hexadecane and 71.88% in xylene (Table 4.10). The highest hydrophobicity in *n*-hexadecane was obtained for *L. pseudomensenteroides* A030 (90.73 %) and in xylene (71.88 %).

Furthermore, *E. faecium* A087 had 75.78 % hydrophobicity in *n*-hexadecane solvent but had no affinity for xylene. In addition, *L. rhamnosus* A012 had 25.0% hydrophobicity in *n*-hexadecane and 15.1% in xylene, *L. plantarum* A011 had 9.2% hydrophobicity in *n*-hexadecane and 13.8 % hydrophobicity in xylene. Meanwhile, *L. pentosus* B1b had 7.25% affinity towards *n*-hexadecane but did not have any affinity for xylene solvent, *L. rhamnosus* A072 had 11.1% hydrophobicity in *n*-hexadecane and 7.4% hydrophobicity in xylene. The least value in cell surface hydrophobicity was obtained with *L. plantarum* A084, which had 0.0% in *n*-hexadecane and *L. pentosus* (0.36 %) in xylene solvent (Table 4.10).

Ability of the lactic acid bacteria to form cellular aggregate through autoaggregation helps the bacteria to persist in the intestine. The autoaggregation result of 15 LAB strains obtained showed that *L. rhamnosus* A072 has the highest percentage (46.37 %) followed by *L. pentosus* A028 (38.20 %) (Figure 4.14). Furthermore, the autoaggregation result also showed that *L. xianqfangensis* B1a2 had 37.06 %, *L. rhamnosus* A012 had 32.50 %, *W. cibaria* B3a had 32.68 and *L. plantarum* A011 had 20.83 % (Figure 4.14). Although, *L. rhamnosus* A072 has the highest percentage value of autoaggregation of 46.37 %, but its autoaggregation value decreased from 47.1 % at the 4^{th} h to 46.37 at the 5^{th} h (Figure 4.14). In addition, *L. pentosus* A4c had reduction in autoaggregation value from from 31.16 % at 4^{th} h to 24.28 % at 5^{th} h (Figure 4.14).

Coaggregation of 11 lactic acid bacteria strains with different strins of *E. coli*; ETEC H40B, EPEC H62E, EIEC H68D, EAEC H40C and STEC H77E showed different range in the percentage of co-aggregation. The co-aggregation of *L. rhamnosus* A012 with EPEC H40C, EIEC H68D and STEC H77E were 33.4%, 0.9% and 2.2% respectively. In addition, *P. pentosaceus* A074 had 30.2% when co-aggregated with EPEC H62E, *L.*

plantarum A011 had 19.2% when co-aggregated with EPEC H62E and *L. pentosus* A028 had 18.3% of co-aggregation with EPEC H62E (Figure 4.15). Moreover, the co-aggregation of EPEC H62E with *L. plantarum* A011 was 19.16%, *L. plantarum* A046 was 11.07%, *L. pentosus* A4c was 26.69%, and *L. pentosus* B1b was 24.96% while, *L. pseudomensenteroides* A044, *L. plantarum* A084, *L. plantarum* A1c, and *W. cibaria* B3a were unable to form a co-aggregation reaction with EPEC H40C (Figure 4 .15). Suprisingly, none of the strains evaluated formed co-aggregation with EAEC H40C. Co-aggregation values of LAB with EIEC H68D were; *L. rhamnosus* A012 (0.94 %), *L. plantarum* A046 (6.64%), *L. plantarum* A084 (4.36 %), *L. pentosus* B1b (16.99%) and *P. pentosaceus* A074 (6.50 %). It was also observed that only *L. rhamnosus* A012 (0.94%) was able to competitively coaggregate with EPEC H62E, EIEC H68D and STEC H77B (Figure 4 .15).

Table 4.10. Affinity of lactic acid bacteria to n-hexadecane and xylene

Name of Isolates	Hexadecane (initial [nm])	Hexadecane (final [nm])	Hydrophobicity (%)	Xylene (initial [nm])	Xylene (final [nm])	Hydrophobicity (%)
L. plantarum A002	0.997	0.734	26.38	0.997	0.837	16.05
E. faecium A003	0.796	0.752	5.53	0.788	0.796	NEG
E .faecium A008	0.951	0.934	1.79	0.954	1.012	NEG
L. pseudomenseteroides A010	0.898	0.866	3.56	0.898	0.918	NEG
L. plantarum A011	0.808	0.734	9.20	0.809	0.697	13.84
L. rhamnosus A012	0.832	0.624	25.00	0.832	0.703	15.50
E. durans 013	0.794	0.814	NEG	0.791	0.8	NEG
L. plantarum A014	0.873	0.795	8.93	0.871	0.777	10.79
L. pentosus A016	0.870	0.821	5.63	0.870	0.831	4.48
L. paracasei 017	0.926	0.694	25.05	0.926	0.721	22.13
E. faecium A018	0.961	0.984	NEG	0.849	0.896	NEG
L. pseudomenseteroides A019	0.914	0.778	14.88	0.911	0.799	12.29
E. faecium A022	0.975	1.019	NEG	0.975	1.059	NEG
L. plantarum A023	0.794	0.612	22.92	0.794	0.714	10.08
E. durans A024	1.001	1.005	NEG	0.991	1.073	NEG
L. pseudomenseteroides A026	0.909	0.613	32.56	0.916	0.63	31.22
E. thailandicus A027	0.935	0.896	4.17	0.948	0.962	NEG
L. pentosus A028	0.824	0.684	16.99	0.825	0.822	0.36
L. pseudomenseteroides A030	0.852	0.079	90.73	0.85	0.239	71.88
E. thailandicus A031	0.785	0.853	NEG	0.778	0.98	NEG
L. plantarum A033	0.763	0.743	2.62	0.776	0.862	NEG
L. plantarum A034	0.896	0.811	9.49	0.901	0.777	13.76
L. plantarum A035	0.888	0.829	6.64	0.891	0.891	0
L. plantarum A036	0.857	0.953	NEG	0.857	1.004	NEG
L. plantarum A037	0.949	0.958	NEG	0.949	0.935	1.48
E. durans A038	0.779	0.836	NEG	0.766	0.88	NEG
E. faecium A039	0.915	0.881	3.72	0.917	1.045	NEG
E. faecium A040	0.705	0.744	NEG	0.834	0.894	NEG
L. plantarum A041	0.924	0.448	51.52	0.938	0.85	9.38
E. durans A043	0.694	0.692	0.29	0.694	0.7	NEG
L. pseudomenseteroides A044	0.994	0.911	8.35	0.994	0.927	6.74
L. plantarum A046	0.814	0.714	12.29	0.814	1.384	NEG
L. plantarum A047	0.791	0.713	9.86	0.791	0.774	2.149
L. plantarum A048	0.96	0.939	2.19	0.96	0.95	1.04
E. faecium A049	0.536	0.602	NEG	0.62	0.64	NEG
E. durans A050	0.912	0.99	NEG	0.905	1.083	NEG
L. plantarum A051	0.612	0.552	9.80	0.612	0.556	9.15
E. lactis A052	0.543	0.425	21.73	0.572	0.351	38.64
E. faecalis A058	0.758	0.8	NEG	0.773	0.857	NEG

Table 4.10. cont.

Name of Isolates	Hexadecane (initial)	Hexadecane (final)	Hydrophobicity (%)	Xylene (initial)	Xylene (final)	Hydrophobicity (%)
L. plantarum A059	0.881	0.902	NEG	0.874	0.931	NEG
E. lactis A060	0.565	0.584	NEG	0.795	0.539	32.20
L. pseudomenseteroides A064	0.767	0.568	25.95	0.772	0.768	0.52
E. faecium A066	0.846	0.982	NEG	0.907	0.983	NEG
L. plantarum A071	0.879	0.886	NEG	0.92	0.84	8.70
L. rhamnosus A072	0.704	0.626	11.10	0.704	0.652	7.39
P. pentosaceus A074	0.993	0.917	7.65	0.993	0.982	1.11
L. plantarum A075	0.909	0.697	23.32	0.919	0.802	12.73
E. faecalis A077	0.79	0.74	6.33	0.827	0.962	NEG
L. plantarum A079	0.862	0.842	2.32	0.862	0.89	NEG
E. faecium A080	0.949	0.93	2.00	0.998	1.012	NEG
E. lactis A081	0.98	0.994	NEG	0.987	0.991	NEG
L. pseudomenseteroides A082	0.888	0.726	18.24	0.902	0.772	14.41
E. faecium A083	0.962	0.974	NEG	0.956	0.926	3.14
L. plantarum A084	0.91	0.91	0	0.91	0.899	1.21
E. faecium A087	0.702	0.17	75.78	0.702	0.757	NEG
L. pseudomenseteroides A089	0.907	0.895	1.32	0.907	0.883	2.65
E. faecium A090	0.902	0.766	15.08	0.921	0.709	23.02
L. pseudomenseteroides A093	0.679	0.546	19.59	0.556	0.37	33.45
L. plantarum A094	0.788	0.82	NEG	0.84	0.874	NEG
E. durans A095	0.904	0.864	4.42	0.972	1.114	NEG
E. lactis A096	0.798	0.781	2.13	0.923	0.985	NEG
E. durans A097	0.938	0.943	NEG	0.938	0.933	0.53
E. durans A098	0.851	0.865	NEG	0.91	0.979	NEG
E. faecalis A4D	0.99	0.986	0.40	0.905	1.002	NEG
W. cibaria B3b	0.948	0.785	17.19	0.959	0.913	4.80
L. pentosus A1d	0.761	0.73	4.07	0.775	0.758	2.19
W. cibaria B3a	0.82	0.72	12.20	0.888	0.852	4.05
L. fermentum A3b	0.858	0.565	34.15	0.939	1.103	NEG
L. plantarum B3c	0.937	0.903	3.62	0.925	0.905	2.16
L. plantarum A4b	0.94	0.725	22.87	0.955	0.756	20.84
L. plantarum B1b2	0.877	0.985	NEG	0.827	0.857	NEG
L. plantarum A1c	0.866	1.06	NEG	0.942	0.892	5.31
W. cibaria B4a	0.983	1.034	NEG	0.88	0.862	2.05
L. pentosus A4f	0.891	0.908	NEG	0.912	1.093	NEG
W. cibaria B1e	0.897	0.972	NEG	0.878	1.13	NEG
L. fermentum A3a	0.983	0.837	14.85	0.943	0.795	15.69
W. cibaria B4c	0.922	0.956	NEG	0.921	1.028	NEG
L. pentosus A4c	0.889	0.792	10.91	0.996	0.836	16.06
W. confusa B1d	0.902	0.812	9.98	0.897	0.822	8.36
L. pentosus A1a	0.821	0.751	8.53	0.887	0.889	NEG
L. plantarum B1f	0.741	0.738	0.40	0.908	0.85	6.39
L. fermentum A3d	0.644	0.622	3.42	0.595	0.56	5.88
L. plantarum B1c	0.997	0.864	13.34	0.967	0.926	4.24
L. fermentum A3c	0.82	0.843	NEG	0.895	0.902	NEG

Table 4.10. cont'd

Name of Isolates	Hexadecane (initial)	Hexadecane (final)	Hydrophobicity (%)	Xylene (initial)	Xylene (final)	Hydrophobicity (%)
L. pentosus B1b	0.8	0.742	7.25	0.851	0.851	0
L. pentosus A1e	0.993	0.803	19.13	0.9	0.955	NEG
L. plantarum B1a	0.864	0.75	13.19	0.861	0.788	8.48
L. Xianqfangensis B1a2	0.59	0.513	13.05	0.58	0.558	3.79
E. durans A004	0.936	0.95	NEG	0.923	0.909	1.52
E. faecalis A020	0.937	0.779	16.86	0.911	0.823	9.66
E. faecalis A029	0.875	0.823	5.94	0.875	0.874	0.11
E. faecalis A063	0.688	0.951	NEG	0.857	0.985	NEG
E. faecalis A078	0.953	0.913	4.20	0.96	1.028	NEG

Note:

Initial reading is the first absorbance reading in nm

Final reading is the second and last absorbance reading in nm

NEG: means the strains did not have affinity for the hydrocarbon (the final reading is greater than the initial reading).

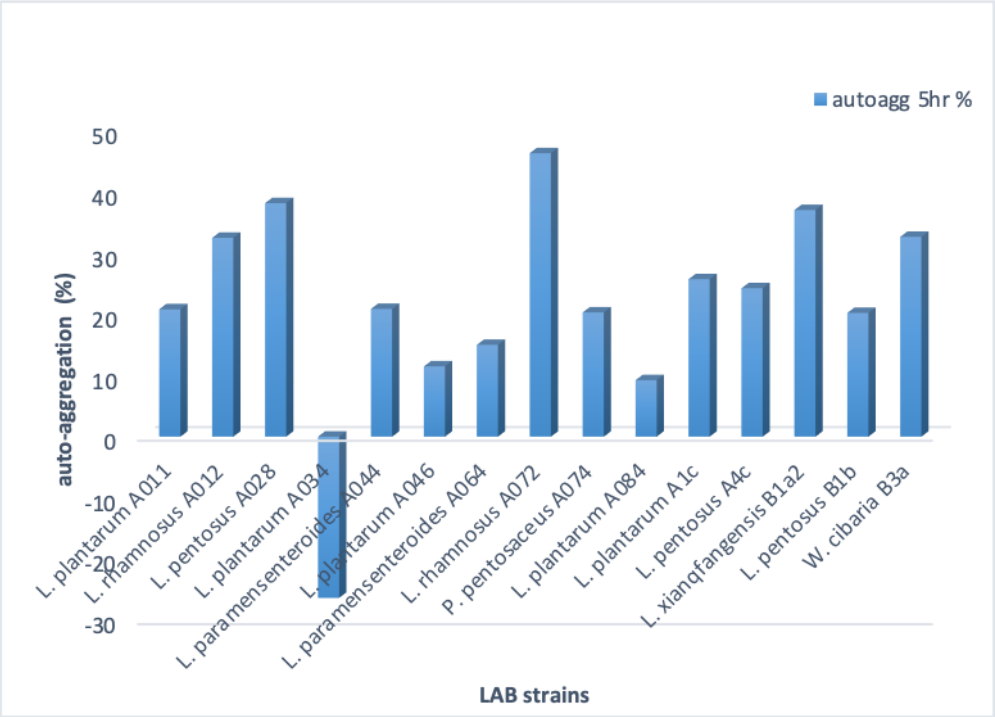

Figure 4.14. Autoaggregation of lactic acid bacteria strains at 5th h (%)

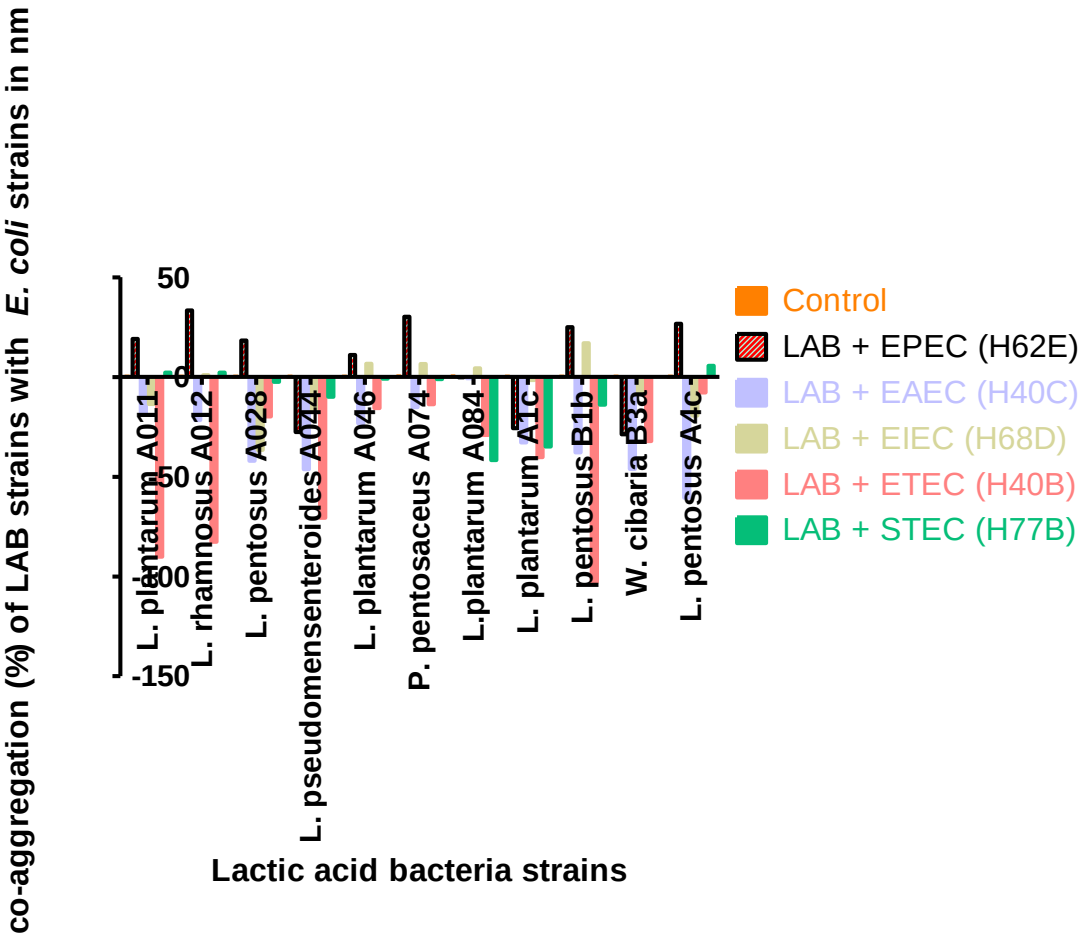

Figure 4.15. Percentage of co-aggregation of lactic acid bacteria strains with diarrhoeagenic *E. coli* strains

Note
EPEC – Enteropathogenic *E. coli* H62E
EAEC – Enteroaggregative *E. coli* H40C
EIEC – Enteroinvasive *E. coli* H68D
ETEC – Enterotoxigenic *E. coli* H40B
STEC – Shiga-toxin *E. coli* H77B

4.5. Immunopotential activity of lactic acid bacteria

The weight of all the mice in each group was monitored five times throughout the experiment. At the initial stage, there was no disparity in the body weight of the experimental mice in all the groups (Figure 4.16). However, after the intraperitoneal administration of cyclophosphamide to group one, group two, group three and group four (immunosuppressed groups), there was a drastic weight loss when compared with group five, group 6 and group 7 (healthy and immunocompetent groups). However, after oral administration of lactobacilli to group three and group four (CTX + *L. rhamnosus* A012 and CTX + *L. plantarum* A011 and groups), therapeutic effect was observed as there was a significant increase in body weight compared with the group one; that is, CTX treated with phosphate buffer saline (negative control group). Consistently, the mice in lactobacilli treatment groups showed an increase in weight throughout the assay than negative control group (Figure 4.16) and the mice in positive control group; group 2 (levamisole + CTX) also showed increase in weight. However, there was no significant decrease in the weight of immunocompetent mice (group five, group six and group seven). The mean of the weight of mice in all groups was reported (Figure 4.16).

The spleen of mice in each group was weighed and the spleen index was reported (Table 4.12 and Figure 4.17). From the result, the spleen indices in CTX induced + *L. rhamnosus* A012 and *L. plantarum* A011 treatment groups (immunosuppressed treated groups), Levamisole hydrochloride + CTX group (standard drug + cyclophosphamide group) *L. rhamnosus* A012 and *L. plantarum* A011 healthy groups (immunocompetent groups) and PBS alone group (healthy group) increased significantly (p <0.05) as compared to CTX + PBS group (negative control group).

The level and quantity of IL-10, TNF-α and IL-6 in mouse body fluids (i.e. the blood serum and spleen homogenate) are reported (Figure 4.18 to Figure 4.23, Table 4.11). The administration of cyclophosphamide to group one mice (CTX + PBS) drastically reduced the level of IL-10 cytokine produced in mice spleen and blood with a p-value less than 0.05 (p<0.05); (Figure 4.18 and Figure 4.22). Meanwhile, the administration of levamisole hydrochloride (standard drug), *L. rhamnosus* A012 and *L. plantarum* A011 to group two (CTX + Lev), group three (CTX + *L. rhamnosus* A012) and group four (CTX + *L. plantarum* A011) up-regulate the level of IL-10 cytokines in the spleen

and blood of the mice, showing the ameliorating effect of the standard drug and probiotic strains. The level of IL 10 cytokine was higher in group three (CTX + *L. rhamnosus* A012) than group two (CTX + Lev), group four (CTX + *L. plantarum* A011) and immunocompetent mice (group five, group six, and group 7) (Figure 4.18 and Figure 4.22).

Simultaneously, the levels of IL-6 and TNF-α in the spleen and blood of experimental mice treated with levamisole hydrochloride, *L. plantarum* A011 and *L. rhamnosus* A012 (group two, three and four) significantly decreased in contrast to CTX +PBS (group one) which had high level of IL-6 in the spleen (Figure 4.19) and in the blood (Figure 4.23), TNF-α in the spleen (Figure 4.20) and in the blood (Figure 4. 21). Besides, the values of IL-6 and TNF-α produced by group three mice (CTX + *L. rhamnosus* A012 treated group), was the lowest, followed by, group four (CTX + *L. plantarum* A011 treated group), and group two (CTX + levamisole hydrochloride treated group). In addition, group five experimental mice (*L. rhamnosus* A012 without CTX), group six (*L. plantarum* A011 without CTX), and group 7 (PBS without CTX) had low production of IL-6 and TNF-α (Figure 4.20, 4.21 and 4.23).

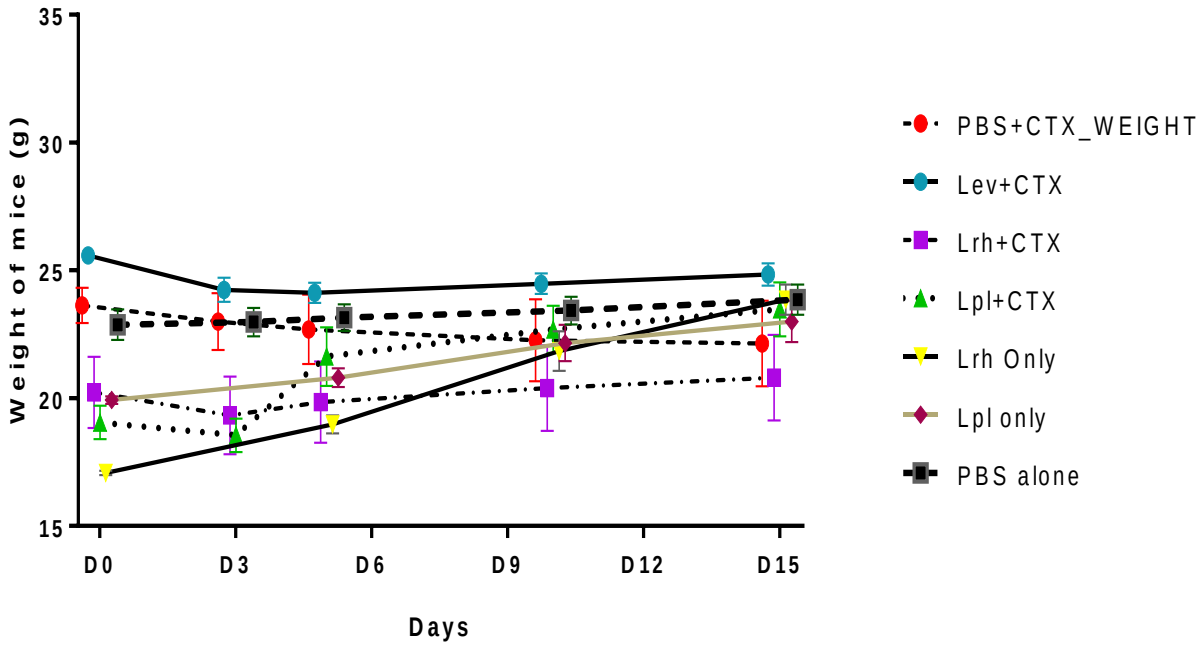

Figure 4.16. The body-weight of the experimental animal.

NOTE:

CTX + PBS: group one, received cyclophosphamide 20 mg/kg, i.p. + PBS, oral;

Lev + CTX: group two, received cyclophosphamide 20 mg/kg, i.p.+levamisole hydrochloride 40 mg/kg, oral.

L. rh + CTX: group three, received cyclophosphamide 20 mg/kg, i.p. + *L. rhamnosus* A012 (1x 10^8 CFU/ml}, oral.

L. pl + CTX- group four, received CTX + *L. plantarum* A011 {1x 10^8 CFU/ml}, oral.

L. rh only: group five, received *L. rhamnosus* A012 (1x 10^8 CFU/ml) oral.

L. pl only: group six, received *L. plantarum* A011 (1x 10^8 CFU/ml}, oral.

PBS only – group seven received PBS, oral.

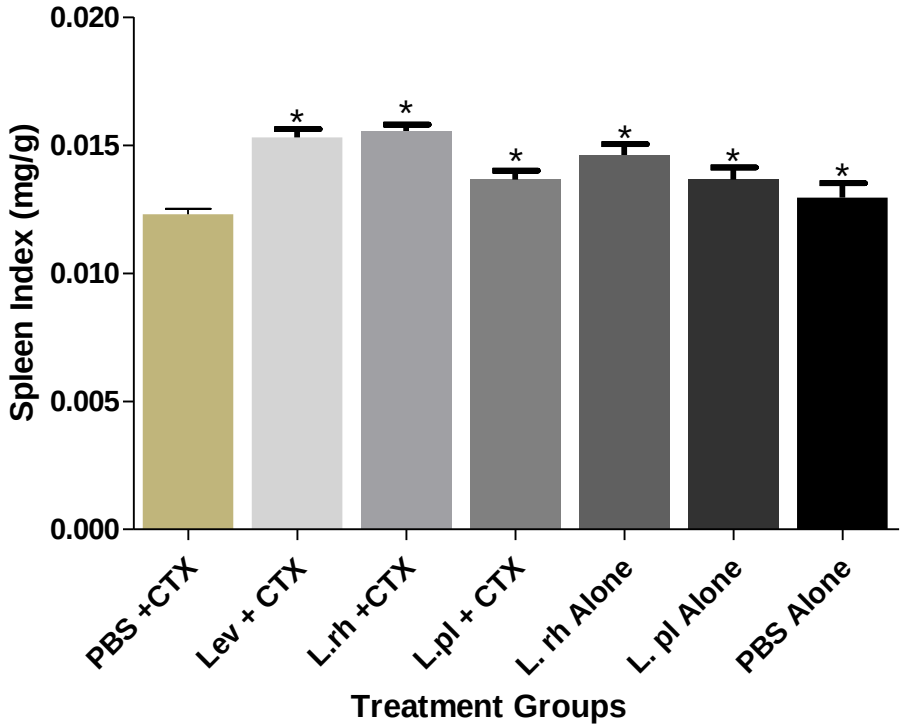

Figure 4.17. Spleen index of treated mice.

NOTE:

CTX + PBS: group one, received cyclophosphamide 20 mg/kg, i.p. + PBS, oral;

Lev + CTX: group two, received cyclophosphamide 20 mg/kg, i.p.+levamisole hydrochloride 40 mg/kg, oral.

L. rh + CTX: group three, received cyclophosphamide 20 mg/kg, i.p.+ *L. rhamnosus* A012 (1x 10^8 CFU/ml}, oral.

L. pl + CTX- group four, received CTX + *L. plantarum* A011 {1x 10^8 CFU/ml}, oral.

L. rh only: group five, received *L. rhamnosus* A012 (1x 10^8 CFU/ml) oral.

L. pl only: group six, received *L. plantarum* A011 (1x 10^8 CFU/ml}, oral.

PBS only – group seven received PBS, oral.

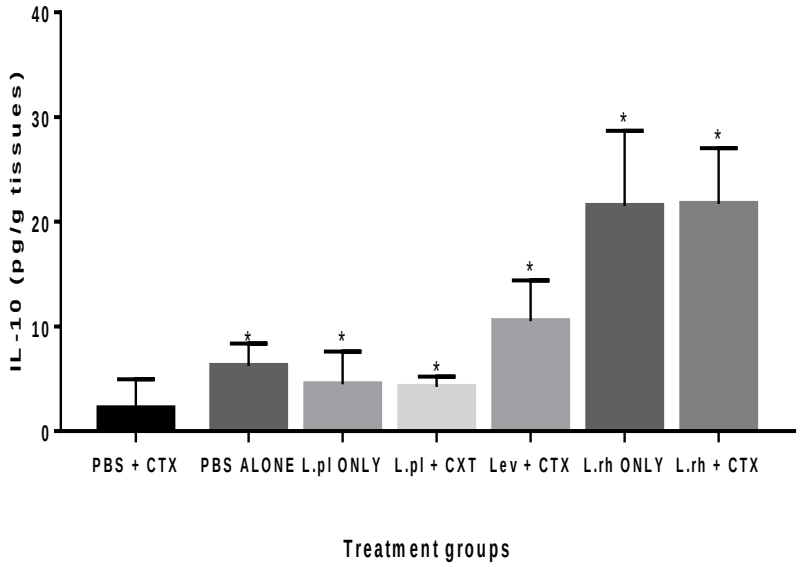

Figure 4.18. Effect of *L. rhamnosus* A012 and *L. plantarum* A011 on IL 10 concentration in the spleen of CTX- treated and untreated mice.

Each column indicates mean ± SD (n = 4). *- significant difference between PBS treated immunosuppressed mice compared to immunosuppressed mice treated with *Lactobacillus* strains or levamisole hydrochloride (positive control), P< 0.0001, compared to immunocompetent mice are indicated with * above the bar (One-way ANOVA followed by Student-Newman-Keuls post-hoc test).

NOTE:

CTX + PBS: group one, received cyclophosphamide 20 mg/kg, i.p. + PBS, oral;

Lev + CTX: group two, received cyclophosphamide 20 mg/kg, i.p.+levamisole hydrochloride 40 mg/kg, oral.

L. rh + CTX: group three, received cyclophosphamide 20 mg/kg, i.p.+ *L. rhamnosus* A012 (1x 10^8 CFU/ml}, oral.

L. pl + CTX- group four, received CTX + *L. plantarum* A011 {1x 10^8 CFU/ml}, oral.

L. rh only: group five, received *L. rhamnosus* A012 (1x 10^8 CFU/ml) oral.

L. pl only: group six, received *L. plantarum* A011 (1x 10^8 CFU/ml}, oral.

PBS only – group seven received PBS, oral.

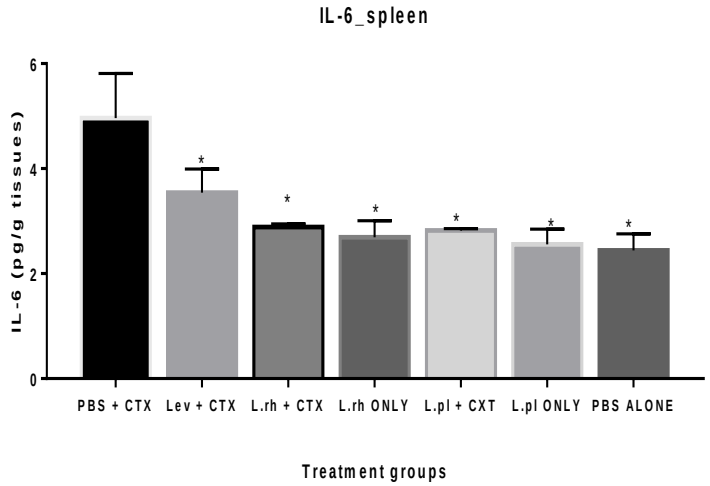

Figure 4.19. Effect of *L. rhamnosus* A012 and *L. plantarum* A011 on IL-6 concentration in the spleen of CTX- treated and untreated mice.

Each column indicates mean ± SD (n = 4). *- significant difference between PBS treated immunosuppressed mice compared to immunosuppressed mice treated with *Lactobacillus* strains or levamisole hydrochloride (positive control), P< 0.05, compared to immunocompetent mice are indicated with * above the bar (One-way ANOVA followed by Student-Newman-Keuls post-hoc test).

NOTE:

CTX + PBS: group one, received cyclophosphamide 20 mg/kg, i.p. + PBS, oral;

Lev + CTX: group two, received cyclophosphamide 20 mg/kg, i.p.+levamisole hydrochloride 40 mg/kg, oral.

L. rh + CTX: group three, received cyclophosphamide 20 mg/kg, i.p.+ *L. rhamnosus* A012 (1x 10^8 CFU/ml}, oral.

L. pl + CTX- group four, received CTX + *L. plantarum* A011 {1x 10^8 CFU/ml}, oral.

L. rh only: group five, received *L. rhamnosus* A012 (1x 10^8 CFU/ml) oral.

L. pl only: group six, received *L. plantarum* A011 (1x 10^8 CFU/ml}, oral.

PBS only – group seven received PBS, oral.

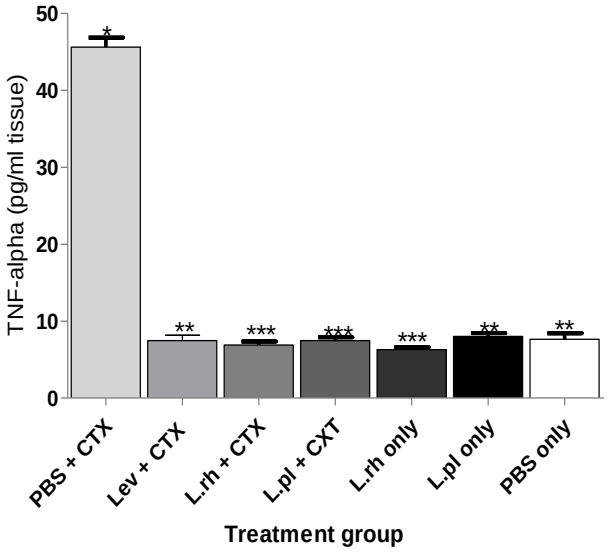

Figure 4.20. Effect of *L. rhamnosus* A012 and *L. plantarum* A011 on TNF-α concentration in the spleen of CTX- treated and untreated mice.

Each column indicates mean ± SD (n = 4). *- significant difference between PBS treated immunosuppressed mice compared to immunosuppressed mice treated with *Lactobacillus* strains or levamisole hydrochloride (positive control), P< 0.0001, compared to immunocompetent mice are indicated with * above the bar (One-way ANOVA followed by Student-Newman-Keuls post-hoc test).

NOTE:

CTX + PBS: group one, received cyclophosphamide 20 mg/kg, i.p. + PBS, oral;

Lev + CTX: group two, received cyclophosphamide 20 mg/kg, i.p.+levamisole hydrochloride 40 mg/kg, oral.

L. rh + CTX: group three, received cyclophosphamide 20 mg/kg, i.p.+ *L. rhamnosus* A012 (1x 10^8 CFU/ml}, oral.

L. pl + CTX- group four, received CTX + *L. plantarum* A011 {1x 10^8 CFU/ml}, oral.

L. rh only: group five, received *L. rhamnosus* A012 (1x 10^8 CFU/ml) oral.

L. pl only: group six, received *L. plantarum* A011 (1x 10^8 CFU/ml}, oral.

PBS only – group seven received PBS, oral.

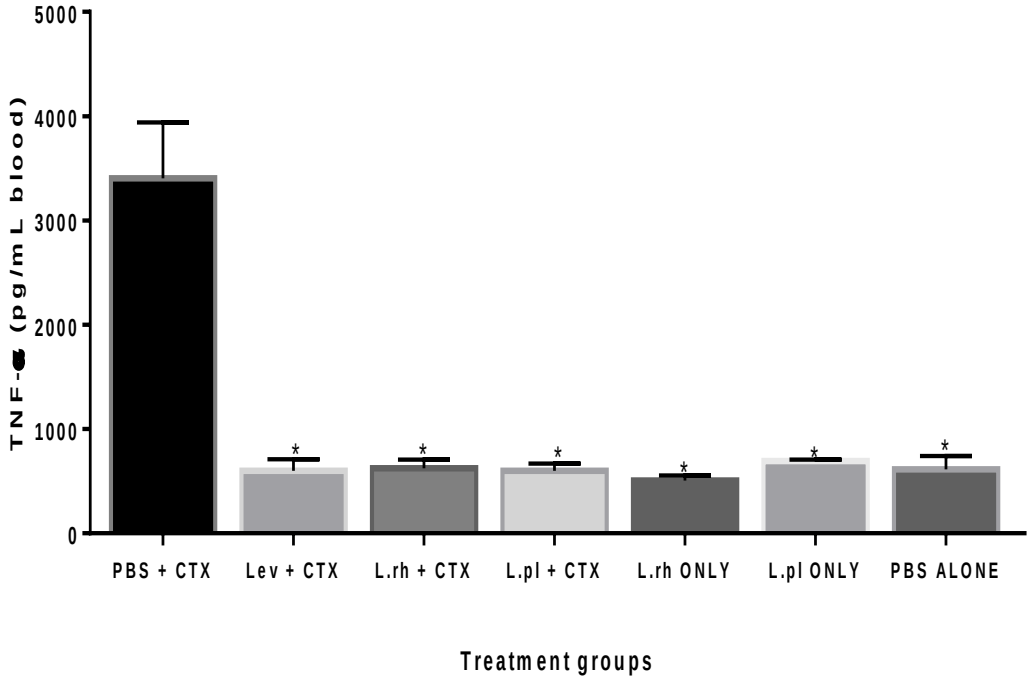

Figure 4.21. Effect of *L. rhamnosus* A012 and *L. plantarum* A011 on TNF-α concentration in the blood of CTX- treated and untreated mice.

Each column indicates mean ± SD (n = 4). *- significant difference between PBS treated immunosuppressed mice compared to immunosuppressed mice treated with *Lactobacillus* strains or levamisole hydrochloride (positive control), P< 0.0001, compared to immunocompetent mice are indicated with * above the bar (One-way ANOVA followed by Student-Newman-Keuls post-hoc test).

NOTE:

CTX + PBS: group one, received cyclophosphamide 20 mg/kg, i.p. + PBS, oral;

Lev + CTX: group two, received cyclophosphamide 20 mg/kg, i.p. + levamisole hydrochloride 40 mg/kg, oral.

L. rh + CTX: group three, received cyclophosphamide 20 mg/kg, i.p.+ *L. rhamnosus* A012 (1x 10^8 CFU/ml}, oral.

L. pl + CTX- group four, received CTX + *L. plantarum* A011 {1x 10^8 CFU/ml}, oral.

L. rh only: group five, received *L. rhamnosus* A012 (1x 10^8 CFU/ml) oral.

L. pl only: group six, received *L. plantarum* A011 (1x 10^8 CFU/ml}, oral.

PBS only – group seven received PBS, oral.

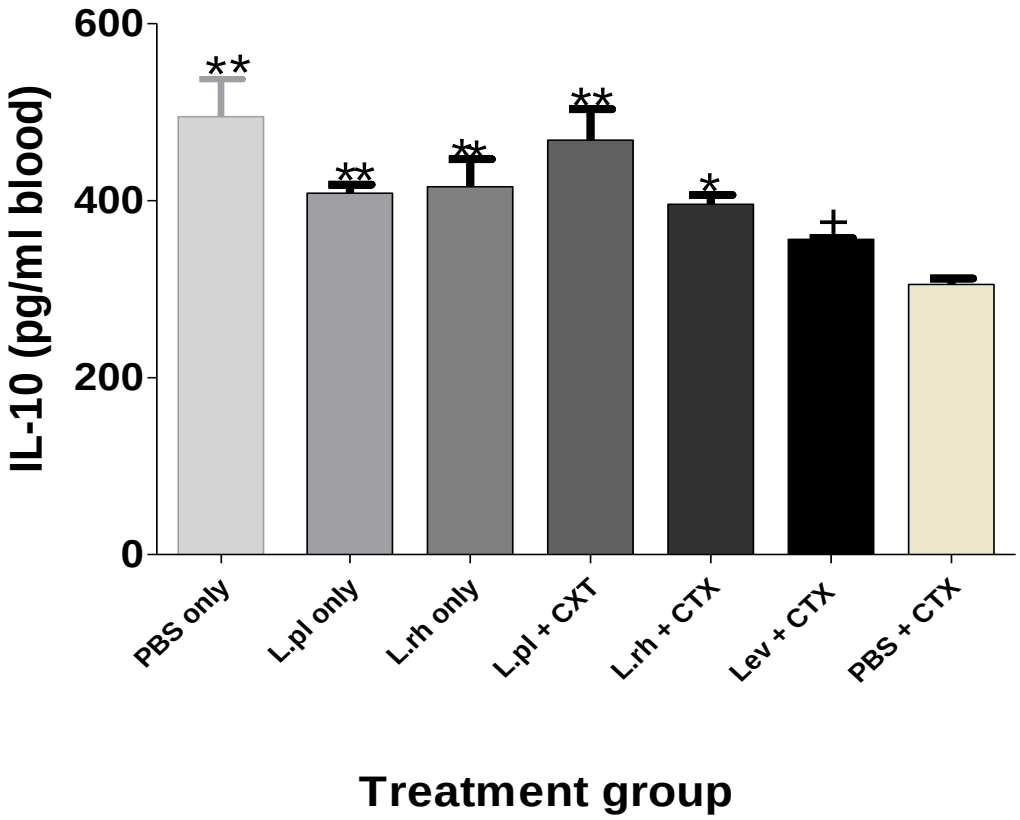

Figure 4.22. Effect of *L. rhamnosus* A012 and *L. plantarum* A011 on IL 10 concentration in the blood of CTX- treated and untreated mice.

Each column indicates mean ± SD (n = 4). *- significant difference between PBS treated immunosuppressed mice compared to immunosuppressed mice treated with *Lactobacillus* strains or levamisole hydrochloride (positive control), P< 0.001, compared to immunocompetent mice are indicated with * above the bar (One-way ANOVA followed by Student-Newman-Keuls post-hoc test).

NOTE:

CTX + PBS: group one, received cyclophosphamide 20 mg/kg, i.p. + PBS, oral;

Lev + CTX: group two, received cyclophosphamide 20 mg/kg, i.p.+levamisole hydrochloride 40 mg/kg, oral.

L. rh + CTX: group three, received cyclophosphamide 20 mg/kg, i.p.+ *L. rhamnosus* A012 (1x 10^8 CFU/ml}, oral.

L. pl + CTX- group four, received CTX + *L. plantarum* A011 {1x 10^8 CFU/ml}, oral.

L. rh only: group five, received *L. rhamnosus* A012 (1x 10^8 CFU/ml) oral.

L. pl only: group six, received *L. plantarum* A011 (1x 10^8 CFU/ml}, oral.

PBS only – group seven received PBS, oral.

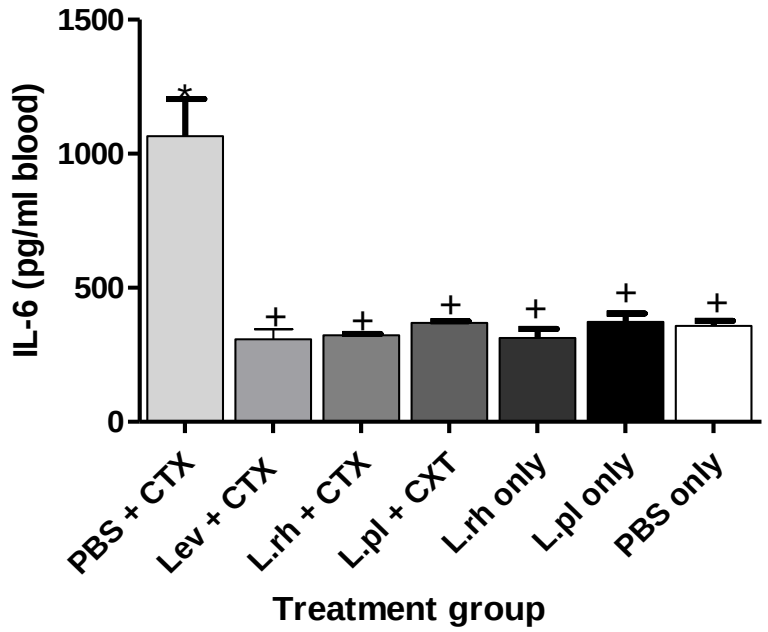

Figure 4.23. Effect of *L. rhamnosus* A012 and *L. plantarum* A011 on IL-6 concentration in the blood of CTX- treated and untreated mice.

Each column indicates mean ± SD (n = 4). *- significant difference between PBS treated immunosuppressed mice compared to immunosuppressed mice treated with *Lactobacillus* strains or levamisole hydrochloride (positive control), P< 0.001, compared to immunocompetent mice are indicated with * above the bar (One-way ANOVA followed by Student-Newman-Keuls post-hoc test).

NOTE:

CTX + PBS: group one, received cyclophosphamide 20 mg/kg, i.p. + PBS, oral;

Lev + CTX: group two, received cyclophosphamide 20 mg/kg, i.p.+levamisole hydrochloride 40 mg/kg, oral.

L. rh + CTX: group three, received cyclophosphamide 20 mg/kg, i.p.+ *L. rhamnosus* A012 (1x 10^8 CFU/ml}, oral.

L. pl + CTX- group four, received CTX + *L. plantarum* A011 {1x 10^8 CFU/ml}, oral.

L. rh only: group five, received *L. rhamnosus* A012 (1x 10^8 CFU/ml) oral.

L. pl only: group six, received *L. plantarum* A011 (1x 10^8 CFU/ml}, oral.

PBS only – group seven received PBS, oral.

As shown in Fig.4.24 below, there was a significant reduction in white blood cells counts of group one (CTX + PBS) in contrast to group two (CTX + lev), group three (CTX + *L. rhamnosus* A012) group four (CTX + *L. plantarum* A011), group five (*L. rhamnosus* A012 immunocompetent), group six (*L. plantarum* A011 immunocompetent) and group seven (PBS immunocompetent).

The result obtained from the histological study of jejunum sections of the small intestine of both CTX treated mice (immunosuppressed group) and untreated healthy mice (immunocompetent group) showed that there was a normal histological morphology of the healthy immunocompetent group. The epithelium villi of these groups were highly columnar and arranged (Figure 4.25). However, the experimental mice in group one (CTX + PBS), negative control) had a decrease in goblet cells and inflammation reaction with the evidence of oedema, (Figure, 4.25) as compared to group 7 (PBS only, healthy group). In group three and group four (CTX + *L. rhamnosus* A012 and CTX + *L. plantarum* A011), there was a minor injury in the jejunum of the small intestine. Moreover, compared to experimental mice in CTX + PBS group, (group one), and CTX + *L. rhamnosus* A012 with CTX + *L. plantarum* A011 groups (group three and group four), numerous goblets cells are seen. The inflammations are not pronounced and the vessels appeared apparently normal (Figure 4.25).

Table 4.11. Column statistics of the quantity of cytokines produced in tissue and blood of experimental mice

IL-10 tissue

Treatment	PBS + CTX	Lev + CTX	L.rh + CTX	L.pl + CXT	L.rh only	L.pl only	PBS only
Mean	1.276	12.76	23.50	16.30	24.46	16.73	27.65
Std. Deviation	1.294	1.009	1.682	3.057	2.049	1.417	0.8401
Std. Error	0.6472	0.5045	0.8411	1.528	1.024	0.7085	0.4201

P value-< 0.0001

IL-6 tissue

Treatment	PBS + CTX	Lev + CTX	L.rh + CTX	L.pl + CXT	L.rh only	L.pl only	PBS only
Mean	4.964	3.638	2.843	2.775	2.510	2.577	2.529
Std. Deviation	0.8495	0.4238	0.09929	0.09430	0.2842	0.2432	0.2605
Std. Error	0.4248	0.2119	0.04965	0.04715	0.1421	0.1216	0.1303

P value-< 0.05

TNF-alpha tissue

Treatment	PBS + CTX	Lev + CTX	L.rh + CTX	L.pl + CXT	L.rh only	L.pl only	PBS only
Mean	45.63	7.467	6.883	7.467	6.300	8.008	7.633
Std. Deviation	2.483	1.394	0.9685	0.8498	0.5932	0.8753	1.587
Std. Error	1.242	0.6972	0.4842	0.4249	0.2966	0.4377	0.7935

P value-< 0.0001

IL-10 blood

Treatment	PBS + CTX	Lev + CTX	L.rh + CTX	L.pl + CXT	L.rh only	L.pl only	PBS only
Mean	305.1	356.0	395.7	468.3	414.0	408.3	493.3
Std. Deviation	9.742	2.188	21.10	69.65	44.23	19.47	35.06
Std. Error	4.871	1.094	10.55	34.83	22.11	9.735	17.53

P value-< 0.001

IL-6 blood

Treatment	PBS + CTX	Lev + CTX	L.rh + CTX	L.pl + CXT	L.rh only	L.pl only	PBS only
Mean	1109	315.2	325.7	368.6	309.5	377.1	359.0
Std. Deviation	215.1	54.71	10.08	8.447	46.90	44.54	27.00
Std. Error	107.6	27.36	5.040	4.224	23.45	22.27	13.50

P value-< 0.001

TNF-alpha blood

Treatment	PBS + CTX	Lev + CTX	L.rh + CTX	L.pl + CXT	L.rh only	L.pl only	PBS only
Mean	3403	600.0	623.3	600.0	506.7	706.7	613.3
Std. Deviation	538.0	111.6	83.18	67.99	47.45	28.80	127.0
Std. Error	269.0	55.78	41.59	33.99	23.73	14.40	63.48

P value-< 0.0001

Table 4.12. The effect of CTX, *L. rhamnosus* and *L. platarum* on spleen indices of experimental mice

Treatment Groups	Group 1 (PBS+CTX)	Group 2 (LEV+CTX)	Group 3 (L.rh+CTX)	Group 4 (L.pl+CTX)	Group 5 (L.rh Alone)	Group 6 (L.pl Alone)	Group 7 (PBS Alone)
Mean	0.01231	0.01530	0.01556	0.01365	0.01462	0.01365	0.01295
Std. Deviation	0.0004060	0.0006612	0.0004859	0.0007033	0.0008476	0.0009721	0.001139
Std. Error	0.000203	0.0003306	0.002429	0.0003517	0.0004238	0.0004860	0.0005694
No of Values	4	4	4	4	4	4	4

P-value=0.0029, P <0.05

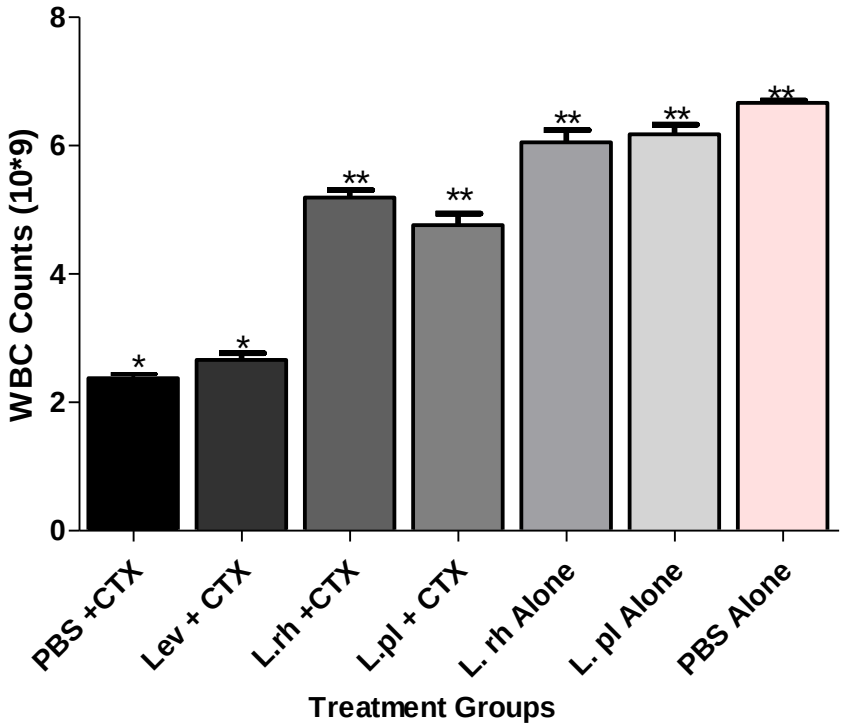

Figure 4. 24. The effect of cyclophosphamide, levamisole HCl and *Lactobacillus spp* on white blood cell counts of experimental mice

NOTE:

CTX + PBS: group one, received cyclophosphamide 20 mg/kg, i.p. + PBS, oral;

Lev + CTX: group two, received cyclophosphamide 20 mg/kg, i.p.+levamisole hydrochloride 40 mg/kg, oral.

L. rh + CTX: group three, received cyclophosphamide 20 mg/kg, i.p.+ *L. rhamnosus* A012 (1x 10^8 CFU/ml}, oral.

L. pl + CTX- group four, received CTX + *L. plantarum* A011 {1x 10^8 CFU/ml}, oral.

L. rh only: group five, received *L. rhamnosus* A012 (1x 10^8 CFU/ml) oral.

L. pl only: group six, received *L. plantarum* A011 (1x 10^8 CFU/ml}, oral.

PBS only – group seven received PBS, oral.

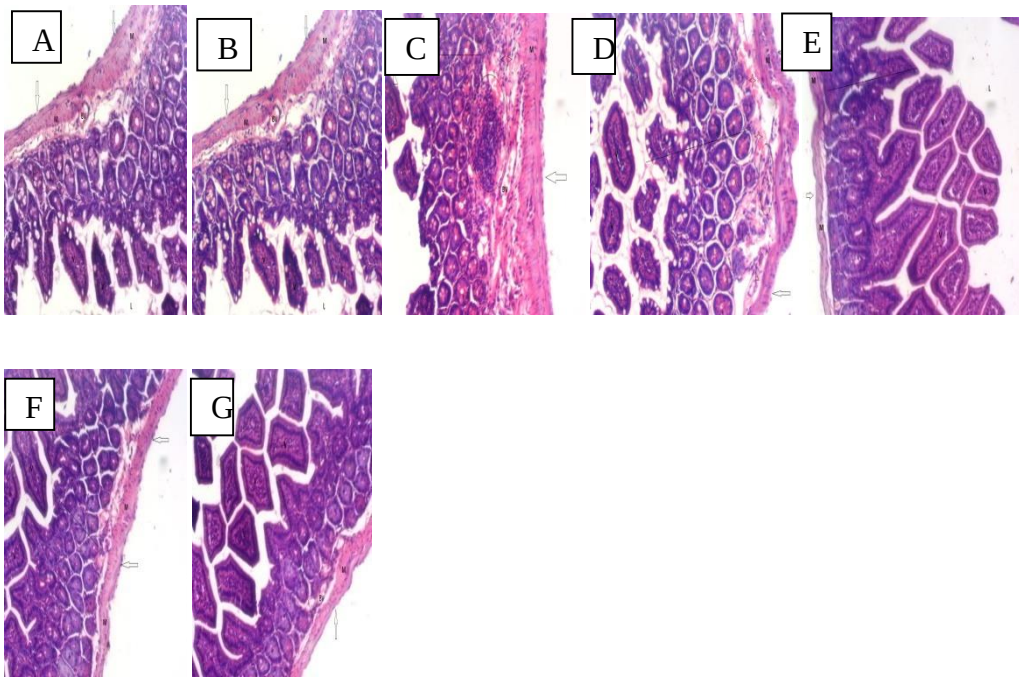

Figure 4.25. Effect of CTX, *L. rhamnosus* A012 and *L. plantarum* A011 on ileum of treated and untreated mice.

The ileum of mice; treated with cyclophosphamide, (20mg/kg; immunosuppressed group) and untreated with cyclophosphamide (immunocompetent group). A- CTX + PBS (20mg/kg cyclophosphamide (i.p.), B- CTX + Lev (40 mg/kg, levamisole hydrochloride, oral.), C (CTX + 1x 10^8 CFU/ml *L. rhamnosus* A012, oral.); D, (CTX + 1x 10^8 CFU/ml *L. plantarum* A011, oral.)), E, (1x 10^8 CFU/ml *L. rhamnosus* A012, oral) F (1x 10^8 CFU/ml, *L. plantarum* A011, oral.)), G (phosphate buffer saline, oral). Scale bar =50 μm.

CHAPTER FIVE

DISCUSSION AND CONCLUSION

5.1. Discussion

5.1.1. Diversity of lactic acid bacteria in breast milk and faeces

Breast milk has always been thought to be sterile until recently when new facts emerge that human breast milk is full of bacteria even when it is collected in an aseptic environment (Lackey *et al*., 2020). The microbiome of breast milk in healthy mothers is very important to child health as this determines and influences the continuously present microbial communities that colonize the GIT of the neonates (Martin *et al.,* 2012b). The diversity of microbiome communities in the GIT of the neonate determines how rich and healthy the gut will be and its ability to prevent the child from having infections (Stiemsma and Michels, 2018). Not all of bacteria present in the gut confer a health benefit to their host. Therefore, proper identification of LAB to specie or strain level (WHO-FAO, 2002) is expedient in linking the probiotic attribute to a particular strain.

The phylogenetic tree constructed in this study showed the degree of evolutionarily relatedness across the strains of LAB isolated from human breast milk and neonates faeces as they clustered closely in accordance with taxonomy in existence. In this study also, the ninety-three LAB isolated from breast milk and infants' faeces belongs to the genera enterococci, *Weissella*, pediococci, lactobacilli, and *Leuconostoc*. These genera are not shared in homogenous way between the two environments; there is therefore diversity in their distribution with all the five genera represented in the breast milk while the faeces have genera enterococci, lactobacilli and *Leuconostoc*. However, some of the species identified in the breast milk are still an indicator of the microbiomes of infants' faeces. The microbial composition of the faeces of the breastfed infant usually reflects their diet especially the breast milk (Medjaoui *et al*., 2016; Taghizadeh *et al*., 2017). The

five genera isolated from the two samples belong to 15 LAB species with *Lactobacillus plantarum* being the predominant species in breast milk (36.0%) and in faeces (18.0%). However, this result is not in correlation with the findings of Medjaoui *et al*. (2016) where *S. epidermidis* is predominant in breast milk and faeces of mother-infant pair in Algeria. In addition, Jimenez *et al*. (2010) reported that *L. salivarius* was predominant species in their study. Okoro *et al*. (2017) reported the dominance of *Lactobacillus plantarum* in infants faeces, whereas Ogunshe, (2018) reported *L. reuteri* as being dominant in infants faeces.

The identification of *Lactobacillus* as predominant genus in both samples still correlates with existing reports. However, isolation of *L. rhamnosus* from breast milk or faecal sample has not been reported in Nigeria before now. The isolation of *Lactobacillus* specie from mothers' breast milk and neonates' faeces in this study also correlates with the report of Osmangaoglu *et al*. (2013) on human milk; *L. casei* was isolated as dominant specie while *L. rhamnosus* was identified in a report from Tulumoglus *et al*. (2013). *Enterococcus* spp was the second most predominant species identified, this correlates with the finding of Medjaoui *et al*. (2016) which report *Enterococcus* specie as the second predominant genus in mothers breast milk and child' faeces (mother-child pair). Moreover, *E. thailandicus* was isolated from an infant' faeces while *L. xianqfangensis* was isolated from a mothers' breast milk. *E. thailandicus* was first isolated in sausage (Tanasupawat *et al*., 2008) and bovine faeces (Beukers *et al*., 2016), no report of its isolation is available yet in Nigeria. Meanwhile, *L. xianqfangensis* was first isolated in Chinese pickles (Gu *et al*., 2012), also, no report of its isolation yet in Nigeria. Therefore, human milk-faecal microbiome consists of thousands of species of microorganisms that are yet to be identified (Lackey *et al*., 2020).

5.1.2. The antimicrobial activity of lactic acid bacteria

Human breast milk and infants' faeces consist of LAB. The LAB must possess antimicrobial activities (an essential criterion for the selection of probiotic strains) against a different array of pathogens including diarrhoeal causing pathogens. Diarrhoea diseases are responsible for high mortality rate among children globally as more than 4 million of lives are lost annually (Mokomane *et al*., 2018). In Nigeria, above 315,000 deaths were recorded among pre-school age due to diarrhoea disease (Akinnibosun *et*

al., 2015*).* Diarrhoea causes mucosa inflammation and motility disturbance, on the other hand, probiotic strains have been reported to possess an inhibitory effect against enteric pathogens, they protect the mucosal layer of the intestine by competing with enteric pathogens for attachment, and display the pathogens from the intestine, thereby, reducing the use of antibiotics (Kerna and Brown, 2018; Kwasi *et al*., 2019).

The cell-free supernatant of 39.8% LAB strains identified in this study produced antimicrobial action against all the seven strains of five different pathotypes of *E. coli* used, although, larger percentage produce inhibitory action against at least four test microorganisms. The inhibitory effect of these lactic acid bacteria strains were made possible because of their ability to produce antimicrobial compounds and their competitive exclusion of the pathogens. The range of antimicrobial activity against all the seven strains of diarrhoeagenic *E. coli* displayed by *L. pentosus* B1b, *L. pentosus* A028 *L. rhamnosus* A012, *L. rhamnosus* A072, *P. pentosaceus* A074, *L. plantarum* A011, *L. pentosus* A4C and *L. plantarum* A1c is very broad. This is in correlation with Kwasi *et al*. (2019) report in which LAB with fermented food displayed antimicrobial activity against different pathotypes of diarrhoeagenic *E. coli* strain. It also correlates with Sertac *et al*. (2016) in which the supernatant of *E. faecalis* isolated from breast milk produced an antagonistic effect against *L. monocytogenes.* Lactic acid bacteria isolated from breast milk and infant faeces has reportedly displayed antimicrobial activity against pathogens (Asan-Ozusaglam and Gunyakti, 2018; Maryam and Abubakr, 2018; Talashi and Sharma, 2019; Bhola *et al*., 2019).

The analysis of the antimicrobial activity of the viable cells of LAB in this study showed that *L. rhamnosus* A012, *L. rhamnosus* A072, *P. pentosaceus* A074, *L. plantarum* A011, *L. pentosus* A4C have produced significant antimicrobial activity against the test microorganisms. The efficacy of these strains against the test pathogens showed that viable cells can also inhibit pathogens. This supports the report of Adetoye *et al*. (2018) in which the viable cells of *L. salivarus* C86 and *L. amylovorus* C94 strains from cow faeces exhibited antimicrobial activity against *Salmonella* sp. Therefore, the supernatants of LAB isolates in this study produced antimicrobial activity, as well as the viable cells of LAB isolates.

Lactic acid bacteria has been shown to competitively inhibit the pathogenic microorganisms when co-cultured (Afolayan Ayeni 2017; Alebiosu *et al.,* 2017). It is noteworthy that, *L. plantarum* A011, *L. plantarum* A084, *L. pentosus* A028, *L. pentosus* B1b, *L. rhamnosus* A012, *L. rhamnosus* A072 and *P. pentosaceus* A074 completely killed all the test *E. coli* strains between 8 h and 16 h of contact. This correlates with other authors findings who also reported total inactivation of *E. coli* strains by LAB in co-culture at 24 h of contact (Alebiosu *et al.,* 2017; Kwasi *et al*, 2019). The LAB produced antimicrobial substances during fermentation as their by-product. In this study as well, the selected LAB produced lactic and acetic acid, this, therefore, correlates with the findings of Adetoye *et al*. (2018) in which the LAB isolates from cow produced large quantities of organic acids. These organic acids produced by LAB produce inhibitory effect against the growth of the test microorganisms.

Forty out of the 93 LAB CFS in this study have antimicrobial effect against *S. aureus* ATCC 29213. Among the isolates are *E. thailandicus* A027, *L. plantarum* B3C, *E. lactis* A060, *L. pseudomesenteroides* A010, *P. pentosaceus* A074, *L. plantarum* A011, *L. plantarum* A1c *L. plantarum* B1a2, *L. rhamnosus* A012 and *E. lactis* A052. This indicates that some of the LAB isolates have a broad spectrum of activity against both Gram-positive and Gram-negative microorganisms. This corroborated the study of Arqués *et al*. (2015) in which the LAB isolates from dairy product and gut displayed antimicrobial activity against the various classes of pathogenic microorganisms. The isolated LAB also produced antimicrobial substance which are proteinaceous and bacteriocin-like, exerting antimicrobial effect against closely related species (Arqués *et al*., 2015; Maldonado-Barragán *et al*., 2016; Meade *et al*., 2020). Presently, certain *Lactobacillus* strains produce bacteriocin like substance and have ability to combat antibiotic-resistant pathogens (Arqués *et al*., 2015; Maldonado-Barragán *et al*., 2016; Meade *et al*., 2020). Bacteriocins exert cidal effect against pathogens by causing pore formation on cell membrane, cause cells lysis and eventually lead to the death of bacteria cells. They also act by inhibiting the specific enzymes that serve as metabolic pathway for the target bacteria (Arqués *et al*., 2015). Therefore, in this study, bacteriocin like substances from *L. plantarum* A011 and *L. plantarum* A1c significantly showed inhibitory activity against closely related *S. aureus* strain.

Inhibition of biofilm-forming pathogens is an important attribute of LAB showing the effectiveness in combating resistance strain (Bjarnsholt *et al.*, 2013, Kaur *et al.*, 2018; Barzegari *et al*. (2020). Biofilm refer to the microorganisms that secret a slimy glue-like substance and form aggregates in a self-produced polymeric matrix in a sessile state (Barzegari *et al*., 2020). The ability of pathogenic strains to form biofilm allows them to become resistant to the action of antimicrobial agents. Interestingly, LAB have the potential to inhibit the formation of biofilm (Abdelhamid *et al.*, 2018; Kaur *et al.*, 2018; Barzegari *et al*., 2020). The mechanism of antibiofilm activity of LAB is by hindering the activity of biofilm forming bacteria, which include their adhesion to surfaces. The antibiofilm LAB achieve this by preventing the biofilm formation; interfere with biofilm integrity and thereby preventing the survival of biofilm microorganisms. In this study, *L. plantarum* A1c and *L. plantarum* A011 have excellent anti-biofilm potential at both lower and higher concentration respectively. The two LAB strains inhibit the biofilm produced by enteroaggregative *E. coli*. Therefore, *L. plantarum* A011 and *L. plantarum* A1c are very effective to inhibit biofilm formation. Abdelhamid *et al*. (2018), also report that CFS of *Lactobacillus* and *Bifidobacterium* strains possess antibiofilm activities against the pathotypes of *E. coli* that are highly resistant to antibiotics.

This also supports the report of Pelyuntha *et al*. (2019), in which the CFS of LAB hindered the biofilm activity of food-borne pathogens due to the production of organic acids by the LAB strains. In other studies, probiotic bacteria producing bacteriocins exert antibiofilm property against pathogenic strains by destroying their cell walls and prevent the pathogens from forming biofilm. (Abdelhamid *et al.*, 2018; Kaur *et al.*, 2018; Pelyuntha *et al*., 2019; Barzegari *et al*., 2020).

Therefore, LAB strains isolated from mothers' breast milk and infants' faeces possess antimicrobial activity against *E. coli* strains; ETEC H40B, EPEC H62E, EIEC H68D, STEC H77E, and EAEC H40C. The viable cells and the CFS of *P. pentosaceus* A074, *L. plantarum* A011, *L. plantarum* A1c, *L. rhamnoss* A012, *L. pentosus* B1b, *L. pentosus* A4c *L. rhamnoss* A072 isolated from breast milk and infants' faeces inhibit the growth of *E. coli* strains (ETEC H40B, EPEC H62E, EIEC H68D, STEC H77E, EAEC H40C. These LAB strains kill the indicated microorganisms in co-culture and inhibit the biofilm-forming pathogens. The LAB strains in this study produced organic acid and bacteriocin-like substance.

5.1.3. Survival in gastrointestinal condition and adhesion properties of lactic acid bacteria isolated

Tolerance to low acid and bile concentrations is an important and a desirable characteristic of oral probiotic strains (FAO/WHO, 2006). The potential probiotic strains must be able to thrive and survive in these conditions (Cervantes-Elizarrarás *et al*., 2019). Most of the LAB strains tested in this study survived pH 3.0 including the isolates from breast milk. However, most of the LAB failed to survive at pH 2.0, although, some survived the condition with three log reduction in their viable cells from 1.15 x 10^8 to 1.8 x 10^5 CFU/ml. Also, some strains were able to survive the bile supplementation of 0.3%, normally found in the human intestine. *L. plantarum* A011, *L. rhamnosus* A012, *L. rhamnosus* A072, *L. pentosus* A4c and *P. pentosaceus* A074 were among the strains observed to demonstrate resistance to the GIT conditions. After subjecting the LAB isolates to GIT challenge, their viable cells were between 1.13 x 10^8 and 1.44 x 10^8 at pH 3.0 (with 0.3% bile), while at pH 2.0, they ranged between 1.15 x 10^8 and 1.8 x 10^5 CFU/ml. It is worthy of note, that LAB isolates from breast milk also survived the harsh condition of GIT. This result correlates with the findings of Cervantes-Elizarrarás *et al*. (2019), in which, the isolated LAB in their study also possesses a high number of viable cells at pH 2.0. The exposure of selected LAB isolates to consecutive acid and bile transit showed a minimum of 2 log reduction between the ranges of 2.65 x 10^8 to 6.0x10^6. Therefore, this present result is in agreement with other studies where isolated LAB from breast milk and neonates' faeces were able to survive the adverse gastrointestinal environment (Kirtzalidou *et al.,* 2011; Kozak *et al.,* 2015, Asan-Ozusaglam and Gunyakti, 2018).

Lactic acid bacteria naturally produce organic acid, which make them tolerant to the hostile acidic condition of the stomach. Besides, LAB strains express bile salt hydrolase1 and 2 (bsh-1 and bsh-2) housekeeping genes, thereby using the evolutionary stress-sensing system defence mechanism that assists the strains to survive the bile toxicity and migrate across the intestine (Diana *et al*., 2013).

The viable counts of selected LAB strains in consecutive GIT transit range between 1.13 x 10^8 and 1.44 x 10^8 cf/ml. Purphan (2015) reported that the quantity of bacteria in the gut that could produce probiotic effect is between 1.0 x 10^6 and 1.0 x 10^{10}. Therefore, *L. plantarum* A011, *L. rhamnosus* A012, *L. rhamnosus* A072, *L. pentosus* A4c and *P.*

pentosaceus A074 were able to survive the etreme condition of the GIT and maintain their viability at this range; this indicates that, these strains are potential probiotics.

Another essential criterium in the selection of probiotic strain is its adherence ability to mucus and/or human epithelial cells and cell lines. Glycocalyx layer covers the intestinal epithelial cells, which consists of glycolipids and glycoproteins (Monteagudo-Mera *et al*., 2019). The viscosity of this mucus layer protects the intestinal epithelium from bacterial infection and also from mechanical damage. The *Lactobacillus* and *Bifidobacterium* species contain surface molecules like lipoteichoic acid (LTA), surface layer associated proteins (SLAPs) and mucin binding proteins (Mubs) that interact with mucus layer of intestinal epithelium cells (Monteagudo-Mera *et al*., 2019). The surface properties of LAB vary, therefore their adherence to the mucus layer of the intestine use specific and non-specific mechanisms to exclude the pathogenic organism (Zhang *et al*., 2016). A cell surface property such as cell hydrophobicity affects non-specific adherence, and it is used to assess the adherence property of the LAB. Therefore, in this study, evaluation of the surface hydrophobic nature of LAB strains to the two hydrocarbons used showed that *L. pseudomesenteroides* A030 had the highest percentage of cell surface hydrophobicity, (90.7% affinity to *n*-Hexadecane, and 71.9% to xylene). *L. plantarum* A041, *L. fermentum* A3b, *L. pseudomesenteroides* A026, *L. plantarum* A002, *L. plantarum* A011 and *L. rhamnosus* A012 showed non-specific adherence through cell surface hydrophobicity with 51.52%, 34.15%, 32.56%, 26.40%, 9.23% and 25.53% affinity to *n*-Hexadecane hydrocarbon. However, the affinity of LAB isolates to xylene was lower with *L. plantarum* A002, *L. rhamnosus* A012 and *L. plantarum* A011 having 16.1%, 15.3% and 13.76% values respectively. It is noteworthy that the LAB strains with surface hydrophobicity are mostly from faecal samples, therefore, our result correlates with the report of Monteagudo-Mera *et al*. (2019), that LAB from faeces usually showed specific and non-specific adherence property to intestinal epithelial layer and cell lines of the host. Furthermore, the adherence property of the LAB strains isolated in this study enhance their competitive ability to display pathogenic microorganisms such as enteritis pathogens, inhibit their growth and exclude these pathogens from causing infection in the GIT through adherence (Ayeni *et al.,* 2011).

According to Monteagudo-Mera *et al*. (2019), the adhesion property of LAB strains is not to the host cell alone but also to bacteria cell of the same species (autoaggregation), and other cells of different species (co-aggregation). Therefore, autoaggregation of our LAB strains is necessary to examine their adhesion property to their cell *in-vitro* and their ability to coaggregate and form a barrier against pathogenic microorganisms (Tuo *et al.,* 2013; Grajek *et al.,* 2016). The present study revealed a positive correlation between cell surface hydrophobicity and adhesion properties of selected LAB tested. The auto-aggregation of LAB ranged from 21.8 % to 46.37 %, with the highest levels of autoaggregation obtained in *L. rhamnosus* A072 after 5 h. *L. rhamnosus* A012 had 32.52 % auto-aggregation while *L. plantarum* A011 had 20.83% auto-aggregation respectively. *L. rhamnosus* A012 was able to exclude the growth of enteropathogenic, enteroinvasive and Shiga toxin-producing *E. coli* in the coaggregation assay, while *L. pentosus* B1b and *P. pentosaceus* A074 competitively displayed enteropathogenic and enteroinvasive *E. coli* strains in the coaggregation assay. This indicates that LAB strains used in this study can protect the intestinal epithelium from infection caused by diarrheagenic *E. coli*.

5.1.4. Safety of probiotic strains

The safety of any strain used as probiotic is indispensable. One of the safety considerations for probiotic strains is to verify that a potential probiotic strain does not carry any transferrable antibiotic resistance gene. Transferrable resistance of probiotic strain to antibiotic might pose a potential risk on human health, as there could be a horizontal transfer of the resistance gene to pathogenic microorganisms (Asan-Ozusaglam and Gunyakti 2018). In this study, all the tested LAB strains are susceptible to gentamicin. This correlates with Tulumoglu *et al*. (2013) report where 96% of the LAB used showed sensitivity to gentamicin. Moreover, all the LAB strains are susceptible to vancomycin, unlike the report that *Lactobacillus* has a natural resistance to vancomycin (Tulini *et al*., 2013), but correlates with the report of Zhang *et al*. (2016), in which 100% of LAB strain in the study showed sensitivity to vancomycin. Therefore, this study`s report contradicts the fact that all *Lactobacillus* species have a natural resistance to vancomycin.

Furthermore, 73.40% of LAB strains in this study showed sensitivity to tetracycline All the *Lactobacillus* species are susceptible to tetracycline but *Pediococcus*, *Leuconostoc* and *Weisella* species showed resistance. This has also been reported by Zhang *et al*. (2016) in which 70% of LAB strains obtained from Yak milk showed resistance to tetracycline, in addition, 100% of LAB isolated from infant faeces were also susceptible to tetracycline (Tulumoglu *et al*., 2013). Ampicillin can effectively inhibit the growth of Gram-positive microorganisms. In this study, only 20% of the potential probiotic strains were sensitive to ampicillin, while, 80% were resistance. The observed resistance of LAB to ampicillin, a ß-lactam antibiotic correlates with Lavanya *et al*. (2011)'s report, where 90% of the LAB isolated from fermented milk are resistance to ampicillin. There was 86.6% susceptibility of LAB strains isolated in this study to clindamycin while 66.67% of the LAB strains isolated were susceptible to erythromycin, this, therefore, support the report of Campedelli *et al*. (2018) that *Lactobacillus* species are susceptible to erythromycin, clindamycin and tetracycline. Meanwhile, this study is in contrast with the work of Sukmarini *et al*. (2014), where LAB isolated from fermented foods were reistance to erythromycin and chloramphenicol. Thumu and Halami (2012) also claimed that LAB isolated from fermented foods carry erythromycin resistance genes. However, the result from this study revealed that LAB isolated from human (mothers' breast milk and neonates' faeces) are susceptible to erythromycin.

Interestingly, *L. rhamnosus* A012 was susceptible to all the 21 antibiotics tested,. This has also been reported by Drago *et al*. (2011), in which *L. rhamnosus* GG showed no resistance to any antibiotic and considered as GRAS. However, *L. rhamnosus* A072 which is, another strain of *L. rhamnosus* isolated in this study was resistant to ampicillin, erythromycin, streptomycin and clindamycin. This strengthens the fact that probiotic strains safety is strain specific and not genus specific. The antimicrobial susceptibility results in this study showed that *L. rhamnosus* A012, *L. pentosus* A4c, *L. pentosus* B1b and *L. plantarum* A011 strains could be potential probiotics based on the possible absence of resistance to selected antibiotics and these strains may not possibly transfer antibiotic resistance.

Another important safety requirement for a probiotic strain is the evaluation of the haemolytic activity of potential probiotic strain (FAO/WHO, 2002). In this study, none of the LAB strains lyses the red blood cell; this showed they are non-haemolytic. This

result is in correlation with Asan-Ozusaglam and Gunyakti (2018) and García *et al*, (2017) reports, where *L. fermentum* strains and other *Lactobacillus* species used in both studies are non-haemolytic. In this study, LAB isolates from mothers' breast milk and infants' faeces do not possess haemolytic properties.

5.1.5. *Invivo* immunomodulatory property of lactic acid bacteria as probiotics

One of the major criteria of probiotic strains is the ability to modulate immune responses. Immune systems are crucial; they comprise of numerous immune cells that defend the host against infection and maintain the homeostasis condition of the host. Dysfunction of the immune system due to an underlying illness, aging, mental stress, chronic diseases and cancer therapy could lead to immunological alterations resulting in inflammatory disease and damaging the immune cells (Mendes *et al*., 2019).

The gastrointestinal tract is a large organ consisting of innumerable microbes co-existing together in the host intestine, also, it comprises of the immune system with various organs such as bone marrow, spleen, thymus and lymph node that serve as the powerhouse of the immune cells. Bifidobacteria and lactic acid bacteria used as probiotic contribute to the defensive function of the immune system thereby playing a crucial role in host defence mechanism through modulating, stimulating and regulating immune system through the innate and adaptive immune cell response (Bajagai *et al.*, 2016; Ding *et al.*, 2017: Mendes *et al*., 2019). Since probiotics are strain sensitive, it is therefore imperative to evaluate the protective role and immunomodulatory activity of potential probiotic strains in cyclophosphamide induce immunosuppressive mice.

Cyclophosphamide is used in chemotherapy treatment of cancer, its clinical effects as an immunosuppressive agent, alteration in the response of immune cells, organ impairment and damaging of the immune cells from its use have been reported by differentr authors (Kwon *et al*., 2018; Meng *et al*., 2018; Zhou *et al*., 2018). Therefore, the selection of cyclophosphamide to modelling immunosuppression was based on this clinical effect. This study showed a significant weight loss in immunosuppressive mice during three days of cyclophosphamide treatment in all the groups (group 1, 2, 3, 4), except the group without the treatment (group 5, 6, 7), as compared to their weight before the treatment, indicating the toxicity effect of the drug. Cyclophosphamide produce

toxins and has been known to cause weight loss, diarrhea, darkening of the skin/nails, alopecia (hair loss). The induced mice reduced greatly in weight as a result of toxin that was released from the administration of cyclophosphamide (Zhou *et al*. 2018) in which the weight of the mice used for the study significantly reduced after the treatment. During the intervention, the experimental mice induced and treated with levamisole HCl (group 2) treated with lactobacilli (group 3, , and group 4), gradually had a weight increase in contrast with the experimental mice in group, (CTX+PBS). Also, reduction in white blood cell counts, loss of appetite, an uncontrollable turning of neck and diarrhoea were observed in group 1 (cyclophosphamide treated mice without intervention). However, in contrast with group 1 mice, group 2 (induced and treated with levamisole hydrochloride), group 3 (induced and treated with *L. rhamnosus* A012) and group 4 mice (induced and treated with *L. plantarum* A011) have no significant reduction in their WBC as compared to immunocompetent group, that is the normal group (group 7), group 5 (*L. rhamnosus* A012) and group 6 (*L. plantarum* A011). This shows the protective effect of *Lactobacillus* specie from immunological damage.

Also, group 1 mice (CTX + PBS) were down with diarrhoea, indicating their intestinal epithelial cells were damaged by the toxic effect of cyclophosphamide. However, the mice in *Lactobacillus* treatment groups were restored from the gastrointestinal motility caused by the alkalating agent through the intervention of *Lactobacillus* strains and became stabilized as group 7 (healthy mice) and immunocompetent groups (*L. rhamnosus* A012 only and *L. plantarum* A011 only), indicating the ability of *Lactobacillus* strains to protect the intestine.

Spleen accommodates all the immune cells that protect the host and plays a major role in regulating the immune responses that are harmful to the host. It can accumulate immunosuppressive myeloid cells (Bronte *et al*., 2013). However, Sabry *et al*. (2015) reported the ability of cyclophosphamide to increase the fraction of suppressive myeloid cells in spleen and blood, and this affects the function of the immune system. Experimental induced mice have a reduction in spleen index in comparison with the healthy mice (untreated mice), indicating the toxic effect of cyclophosphamide. Nevertheless, the spleen index increased in induced and treated with *Lactobacillus* sp in comparison with group 1 (induced mice) indicating the ameliorating effect of *Lactobacillus* from depletion of the spleen. This study is in correlation with the study of

Kwon *et al*. (2018) and Meng *et al*. (2018) in which the *Lactobacillus* strain used in their studies made the spleen recover from damage caused by cyclophosphamide in immunosuppressed mice as the lymph and spleen index significantly increased compared to the model group.

Moreover, the study evaluates the ability of *L. rhamnosus* A012 and *L. plantarum* A011 to stimulate and regulate the production of pro-inflammatory and anti-inflammatory cytokines. TNF-α is a pro-inflammatory cytokine, it is activated and produced in the macrophage and dendritic cells and inflammatory condition like intestinal inflammation (Mendes *et al*., 2019). As a cell signal cytokine, TNF-α responds to inflammatory condition by triggering the molecules of the immune system and induce the neutrophil activation, which is a defining step in the inflammatory response (Mendes *et al*., 2019). TNF-α is hardly noticed in a healthy individual but can be found as an endogenous pyrogen, involving in the dysfunction and destruction of the intestinal epithelial barrier, inducing epithelial cells apoptosis, and play a key role in the entry and colonization of bacterial pathogens (Lee *et al*., 2017). Tumor necrotic factor alpha were significantly upregulated in experimental induced mice, in contrast, it was ameliorated in induced mice treated with *Lactobacillus* sp, As a consequence, the production of TNF-α was regulated to a moderate level.

IL-6 has a wide influence on the immune system as its response triggers with either a pro-inflammatory or an anti-inflammatory profile. The stimulation of IL-6 triggers when aggravated by acute intestinal inflammation similar to other pro-inflammatory immune response (Kittana *et al*., 2018). Also, IL-6 was upregulated in experimental induced mice but when treated with *Lactobacillus* sp the proinflammatory cytokines was moderately upregulated this shows that *Lactobacillus* sp ameliorated the effect of cyclophosphamide in induced mice. In contrast to TNF-α and IL-6, IL-10 is an anti-inflammatory cytokine; it maintains the balance in the immune system by regulating the production of pro-inflammatory cytokines. It binds to IL-10R1, a specific receptor for IL-10, where it exerts its mechanism of action by initiating the signal transduction activity using Jak-STAT pathway, thereby controlling the proliferation and differentiation of macrophages (Mendes *et al*., 2019). Through the signally transduction, it strengthens the intestinal epithelial barrier and control the permeability of the microbes and therefore serve as the central regulator of the inflammatory response and mucosa immune system (Kittana *et al*., 2018; Mendes *et al*., 2019). In this study therefore, IL-10 was down-regulated in

experimental induced mice but was significantly upregulated in mice treated with *Lactobacillus* and lower the expression of pro-inflammatory cytokines (such as TNF-α and IL-6.

In this study, the induced mice produced a high level of TNF-α compare to other groups at $p<0.001$. The increase indicates the cell signalling of TNF-α, as it sends a signal to other immune systems. Meanwhile, the increase in the stimulation of TNF-α can cause a deleterious effect on the eperimental mice in group 1, as a result of the involvement of TNF-α in the destruction of the intestinal barrier leading to apoptosis of epithelial cell (Mendes *et al*., 2019). However, treatment with levamisole hydrochloride and *Lactobacillus* strains ameliorate the effect of cyclophosphamide on the mice in group 2,3 and 4, the standard drug, *L. rhamnosus* A012 and *L. plantarum* A011 down-regulated the production of TNF-α and lower its expression. The level of TNF-α in the levamisole hydrochloride treatment group and the two *Lactobacillus* strains treatment group significantly reduced compared to PBS treatment group (group 1). Meanwhile, the level of TNF-α in healthy and immunocompetent mice was low to support the report that, TNF-α is not usually detected in a healthy individual. *L. rhamnosus* A012 and *L. plantarum* A011 lowered the expression of pro-inflammatory cytokines in untreated mice in contrast to Kwon *et al*. 2018 report where the production of TNF-α was elevated by *Lactobacillus* strains used. However, this study correlates with the findings of Liu *et al*. (2017) and Mendes *et al*. (2019), where *Lactobacillus* strains decreased the expression of TNF-α.

The level of IL-6 significantly increased in induced mice compared to induced but treated with *Lactobacillus* ($p<0.05$). Meanwhile, its production was higher in induced but treated with *Lactobacillus* than the group not induced, indicating that, the standard drug, *L. plantarum* A011 and *L. rhamnosus* A012 modulate the stimulatory and regulatory response of IL-6 cytokine since its response is triggered in both pro-inflammatory and anti-inflammatory profile. This finding correlates with the report of Liu *et al*. (2017) and Kwon *et al*. (2018), in which the *Lactobacillus* strains modulate the production and regulation of IL-6 cytokines.

Our findings showed that *L. plantarum* A011 and *L. rhamnosus* A012 modulate the production of IL-10 cytokines; the cytokine was upregulated in groups 3 and 4 as the

level was significantly higher than group one. The standard drug also modulated the production of this anti-inflammatory cytokine but not as much as the two strains of *Lactobacillus* species. Comparing the production of IL-10 in group 2, group 3, and group 4, (intervention groups) with the healthy group, the response of this cytokine was up regulated in intervention groups, indicating the action of *Lactobacillus* strain in restoring the gut microbiota and strengthen the intestinal epithelial barrier that was damaged through the action of cyclophosphamide, defending the host. Therefore, this study supports the report of Xie *et al*. (2015), Kwon *et al*. (2018), Meng *et al*. (2018) and Mendes *et al*. (2019), in which *Lactobacillus* strains modulate the response of IL- 10 in immunosuppressed mice.

The normal cellular and humoral immune responses in the host is essential, in order to maintain this, Th1 and Th2 must function at a stable equilibrium condition. The T-helper 1 cells secrete the production of IFN-γ and TNF-α. They play a principal role in cell-mediated immunity; on the other hand, Th2 cells secrete the production of IL-6 and IL-10 to regulate the humoral immune system (Meng *et al*., 2018). In this study, administration of cyclophosphamide cause reduction in immune system which generally leads to imbalance in Th1/Th2 cells resulting in immunosuppression, this supports the study of Kawashima *et al*. (2017) and Kwon *et al*. (2018) where the same effect of imbalance in immune cells of cyclophosphamide treated mice was reported. Nevertheless, *L. plantarum* A011 and *L. rhamnosus* A012 used in this study, successfully moderate the immune responses, this supports the claims from previous experimental works, in which different probiotic strains moderate the Th1/Th2 immune responses (Xie *et al*., 2016; Meng *et al*., 2018); Kwon *et al*., 2018). Therefore, the modulatory effect demonstrated by *L. plantarum* A011 and *L. rhamnosus* A012 in this study indicates that these potential probiotic strains can maintain the intestinal immune balanced by contributing to the stimulation and regulation of cytokines (TNF-α, IL-6 and IL-10) and thereby regulating the Th1/Th2 balance.

Furthermore, histological examination of ileum showed cell infiltration and inflammation in induced experimental mice, (CTX+PBS). Also, treatment with cyclophosphamide affected the dividing hematopoietic cells indicating neutropenia, which results in a decrease of immune cells. This report correlates with the report of Sabry *et al*. (2015) in which treatment with cyclophosphamide affect the lymphoid organ

and caused neutropenia and lymphopenia in mice. However, the treatment with the standard drug and *Lactobacillus* strains reversed the effect of intervention groups (group 2, 3 and 4), where the villus height and crypt depth of intestinal epithelial cell significantly improved compared to group one. The result is in agreement with a report in which *L. plantarum* isolate from a vegetable reverse the damage of crypt depth and villus height by cyclophosphamide (Xie *et al*., 2016).

5.2. Conclusion

Infantile diarrhoea poses a health risk in Nigeria. The outcome of this research indicates that lactic acid bacteria isolated from human breast milk and neonate's faeces are potential therapeutic agents against diarrhoeagenic *E. coli* infections. The antimicrobial activity displayed by the LAB species against diarrhoeagenic *E. coli* strains in this work are due to the metabolites produced by LAB and the cells themselves through adherence and competitive exclusion. *L. plantarum* A011 and *L. rhamnosus* A012 of faecal origin were able to survive the simulated gastrointestinal environment in the gut. Both strains inhibit the production of antibiofilm by enteroaggregative *E. coli* strain indicating the ability of the two potential probiotic strains to intercept the resistance tactics of the pathogen. *L. rhamnosus* A012 and *L. plantarum* A011 are non-hemolytic and *L. rhamnosus* A012 is susceptible to all tested antibiotics. The two strains possess immunomodulatory properties, as they significantly stimulate and upregulate the anti-inflammatory cytokine (IL 10), and downregulating the stimulation of pro-inflammatory cytokines (TNF-α and IL 6). These properties make these bacteria to be good potential probiotics that could be used for treatment of diarrhoeagenic *E. coli* infections.

5.3. Contribution to knowledge

- To the best of our knowledge, this study is the first to determine the diversity of LAB isolated from from mother-child pair in Nigeria and it is the first to report *L. rhamnosus, E. thailandicus* and *L. xianqfangensis* from mothers' breast milk and infants' faeces in Nigeria.
- This study is the first to report the immunomodulatory ability of *Lactobacillus* strains isolated from mothers' breast milk and infants' faeces in Nigeria.
- The *L. plantarum* A011 and *L. rhamnosus* A012 strains isolated from mothers' breast milk and neonate faeces in Nigeria could be potential probiotic strains.

5.4. Recommendations

It is recommended that the Nigerian government should make probiotic products available, especially, to the infant's that are not being breast fed through complication that may arise during childbirth.

There should be more awareness of the benefit of probiotics as there is a paucity of information on probiotic products.

Nursing mothers are encouraged to exclusively breastfed their children as this can also manage diarrhoeal infection through resident beneficial microbes and serve as a good alternative to antibiotics.

Future Directions

Studies that are more clinical should be carried out on *L. rhamnosus* A012 and *L. plantarum* A011 strains, using experimental animal and human trials, to fully ascertain its candidacy as probiotic.

Further information on the genomics of *L. rhamnosus* A012 and *L. plantarum* A011 are essential using metagenomics analysis to have better information on the strains.

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APPENDIX I

Equipment and consumables

Hot air oven

Bunsen burner

Incubator

Autoclave

Innoculating loop

Centrifuge

Microscope

Refrigerator

Weighing balance

Test tube racks

Universal bottles

Vortex

Micropipette

Eppendorf tubes and tips

Water bath

Agarose gel casting trays and combs

Gel chambers

Power suppliers

Gel reader

Petri dish

pH Meter

Spectrophotometer

Cycler Thermal Cycler

AnaeroGen

Anaerobic Jar

Refrigerator (-80o)

0.45 pore size filter

Gene sequencer ABI PRISM (Applied Biosystem, Foster City, CA, USA).

APPENDIX II

Enzymes, Standard solutions Chemicals and Kit

Ethanol

Glycerol

Isopropanol

RNase

Proteinase K

AnaerogenGen^{TM}Oxoid, UK

Accu® Prep Genomic DNA extraction kit. (BIONEER).

JENA Bioscience Genomic DNA extraction kit

BioLegend IL-6 Cytokine kit

BioLegend IL-10 Cytokine kit

BioLegend TNF-α Cytokine kit

Phosphate Buffer Solution

Cyclophosphamice compound

Levamisole HCl

APPENDIX III

Growth media, reagent and buffers

The bacterial growth media, reagent are listed below with their respective preparation for cultivation of bacteria.

MRS (Mann Rogosa Sharpe) Broth (Oxoid)

Polypeptone	10.0 g
Meat extract	10.0 g
Yeast extract	5.0 g
Glucose	20.0 g
Tween 80	1.08 ml
Di-potassium phosphate	2.0 g
Sodium acetate	5.0 g
Ammonium citrate	2.0 g
Magnesium sulphate	0.2 g
Manganese	0.05 g
pH	6.4

Suspend the 52 g in 1L of distill water. Mix thoroughly. Heat with frequent agitation and boil until all the powders have completely dissolved. Autoclave at 121oC for 15 minutes.

MRS agar supplemented with 0.5g of L-cysteine HCl in 1L

Mueller Hinton Agar (Becton, Dickson and Co, Spark, MD, USA)

Suspend 38.0g of the powder in 1L of distilled water. Mix thoroughly, heat with frequent agitation and boil until all the powder completely dissolved. Autoclave at 121^{o} for 15 minutes.

Phosphate Buffer Saline (PBS)

NaCl	9.0
Na2HPO4	1.15
KH2PO4	0.3
pH	7.2

APPENDIX IV

Identification of lactic acid bacteria by partial sequencing of 16S rRNA genes

Code of Isolates	IDENTITY OF ISOLATES	TOTAL SCORE	% SIMILARITY	Accessions No
ATO002	*L. plantarum*	1464	100.00%	SRR11626722
A003	*E. thailandicus*	75	100.00	SRR11626711
A008	*E. faecium*	71.3	98.00	SRR11626751
A010	*L. pseudomesenteroides*	324	94.00	SRR11626750
A011	*L. plantarum*	3719	100.00	SRR11626749
A012	*L. rhamnosus*	2576	100.00	SRR11626748
A013	*E. durans*	946	99.00	SRR11626747
A014	*L. plantarum*	587	100.00	
A016	*L. pentosus*	1040	99.00	SRR11626746
A017	*L. paracasei*	4299	99.79	SRR11626745
A018	*E. faecium*	4039	99.20	SRR11626743
A019	*L. pseudomesenteroides*	1200	97.00	SRR11626742
A022	*E. faecium*	7299	98.97	SRR11626740
A023	*L. plantarium*	5473	99.83	SRR11626739
A024	*E. faecium*	7520	98.06	SRR11626738
A026	*L. pseudomesenteroides*	839	99.00	SRR11626737
A027	*E. thailandicus*	1181	93.50	
A028	*L. pentosus*	1209	99.70	SRR11626736
A030	*L. pseudomesenteroides*	1103	99.00	SRR11626734
A031	*E. thailadicus*	58.4	96.79	
A033	*L. plantarum*	4744	100.00	SRR11626732
A034	*L. plantarum*	5708	99.37	SRR11626731
A035	*L. plantarum*	5689	99.84	SRR11626730
A036	*L. plantarum*	6123	100.00	SRR11626729
A037	*L. plantarum*	5048	98.43	SRR11626728
A038	*E. durans*	1103	99.00	SRR11626727
A039	*E. faecium*	4728	99.77	SRR11626726
A040	*E. faecium*	7121	98.37	SRR11626725
A041	*L .plantarum*	5307	100.00	SRR11626724
A043	*E. faecalis*	1101	99.00	SRR11626723
A044	*L. pseudomesenteroides*	1110	99.00	SRR11626721
A046	*L. plantarum*	6520	99.58	SRR11626720
A047	*L. plantarum*	5381	99.49	SRR11626719
A048	*L. plantarum*	5800	99.53	SRR11626718
A049	*E. faecium*	6069	98.78	SRR11626717
A050	*E. durans*	1106	99.72	SRR11626716
A051	*L. plantarum*	5242	99.48	SRR11626715
A052	*E. lactis*	1155	99.02	SRR11626714
A058	*E. faecalis*	4290	99.49	SRR11626713
A059	*L. plantarum*	6206	99.41	SRR11626712

Code of Isolates	*IDENTITY OF ISOLATES*	TOTAL SCORE	% SIMILARITY	Accessions No
A060	*E. lactis*	1101	99.00	SRR11626710
A064	*L. pseudomesenteroides*	1150	99.00	SRR11626709
A066	*E. faecium*	4518	99.76	SRR11626708
A071	*L. plantarum*	5325	100.00	SRR11626707
A072	*L. rhamnosus*	4247	98.94	SRR11626706
A074	*P. pentosaceus*	4847	100.00	SRR11626705
A075	*L. plantanum*	5717	99.52	SRR11626704
A077	*E. faecalis*	4125	99.55	SRR11626703
A079	*L. plantarum*	6326	99.57	SRR11626701
A080	*E .faecium*	7498	99.56	SRR11626699
A081	*E. lactis*	1269	99.00	SRR11626698
A082	*L. Pseudomesenteroides*	277	95.00	SRR11626697
A083	*E. faecium*	7144	98.52	SRR11626696
A084	*L. plantarum*	5150	99.82	SRR11626695
A087	*E. faecium*	6043	99.64	SRR11626694
A089	*L. Pseudomesenteroides*	1127	99.00	SRR11626693
A090	*E. faecium*	6778	99.05	SRR11626692
A093	*L. Pseudomesenteroides*	1219	99.00	SRR11626691
A094	*L. plantarum*	5648	100.00	SRR11626690
A095	*E. durans*	1251	99.00	SRR11626688
A096	*E. lactis*	1199	99.00	SRR11626687
A097	*E. durans*	1122	98.58	SRR11626686
A098	*E. durans*	848	99.00	SRR11626685
A4d	*E. faecalis*	5442	94.6	
B3b	*W. cibaria*	12285	99.78	SRR11626675
A1d	*L. pentosus*	8145	99.78	SRR11626744
B3a	*W. cibaria*	13157	99.45	SRR11626676
A3b	*L. fermentum*	8121	99.55	SRR11626689
B3c	*L. plantarum*	6489	98.42	
A4b	*L. plantarum*	6412	98.52	
B1b2	*L. plantarum*	5268	95.54	
A1c	*L. plantarum*	7414	96.22	SRR11626670
B4a	*W. cibaria*	12891	98.89	SRR11626674
A4F	*L. pentosus*	8090	99.11	SRR11626752
B1e	*W. cibaria*	13142	99.34	SRR11626679
A3a	*L. fermentum*	8204	99.67	SRR11626700
B4c	*W. cibaria*	13275	99.24	SRR11626673
A4c	*L. pentosus*	8099	99.66	SRR11626753
B1d	*W. confusa*	11546	95.53	SRR11626680
A1a	*L. pentosus*	8108	99.33	SRR11626671
B1f	*L. plantarum*	8090	99.66	SRR11626677
A3d	*L. fermentum*	6598	92.41	SRR11626672
B1c	*L. plantarum*	8042	98.89	SRR11626681
A3c	*L. fermentum*	8104	99.00	SRR11626678
B1b	*L. pentosus*	8127	99.66	SRR11626682
A1e	*L. pentosus*	8117	99.77	SRR11626733

Code of Isolates	*IDENTITY OF ISOLATES*	TOTAL SCORE	% SIMILARITY	Accessions No
B1a	*L. plantarum*	6298	94.82	SRR11626684
B4b	*L. pentosus*	1156	98.20	
B1a2	*L. xianqfangensis*	383	85.25	SRR11626683
A078	*E. faecalis*	5479	93.28	SRR11626702
A004	*E. durans*	9603	99.77	SRR11626669
A020	*E. durans*	9614	99.66	SRR11626741
A029	*E. durans*	9516	99.06	SRR11626735
A063	*E. faecalis*	9428	99.52	

APPENDIX V

Antimicrobial activity assay of viable cells of lactic acid bacteria on pathotypes of *E. coli*

Name of Isolates	Source of the Isolates	ETEC (H40B)	EPEC (H62E)	EIEC (H68D)	STEC (H77E)	EAEC (H40C)	EAEC (D49B)	EAEC (D47C)
L. plantarum A002	Breast Milk	20. ±0.0	0	8±0.2	13±0..0	9±0..0	8±0..0	8±0..0
E. faecium A003	Breast Milk	16±0..0	10±0..0	11±0..0	13±0..0	14±0..0	12±0..0	10±0..0
E. faecium A008	Breast Milk	14±0..0	8±0..0	0	16±0..0	15±0..0	12±0..0	12±0.
L. pseudomenseteroides A010	Breast milk	16±0..0	7±0..0	8±0..1	18±0..1	14±0..2	9±0..0	8±0..0
L. plantarum A011	Faeces	15±0..1	12±0..3	10±0..2	21±0..0	18±0..1	10±0..0	10±0..0
L. rhamnosus A012	Faeces	24±0..2	10±0..0	14±0..0	17±0..0	16±0..1	12±0..0	14±0..0
E. durans A013	Faeces	24±0..1	12±0..4	8±0..3	17±0..1	16±0..3	10±0..0	8±0..0
L. plantarum A014	Faeces	12±0..2	14±0..2	14±0..1	14±0.1	18±0..1	14±0..0	14±0..0
L. pentosus A016	Breast Milk	14±0..4	11±0..3	0	18±0..2	15±0..1	0	0
L. paracasei A017	Breast Milk	18±0..1	0	0	15±0.	16±0..0	10±0..0	12±0..0
E. faecium A018	Breast Milk	9±0..3	12±0..1	10±0..2	19±0..1	16±0..3	15±0..0	12±0..0
L. pseudomenseteroides A019	Breast Milk	14±0..1	0	0	21±0..2	19±0..1	0	8±0..0
E. faecium A022	Breast Milk	13±0..6	0	10±0..0	14±0..0	14±0..2	22±0..0	14±0..0
L. plantarum A023	Feaces	21±0..2	12±0..1	14±0..1	16±0..3	11±0..2	19±0..0	14±0..0
E. durans A024	Feaces	16±0..2	12±0..0	12±0..0	19±0..1	20±0..2	0	17±0..0
L. pseudomenseteroides A026	Faeces	12±0..2	13±0..2	14±0..1	14±0..2	16±0..1	0	0
E. .thailandicus A027	Faeces	10±0..1	16±0..0	11±0..0	12±0..1	21±0..2	12±0..0	14±0..0
L. pentosus A028	Breast Milk	16±0..1	16±0..2	10±0.1	16±0..1	13±0..2	12±0..0	12±0..0
L. pseudomenseteroides A030	Feaces	15±0..3	0	0	12±0..2	16±0..0	0	12±0..0
E. thailandicus A031	Faeces	10±0..1	0	0	14±0..2	12±0..1	0	8±0..0
L. plantarum A033	Breast Milk	10±0..3	0	0	21±0..2	16±0..3	10±0..0	8±0..0
L. plantarum A034	Breast Milk	16±0..0	10±0..2	12±0..0	18±0..1	18±0..0	11±0..0	11±0..0
L. plantarum A035	Breast Milk	12±0..1	16±0..3	14±0..1	17±0..0	18±0..6	0	7±0..0
L. plantarium A036	Breast Milk	15±0..1	12±0..0	0	16±0..1	12±0..1	15±0..0	14±0..0
L. plantarium A037	Breast Milk	13±0..2	0	0	16±0..1	15±0..2	8±0..0	7±0..0
E. durans A038	Breast Milk	11±0..1	14±0..0	0	14±0..2	14±0..1	0	0
E. faecium A039	Breast Milk	24±0..1	18±0..1	10±0..1	21±0..0	16±0..6	0	10±0..0
E. faeciuum A040	Breast Milk	18±0..2	14±0..1	12±0..4	14±0..0	17±0..4	8±0..0	7±0..0
L. plantarium A041	Breast Milk	19±0..1	0	0	18±0..1	16±0..2	12±0..0	11±0..0
E. durans A043	Breast Milk	12±0..2	0	0	14±0..0	8±0..4	8±0..0	0
L. pseudomenseteroides A044	Breast Milk	16±0..2	12±0..4	13±0..2	17±0..1	19±0..0	13±0..0	13±0..0
L. plantarium A046	Breast Milk	20±0..1	16±0..3	10±0..5	15±0..2	19±0..3	14±0..0	10±0..0
L. plantarium A047	Breast Milk	19±0..2	17±0..1	0	14±0..1	10±0..4	8±0..0	16±0..0
L. plantarium A048	Breast Milk	15±0..1	14±0..3	0	10±0..0	12±0..1	8±0..0	12±0..0
E. faecium A049	Breast Milk	12±0..1	0	0	16±0..3	16±0..0	8±0..0	0
E. durans A050	Breast Milk	21±0..1	14±0..4	14±0..1	16±0..1	18±0..2	10±0.1	0
L. plantarium A051	Breast Milk	18±0..1	14±0..2	8±0..3	21±0..3	18±0..1	12±0..0	12±0..0
E. lactis A052	Breast Milk	10±0..1	12±0..1	0	19±0..3	21±0..1	14±0..0	14±0..0

Name of Isolates	Source of the Isolates	ETEC (H40B)	EPEC (H62E)	EIEC (H68D)	STEC (H77E)	EAEC (H40C)	EAEC (D49B)	EAEC (D47C)
E. faecalis A058	Faeces	14±0..1	0	0	0	21±0..2	0	12±0..0
L. plantarium A059	Faeces	23±0..0	10±0..1	10±0..0	22±0..1	23±0..1	8±0..0	10±0..0
E. lactis A060	Faeces	13±0..2	0	0	11±0..1	14±0..5	0	7±0..0
L. pseudomenseteroides A064	Faeces	21±0..1	12±0..0	10±0..0	16±0..3	22±0..1	16±0..0	17±0..0
E. faecium A066	Faeces	20±0..1	11±0..2	12±0..2	20±0..1	12±0..3	10±0..0	8±0..0
L. plantarum A071	Breast Milk	13±0..2	12±0..2	0	11±0..2	13±0..1	8±0..0	8±0..0
L. rhamnosus A072	Breast Milk	20±0..3	16±0..4	10±0..1	17±0..0	16±0..1	11±0..0	8±0..0
P. pentosaceus A074	Breast Milk	21±0..1	15±0..2	10±0..2	22±0..1	23±0..1	12±0..0	10±0..0
L. plantarum A075	Breast Milk	16±0..0	17±0..0	11±0..2	16±0..2	16±0..0	8±0..0	0
E. faecalis A077	Faeces	25±0..1	14±0..2	8±0..2	21±0..1	18±0..2	8±0..0	0
L. plantarum A079	Faeces	13±0..2	10±0..1	0	19±0..1	16±0..1	13±0..0	8±0..0
E. faecium A080	Breast Milk	18±0..1	15±0..2	8±0..1	20±0..2	20±0..3	15±0..0	14±0..0
E. lactis A081	Breast Milk	21±0..1	14±0..3	0	23±0..1	14±0..1	13±0..0	14±0..0
L. pseudomenseteroides A082	Breast Milk	16±0..1	14±0..1	8±0.2	14±0..0	12±0..0	10±0..0	10±0..0
E. faecium A083	Breast Milk	21±0..2	15±0..1	10±0..1	20±0..0	18±0..0	0	8±0..0
L. plantarium A084	Breast Milk	14±0..0	10±0..1	12±0..0	19±0..0	11±0..0	9±0..0	14±0..0
E. faecium A087	Faeces	18±0..0	16±0..0	10±0..0	20±0..0	19±0..0	12±0..0	10±0..0
L. pseudomenseteroides A089	Faeces	14±0..0	12±0..0	14±0..0	14±0..0	16±0..1	14±0..0	15±0..0
E. faecium A090	Faeces	19±0..0	10±0..2	0	23±0..1	21±0..2	0	0
L. pseudomenseteroides A093	Breast Milk	26±0..0	16±0..0	7±0..0	10±0..0	11±0..0	12±0..0	10±0..0
L. plantarium A094	Faeces	10±0..0	14±0..0	0	14±0..1	11±0..2	8±0..0	10±0..0
E. durans A095	Faeces	21±0..0	17±0..0	0	16±0..0	23±0..0	15±0..0	14±0..0
E. lactis A096	Faeces	14±0..0	0	0	14±0..0	12±0..2	8±0..0	0
E. durans A097	Faeces	18±0..0	12±0..0	0	17±0..0	19±0..0	14±0..0	15±0..0
E. durans A098	Faeces	12±0..0	17±0..0	10±0..0	17±0..0	13±0..0	15±0..0	15±0..0
E. faecalis A4D	Faeces	14±0..0	12±0..0	12±0..0	8±0..0	14±0..0	16±0..0	14±0..0
W. cibaria B3b	Breast Milk	10±0.2	10±0..0	0	0	10±0..0	16±0..0	14±0..0
L. pentosus A1d	Faeces	8±0..0	8±0..0	0	0	0	0	0
W. cibaria B3a	Breast Milk	10±0..0	12±0..0	14±0..0	14±0..0	12±0..0	14±0..0	15±0..0
L. fermentum A3b	Faeces	0	0	8±0..0	8±0..0	0	10±0..0	11±0..0
L. plantarum B3c	Breast Milk	16±0..0	8±0..0	14±0..0	14±0..0	10±0..0	16±0..0	13±0..0
L .plantarum A4b	Faeces	13±0..0	12±0..0	8±0..0	10±0..0	12±0..0	16±0..0	18±0..0
L. plantarum B1b2	Breast Milk	14±0..0	0	18±0..0	0	17±0..0	20±0..0	18±0..0
L. plantarum A1c	Faeces	13±0..0	12±0..0	15±0..0	13±0..0	9±0..0	14±0..0	15±0..0
W. cibaria B4a	Breast Milk	15±0..0	0	12±0..0	0	0	8±0..0	11±0..0
L. pentosus A4f	Faeces	12±0..0	0	10±0..0	16±0..0	14±0..0	12±0..0	12±0..0
W. cibaria B1e	Breast Milk	12±0..0	0	0	0	8±0..0	11±0..0	0
L. fermentum A3a	Faeces	8±0..0	0	14±0..0	13±0..0	10±0..0	13±0..0	10±0..0
W. cibaria B4c	Breast Milk	8±0..0	0	0	0	0	0	0
L. pentosus A4c	Faeces	15±0..0	14±0..0	13±0..0	15±0..0	14±0..0	12±0..0	11±0..0

Name of Isolates	Source of the Isolates	ETEC (H40B)	EPEC (H62E)	EIEC (H68D)	STEC (H77E)	EAEC (H40C)	EAEC (D49B)	EAEC (D47C)
W. confusa B1d	Breast Milk	8±0..0	0	0	0	0	0	0
L. pentosus A1a	Faeces	8±0..0	8±0..0	10±0..0	14±0..0	15±0..0	0	15±0..0
L. plantarum B1f	Breast Milk	0	0	8±0..0	8±0..0	7±0..0	0	0
L. fermentum A3d	Faeces	0	0	8±0..0	0	7.0	11±0..0	14±0..0
L. plantarum B1c	Breast Milk	10±0..0	10±0..0	16±0..0	9±0..0	16±0..0	13±0..0	17±0..0
L. fermentum A3c	Faeces	0	14±0..0	12±0..0	11±0..0	8±0..0	12±0..0	14±0..0
L. pentosus B1b	Breast Milk	12±0..0	17±0..0	26±0..0	26±0..0	14±0..0	10±0..1	16±0..0
L. pentosus A1e	Faeces	10±0..0	0	20±0..0	15±0..0	14±0..0	8±0..0	0
L. plantarum B1a	Breast Milk	14±0..0	0	12±0..0	13±0..0	14±0..0	12±0..0	16±0..0
L. Xianqfangensis B1a2	Breast Milk	0	12±0..0	12±0..0	0	11±0..0	22±0..0	20±0..0
E. durans A004	Breast Milk	0	8.0	0	16.0	14.0	0	7.0
*E. faecalis*A020	Breast Milk	18±0..0	12±0..0	10±0..0	15±0..0	16±0..0	0	12±0..0
E. faecalis A029	Breast Milk	18±0..0	15±0..0	8±0..0	18±0..0	16±0..0	15±0..0	8. ±0.0
E. faecalis A063	Faeces	0	0	0	16±0..0	0	0	7±0..0
E. faecalis A078	Faeces	18±0..0	0	0	0	21±0..0	0	0

Note:
The diameter of the zone of inhibition of LAB is in mm

APPENDIX VI

Cytokines standard cuves

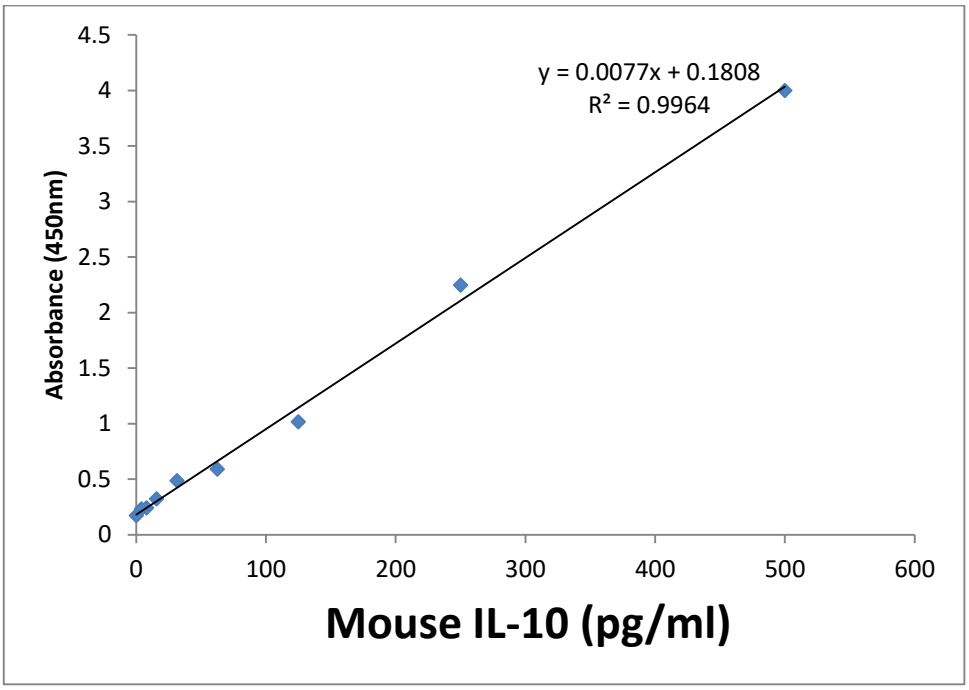

Figure A. 1. IL-10 cytokine' standard curve

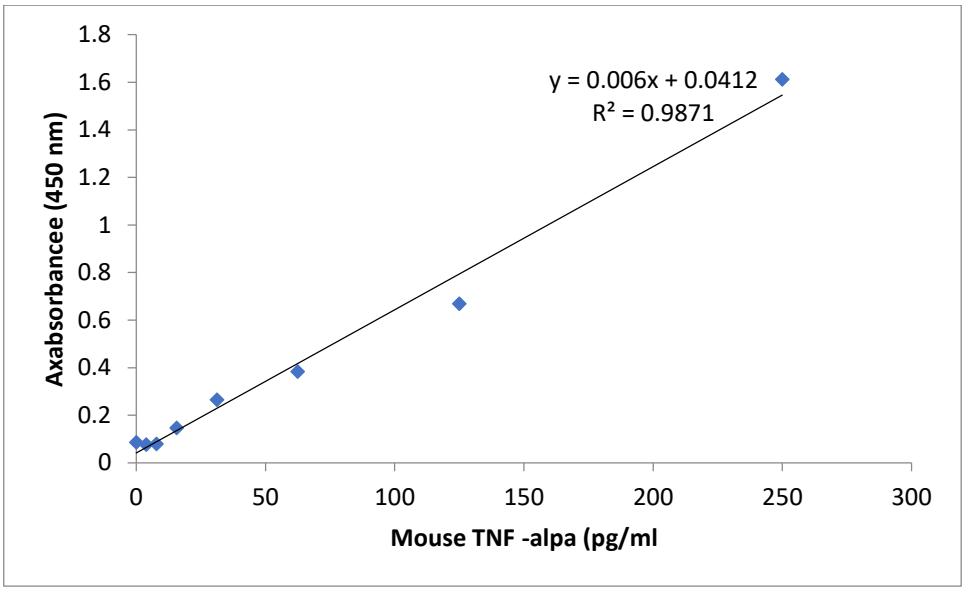

Figure A.2. TNF-alpha cytokine' standard curve

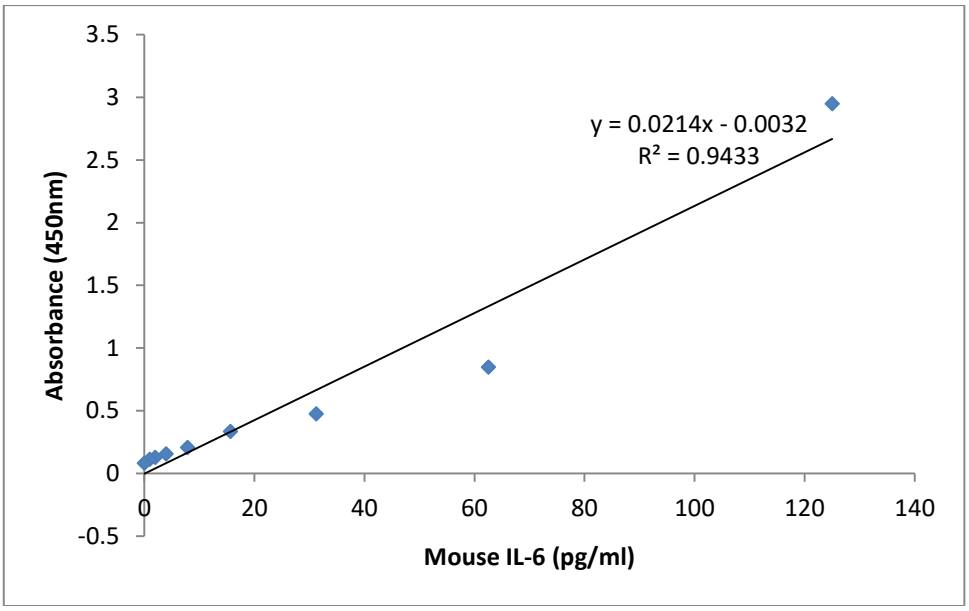

Figure A.3. Interleukins 6 cytokine' standard curve

APPENDIX VII

Phylogenetic tree

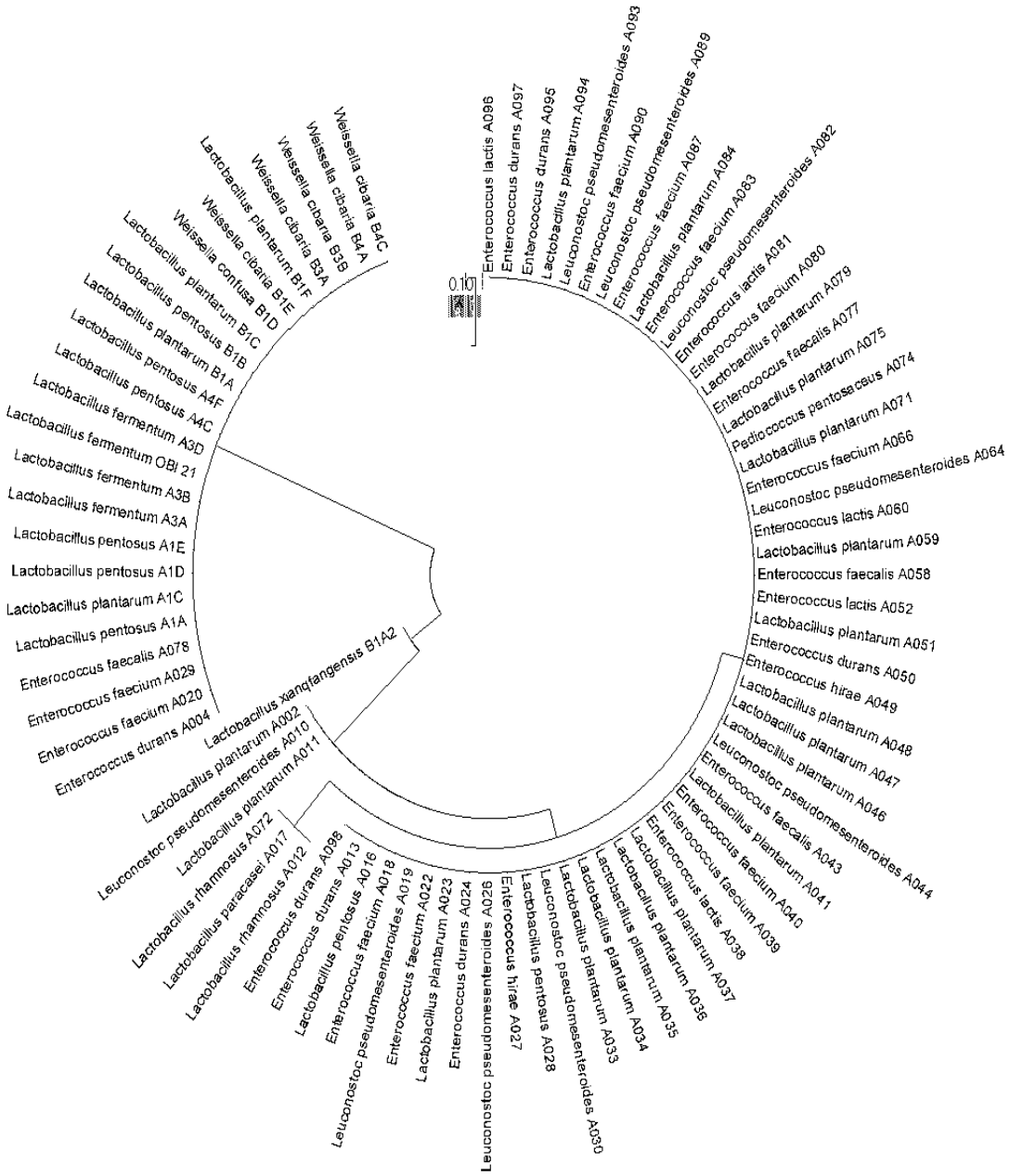

Figure A.4. The phylogenetic tree of the lactic acid bacteria isolated from human breast milk and neonates'faeces

APPENDIX VIII

16S rRNA Sequences of lactic acid bacteria identified in this study

Weissellacibaria B3b

CCAGGGCGGAGTGCTTAATGCGTTAGCTGCGGCACTKAAGGGCGGAAACC
CTCMAACACYTAGCAYTCATCGTTTACGGTGTGGACTACCAGGGTATCTAA
TCCTGTTTGCTACCCAYACTTTCGAGCCTCAACGTCAGTTACAGWCCAGAA
AGCCGCCTTCGCCACTGGTGTTCTTCCATATATCTACGCATTTCACCGCTAC
ACATGGAGTTCCACTKTCCTCTACTGCACTCAAGTCATCCAGTTTCCAAAG
CAATTCCTCAGTTGAGCTGAGGGCTTTCACTTCAGACTTAAATAACCGTCT
GCGCTCGCTTTACGCCCAATAAATCCGGATAACGCTTGGAACMTACGTATT
ACCGCGGCTGCTGGCACGTATTTAGCCGTKCCTTTCTGGTAARATACCGTC
ACWMMTTGAACAGTTACTCTCAAWGWYRTTCTTCTCTTACAACAGWGTTT
TACGAGCCGAAACCCTTCWTCACWCACGCGGCGTTGCTCCATCAGGCTTT
CGCCCATTGTGGAAGATTCCCTACTGCTGCCTCCCGTAGGAGTATGGGCCG
TGTCTCAGTCCCATTGTGGCCGATCASTCTCTCAASTCGGCTATGCATCATC
GTCTTGGTGAGCCATTACCTCACCAWCTAACTAATGCACCGCGGGACCATC
TCTTAGTGATAGCAGAACCATCTTTTAAGTAKCAACCATGCGGTTGCTATT
GTTATACGGTATTAGCATCTGTTTCCAARTGTTATCCCCTGCTAAGAGGTAG
GTTTCCCACGTGTTACTCACCCGTTCGCCACTCTTTGCAATGTCCATCGTCA
TATCTGAGCAAGCTCTTCAAATCAGTTGAACCACAWAGCGTTCGACTTGCA
TGTATAGGCACGCCSCCRGSKYCATCCTGACCCACATWCMAAACTCTCA

Lactobacillus pentosus A1d

GGGCGGATGCTTAATGCGTTAGCTGCAGCACTGAAGGGCGGAAACCCTCC
AACACTTAGCATTCATCGTTTACGGTATGGACTACCAGGGTATCTAATCCT
GTTTGCTACCCATACTTTCGAGCCTCAGCGTCAGTTACAGACCAGACAGCC
GCCTTCGCCACTGGTGTTCTTCCATATATCTACGCATTTCACCGCTACACAT
GGAGTTCCACTGTCCTCTTCTGCACTCAAGTTTCCCAGTTTCCGATGCACTT
CTTCGGTTGAGCCGAAGGCTTTCACATCAGACTTAAAAAACCGCCTGCGCT
CGCTTTACGCCCAATAAATCCGGACAACGCTTGCCACCTACGTATTACCGC
GGCTGCTGGCACGTAGTTAGCCGTGGCTTTCTGGTTAAATACCGTCAATAC
CTGAACAGTTACTCTCAGATATGTTCTTCTTTAACAACAGAGTTTTACGAGC
CGAAACCCTTCTTCACTCACGCGGCGTTGCTCCATCAGACTTTCGTCCATTG
TGGAAGATTCCCTACTGCTGCCTCCCGTAGGAGTTTGGGCCGTGTCTCAGT
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GAGCCGTTACCCCACCATCTAGCTAATACGCCGCGGGACCATCCAAAAGTG
ATAGCCGAAGCCATCTTTCAAACTYGGACCATGCGGTCCAAGTTGTTATGC
GGTATTAGCATCTGTTTCCAGGTGTTATCCCCCGCTTCTGGGCAGGTTTCCC
ACGTGTTACTCACCAGTTCGCCACTCACTCAAATGTAAATCATGATGCAAG
CACCAATCAATACCAGAGTTCGTTCGACTTGCATGTATTAGGCACGCCGCC
AGCGTTCGTCCTGAGCCAGATWYMAAACTYTCACCC

Weissellacibaria B3a

CCACGCGGAGTGCTTAATGCGTTAGCTGCGGCACTTAAGGGCGGAAACCCT
CAAACACCTAGCACTCATCGTTTACGGTGTGGACTACCAGGGTATCTAATC
CTGTTTGCTACCCACACTTTCGAGCCTCAACGTCAGTTACAGTCCAGAAAG
CCGCCTTCGCCACTGGTGTTCTTCCATATATCTACGCATTTCACCGCTACAC
ATGGAGTTCCACTTTCCTCTACTGCACTCAAGTCATCCAGTTTCCAAAGCA
ATTCCTCAGTTGAGCTGAGGGCTTTCACTTCAGACTTAAATAACCGTCTGC
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CGCGGCTGCTGGCACGTATTTAGCCGTTCCTTTCTGGTAAGATACCGTCAC
ACATTGARCAGTTACTCTCAATGTCATTCTTCTCTTACAACAGTGTTTTACG
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ACGGTATTAGCATCTGTTTCCAAATGTTATCCCCTGCTAAGAGGTAGGTTTC
CCACGTGTTACTCACCCGTTCGCCACTCTGTGCAATGTYCATCGTCATATCT
GAGCAAGCTCTTCAAATCAGTTGAACCACAAAGCGTTCGACTTGCATGTAT
TAGCACGCCGCCAGCGTTCATCCTGAGCCAGATWMMMAAAMTYTCAA

Lactobacillus fermentum A3b

AGGGCGGGGAGTGCTTAATGCGTTAGCTCCGGCACTGAAGGGCGGAAACC
CTCCAACACCTAGCACTCATCGTTTACGGCATGGACTACCAGGGTATCTAA
TCCTGTTCGCTACCCATGCTTTCGAGTCTCAGCGTCAGTTGCAGACCAGGT
AGCCGCCTTCGCCACTGGTGTTCTTCCATATATCTACGCATTCCACCGCTAC
ACATGGAGTTCCACTACCCTCTTCTGCACTCAAGTTATCCAGTTTCCGATGC
ACTTCTCCGGTTAAGCCGAAGGCTTTCACATCAGACTTAGAAAACCGCCTG
CACTCTCTTTACGCCCAATAAATCCGGATAACGCTTGCCACCTACGTATTA
CCGCGGCTGCTGGCACGTAGTTAGCCGTGACTTTCTGGTTAAATACCGTCA
ACGTATGAACAGTTACTCTCATACGTGTTCTTCTTTAACAACAGAGCTTTAC
GAGCCGAAACCCTTCTTCACTCACGCGGTGTTGCTCCATCAGGCTTGCGCC
CATTGTGGAAGATTCCCTACTGCTGCCTCCCGTAGGAGTATGGGCCGTGTC
TCAGTCCCATTGTGGCCGATCAGTCTCTCAACTCGGCTATGCATCATCGCCT
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AGTGATAGCGAGAAGCCATCTTTTAAGCGTTGTTCATGCGAACAACGYTGT
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TACCTACGTGTTACTCACCCGTCCGCCACTCGTTGGCGACCAAAATCAATC
AGTGCAAGCACCATCAATCAATTGGGCCAACGCGTTCGACTTGCATGTATT
AGGCACACCGCCAGCGTTCATCCTGAGCAGATWYMMAAAMYYCYCAA

Lactobacillus plantarum A1c

TSGGGAAAWTGCTTWAATGCGTTAGCTGCAGCACTGAAGGGCGGAAACCC
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CTGTTTGCTACCCATACTTTCSAGCCTCAGCGTCAGTTACAGACCAGACAG
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ATGGAGTTCCACTGTCCTCTTCTGCACTCAAGTTTCCCAGTTTCCGATGCAC
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CTCGCTTTACGCCCAATAAATCCGGACAACGCTTGCCACCTACGTATTACC
GCGGCTGCTGGCACGTAGTTAGCCGTGGCTTTCTGGTTAAATACCGTCAAT
ACCTGAACAGTTACTCTCAGATATGTTCTTCTTTAACAACAGAGTTTTTACG
AGCCGAAACCCTTCTTCATCACGCGGCGTTGCTCCATCAGACTTTCGTCCAT
TGTGGAAGATTCCCTACTGCTGCCTCCCGTAGGAGTTTGGGCCGTGTCTCA
GTCCCAATGYGGCCGATTACCCTCTCAGGTCGGCTACGTATCATTGCCATG
GTGAGCSGTTACCCCACCCATCTAGCTAATACGCCGCGGGACCATCCAAAA
GTGATAGCCRAAGCCATCTTTCAARCTTGGGACCATGCGGTCCMAAGTTGT
TATGCGGTATTAGCATCTGTTTCCCAGGCGTTATCCCCCCGCTTTCTGGGCA
GGTTTYCCCACSYGGTWMCTCMCCAGTTCSCCACTCACTCAAATGTAAATC
ATGATGSCAAGCMCCAATCAATACARAGTTCGTTCGACTTKGCATGTATTA
AGGAACGCCGCCAGCGGTCCGTCCCTGAGCCAGGAAYCAAAMCTMWWAG
G

*Weissellacibaria*B4a

AGCGGGARGTGSTTAATGCCGTTAGCTGCGGCACTTAAGGGCGGAAACCCT
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CCGCCTTCGCCACTGGTGTTCTTCCATATATCTACGCATTTCACCGCTACAC
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GCTCGCTTTACGCCCAATAAATCCGGATAACGCTTGGAACATACGTATTAC
CGCGGCTGCTGGCACGTATTTAGCCGTTCCTTTCTGGTAAGATACCGTCAC
ACATTGAACAGTTACTCTCAATGTCATTCTTCTCTTACAACAGTGTTTTACG
AGCCGAAACCCTTCATCACACACGCGGCGTTGCTCCATCAGGCTTTCGCCC
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CAGTCCCATTGTGGCCGATCAGTCTCTCAACTCGGCTATGCATCATCGTCTT
GGTGAGCCATTACCTCACCAACTAACTAATGCACCGCGGGACCATCTCTTA
GTGATAGCAGAACCATCTTTTAAGTAGCAACCATGCGGTTGCTATTGTTAT
ACGGTATTAGCATCTGTTTCCAAATGTTATCCCCTGCTAAGAGGTAGGTTTC
CCACGTGTTACTCACCCGTTCGCCACTCTTTGCAATGTCCATCGTCATATCT
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AGGCACGCCGCCAGCGTTCATCCTGAGCATGAATMAAACWCTACCMC

Lactobacillus pentosus A4f

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GACAGCCGCCTTCGCCACTGGTGTTCTTCCATATATCTACGCATTTCACCGC
TACACATGGAGTTCCACTGTCCTCTTCTGCACTCAAGTTTCCCAGTTTCCGA
TGCACTTCTTCGGTTGAGCCGAAGGCTTTCACATCAGACTTAAAAAACCGC
CTGCGCTCGCTTTACGCCCAATAAATCCGGACAACGCTTGCCACCTACGTA
TTACCGCGGCTGCTGGCACGTAGTTAGCCGTGGCTTTCTGGTTAAATACCG
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TACGAGCCGAAACCCTTCTTCACTCACGCGGCGTTGCTCCATCAGACTTTC
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GCCATGGTGAGCCGTTACCCCACCATCTAGCTAATACGCCGCGGGACCATC
CAAAAGTGATAGCCGAAGCCATCTTTCAAACTCGGACCATGCGGTCCAAGT
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AGGTTTCCCACGTGTTACTCACCAGTTCGCCACTCACTCAAATGTAAATCA
TGATGCAAGCACCAATCAATACCAGAGTTCGTTCGACTTGCATGTATTAGG
CACGCCGCCAGCGTTTCGTCCTGAGCCATGAATCAAACTCTASCMCMSCSC
CCCCCSMCWCTSCKK

Weissellacibaria B1e

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TCCAGAAAGCCGCCTTCGCCACTGGTGTTCTTCCATATATCTACGCATTTCA
CCGCTACACATGGAGTTCCACTTTCCTCTACTGCACTCAAGTCATCCAGTTT
CCAAAGCAATTCCTCAGTTGAGCTGAGGGCTTTCACTTCAGACTTAAATAA
CCGTCTGCGCTCGCTTTACGCCCAATAAATCCGGATAACGCTTGGAACATA
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TCGTCTTGGTGAGCCATTACCTCACCAACTAACTAATGCACCGCGGGACCA
TCTCTTAGTGATAGCAGAACCATCTTTTAAGTAGCAACCATGCGGTTGCTA
TTGTTATACGGTATTAGCATCTGTTTCCAAATGTTATCCCCTGCTAAGAGGT
AGGTTTCCCACGTGTTACTCACCCGTTCGCCACTCTTTGCAATGTYCATCGT
CATATCTGAGCAAGCTCTTCAAATCAGTTGAACCACAAAGCGTTCGACTTG
CATGTATTAGGCACGCCGCCAGCGTTCATCCTGAGCATGAAWTCAAAMMT
MMTAGGGACGCGG

Lactobacillus fermentum A3a

CAGGCGGAGTGCTTAATGCGTTAGCTCCGGCACTGAAGGGCGGAAACCCT
CCAACACCTAGCACTCATCGTTTACGGCATGGACTACCAGGGTATCTAATC
CTGTTCGCTACCCATGCTTTCGAGTCTCAGCGTCAGTTGCAGACCAGGTAG
CCGCCTTCGCCACTGGTGTTCTTCCATATATCTACGCATTCCACCGCTACAC
ATGGAGTTCCACTACCCTCTTCTGCACTCAAGTTATCCAGTTTCCGATGCAC
TTCTCCGGTTAAGCCGAAGGCTTTCACATCAGACTTAGAAAACCGCCTGCA
CTCTCTTTACGCCCAATAAATCCGGATAACGCTTGCCACCTACGTATTACC
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GTATGAACAGTTACTCTCATACGTGTTCTTCTTTAACAACAGAGCTTTACGA
GCCGAAACCCTTCTTCACTCACGCGGTGTTGCTCCATCAGGCTTGCGCCCA
TTGTGGAAGATTCCCTACTGCTGCCTCCCGTAGGAGTATGGGCCGTGTCTC
AGTCCCATTGTGGCCGATCAGTCTCTCAACTCGGCTATGCATCATCGCCTTG
GTAGGCCRTTACCCCACCAACAAGCTAATGCACCGCAGGTCCATCCAGAA

GTGATAGCGAGAAGCCATCTTTTAAGCGTTGTTCATGCGAACAACGYTGTT
ATGCGGTATTAGCATCTGTTTCCAAATGTTGTCCCCCGCTTCTGGGCAGGTT
ACCTACGTGTTACTCACCCGTCCGCCACTCGTTGGCGACCAAAATCAATCA
GGTGCAAGCACCATCAATCAATTGGGCCAACGCGTTCGACTTGCATGTATT
AGGCACACCGCCAGCGTTCATCCTGAGCAGRAAWCAAAMYWMWAGG

Weissellacibaria B4c
CCMAGGCSGGAGTGCTTAATGCGTTAGCTGCGGCACTTAAGGGCGGAAAC
CCTCAAACACCTAGYWCTCATCGTTTACGGTGTGGACTACCAGGGTATCTA
ATCCTGTTTGCTACCCACACTTTCGAGCCTCAACGTCAGTTACAGTCCAGA
AAGCCGCCTTCGCCACTGGTGTTCTTCCATATATCTACGCATTTCACCGCTA
CACATGGAGTTCCACTTTCCTCTACTGCACTCAAGTCATCCAGTTTCCAAAG
CAATTCCTCAGTTGAGCTGAGGGCTTTCACTTCAGACTTAAATAACCGTCT
GCGCTCGCTTTACGCCCAATAAATCCGGATAACGCTTGGAACATACGTATT
ACCGCGGCTGCTGGCACGTATTTAGCCGTTCCTTTCTGGTAAGATACCGTC
ACACATTGARCAGTTACTCTCAATGTCATTCTTCTCTTACAACAGTGTTTTA
CGAGCCGAAACCCTTCATCACACACGCGGCGTTGCTCCATCAGGCTTTCGC
CCATTGTGGAAGATTCCCTACTGCTGCCTCCCGTAGGAGTATGGGCCGTGT
CTCAGTCCCATTGTGGCCGATCAGTCTCTCAACTCGGCTATGCATCATCGTC
TTGGTGAGCCATTACCTCACCAACTAACTAATGCACCGCGGGACCATCTCT
TAGTGATAGCAGAACCATCTTTTAAGTAGCAACCATGCGGTTGCTATTGTT
ATACGGTATTAGCATCTGTTTCCAAATGTTATCCCCTGCTAAGAGGTAGGT
TTCCCACGTGTTACTCACCCGTTCGCCACTCTTTGCAATGTCCATCGTCATA
TCTGAGCAAGCTCTTCAAATCAGTTGAACCACAAAGCGTTCGACTTGCATG
TATTAGGCACGCCGCCAGCGTTCATCCTGAGCCATGAATCAAACTMWAGG

Lactobacillus pentosus A4c

CMGGGCGGGGATGCTTAATGCGTTAGCTGCAGCACTGAAGGGCGGAAACC
CTCCAACACTTAGCATTCATCGTTTACGGTATGGACTACCAGGGTATCTAA
TCCTGTTTGCTACCCATACTTTCGAGCCTCAGCGTCAGTTACAGACCAGAC
AGCCGCCTTCGCCACTGGTGTTCTTCCATATATCTACGCATTTCACCGCTAC
ACATGGAGTTCCACTGTCCTCTTCTGCACTCAAGTTTCCCAGTTTCCGATGC
ACTTCTTCGGTTGAGCCGAAGGCTTTCACATCAGACTTAAAAAACCGCCTG
CGCTCGCTTTACGCCCAATAAATCCGGACAACGCTTGCCACCTACGTATTA
CCGCGGCTGCTGGCACGTAGTTAGCCGTGGCTTTCTGGTTAAATACCGTCA
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GAGCCGAAACCCTTCTTCACTCACGCGGCGTTGCTCCATCAGACTTTCGTC
CATTGTGGAAGATTCCCTACTGCTGCCTCCCGTAGGAGTTTGGGCCGTGTC
TCAGTCCCAATGTGGCCGATTACCCTCTCAGGTCGGCTACGTATCATTGCC
ATGGTGAGCCGTTACCCCACCATCTAGCTAATACGCCGCGGGACCATCCAA
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TATGCGGTATTAGCATCTGTTTCCAGGTGTTATCCCCCGCTTCTGGGCAGGT
TTCCCACGTGTTACTCACCAGTTCGCCACTCACTCAAATGTAAATCATGAT
GCAAGCACCAATCAATACCAGAGTTCGTTCGACTTGCATGTATTAGGCACG
CCGCCAGCGTTCGTCCTGAGCATGAAWWYMAAACTWMTAGGG

Weissellaconfusa B1d

CCMRAGCCGGAGTGCTTAATGCGTTAGCTGCGGCACTTAAGGGCGGAAAC
CCTCAAACACCTASCAYTCATCGTTTACGGTGTGGACTACCAGGGTATCTA
ATCCTGTTTGCTACCCACACTTTCGAGCCTCAACGTCAGTTACAGTCCAGA
AAGCCGCCTTCGCCACTGGTGTTCTTCCATATATCTACGCATTTCACCGCTA
CACATGGAGTTCCACTTTCCTCTACTGCACTCAAGTCATCCAGTTTCCAAAG
CAATTCCTCAGTTGAGCTGAGGGCTTTCACTTCAGACTTAAATAACCGTCT
GCGCTCGCTTTACGCCCAATAAATCCGGATAACGCTTGGAACATACGTATT
ACCGCGGCTGCTGGCACGTATTTAGCCGTTCCTTTCTGGTAAGATACCGTC
ACACATTGAACAGTTACTCTCAATGTCATTCTTCTCTTACAACAGTGTTTTA
CGAGCCGAAACCCTTCATCACACACGCGGCGTTGCTCCATCACGCTTTCGC
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TGGTGAGCCATTACCTCACCAACTAACTAATGCACCGCGGGACCATCTCTT
AGTGATAGCAGACCCATCTTTAAGTAGCAACCATGCCGTGCTATTGTAWAC
GGGATTASCATCTGTTCCAAACTGTWATCCCCTGCTAARAGGTAGTTTCCM
CGTGTACYCMCCCGGTCGCCMCCYCTTTGCAATGTCCATCGTCATATCTGR
ACAAGCCYCTCAAATCAGGTGAACCCMAAAGCGTTCGACTTGCATGATTA
AGACGCGCAAGCGTTCATCTTGRRCRRGATYAMAMYYMTAAGG

Lactobacillus pentosus A1a

CGGCCGGGGAATGSTTAATGCGTTAGCTGCAGCACTGAAGGGCGGAAACC
CTCCAACACTTAGCWTTCATCGTTTACGGTATGGACTACCAGGGTATCTAA
TCCTGTTTGCTACCCATACTTTCGAGCCTCAGCGTCAGTTACAGACCAGAC
AGCCGCCTTCGCCACTGGTGTTCTTCCATATATCTACGCATTTCACCGCTAC
ACATGGAGTTCCACTGTCCTCTTCTGCACTCAAGTTTCCCAGTTTCCGATGC
ACTTCTTCGGTTGAGCCGAAGGCTTTCACATCAGACTTAAAAAACCGCCTG
CGCTCGCTTTACGCCCAATAAATCCGGACAACGCTTGCCACCTACGTATTA
CCGCGGCTGCTGGCACGTAGTTAGCCGTGGCTTTCTGGTTAAATACCGTCA
ATACCTGAACAGTTACTCTCAGATATGTTCTTCTTTAACAACAGAGTTTTAC
GAGCCGAAACCCTTCTTCACTCACGCGGCGTTGCTCCATCAGACTTTCGTC
CATTGTGGAAGATTCCCTACTGCTGCCTCCCGTAGGAGTTTGGGCCGTGTC
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AAGTGATAGCCGAAGCCATCTTTCAAACTYGGACCATGCGGTCCAAGTTGT
TATGCGGTATTAGCATCTGTTTCCAGGTGTTATCCCCCGCTTCTGGGCAGGT
TTCCCACGTGTTACTCACCAGTTCGCCACTCACTCAAATGTAAATCATGAT
GCAAGCACCAATCAATACCAGAGTTCGTTCGACTTGCATGTATTAGGCACG
CCGCCAGCGTTCGTCCTGAGCCATGAAATCAAAACTMMTAGGG

Lactobacillus plantarum B1f

CCGGGCGGGATGCTTAATGCGTTAGCTGCAGCACTGAAGGGCGGAAACCC
TCCAACACTTAGCATTCATCGTTTACGGTATGGACTACCAGGGTATCTAAT
CCTGTTTGCTACCCATACTTTCGAGCCTCAGCGTCAGTTACAGACCAGACA
GCCGCCTTCGCCACTGGTGTTCTTCCATATATCTACGCATTTCACCGCTACA

CATGGAGTTCCACTGTCCTCTTCTGCACTCAAGTTTCCCAGTTTCCGATGCA
CTTCTTCGGTTGAGCCGAAGGCTTTCACATCAGACTTAAAAAACCGCCTGC
GCTCGCTTTACGCCCAATAAATCCGGACAACGCTTGCCACCTACGTATTAC
CGCGGCTGCTGGCACGTAGTTAGCCGTGGCTTTCTGGTTAAATACCGTCAA
TACCTGAACAGTTACTCTCAGATATGTTCTTCTTTAACAACAGAGTTTTACG
AGCCGAAACCCTTCTTCACTCACGCGGCGTTGCTCCATCAGACTTTCGTCC
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CAGTCCCAATGTGGCCGATTACCCTCTCAGGTCGGCTACGTATCATTGCCA
TGGTGAGCCGTTACCCCACCATCTAGCTAATACGCCGCGGGACCATCCAAA
AGTGATAGCCGAAGCCATCTTTCAAACTYGGACCATGCGGTCCAAGTTGTT
ATGCGGTATTAGCATCTGTTTCCAGGTGTTATCCCCCGCTTCTGGGCAGGTT
TCCCACGTGTTACTCACCAGTTCGCCACTCACTCAAATGTAAATCATGATG
CAAGCACCAATCAATACCAGAGTTCGTTCGACTTGCATGTATTAGCACGCC
GCCAGCGTTCGTCCTGAGCAGTWYWMAAAAACYYCYMA

Lactobacillus fermentum A3d

CCARGCGGGAGTGCTTAATGCGTTAGCTSCRGCACTGAAGGGCGGAAACCC
TCCAACACYTASCWYTCATCGTTTACGGYATGGACTACCAGGGTATCTAAT
CCTGTTYGCTACCCATGCTTTCGAGYCTCAGCGTCAGTTRCAGACCAGRYA
GCCGCCTTCGCCACTGGTGTTCTTCCATATATCTACGCATTYCACCGCTACA
CATGGAGTTCCACTRCCCTCTTCTGCACTCAAGTTWYCCAGTTTCCGATGC
ACTTCTYCGGTTRAGCCGAAGGCTTTCACATCAGACTTARAAAACCGCCTG
CRCTCKCTTTACGCCCAATAAATCCGGAYAACGCTTGCCACCTACGTATTA
CCGCGGCTGCTGGCACGTAGTTAGCCGTGRCTTTCTGGTTAAATACCGTCA
AYRYMTGAACAGTTACTCTCATAYRTGTTCTTCTTTAACAACAGAGYTTTA
CGAGCCGAAACCCTTCTTCACTCACGCGGYGTTGCTCCATCAGRCTTKCGY
CCATTGTGGAAGATTCCCTACTGCTGCCTCCCGTAGGAGTATGGGCCGTGT
CTCAGTCCCATTGTGGCCGATYASYCTCTCAASTCGGCTATGYATCATCGCC
TTGGTRAGCCRTTACCCCACCAWCWAGCTAATGCACCGCAGGWCCATCCA
GAAGTGATAGCSAGAAGCCATCTTTYTAAGYGTTGYTCATGCGAACAACGT
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GGTTACCTACGTGTTACTCACCCGTCCGCCACTCSTYGGMKACCAATATCR
ATSAGTGCAMGCWCCATCAATCAGTTGGGTCAACGCGYWYGACTTRCRTG
ATCGCACCGCCAGCTGATCATCGGGCATAATCAAACTCAGAC

Lactobacillus plantarum B1c

ACGCATGCTTAATGCGTTAGCTGCAGCACTGAAGGGCGGAAACCCTCCAA
CACTTTTCATCGTTTACGGTATGGACTACCAGGGTATCTAATCCTGTTTGCT
ACCCATACTTTCGAGCCTCAGCGTCAGTTACAGACCAGACAGCCGCCTTCG
CCACTGGTGTTCTTCCATATATCTACGCATTTCACCGCTACACATGGAGTTC
CACTGTCCTCTTCTGCACTCAAGTTTCCCAGTTTCCGATGCACTTCTTCGGT
TGAGCCGAAGGCTTTCACATCAGACTTAAAAAACCGCCTGCGCTCGCTTTA
CGCCCAATAAATCCGGACAACGCTTGCCACCTACGTATTACCGCGGCTGCT
GGCACGTAGTTAGCCGTGGCTTTCTGGTTAAATACCGTCAATACCTGAACA
GTTACTCTCAGATATGTTCTTCTTTAACAACAGAGTTTTACGAGCCGAAAC
CCTTCTTCACTCACGCGGCGTTGCTCCATCAGACTTTCGTCCATTGTGGAAG

ATTCCCTACTGCTGCCTCCCGTAGGAGTTTGGGCCGTGTCTCAGTCCCAATG
TGGCCGATTACCCTCTCAGGTCGGCTACGTATCATTGCCATGGTGAGCCGT
TACCCCACCATCTAGCTAATACGCCGCGGGACCATCCAAAAGTGATAGCCG
AAGCCATCTTTCAACTYGGACCATGCGGTCCAAGTTGTTATGCGGTATTAG
CATCTGTTTCCAGGTGTTATCCCCCGCTTCTGGGCAGGTTTCCCACGTGTTA
CTCACCAGTTCGCCACTCACTCAAATGTAAATCATGATGCAAGCACCAATC
AATACCAGAGTTCGTTCGACTTGCATGTATTAGGCACGCCGCCAGCGTTCG
TCCTGAGCCATGAATCAAACTCTAAGGACCCCC

Lactobacillus pentosus B1b

CCRGGCGGATGCTTAATGCGTTAGCTGCAGCACTGAAGGGCGGAAACCCT
CCAACACTTAGCATTCATCGTTTACGGTATGGACTACCAGGGTATCTAATC
CTGTTTGCTACCCATACTTTCGAGCCTCAGCGTCAGTTACAGACCAGACAG
CCGCCTTCGCCACTGGTGTTCTTCCATATATCTACGCATTTCACCGCTACAC
ATGGAGTTCCACTGTCCTCTTCTGCACTCAAGTTTCCCAGTTTCCGATGCAC
TTCTTCGGTTGAGCCGAAGGCTTTCACATCAGACTTAAAAAACCGCCTGCG
CTCGCTTTACGCCCAATAAATCCGGACAACGCTTGCCACCTACGTATTACC
GCGGCTGCTGGCACGTAGTTAGCCGTGGCTTTCTGGTTAAATACCGTCAAT
ACCTGAACAGTTACTCTCAGATATGTTCTTCTTTAACAACAGAGTTTTACGA
GCCGAAACCCTTCTTCACTCACGCGGCGTTGCTCCATCAGACTTTCGTCCAT
TGTGGAAGATTCCCTACTGCTGCCTCCCGTAGGAGTTTGGGCCGTGTCTCA
GTCCCAATGTGGCCGATTACCCTCTCAGGTCGGCTACGTATCATTGCCATG
GTGAGCCGTTACCCCACCATCTAGCTAATACGCCGCGGGACCATCCAAAAG
TGATAGCCGAAGCCATCTTTCAAACTYGGACCATGCGGTCCAAGTTGTTAT
GCGGTATTAGCATCTGTTTCCAGGTGTTATCCCCCGCTTCTGGGCAGGTTTC
CCACGTGTTACTCACCAGTTCGCCACTCACTCAAATGTAAATCATGATGCA
AGCACCAATCAATACCAGAGTTCGTTCGACTTGCATGTATTAGGCACGCCG
CCAGCGTTCGTCCTGAGCGAAAAACG

Lactobacillus pentosus A1e

CTGGGCGGATGCTTAATGCGTTAGCTGCAGCACTGAAGGGCGGAAACCCT
CCAACACTTAGCATTCATCGTTTACGGTATGGACTACCAGGGTATCTAATC
CTGTTTGCTACCCATACTTTCGAGCCTCAGCGTCAGTTACAGACCAGACAG
CCGCCTTCGCCACTGGTGTTCTTCCATATATCTACGCATTTCACCGCTACAC
ATGGAGTTCCACTGTCCTCTTCTGCACTCAAGTTTCCCAGTTTCCGATGCAC
TTCTTCGGTTGAGCCGAAGGCTTTCACATCAGACTTAAAAAACCGCCTGCG
CTCGCTTTACGCCCAATAAATCCGGACAACGCTTGCCACCTACGTATTACC
GCGGCTGCTGGCACGTAGTTAGCCGTGGCTTTCTGGTTAAATACCGTCAAT
ACCTGAACAGTTACTCTCAGATATGTTCTTCTTTAACAACAGAGTTTTACGA
GCCGAAACCCTTCTTCACTCACGCGGCGTTGCTCCATCAGACTTTCGTCCAT
TGTGGAAGATTCCCTACTGCTGCCTCCCGTAGGAGTTTGGGCCGTGTCTCA
GTCCCAATGTGGCCGATTACCCTCTCAGGTCGGCTACGTATCATTGCCATG
GTGAGCCGTTACCCCACCATCTAGCTAATACGCCGCGGGACCATCCAAAAG
TGATAGCCGAAGCCATCTTTCAAACTYGGACCATGCGGTCCAAGTTGTTAT
GCGGTATTAGCATCTGTTTCCAGGTGTTATCCCCCGCTTCTGGGCAGGTTTC
CCACGTGTTACTCACCAGTTCGCCACTCACTCAAATGTAAATCATGATGCA

AGCACCAATCAATACCAGAGTTCGTTCGACTTGCATGTATTAGGCACGCCG
CCAGCGTTCGTCCTGAGCGAAAAATTAGG

Lactobacillus plantarum B1a

AGGGCGGGGAAKGCCTTAATGCGTTAGCTGCAGCACTGAAGGGCGGAAAC
CCTCCAACACTTASCWTTCATCGTTTACGGTATGGACTACCAGGGTATCTA
ATCCTGTTTGCTACCCATACTTTCGAGCCTCAGCGTCAGTTACAGACCAGA
CAGCCGCCTTCGCCACTGGTGTTCTTCCATATATCTACGCATTTCACCGCTA
CACATGGAGTTCCACTGTCCTCTTCTGCACTCAAGTTTCCCAGTTTCCGATG
CACTTCTTCGGTTGAGCCGAAGGCTTTCACATCAGACTTAAAAAACCGCCT
GCGCTCGCTTTACGCCCAATAAATCCGGACAACGCTTGCCACCTACGTATT
ACCGCGGCTGCTGGCACGTAGTTAGCCGTGGCTTTCTGGTTAAATACCGTC
AATACCTGAACAGTTACTCTCAGATATGTTCTTCTTTAACAACAGAGTTTTA
CGAGCCGAAACCCTTCTTCACTCACGCGGCGTTGCTCCATCAGACTTTCGT
CCATTGTGGAAGATTCCCTACTGCTGCCTCCCGTAGGAGTTGGGCCGTGTC
TMGTCCAATGYGCCGATTACCCTCTCAGYCGCTACGTATCATTGCCATGGT
GAGCYGTTACCCCACCATCTAGCTAATACSCCGCGGGACCATCCAAAGKGA
TAGCCGAAGCCATYTTTTYAACTTCGGACCATGCGGTCCAAGTKGTATKGC
GGTATTAGCATYCTKTTCCCAGGKGTWWTCCCCSSTTYYKGGGCAGGGTTC
CCACTGGTTACTCMCCAGTTCSCCMCCYTMCTYAATGTAAAYTCKGATKG
CAAGCCCCATTCATCCAARGTTCTTCAATTGCWRTGATTAAGGMA

Lactobacillus xianqfangensis B1a2

GGKATCTAGCACKTTCCACTTGCACTTCTTCGAAKAARTCTAARGMTTTCA
CATCAKACTTAAAAAACCGCCTGCGCTCGCTTTACSCCCAATAAATCCGGA
CAACGCTTGCCACCTACRTATTACCGCGGCTGCTGGCACGTAATTTATTGG
TGGTTTTCTGGTTAAATACCGTCAATACCTGAACAGTTACTCTCCTATATGT
TCTTCTTTAACAACAGAGTTTTACTGGCCGAAACCCTTCTTCACTACGCRGY
GKAACTCCATGTGTATYGGTGMCWTTGTGGAAGATTCCCTWMTGCTGCCT
CCMGKARGAGTTKGGTCCRTGCYTCAGGCCCAATGTGGAAGATTACCCTC
CAAATATGWCTATTTTTTGTCTTTATGACGATCGGAACTGCCGTTCGWGCA
GGGATCACTCCCGATAACTTACTCTCCATGAGYACCGGYCA

Lactobacillus plantarum B1b2

GTCGAACGAACTCTGGTATTGATTGGTGCTTGCATCATGATTTACATTTGA
GTGAGTGGCGAACTGGTGAGTAACACGTGGGAAACCTGCCCAGAA**CC**AGG
CCGGGAAGTTGCTTAATGCGTTAGCTGCAGCACTGAAGGGCGGAAACCCT
CCAACTTCCACTCTCCTCTTCTGCACTCAACTTMKTYACTCATCGTTTACGG
CGTGGACTACCAGGGTATCTAATCCTGTTTGCTCCCCACGCTTTCGAGCCTC
AGCGTCAGTTACAGACCAGAGAGCCGCCTTCGCCACTGGTGTTCCTCCATA
TATCTACGCATTTCACCGCTACACATGGAATTCCACTCTCCTCTTCTGCACT
CAAGTCTCCCAGTTTCCAATGACCCTCCCCGGTTGAGCCGGGGGCTTTCAC
ATCAGACTTAAGAAACCGCCTGCGCTCGCTTTACGCCCAATAAATCCGGAC

AACGCTTGCCACCTACGTATTACCGCGGCTGCTGGCACGTAGTTAGCCGTG
GCTTTCTGGTTAGATACCGTCAAGGGATGAACAGTTACTCTCATCCTTGTTC
TTCTCTAACAACAGAGTTTTACGATCCGAAAACCTTCTTCACTCACGCGGC
GTTGCTCGGTCAGACTTTCGTCCATTGCCGAAGATTCCCTACTGCTGCCTCC
CGTAGGAGTTTGGGCCGTGTCTCAGTCCCAATGTGGCCGATCACCCTCTCA
GGTCGGCTATGCATCGTGGCCTTGGTGAGCCGTTACCTCACCAACTAGCTA
ATGCACCGCGGGTCCATCCATCAGCGACACCCGAAAGCGCCTTTCAAATCA
AAACCATGCGGTTTCGATTGTTATACGGTATTAGCACCTGTTTCCAAGTGTT
ATCCCCTTCTGATGGGCAGGTTACCCACGTGTTACTCACCCGTTCGCCACTC
TTCTTTTTCCGGTGGAGCAAGCTCCGGTGGAAAAAGAAGCGTACGACTTGC
ATGTATTAGGCMCSCCGCCCAGCGTTCGTCCTGAGCCAGGATCAAACCTCT
CAA

Enterococcus faecalis A4d

CATCGTTCTTTAAATTGGGGTTTAATTTGSWGKGGAGKAGAGGGGGKATCC
TGWAAAMAAATCAAATAAGGTGTAACCGGWTTGCCTCCCYAAARTTTKT
WGATTGTTTCCTGCTAAAAAATACAGACAGGCTTKATARATTTGTTGGGGG
TTTAAGGATYAAAACGGTATTTTWAGTAAATTTWAWTGSCATCTGKTCTK
TGAAAAAAAGATTTAAMTTGGGAAATTTCRTSTCATTAGGGAGTAACTTAG
GAATAGCGGTTGCTTAAAAATTMGTCARGTAAGTCTTTTTATGTTGAAAMA
CAGTAAGAGATCGGTKGATTCTGGTAAGTTGAA

Enterococcus faecalis A078
CCGGGSRRGGRGKGSTTAATGCGTTTGCTGCAGCACTGAAGGGCGGAAACC
CTCCAACACTTASYWYTCATCGTTTACGGCGTGGACTACCAGGGTATCTAA
TCCTGTTTGCTCCCCACGCTTTCGAGCCTCAGCGTCAGTTACAGACCAGAG
AGCCGCCTTCGCCACTGGTGTTCCTCCATATATCTACGCATTTCACCGCTAC
ACATGGAATTCCACTCTCCTCTTCTGCACTCAAGTCTCCCAGTTTCCAATGA
CCCTCCCCGGTTGAGCCGGGGGCTTTCACATCAGACTTAAGAAACCGCCTG
CGCTCGCTTTACGCCCAATAAATCCGGACAACGCTTGCCACCTACGTATTA
CCGCGGCTGCTGGCACGTAGTTAGCCGTGGCTTTCTGGTTAGATACCGTCA
GGGGACGTTCAGTTACTAACGTCCTTGTTTCTTCTCTAACAACAGAGTTTTA
CGATCCGAAAACCTTCTTCACCTCACGCGGCGTTGCTCGGTCAGACTTTCG
TCCATTGCCGAAGATTCCCTACTGCTGCCTCCCGTAGGAGTCTGGGCCGKG
TCTCAKTCCCRGGGKGGCCGATCACCCYTTTCGGGKCGGTAATGCATCGW
GGCTTKGGKGAGSCGTTACCCCACCMACCMMCCCAMCSCACCGCGGGGTC
CATCCATCAGCGAMACCCGAAAGCGCCTTTCACTCTTATGCCATGCGGAW
TAAACTGTTATGCGGWWTWAGCACCTGTTTTCAAGKGTTATCCCCCTYTG
ATGGGWAGGTWACCCMCGTGTTACTCACCCGTCCGCMAYTCTCTTTCCAA
TTGAGTGCAASCACTCGGGARGAAARAAGCGTTCGAMTTGGMATKTTTAG
GMAGCCGCCAGCGTTCGTCCTGAGCAGGAYCAAACTCTCAGGGG

Enterococcus durans A004

CCCRGGGCGGGGAGTGCTTAATGCGTTAGCTGCAGCACTGAAGGGCGGAA
ACCCTCCAACACTTAGCACTCATCGTTTACGGCGTGGACTACCAGGGTATC
TAATCCTGTTTGCTCCCCACGCTTTCGAGCCTCAGCGTCAGTTACAGACCA
GAGAGCCGCCTTCGCCACTGGTGTTCCTCCATATATCTACGCATTTCACCGC

TACACATGGAATTCCACTCTCCTCTTCTGCACTCAAGTCTCCCAGTTTCCAA
TGACCCTCCCCGGTTGAGCCGGGGGCTTTCACATCAGACTTAAGAAACCGC
CTGCGCTCGCTTTACGCCCAATAAATCCGGACAACGCTTGCCACCTACGTA
TTACCGCGGCTGCTGGCACGTAGTTAGCCGTGGCTTTCTGGTTAGATACCG
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TACGATCCGAAAACCTTCTTCACTCACGCGGCGTTGCTCGGTCAGACTTTC
GTCCATTGCCGAAGATTCCCTACTGCTGCCTCCCGTAGGAGTTTGGGCCGT
GTCTCAGTCCCAATGTGGCCGATCACCCTCTCAGGTCGGCTATGCATCGTG
GCCTTGGTGAGCCGTTACCTCACCAACTAGCTAATGCACCGCGGGTCCATC
CATCAGCGACACCCGAAAGCGCCTTTCAAATCAAAACCATGCGGTTTCGAT
TGTTATACGGTATTAGCACCTGTTTCCAAGTGTTATCCCCTTCTGATGGGCA
GGTTACCCACGTGTTACTCACCCGTTCGCCACTCTTCTTTTTCCGGTGGAGC
AAGCTCCGGTGGAAAAAGAAGCGTACGACTTGCATGTATTAGGCACGCCG
CCAGCGTTCGTCCTGAGCAGTWYYMAAAAMYYYYCA

Enterococcus faecium A020

CTTCCYCCMGGCGGAGTGCTTAATGCGTTAGCTGCAGCACTGAAGGGCGG
AAACCCTCCAACACTTAGCACTCATCGTTTACGGCGTGGACTACCAGGGTA
TCTAATCCTGTTTGCTCCCCACGCTTTCGAGCCTCAGCGTCAGTTACAGACC
AGAGAGCCGCCTTCGCCACTGGTGTTCCTCCATATATCTACGCATTTCACC
GCTACACATGGAATTCCACTCTCCTCTTCTGCACTCAAGTCTCCCAGTTTCC
AATGACCCTCCCCGGTTGAGCCGGGGGCTTTCACATCAGACTTAAGAAACC
GCCTGCGCTCGCTTTACGCCCAATAAATCCGGACAACGCTTGCCACCTACG
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GTGTCTCAGTCCCAATGTGGCCGATCACCCTCTCAGGTCGGCTATGCATCG
TGGCCTTGGTGAGCCGTTACCTCACCAACTAGCTAATGCACCGCGGGTCCA
TCCATCAGCGACACCCGAAAGCGCCTTTCAAATCAAAACCATGCGGTTTCG
ATTGTTATACGGTATTAGCACCTGTTTCCAAGTGTTATCCCCTTCTGATGGG
CAGGTTACCCACGTGTTACTCACCCGTTCGCCACTCTTCTTTTTCCGGTGGA
GCAAGCTCCGGTGGAAAAAGAAGCGTACGACTTGCATGTATTAGGCACGC
CGCCAGCGTTCGTCCTGAGCCAGTAYMAAAACTCTCAA

Enterococcus faecium A029

CCRGGCGGGGRGTGCTTAATGCGTTAGCTGCAGCACTGAAGGGCGGAAAC
CCTCCAACACTTAGCACTCATCGTTTACGGCGTGGACTACCAGGGTATCTA
ATCCTGTTTGCTCCCCACGCTTTCGAGCCTCAGCGTCAGTTACAGACCAGA
GAGCCGCCTTCGCCACTGGTGTTCCTCCATATATCTACGCATTTCACCGCTA
CACATGGAATTCCACTCTCCTCTTCTGCACTCAAGTCTCCCAGTTTCCAATG
ACCCTCCCCGGTTGAGCCGGGGGCTTTCACATCAGACTTAAGAAACCGCCT
GCGCTCGCTTTACGCCCAATAAATCCGGACAACGCTTGCCACCTACGTATT
ACCGCGGCTGCTGGCACGTAGTTAGCCGTGGCTTTCTGGTTAGATACCGTC
AAGGGATGAACAGTTACTCTCATCCTTGTTCTTCTCTAACAACAGAGTTTTA
CGATCCGAAAACCTTCTTCACTCACGCGGCGTTGCTCGGTCAGACTTTCGT
CCATTGCCGAAGATTCCCTACTGCTGCCTCCCGTAGGAGTTTGGGCCGTGT

CTCAGTCCCAATGTGGCCGATCACCCTCTCAGGTCGGCTATGCATCGTGGC
CTTGGTGAGCCGTTACCTCACCAACTAGCTAATGCACCGCGGGTCCATCCA
TCAGCGACACCCGAAAGCGCCTTTCAAATCAAAACCATGCGGTTTCGATTG
TTATACGGTATTAGCACCTGTTTCCAAGTGTTATCCCCTTCTGATGGGCAGG
TTACCCACGTGTTACTCACCCGTTCGCCACTCTTCTTTTTCCGGTGGAGCAA
GCTCCGGTGGAAAAAGAAGCGTACGACTTGCATGTATTAGGCACGCCGCC
AGCGTTCGTCCTGAGCCAGTWYWMAAAACMYYCACA

Lactobacillus plantarum
AACTCTGTGATTGGTGCTTGCATCATGATTTACATTTGAGTGAGTGGCGAA
CTGGTGAGTAACACGTGGGAAACCTGCCCAGAAGCGGGGGATAACACCTG
GAAACAGATGCTAATACCGCATAACAACTTGGACCGCATGGTCCGAGTTTG
AAAGATGGCTTCGGCTATCACTTTTGGATGGTCCCGCGGCGTATTAGCTAG
ATGGTGGGGTAACGGCTCACCATGGCAATGATACGTAGCCGACCTGAGAG
GGTAATCGGCCACATTGGGACTGAGACACGGCCCAAACTCCTACGGGAGG
CAGCAGTAGGGAATCTTCCACAATGGACGAAAGTCTGATGGAGCAACGCC
GCGTGAGTGAAGAAGGGTTTCGGCTCGTAAAACTCTGTTGTTAAAGAAGA
ACATATCTGAGTAACTGTTCAGGTATTGACGGTATTTAACAAAGCCTATGC
CGCCGcGGTAATACGTAAGCGTCCGGATTTAT

Lactobacillus plantarum

TCGAACTCTGGTATTGATTGGTGCTTGCATCATGATTTACATTTGAGTGAGT
GGCGAACTGGTGAGTAACACGTGGGAAACCTGCCCAGAAGCGGGGGATAA
CACCTGGAAACAGATGCTAATACCGCATAACAACTTGGACCGCATGGTCC
GAGTTTGAAAGATGGCTTCGGCTATCACTTTTGGATGGTCCCGCGGCGTAT
TAGCTAGATGGTGGGGTAACGGCTCACCATGGCAATGATACGTAGCCGAC
CTGAGAGGGTAATCGGCCACATTGGGACTGAGACACGGCCCAAACTCCTA
CGGGAGGCAGCAGTAGGGAATCTTCCACAATGGACGAAAGTCTGATGGAG
CAACGCCGCGTGAGTGAAGAAGGGTTTCGGCTCGTAAAACTCTGTTGTTAA
AGAAGAACATATCTGAGAGTAACTGTTCAGGTATTGACGGTATTTAACCAG
AAAGCCACGGCTAACTACGTGCCAGCAGCCGCGGTAATACGTAGGTGGCA
AGCGTTGTCCGGATTTATTGGGCGTAAGCGCAGGCGGTTTTTTAAGTCTGA
TGTTTCGGCTCAAAACTGGGGAAAC

Enterococcus durans

CTACGCTTCTTTTTCACCGGAGCTTGCCGGAAAAAGAAGAGTGGCGAACGG
GTGAGTAACACGTGGGTAACCTGCCCATCAGAAGGGGATAACACTTGGAA
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GGATGAGAGTAACTGTTCATCCCTTGACGGTATCTAACCAGAAAGCCACGG
CTAACTACGTGCCAGCAGCCGCGGTAATACGTAGGTGGCAAGCGTTGTCCG
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CCGGCTCAA

Enterococcus faecium

GTCGTACGCTTCTTTGAGCTTGCCGGAAAAAGAAGAGTGGCGAACGGGTG
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CGGCCACATTGGGACTGAGACACGGCCCAAACTCCTACGGGAGGCAGCAG
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GTGAAGAAGGTTTTCGGATAAACTCTGTTGTTAGAGAACAAGGATGAACT
GTTCATCCCTTGACGTCTAACCAGAAAGCG

Enterococcus durans A098

AGTCGTACGCTTCTTTGAGCTTGCGGAAAAAGAAGAGTGGCGAACGGGTG
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GGTGCTAATACCGTATAACAATCGAAACCGCATGGTTTTGATTTGAAAGGC
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AGGTAACGGCTCACCAAGGCCACGATGCATAGCCGACCTGAGAGGGTGAT
CGGCCACATTGGGACTGAGACACGGCCCAAACTCCTACGGGAGGCAGCAG
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GTGAAGAAGGTTTTCGGATCGTAAAACTCTGTTGTTAGAGAAGAACAAGG
ATGAGAGTAACTGTTCATCCCTTGACGGTATCTAACCAGAAAGCCACGGCT
A

Enterococcus durans A097

CAGTCGTACGCTTCTTTGAGCTTGCCGGAAAAAGAAGAGTGGCGAACGGG
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CAGGTGCTAATACCGTATAACAATCGAAACCGCATGGTTTTGATTTGAAAG
GCGCTTTCGGGTGTCGCTGATGGATGGACCCGCGGTGCATTAGCTAGTTGG
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Enterococcus lactis A096

GCAGTCGTACGCTTCTTTTTCCACCGGAGCTTGCGGAAAAAGAAGAGTGGC
GAACGGGTGAGTAACACGTGGGTAACCTGCCCATCAGAAGGGGATAACAC
TTGGAAACAGGTGCTAATACCGTATAACAATCGAAACCGCATGGTTTTGAT
TTGAAAGGCGCTTTCGGGTGTCGCTGATGGATGGACCCGCGGTGCATTAGC
TAGTTGGTGAGGTAACGGCTCACCAAGGCCACGATGCATAGCCGACCTGA
GAGGGTGATCGGCCACATTGGGACTGAGACACGGCCCAAACTACGGGAGG
CAGCAGTAGGGAATCTTCGGCAATGGACGAAAGTCTGACCGAGCAACGCC
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ACAAGGATGAGAGTAACTGTTCATCCCTTGACGGTATCTAACCAGAAAGCC
ACGGCTAACATGCCAGCAGCCGCGGTAATACGTAGGTGGCAAGCGTTGTC
CGGATTTATTGGGCGTAAAGCGAGCGCAGGCGGTTTCTTAAGTCTGATGTG
AAAGCCCCCGGCTCAACCGGGGAGGGTCATTGGAAACTGGGAGACTTGAG
TGCAGAGAGTGGAATTCCATGTGTGAAA

Enterococcus durans A095

CAGTCGTACGCTTCTTTGAGCTTGCCGGAAAAAGAAGAGTGGCGAACGGG
TGAGTAACACGTGGGTAACCTGCCCATCAGAAGGGGATAACACTTGGAAA
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GGATGAGAGTAACTGTTCATCCCTTGACGGTATCTAACCAGAAAGCCACGG
CTAACTACGTGCCAGCAGCCGCGGTAATACGTAGGTGGCAAGCGTTGTCCG
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AGCCCCCGGCTCAACCGAGGGTCATTGGAAACTGGGAGACTTGAGTGCAG
AAGAGGAATTCCATGTGTAGCGGTGAAATGCGAGGAGGAACACCAGTGGC
G

Lactobacillus plantarum A094

CGAACGAACTCTGGTATTGATTGGTGCTTGCATCATGATTTACATTTGAGT
GAGTGGCGAACTGGTGAGTAACACGTGGGAAACCTGCCCAGAAGCGGGGG
ATAACACCTGGAAACAGATGCTAATACCGCATAACAACTTGGACCGCATG
GTCCGAGTTTGAAAGATGGCTTCGGCTATCACTTTTGGATGGTCCCGCGGC
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GACCTGAGAGGGTAATCGGCCACATTGGGACTGAGACACGGCCCAAACTC
CTACGGGAGGCAGCAGTAGGGAATCTTCCACAATGGACGAAAGTCTGATG
GAGCAACGCCGCGTGAGTGAAGAAGGGTTTCGGCTCGTAAAACTCTGTTGT
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CAGAAAGCCACGGCTAACTACGTGCCAGCAGCCGCGGTAATACGTAGGTG
GCAAGCGTTGTCCGGATTTATTGGGCGTAAAGCGAGCGCAGGCGGTTTTTT
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Leuconostoc pseudomesenteroides A093

GTCGTACGCTTCTTTGAGCTTGCCGGAAAAAGAAGAGTGGCGAACGGGTG
AGTAACACGTGGGTAACCTGCCCATCAGAAGGGGATAACACTTGGAAACA
GGTGCTAATACCGTATAACAATCGAAACCGCATGGTTTTGATTTGAAAGGC
GCTTTCGGGTGTCGCTGATGGATGGACCCGCGGTGCATTAGCTAGTTGGTG
AGGTAACGGCTCACCAAGGCCACGATGCATAGCCGACCTGAGAGGGTGAT
CGGCCACATTGGGACTGAGACACGGCCCAAACTACGGGAGGCAGCAGTAG
GGAATCTTCGGCAATGGACGAAAGTCTGACCGAGCAACGCCGCGTGAGTG
AAGAAGGTTTTCGGATCGTAAAACTCTGTTGTTAGAGAAGAACAAGGATG
AGAGTAACTGTTCATCCCTTGACGGTATCTAACCAGAAAGCCACGGCTAAC
TACGTGCCAGCAGCCGCGGTAATACGTAGGTGGCAAGCGTTGTCCGGATTT
ATTGGGCGTAAAGCGAGCGCAGGCGGTTTCTTAAGTCTGATGTGAAAGCCC
CCGGCTCAACCGGGGAGGGTCATTGGAAACTGGGAGACTTGAGTGCAGAA
GAGGAGAGTGGAATTCCATGTGTAGTGAAATGCGTAGGAAC

Enterococcus faecium A090

GAGCTTGCTCCACCGGAAAAAGAAGAGTGGCGAACGGGTGAGTAACACGT
GGGTAACCTGCCCATCAGAAGGGGATAACACTTGGAAACAGGTGCTAATA
CCGTATAACAATCGAAACCGCATGGTTTTGATTTGAAAGGCGCTTTCGGGT
GTCGCTGATGGATGGACCCGCGGTGCATTAGCTAGTTGGTGAGGTAACGGC
TCACCAAGGCCACGATGCATAGCCGACCTGAGAGGGTGATCGGCCACATT
GGGACTGAGACACGGCCCAAACTCCTACGGGAGGCAGCAGTAGGGAATCT
TCGGCAATGGACGAAAGTCTGACCGAGCAACGCCGCGTGAGTGAAGAAGG
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TGTTCATCCCTTGACGGTATCTAACCAGAAAGCCACGGCTAACTACGTGCC
AGCAGCCGCGGTAATACGTAGGTGGCAAGCGTTGTCCGGATTTATTGGGCG
TAAAGCGAGCGCAGGCGGTTTCTTAAGTCTGATGTGAAAGCCCCCGGCTCA
ACCGGGGAGGGTCATTGGAAACTGGGAGTGCAGAGGATTCTTAGCGGTG

Leuconostoc pseudomesenteroides A089

GTCGTACGCTTCTTTGAGCTTGCTCCAAAAGAAGAGTGGCGAACGGGTGAG
TAACACGTGGGTAACCTGCCCATCAGAAGGGGATAACACTTGGAAACAGG
TGCTAATACCGTATAACAATCGAAACCGCATGGTTTTGATTTGAAAGGCGC
TTTCGGGTGTCGCTGATGGATGGACCCGCGGTGCATTAGCTAGTTGGTGAG
GTAACGGCTCACCAAGGCCACGATGCATAGCCGACCTGAGAGGGTGATCG
GCCACATTGGGACTGAGACACGGCCCAAACTCctACGGGAGGCAGCAGTAG
GGAATCTTCGGCAATGGACGAAAGTCTGACCGAGCAACGCCGCGTGAGTG
AAGAAGGTTTTCGGATCGTAAAACTCTGTTGTTAGAGAAGAACAAGGATG
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TACGTGCCAGCAGCCGCGGTAATACGTAGGTGGCAAGCGTTGTCCGGATTT
ATTGGGCGTAAAGCGAGCGCAGGCGGTTTCTTAAGTCTGATGTGAAAGCCC
CCGGCTCAACCGGGGAGGGTCATTGGAAACTGGGAGAAGT

Enterococcus faecium A087

AAAGAAGAGTGGCGAACGGGTGAGTAACACGTGGGTAACCTGCCCATCAG
AAGGGGATAACACTTGGAAACAGGTGCTAATACCGTATAACAATCGAAAC

CGCATGGTTTTGATTTGAAAGGCGCTTTCGGGTGTCGCTGATGGATGGACC
CGCGGTGCATTAGCTAGTTGGTGAGGTAACGGCTCACCAAGGCCACGATG
CATAGCCGACCTGAGAGGGTGATCGGCCACATTGGGACTGAGACACGGCC
CAAACTACGGGAGGCAGCAGTAGGGAATCTTCGGCAATGGACGAAAGTCT
GACCGAGCAACGCCGCGTGAGTGAAGAAGGTTTTCGGATCGTAAAACTCT
GTTGTTAGAGAAGAACAAGGATGAGAGTAACTGTTCATCCCTTGACGGTAT
CTAACCAGAAAGCCACGGCTAACTACGTGCCAGCAGCCGCGGTAATACGT
AGGTGGCAAGCGTTGTCCGGATTTATTGGGCGTAAAGCGAGCGCAGGCGG
TTTCTTAAGTCTGATGTGAAAGCCCCCGGCTCAAACCGGGGAGGGT

Lactobacillus plantarum A084

GTCGaaCGAACTCTGGTATTGATTGGTGCTTGCATCATGATTTACATTTGAG
TGAGTGGCGAACTGGTGAGTAACACGTGGGAAACCTGCCCAGAAGCGGGG
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GGTCCGAGTTTGAAAGATGGCTTCGGCTATCACTTTTGGATGGTCCCGCGG
CGTATTAGCTAGATGGTGGGGTAACGGCTCACCATGGCAATGATACGTAGC
CGACCTGAGAGGGTAATCGGCCACATTGGGACTGAGACACGGCCCAAACT
CCTACGGGAGGCAGCAGTAGGGAATCTTCCACAATGGACGAAAGTCTGAT
GGAGCAACGCCGCGTGAGTGAAGAAGGGTTTCGGCTCGTAAAACTCTGTT
GTTAAAGAAGAACATATCTGAGAGTAACTGTTCAGGTATTGACGGTATTTA
ACCAGAAAGCCACGGCTAACTACGTGCCAGCAGCCGCGGTAATACGTAGG
TGGCAAGCGTTGTCCGGATTTATTGGGCGTAAAGCGAGGCGTT

Enterococcus faecium A083

GTCGTACGCTTCTTTTTCCACCGGAGCTTGCCGGAAAAAGAAGAGTGGCGA
ACGGGTGAGTAACACGTGGGTAACCTGCCCATCAGAAGGGGATAACACTT
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GAAAGGCGCTTTCGGGTGTCGCTGATGGATGGACCCGCGGTGCATTAGCTA
GTTGGTGAGGTAACGGCTCACCAAGGCCACGATGCATAGCCGACCTGAGA
GGGTGATCGGCCACATTGGGACTGAGACACGGCCCAAACTACGGGAGGCA
GCAGTAGGGAATCTTCGGCAATGGACGAAAGTCTGACCGAGCAACGCCGC
GTGAGTGAAGAAGGTTTTCGGATCGTAAAACTCTGTTGTTAGAGAAGAAC
AAGGATGAGAGTAACTGTTCATCCCTTGACGGTATCTAACCAGAAAGCCAC
GGCTAACTACGTGCCAGCAGCCGCGGTAATACGTAGGTGGCAAGCGTTGT
CCGGATTTATTGGGCGTAAAGCGAGCGCAGGCGGTTTCTTAAGTCTGATGT
GAAAGCCCCCGGCTCAACCGGGGAGGGTCATTGGGAGACTTGAGTGCAAG
TGGAATTCCATTGTAGCGGTGAAATGCTT

Enterococcus lactis A081

GCAGTCGTACGCTTCTTTGAGCTTGCCGGAAAAAGAAGAGTGGCGAACGG
GTGAGTAACACGTGGGTAACCTGCCCATCAGAAGGGGATAACACTTGGAA
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GGCGCTTTCGGGTGTCGCTGATGGATGGACCCGCGGTGCATTAGCTAGTTG

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TAGGGAATCTTCGGCAATGGACGAAAGTCTGACCGAGCAACGCCGCGTGA
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GCCCCCGGCTCAACCGGGGAGGGTCATTGGAAACTGGGAGACTTGAGTGC
AGAAGAGGAGAGTGGAATTCCATGTGTAGCGGTGAAATGCGTAGATATAT
GGAGGAACACCTGCGAACTCTGGTGACG

Enterococcus faecium A080

CGGAAAAAGAAGAGTGGCGAACGGGTGAGTAACACGTGGGTAACCTGCCC
ATCAGAAGGGGATAACACTTGGAAACAGGTGCTAATACCGTATAACAATC
GAAACCGCATGGTTTTGATTTGAAAGGCGCTTTCGGGTGTCGCTGATGGAT
GGACCCGCGGTGCATTAGCTAGTTGGTGAGGTAACGGCTCACCAAGGCCA
CGATGCATAGCCGACCTGAGAGGGTGATCGGCCACATTGGGACTGAGACA
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GAAAGTCTGACCGAGCAACGCCGCGTGAGTGAAGAAGGTTTTCGGATCGT
AAAACTCTGTTGTTAGAGAAGAACAAGGATGAGAGTAACTGTTCATCCCTT
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CAGGCGGTTTCTTAAGTCTGATGTGAAAGCCCCCGGCTCAACCGGGGAGG
GTCATTGGAAACTGGGAGACTTGAGTGCAGAAGAGGAGAGTGGAATTCCA
TGTGTAGCGGTGAAATGCGTAGATTCACCAGTGGCGCTCTCTGGTCTGTCT
GACTCT

Lactobacillus plantarum A079

TCCTCTGGTATTGATTGGTGCTTGCATCATGATTTACATTTGAGTGAGTGGC
GAACTGGTGAGTAACACGTGGGAAACCTGCCCAGAAGCGGGGGATAACAC
CTGGAAACAGATGCTAATACCGCATAACAACTTGGACCGCATGGTCCGAT
GAAAGATGGCTTCGGCTATCACTTTTGGATGGTCCCGCGGCGTATTAGCTA
GATGGTGGGGTAACGGCTCACCATGGCAATGATACGTAGCCGACCTGAGA
GGGTAATCGGCCACATTGGGACTGAGACACGGCCCAAACTCCTACGGGAG
GCAGCAGTAGGGAATCTTCCACAATGGACGAAAGTCTGATGGAGCAACGC
CGCGTGAGTGAAGAAGGGTTTCGGCTCGTAAAACTCTGTTGTTAAAGAAG
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AAGACAGTGGAACTCCATGGTGAAATGCGTATGGAAGAACACCAGTGGCG
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Enterococcus faecalis A077

ACGCTTCTTTCCTCCCGAGTGCTTGCACTCAATTGGAAAGAGGAGTGGCGG
ACGGGTGAGTAACACGTGGGTAACCTACCCATCAGAGGGGGATAACACTT
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ATGTGAAAGCCCCCGGCTCaaccGGGGAGGGTCATTGGAAACTGGGAGACTT
GAGTGCAGAAGAGGAGAGTCATGTGTAGCGGTGAAATGCGTATATATGGA
GGAAC

Lactobacillus plantarum A075

TCGAACGAACTCTGGTATTGATTGGTGCTTGCATCATGATTTACATTTGAGT
GAGTGGCGAACTGGTGAGTAACACGTGGGAAACCTGCCCAGAAGCGGGGG
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GACCTGAGAGGGTAATCGGCCACATTGGGACTGAGACACGGCCCAAACTC
CTACGGGAGGCAGCAGTAGGGAATCTTCCACAATGGACGAAAGTCTGATG
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CAGAAAGCCACGGCTAACTACGTGCCAGCAGCCGCGGTAATACGTAGGTG
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Pediococcus pentosaceus A074

TAATTGATTATGACGTACTTGTACTGATTGAGATTTTAACACGAAGTGAGT
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CACCTGGAAACAGATGCTAATACCGTATAACAGAGAAAACCGCATGGTTT
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AGCTAGTTGGTGAGGTAAAGGCTCACCAAGGCAGTGATACGTAGCCGACC
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AGCGTTATCCGGATTTAT

Lactobacillus plantarum
GAACTCTGGTATTGATTGGTGCTTGCATCATGATTTACATTTGAGTGAGTG
GCGAACTGGTGAGTAACACGTGGGAAACCTGCCCAGAAGCGGGGGATAAC
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GAGGCAGCAGTAGGGAATCTTCCACAATGGACGAAAGTCTGATGGAGCAA
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GCCACGGCTAACTACGTGCCAGCCGCGGTAATACGTAGGTGGCAAGCGTT
GTCCGGATTTATTGGA

Lactobacillus rhamnosus A072

CGAGTTCTGATTATTGAAAGGTGCTTGCATCTTGATTTAATTTTGAACGAGT
GGCGGACGGGTGAGTAACACGTGGGTAACCTGCCCTTAAGTGGGGGATAA
CATTTGGAAACAGATGCTAATACCGCATAAATCCAAGAACCGCATGGTTCT
TGGCTGAAAGATGGCGTAAGCTATCGCTTTTGGATGGACCCGCGGCGTATT
AGCTAGTTGGTGAGGTAACGGCTCACCAAGGCAATGATACGTAGCCGAAC
TGAGAGGTTGATCGGCCACATTGGGACTGAGACACGGCCCAAACTCCTAC
GGGAGGCAGCAGTAGGGAATCTTCCACAATGGACGCAAGTCTGATGGAGC
AACGCCGCGTGAGTGAAGAAGGCTTTCGGGTCGTAAAACTCTGTTGTTGGA
GAATGGTCGGCAGagtAACTGTTGTTATCCACAGAAAGC

Lactobacillus plantarum A071

GAACTCTGGTATTGATTGGTGCTTGCATCATGATTTACATTTGAGTGAGTG
GCGAACTGGTGAGTAACACGTGGGAAACCTGCCCAGAAGCGGGGGATAAC
ACCTGGAAACAGATGCTAATACCGCATAACAACTTGGACCGCATGGTCCG
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GAGGGTAATCGGCCACATTGGGACTGAGACACGGCCCAAACTCCTACGGG
AGGCAGCAGTAGGGAATCTTCCACAATGGACGAAAGTCTGATGGAGCAAC
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CCACGAACTACGTGCCAGCAGCCGCGGTAATACGTAGGTGGCAAGCGTTG
TCCGGATTTATTGGGCGTAAACGCGGGTTTTTTAAGTCTGA

Enterococcus faecium A066

CGGAAAAAGAAGAGTGGCGAACGGGTGAGTAACACGTGGGTAACCTGCCC
ATCAGAAGGGGATAACACTTGGAAACAGGTGCTAATACCGTATAACAATC
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CGATGCATAGCCGACCTGAGAGGGTGATCGGCCACATTGGGACTGAGACA
CGGCCCAAACTCCTACGGGAGGCAGCAGTAGGGAATCTTCGGCAATGGAC
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Leuconostoc pseudomesenteroides A064

CGGAAAAAGAAGAGTGGCGAACGGGTGAGTAACACGTGGGTAACCTGCCC
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GAAACCGCATGGTTTTGATTTGAAAGGCGCTTTCGGGTGTCGCTGATGGAT
GGACCCGCGGTGCATTAGCTAGTTGGTGAGGTAACGGCTCACCAAGGCCA
CGATGCATAGCCGACCTGAGAGGGTGATCGGCCACATTGGGACTGAGACA
CGGCCCAAACTCCTACGGGAGGCAGCAGTAGGGAATCTTCGGCAATGGAC
GAAAGTCTGACCGAGCAACGCCGCGTGAGTGAAGAAGGTTTTCGGATCGT
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CAGGCGGTTTCTTAAGTCTGATGTGAAAGCCCCCGGCTCAACCGGGGAGG
GTCATTGGAAACTGGGAGACTTGAGTGCAAGATGGAATCCATGTGTAGCG
GTGAAATGCGTAGATATATGGAGGAA

Enterococcus lactis A060

GTCGTACGCTTCTTTTGAGCTTGCTCCAAAAGAAGAGTGGCGAACGGGTGA
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CTTTCGGGTGTCGCTGATGGATGGACCCGCGGTGCATTAGCTAGTTGGTGA
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GGCCACATTGGGACTGAGACACGGCCCAAACTCCTACGGGAGGCAGCAGT
AGGGAATCTTCGGCAATGGACGAAAGTCTGACCGAGCAACGCCGCGTGAG
TGAAGAAGGTTTTCGGATCGTAAAACTCTGTTGTTAGAGAAGAACAAGGA
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ACTACGTGCCAGCAGCCGCGGTAATACGTAGGTGGCAAGCGTTGTCCGGA
TTTATTGGGCGTAAAGCGAGCGCAGGCGGTTTCTTAAGTCTGATGTGAAAG
CCCCCGGCTCAACCGGGGAGGGTCATTGGAAACTGGGACTTGAGTGCAG

Lactobacillus plantarum A059

GCGAACTCTGGTATTGATTGGTGCTTGCATCATGATTTACATTTGAGTGAGT
GGCGAACTGGTGAGTAACACGTGGGAAACCTGCCCAGAAGCGGGGGATAA
CACCTGGAAACAGATGCTAATACCGCATAACAACTTGGACCGCATGGTCC
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GCTAGATGGTGGGGTAACGGCTCACCATGGCAATGATACGTAGCCGACCT
GAGAGGGTAATCGGCCACATTGGGACTGAGACACGGCCCAAACTCCTACG

GGAGGCAGCAGTAGGGAATCTTCCACAATGGACGAAAGTCTGATGGAGCA
ACGCCGCGTGAGTGAAGAAGGGTTTCGGCTCGTAAAACTCTGTTGTTAAAG
AAGAACATATCTGAGAGTAACTGTTCAGGTATTGACGGTATTTAACCAGAA
AGCCACGGCTAACTACGTGCCAGCAGCCGCGGTAATACGTAGGTGGCAAG
CGTTGTCCGGATTTATTGGGCGTAAAGCGAGCGCAGGCGGTTTTTTAAGTC
TGATGTGAAAGCCTTCGGCTCAACCGAAGAAGTGCATCGGAAACTGGGAA
ACTTGAGTGCAGAAGAGGACAGgAACTCCATGTGTAGCGGTGAAATTATG
GA

Enterococcus faecalis A058

TCTCCGAGTGCTTGCACTCAATTGGAAAGAGGAGTGGCGGACGGGTGAGT
AACACGTGGGTAACCTACCCATCAGAGGGGGATAACACTTGGAAACAGGT
GCTAATACCGCATAACAGTTTATGCCGCATGGCATAAGAGTGAAAGGCGC
TTTCGGGTGTCGCTGATGGATGGACCCGCGGTGCATTAGCTAGTTGGTGAG
GTAACGGCTCACCAAGGCCACGATGCATAGCCGACCTGAGAGGGTGATCG
GCCACACTGGGACTGAGACACGGCCCAGACTCCTACGGGAGGCAGCAGTA
GGGAATCTTCGGCAATGGACGAAAGTCTGACCGAGCAACGCCGCGTGAGT
GAAGAAGGTTTTCGGATCGTAAAACTCTGTTGTTAGAGAAGAACAAGGAC
GTTAGTAACTGAACGTCCCCTGACGGTATCTAACCAGAAAGCCACGGCTAA
CTACGTGCCAGCAGCCGCGGTAATACGTAGGTGGCAAGCGTTGTCCGGATT
TATTGCGTAAAGCGAGCGCAGGCGGTTTCTTAAGTCTGATGTGAAAGCCCC
CGGctcAACCGGG

Enterococcus lactis A052

GCAGTCGTACGCTTCTTTGGAGCTTGCCGGAAAAAGAAGAGTGGCGAACG
GGTGAGTAACACGTGGGTAACCTGCCCATCAGAAGGGGATAACACTTGGA
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AGGCGCTTTCGGGTGTCGCTGATGGATGGACCCGCGGTGCATTAGCTAGTT
GGTGAGGTAACGGCTCACCAAGGCCACGATGCATAGCCGACCTGAGAGGG
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GATTTATTGGGCGTAAAGCGAGCGCAGGCGGTTTCTTAAGTCTGATGTGAA
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CAGAGAGAGTGGATCCATGC

Lactobacillus plantarum A051

TCGaACGAACTCTGGTATTGATTGGTGCTTGCATCATGATTTACATTTGAGT
GAGTGGCGAACTGGTGAGTAACACGTGGGAAACCTGCCCAGAAGCGGGGG
ATAACACCTGGAAACAGATGCTAATACCGCATAACAACTTGGACCGCATG
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GTATTAGCTAGATGGTGGGGTAACGGCTCACCATGGCAATGATACGTAGCC
GACCTGAGAGGGTAATCGGCCACATTGGGACTGAGACACGGCCCAAACTC
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GAGCAACGCCGCGTGAGTGAAGAAGGGTTTCGGCTCGTAAAACTCTGTTGT
TAAAGAAGAACATATCTGAGAGTAACTGTTCAGGTATTGACGGTATTTAAC
CAGAAAGCCACGGCTAACTACGTGCCAGCAGCCGCGGTAATACGTAGGTG
GCAAGCGTTGTCCGGATTTATTGGGCGTAAAGCGCGCGTTTTTTTGA

Enterococcus durans A050

CAGTCGTACGCTTCTTTTTGAGCTTGCGGAAAAAGAAGAGTGGCGAACGG
GTGAGTAACACGTGGGTAACCTGCCCATCAGAAGGGGATAACACTTGGAA
ACAGGTGCTAATACCGTATAACAATCGAAACCGCATGGTTTTGATTTGAAA
GGCGCTTTCGGGTGTCGCTGATGGATGGACCCGCGGTGCATTAGCTAGTTG
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GATCGGCCACATTGGGACTGAGACACGGCCCAAACTACGGGAGGCAGCAG
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AACTACGTGCCAGCAGCCGCGGTAATACGTAGGTGGCAAGCGTTGTCCGG
ATTTATTGGGCGTAAAGCGAGCGCAGGCGGTTTCTTAAGTCTGATGTGAAA
GCCCCCGGCTCAACCGGGGAGGGTCATAACTGGGAGACTTG

Enterococcus hirae A049

CAGTCGTACGCTTCTTTTTCCACCGGAGCTTGCCGGAAAAAGAAGAGTGGC
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TTGGAAACAGGTGCTAATACCGTATAACAATCGAAACCGCATGGTTTTGAT
TTGAAAGGCGCTTTCGGGTGTCGCTGATGGATGGACCCGCGGTGCATTAGC
TAGTTGGTGAGGTAACGGCTCACCAAGGCCACGATGCATAGCCGACCTGA
GAGGGTGATCGGCCACATTGGGACTGAGACACGGCCCAAACTCCTACGGG
AGGCAGCAGTAGGGAATCTTCGGCAATGGACGAAAGTCTGACCGAGCAAC
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GAACAAGGATGAGAGTAACTGTTCATCCCTTGACGGTATCTAACCAGAAA
GCCACGGCTAACTACGTGCCAGCAGCCGCGGTAATACGTAGGTGGCAAGC
GTTGTCCGGATTTATTGGGCGTAAAGCCGCAGGCGGTTTCTG

Lactobacillus plantarum A048

TCGAACGAACTCTGGTATTGATTGGTGCTTGCATCATGATTTACATTTGAGT
GAGTGGCGAACTGGTGAGTAACACGTGGGAAACCTGCCCAGAAGCGGGGG
ATAACACCTGGAAACAGATGCTAATACCGCATAACAACTTGGACCGCATG
GTCCGAGTTTGAAAGATGGCTTCGGCTATCACTTTTGGATGGTCCCGCGGC
GTATTAGCTAGATGGTGGGGTAACGGCTCACCATGGCAATGATACGTAGCC
GACCTGAGAGGGTAATCGGCCACATTGGGACTGAGACACGGCCCAAACTC
CTACGGGAGGCAGCAGTAGGGAATCTTCCACAATGGACGAAAGTCTGATG
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CAGAAAGCCACGGCTAACTACGTGCCAGCAGCCGCGGTAATACGTAGGTG
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TGAGTGCAG

Lactobacillus plantarum A047

GATTGGTGCTTGCATCATGATTTACATTTGAGTGAGTGGCGAACTGGTGAG
TAACACGTGGGAAACCTGCCCAGAAGCGGGGGATAACACCTGGAAACAGA
TGCTAATACCGCATAACAACTTGGACCGCATGGTCCGAGTTTGAAAGATGG
CTTCGGCTATCACTTTTGGATGGTCCCGCGGCGTATTAGCTAGATGGTGGG
GTAACGGCTCACCATGGCAATGATACGTAGCCGACCTGAGAGGGTAATCG
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GGGAATCTTCCACAATGGACGAAAGTCTGATGGAGCAACGCCGCGTGAGT
GAAGAAGGGTTTCGGCTCGTAAAACTCTGTTGTTAAAGAAGAACATATCTG
AGAGTAACTGTTCAGGTATTGACGGTATTTAACCAGAAAGCCACGGCTAAC
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ATTGGGCGTAAAGCGAGCGCAGGCGGTTTTTTAAGTCTGTGAAAGCCTTCA
ACCGAAGAAGTGCATCGGAA

Lactobacillus plantarum A046

GTGAACTCTGGTATTGATTGGTGCTTGCATCATGATTTACATTTGAGTGAGT
GGCGAACTGGTGAGTAACACGTGGGAAACCTGCCCAGAAGCGGGGGATAA
CACCTGGAAACAGATGCTAATACCGCATAACAACTTGGACCGCATGGTCC
GAGTTTGAAAGATGGCTTCGGCTATCACTTTTGGATGGTCCCGCGGCGTAT
TAGCTAGATGGTGGGGTAACGGCTCACCATGGCAATGATACGTAGCCGAC
CTGAGAGGGTAATCGGCCACATTGGGACTGAGACACGGCCCAAACTCCTA
CGGGAGGCAGCAGTAGGGAATCTTCCACAATGGACGAAAGTCTGATGGAG
CAACGCCGCGTGAGTGAAGAAGGGTTTCGGCTCGTAAAACTCTGTTGTTAA
AGAAGAACATATCTGAGAGTAACTGTTCAGGTATTGACGGTATTTAACCAG
AAAGCCACGGCTAACTACGTGCCAGCAGCCGCGGTAATACGTAGGTGGCA
AGCGTTGTCCGGATTTATTGGGCGTAAAGCGAGCGCAGGCGGTTTTTTAAG
TCTGATGTGAAAGCCTTCGGCTCAACCGAAGAAGTGCATCGGaaACTGGGA
AACTTGAGTGCAGAAGAGGACAGTGTCCATGTGTAGCGGTGAAATGCGTA
GATATATGGAACCACGGCTGTCTTCT

Leuconostoc pseudomesenteroides A044

GTCGTACGCTTCTTTTGAGCTTGCCGGAAAAAGAAGAGTGGCGAACGGGT
GAGTAACACGTGGGTAACCTGCCCATCAGAAGGGGATAACACTTGGAAAC
AGGTGCTAATACCGTATAACAATCGAAACCGCATGGTTTTGATTTGAAAGG
CGCTTTCGGGTGTCGCTGATGGATGGACCCGCGGTGCATTAGCTAGTTGGT
GAGGTAACGGCTCACCAAGGCCACGATGCATAGCCGACCTGAGAGGGTGA
TCGGCCACATTGGGACTGAGACACGGCCCAAACTACGGGAGGCAGCAGTA

GGGAATCTTCGGCAATGGACGAAAGTCTGACCGAGCAACGCCGCGTGAGT
GAAGAAGGTTTTCGGATCGTAAAACTCTGTTGTTAGAGAAGAACAAGGAT
GAGAGTAACTGTTCATCCCTTGACGGTATCTAACCAGAAAGCCACGGCTAA
CTACGTGCCAGCAGCCGCGGTAATACGTAGGTGGCAAGCGTTGTCCGGATT
TATTGGGCGTAAAGCGAGCGCAGGCGGTTTCTTAAGTCTGATGTGAAAGCC
CCCGGCTCAACCGGGGAGGGTCATTAACTGGGAGACTTGAGTGCA

Enterococcus faecalis A043

AAAGAAGAGTGGCGAACGGGTGAGTAACACGTGGGTAACCTGCCCATCAG
AAGGGGATAACACTTGGAAACAGGTGCTAATACCGTATAACAATCGAAAC
CGCATGGTTTTGATTTGAAAGGCGCTTTCGGGTGTCGCTGATGGATGGACC
CGCGGTGCATTAGCTAGTTGGTGAGGTAACGGCTCACCAAGGCCACGATG
CATAGCCGACCTGAGAGGGTGATCGGCCACATTGGGACTGAGACACGGCC
CAAACTACGGGAGGCAGCAGTAGGGAATCTTCGGCAATGGACGAAAGTCT
GACCGAGCAACGCCGCGTGAGTGAAGAAGGTTTTCGGATCGTAAAACTCT
GTTGTTAGAGAAGAACAAGGATGAGAGTAACTGTTCATCCCTTGACGGTAT
CTAACCAGAAAGCCACGGCTAACTACGTGCCAGCAGCCGCGGTAATACGT
AGGTGGCAAGCGTTGTCCGGATTTATTGGGCGTAAAGCGAGCGCAGGCGG
TTTCTTAAGTCTGATGTGAAAgcCCCCGGCTCAACCGGGGAGGGTCATTGG
GGAGACTTGTGCAGAAGAGGAGAGAATTCCATGTG

Lactobacillus plantarum A041

TGAACTCTGTTGATTGGTGCTTGCATCATGATTTACATTTGAGTGAGTGGCG
AACTGGTGAGTAACACGTGGGAAACCTGCCCAGAAGCGGGGGATAACACC
TGGAAACAGATGCTAATACCGCATAACAACTTGGACCGCATGGTCCGAGTT
TGAAAGATGGCTTCGGCTATCACTTTTGGATGGTCCCGCGGCGTATTAGCT
AGATGGTGGGGTAACGGCTCACCATGGCAATGATACGTAGCCGACCTGAG
AGGGTAATCGGCCACATTGGGACTGAGACACGGCCCAAACTCCTACGGGA
GGCAGCAGTAGGGAATCTTCCACAATGGACGAAAGTCTGATGGAGCAACG
CCGCGTGAGTGAAGAAGGGTTTCGGCTCGTAAAACTCTGTTGTTAAAGAAG
AACATATCTGAGAGTAACTGTTCAGGTATTGACGGTATTTAACCAGAAAGC
CACGGCTAACTACGTGCCAGCAGCCGCGGTAATACGTAGGTGGCAAGCGT
TGTCCGGATTTATTGGGCGTAAAGCGAGCGCAGGCGGTTTTTTAAGTCG

Enterococcus faecium A040

ATGCAGTCGTCTCCGGAGCTTGCCGGAAAAAGAAGAGTGGCGAACGGGTG
AGTAACACGTGGGTAACCTGCCCATCAGAAGGGGATAACACTTGGAAACA
GGTGCTAATACCGTATAACAATCGAAACCGCATGGTTTTGATTTGAAAGGC
GCTTTCGGGTGTCGCTGATGGATGGACCCGCGGTGCATTAGCTAGTTGGTG
AGGTAACGGCTCACCAAGGCCACGATGCATAGCCGACCTGAGAGGGTGAT
CGGCCACATTGGGACTGAGACACGGCCCAAACTCCTACGGGAGGCAGCAG
TAGGGAATCTTCGGCAATGGACGAAAGTCTGACCGAGCAACGCCGCGTGA

GTGAAGAAGGTTTTCGGATCGTAAAACTCTGTTGTTAGAGAAGAACAAGG
ATGAGAGTAACTGTTCATCCCTTGACGGTATCTAACCAGAAAGCCACGGCT
AACTACGTGCCAGCAGCCGCGGTAATACGTAGGTGGCAAGCGTTGTCCGG
ATTTATTGGGCGTAAAGCGAGCGCAGGCGGTTTCTTAAGTCTGATGTGAAA
GCCCCCGGCTCAACCGGGGAGGGTCATTGGAAACTGGGAGACtTGAGTGCA
GAAGAGGAGAGTGGAATTCCATGTGTAGCGGTGAAATGC

Enterococcus faecium A039

CGGAAAAAGAAGAGTGGCGAACGGGTGAGTAACACGTGGGTAACCTGCCC
ATCAGAAGGGGATAACACTTGGAAACAGGTGCTAATACCGTATAACAATC
GAAACCGCATGGTTTTGATTTGAAAGGCGCTTTCGGGTGTCGCTGATGGAT
GGACCCGCGGTGCATTAGCTAGTTGGTGAGGTAACGGCTCACCAAGGCCA
CGATGCATAGCCGACCTGAGAGGGTGATCGGCCACATTGGGACTGAGACA
CGGCCCAAACTCCTACGGGAGGCAGCAGTAGGGAATCTTCGGCAATGGAC
GAAAGTCTGACCGAGCAACGCCGCGTGAGTGAAGAAGGTTTTCGGATCGT
AAAACTCTGTTGTTAGAGAAGAACAAGGATGAGAGTAACTGTTCATCCCTT
GACGGTATCTAACCAGAAAGCCACGGCA

Enterococcus lactis A038

GCAGTCGTACGCTTCTTTGGAGCTTGCGGAAAAAGAAGAGTGGCGAACGG
GTGAGTAACACGTGGGTAACCTGCCCATCAGAAGGGGATAACACTTGGAA
ACAGGTGCTAATACCGTATAACAATCGAAACCGCATGGTTTTGATTTGAAA
GGCGCTTTCGGGTGTCGCTGATGGATGGACCCGCGGTGCATTAGCTAGTTG
GTGAGGTAACGGCTCACCAAGGCCACGATGCATAGCCGACCTGAGAGGGT
GATCGGCCACATTGGGACTGAGACACGGCCCAAACTCCtACGGGAGGCAGC
AGTAGGGAATCTTCGGCAATGGACGAAAGTCTGACCGAGCAACGCCGCGT
GAGTGAAGAAGGTTTTCGGATCGTAAAACTCTGTTGTTAGAGAAGAACAA
GGATGAGAGTAACTGTTCATCCCTTGACGGTATCTAACCAGAAAGCCACGG
CTAACTAGCCGCAGCCGCAATACGTAGGTGGCAAGCGTTGTCCGGATTTAT
TGGGCGTAAAGCGAGCGCCGGTTTCTTAAGTCTGATGTGCCCCCGGCTCAA
CCGGTCTAACTGGGAGACT

Lactobacillus plantarum A037

AGTGAACTCTGGTATTGATTGGTGCTTGCATCATGATTTACATTTGAGTGA
GTGGCGAACTGGTGAGTAACACGTGGGAAACCTGCCCAGAAGCGGGGGAT
AACACCTGGAAACAGATGCTAATACCGCATAACAACTTGGACCGCATGGT
CCGAGTTTGAAAGATGGCTTCGGCTATCACTTTTGGATGGTCCCGCGGCGT
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ACCTGAGAGGGTAATCGGCCACATTGGGACTGAGACACGGCCCAAACTCC
TACGGGAGGCAGCAGTAGGGAATCTTCCACAATGGACGAAAGTCTGATGG
AGCAACGCCGCGTGAGTGAAGAAGGGTTTCGGCTCGTAAAACTCTGTTGTT
AAAGAAGAACATATCTGAGAGTAACTGTTCAGGTATTGACGGTATTTAACC
AGAAAGCCACACTACGTGCCAGCAGCCGTAATACGGTGGCAAGCGTTTGT
CACGGTTTTTTAAG

Lactobacillus plantarum A036

GTCGCGAACTCTGGTATTGATTGGTGCTTGCATCATGATTTACATTTGAGTG
AGTGGCGAACTGGTGAGTAACACGTGGGAAACCTGCCCAGAAGCGGGGGA
TAACACCTGGAAACAGATGCTAATACCGCATAACAACTTGGACCGCATGG
TCCGATTGAAAGATGGCTTCGGCTATCACTTTTGGATGGTCCCGCGGCGTA
TTAGCTAGATGGTGGGGTAACGGCTCACCATGGCAATGATACGTAGCCGA
CCTGAGAGGGTAATCGGCCACATTGGGACTGAGACACGGCCCAAACTCCT
ACGGGAGGCAGCAGTAGGGAATCTTCCACAATGGACGAAAGTCTGATGGA
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AAGAAGAACATATCTGAGAGTAACTGTTCAGGTATTGACGGTATTTAACCA
GAAAGCCACGGCTAACTACGTGCCAGCAGCCGCGGTAATACGTAGGTGGC
AAGCGTTGTCCGGATTTATTGGGCGTAAAGCGAGCGCAGGCGGTTTTTTAA
GTCTGATGTGAAAGCCTTCGGCTCAACCGAAGAAGTGCAGAACAACTTGA
GTGCAGAAGAGGACAGTCTCCATGTGTAGCGGTGAAATG

Lactobacillus plantarum A035

CAGTGAACTCTGGTATTGATTGGTGCTTGCATCATGATTTACATTTGAGTGA
GTGGCGAACTGGTGAGTAACACGTGGGAAACCTGCCCAGAAGCGGGGGAT
AACACCTGGAAACAGATGCTAATACCGCATAACAACTTGGACCGCATGGT
CCGAGTTTGAAAGATGGCTTCGGCTATCACTTTTGGATGGTCCCGCGGCGT
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TACGGGAGGCAGCAGTAGGGAATCTTCCACAATGGACGAAAGTCTGATGG
AGCAACGCCGCGTGAGTGAAGAAGGGTTTCGGCTCGTAAAACTCTGTTGTT
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AGAAAGCCACGGCTAACTACGTGCCagcAGCCGCGGTAATACGTAGGTGGC
AAGCGTTGTCCGGATTTATTGGGCGTAAAGCGAGCGCAGGCGGTTTTTTAA
GTCTGATGTGAACTTCGGCTCAACCGAAGTGCATCGGAAACTGGGAACTT

Lactobacillus plantarum A034

CAGTGAACGAACTCTGGTATTGATTGGTGCTTGCATCATGATTTACATTTG
AGTGAGTGGCGAACTGGTGAGTAACACGTGGGAAACCTGCCCAGAAGCGG
GGGATAACACCTGGAAACAGATGCTAATACCGCATAACAACTTGGACCGC
ATGGTCCGATTGAAAGATGGCTTCGGCTATCACTTTTGGATGGTCCCGCGG
CGTATTAGCTAGATGGTGGGGTAACGGCTCACCATGGCAATGATACGTAGC
CGACCTGAGAGGGTAATCGGCCACATTGGGACTGAGACACGGCCCAAACT
CCTACGGGAGGCAGCAGTAGGGAATCTTCCACAATGGACGAAAGTCTGAT
GGAGCAACGCCGCGTGAGTGAAGAAGGGTTTCGGCTCGTAAAACTCTGTT
GTTAAAGAAGAACATATCTGAGAGTAACTGTTCAGGTATTGACGGTATTTA
ACCAGAAAGCCACGGCTAACTACGTGCCAGCAGCCGCGGTAATACGTAGG

TGGCAAGCGTTGTCCGGATTTATTGGGCGTAAAGCGAGCGCAGGCGGTTTT
TTAAGTCTGATGTGAAAGCCTTCCTCAACCGAAGCAGAACTGGGTGT

Lactobacillus plantarum A033

GACTCTGGTATTGATTGGTGCTTGCATCATGATTTACATTTGAGTGAGTGGC
GAACTGGTGAGTAACACGTGGGAAACCTGCCCAGAAGCGGGGGATAACAC
CTGGAAACAGATGCTAATACCGCATAACAACTTGGACCGCATGGTCCGATT
GAAAGATGGCTTCGGCTATCACTTTTGGATGGTCCCGCGGCGTATTAGCTA
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GGGTAATCGGCCACATTGGGACTGAGACACGGCCCAAACTCCTACGGGAG
GCAGCAGTAGGGAATCTTCCACAATGGACGAAAGTCTGATGGAGCAACGC
CGCGTGAGTGAAGAAGGGTTTCGGCTCGTAAAACTCTGTTGTTAAAGAAG
AACATATCTGAGAGTAACTGTTCAGGTATTGACGGTATTTAACCAGAAAGC
CACGCTAACTATGCCAGCAGCGCGGTAATACGGCAAGCGTT

Leuconostoc pseudomesenteroides A030

TCGTACGCTTCTTTGAGCTTGCCGGAAAAAGAAGAGTGGCGAACGGGTGA
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GTGCTAATACCGTATAACAATCGAAACCGCATGGTTTTGATTTGAAAGGCG
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GGCCACATTGGGACTGAGACACGGCCCAAACTCCTACGGGAGGCAGCAGT
AGGGAATCTTCGGCAATGGACGAAAGTCTGACCGAGCAACGCCGCGTGAG
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TTTATTGGGCGTAAAGCGAGCGCAGGCGGTTTCTTAAGTCTGATGTGACCC
GGCTCAACCGGGGAGGGTCATTGGAAACTGGGAGACTTGAGTGCGGA

Pediococcus pentosaceus
TTGATTATGACGTACTTGTACTGATTGAGATTTTAACACGAAGTGAGTGGC
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CCACGGCTAACTACGTGCCAGCAGCCGCGGTAATACGTAGGTGGCAAGCG
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Lactobacillus pentosus A028

TCGAACGAACTCTGGTATTGATTGGTGCTTGCATCATGATTTACATTTGAGT
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GTCCGAGTTTGAAAGATGGCTTCGGCTATCACTTTTGGATGGTCCCGCGGC
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GACCTGAGAGGGTAATCGGCCACATTGGGACTGAGACACGGCCCAAACTC
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GCAAGCGTTGTCCGGATTTATTGGGCGTAAAGCGAGCGCAGGCGGTTTTTT
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Leuconostoc pseudomesenteroides A026
GTCGTACGCTTCTTTGAGCTTGCTCCAAAAGAAGAGTGGCGAACGGGTGAG
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TGCTAATACCGTATAACAATCGAAACCGCATGGTTTTGATTTGAAAGGCGC
TTTCGGGTGTCGCTGATGGATGGACCCGCGGTGCATTAGCTAGTTGGTGAG
GTAACGGCTCACCAAGGCCACGATGCATAGCCGACCTGAGAGGGTGATCG
GCCACATTGGGACTGAGACACGGCCCAAACTCCtACGGGAGGCAGCAGTA
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Enterococcus durans A024

CAGTCGTACGCTTCTTTGGAGCTTGCCGGAAAAAGAAGAGTGGCGAACGG
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ACAGGTGCTAATACCGTATAACAATCGAAACCGCATGGTTTTGATTTGAAA
GGCGCTTTCGGGTGTCGCTGATGGATGGACCCGCGGTGCATTAGCTAGTTG
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GATCGGCCACATTGGGACTGAGACACGGCCCAAACTcctACGGGAGGCAGC
AGTAGGGAATCTTCGGCAATGGACGAAAGTCTGACCGAGCAACGCCGCGT
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CCCCGGCTCAACCGGGGAGGGTCATTGGAAACTGGGAGACTTGAGTGCAG
AAGAGGAGAGTTCCATGTGTAGCGGTGAAATGCGTAGGAACACCAGAGGT
CTCTGGTC

Lactobacillus plantarum A023

CAGTGAACTCTGGTATTGATTGGTGCTTGCATCATGATTTACATTTGAGTGA
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CTGAGAGGGTAATCGGCCACATTGGGACTGAGACACGGCCCAAACTCCTA
CGGGAGGCAGCAGTAGGGAATCTTCCACAATGGACGAAAGTCTGATGGAG
CAACGCCGCGTGAGTGAAGAAGGGTTTCGGCTCGTAAAACTCTGTTGTTAA
AGAAGAACATATCTGAGAGTAACTGTTCAGGTATTGACGGTATTTAACCAG
AAAGCCACGGCTAACTACGTGCCAGCAGCCGCGGTAATACGTAGGTGGCA
AGCGTTGTCCGGATTTATTGGGCGTAAAGCGAGCGCACGGTTTTTTAAGTC
TGATGTGAAAGCCTTCGGCTA

Enterococcus faecium A022

CGTACGCTTCTTTTTCCACCGGAGCTTGCTCCAAAAAGAAGAGTGGCGAAC
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AAACAGGTGCTAATACCGTATAACAATCGAAACCGCATGGTTTTGATTTGA
AAGGCGCTTTCGGGTGTCGCTGATGGATGGACCCGCGGTGCATTAGCTAGT
TGGTGAGGTAACGGCTCACCAAGGCCACGATGCATAGCCGACCTGAGAGG
GTGATCGGCCACATTGGGACTGAGACACGGCCCAAACTCCTACGGGAGGC
AGCAGTAGGGAATCTTCGGCAATGGACGAAAGTCTGACCGAGCAACGCCG
CGTGAGTGAAGAAGGTTTTCGGATCGTAAAACTCTGTTGTTAGAGAAGAAC
AAGGATGAGAGTAACTGTTCATCCCTTGACGGTATCTAACCAGAAAGCCAC
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CCGGATTTATTGGGCGTAAAGCGAGCGCAGGCGGTTTCTTAAGTCTGATGT
GAAAGCCCCCGGCTCAACCGGGGAGGGTCATTGGAAACTGGGAGACTTGA
GTGCAGAAGAGGAGAGTGGAATTCCATGTTAGCGGTGAAATGCGATATGG
AGGAACACc

Leuconostoc pseudomesenteroides A019

TCGTACGCTTCTTTTTCACCGGAGCTTGCTCCAAAAGAAGAGTGGCGAACG
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AACAGGTGCTAATACCGTATAACAATCGAAACCGCATGGTTTTGATTTGAA
AGGCGCTTTCGGGTGTCGCTGATGGATGGACCCGCGGTGCATTAGCTAGTT
GGTGAGGTAACGGCTCACCAAGGCCACGATGCATAGCCGACCTGAGAGGG
TGATCGGCCACATTGGGACTGAGACACGGCCCAAACTACGGGAGGCAGCA
GTAGGGAATCTTCGGCAATGGACGAAAGTCTGACCGAGCAACGCCGCGTG
AGTGAAGAAGGTTTTCGGATCGTAAAACTCTGTTGTTAGAGAAGAACAAG
GATGAGAGTAACTGTTCATCCCTTGACGGTATCTAACCAGAAAGCCACGGC
TAACTACGTGCCAGCAGCCGCGGTAATACGTAGGTGGCAAGCGTTGTCCG
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AGCCCCCGGCTCAACCGGGGAGGGTCATTGGAAACTGGGAGACTTGAGTG
CAGAAGTGGAATTCCATGTGTAATGCGATATGGAGGAACGCCTCTGGTCT

Enterococcus faecium A018

AAAAGAAGAGTGGCGAACGGGTGAGTAACACGTGGGTAACCTGCCCATCA
GAAGGGGATAACACTTGGAAACAGGTGCTAATACCGTATAACAATCGAAA
CCGCATGGTTTTGATTTGAAAGGCGCTTTCGGGTGTCGCTGATGGATGGAC
CCGCGGTGCATTAGCTAGTTGGTGAGGTAACGGCTCACCAAGGCCACGAT
GCATAGCCGACCTGAGAGGGTGATCGGCCACATTGGGACTGAGACACGGC
CCAAACTCCTACGGGAGGCAGCAGTAGGGAATCTTCGGCAATGGACGAAA
GTCTGACCGAGCAACGCcgCGTGAGAAGGTTTTCGGATTCTGTTGtTAGAGA
A

Lactobacillus paracasei A017

CAGTCGAACGAGTTCTtGATGATCGGTGCTTGCACCGAGATTCAACATGGA
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TGGTTCTTGGCTGAAAGATGGCGTAAGCTATCGCTTTTGGATGGACCCGCG
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CCGAACTGAGAGGTTGATCGGCCACATTGGGACTGAGACACGGCCCAAAC
TCCTACGGGAGGCAGCAGTAGGGAATCTTCCACAATGGACGCAAGTCTGA
TGGAGCAACGCCGCGTGAGTGAAGAAGGCTTTCGGGTCGTAAAACTCTGTT
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Lactobacillus pentosus A016

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GTCCGAGTTTGAAAGATGGCTTCGGCTATCACTTCTGGATGGTCCCGCGGC
GTATTAGCTAGATGGTGAGGTAACGGCTCACCATGGCAATGATACGTAGCC
GACCTGAGAGGGTAATCGGCCACATTGGGACTGAGACACGGCCCAAACTC
CTACGGGAGGCAGCAGTAGGGAATCTTCCACAATGGACGAAAGTCTGATG
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CAGAAAGCCACGGCTAACTACGTGCCAGCAGCCGCGGTAATACGTAGGTG
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Enterococcus durans A013

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GTAGGGAATCTTCGGCAATGGACGAAAGTCTGACCGAGCAACGCCGCGTG
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GATGAGAGTAACTGTTCATCCCTTGACGGTATCTAACCAGAAAGCCACGGC
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Lactobacillus rhamnosus A012

CGAACGAGTTCTGATTATTGAAAGGTGCTTGCATCTTGATTTAATTTTGAAC
GAGTGGCGGACGGGTGAGTAACACGTGGGTAACCTGCCCTTAAGTGGGGG
ATAACATTTGGAAACAGATGCTAATACCGCATAAATCCAAGAACCGCATG
GTTCTTGGCTGAAAGATGGCGTAAGCTATCGCTTTTGGATGGACCCGCGGC
GTATTAGCTAGTTGGTGAGGTAACGGCTCACCAAGGCAATGATACAGCACT
GAGGTTGAT

Lactobacillus plantarum A011

AACTCTGGTATTGATTGGTGCTTGCATCATGATTTACATTTGAGTGAGTGGC
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CTGGAAACAGATGCTAATACCGCATAACAACTTGGACCGCATGGTCCGAG
TTTGAAAGATGGCTTCGGCTATCACTTTTGGATGGTCCCGCGGCGTATTAG
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GAGGCAGCAGTAGGGAATCTTCCACAATGGACGAAAGTCTGATGGAGCAA
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Leuconostoc pseudomesenteroides A010

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GACCCGCGGTGCA

Lactobacillus plantarum A002

CGCGAACTATTGATTGGTGCTTGCATCATGATTTACATTTGAGTGAGTGGC
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GAAACAGATGCTAATACCCATAACAACTTGGAGCATGCC

APPENDIX IX

Component of calibration report

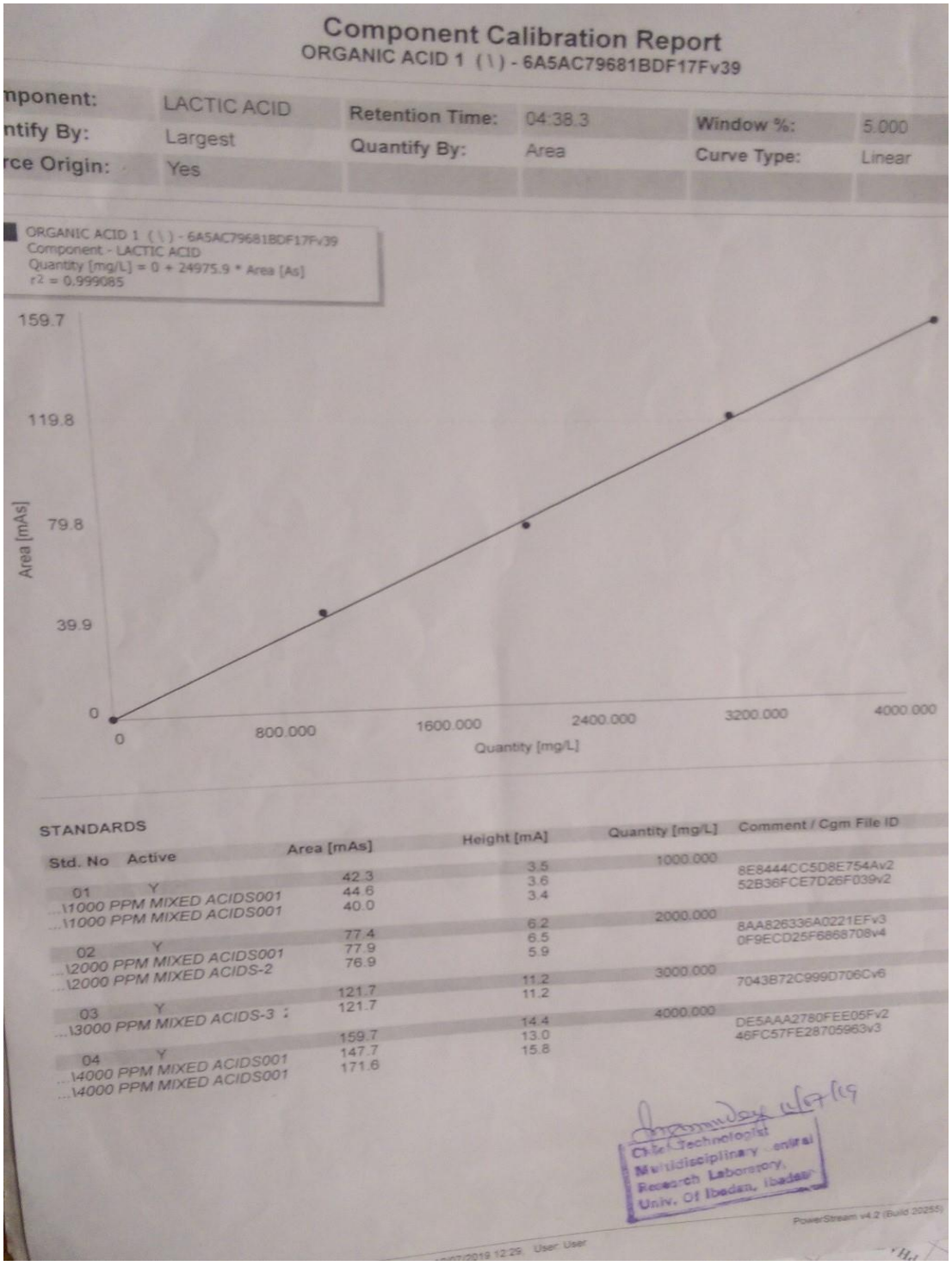


Figure A.5. Component of calibration report

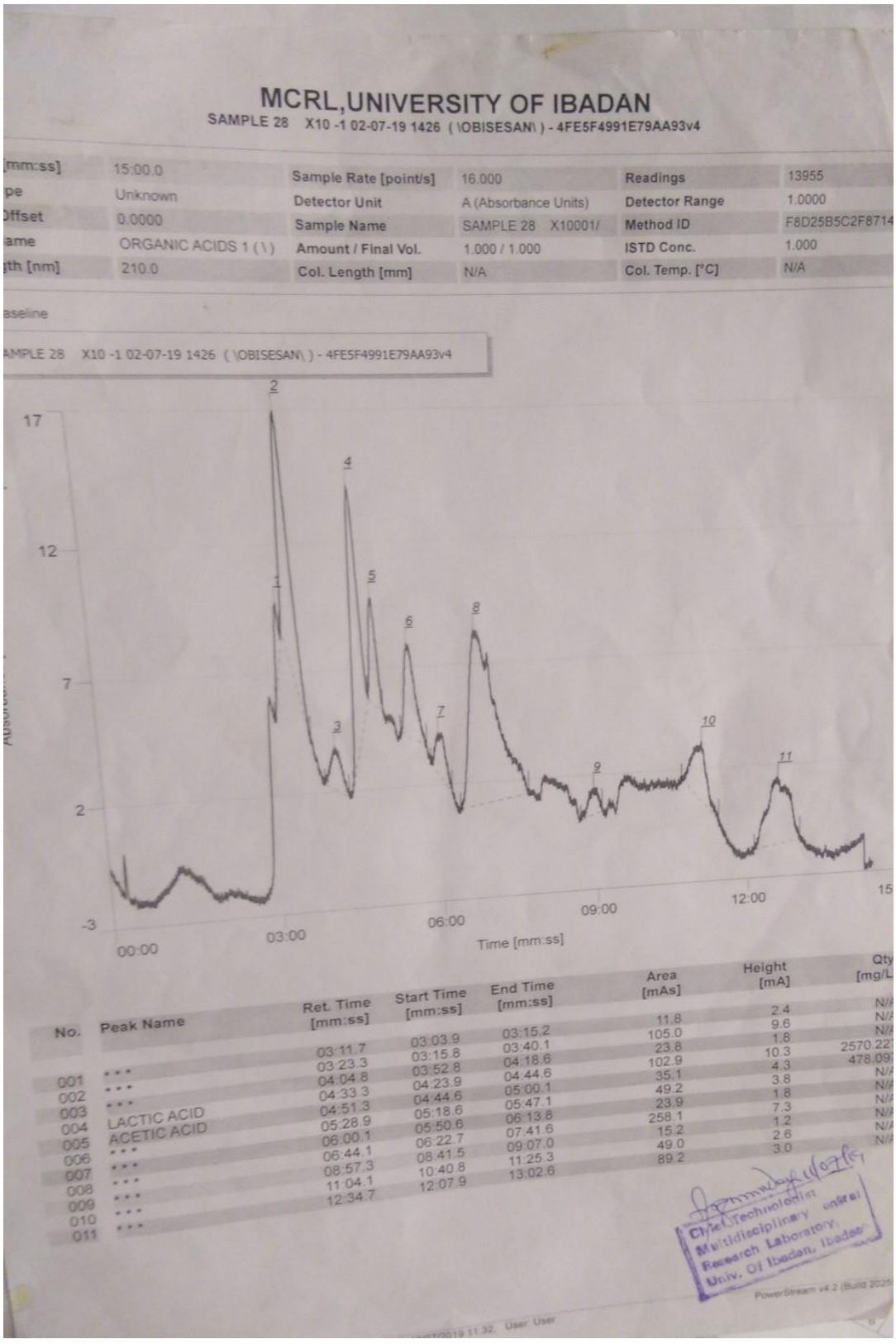
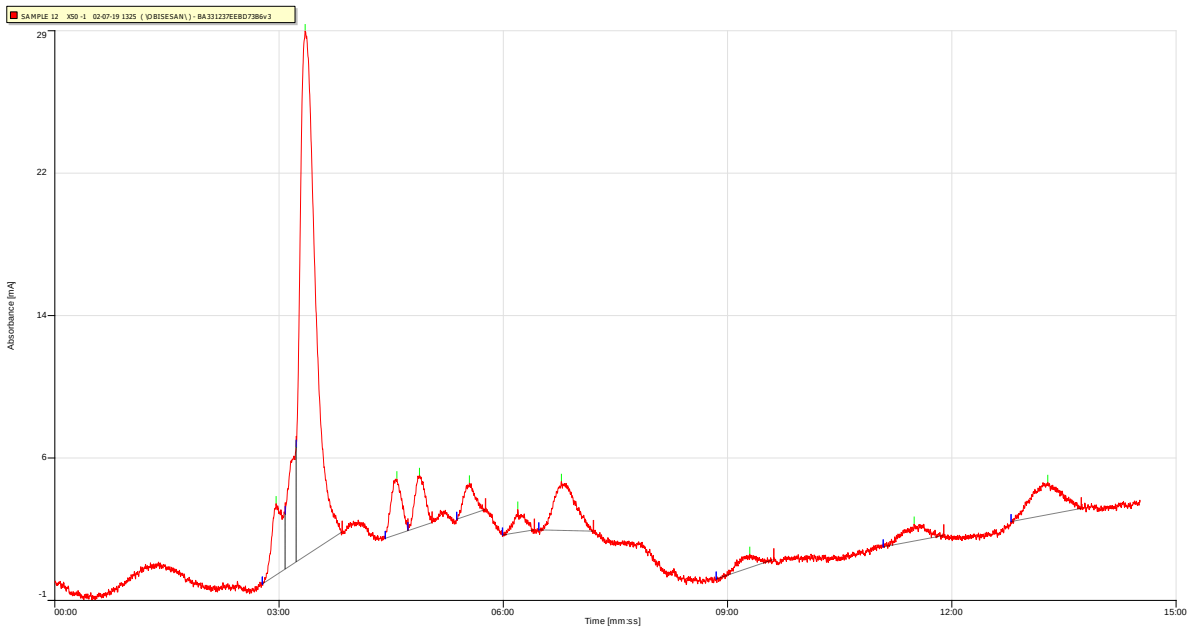


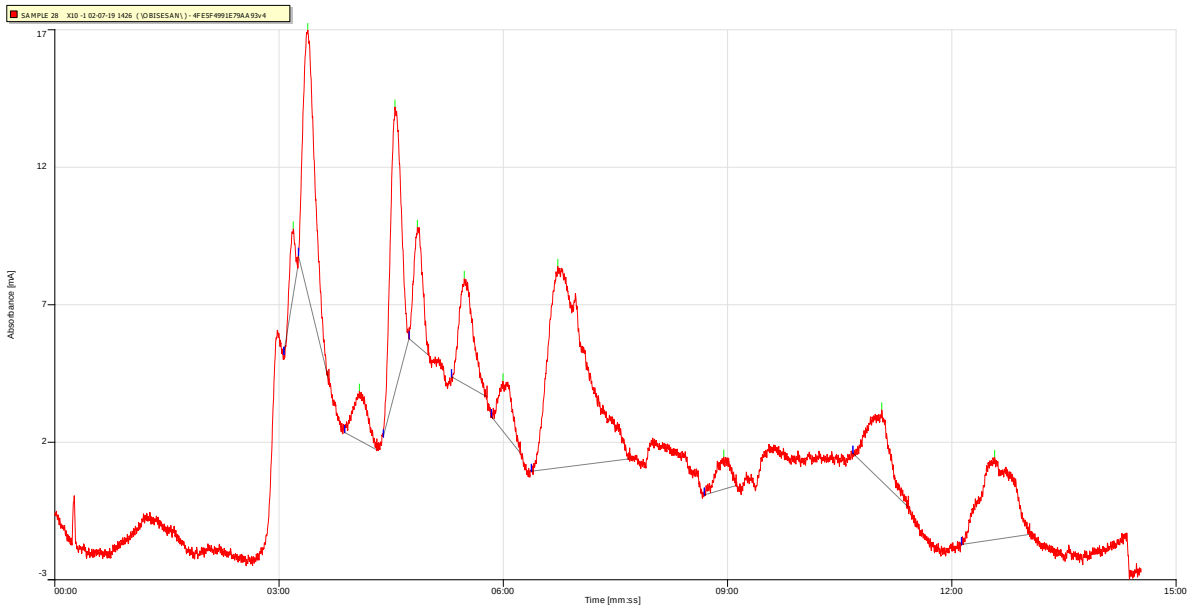
Figure A.6. Analysis of area of curve produced by organic acid prensent in the lactic acid bacteria

APENDIX X

Chromatogram for quantification of organic acid produced by lactic acid bacteria

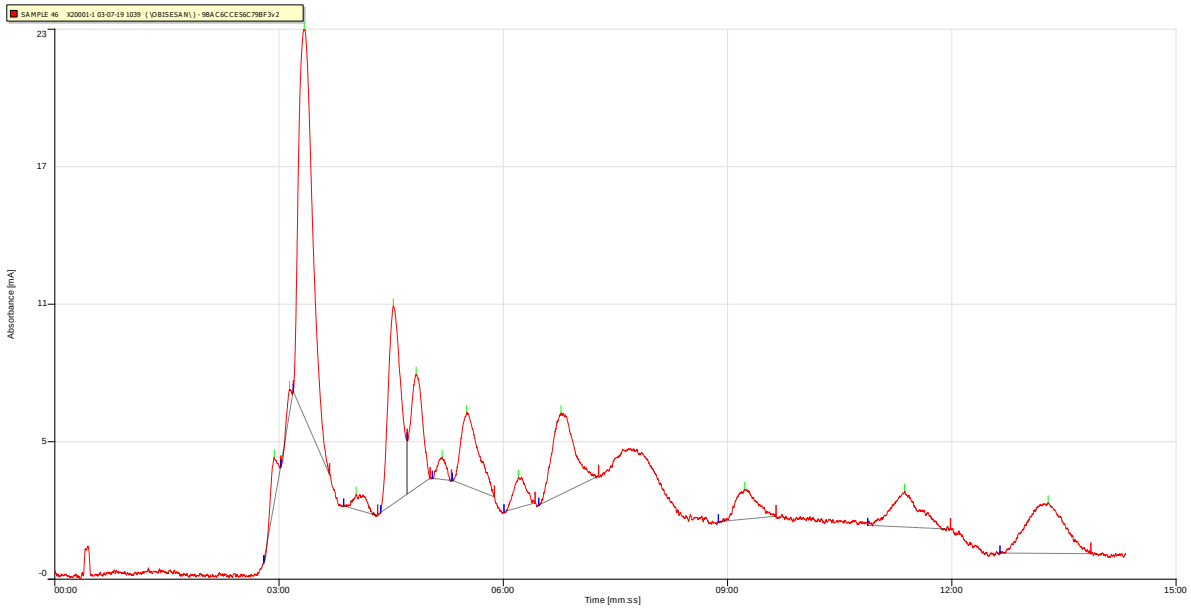


Lactobacillus rhamnosus A012

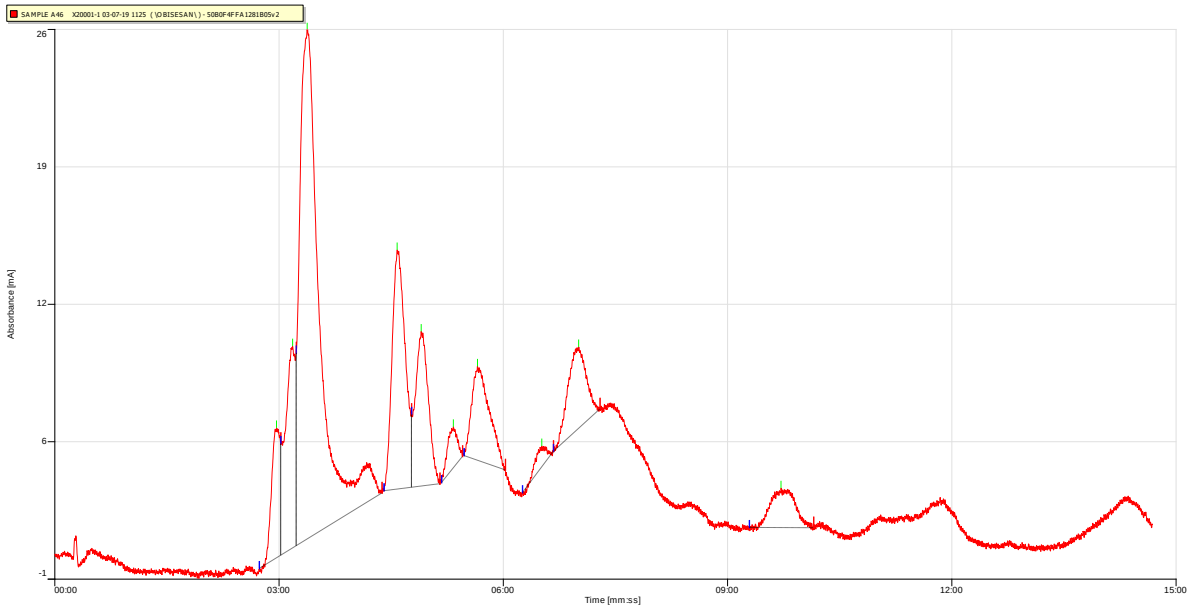


Lactobacillus pentosus A028

Figure A.7. Chromatogram for quantification of organic acid produced by lactic acid

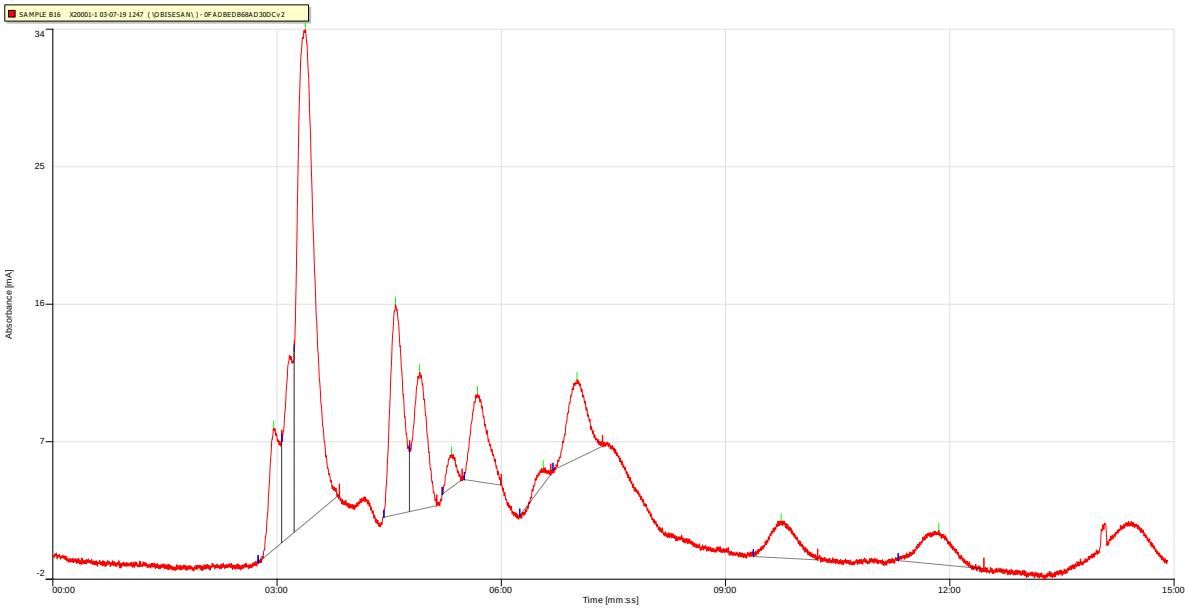


Lactobacillus plantarum A0 11

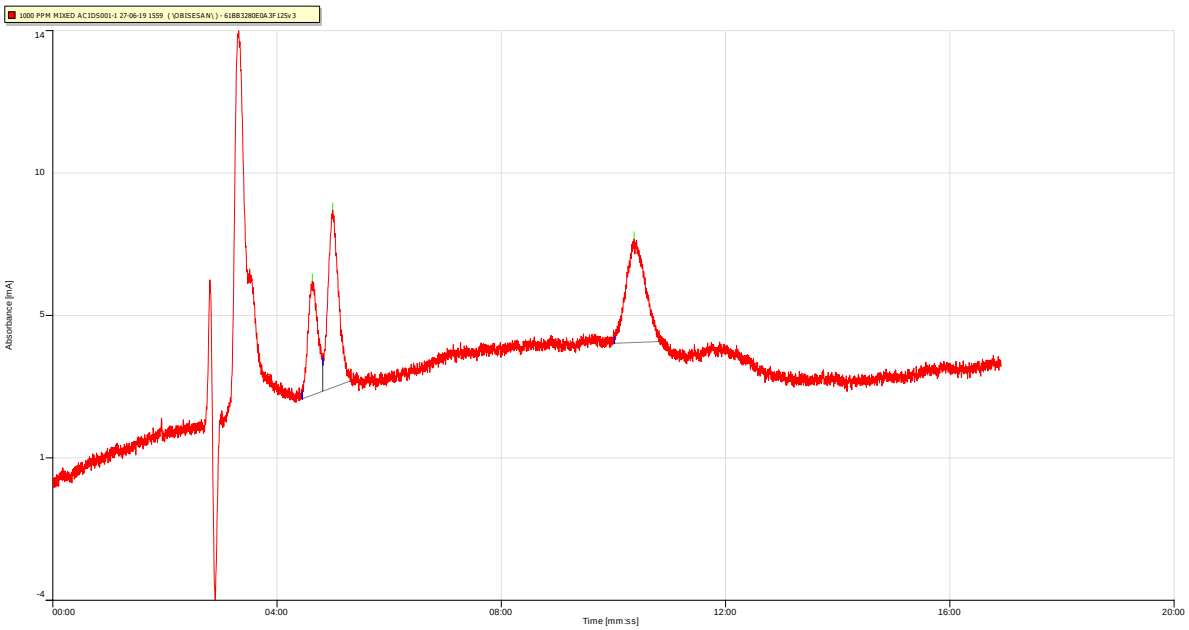


Lactobacillus pentosus A4C

Figure A.8. Chromatogram for quantification of organic acid produced by lactic acid



Lactobacillus pentosus B1b



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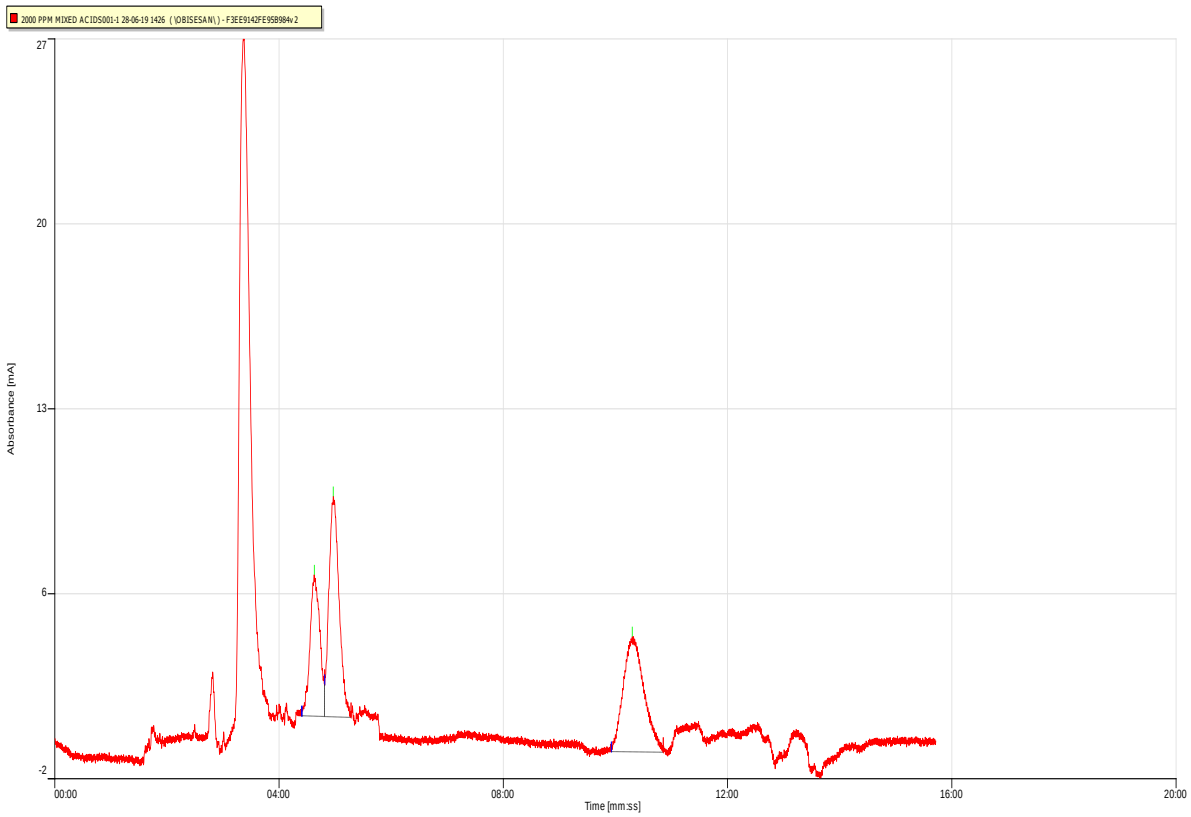

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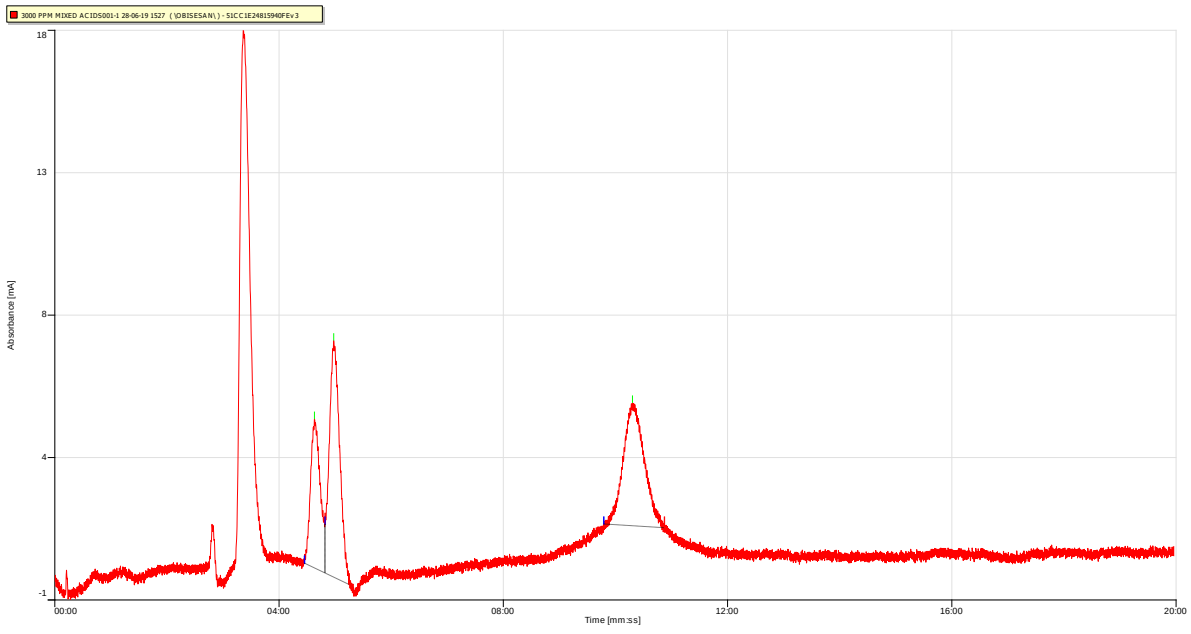

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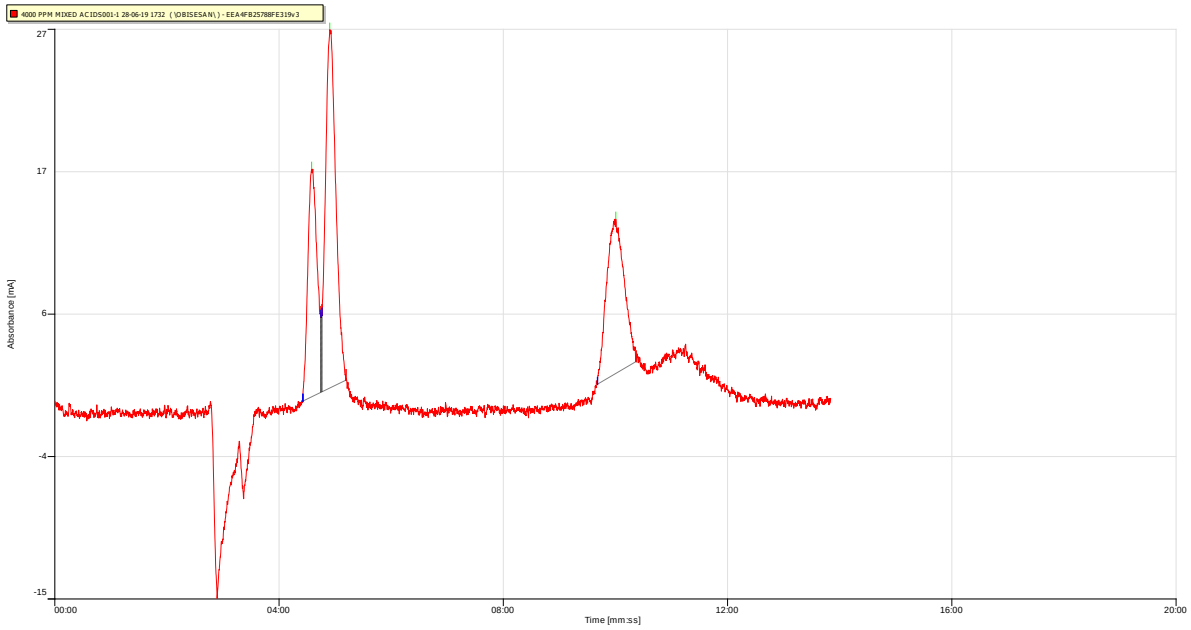
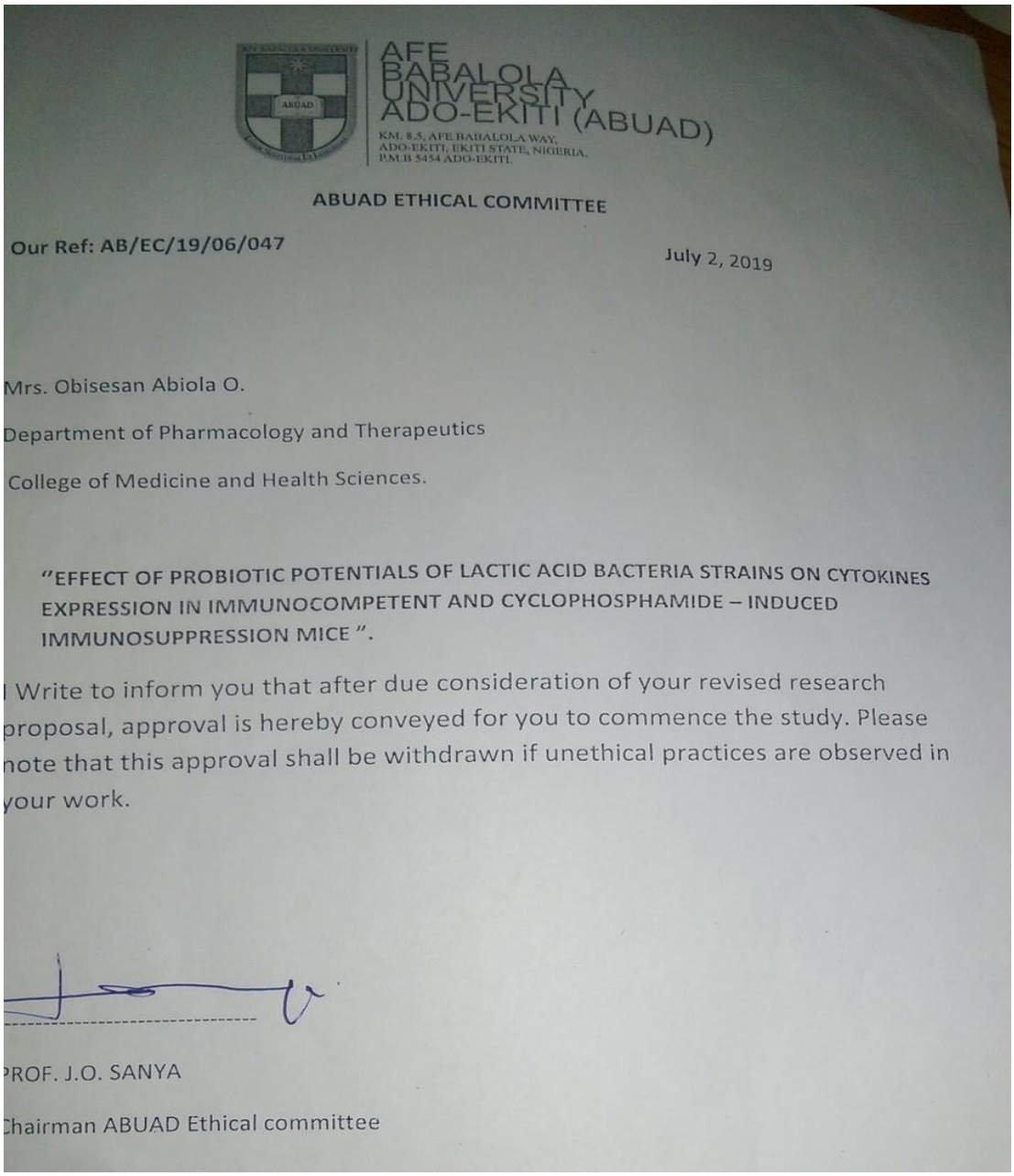

4000 PPM MIXED ACIDS001-1 28-06-19 1732 (\DBISESAN\) - EEA4FB2588FE319v3
27
17
6
-4
-15
Absorbance [mA]
00:00
04:00
08:00
12:00
16:00
20:00
Time [mm:ss]

4000 PPM

.

APPENDIX XI

ETHICAL APPROVAL FOR INVIVO STUDIES



AFE BABALOLA UNIVERSITY
ADO-EKITI (ABUAD)

KM. 8.5, AFE BABALOLA WAY,
ADO-EKITI, EKITI STATE, NIGERIA.
P.M.B 5454 ADO-EKITI.

ABUAD ETHICAL COMMITTEE

Our Ref: AB/EC/19/06/047

July 2, 2019

Mrs. Obisesan Abiola O.

Department of Pharmacology and Therapeutics

College of Medicine and Health Sciences.

"EFFECT OF PROBIOTIC POTENTIALS OF LACTIC ACID BACTERIA STRAINS ON CYTOKINES EXPRESSION IN IMMUNOCOMPETENT AND CYCLOPHOSPHAMIDE – INDUCED IMMUNOSUPPRESSION MICE ".

I Write to inform you that after due consideration of your revised research proposal, approval is hereby conveyed for you to commence the study. Please note that this approval shall be withdrawn if unethical practices are observed in your work.

PROF. J.O. SANYA

Chairman ABUAD Ethical committee

Figure A.9. Ethical approval for invivo studies

APPENDIX XII:

Antibiofilm reaction of LAB before staining with Gentamicin Crystal violet

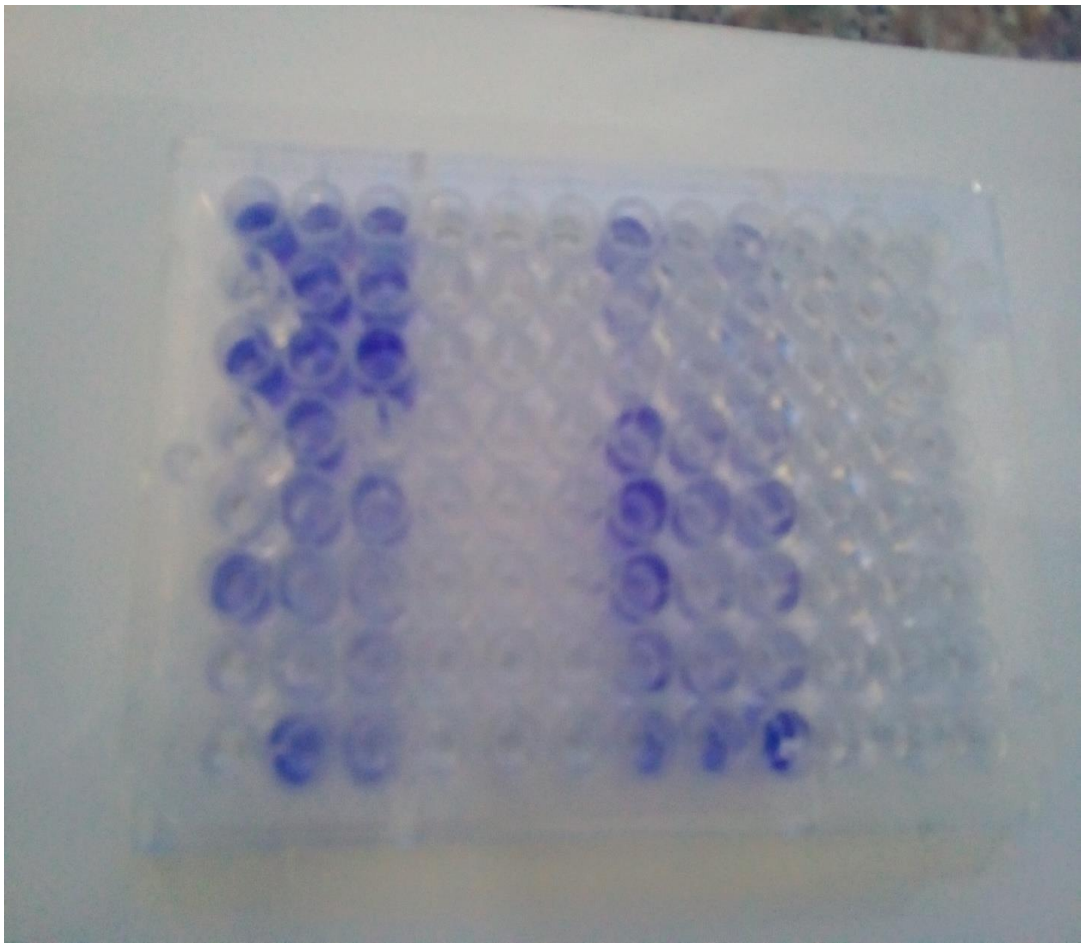


Figure A.10. Antibiofilm reaction of LAB before staining with gentamicin crystal violet

APPENDIX XIII:

Antibiofilm reactions of LAB after staining with Gentamicin Crystal violet

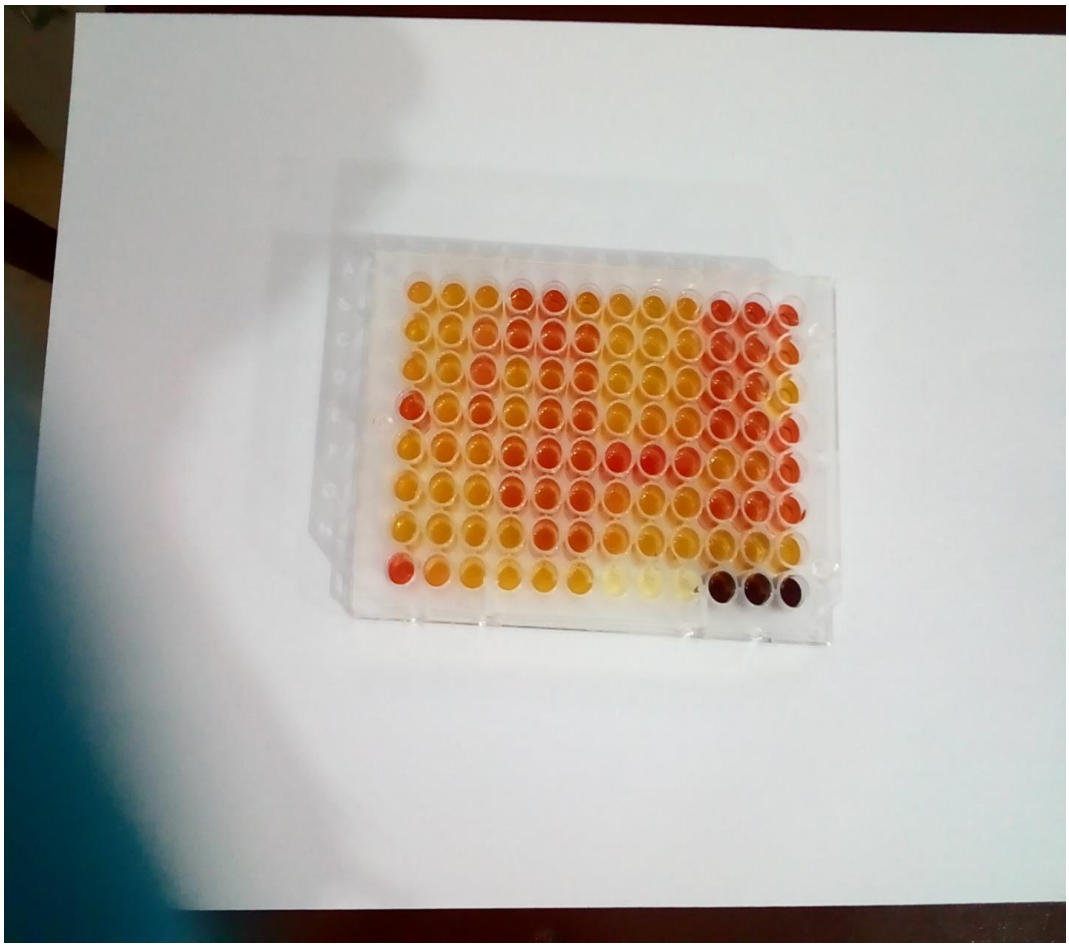


Figure A.11. Antibiofilm reactions of LAB after staining with gentamicin crystal violet**.**

APPENDIX XIV

Quantification of cytokines in tissue and body fluid of experimental in 450nm absorbance

Figure A.12. Quantification of cytokines in tissue and body fluid of experimental in 450nm absorbance