THE STUDY OF THE DESORPTION EFFECT OF SOLUBULIZING AGENTS ON ADHERED LACTOBACILLUS REUTERI

BY

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DEPARTMENT OF PHARMACEUTICAL MICROBIOLOGY AND BIOTECHNOLOGY

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DECEMBER 2022.

DECLARATION

I, CHINEDU TONY EZEH with the matriculation number PHA1405716 hereby declare that this project work entitled THE STUDY OF THE DESORPTION EFFECT OF SOLUBULIZING AGENTS ON ADHERED LACTOBACILLUS REUTERI is the original research work carried out by me in the department of Pharmaceutical Microbiology, Faculty of Pharmacy, University of Benin, Benin city, Nigeria.

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CHINEDU TONY EZEH DATE

CERTIFICATION

This is to certify that this is an original research work carried out by CHINEDU TONY EZEH in the Department of Pharmaceutical Microbiology, Faculty of Pharmacy, University of Benin, in partial fulfillment of the requirements for the award of Doctor of Pharmacy (Pharm.D) degree.

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DR E. OLOTON DATE

(PROJECT SUPERVISOR)

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DR E. OLOTON DATE

(HEAD OF DEPARTMENT)

DEDICATION

To my father and mother, Mr Christopher and Christiana Ezeh. To Family and friends, and to more amazing research in Pharmaceutical Microbiology.

ACKNOWLEDGEMENT.

I want to appreciate God Almighty for the strength, wisdom, direction and the gift of men and women He has placed in my life through out this journey.

From My parents(Mr and Mrs Ezeh) to my Brothers (Austin, Daniel, Promise - glee) and my favourite sister, Ada.i am grateful.

To friend in Christian Union (CU), to HOP - I love you guys.

To Seyi, Nosa, Elvis, Omo, Moses, Esther, Francis, Osas, Beulah, Chi Chi.

To Praedita erudites, to my course mates.

I am grateful.

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For being a father to me in this project, for guidance, Thank you, sir and may God continue to bless you and your family.

I want to Appreciate my project colleagues(Abigail and Valentina), you ladies are amazing.

ABSTRACT

There are different methods used in experimentally assessing microbial adhesion. They include the direct method and indirect methods. The direct methods are laborious and the bacteria have to remain culture able. while the indirect method are less have low through put ,less accurate, less sensitive.In this experiment, the desorption effect of solubilizing agents(propylene glycol and tween 80) on adhered *Lactobacillus reuteri* was studied. .My aim was to observe the effect of contact time and different concentrations (100%, 75%,50%,25%,12.5%) of solubilizing agents (Propylene glycol and Tween 80) on the growth of *Lactobacillus reuteri*. The growth inhibitory effect different concentrations (100%, 75%,50%,25%,12.5%) of solubilizing agents (Propylene glycol and Tween 80) on the growth of *Lactobacillus reuteri* was determined at different contact time.A growth inhibitory test and then we went further to determine the determine the desorption effect of the solubilizing agents by testing the effect of different contact time (2 ½ minutes to 30 minutes) and different concentrations of the solubilizing agents on *Lactobacillus.*

The result of this experiment showed that the solubilizing agents used had no growth inhibitory effect on the adhered *lactobacillus reuteri* and so would not affect the validity of the experiment. The desorption effect Tween 80 and Propylene glycol were not concentration dependent but can be said to be time dependent at an optimum desorption concentration(which was 25% in this experiment).Tween 80 had the highest desorption effect with a yield of at a contact time of 15 minutes at 25% concentration.

Probiotics mechanism of action is mainly by adhesion and colonization on the surface of the host. They do this and increase in number before they can exert any health benefits such as maintenance of microbial flora and prevent of the growth of pathogenic microorganisms.

Table of Contents

CHAPTER ONE

1.1 INTRODUCTION

The meaning of probiotics has been altered with expanding information in the field of how they work. (Thantsha, 2012) The term is gotten from the Greek language meaning 'for life. In the past, there have been many endeavors to characterize the term probiotic, one of the first being portrayed by Lilly and Stillwell in 1965 (Thantsha, 2012) . They defined probiotics as "substances secreted by one microorganism, which stimulates the growth of another". The focal point of this definition was to recognize them and clarify that they are something contrary to antibiotics. Hence, in 1974, Parker characterized them as "organisms and substances which contribute to intestinal microbial balance" (Schrezenmier & de Vrese, 2001). In 1989, Fuller tried to improve on Parker's definition by proposing the following definition: "live microbial feed supplement, which beneficially affects the host (animal or human) by improving its intestinal microbial balance" (Salminen *et al*, 1999; Vilsiljevic & Shah, 2008). Then, Havenaar & Huis In't Veld (1992) defined probiotics acceptably as 'a viable mono- or mixed culture of microorganisms which applies to animal or man, beneficially affects the host by improving the properties of the indigenous microflora'. Schrezenmeir & de Vrese (2001) defined the term probiotic as "a preparation of or a product containing viable, defined microorganisms in sufficient numbers, which alter the microflora by implantation or colonization, in a compartment of the host and by that, exert beneficial effects on host health". Among these depictions and definitions, there were numerous others, until the Food and Agriculture Organization of the United Nations-World Health Organization (FAO-WHO) formally defined probiotics as: "live microorganisms that when administered in adequate amounts confer a significant health benefit on the host" (FAO/WHO, 2001).

This definition was subsequently embraced by the International Scientific Association for Probiotics and Prebiotics (ISAPP) and is at present the most acknowledged meaning of probiotics by researchers around the world Probiotic food societies have become famous because of their enthusiasm for their contributions to good health (Reid, 2006). In probiotic treatment, these advantageous microorganisms are ingested and accordingly introduced to the digestive microflora purposefully. This results in big quantities of helpful microorganisms to partake in rivalry for supplements with and starving off unsafe microscopic organisms (Mombelli and Gismondo, 2000).The valuable impacts of the ingested probiotic microbes are given by those organisms that adhere to the intestinal epithelium (Salminen *et al*., 1998). The presence and adherence of probiotics to the mucous layer of the intestinal tract develop a solid natural biological barrier for some pathogenic microorganisms. Adhesion is accordingly viewed as the first step to colonization. Adhesion to the epithelium can be specific, including bond of microorganisms and receptor particles on the epithelial cells, or none specific, based on physicochemical variables.

1.2 BENEFICIAL HEALTH EFFECTS OF PROBIOTICS

Dairy strains of lactic acid bacteria (LAB) have a long history of usage. LAB, including different kinds of Lactobacillus and Enterococcus species, that have been drunk every day since people began to use fermented milk as food. Probiotic effects are strain-specific, thus what works for one strain may not work for others, and each Probiotic bacterial strain has its own set of medical benefits. The major beneficial effects are correlated to various disease conditions. Probiotics have a giant centrality and application in controlling various types of microbial diseases.

Probiotics are relevant to human well-being improvement, contamination control, disease treatment and management

Table 1. Role of probiotics in health improvement, infection control and disease treatment (Abatenh *et al*,2018)

Probiotic Strains	Types of diseases or disorders	Probiotic outcomes/results
Oxalobacter formigenes Lactobacillus and Bifidobacterium species, (Lactobacillus plantarum \aaPBS067, Lactobacillus acidophilus LA-14, Bifidobacterium breve PBS077, Bifidobacterium longum PBS078)	Kidney/Urinary stones	(i)Modify or utilize several types of urinary stone. (ii) Act as a key tool to manipulate, metabolize and degrade a toxic compound.
Lactobacillus GG, L. rhamnosus Lactis, Lactobacillus fermentum, Bifidobacterium bifidum, Bifidobacterium lactis, L. acidophilus, L. casei, L. salivarius and Lactococcus lactis	Atopic Diseases	(i) Atopic eczema reduction is observed, and skin condition also improved. (ii)Atopic dermatitis symptoms are removed from infants who found in moderateto severe condition. It is mainly depends on the selection of specific probiotic strains, time of administration (on set time), duration of exposure, and dosage.
L. casei, L. rhamnosus, S. thermophilus,B. breve, L. acidophilus, B.infantis, L.delbrueckii subsp. Bulgaricus, L. reuteri DSM 17938	Colic	Very effective in reducing colic in breastfed infants and children.
Lactobacillus, Bifidobacerium and L. johnsoni	*Helicobacter pylori* infection	Destruction the adverse effects of H. pylori through the release of bacteriocins, production of organic acids, and competitive

		colonization in epithelial or mucosal cells. At that time can hinder its growth, adhesion and bacterial load.
Lactobacillus rhamnosus, L. rhamnosus GG, B. animalis subsp. lactis alone or in combination with S treptococcus.thermophilus,and L. reuteri, L. rhamnosus (not GG),and L. acidophilus, Saccharomyces boulardii. Lactobacillus casei	Acute and antibiotic associated diarrhea	(i)Competitive blockage of receptor site signals regulating secretory and motility defenses. (ii) Enhancement of the immune response, and production of substances that directly inactivate the viral particles. (iii)Inhibit the growth by preventing adhesion and invasion of pathogens.
Lactobacillus rhamnosus, Lactobacillus reuteri, *Propionibacterium freudenreichii*	Candida infection	Used as a therapeutic option to combat fungal pathogen.
Bifidobacterium species, Bifidobacterium lactis, Bifidobacterium longum, Bifidobacterium breve, Bifidobacterium infantis, Lactobacillus casei, Lactobacillus rhamnosus, Streptococcus thermophiles, Lactobacillus acidophilus, Lactobacillusbulgaricus	Constipation	(i)Altering microflora and restoring disturbed community in side GIT, (ii)Participating and solving undesired gastro intestinal problems. (iii)Improving/managing whole gut transit time, stool frequency and consistency.
L. acidophilus, L. plantarum, L. casei, B.lactis, S cerevisiae	Irritable bowel syndrome	(i) Reduction of irritable bowel syndrome symptoms. (ii) Effective in alleviating and managing symptoms of this unpleasant condition.
L. GG, L. casei Shirota, L. acidophilus, B. bifidum, L. rhamnosus, L. plantarum and L. paracase, Streptococcus salivarius, Bifidobacterium animalis subsp. Lactis	Acute viral upper respiratory infections	(i)Colonizing epithelial cells and keep away from adherence of pathogens. (ii)Create adhesion, binding sites, nutrients and space competition lastly able to avoid risk of upper respiratory track

		completely.
B. animalis subsp. Lactis, L. lactis subsp.actis	Modulation of gut –brain axis	(i) Modulation of brain activity and Provide mental health. (ii) Maintaining the functionality of the central nervous system through metabolic, neuroendocrine and immune pathways. (iii) Contribute to the early development of normal social and cognitive behaviors. (iv) Useful strains having positive direct effect on central nervous system and also solve disorders.
Lactic acid bacteria	Colon Cancer	(i) Comprise modification of the metabolic activities of intestinal micro flora and alteration of physicochemical conditions in the colon as well as binding site. (ii) Biodegradation of potential carcinogens. (iii) Production of anti-tumorigenic or mutagenic compounds due to the ability to decrease the activity of enzyme called β glucuronidase. (iv) Increasing the host immune response, by alteration in procancerous enzymatic activity of colonic microorganisms.
Lactobacillus acidophilus NCFM, Lactobacillus gasseri SBT2055, L. rhamnosus CGMCC1.3724	Diabetes and Obesity	(i) Decrease the risk of type two diabetes mellitus and insulin resistance. (ii) By improving and maintaining the metabolic equilibrium of the host then actual weight loss is observed significantly.

Probiotics and Allergy

Allergies are misguided reactions of the immune system in response to (what should be harmless) particles. Allergies are treated with probiotics by repairing your damaged digestive system, which reduces inflammation, stabilizes your immune system, and strengthens your gut lining.

A hypersensitive reaction triggered by immunological systems is known as an allergy. Probiotics modify the structure of antigens, reduce their immunogenicity, intestinal permeability and the generation of pro-inflammatory cytokines that are eminent in patients with a diversity of allergic disorders. *Lactobacillus GG and Lactobacillus rhamnosus GG* help to alleviate the symptoms of food allergies while also lowering the chance of acquiring allergic illnesses. Manoj *et al, 201;* Licciardi *et al, 2013*). Well-known techniques for treating allergic diseases include: Minimizing antigen translocation into the bloodstream, enhancing mucosal barrier function, and preventing excessive immunologic responses to enhanced antigen stimulation of the gut.

Probiotics and Blood Pressure

It's also been proven that probiotics and their products can lower blood pressure through improving total mechanisms such as cholesterol and low-density lipoprotein cholesterol levels. (Patel *et al*, 2010; Guo *et al*, 2011) . Diminishing blood glucose level and insulin obstruction, controlling the renin-angiotensin framework and huge decrease happens in blood or serum cholesterol when cholesterol is raised. Interestingly, probiotic supplementation might positively help in reducing Blood pressure in the hypertensive conditions. *Lactobacillus helveticus Saccharomyces cerevisiae, Lactobacillus rhamnosus GG, Lactobacillus casei, Lactobacillus acidophilus, Lactobacillus rhamnosus, Lactobacillus bulgaricus, Bifidobacterium breve,* *Bifidobacterium longum Streptococcus thermophiles, Lactobacillus delbrueckii ssp. Bulgaricus, Lactobacillus kefiri* are the normal ones utilized against hypertension ((Rerksuppaphol & Rerksuppaphol, 2015 ;Golnaz *et al*, 2017)

Probiotics and Inflammatory Bowel Disease

By reducing the production of local pro-inflammatory cytokines, probiotic bacteria can help to maintain the immunological barrier in the gut mucosa. Inflammatory bowel diseases such as ulcerative colitis, Crohn's disease, and Pouchitis are treated with probiotics. Suppression of growth or epithelial binding and invasion by harmful bacteria, generation of antimicrobial compounds, enhanced epithelial barrier function, and immunoregulation are some of the possible mechanisms. The effects of probiotics are probably both strain-dependent and dose dependent (Momir *et al,* 2014)

Probiotics and Urogenital infections (Bacterial vaginitis)

Bacterial vaginosis is a vaginal abnormality marked by vaginal secretions and caused by an overgrowth of unusual bacteria in the vagina.

An infection of the kidneys, ureters, bladder, or urethra is known as a urinary tract infection. Urine goes through these structures before being removed from the body. Urogenital infection is caused by changes in the vaginal environment, such as a decrease in lactobacilli concentrations or the absence of lactobacilli. Lactobacillus spp, are the noticeable microbial variables that oversees the presence, development, colonization and tirelessness of non-endogenous microorganisms in the vagina.As the Lactobacillus spp. count decreases, the protection provided by them against uropathogens also decreases. Lactobacilli are also thought to create biofilms that cover the urogenital cells. Clinical experiments have shown that lactobacilli are effective in treating bacterial vaginosis. Probiotic capsules for example *Lactobacillus rhamnosus, Lactobacillus crispatus, Lactobacillus gasseri, Lactobacillus vaginalis, Lactobacillus acidophilus, Lactobacillus reuteri and Streptococcus thermophilus* are effectiveness for recurrent bacterial vaginosis prevention (Ya *et al,* 2010;Rukshana *et al,* 2017; Lorenzo *et al,* 2017).The principal mechanisms by which lactobacilli exert their protective functions in urogenital health care are:

1. Stimulation of the immune system.

2. Competition with other microorganisms for nutrients and for adherence to the vaginal epithelium, urinary and vaginal tract cells.

3. Reduction of the vaginal pH by the production of organic acids, especially lactic acid.

4. Production of antimicrobial substances and competitive exclusion Inhibitor production, such as bacteriocins, and hydrogen peroxide. (Abatenh *et al*,2018)

Probiotics and Liver Diseases

Micro flora resident in intestinal lumen plays a significant role in hepatocytes function. Cirrhosis, nonalcoholic fatty liver disease, alcoholic liver disease, and hepatic encephalopathy are all major and dangerous liver dysfunctions that can be caused by changes in the type and amount of bacteria that live in the digestive system. Probiotic is used as a novel treatment strategy against liver disease in a mechanism of regulation, restoration and alteration of gut microflora and immune function (Lunia *et al*, 2014;Leila *et al*,2017).

Probiotics are beneficial in the treatment of chronic liver illnesses because they increase the strength of the intestinal barrier, which prevents microorganisms from entering the bloodstream and eventually the liver.

Probiotics and Cholesterol Assimilation

Probiotic strains, especially lactic acid microscopic organisms (bacteria), have an important role in lowering cholesterol levels by reducing the mechanism. Probiotics can help lower cholesterol levels in both direct and indirect ways. Inhibition of DE novo synthesis or a decrease in dietary cholesterol intestine absorption are the direct mechanisms. There are three approaches to reducing dietary cholesterol retention: assimilation, binding, and degradation. Probiotic strains absorb the cholesterol for their own particular digestion .Probiotic strains can attach to the cholesterol particle, and they are capable for debasing cholesterol to its catabolic products. The cholesterol level can be decreased in an indirect way by deconjugating the cholesterol to bile acids, in this way lessening the aggregate body pool. Reduction of total cholesterol to be done in *B. animalis subsp. lactis MB 202/DSMZ 23733*, *B.bifidum, B. breve* (Bordoni *et al* 2013). Hypercholesterolemia (high blood cholesterol levels) is thought to be a major risk factor for coronary heart disease. As a result, reducing blood cholesterol levels is critical for disease prevention.The cholesterol removing ability of LAB isolates was assessed *in vitro* and *in vivo* mechanisms. *Lactobacillus pentosus* LP05, *L.brevis LB32*, *L. reuteri* and *L. plantarum* are powerful (Catherine *et al*, 2014; Naheed *et al*, 2015; Vaishnavi *et al,* 2016).

Probiotics and Dental Caries

Dental caries is a bacterially-caused multifactorial illness characterized by corrosive demineralization of tooth enamel. It appears that alterations in the oral environment's homeostasis prompted the growth of a bacterial biofilm, which included streptococci from the mutant group.. A probiotic must be able to attach to tooth surfaces and coordinate the bacterial groups that make up the dental biofilm in order to be effective in preventing or reducing dental caries. It must also compete with and antagonize cariogenic bacteria to stop them from multiplying. Finally, the probiotic's digestion of food-grade carbohydrates should result in a minimal acid generation. The ability of probiotics to neutralize acidic situations is one of the benefits of integrating them into dairy products. Cheese, for example, has recently been discovered to inhibit enamel demineralization and promote remineralization (Tandon *et al*, 2015; Haukioja, 2010)

Probiotics and Orthodontic Treatment

Streptococcus mutants create white spot lesions, which are the most common scars seen during and after orthodontic treatment. Microbes that promote good health can correct the imbalance in the oral biofilm by actively inhibiting pathogens and shifting the oral milieu to a higher pH, thus reversing demineralization. Fixed orthodontic appliances are thought to pose a risk to dental health because of the accumulation of microorganisms that can lead to enamel demineralization, which is clinically obvious as white spot lesions. Furthermore, the intricate design of orthodontic bands and brackets may create a biological environment that promotes the formation and growth of cariogenic mutant streptococci strains. The occurrence of white spot lesions can be considered as an imbalance between mineral loss and gain, and the most recent orderly audits have looked into ways to avoid this adverse effect of orthodontic treatment. Probiotics must be studied further to see if they can be effective as an alternate way of preventing demineralization and white spots. *S. mutans* levels in plaque around orthodontic brackets could be reduced by taking a lozenge tablet containing *Lactobacilli brevis, Bifidobacterium animalis subsp. Lactis BB-12* and a *Bifidobacterium lactis* derived probiotic. (Widyarman, *et al*., 2018; Saurav *et al* 2016)

Probiotics and Oral Health

The reduction of inflammation is one of the most important advantages of probiotics in the oral cavity. Probiotics can aid in the destruction of pathogenic microorganisms in the mouth by combating them, as well as the maintenance of healthy gums and teeth. Probiotics should have no negative side effects because they are an all-natural medication. Antifungal activities are widely documented in *Lactobacillus acidophilus* and *Bifidobacterium lactis*. (Lesan, *et al*., 2017)

Probiotics and Voice Prosthesis

Pathogenic bacteria are significantly reduced in voice prosthesis biofilms when probiotics are used. Biofilm formation on indwelling vocal prostheses is effectively eliminated, possibly due to the presence of *Streptococcus thermophiles* and *Lactobacillus bulgaricus*. (Divya, 2016)

Probiotics and Halitosis

Bad Breath, also known as halitosis, is a condition in which the breath has an unpleasant odour. It can be caused by a variety of factors, including the consumption of certain foods, metabolic abnormalities, respiratory tract infections, and an abnormality of the oral cavity's commensal bacteria. It all begins with anaerobic bacteria degrading salivary and dietary proteins to produce amino acids, which are then converted into volatile sulphur compounds such as hydrogen sulfide and methanethiol. *Streptococcus salivarius* is a commensal oral probiotic that is screened and detected in people who do not have halitosis. Bacteriocins are produced by *S. salivarius*, which could help reduce the number of microscopic organisms that produce volatile sulphur compounds. Regular use of *S. salivarius K12* (BLIS Technologies Ltd., Dunedin, New Zealand) gum or capsules showed lower levels of volatile sulphur compounds in patients with halitosis who also took probiotic supplements regularly (Bonifait, *et al*., 2009) There is evidence that it aids in the control of pathogenic microorganism growth. *S. salivariu, L. salivarius, L. reuteri*, *L.casei*, and *W. Cibaria* were provided as a management option ((Zohreh, *et al.*, 2017)

Probiotics and Periodontal Diseases

Lactobacilli, particularly *Lactobacillus gasseri* and *Lactobacillus fermentum*, were shown to be more prevalent in the oral cavity in healthy people than in patients with chronic periodontitis, according to studies. Lactobacilli's ability to suppress the development of periodontal pathogens such as *P. gingivalis, Prevotella intermedia,* and *A. actinomycetemcomitans* has been studied extensively. These findings suggest that lactobacilli in the oral cavity may play a role in maintaining the oral ecological balance (Gupta, 2011),.*L. brevis, L. casei, L. salivarius, reuteri strain, Bacillus subtilis, L. reuteri* and *L. brevis* the involvement cared out in anti-inflammatory activity decreasing the number of pathogens in periodontal tissues (Monica, *et al*., 2013)

Immunologic Enhancement/ Immunity Stimulation

Probiotics have a biological influence on the immune system. Probiotics' immunological advantages can be attributed to local macrophage activation and modification of IgA synthesis both locally and systemically, as well as changes in pro/anti-inflammatory cytokine profiles and manipulation of food antigen responses. Lactobacilli are interesting for health applications because of their inherent ability to influence the immune system. The proposed systems involved reinforcing nonspecific and antigen-specific defence against infection and tumours, adjuvant effect in antigen-specific immune responses, regulating/affecting Th1/Th2 cells, production of anti-inflammatory cytokines, improving phagocytic action of granulocytes, cytokine discharge in lymphocytes, and increasing immunoglobulin-emitting cells in the blood to scale up antibody production. These are typical probiotic reactions, all of which indicate immune system alterations. (Abatenh *et al*,2018). An inflammatory immune response brought cytokine-activated monocytes and macrophages to the body, resulting in the arrival of cytotoxic particles capable of lysing tumour cells and pathogens. (Abatenh *et al*,2018)

Probiotics and HIV

Probiotics appear to help maintain a healthy gut epithelial layer, improve gut barrier function, and stimulate innate immunity, which acts as the first line of defense against viral and bacterial pathogen translocation. The immune system can prevent HIV replication and reduce the progression of AIDS in the host when it is well developed. People living with HIV might raise their CD4 count by taking probiotics on a daily basis for a long time. A study of saliva samples from numerous participants revealed that some Lactobacillus strains produce proteins that are capable of binding a specific sort of sugar present on the HIV envelope called mannose. The sugar binds to the mucosal covering of the mouth and stomach system, allowing microscopic organisms (bacteria) to stick to it and colonize it. One of the strains released a large number of mannose-binding protein particles into the environment, which bind to the sugar coating and therefore neutralizes HIV. It has also been discovered that when immune cells are trapped by lactobacilli, clumps form, immobilizing any immune cells carrying HIV and preventing them from infecting other cells ((Pranay & Priyanka, 2012)

Probiotics and Lactose Intolerance

Lactose intolerance is the inability to hydrolyze lactose into the monosaccharides glucose and galactose due to a genetically programmed beta-galactosidase deficiency. When undigested lactose reaches the big intestine, bacterial enzymes breakdown it, causing osmotic diarrhoea. Infection with rotavirus, which infects lactase-producing cells, and short bowel syndrome are all acquired, typically reversible causes of beta-galactosidase insufficiency. When lactose intolerant people consume milk or milk products, they experience diarrhoea, abdominal discomfort, and flatulence. Although conventional yoghurt preparations containing S. thermophilus and L. delbrueckii ssp. Bulgaricus are more effective in this regard, partly due to higher beta-galactosidase activity, lactose metabolism improvement is a claimed health benefit attributed to probiotics, and it appears to involve certain strains more than others and in specific concentrations. As a result, and because some people have responded well to probiotic supplementation, clinicians should explore it as a therapeutic option (de Vrese, *et al*., 2001); Levri, *et al*., 2005).

1.3 Mechanism of action

Probiotics can have direct or indirect positive effects on a host (Hemaiswarya, *et al*., 2013) The mechanisms of action of probiotics remain an important topic regarding their clinical usage; the broad definition of probiotics makes studying their mode of action difficult; yet, scientists have examined and proposed several mechanisms of action for probiotics throughout the last decades. Guarner *et al* (2011) reported immunologic and non-immunologic mechanisms of action, whereas Patel and DuPont (Patel & DuPont, 2015), identified three main mechanisms of action; antimicrobial activity, immunological modulation, and improvement of mucosal barrier integrity.

Binns (2013) proposed two basic routes of action: microorganisms' or their metabolites' impact on the gastrointestinal tract and microbiota, and contact with the host's cells and immune system. However, depending on the function involved, any one of the mechanisms could be further differentiated. The following are the proposed mechanisms of probiotics' effect on host health in order to prevent or cure certain diseases:

1.3.1 Competition with pathogens on adhesion sites and nutrients

The first is a competition for nutrients and ecological niche; at this time, the presence of indigenous anaerobic flora in the digestive tract inhibits the concentration of potentially harmful flora. Probiotics can have a direct effect on other microorganisms by inhibiting pathogen adhesion. This type of major defense mechanism is used to keep internal health in check. Lactobacilli and bifidobacteria have been proven to inhibit a wide variety of diseases by colonizing pathogenic bacteria and then acting as antagonists against gastrointestinal pathogens. In many circumstances, this idea is important for infection prevention and treatment, as well as gut microbial balance restoration.

1.3.2 Production of antimicrobial agents

Antimicrobial compounds, bacteriocins, toxins, organic acids, short-chain fatty acid synthesis, and a reduction in gut pH are all part of this mechanism. Other hazardous bacteria, including foodborne pathogens and spoilage organisms, are prevented from growing in the GIT environment by these compounds. By generating antagonistic conditions, these compounds cause the pathogen to die, and this process may cause toxins to become inactive. One of the most important examples of probiotics' antimicrobial effect is the effect of Lactobacillus spp. on Helicobacter pylori infection of the gastric mucosa (Boirivanta & Strober, 2007)

1.3.3 Stimulation of Specific and non-specific immune responses.

This mechanism involves the stimulation or modulation of specific and nonspecific immune responses by T-cell activation and cytokine production and through immunomodulation by inducing phagocytosis and IgA secretion, modifying T-cell responses, enhancing Th1 responses, and attenuating Th2 responses. This mechanism of action is probably crucial in the prevention and treatment of infectious illnesses (Thomas & Greer, 2010; Boyle, *et al*., 2006; Vitetta, *et al*, 2014). Probiotic bacteria have the ability to modulate the immune system. These bacteria have the ability to interact with epithelium and dendritic cells (DCs), as well as monocytes/macrophages and lymphocytes. They interact and affect the immune system in a positive way through several ways. Probiotics' immunological benefits may be due to local macrophage activation and modification of IgA synthesis locally and systemically, changes in pro/anti-inflammatory cytokine profiles, or modulation of food antigen response (Hemaiswarya *et al*,2013, Vanderpool *et al*, 2008).

1.4 Species used As Probiotics

Probiotics can come from a variety of microbial species. Yeast, bacteria, and molds are all possibilities. Bacterial species, however, are the most common.

Table 2. Species used as probiotics (Abatenh *et al*,2018)

List of probiotic species	Group of Microbes
Lactobacillus sacidophilus, Lactobacillus bulgaricus, Lactobacillus casei, Lactobacillus fermentum, Lactobacillus lactis, Lactobacillus acidophilus, Lactobacillus paracasei, L. rhamnosus, L. delbrueckii subsp. bulgaricus, L. brevis, L. johnsonii, Lactobacillus	Lactic acid producing bacteria

plantarum, Lactobacillus salivarius, Lactobacillus fermentum, Lactobacillus kefir	
Enterococcus faecalis, Enterococcus faecium, Esherichia coli Nissle, Streptococcus thermophiles, Propinobacterium	Non lactic acid producing bacteria
Bifidobacterium adolescentis, Bifidobacterium bifidum, Bifidobacterium breve, Bifidobacterium lactis, Bifidobacterium longum, Bifidobacterium infantis, B. animalis subsp animalis, B. animalis subsp lactis, B. bifidum	Bifidobacterium species
Saccharomyces boulardii	Nonpathogenic yeast
Coccobacillus, Lactobacillus, Streptococcus, Leuconostoc, Lactococcus lactis subsp. Lactis, Pediococcus, Propionibacterium, Enterococcus, Enterococcus durans, Bifidobacterium, Bacillus, Bacillus coagulans, Bacillus subtilis, Saccharomyces cerevisiae, Candida pintolopesii, Aspergillus niger, A. oryzae, Bacillus lichenformis, B. cereus var. toyoi, B. clausii, B. coagulans, B. laterosporus, B. pumilus, B. racemilacticus, Streptococcus sthermophiles	Non-spore forming

1.5 Possible harmful side effects of probiotics; Disadvantages

Probiotics may be responsible for four types of side effects in susceptible individuals: systemic infections, deleterious metabolic activities, excessive immune stimulation, and gene transfer. Some disadvantages include :

1. Bacteria have the potential to translocate/transmigrate across the gastrointestinal tract, causing invasive infection. Intestinal bacteria translocation is aided by a number of factors, including intestinal mucosal injury, immunodeficiency, gut preterm, aberrant bacterial flora, and bacterial adhesion to the mucosal surface. (Abatenh *et al*,2018)

2. Antibiotic resistance could be passed down from probiotic bacteria to other potentially dangerous bacteria if some probiotic life forms have protection against anti-infection drugs (antibiotics). Because the antibiotic resistance gene can be shared through conjugation, transduction, or transformation, such organisms may harbor genes that can contribute to opportunistic infections. (Abatenh *et al*,2018)

3. Probiotics' metabolic activity and immunologic effects may have negative metabolic consequences and excessive immunological activation. (Abatenh *et al*,2018).

4. This is related to product quality, as items that do not contain the probiotic listed on the label or that contain contaminants may also endanger the consumer. (Abatenh *et al*,2018)

Because of the potential impact of probiotics on gastrointestinal physiology, gastrointestinal toxicity studies should be investigated as part of the safety concern, as there is a chance that undesirable metabolites will be produced, as well as a chance that the probiotic bacteria will cause, encourage, or increase the risk of various physiological and anatomical issues ((Shira & David, 2015; Suresh, *et al.*, 2013)

1.6 Advantage Of Probiotics over other Anti-infective

Probiotics are used to treat infections as an alternative to antibiotics.

Probiotics have been shown to help with the treatment and prevention of infectious disorders (Yang *et al*, 2020). Antibiotics are routinely used to treat infectious diseases today. Antibiotic overuse, on the other hand, can have negative repercussions for patients, such as drug-specific side effects, and for public health, such as the selection of multidrug-resistant microorganisms (

(Yang, *et al*., 2020). As a result, novel antimicrobial therapy options are desperately needed, with a particular focus on natural product-based medicines (Silva *et al,* 2019). Probiotics have been proven to be useful in the treatment of constipation, diarrhoea, polycystic ovarian syndrome, ulcerative colitis, stress and anxiety, inflammatory bowel disease, breast cancer, and diabetes in clinical trials (Kechagia *et al,* 2013).

Despite probiotics' demonstrated biological features, such as antibacterial activity, research in this area is still in its early stages and need further discussion.

1.6.1 Probiotics as potential substitute for Antimicrobial Resistance (AMR)

Antimicrobials, such as antibiotics that target bacteria, kill or prevent the growth of microorganisms. Antimicrobial resistance is an increasing worldwide health problem due to the widespread and sometimes improper use of antibiotics in humans, animals, and the environment (AMR). This is the bacteria's natural adaption to survive an antibiotic onslaught. When resistance is gained, it can easily spread between species. It is extremely difficult to eradicate such resistance mechanisms once they have been established. MDR microorganisms have emerged, making therapeutic treatments more difficult, and some of them may become untreatable. AMR is a critical concern for EU and global healthcare sustainability. Antibiotic use and AMR are linked to veterinary medicine, agricultural animal management, and food production, among other fields. Probiotics as a potential antibiotic alternative are becoming more popular in human, veterinary, and environmental settings as antibiotic-resistant strains become more frequent. To tackle AMR, researchers have looked into treating infectious and non-infectious disorders with probiotics rather than antibiotics. In a nutshell, instead of employing antibiotics to eliminate pathogenic microorganisms, the creation of commensal and occasionally mutualistic microbes in the same host-microbial environment may inhibit the growth of disease-causing microbes. Probiotic use may aid to reduce the pace of emergence of antibiotic-resistant strains caused by widespread antibiotic use by restricting antibiotic use. Furthermore, there is evidence that maintaining what is deemed a "normal" microbiota for specific host-microbial environments can help avoid disease – even if the cause is not infectious – and enhance overall health.

1.6.2 Probiotics have low cost and side effects compared to Other Antimicrobials

Antimicrobial medications now used in clinical practise are effective, but they are expensive, have side effects, and are resistant to treatment (Graham & Fischbach, 2010) When compared to probiotics, antibacterial medicines have greater adverse effects and are more expensive. Resistance to antibiotics has also been documented. Probiotics therapy can be used alone or in combination with other treatments to achieve better results.

As a result, the use of a combination of probiotic microbes and traditional medications has been investigated. The following are some of the synergistic benefits:

(i)faster healing; (ii) half dose of conventional drug needed; (iii) reduction of side effects caused by classical therapy; and (iv) increasing eradication rates of some microbial infections (Kosgey, *et al*., 2019)..

Lesbros-Pantoflickova, (Lesbros-Pantoflickova, *et al*., 2007) examined nine studies that looked at the benefits of combining probiotics and antibiotics for the treatment of H. pylori infections, especially in terms of reducing side effects and increasing eradication rates.\

1.7 Formulations for the Effective Delivery of Probiotics

To enhance the therapeutic advantages of probiotics, many dose forms and formulation methodologies have been tried via various routes of administration, as shown in Table 3. (Baral, *et al*., 2021)

*Dep*ending on anatomical characteristics, environment, and individual physiological parameters, each administration route has its own set of constraints. As a result, formulation methods differ depending on administration routes, probiotic strains, and therapeutic regions.

Table 3. different probiotics, dosage forms and routes of administration (Baral, *et al*., 2021).

Probiotic	Dosage form	Route
Enterococcus faecium	Oral film	Oral
Levi lactobacillus brevis	Buccal film	Oral
Lactobacillus acidophilus	Granule	Oral
Limos lactobacillus reuteri	Tablet	Oral
Lactiplantibacillus plantarum	Orodispersible	Oral
Lactobacillus strains	Capsule	Oral
Lactiplantibacillus plantarum	Hydrogel	Oral
Streptococcus salivarius	Nasal spray	Nasal
Lacticaseibacillus casei	Nasal spray	Nasal
Lactobacillus	Micro needle	Transdermal
Lactobacillus subsp.	Tablet	Vaginal
Lactobacillus gasseri	In situ gel	Vaginal
Lactobacillus acidophilus	Suppository	Vaginal
Limosilactobacillus reuteri	Enema	Rectal

1.7.1 Oral Delivery

Oral formulations are the most popular dose form of probiotics due to excellent patient compliance, cost-effectiveness, and convenience of mass production. As a result, oral formulations are most commonly used for prophylactic or therapeutic probiotics, especially for the treatment of Crohn's disease, ulcerative colitis, and irritable bowel syndrome, the maintenance of intestinal microflora after antibiotic therapy, and tumour growth suppression (Teruel, *et al*., 2020) . Probiotics can be delivered orally in a variety of dose forms, including tablets, capsules, oral films, and hydrogels. Microencapsulation and surface coating technologies are frequently used to improve probiotic stability in the GI tract.

Some selected formulation techniques for oral delivery of probiotics are discussed as follows.

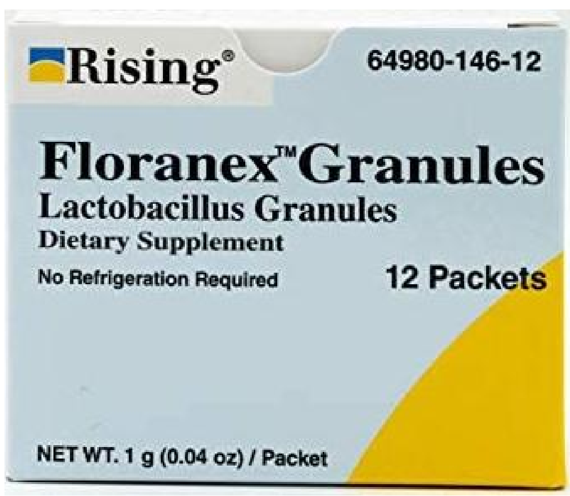
Oral Films

Orally dissolving or disintegrating films (ODFs) have received a lot of interest in recent years as a patient-centric formulation. ODFs hydrate in the saliva shortly after being placed on the tongue, then disintegrate and release the active ingredients in the mouth quickly. (Irfan *et al*, 2016). ODFs are often made up of active chemicals, film-forming polymers, and plasticizers, as well as functional excipients such as sweeteners, flavours, and colours, depending on the application. (Lee *et al.*, 2017) Since ODFs dissolve in the tongue, taste masking is essential, especially for bitter or disagreeable flavors. ODFs have a number of therapeutic advantages, including convenience of administration without the need for water, no risk of choking, convenient transportation, and a quick onset of action, all of which provide a marketing advantage by boosting patient compliance. ODFs are attracting the attention of pediatrics, geriatrics, immobile patients, and those with functional dysphagia. Furthermore, ODFs can bypass the GI tract, limiting the degradation of active components. As a result, ODFs are being studied as a viable oral dosage form for providing viable probiotics to support oral health. (Heinemann *et al*, 2013)., for example, developed ODF for probiotic delivery, encasing Lactobacillus acidophilus or

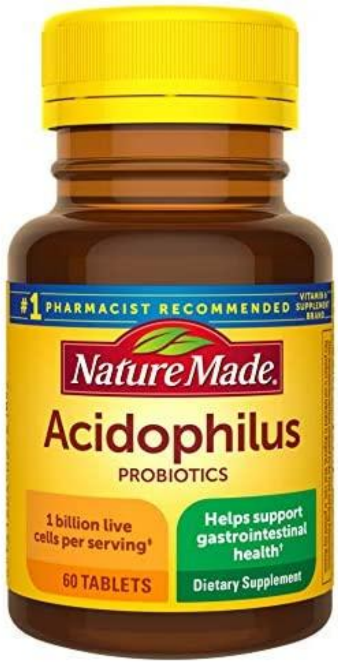
Bifidobacterium animalis subsp. lactis in a matrix of starch, gelatin, and carboxymethyl cellulose. The probiotics in this ODF formulation lasted 90 days in storage (Heinemann *et al*, 2013)

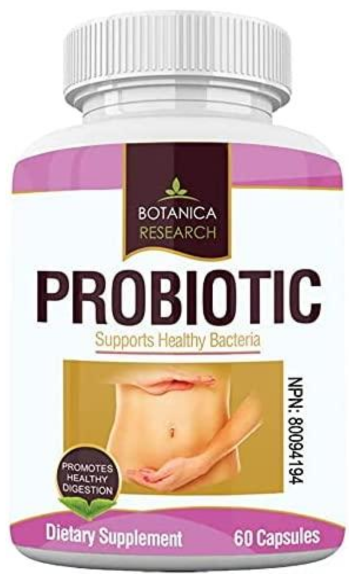
1.7.2 Powder/Granules

The majority of commercially available probiotics are in powder or granule form. Powders have several advantages, including the ability to provide a large dose of probiotics, quick dissolution, low cost, and flexibility in mixing solids. Probiotic granules are also widely available. Wet or dry granulation is used to produce free-flowing agglomerated particles with desirable physical qualities such as size, hardness, porosity, density, and particle distribution uniformity. (Shanmugam, 2015) Granules are a practical and effective way to provide a big dose of probiotics. Granules are more convenient to work with than powders since they don't produce dust and have greater flowability and compressibility. To improve the stability of acid-labile probiotics or modulate their release rate, granules can be coated with different polymers. For example, Pyar(2014) developed Lactobacillus acidophilus ATCC 4962 enteric-coated granules. (Pyar & Peh, 2014).



Tablet and Capsule





Probiotics are delivered in tablets and capsules, which are handy solid dosage forms. These formulations enable the use of a variety of functional excipients to improve shelf life, GI stability, and cell viability, as well as modulate probiotic release rates and target sites. Kim and his colleagues (Kim, *et al*., 2019) recently developed L. reuteri LRT18 tablets that use a pH-sensitive phthalyl inulin to protect probiotics from stomach acid. The phthalyl inulin-coated pills increased cell viability in tough stomach circumstances while also releasing probiotics quickly into the intestinal fluids. These tablets could keep probiotics alive for up to six months in the refrigerator. (Kim, *et al*., 2019)

1.7.3 Nasal Spray.

Nasal delivery devices are non-invasive, simple to use, and provide a quick start of effect. They can also avoid the GI tract's severe acidic environment. In general, the nasal route of administration is appropriate for local and systemic drug delivery in the form of solutions, gels, sprays, suspensions, emulsions, liposomes, and micro/Nanoparticles. The residency period and host-microbiota interaction can be affected by physicochemical parameters of formulations (particle size, osmolality, viscosity, muco adhesiveness, and so on). Membrane permeability, pH, nasal blood flow, nasal enzymes, mucin secretion from goblet glands, and mucociliary clearance are all physiological and anatomical parameters that can affect the effectiveness of probiotic distribution through the nose. (Chaturvedi *et al*, 2011; Xu *et al*, 2020).

Nasal atrophic rhinitis and severe vasomotor rhinitis are two diseases that may affect the degree of nasal medication delivery (Baral, *et al*., 2021) . As a result, several pharmacological and physiological aspects should be considered while formulating probiotics for nasal delivery. Nasal spray is the most often utilized nasal formulation in clinical practice. A nasal spray of *Streptococcus salivarius* 24SMB lowers the incidence of acute otitis media in otitis-prone youngsters.(Marchisio *et al*, 2015).

1.7.4 Topical Skin Delivery

Probiotics delivered through the skin are appealing because they are non-invasive and have a number of benefits, including avoiding GI degradation, regulated drug release, simplicity of self-medication, and high patient compliance. (Ammar *et al*, 2020) Given the importance of skin microorganisms in the epidermis and dermis in maintaining skin health and avoiding pathogen invasion, efficient dermal distribution of probiotics could help to modulate local skin immunity.

Viable probiotics administered through the skin, in particular, can produce substantial amounts of lactic acid and lower the pH of the skin, limiting pathogenic bacteria development and increasing skin immunity. (Proksch .2018). The use of absorption enhancers, iontophoresis, electroporation, sonophoresis, and microneedles have all been tried to improve macromolecule permeation through the skin. Microneedles, for example, have the ability to administer probiotics effectively through the skin. Microneedles are noninvasive or minimally invasive devices with 50–900 millimetre needles. Depending on the needle length, they can penetrate the stratum corneum and release probiotics in the epidermis or dermis, increasing skin penetration and local probiotic delivery. (Cho Lee Ae-Ri, 2019)

1.7.5 Vaginal Delivery

To restore the normal vaginal micro- biota and prevent infections, probiotics can be injected into the vaginal canal. (Ma *et al* 2017; Wijgert & Verwijs 2020). Below are some of the formulation approaches for vaginal distribution of probiotic.

.In-situ Gels

To provide therapeutic effects, vaginal probiotic formulations must sustain an extended residence period in the vagina. For the local administration of several treatments, in situ gelling methods have been offered as an alternative. These systems are fluids before administration, but when they come into contact with biological fluids, they go through an in situ sol-gel transition in response to numerous stimuli, such as changes in pH, temperature, and ion concentration. (Vigani, *et al*., 2020)

Tablets

Vaginal tablets have a number of advantages, including precise dosing, convenience of handling and storage, low manufacturing costs, and ease of administration. Vaginal pills, as opposed to oral tablets, can improve probiotic stability since they are given directly to the target region and avoid the hostile environment of the GI tract. Using diverse functional excipients and formulation processes, vaginal tablets can be made with extra properties such as bio adhesive, sustained release of active components, and quick disintegration, just like oral tablets. For the treatment of vulvovaginal infections, Sanchez developed a bilayered mucoadhesive vaginal tablet containing Lactobacillus subsp. bacteria.(Sánchez *et al*, 2018)

Suppository

Vaginal suppositories are popular because they are simple to use and do not require an applicator. Excipients such as cocoa butter, oils and fats, glycerinated gelatin, and polyethylene glycols can be used to make these suppositories. Suppositories are divided into two categories based on the mix of these excipients: lipophilic and hydrophilic suppositories. (Ham and Buckheit, 2017) Suppositories dissolve more quickly than vaginal tablets, resulting in a quicker beginning of action. (Darwish *et al* 2007) For the vaginal delivery of Lactobacillus acidophilus, Rodrigues and his colleagues produced both solid body and hollow-type suppositories (Rodrigues, *et al*., 2015)

Rectal Delivery

In genetically sensitive individuals, inflammatory bowel disease (IBD) may be triggered by an incorrect immune response to intestinal bacteria and an imbalance in the enteric micro- biota. (Ghouri *et al*, 2014). As a result, the gut micro- biota plays an essential role in IBD treatment, and probiotic bacteria consumption should be advantageous for IBD treatment initiation and maintenance. Rectal administration, especially for ulcerative colitis, can be a promising route of delivery for probiotics to reach the inflamed colon. It can avoid destabilizing probiotics before reaching the colon by bypassing the GI tract. (Baral, *et al*., 2021)

1.8 SOLUBULIZING AGENTS.

The term "solubilization" has evolved to mean "the creation of a thermodynamically stable isotropic solution of a chemical that is ordinarily insoluble or very marginally soluble in a particular saddling by adding an extra amphiphilic component or components.

Surfactants are excipients that are used as solubilizers in pharmaceutical formulations to improve the solubility of medications that aren't very soluble. This increases the bioavailability of the active pharmaceutical ingredient API.. (Merritt & An, 2000) (Tadros, 2014)

Surfactants are molecules that are amphiphilic or amphipathic, with a polar or ionic component linked to a non-polar hydrophobic portion. These molecules are typically hydrocarbon or fluorocarbon chains with 8–18 carbon atoms that are straight or branching (hydrophilic). The word "amphiphilic," which translates from the Greek as "both," refers to the fact that every surfactant molecule has two parts, one of which is soluble in a particular liquid, such water (the hydrophilic component), and the other of which is insoluble in water (the insoluble part) (the hydrophobic part). Nonionic, ionic, or zwitterionic hydrophilic portions can all be accompanied by counter ions in the last two situations. The hydrocarbon chain interacts with water molecules in an aqueous environment rather weakly, whereas the polar or ionic head group interacts with water molecules considerably through dipole or ion-dipole interactions. The surfactant becomes soluble in water as a result of this close interaction with the water molecules. Instead of coming into contact with the hydrophobic chain, the water molecules avoid it, and their combined effect of dispersion and hydrogen bonding tries to squeeze the hydrocarbon chain out of the water by aggregating at interfaces and producing aggregation units known as micelles in solution. (Tadros, 2014).

1.9 Application of Surfactants

Pharmaceutical Application of surfactants

(a) Surfactants as enhancers for percutaneous absorption:

The application of specific adjuvants known as enhancers can accelerate the transport of molecules through the skin. Ionic surfactants increase transdermal absorption by disrupting the stratum corneum's lipid layer and denaturing keratin. Enhancers may improve medication penetration by inducing the stratum corneum to thicken and/or leach away some structural components, lowering diffusional resistance and boosting skin permeability (Muthuprasanna, *et al*., 2009)

1.9.1 Surfactants as flocculating agents:

To prevent the floccules from sedimenting, a suspending agent is frequently used. Carboxy methylcellulose, carbopol 934, veegum, tragacanth, or bentonite are examples of such agents that can be used alone or in combination. (Muthuprasanna, *et al*., 2009)

1.9.2 Surfactants in mouth washes:

Mouthwashes are aqueous solutions that typically come in concentrated form and contain one or more active ingredients called excipients. They work by swishing the liquid around in the mouth.. (Muthuprasanna, *et al*., 2009)

1.9.3 Surfactants in respiratory distress therapy:

Premature newborns with neonatal respiratory distress syndrome are treated with surfactant formulations as a replacement therapy (also known as hyaline membrane disease).

1.9.4 Surfactants in suppository bases:

Several nonionic surface-active chemicals that are chemically related to polyethylene glycols have been developed as suppository carriers. Many of these bases can be used to create drugs that are both oil- and water-soluble. The most often used surfactants in suppository formulations are polyoxyethylene sorbitan fatty acid esters (tween), polyoxyethylene stearates, and sorbitan fatty acid esters (Span and Arlacel). When using surfactants, vigilance should be exercised. Some papers claim that the rate of drug absorption has accelerated, while others contend that these surface active compounds interact with medications and reduce their therapeutic effect. (Muthuprasanna, *et al*., 2009)

1.9.5 Surfactants in suspension aerosols:

The use of surfactants in aerosol suspensions has shown to be quite effective. These surfactants work by coating each particle in suspension and orienting themselves at the solid-liquid interface. Agglomeration is minimised, resulting in increased stability due to the presence of a physical barrier.

1.9.6 Surfactants for contact lens cleaning:

Surfactants clean contact lenses by emulsifying accumulated oils, lipids, and inorganic substances. Surfactant agents are used in a mechanical cleaning device or by gently rubbing the lens back and forth with the thumb and forefinger or by placing the lens in the palm and softly rubbing with a fingertip (about 20 to 30 seconds). A nonionic detergent, wetting agent, buffers, and preservatives are commonly found in these cleansers. (Muthuprasanna, *et al*., 2009)

1.9.7 Surfactants in microbiology:

A surfactant can either influence the activity of a medicine or act as a drug. In the first scenario, the addition of a low quantity of surfactant increases the penetration of hexylresorcinol into the pinworm Ascaris. The lowering of interfacial tension between the liquid phase and the organism's cell wall causes this potentiation of activity. As a result, the adsorption and diffusion of hexylresorcinol over the surface of the organism is facilitated. The rate of penetration of the anthelmentic drops to nearly zero when the concentration of surface active agent exceeds that required to form micelles. Because the medication has been partitioned between the micelles and the aqueous phase, the effective concentration has been reduced. (Muthuprasanna, *et al*., 2009)

Other possibilities include:

Surfactants' practical and theoretical importance in biochemistry can be illustrated by the following examples: Surfactants, which act as solubilizers and probes for hydrophobic binding sites, have made it possible to study the molecular characteristics of membrane proteins and lipoproteins (Righetti, 1983).

In high-performance affinity chromatography, affinity surfactants have been utilized as reversibly bound ligands. (Muthuprasanna, *et al*., 2009)

1.10 Classification of surfactants:

Surfactants are categorised using the charged groups that are present on their heads. A nonionic surfactant has no charge groups on its surface. An ionic surfactant has a net charge on its head. The surfactant is referred to be anionic or cationic depending on whether it has a positive or negative charge. A surfactant is referred to as a zwitterion if its head has two oppositely charged groups.. (Muthuprasanna, *et al*., 2009)

.(a) Anionic surfactants:

In solution, the head has a negative charge. These surfactants are the most commonly used type of surfactant in shampoo formulation due to their excellent cleansing and hair conditioning capabilities. Anionic surfactants have a significant role in oily cleaning and oil/clay suspension. They can still partially deactivate by reacting with the calcium and magnesium ions, which are positively charged water hardness ions, in the wash water. The most widely used anionic surfactants are soaps, alkyl sulphates, and alkyl ethoxylate sulphates. The bulk of anionic surfactants are made up of carboxylate, sulphate, and sulfonate ions. (Remington, 1995) . The straight chain is a C12-C18 aliphatic group that is saturated or unsaturated. The presence of double bonds determines the surfactant's water solubility potential (G.Zagrafti, 1995)

Anionic surfactants come in five main varieties. Alkyl sulphates, amine soaps, alkyl phosphate anionic surfactants, divalent and trivalent metal soaps, alkali metal and ammonium soaps, and alkyl sulphate surfactants are some of the options available. Alkali metal soaps, which contain long-chain fatty acids like oleic, stearic, and ricinoleic acid, generate oil-in-water emulsions when they are hydrated with sodium, potassium, or ammonium. Though they are stable above pH 10, the emulsion splits when exposed to acid. To emulsify water in oil, surfactants containing divalent and trivalent cations are utilised. Both their alkalinity and their acid sensitivity are reduced. Triethanolamine is the amine surfactant of choice for medicinal purposes.. (Muthuprasanna, *et al*., 2009)

(B) Cationic Surfactants:

In solution, the head of the cationic surfactant is positively charged. Due to their high bactericidal activities, cationic surfactants—quaternary ammonium compounds—are typically used for their disinfectant and preservation properties. They are applied to cleanse skin burns or wounds. The most widely used cationic surfactant is cetrimide, which is composed primarily of tetradecyl trimethyl ammonium bromide and a trace amount of dodecyl and hexadecyl compounds. Examples of cationic surfactants include benzalkonium chloride, cetylpyridinium chloride, and others.

(c) Non-Ionic Surfactants:

These surfactants are not deactivated by hardness of the water since they don't carry an electrical charge. As opposed to other anionic or cationic surfactants, they don't irritate the skin as much. The hydrophilic part contains derivatives of polyols, polyoxyethylene, and polyoxypropylene. The component that is hydrophobic is made up of fatty acids, whether they are saturated or unsaturated, as well as fatty alcohols. Both as emulsifiers and oil/grease removers, they are incredibly effective. The hardness sensitivity of the surfactant system can be lessened with the aid of nonionic surfactants. Non-ionic surfactants can be classified as polyol esters, polyoxyethylene esters, and poloxamers. Polyol esters include substances like glycerol and glycol esters as well as sorbitan derivatives. The polyoxyethylene ester polyethylene glycol is one example (PEG 40, PEG -50, PEG- 55). Ethers of fatty alcohols are the most widely utilized non-ionic surfactants. (Muthuprasanna, *et al*., 2009)

.(d) Amphoteric/Zwitterionic Surfactants:

These surfactants are perfect for use in personal care products for people with sensitive skin because they are gentler than most other surfactants. They come in three different charge states in solution: anionic (negatively charged), cationic (positively charged), and non-ionic (no charge), depending on the acidity or pH of the water. It is possible that those surfactants include two charged groups of various signs. In general, ammonium is always the positive charge, but the source of the negative charge can change (carboxylate, sulphate, sulphonate). The dermatological characteristics of these surfactants are outstanding. Because of their great foaming capabilities, they are commonly used in shampoos and other cosmetic goods, as well as hand dishwashing liquids. (Muthuprasanna, *et al*., 2009)

1.11 Characteristics Of Surfactants

Intrinsic Antimicrobial Property.

The term "surface active agents" refers to a vast group of chemical molecules. Because of their structure, surfactants are split into four categories. Nonionic, anionic, cationic, and amphoteric surfactants fall within this category (Daria, *et al*., 2014) . They have a wide range of unique characteristics. The capacity to lower surface tension, wettability, foamability, good solubility in water, detergency, and biocidal activity are the most significant characteristics. (Daria, *et al*., 2014)

Many chemicals are classed as surfactants, and they are divided into two groups: nonionic and ionic (anionic, cationic and amphoteric). They have diverse chemical structures, which means they have different qualities and are utilized to make a wide range of . (Staszak, *et al*., 2013).

Compounds that produce positively charged organic ions responsible for surface action in aqueous solution are classified as cationic surfactants. Quaternary ammonium compounds are one of the most common types of cationic surfactants (QACs). They've been used in a variety of industries, including cosmetics and medicine (Buffet-Bataillon *et al*., 2012), wood protection, and domestic products. They have bactericidal (Caillier, *et al*., 2009) , fungicidal (Chen, *et al*., 2011) , and antiviral activity, and are utilized as disinfectants on a broad basis (Daria, *et al*., 2014) . In the medical, food, detergent, and glue sectors, quaternary ammonium compounds are extensively utilized as disinfectants (Wong, *et al*., 2012)]. McDonnell and Russell (1999) found them as an ingredient in a variety of pharmaceutical products and medicines. Despite the fact that QACs cover a wide variety of chemicals, the first reports of their biocidal action originate from the 1920s (Walker, 2003). The synthesis and characteristics of new QACs have been reported in recent papers (Daria, *et al*., 2014). It also includes antimicrobial activity and the mechanisms by which they target microorganisms, as well as the mechanisms by which they are biologically degraded (He, *et al*., 2011) .

Benzalkonium chloride (BAC), cetylpyridinium chloride (CPC), and cetyltrimethylammonium bromide (CTAB) are all QACs.

In Removal of Cells from Surfaces.

Mechanical forces experienced during washing and the microbe's surface energetics—surface contact—can both affect how easily bacteria detach from plant surfaces or other surfaces. Surface energetics can be influenced by a number of factors, including produce-water contact, bacterial dispersion on the surface of the plant, and surface roughness. ((Faille, *et al*., 2013) . Only a small amount of study has been done to demonstrate how surface hydrophobicity influences microorganism separation from the surface. Ukuku and Fett (2002) found that the amount of bacterial adhesion to cantaloupe surfaces correlated with the hydrophobicity of bacterial cell surfaces (Ukuku & Fett, 2002). The partitioning of cells to the octan-water interface was used in this work to evaluate the hydrophobicity of the cell surface. Surfactants are one way that could be used to modify the surface energetics of bacteria-produce interactions. Surfactants are surface-active chemicals that can reduce liquid-solid interfacial tension (Banat, *et al*., 2000;

Mukherjee & Das, 2010). This property of surfactants allows for the release of surface-bound pollutants like foodborne pathogens, which prompted us to hypothesise that surfactants could assist in the removal of foodborne pathogens from fresh fruit. From a variety of ionic and nonionic surfactants, sodium dodecyl sulphate (SDS), Tween-20, and lauric arginate (LAE) were selected for this study. While LAE is a cationic surfactant, tween-20 is a nonionic surfactant. Both drugs are GRAS (generally recognized as safe). SDS is an anionic surfactant and food additive that has received FDA approval. The results of this investigation showed that the addition of surfactants increased bacterial clearance from the plant surface. The addition of surfactants to wash water reduced the contact angle between the wash water and the leaf surface. (Kang & Nitin, 2017)

1.12 Adhesion mechanisms of probiotics to intestinal mucosa

The probiotic bacteria Lactobacillus and Bifidobacterium species are the most prevalent. Both genera of lactic acid bacteria are Gram-positive, and they have surface molecules such lipoteichoic acid (LTA), surface layer associated proteins (SLAPs), and mucin binding proteins (Mubs) that play an important role in interacting with mucus constituents (Lebeer, *et al*., 2010).Bacterial adherence to intestinal surfaces could be triggered by non-specific physical contacts, such as hydrophobic interactions, followed by adhesion by specific cell wall components in a second step. Hydrophobicity and adhesion have been linked by some scientists (Pan, *et al*., 2006) . As shown by studies on lactic acid bacteria (Muoz-Provencio *et al*, 2012; Zhang *et al*., 2015; Radziwill-Bienkowska *et al*., 2017), some surface proteins, such as cell wall-anchored proteinases, can enhance hydrophobicity and adherence. Bacterial cell walls include adhesins, which facilitate bacterial adhesion to the stomach.

Mucus-binding proteins are surface adhesive proteins with Mub and/or MucBP (MUCin-Binding Protein) domains that can bind mucins and a C-terminal Leu-Pro-any-Thr-Gly motif that links them to the peptidoglycan cell wall (LPxTG). Listeria monocytogenes and other dangerous bacteria have been shown to contain MucBP domains (Popowska et al., 2017), however lactic acid bacteria isolated from the human gastrointestinal system are the only known source of Mub domains (Boekhorst, et al., 2006;Van Tassell & Miller, 2011).. Fimbriae and pili, which are the thin, protein-rich extensions of bacterial cells, can also aid in adhesion. Type IV pili in Gram-negative bacteria have been the subject of in-depth research. Recent studies have revealed that Gram-positive bacteria like Bifidobacterium may also express this type of pili, which aids bacteria in colonising mucosal surfaces (O'Connel Motherway *et al*., 2011; Piepenbrink & Sundberg, 2016). SpaCBA pili can also be produced by some Lactobacillus species (Reunanen, *et al*., 2012; Toh*, et al*., 2013). This form of pili (originally identified and defined in the probiotic strain Lactobacillus rhamnosus LGG) is made up of three subunits that are encoded by the SpaCBA cluster and assembled by a sortase. While SpaB and SpaC are secondary fibre components, SpaA is the pilus' main fibre. The great ability of Lactobacillus rhamnosus LGG to adhere to surfaces is due to the mucus-binding protein SpaC, which is located at the tip of the pili (Reunanen *et al*., 2012)

In addition to mucus-binding proteins and pili, other surface proteins, such as fibronectin-binding proteins (FBPs) and surface layer proteins (SLPs), can aid bacterial adhesion to the intestinal mucosa.Fibronectin is an extracellular matrix glycoprotein present in the intestine in an insoluble state. Gram-negative and Gram-positive bacteria have both been found to have FBPs, due to their ability to invade host epithelial cells, the presence of these proteins has been linked to pathogen virulence. Contrarily, FBPs may help probiotic bacteria by improving their capacity to adhere to host cells, thereby excluding pathogens. (Lehri, *et al*., 2015; Hymes, *et al*., 2016). SLPs, in contrast, are extracellular para-crystalline proteins that cover the surface of bacteria's cells and serve a number of purposes, including those of structural components, pathogenicity, antifouling coating, and adhesion promoters. (Sleytr, *et al*., 2014) . Although SLPs vary in type and distribution among strains, they appear to be crucial for probiotic bacteria adhesion to intestinal cells because adhesion has been decreased when SLPs were eliminated through chemical treatments (Tallon, *et al*., 2007; Zhang *et al*., 2013). Because SLPs interact with host intestinal receptors to trigger an immunological response, they may also function as an immunomodulatory component in probiotic bacteria (Konstantinov *et al*., 2008).

Probiotic bacteria adhesion is routinely evaluated using in-vitro tests. Mucin adsorbs to abiotic surfaces and human tumor-causing cell lines including Caco-2 and HT-29. (Lebeer*, et al*., 2012;Monteagudo-Mera, *et al*., 2012; Toh*, et al*., 2013; Garriga, *et al*., 2015)were utilized to imitate the attachment to intestinal epithelial cells using the cell line HT-29 recently discovered a new surface layer protein (choline-binding protein A) that is required for the adhesion of the novel probiotic strain Lactobacillus salivarius REN.in this investigation (IECs). The usage of epithelial cell lines has been extremely helpful in identifying adhesion mechanisms and molecules. Wang and associates' 2017 study (Wang, *et al*.)

CHAPTER TWO

MATERIALS AND METHOD.

2.1 MATERIALS

All materials used during this experiment were sterilized before and after use.

2.1.1 Equipment

Inoculating wire loop, autoclave, bacteriological incubator, digital weighing balance, Bunsen burner, refrigerator, gas cylinder, hot air oven, microscope, centrifuge, spectrophotometer, anaerobic jars, micropipettes (1000 microliter and 200 microliter micropipettes).

2.1.2 Glass wares

Measuring cylinders, disposable petri dishes, universal bottles, pipettes (1ml, 5ml, 10ml), Bijou bottles, disinfectant jar, glass stirrer.

Other materials include cotton wool, grease pencil, marker pens, masking tape, gloves, face mask, candle, spatula, forceps.

2.1.3 Microbiological media

MRS agar, MRS broth.

2.1.4 Reagents and Chemicals

Phosphate buffer saline (sodium hydrogen phosphate + disodium hydrogen phosphate + sodium chloride + water), polyethylene glycol, Tween 80,,crystal violet, absolute ethanol, methylated spirit, disinfectant liquid.

2.1.5 Organism

Lactobacillus reuteri strain

Source: The organism was obtained from the Pharmaceutical Microbiology Laboratory.

2.2 METHOD

2.2.1 Study area

The study was conducted at the Post Graduate Pharmaceutical Microbiology laboratory of the University of Benin, Benin city.

2.2.2 Preparation of solubilizing Agents

All procedures were carried out aseptically and in a sterile zone.

Concentrations of 12.5%, 25%, 50%, 75% and 100% of each solubilizing agent (tween 80, Propylene glycol) was prepared, to a total volume of 5ml for each concentration. The concentrations were labelled A (100%), B (75%), C (50%), D (25%) and E (12.5%).

2.2.3 Growth inhibitory test.

20 microliters of *Lactobacillus reuteri* was sub cultured in 5 universal bottles containing 10ml of MRS Broth. The universal bottles were incubated for 48 hours in a microaerophilic condition at 37^0.After incubation the culture of the MRS broth were discarded while the bottle was aseptically rinsed 3 times with 10ml sterile Phosphate Buffer saline .The individual bottles were homogenized in 5ml of specific concentration of the solubilizing agents (Propylene glycol or Tween 80). At an interval of 2 ½ minutes to 30 minutes.20 microliters were sub cultured in 5 ml of sterile MRS broth and incubated for 48 hours in microaerophilic condition.The endpoint was documented as growth (for change in turbidity) and no growth when the broth retrained it's original state.

2.2.4 Determination of the effect of contact time and concentration of solubilizing agents on adhered *lactobacillus reuteri*.

20 microliters of *Lactobacillus reuteri* was sub cultured in 5 universal bottles containing 10ml of MRS Broth. The universal bottles were incubated for 48 hours in a microaerophilic condition at 37^0. After incubation the culture of the MRS broth were discarded while the bottle was aseptically rinsed 3 times with 10ml sterile Phosphate Buffer saline saline.The individual bottles were homogenized in 5ml of specific concentration of the solubilizing agents (Propylene glycol or Tween 80). At an interval of 2 ½ minutes to 30 minutes.20 microliters were sub cultured in 5 ml of sterile MRS broth and incubate for 48 hours in microaerophilic condition.After incubation the culture of the MRS broth were discarded while the bottle was aseptically rinsed 3 times with 10ml sterile Phosphate Buffer saline .

The individual bottles were homogenized in 5ml of specific concentration of the solubilizing agents (Propylene glycol or Tween 80). At an interval of 2 ½ minutes to 30 minutes.20 serial dilutions were carried out.20 microliters of the dilution were placed in MRS agar in triplicate. The plate were incubated for 48 hours at 37^o. The number of colonies were used to determine the Colony forming unit(CFU)/ml of various concentrations with respective time intervals.

CHAPTER THREE

3.1 RESULT

Table 3.1 is a representation of the growth in inhibitory effect of the solubilizing agents on *Lactobacillus reuteri* at a contact time of 2 ½ minutes.Tween 80 had all growth at the different concentrations.Propylene glycol showed all growth of *Lactobacillus reuteri* at the different concentrations.

Table 3.2 is a representation of the growth inhibitory effect of the different concentrations of solubilizing agents between a contact time of 2 ½ minutes to 30 minutes.Propylene glycol showed all growth.

Table 3.3 is a representation of the growth inhibitory effect of the different concentrations of solubilizing agents between a contact time of 2 ½ minutes to 30 minutes.Tween 80 showed all growth.

Table 3.4.1 is a representation of the effect of different concentrations of Propylene glycol and contact time (2 ½ minutes to 15 minutes) on the desorption of the adhered lactobacillus reuteri. The highest Colonies of Lactobacillus reuteri was at 25% concentration with $8.0x10^4$CFU/ml at 12 ½ minutes and the lowest yield was $1.66x10^2$ at 2 ½ minutes, at 50% concentration.

Table 3.4.2 is a representation of the effect of different concentrations of Tween 80 and contact time (2 ½ minutes to 15 minutes) on the desorption of the adhered lactobacillus reuteri.The highest Colonies of Lactobacillus reuteri was at 25% concentration with $6.0x10^6$CFU/ml at 15 minutes and the lowest yield was $1.7x10^2$ at 7 ½ minutes, at 100% concentration.

Table 3.5 served as a control of the experiment.It represents the effect of contact time and 100% sterile distilled water on the desorption of adhered lactobacillus reuteri.The highest yield was 3.33×10^{3} CFU/ml at 2 ½ minutes and the lowest yield was 3.33×10^{2} CFU/ml at 5 minutes.

TABLE 3.1 : GROWTH INHIBITORY EFFECT OF SOLUBILIZING AGENTS ON LACTOBACILLUS REUTERI AT 2 ½ MINUTES

	SOLUBILIZING AGENT	
CONCENTRATION (%)	PROPYLENE GLYCOL	TWEEN 80
100	G	G
75	G	G
50	G	G
25	G	G
12.5	G	G

KEY;

NG= NO GROWTH

G= GROWTH

TABLE 3.2; GROWTH INHIBITION OF LACTOBACILLUS REUTERI USING PROPYLENE GLYCOL

TIME (MIN)	**A**	**B**	**C**	**D**	**E**
2 ½	G	G	G	G	G
5	G	G	G	G	G
7 ½	G	G	G	G	G
10	G	G	G	G	G
12 ½	G	G	G	G	G
15	G	G	G	G	G
17 ½	G	G	G	G	G
20	G	G	G	G	G
22 ½	G	G	G	G	G
25	G	G	G	G	G
27 ½	G	G	G	G	G
30	G	G	G	G	G

KEY;

NG= NO GROWTH

G= GROWTH

A= concentration of solubilizing at 100%

B= concentration of solubilizing agent at 75%

C = concentration of solubilizing agent at 50%

D= concentration of solubilizing agent at 25%

E = concentration 0f solubilizing at 12.5%

TABLE 3.3; GROWTH INHIBITION OF LACTOBACILLUS USING TWEEN 80

TIME (MIN)	**A**	**B**	**C**	**D**	**E**
2 ½	G	G	G	G	G
5	G	G	G	G	G
7 ½	G	G	G	G	G
10	G	G	G	G	G
12 ½	G	G	G	G	G
15	G	G	G	G	G
17 ½	G	G	G	G	G
20	G	G	G	G	G
22 ½	G	G	G	G	G
25	G	G	G	G	G
27 ½	G	G	G	G	G
30	G	G	G	G	G

KEY;

NG= NO GROWTH
G= GROWTH
A= concentration of solubilizing at 100%
B= concentration of solubilizing agent at 75%
C = concentration of solubilizing agent at 50%
D= concentration of solubilizing agent at 25%
E = concentration 0f solubilizing at 12.5%

TABLE 3.4: EFFECT OF CONCENTRATION AND CONTACT TIME ON DESORPTION OF LACTOBACILLUS

TABLE 3.4.1: Effect of different concentrations and contact time of propylene glycol

COLONY FORMING UNIT (CFU/ML)

	TWEEN 80				
TIME(MIN)	100 %	75%	50%	25%	12.5%
2 ½	3.3×10^2	3.8×10^3	1.66×10^2	5.3×10^4	3.8×10^3
5	1.7×10^2	6.5×10^3	1.0×10^3	6.0×10^4	3.8×10^3
7 ½	1.3×10^3	6.0×10^3	1.7×10^2	5.2×10^4	2.5×10^3
10	5.0×10^2	6.8×10^3	1.0×10^3	1.5×10^4	2.8×10^3
12 ½	6.7×10^2	4.0×10^3	1.7×10^2	8.0×10^4	4.7×10^3
15	1.8×10^3	2.7×10^3	1.0×10^3	7.5×10^4	4.10×10^3

TIME(MIN)	100 %	75%	50%	25%	12.5%
2 ½	---------	7.3×10^4	6.0×10^5	1.0×10^6	9.7×10^5
5	5.0×10^2	1.2×10^5	1.2×10^6	2.5×10^6	9.5×10^5
7 ½	1.7×10^2	6.5×10^4	5.8×10^5	1.0×10^6	9.8×10^5
10	----------	4.8×10^4	7.7×10^5	4.5×10^6	7.3×10^5
12 ½	----------	3.0×10^4	3.4×10^5	1.0×10^6	4.0×10^5
15	----------	7.3×10^4	1.3×10^5	6.0×10^6	3.0×10^5

TABLE 3.4.2: Effect of different concentrations and contact time of Tween 80

TABLE 3.5 ;CONTROL

TIME(MIN)	COLONY FORMING UNIT (Cfu/ml)
	WATER
	100%
2 ½	3.33×10^3
5	3.33×10^2
7 ½	2.8×10^3
10	2.3×10^3
12 ½	2.4×10^3
15	3.0×10^3

CHAPTER FOUR

DISCUSSION

Microbial adhesion can be assessed experimentally, however, there are still few techniques to measure it (Satu, *et al*., 2005) .The techniques used include direct and indirect methods. The direct methods are laborious and the bacteria have to remain culture able. while the indirect method are less have low through put ,less accurate, less sensitive. (Merritt & An, 2000)

Some examples of direct method include light microscopy , Transmission electron (TEM), counting (colony forming unit- CFU), coulter counter, flow cytometry, atomic force(AFM), radio labelling etc. .Indirect methods include Spectrophotometric methods, bio-timer assay (BTA), Adenosine triphosphate (ATP) assay , quartz crystal microbalance etc. .Indirect methods are less sensitive, less accurate and have low through put. (Meireles, *et al*., 2015).

In this experiment, I experimentally assessed the microbial adhesion of adhered Lactobacillus reuteri on an abiotic surface. This was done by studying the desorption effect of different concentrations of Tween 80 and Propylene glycol at different contact time on adhered *lactobacillus reuteri* (*L.reuteri*).This is a direct method of experimentally assessing microbial adhesion. And on the long run I want to design an experimental model for assessing microbial adhesion of probiotics on vaginal epithelium *in vitro.*

Do Carmo and his colleagues (do Carmo, *et al*., 2016) conducted an experiment where they experimentally assessed microbial adhesion of different lactobacillus species on Human cervical epithelial cells (HeLa).They investigated the ability of Lactobacillus species to adhere to Human cervical epithelial (HeLa) cells.This was done by a method called the " adhesion assay to eukaryotic cells" it was a modification of the cell adhesion assay (Kaewnopparat, *et al*., 2013)

The results showed that high yield of most Lactobacillus species used.this yield range from 10^7 to 10^8 CFU/ml.Some of the lactobacillus species used include *Lactobacillus brevis ATCC367,L.delbrueckii spp. delbrueckii ATCC 9645, L. fermentum ATCC23271, L.rhamnosus ATCC 8014* etc.This meant that there was high binding with mucin , which is a major component of cervical mucus on the epithelium of the uterus and vaginal tract .This high binding with mucin is very important in it's ability to impair pathogens binding to the genital tract. (do Carmo, *et al*., 2016)

From the result showing the growth inhibitory effect of different concentrations of the propylene glycol and Tween 80 (100%, 75%, 50%, 25%,12.5%). This result was observed at a contact time of 2 ½ minutes to 30 minutes . From the result, it was observed that the two solubilizing agents at this contact time and at the different concentrations, had no growth inhibitory effect on the *lactobacillus reuteri*, and an unhindered growth of the *lactobacillus reuteri* through out the different concentrations was observed.

The growth inhibitory test is necessary in this experiment. A characteristics that is ideal for any solubilizing agents to be used to quantify the desorption of *Lactobacillus reuteri* from the walls of surfaces is it's non- inhibitory property or the ability not to cause growth inhibition of the *lactobacillus* strain. Causing growth inhibition would affect the number of viable colonies and therefore reduce the number of colonies that would grow on the MRS(de Man Rogosa Sharpe) agar plate. This would affect the validity of our experiment. From the results showing the effect of different concentrations of the solubilizing agents (propylene glycol and Tween 80) at different contact time (2 ½ min, 5 min, 7 ½ min, 10 min,12 ½ min,15 min) on the desorption of *Lactobacillus reuteri* from the walls of the abiotic surface, analyzing the result of propylene glycol vertically, at a 100% concentration , the higher yield of *Lactobacillus reuteri* was 1.8x 10^3 at 15 minutes and the lowest yield was 1.7x 10^2 at 5 minutes.

At a concentration of 75% , the highest yield was 6.8x 10^3 at 10 minutes and the lowest 2.7x10^3 at 15 minutes.

At a concentration of 50%, the highest yield was 1.0x103 observed at 5,10 and 15 minutes. The lowest is 1.66 x10^2 at 2 ½ minutes

At 25%, the highest yield was 8.0x10^4 at 15 minutes and the lowest yield was 1.5x10^4 at 10 minutes.

At a 12.5%, the highest yield was 8.3x10^3 at 2 ½ minutes and the lowest yield was 1.2x10^3 at 5 minutes. The desorption effect of Propylene glycol is not concentration dependent because an optimal yield of 8.0x10^4 CFU/ml was observed at 15 minutes at a concentration of 25%.we could concluded that it is time dependent only at an optimum concentration (which in this case is 25%). Therefore, when using propylene glycol as a desorption agent in assessing microbial adhesion of adhered *lactobacillus reuteri*, at an optimum concentration, more time will bring about more desorption effect of propylene glycol.

Analyzing the result of Tween 80 vertically, at a 100% concentration , the higher yield of *Lactobacillus reuteri* was 5.0x 10^2 CFU /ml at 5 minutes and the lowest yield was 1.7x10^2 CFU/ml at 7 ½ minutes.

At a concentration of 75% , the highest yield was 1.2x 10^5 at 5 minutes and the lowest 3.0x10^4 at 12 ½ minutes.

At a concentration of 50%, the highest yield was 1.2x10^6 observed at 5 minutes. The lowest is 1.3 x10^5 at 15 minutes

At 25%, the highest yield was 6.0x10^6 and the lowest yield was 1.0x10^6 at 2 ½ minutes , 7 ½ and 12 ½ at minutes.

At a 12.5%, the highest yield was 9.8x10^5 at 7 ½ minutes and the lowest yield was 3.0x10^5 at 15 minutes. The desorption effect of Tween 80 is not concentration dependent because an optimal yield of 6.0x10^6 CFU/ml was observed at 15 minutes at a concentration of 25%.we could concluded that it is time dependent only at an optimum concentration (which in this case is 25%). Therefore, when using Tween 80 as a desorption agent in assessing microbial adhesion of adhered *lactobacillus reuteri*, at an optimum concentration, more time will bring about more desorption effect of Tween 80.

Comparing the contact time of both solubilizing agent. from the result, the desorption effect was seen to begin at time 2 ½ minutes for each solubilizing agents. Increase in the contact time didn't have much effect on desorption of *Lactobacillus reuteri* but there is possibility of increase in desorption effect when the concentration is optimum for desorption. At this optimum concentration of solubilizing agent, we could say more contact time would bring about better desorption of *Lactobacillus reuteri*. This was observed for both results of the solubilizing agents (propylene glycol and Tween 80) at 25% concentration. An increase in colonies of *Lactobacillus reuteri* as the contact time increased. Therefore from the result it could be deducted that the desorption effect of Propylene glycol and Tween 80 on adhered *Lactobacillus reuteri* was not concentration dependent. It could be time dependent if this desorption effect was observed at an optimum desorption concentration.

Propylene glycol had the highest yield of *lactobacillus reuteri* at 25% with 8.0×10^4 CFU/ml at 15 minutes and the lowest yield at 50% with 1.66×10^2 CFU/ml at 2 ½ minutes.

Tween 80 had the highest yield of *lactobacillus reuteri* at 25% with 6.0×10^6 CFU/ml at 15 minutes and the lowest yield at 100% with 1.72×10^2 CFU/ml at 7 ½ minutes.

The two solubilizing agents had desorption effect on adhered *lactobacillus reuteri* but when comparing the two solubilizing agent, Tween 80 (6.0×10^6) CFU/ml produced a higher number *lactobacillus reuteri* compared to propylene glycol. (8.0×10^4 CFU/ml). Therefore, had a higher desorption effect on adhered *lactobacillus reuteri*.

There are many other techniques for experimentally assessing microbial adhesion. This has been classified to direct and indirect methods. Microbial adhesion is very important attribute in probiotics as it is required in colonization as well as in the modification of the immune system and antagonism against pathogens. Simulating experimental models to assess probiotic adhesion on the epithelial of the vaginal in-vitro will help us understand probiotic adhesion and simulate better conditions that will facilitate better adhesion and colonization of the vaginal epithelium thus maintain vaginal micro biome and reduce growth and effect of pathogenic organisms. (Bijender kumar, *et al*., 2015)

CHAPTER FIVE

CONCLUSION

In conclusion, the different solubilizing agents used (propylene glycol and Tween 80) at different concentrations and various contact time had no growth inhibitory effect on but had desorption effect on adhered *Lactobacillus reuteri.*

In the desorption of *Lactobacillus reuteri*, an optimum concentration of the solubilizing agents is paramount. contact time is not of much relevance but can only be put into consideration when an optimum concentration for desorption is used. At this concentration, an increase in contact time equates an increase in the desorption by resulting in higher counts of the viable *lactobacillus reuteri* on MRS agar plate.

Tween 80 and Propylene glycol have proportionate desorption effect on *Lactobacillus reuteri.* But comparatively, Tween 80 had higher desorption effect on adhered *lactobacillus reuteri*.

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