Studies to determine the efficacy of the commercially available probiotic products

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DECLARATION BY STUDENT

I hereby declare that the data presented in this dissertation entitled "Studies to determine the efficacy of the commercially available probiotic products" is based on the results of investigations carried out by me in the Microbiology programme at the School of Biological Sciences and Biotechnology, Goa University under the supervision of Dr. Milind M. Naik, Assistant Professor in Microbiology, Goa University and the same has not been submitted elsewhere for the award of a degree or diploma by me. Further, I understand that Goa University or its authorities will not be responsible for the correctness of observations/experimental or other findings given in the dissertation.

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COMPLETION CERTIFICATE

This is to certify that the dissertation report entitled **"Studies to determine the efficacy of the commercially available probiotic products"** is a bonafide work carried out by **Ms. Vaishnavi Kashinath Gaude** under my supervision in partial fulfilment of the requirements for the award of the degree of **Master of Science in Microbiology**, in the Microbiology programme at the School of Biological Sciences and Biotechnology, Goa University .

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PREFACE

Probiotics were defined as “living microorganisms when taken in suitable amounts provide a health benefit on host”. Commercially available probiotic formulations and dairy products contains live microorganisms which provides benefits to the host. Probiotics plays an important role in the diet and also provide health benefits such as prevention of lactose intolerance, gut infection, antibiotic associated diarrhoea, control irritable bowel syndrome, control inflammatory bowel disease, lowers the cholestrol. The study was carried out to see if commercially available probiotic products possess all the characteristics of ideal probiotics that was acid tolerance, bile salt tolerance, adherence to epithelial cells, antimicrobial activity against pathogens.

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ABBREVIATIONS USED

Entity	Abbreviation
Antibiotic associated diarrhoea	AAD
Bile salt hydrolase	BSH
Dendritic cells	DC_S
Gastrointestinal tract	GIT
Gram	g
Hydrochloric acid	HCL
Hydrogen peroxide	H_2O_2
Intestinal epithelial cells	IEC_S
Irritable bowel syndrome	IBS
Lactic acid bacteria	LAB
Lactose intolerance	LI
Microliter	µl
Mililiter	ml
Percentage	%
Phosphate buffered saline	PBS
Polycystic ovary syndrome	PCOS
Short chain fatty acids	$SCFA_S$
Transforming Growth factor beta	TGFβ

ABSTRACT

Probiotics were defined as “live microorganisms which when taken in suitable amounts provide a health benefit on host”. The aim of the present study was to determine the efficacy of the commercially available probiotic products. The total four commercially available probiotic formulations (capsule and sachet) and probiotic dairy products (curd and yakult) were collected from local supermarkets or pharmacies. The nine isolates were isolated and identified by morphological and biochemical characterization. All the isolates were characterized in lab for their probiotic properties such as acid tolerance, bile salt tolerance, cell surface hydrophobicity, bile salt hydrolytic activity, enzyme activity, antimicrobial activity against pathogens and quorum quenching potential of probiotics. The isolates were able to tolerate (0.5-2.5%) bile salts, showed best growth at acidic pH and some isolates showed bile salt hydrolytic activity. Three bacterial isolates FI1, FI2, FI3 isolated from Product F showed antimicrobial activity against *S. pyogenes* ATCC 19615. Three isolates namely YC3 isolated from Product Y and FI1, FI2 isolated from Product F showed pigment inhibition against *Serratia marcescens*. Two isolates showed protease enzyme activity. Based on the result obtained we concluded that the some isolates were considered as a potential probiotic microorganisms.

Keywords : Probiotics , probiotic capsule ,acid tolerance, bile salt tolerance, antimicrobial activity, quorum quenching activity

1. INTRODUCTION

1.1 Background

The Normal bacterial flora plays crucial role in health of humans and animals. Some bacteria causes diseases that are fatal, while others act as 'excellent supporters', preventing and treating certain illnesses. Normal bacterial flora, including those found in the colon and vagina, plays vital role in regulating physiological functioning. Regular and excessive use of antibiotics has proven a challenge in certain situations due to improvement of microbial resistance, resulting in ineffectiveness and undesirable adverse effects. So probiotics have become a popular alternative to antibiotic treatment for managing some infections and disorders (Bansal et al., 2008).

Probiotics is a word which originated from Greek language "pro" (favor) and "bios" (life) (Reid et al., 2003). According to Food and Agriculture Organization probiotics were defined as “living microorganisms when taken in suitable amounts provide a health benefit on host” (Collado et al., 2009). The first concept of probiotics was put forward by father of immunology, Elie Metchnikoff who won a Nobel prize in 1908. In 1908, Metchnikoff found that Bulgarian peasants lived longer lives due to the consumption of milk products fermented with *Bacillus*. This reduced the infections caused due to bacterial pathogens indicating a favourable influence of colonic micro flora (Metchnikoff, 1908).

Prebiotics are undigestible food components that helps host by significantly promoting the growth and activity of a particular bacterium or set of related bacteria in colon, hence enhancing the host's health (Ziemer et al., 1998). Prebiotics are beneficial carbohydrates that avoid digestion in upper GIT and change the type of substrate that are available to the gut's residing microbial population such as fructo oligosaccharides, gluco oligosaccharides and inulin (Gibson et al., 1995). This results in an alteration of the gut's bacterial composition. A food ingredient must meet four requirements to be considered a prebiotic : 1) it must be neither hydrolysed or absorbed in upper GIT; 2) it must serve as a selective substrate

for number of beneficial bacteria commensal to the colon, which are encouraged to grow and/or are metabolically activated; 3) it must be able to change the composition of the colonic flora; 4) it must cause systemic effects that is benefits to the host's health (Fooks et al., 1999).

Synbiotics combine probiotics and prebiotics to benefit the gastrointestinal system while also promoting the growth and function of indigenous microorganisms.

1.1.1 Properties of probiotics

Probiotics are selected based on their tolerance to gastrointestinal conditions including gastric acid and bile. Capacity to adhere to the mucosa and competitive exclusion of pathogens. A useful probiotic should meet the following characteristics. (1) Have a demonstrated positive effect on the host. (2) Be non-pathogenic, non-toxic and without substantial negative side effects. (3) Able to survive in the GIT. (4) the product should contains enough live cells to provide the desired health benefit. (5) Ensure compatibility with product matrix, processing and storage conditions to preserve specified qualities and label accurately (Salminen et al., 1999). (6) It should be capable of interacting and sending signals to immune modulators. (7) It should be anti-carcinogenic and anti-mutagenic, decrease cholesterol, maintain mucosal integrity and improve intestinal motility (Pandya, 2016).

1.1.2 Mechanism of probiotics

Probiotics that are consumed must resist the harsh conditions of the GIT and stick to intestinal mucosa. The first process involves release of chemicals and competition for adherence sites, which keep pathogenic bacteria from attaching to host epithelium. Tight junction proteins are stimulated and mucus secretion is increased, to keep the barrier functioning properly. Pathogenic microorganisms can activate T-helper cells 1 and 2 which

release cytokines and stimulate immune cell production. Probiotics increase T-regulatory cells, including IL-10 and TGFβ, which control the strength of the immune response. Probiotics have been proved to boost T-regulatory cells, which are known to limit the immune response in the host. These cells also help with anti-inflammatory responses (Brito et al., 2012).

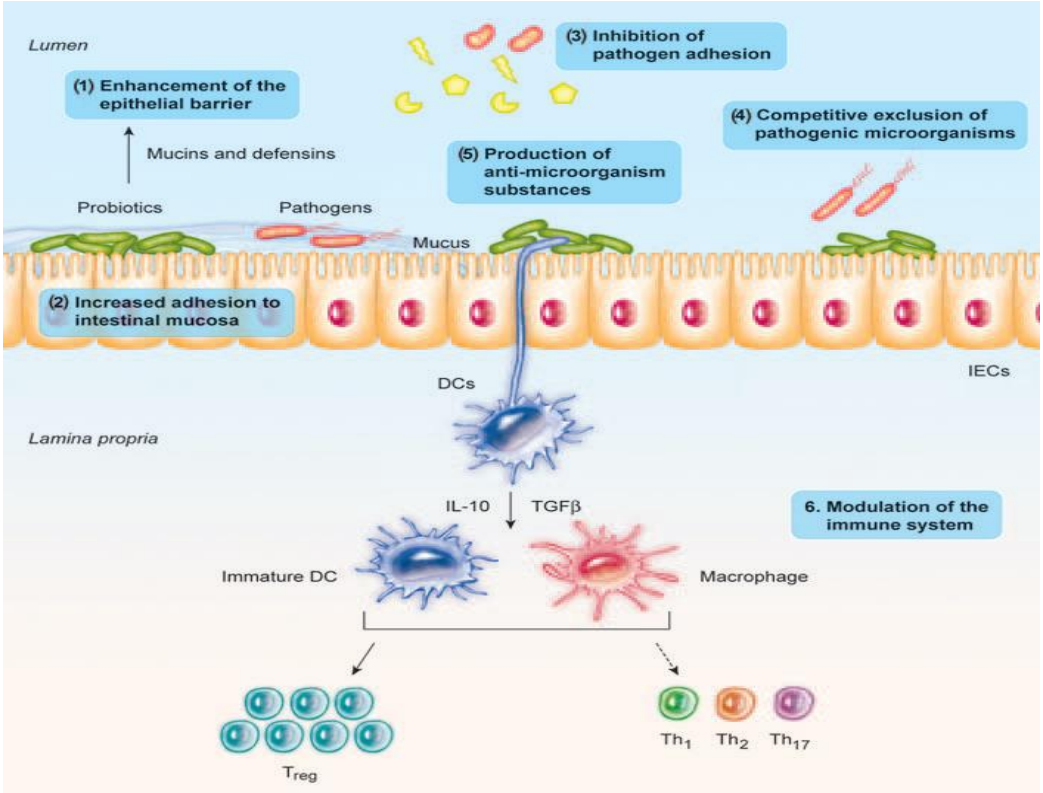


Fig.1.1.2 Mechanism of probiotics (Brito et al., 2012)

Probiotics mechanism of action are illustrated as : (1) enhancing the epithelial barrier,(2) increased adhesion to the intestinal mucosa,(3) inhibiting pathogen adherence,(4) competitive rejection of pathogenic bacteria, (5) modulating the immune system.

1.1.3 Probiotic Microorganisms

Probiotics have lactic acid bacteria (LAB) from the *Bifidobacterium* and *Lactobacillus* genera. The human intestine contains a high concentration of these microorganisms. Probiotic

strains include *Streptococci, Enterococci, Pediococci, Bacilli* and yeast like *Saccharomyces cerevisiae* and *Saccharomyces boulardii* (Vanderhoof et al., 1998).

Lactic acid bacteria are rod shaped, Gram-positive, heterotrophic, non-motile and non-sporulating. LAB are crucial microorganisms in food fermentation, producing antimicrobial compounds like hydrogen peroxide, lactic acid and bacteriocin to prevent spoilage and pathogenic bacteria. They also improve the texture and rate of fermentation (Sharafi et al., 2015).

Table 1.1.3 : List of probiotic microorganisms (Heyman et al., 2002)

Lactobacillus spp	*L. bulgaricus, L. acidophilus, L. plantarum, L. gasseri, L. reuteri, L. rhamnosus, L. salivarius L. casei, L. paracasei, L. lactis*
Bifidobacterium spp	*B. longum, B. breve, B. lactis, B. bifidum,*
Yeast	*Saccharomyces cerevisiae, Saccharomyces boulardii*
Others	*E. coli, Streptococcus thermophilus, Bacillus cereus, Clostridium butyricum, Enterococcus faecalis, Enterococcus faecium,* VSL#3 (three stains of *bifidobacteria*, four strains of *lactobacilli*, one strain of *Streptococcus salivarius* sp. , *Streptococcus thermophilus*)

1.1.4 Sources of probiotic microorganisms

Probiotic bacteria for humans is obtained from gastrointestinal tract and breast milk . Human milk plays crucial role in developing microorganisms in a newborn's gut. Human milk

contain microorganisms that can function as probiotics. Adults, children and newborns contain a large number of probiotic bacteria (Hopkins et al., 2005). Probiotic strains are obtained from a food sources such as raw milk, fermented foods, and plant-based fermented foods (Heller et al., 2001).

1.1.5 Commercial probiotic products

Probiotics are tested for various pharmacological applications such as Irritable bowel syndrome (IBS), ulcerative colitis, abdominal bloating, infantile colic, immunity enhancement, vaginal diseases and cold and flu. Commercial probiotic products available in a variety of forms including powder, capsules, pills, drops, chewing gum, lozenges, straws, stick packs, bottle caps. They combine multiple probiotic strains. Supplement formulations include vitamins, prebiotics, and probiotic strains. Infant probiotics are offered in oil solutions, making them easier to take. Probiotics and oral rehydration salts can help treat acute diarrhoea (Saxelin, 2008).

Table 1.1.5 : Commercially available probiotic formulations

Product	Form	Probiotic bacteria	Uses
1. Eugi	Solid (capsule)	*Lactobacillus acidophilus, Lactobacillus rhamnosus, Bifidobacterium longum, Bifidobacterium bifidum Saccharomyces boulardi Streptococcus thermophilus*	Abnormal digestion, prevention of diarrhoea, reduce lactose intolerance and controls irritable bowel syndrome
2. Yogut	Solid (capsule)	*Lactobacillus acidophilus, Lactobacillus rhamnosus, Bifidobacterium longum, Bifidobacterium infantis*	Prevention from Diarrhoea, reduce lactose intolerance, colitis,prevent urinary tract infections.

3. ProGG$_{RF}$	Solid (powdered sachet)	*Lactobacillus rhamnosus*	Prevention from Diarrhoea
4. Fourrts	Solid (powdered sachet)	*Lactobacillus acidophilus, Lactobacillus rhamnosus, Bifidobacterium longum, Bifidobacterium infantis Saccharomyces boulardi*	Control irritable bowel syndrome and inflammatory bowel disease, prevention of Diarrhoea

1.2 Aims and Objectives

Aim : The aim is to determine the efficacy of the commercially available probiotic products and to find out which is the best commercially available probiotic product available in the market.

Objectives

1. Selecting different commercially available probiotic formulations (capsules and sachets) and probiotic dairy products (curd and yakult).
2. Isolation of probiotic microorganisms from commercially available probiotic formulations (capsules and sachets) and probiotic dairy products (curd and yakult).
3. Screening of the isolates for their probiotic potential.
4. Comparing different commercially available probiotic formulations (capsules and sachets) and probiotic dairy products (curd and yakult) and find out which is the best probiotic product .

1.3 Hypotheses

1. Commercially available probiotics will have probiotic bacteria which will have probiotic potential.
2. We need to check if these bacteria really have probiotic potential or not.
3. After comparing probiotic potential of different probiotic products we can confirm which probiotic product is best.
4. We will also come to know if there is any contamination in probiotic formulations (capsules and sachets).

1.4 Scope

Probiotics plays an important role in the diet and also provide health benefits such as prevention of lactose intolerance, gut infection, antibiotic associated diarrhoea, control

irritable bowel syndrome, control inflammatory bowel disease. In the future, probiotics may be widely used as alternate growth promoting and health enhancing feed additives, due to their regulating effects on animal immune, gut microbiota and dietary intake.

2. LITERATURE REVIEW

2.1 Characterisation of probiotics

2.1.1 Acid and bile salt tolerance

The survivability of the bacteria after consumption is unknown due to unfavourable physiological conditions in the GI tract, such as an acidic environment (Holzapfel et al., 1998). These include variations in acidic environments at various incubation time which simulate the physiological components of the human digestive system. Bacteria must be viable upon consumption and survive in the GI tract to provide health benefits to the host (Koll et al., 2008). To be a good source of probiotics, they must also tolerate high acid levels in the stomach (Huang and Adams 2004). According to (Fernandez et al., 2003) excellent probiotic sources should be able to survive at pH 1.5- 3.0.

(Zavaglia et al., 2002) found that stomach acid that is hydrochloric acid (HCl), is a strong oxidant. As a result it can oxidize and disrupt numerous essential biomolecular components in cells while undergoing reduction. Acids can degrade important biological compounds such as fatty acids, proteins, cholesterol and DNA (Pan et al., 2008).

Every orally administered probiotic for humans or animals must be bile-tolerant due to the harsh environment of the host's gastrointestinal tract (GIT), which contains bile salts. The stress situation in the human GIT begins in the stomach. The stomach transit time is from less than 1 hour to 3-4 hours depending on the individual, diet and upper intestinal tract characteristics. Bile tolerance is a crucial factor when selecting a probiotic strain (Hofmann et al., 1992). Biliary salts help digest lipophilic substances and act as an antibacterial agent by altering the microbiota in the gut. The physiological concentrations of human bile range from 0.3 to 0.5% (Dunne et al., 2001).

According to (Mainville et al., 2005) certain *bifidobacteria* can survive in 0.3% bile salt for 90 minutes. According to (Sahadeva et al., 2011) *L. acidophilus, L. casei Shirota,*

Streptococcus thermophilus and *bifidobacterium* from four commercially cultured milk drink brands exhibited strain- specific bile tolerance at 0.3% bile salt.

2.1.2 Adherence to mucus and/or epithelial cells

Probiotic microorganisms need to stick to intestinal mucosa to colonize and interact with the host. Probiotics adhesion to the gut mucosa helps regulate the immunity and fight against pathogens. Lactic acid bacteria link to intestinal epithelial cells (IECs) and mucus via surface determinants. IECs release mucin, a complex glycoprotein that forms mucous and protects pathogenic microorganisms from sticking to it. Mucous gel also contains lipids, free proteins, immunoglobulins and salt. Probiotic bacteria's surface proteins have a role in competitively excluding pathogens from mucus (Ouwehand et al., 2002).

The adhesion experiment utilized the person intestinal epithelial cell line, Caco-2 (ATCC 2102-CRL). Caco-2 cells were cultivated to 80-85% confluence in Dulbecco's Modified Eagle's Medium with 20% fasting blood sugar, 100 IU/mL penicillin, and 10 mg/mL streptomycin and result found that all nine *Lactobacillus* strains, including the reference strain, were able to attach to Caco2 cells. *L. fermentum* HM3 isolated from the human milk had the highest ($P < 0.05$) adhesion ability while *L. casei* BF1 had the lowest ($P < 0.05$) adherence ability (Shokryazdan et al., 2014).

2.1.3 Antimicrobial and antagonistic activities against pathogens

Antimicrobial activity against pathogens is an important aspect for selection of potential probiotic strains. Antimicrobial compounds produced by LAB are influenced by their species and the growth medium's chemical composition. Homofermentative LAB ferments hexoses producing lactic acid. Heterofermentative LAB ferments the same substrate producing equimolar quantities of lactate, acetate, ethanol as well as carbon dioxide (Corsetti et al.,

2007).

Antimicrobial substances such as formic acid, acetoin, acetaldehyde and diacetyl accumulate during heterofermentation. Malic, lactic and citric acid is converted into antimicrobial compounds such as formic acid, acetic acid and CO_2. LAB produces two different types of antimicrobial substances: non-bacteriocin and bacteriocins. The most important antimicrobial chemicals produced by LAB are organic acids particularly lactic and acetic acids. LAB have been shown to improve the intestine's ecosystem by producing organic acids and lowering pH levels leading to the colonization of beneficial microorganisms and a reduction in pathogens (Aroutcheva et al., 2001). Most heterofermentative LAB species feature flavoprotein oxidase enzyme that lower oxygen and produce hydrogen peroxide. Hydrogen peroxide's antimicrobial effectiveness obtain from its capacity to oxidize bacterial cells and break down basic protein structures (Lindgren et al., 1990). Bacteriocins are peptides or proteins produced in the ribosome by some bacterial strains. Bacteriocins have antibacterial effect against other bacteria however producer cells are resistant to their own bacteriocins (Dicks et al., 2011).

In the study by (Osuntoki et al., 2008) *Lactobacillus* spp. isolated from fermented milk products had shown antibacterial action against clinically significant pathogens, including *Salmonella typhimurium*, *Listeria monocytogenes* and *E. coli* .

Bacteriocins are single polypeptides produced by bacteria that inhibit the growth of bacterial strains (Al-Omari et al., 2022). *L. salivarius* UCC118, which was isolated from the human gut microbiota, generates the bacteriocin Abp118. *Lactococcus lactis* generates the bacteriocin nisin. *Lactobacillus acidophilus* produces the bacteriocin Acidocin B. *Lactobacillus reuteri* produces the bacteriocin reutericin. *Lactobacillus plantarum* generates the bacteriocin plantaricin Wα (Dobson et al., 2012).

2.1.4 Digestive Enzymes produced by probiotics

Enzymes play crucial roles in several biological processes that influence human health. Lysosomes in the gastrointestinal tract digest a wide range of external substances. Enzymes work together to convert carbohydrates, proteins and lipids into monomers that human cells can absorb. Digestive enzymes include amylase and lactase in salivary glands, pepsin in gastric glands, trypsin, pancreatic amylase, lipase and nuclease in pancreas, maltase and lactase in small intestine. Gut-colonizing bacteria produces enzyme that helps to break complex substances during human metabolism (Maske et al., 2021).

2.1.4.1 Lactase

Lactose is the major carbohydrate in milk that serves as a main source of energy for newborns. Lactose is broken down by enzyme lactase also known as lactose-galactose hydrolase. The intestinal cells absorb glucose and galactose. Lactose improves calcium absorption, promotes a healthy gut flora, increases defense against infections and maintains proper feces consistency (Nascimento et al., 2003). Lactose a fermentable carbohydrate and is the primary source of energy for *lactobacilli* including *L. acidophilus*, *L. helveticus*, and *L. johnsonii* as well as *Lactobacillus coryniformis*, *Lactobacillus plantarum* (Maischberger et al., 2010).

2.1.4.2 Protease

Proteases and peptidases break down peptide bonds to release amino acids from polypeptide chains. Endopeptidases break the internal portion of polypeptide chains, while exopeptidases cleave the C- and N-terminals (Broadbent et al., 2011). LAB fermentation processes depend typically on proteases and peptidases. The proteolytic system catalyses proteins, amino acids and oligopeptides for cell advancement and maintenance that results in flavor development, bitterness reduction and release of bioactive peptides. LAB's proteolytic system consists of a

cell envelope-associated proteinase, peptide and amino acid transport mechanisms and cytoplasmic peptidases (Qi et al., 2021).

2.1.4.3 Amylase

Starch is a polysaccharide which is composed of amylose and amylopectin. Amylose is a linear polymer made up of 1000-6000 glucose units with glycosidic linkages (α,1-4). Amylopectin is made up of a short linear chain (α,1-4) having 10-60 glycoside residues and side chains (α,1-6) with 15-45 glucose units. Starch is mostly dissolve in small intestine by pancreatic α-amylases. Factors such as amylose and amylopectin content, particle size and cooking method can impact digestion (Higgins et al., 2013). The gut microbiota ferments starch and yield short chain fatty acids such as acetate, butyrate and propionate. Producing these compounds can provide health benefits including acetate for lipogenesis, butyrate for energy in colon cells and propionate for glycogenesis in the liver (Zampa et al., 2004).

2.1.4.4 Bile salt hydrolases

Bile acids are produced in the liver from cholesterol and are conjugated with glycine or taurine. The gall bladder stores bile acids which are release into the intestine through the bile duct that improves dietary fat absorption (Hofmann et al., 1984). Conjugated bile salts inhibit the growth of Gram-negative and Gram-positive bacteria. The probiotic bacteria *Lactobacillus acidophilus* produce bile salt hydrolase and hydrolyse bile salts. BSH can hydrolyse glycine- and taurine-conjugated bile salts into free deconjugated bile acids and amino acids. Probiotic bacteria are generally selected on the basis of their BSH activity which reduce plasma cholesterol levels (Corzo etal., 1999).

2.2 Applications of Probiotics

2.2.1 Minimise Lactose Intolerance

Lactose intolerance (LI) refers to the inability to assimilate lactose due to inadequate lactase enzyme activity. Colonic bacteria convert unabsorbed lactose into Short chain fatty acids (SCFAs) and the gas and lactase activity drops with age. Nausea, bloating, abdominal cramps and diarrhoea are the signs of lactose intolerance (Mattar et al., 2012). Research indicates that consuming unfermented milk with *L. acidophilus* improves lactose tolerance, digestion and transport in lactose-intolerant individuals (Mustapha et al., 1997). Milk fermented with *Lactobacillus acidophilus* and *L. casei* of human origin can improve lactose digestion in lactose-intolerant individuals, with better tolerance compared to regular milk without these probiotic strains (Gaon et al., 1995).

Zhong et al., 2006 found that supplementing with *B. animalis* led to a significant decrease in lactose intolerance symptoms. Eleven lactose intolerant individuals participated in a trial separated into three phases: 7-day basal, 14-day supplementation and 7-day post supplementation. Two lactose challenge tests were carried out before and after supplementation along with symptom scores and hydrogen tests. Supplementation resulted in a considerable increase in the gut flora. During supplementation fecal samples contained extraneous *B. animalis* from capsules and yogurt indicating the strain's resilience to gastric acid and bile salt. Supplementing with *B. animalis* increased the number of necessary types of microorganisms in the colon.

2.2.2 Prevention of Diarrhoea

Probiotics have shown to lower the incidence and period of certain diarrhoea which constitutes most well-established health benefits. Most research on probiotics in diarrhoea has focused on treating acute infectious diarrhoea in children. Clinical trials have shown that certain strains such as *Lactobacillus* GG, *Lactobacillus reuteri*, *Lactobacillus casei*,

Saccharomyces boulardii, and *Bifidobacterium* can reduce the intensity and term of acute diarrhoea (Guandalini et al., 2000). Probiotics have demonstrated to treat various types of diarrhoea including traveller's diarrhoea and rotavirus-induced diarrhoea in young infants. Administration of probiotics may lower the duration of acute diarrhoea in children.

Probiotic strains have been studied to prevent or treat diarrhoea caused by enteropathogens such *E. coli, Shigella* and *Salmonella* (Adachi et al., 2000). Probiotic strains such as *Bifidobacterium lactis* Bb12 and *Lactobacillus rhamnosus* GG have shown to effectively prevent and treat acute diarrhoea (Isolauri et al., 2003). *Lactobacillus* GG taken in an oral rehydration solution reduced the duration of acute diarrhoea in children. Probiotics, including VLS#3 and *Lactobacillus casei* DN-114 001, reduces the incidence and severity of radiation-induced diarrhoea (Giralt et al., 2008).

2.2.3 Treatment of Antibiotic- and *Clostridium difficile*-Associated Diarrhoea

Antibiotic-associated diarrhoea (AAD) is a characteristic side effect of using antibiotics. It happens when antibiotics disturb the natural balance of bacteria in the digestive tract, resulting in an excess of harmful microorganisms. AAD can arise after using oral or intravenous antibiotics especially for patients with enterohepatic circulation. Antibiotics produce diarrhoea through various mechanisms. Antibiotics including aminopenicillins, cephalosporins and clindamycin inhibit anaerobes in the intestine resulting in deficient glucose metabolism and osmotic diarrhoea. Antibiotics can alter the gut flora, leading to the proliferation of harmful organisms such *Clostridium difficile, Salmonella, Clostridium perfringens* type A, *Staphylococcus aureus,* and *Candida albicans*. Antibiotics can directly impact gastrointestinal motility. *C. difficile* is a spore-producing, anaerobic, Gram-positive bacterium causing gastrointestinal infections such as diarrhoea and colitis. Probiotics, specifically *Lactobacillus* GG and *S. boulardii* use in preventing and treating *C. difficile*-

associated diarrhoea (Szajewska et al., 2015). *Lactobacillus* strains, such as *Lactobacillus* GG can prevent Antibiotic associated diarrhoea in children taking antimicrobial therapy. Antibiotics can cause *Clostridium difficile* infection leading to pseudomembranous colitis (Hickson et al., 2007).

2.2.4 Probiotics protect against *Helicobacter pylori* Infection

Helicobacter pylori is a Gram-negative, spiral-shaped, micro-aerophilic rod that colonizes the gastrointestinal mucosa. *Helicobacter pylori* is a cause of chronic gastritis, gastric cancer and other stomach malignancies (Go et al., 2002). *Bifidobacterium* sp. has been shown to inhibit *H. pylori* growth and attachment through organic acid production, competitive inhibition for binding sites to mucus-producing cell and immunomodulation (Collado et al., 2005). *L. gasseri* OLL2716 effectively suppresses *H. pylori* and reduces stomach mucosal inflammation (Sakamoto et al., 2001).

2.2.5 Prevention from Colorectal cancer

Colorectal cancer is a cancer of the colon and rectum. Colorectal cancer affects the digestive system which processes food and remove waste. The colon is the first portion of the large intestine. It collects water and nutrients from food and stores solid waste. Waste travels from the colon to the rectum the last 6 inches of the large intestine before leaving the body by the anus. Colorectal cancer is primarily caused by polyps that form on the colon or rectum lining. Ingesting probiotics, prebiotics or a combination of both (synbiotics) can improve the gut microbiota by increasing beneficial bacteria and decreasing harmful microorganisms. This strategy prevents abnormal growth by reducing intestinal inflammation, improving immune function and anti-tumor activity (Geier et al., 2006).

Lactobacillus produces antioxidants like glutathione (GSH), superoxide dismutase (SOD) and catalase (CAT) as well as antiangiogenesis factors that reduce DNA damage, inflammation and tumor size. It also inhibits the expression of tumor-specific proteins, polyamine components and procarcinogenic enzymes all of which contribute to cancer prevention and treatment. *L. casei* decreases the hazard of colorectal cancer by reducing mutagens in stool and stimulating the immune system to protect against certain carcinogens. Research suggests that exposure to a carcinogen with *L. casei* and *L. rhamnosus* reduces the danger of developing cancers (Golden, 1996).

2.2.6 Control Irritable Bowel Syndrome

Irritable bowel syndrome is the common gastrointestinal illness with indication such as abdominal discomfort, diarrhoea, constipation and bloating. Probiotics help in controlling IBS symptoms by raising mucosal TGF-β and IL-10 levels and decreasing pro-inflammatory cytokines like IL-12. Studies in adults have shown that *B. infantis, L. rhamnosus* GG, *B. breve* Bb99 and *Propionibacterium freudenreichii* JS can effectively alleviate IBS symptoms (Kajander et al., 2005).

2.2.7 Control Inflammatory Bowel Disease

Inflammatory bowel disease is a chronic and recurring inflammation that affects the colon. Probiotic bacteria can stabilize the intestinal barrier by reducing the output of pro-inflammatory cytokines. Probiotics are used to handle inflammatory bowel disease such as ulcerative colitis, Crohn's disease and Pouchitis. Potential processes include suppressing harmful microorganisms, producing antimicrobial compounds, improving epithelial barrier function and regulating immunity. The VSL#3 probiotic mixture has been shown to effectively manage chronic pouchitis. A study indicated that *Bifidobacterium* spp., *L.*

acidophilus, and VSL#3 can effectively cure mild to moderate active ulcerative colitis (Bibiloni et al., 2005).

2.2.8 Cholesterol-lowering attributes of probiotics

Probiotics specially lactic acid bacteria, play most important role in lowering cholesterol levels. Probiotics can reduce cholesterol levels both directly and indirectly. The direct approach includes inhibiting the intestinal absorption of dietary cholesterol. Dietary cholesterol retention can be reduced in three ways: by assimilation, binding and breakdown. Probiotic strains absorb cholesterol for their own specific digestion. Probiotic strains can adhere to cholesterol particles and degrade cholesterol into catabolic metabolites. The cholesterol level can be reduced indirectly by deconjugating cholesterol into bile acids hence reducing the aggregate body pool (Gilliland et al., 1985).

Pereira & Gibson 2002 discovered that probiotic microorganisms can integrate cholesterol into their membranes when exposed to bile. Gopal et al., 1996 found that *Bifidobacterium* spp. and *L. acidophilus* can effectively remove cholesterol. Probiotics may function by cholesterol assimilation, bile salt deconjugation, cholesterol binding to bacterial cell walls, and reduced cholesterol production.

2.2.9 Probiotics increases immunity of host

Probiotic microorganisms have an immunomodulatory effect. Probiotic bacteria interact with epithelial and dendritic cells (DCs) as well as monocytes, macrophages and lymphocytes. The immune system is separated into innate and adaptive systems. B and T cells that identify specific antigens play a most significant role in the adaptive immune response. The innate immune system recognizes pathogen-associated molecular patterns (Gomez et al., 2010). *L. acidophilus* improve host immunity by forming strong colonization in the digestive system, preventing harmful bacteria from causing damage (Perdigón et al., 1993). *L. acidophilus* can

boost immunity by raising the number of antibody secretor cells and interferon production in the gut epithelium (Cunningham et al., 2000).

2.2.10 Probiotics use in Reproductive improvements

Probiotics use has found to benefit the female reproductive system. Women can use probiotics orally or vaginally to treat gynecological problems such as vaginosis and polycystic ovary syndrome as well as improve their gut microbiome. Probiotics help avoid gynecological problems (Hashem et al.,2022).

Probiotics help to maintain the proper balance of microbes in the vaginal microbiome which is essential for avoiding infections like bacterial vaginosis (BV) and candidiasis . *Lactobacillus* species are the most familiar bacteria in a healthy vaginal microbiome and probiotic bacteria such as *Lactobacillus rhamnosus* and *Lactobacillus acidophilus* have been formed to improve vaginal health by restoring the balance (Chen et al., 2021). Probiotic treatments are becoming a common treatment for bacterial vaginosis. *Lactobacillus* spp. is a well-known probiotic genus that breaks down carbohydrates and maintains an acidic microflora in the vaginal canal. This prevents pathogenic microbes like *Enterobacteria, Escherichia coli, Candida* spp., and *G. vaginalis* from colonizing vaginal canal (Homayouni et al., 2014).

PCOS is a complex gynaecological endocrine disorder that affects the reproductive process (infertility, hyperandrogenism), metabolic processes (insulin resistance, type 2 diabetes mellitus, impaired glucose tolerance) and psychological characteristics (depression, increased anxiety). Probiotics have demonstrated to benefits individual with PCOS (Teede et al., 2010). Deswal et al., 2020 in his clinical trial administered a probiotic capsule with freeze-dried strains of *Lactobacillus acidophilus, Lactobacillus casei* and *Bifidobacterium bifidum* to 60 PCOS patients daily for 12 weeks. The study found that 12 weeks of probiotic treatment

significantly reduced body mass index and weight in PCOS patients. Weight loss resulted in significant decreases in serum triglycerides and serum insulin, fasting plasma glucose.

Studies showed that probiotics supplementation during pregnancy decrease the danger of preterm birth and other pregnancy complications. By promoting a healthy gut microbiota, probiotics could modulate the immune response and inflammation, which are the factors related with preterm labor (Jones et al., 2014). Probiotics influence hormone levels indirectly by modulating gut health. The gut microbiota plays a role in metabolizing hormones such as estrogen and maintaining a healthy gut microbiome may help regulate hormone levels (Ya et al., 2010).

3. MATERIALS AND METHODS

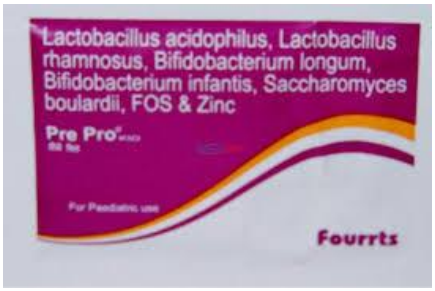
3.1 Sample collection

A total of four commercially available probiotic formulations (probiotic capsule and sachet) and two commercially available probiotic dairy products (curd and yakult) were selected for study. All the samples were bought from local supermarkets and pharmacies. All were stored according to the label and were used for study before their expiry dates. Below are the commercially available probiotics products used in present study.

(a)

(b)

(c)



(d)

(e)

(f)

Fig 3.1 collection of samples (a) Product E (b) Product Y (c) Product P (d) Product F (e) Curd (f) Yakult

3.2 Cultivation of anaerobic/ microaerophilic bacteria

Anaerobic jar was taken and the sample was streaked on De Man-Rogosa-Sharpe agar media plates. Plates were then placed in anaerobic jar and sealed properly. Indicator tablet was placed inside the jar to monitor if anaerobic/ microaerophiic conditions are maintained. Then oxygen was removed from the jar using vacuum pump. Nitrogen gas was then added to create anaerobic condition. The plates were then incubated at temperature 37^0C for 48-72 hours.

Alternatively we use candle jar method. A jar was taken and the sample was streaked on De Man-Rogosa-Sharpe agar media plates. Plates were then placed in jar. The candle was lit and the jar lid was closed. The candle's flame burns until it was extinguished due to oxygen deprivation, leaving the jar with a carbon dioxide-rich but oxygen-poor atmosphere. The plates were then incubated at 37^0C for 48-72 hours.

3.3 Isolation of probiotic bacteria

3.3.1 Curd and Yakult (liquid)

One ml of the sample was serially diluted in nine ml of saline (appendix I) and the dilutions were made up to 10^{-4}. 100 µl sample from last three dilutions (10^{-2},10^{-3},10^{-4}) were spread plated on De Man-Rogosa-Sharpe agar plates (appendix I). The pH of media was maintained as 6.5. The plates were then incubated under microaerophilic condition at 37^0C for 48-72 hours.

3.3.2 Probiotic capsules/sachet

One gram of sample was weighed and dissolved in nine ml of saline and the dilutions were carried out upto 10^{-4}.100 µl sample from last three dilutions (10^{-2},10^{-3},10^{-4}) were spread plated on De Man-Rogosa-Sharpe agar plates. The pH of media was maintained as 6.5. The plates were then incubated under microaerophilic condition for 48-72 hours at 37^0C.

3.4 Purification of cultures

Colonies appeared on De Man-Rogosa-Sharpe agar plates after 48 hours were picked from each plate and were then streaked on fresh MRS agar media plates. The colonies were purified until pure culture were obtained. The pure culture were then maintained by sub-culturing regularly and the cultures were maintained on MRS agar slants at 4^0C in refrigerator.

3.5 Identification of bacterial isolates

Cultural, biochemical and morphological characteristics of the bacterial isolates were studied and isolates were identified by using standard methods described by Bergey's Manual of systematic Bacteriology [Vos et al., 2011]

The isolates grown on specific media were observed to see the colony colour, shape, surface texture, margin, size, elevation etc. The morphological characteristics were observed by Gram staining.

3.5.1 Gram staining

A clean smear of the 24 hour old bacterial culture suspension was prepared, the slide was then air dried and gently passed to the flame for heat fixing. The slide was flooded with crystal violet stain (appendix II) and kept for 1 minute. The excess stain was drained. The slide was then flooded with Grams iodine (appendix II) and kept for 1 minute. Using 95% ethanol the slide was decolourized and the slide was flooded with secondary stain, Safranin (appendix II) for 30 seconds. Excess stain was removed and slide was rinsed slowly under indirect running tap water. The slide was kept for air dried and then the slide was observed under oil immersion lens (100X objective) of microscope.

3.5.2 Biochemical characterisation

3.5.2.1 Determination of Sugar fermentation

The five different sugars i.e glucose, fructose, maltose, sucrose and lactose was dissolved in deionized water (1%) (appendix II). MRS broth containing phenol red (0.01 g/L) (appendix II) use as a pH indicator. The 5 ml broth was added into the tubes and inverted durham's tubes were put and tubes were autoclaved. One ml of the sugar and 50 µl of bacterial culture was inoculated into the tubes and the tubes were kept for incubation at 37^0C under microaerophilic condition for 24 hours.

3.5.2.2 Catalase test

2-3 drops of 3% H_2O_2 (appendix II) were added to a clean, dry and grease free cavity slide. A loopful of 24-48 hours old culture of the isolates was mixed properly with H_2O_2 in the cavity slides. The slide was observed for effervescence.

3.5.2.3 Motility test

Nutrient broth containing 0.5% agar was prepared (appendix I) and 24-48 hours old cultures were stab inoculated into tubes containing the semi-solid nutrient agar medium. The tubes were then incubated under microaerophilic condition at 37^0C for 48-72 hours.

3.5.2.4 Citrate test

A loopful of culture was streaked on simmon citrate slant (appendix I) and incubated under microaerophilic condition at 37^0C for 48-72 hours. Colour change from green to blue is the positive test.

3.5.2.5 Endospore staining

A clean smear of the 24-48 hour old bacterial culture suspension was prepared, the slide was then air dried and gently passed to the flame for heat fixing. The slide was flooded with 0.5% malachite green stain (appendix II) solution and was steamed for 5 minutes over the container of boiling water. The slide was rinsed slowly with tap water. The slide was then flooded with counter stain, Safranin (0.5%) for 30 seconds. The slide was wash lightly under indirect running tap water and then the slide was kept for air dried and observed under the microscope. Endospores appeared as bright green and vegetative cells are brownish red to pink in colour.

3.6 Screening For probiotic properties of bacterial isolates

3.6.1 Acid tolerance test

To study the growth of isolates at various pH levels, a isolated colony was inoculated in 5 ml MRS broth (appendix I) of pH varying between 2-7 in different tubes, adjusted using NaOH (1.0 M) (appendix II) or HCl (1.0 M) (appendix II) and the tubes were incubated under microaerophilic condition at 37^0C for 24-48 hours. Bacterial growth was assessed by taking absorbance at 600 nm against uninoculated broth to study bacterial isolates capacity to survive at various pH levels (Prabhurajeshwar et al., 2019).

3.6.2 Bile salt tolerance test

To check for the tolerance to maximum concentration of bile the isolated colony was picked from MRS agar plate and was inoculated into 5 ml MRS broth of different tubes containing 0.5, 1, 1.5, 2 and 2.5% bile salts. The tubes were then incubated at 37^0C under microaerophilic condition for 24-48 hours. Bacterial growth was measured by taking absorbance at 600 nm. Bile salt free MRS broth was kept as a control (Prabhurajeshwar et al., 2019).

3.6.3 Bile Salt Hydrolytic activity

To assess the Bile Salt Hydrolytic activity of isolates an overnight culture of selected isolates was spot inoculated on MRS agar plates containing 0.37g/l $CaCl_2$ and 0.5% w sodium salt of taurocholic acid. The plates were then incubated under microaerophilic conditions for 72 hours at 37^0C. An inhibitory zone around colonies or white opaque colonies shows BSH activity. The negative control of each isolate in MRS agar without supplementation (Sharafi et al., 2014).

3.6.4 Cell-surface hydrophobicity

Bacterial adhesion was measured to examine microorganisms adhering capacity to surface hydrocarbons which measures the adhesion to epithelial cells of gut. The isolates were inoculated into MRS Broth and incubated at 37^0C under microaerophilic condition for 18- 24 hours. After 18-24 hours growth in MRS Broth, the cultures were centrifuged. The cells were suspendend in PBS and then OD was taken (A_0) at 600 nm . After this 1ml of n-hexane was put in centrifuge tubes and the tubes were incubated at 37^0C for 1 hour and again the OD of upper aqueous phase was taken (A_1) at 600 nm. Following formula is adopted to calculate the % hydrophobicity (Prabhurajeshwar et al., 2019)

$$\% \text{ hydrophobicity} = A_0\text{-}A_1/A_0 * 100$$

Where A_0 : Initial OD

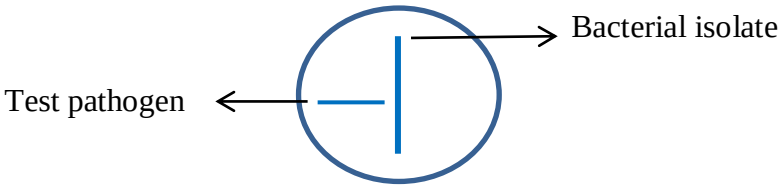
A_1 : final OD

3.6.5 Antimicrobial activity of isolates by cross streak method

Nutrient agar media (appendix I) plates were prepared and isolate was streaked vertically by a single streak on the plate and then the plates were incubated under microaerophilic conditions for 24-48 hours at 37^0C. The test microorganisms were streaked horizontally from edges towards the colony. The plates were then incubated at 37^0C for 24 hours.

3.6.6 Quorum Quenching activity

Nutrient agar media plates were prepared and isolate was streaked vertically by a single streak on the plate and then the plates were incubated under microaerophilic conditions for 24-48 hours at 37^0C. The test microorganisms were streaked horizontally from edges towards the colony. The plates were then incubated for 24 hours at room temperature.



3.6.7 Screening of digestive enzymes produced by probiotics

3.6.7.1 Amylase enzyme

The ability of the isolates to produce amylase was examined by spot inoculating the isolates on MRS Agar medium containing 1% soluble starch. The plates were then incubated under microerophilic condition for 48 hours at 37^0C. After incubation the plates were flooded with iodine solution (appendix II) and were observed for zone of clearance. Culture plate will be dark blue and surrounding colony zone of clearance was seen (Tallapragada et al., 2018).

3.6.7.2 Protease enzyme

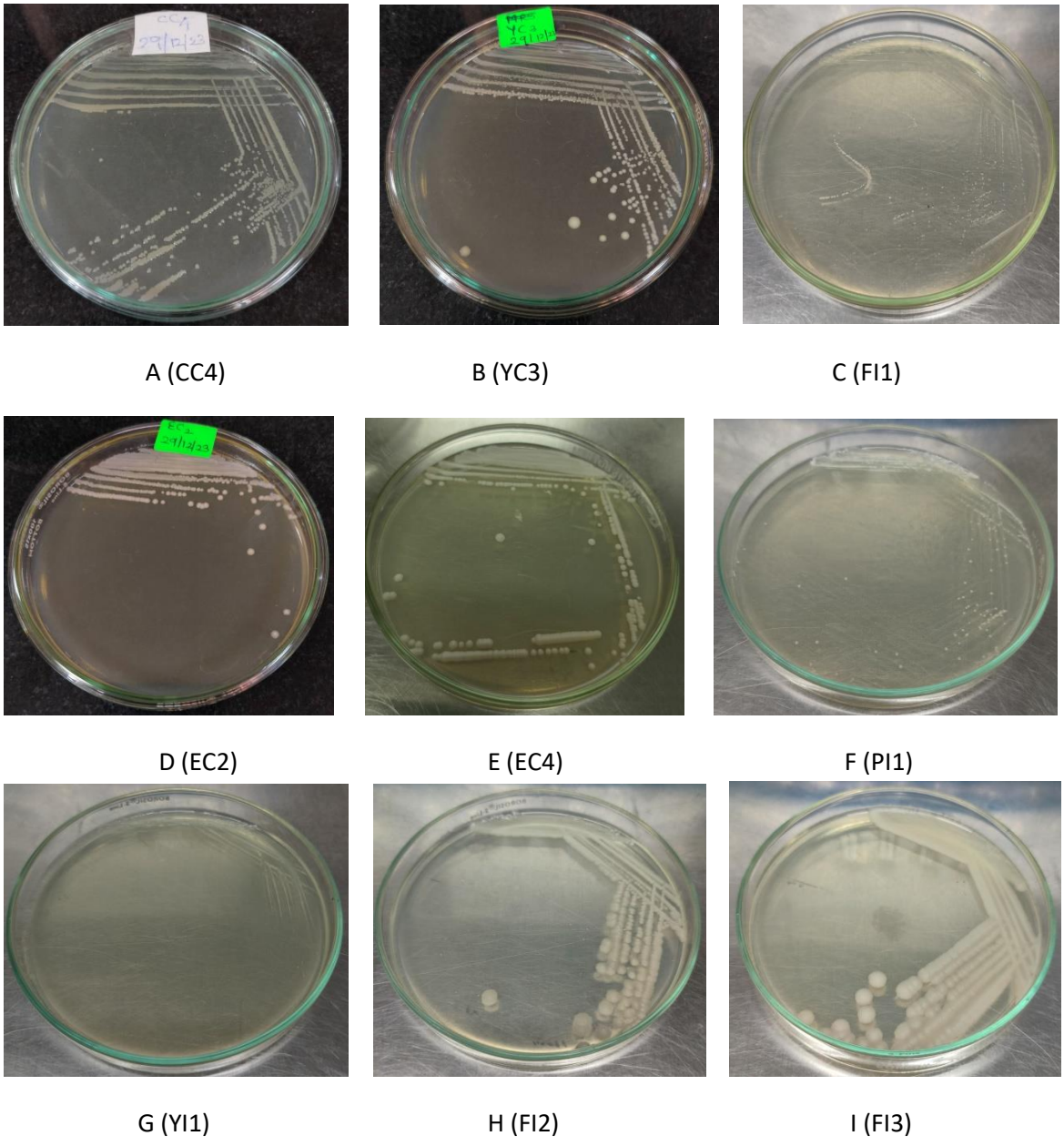
The ability of the isolates to produce protease was checked by spot inoculating the isolates on MRS agar containing 2% skimmed milk. The plates were then incubated at 37^0C under microaerophilic condition for 48 hours. After incubation the plates were checked for zones of clearance (Tallapragada et al., 2018).

3.6.7.3 Lipase enzyme

The ability of the isolates to produce lipase was checked out by spot inoculating the isolates on MRS agar medium containing 1% Tween 80. The plates were then incubated under microaerophilic condition for 48 hours at 37^0C. After incubation the plates were observed for white precipitate around the colonies (Tallapragada et al., 2018).

4. RESULTS AND DISCUSSION

4.1 Isolation and purification of culture



A (CC4) B (YC3) C (FI1)

D (EC2) E (EC4) F (PI1)

G (YI1) H (FI2) I (FI3)

Fig4.1 A- CC4 , B- YC3, C- FI1, D- EC2, E- EC4, F- PI1, G- YI1, H- FI2, I- FI3

1. **Curd**: Off white colour (1 mm) colonies were observed on MRS agar plates. After purification we named them as CC4.
2. **Product Y** : White colour (1 mm) colonies were observed on MRS agar plates. After purification we named the isolate as YC3.

3. **Product F** : White (pinpoint, shiny), creamy white (1 mm) and white colour (1 mm) colonies were observed on MRS agar plates. After purification we named the isolates as FI1, FI2, FI3 respectively.
4. **Product E** : White (small) and white colour (1mm) colonies were observed on MRS agar plates. After purification we named the isolates as EC2 and EC4 respectively .
5. **Product P** : White (pinpoint) colonies were observed on MRS agar plates. After purification we named the isolate as PI1.
6. **Yalult**: White colour shiny, pinpoint colonies were observed on MRS agar plates. After purification we named the isolate as YI1.

4.2 Identification of the isolates

4.2.1 Morphological characterisation of the isolates

The isolates were isolated from probiotic capsules and sachet of different brands (Product E, Product Y, Product P and Product F) and commercially available probiotic dairy products (curd and yakult)

Table 4.2.1 : Morphological characterisation of isolates

characters	Isolates								
	CC4	**YC3**	**FI1**	**EC2**	**EC4**	**PI1**	**YI1**	**FI2**	**FI3**
Size	1mm	1 mm	pinpoint	small	1mm	pinpoint	pinpoint	1mm	1mm
Shape	circular	circular	punctiform	circular	circular	circular	punctiform	circular	circular
Colour	Off white	white	White (shiny)	white	white	White	White	Creamy white	white
Elevation	raised	raised	convex	raised	raised	convex	raised	umbonate	flat
Margin	entire	entire	entire	entire	entire	entire	entire	entire	entire
Surface texture	smooth	smooth	Shiny	smooth	smooth	smooth	shiny	smooth	smooth
Opacity	opaque	opaque	opaque	opaque	opaque	opaque	opaque	opaque	opaque
Gram character	Gram positive	Gram positive	Gram positive	Gram positive	Gram positive	Gram positive	Gram positive	Gram positive	Gram positive

The total 9 isolates were isolated and named as CC4, YC3, FI1, EC2, EC4,PI1, YI1, FI2 and FI3 that formed circular, punctiform, white, off white, creamy white, raised, convex, smooth, shiny, opaque colonies isolated on MRS agar plates.

4.2.1.1. Gram staining of the isolates

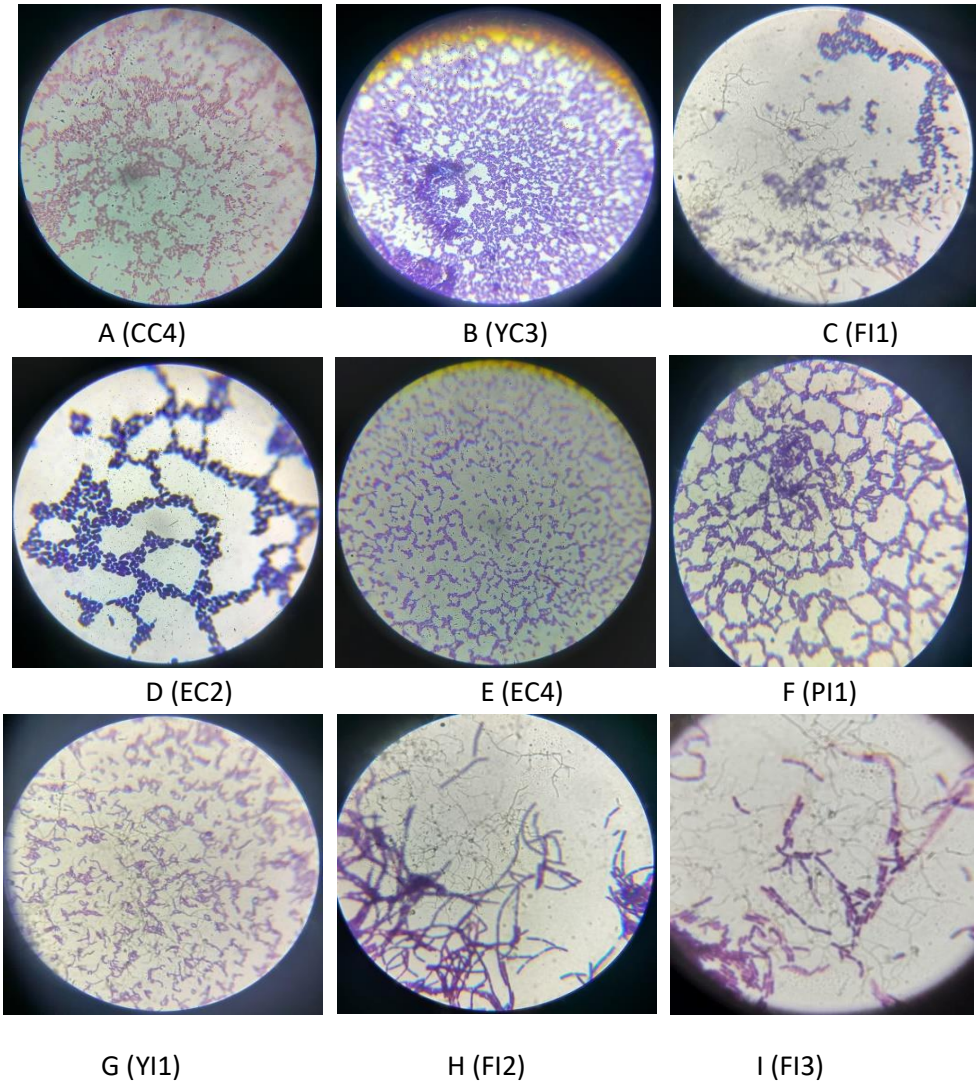


Fig. 4.2.1.1 Gram staining of the isolates: A- Isolate CC4 , B- Isolate YC3, C- Isolate FI1, D- Isolate EC2, E- Isolate EC4, F- Isolate PI1, G- Isolate YI1, H- Isolate FI2, I- Isolate FI3 .

The isolates were observed under the microscope and found that the Isolate CC4 (Gram positive, rod-shaped), Isolate YC3 (Gram positive, rod-shaped), Isolate FI1 (Gram positive, rod-shaped), Isolate EC2 (Gram positive, oval shaped), Isolate EC4 (Gram positive, rod-

shaped), Isolate PI1 (Gram positive, long rods), Isolate YI1 (Gram positive, long rods), Isolate FI2 (Gram positive, long rods) Isolate FI3 (Gram positive, long rods).

4.2.2 Biochemical Characterisation

All the isolates namely CC4, YC3, FI1, EC2, EC4, PI1, YI1, FI2 and FI3 were identified by performing biochemical tests such as motility test, catalase test, citrate test, endospore staining and the fermentation test (using sugars such as sucrose, glucose, lactose, fructose and maltose).

Table 4.2.2: The above table of the biochemical tests performed and the results of the tests

Biochemical test	**CC4**	**YC3**	**FI1**	**EC2**	**EC4**	**PI1**	**YI1**	**FI2**	**FI3**
Gram character	Gram positive	Gram positive	Gram positive	Gram positive	Gram positive	Gram positive	Gram positive	Gram positive	Gram positive
Shape	Rod-shaped	Rod-shaped	Rod-shaped	Oval shaped	Rod-shaped	Long rods	Long rods	Long rods	Long rods
Endospore staining	-	-	-	-	-	-	-	-	-
Motility	-	-	-	-	-	-	-	-	-
Catalase	-	-	-	-	-	-	-	-	-
Citrate	-	-	-	-	-	-	-	-	-
Glucose	+	+	+	+	+	+	+	+	+
Fructose	+	+	+	+	+	+	+	+	+
Maltose	+	+	+	+	+	+	+	+	+
Sucrose	+	+	+	+	+	+	+	+	+
Lactose	+	+	+	+	+	+	+	+	+
identification	*Lactobacilllus* sp	*Lactobacilllus* sp	*Lactobacilllus* sp	*Saccharomyces*	*Lactobacilllus* sp	*Lactobacilllus* sp	*Lactobacilllus* sp	*Lactobacilllus* sp	*Lactobacilllus* sp

Key + : positive - : negative

All the isolates namely CC4, YC3, FI1, EC2, EC4, PI1, YI1, FI2, FI3 were non endospore forming, non- motile, catalase negative, citrate negative, gram positive rods, long rods except isolate EC2 which was oval shaped . All the total 9 isolates were capable of fermenting sugars such as sucrose, glucose, lactose, fructose and maltose. So the above isolates CC4,

YC3, FI1, EC4, PI1, YI1, FI2, FI3 were tentatively identified as *lactobacillus sp* and the isolate EC2 which was tentatively identified as *Saccharomyces*.

4.3 Screening For probiotic properties of isolates

4.3.1 Acid tolerance test

Table 4.3.1 : Survival of the isolates at different pH

Isolates	pH					
	2	3	4	5	6	7
CC4	0.003	0.021	0.668	1.106	1.331	1.257
YC3	0.016	0.365	2.055	2.115	2.084	2.020
FI1	0.009	0.014	0.038	0.565	1.384	1.608
EC2	0.042	0.570	1.088	1.271	1.286	1.140
EC4	0.022	0.200	1.101	1.225	1.256	1.096
PI1	0.001	0.005	1.964	2.100	2.232	2.370
YI1	0.017	0.023	0.193	1.284	2.119	2.450
FI2	0.008	0.009	0.012	0.487	0.625	1.738

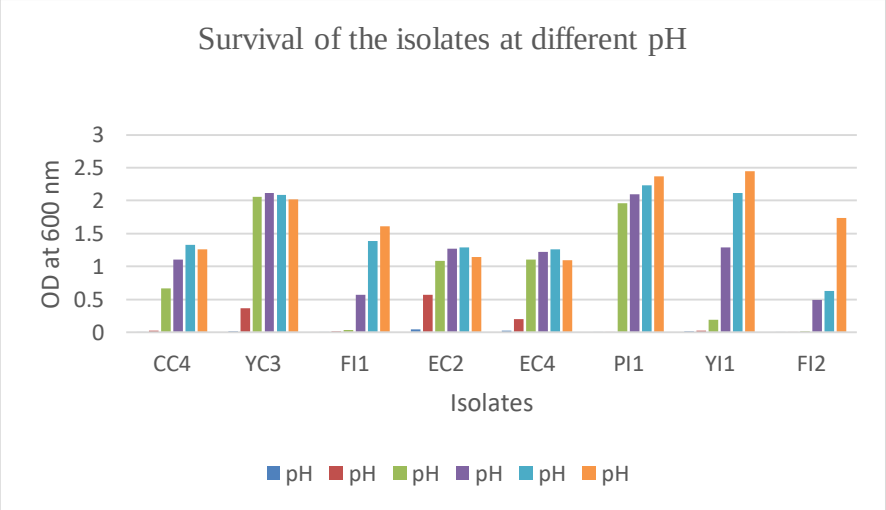


Fig 4.3.1 : Graphical representation of survival of the isolates at different pH

Probiotic organisms should be able to survive at the low pH of human intestine. The result showed that the isolates CC4, YC3, FI1, EC2, EC4, PI1, YI1and FI2 which were isolated from probiotic capsules and sachet of different brands (Product E, Product Y, Product P and Product F) and probiotic dairy products (curd and yakult) shows maximum growth at pH 7(neutral pH) but also the isolates were able to survive at pH 5 (acidic pH).

pH has a significant impact on bacterial growth. To be utilized as probiotics, organisms should survive at the low pH of the human intestine. In this study, all the isolates can tolerate a range of pH (2-7) and also showed growth at acidic pH (5). The growth was decreased with the increase in acidic pH. Some of the above isolates showed good growth and few isolates showed weak growth at pH 4. The isolate YC3 which was isolated from Product Y showed maximum growth at pH 5. Since the isolates in this study showed a growth at acidic pH, so they are likely to be utilized as a source of probiotics.

4.3.2 Bile salt tolerance test

Table 4.3.2: Survival of the isolates at different bile salt concentration

Isolates	Bile salt (%)				
	0.5%	1%	1.5%	2%	2.5%
CC4	0.090	0.140	0.217	0.138	0.134
YC3	1.912	1.966	1.922	1.861	2.030
FI1	1.596	1.568	1.543	1.562	1.570
EC2	0.963	0.895	0.962	0.934	0.925
EC4	0.958	0.966	0.990	1.018	0. 846
PI1	2.294	2.405	2.344	2.294	2.330
YI1	2.460	2.263	2.312	2.068	2.387
FI2	0.466	0.308	0.242	0.292	0.316
FI3	0.228	0.186	0.216	0.221	0.198

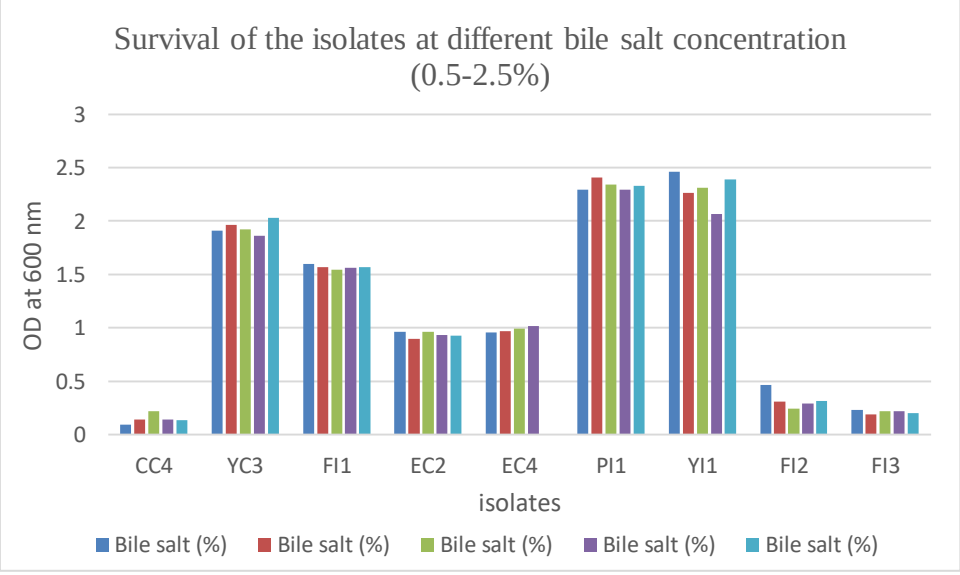


Fig. 4.3.2 : Graphical representation of survival of the isolates at different bile salt concentration

Bile salt tolerance test is a main criteria for choosing of probiotics because the human intestine and colon contain high content of bile salts. Various concentrations of bile salts (0.5, 1, 1.5, 2 and 2.5%) were being used for the study. The isolates were able to grow and survive at 0.5-2.5% Bile salt. The maximum bile salt tolerance was showed by the bacterial isolate PI1 followed by isolates YI1, YC3,FI1, EC4, EC2, FI2, FI3 and least bile salt tolerance was showed by the bacterial isolate CC4.

4.3.3 Cell Surface hydrophobicity test

Table 4.3.3 : % cell surface hydrophobicity of isolates towards n-hexane

Isolates	% hydrophobicity
CC4	32.69
YC3	60.73
FI1	49.55
EC2	14.75
EC4	46.42
PI1	41.66
YI1	25.92
FI2	47.5
FI3	26.80

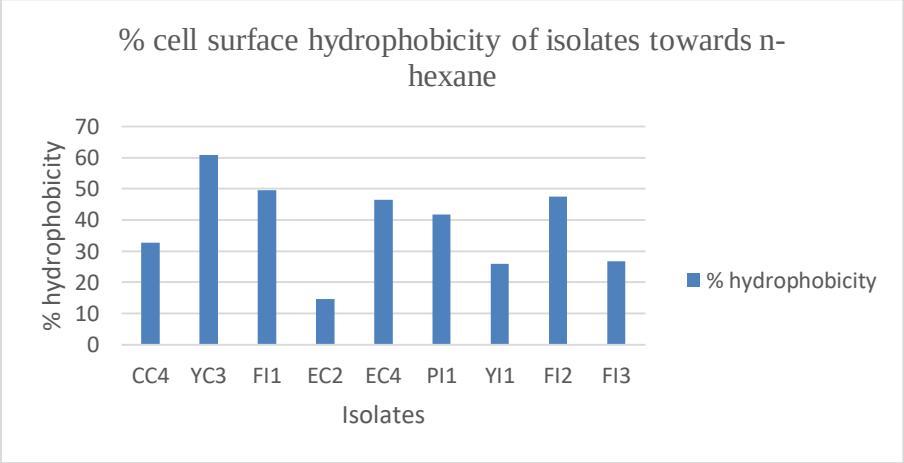
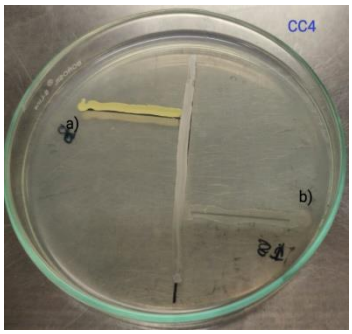


Fig 4.3.3 : Graphical representation of % cell surface hydrophobicity of isolates towards n-hexane

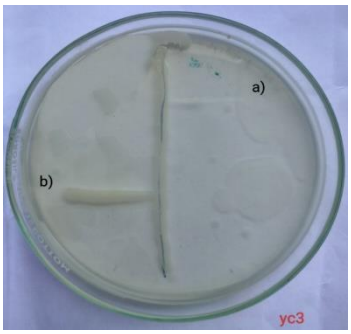
Cell surface hydrophobicity is also an important property for screening probiotic microorganisms, as it provides a competitive advantage in probiotic microbe adherence to the gut epithelium, enhancing colonization and immune system modulation (Powthong et al.,

2015). All of the total 9 isolates had showed cell surface hydrophobicity towards n- hexane. The bacterial isolate YC3 which was isolated from Product Y showed highest % hydrophobicity of 60.73% and isolate EC2 isolated from Product E showed lowest % hydrophobicity with 14.75%.

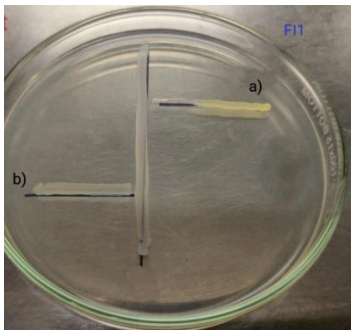
4.3.4 Antimicrobial potential of Probiotics



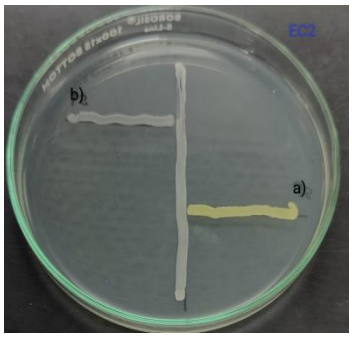
A (CC4)



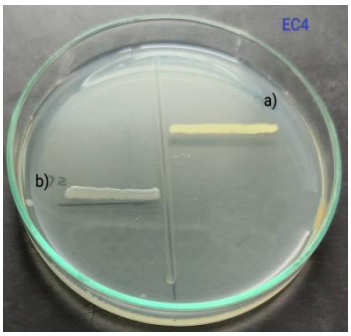
B (YC3)



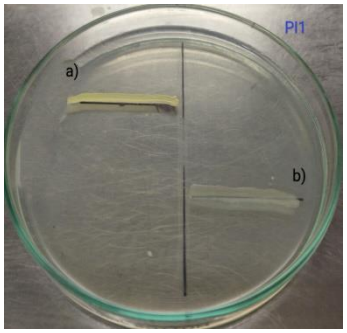
C (FI1)



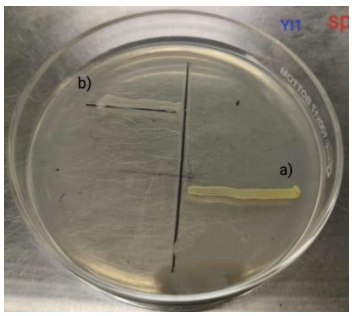
D (EC2)



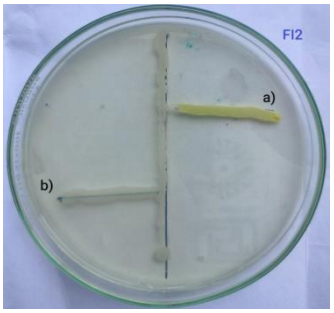
E (EC4)



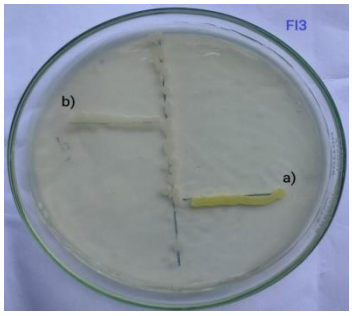
F (PI1)



G (YI1)



H (FI2)

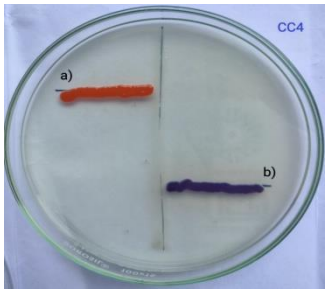


I (FI3)

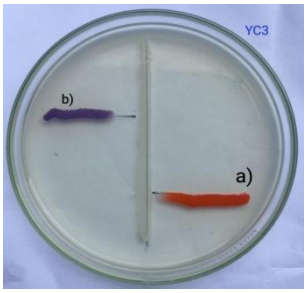
Fig 4.3.4 Antimicrobial activity against pathogens a) *Streptococcus pyogenes* ATCC 19615, b) *S. typhimurium* ATCC 14028. A- CC4, B- YC3, C- FI1, D- EC2, E- EC4, F- PI1, G- YI1, H- FI2, I- FI3

Antimicrobial activity is a key selection criterion for probiotics. LAB produces antimicrobial compounds, including organic acids like lactic, acetic and propionic acids, bacteriocins, diacetyl, low molecular weight antimicrobial compounds, CO_2 and hydrogen peroxide (Dicks et al., 2011). The antimicrobial activity of the isolates were checked against two pathogens namely *S. typhimurium* ATCC 14028 and *Streptococcus pyogenes* ATCC 19615. The bacterial isolates FI1 and FI2 and FI3 which was isolated from Product F showed antimicrobial activity against *Streptococcus pyogenes* ATCC 19615. None of the isolates shows antimicrobial activity against test pathogen *S. typhimurium* ATCC 14028 .

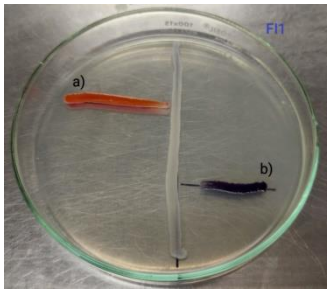
4.3.5 Quorum quenching potential of Probiotics



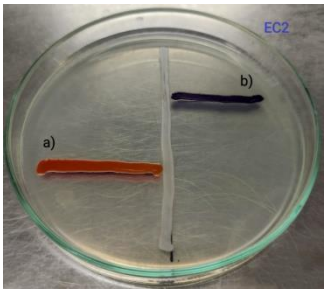
A (CC4)



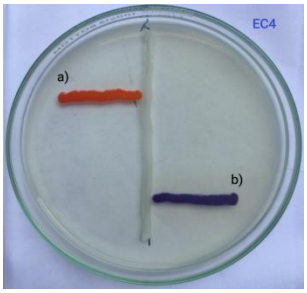
B (YC3)



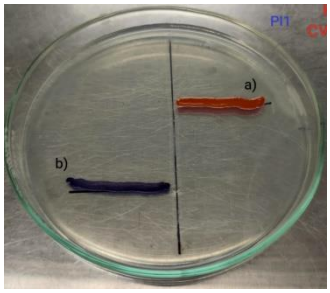
C (FI1)



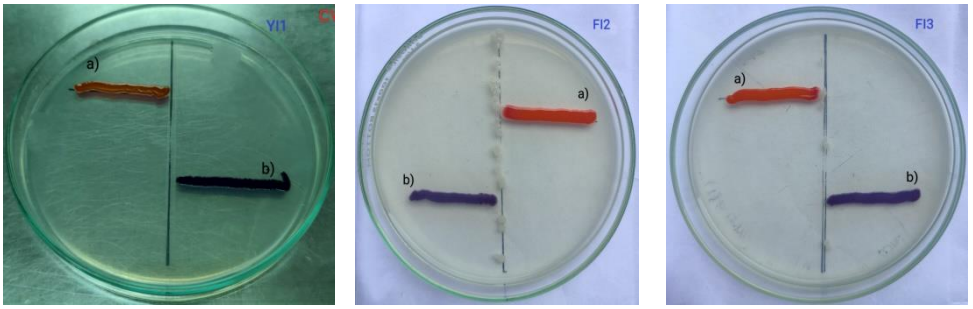
D (EC2)



E (EC4)



F (PI1)

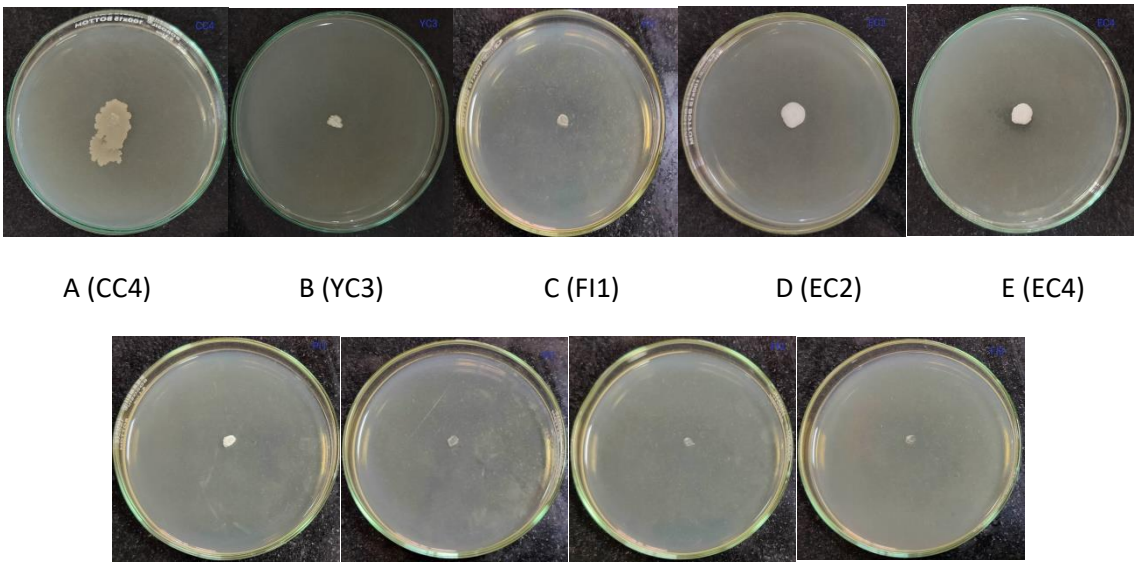


G (YI1) H (FI2) I (FI3)

Fig 4.3.5 Quorum quenching potential of probiotics a) *Serratia marcescens* b) *C. violaceum* A- CC4, B- YC3, C- FI1, D- EC2, E- EC4, F- PI1, G- YI1, H- FI2, I- FI3

In the present study the isolates were checked for quorum quenching potential of probiotics. The bacterial isolates YC3 which was isolated from Yogut capsule and isolate FI1 and FI2 isolated from Product F showed pigment inhibition against *Serratia marcescens* which indicates its quorum quenching potential.

4.3.6 Bile salt hydrolytic activity



A (CC4) B (YC3) C (FI1) D (EC2) E (EC4)

F (PI1) G (YI1) H (FI2) I (FI3)

Fig 4.3.6 bile salt hydrolytic activity A- CC4, B- YC3, C- FI1, D- EC2, E- EC4, F- PI1, G- YI1, H- FI2, I- FI3

The isolates CC4, YC3, FI1, EC2, EC4, PI1, YI1, FI2 and FI3 were screened for bile salt hydrolytic activity. The isolates CC4, EC2, EC4, PI1, YC3, FI1 showed white opaque

colonies indicating positive results, the isolates YI1, FI2, FI3 did not showed white opaque colonies indicating negative results for bile salt hydrolytic activity.

4.3.7 Screening of digestive enzymes produced by probiotics

The nine isolates were isolated from commercially available probiotic products. The isolates were screened for 3 different enzymes: amylase, protease and lipase. The isolates showing results are given in the table below.

Table 4.3.7 : Screening of digestive enzymes produced by probiotics (amylase, protease and lipase)

Isolates	Amylase	Protease	Lipase
CC4	-	-	-
YC3	-	-	-
FI1	-	+	-
EC2	-	-	-
EC3	-	-	-
PI1	-	+	-
YI1	-	-	-
FI2	-	-	-
FI3	-	-	-

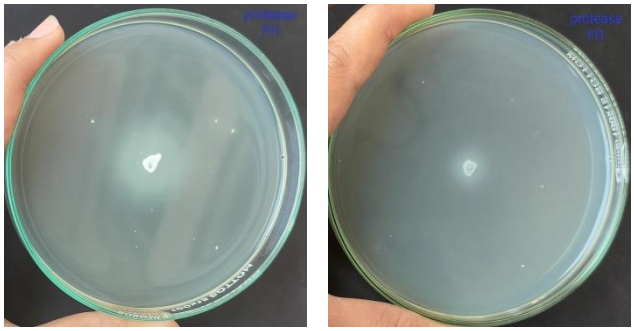
KEY + : Positive enzyme activity - : Negetive enzyme activity

4.3.7.1 Amylase enzyme activity

The isolates were screened for amylase enzyme activity. None of the above isolates showed amylase enzyme activity.

4.3.7.2 Protease enzyme activity

The bacterial isolates FI1 and PI1 showed positive results for protease enzyme activity by showing a zone of clearance surrounding the colony.



A (FI1) B (PI1)

fig. 4.3.7.2 protease enzyme activity A- Isolate FI1 B- Isolate PI1

4.3.7.3 Lipase enzyme activity

The isolates were screened for lipase enzyme activity. None of the isolates showing white precipitate surrounding the colony.

Discussion

Table 4.4 : Compiled results for screening of probiotic properties of isolates obtained from commercially available probiotic formulation and probiotic dairy products.

	Isolates								
	CC4	YC3	FI1	EC2	EC4	PI1	YI1	FI2	FI3
Acid tolerance	++	+++	++	++	++	+++	++	++	-
Bile salt tolerance	++	+++	++	++	++	+++	+++	++	++
Cell surface hydrophobicity	+	+++	++	+	++	++	+	++	+
Antimicrobial activity	-	-	+	-	-	-	-	+	+
Quorum quenching activity	-	+	+	-	-	-	-	+	-
Bile salt hydrolytic activity	+	+	+	+	+	+	-	-	-
Amylase enzyme activity	-	-	-	-	-	-	-	-	-
Protease enzyme activity	-	-	+	-	-	+	-	-	-
Lipase enzyme activity	-	-	-	-	-	-	-	-	-

+++ : Excellent growth ++ : Growth + : Positive - : Negative

The total nine isolates were isolated from commercially available probiotic formulations and probiotic dairy products.

Isolate CC4 which was isolated from curd sample was able tolerate the acidic pH, bile salts and showed 32.69% hydrophobicity. The isolate did not showed antimicrobial activity, quorum quenching activity, bile salt hydrolytic activity, amylase, protease and lipase enzyme activity. This isolate possess few properties of an ideal probiotics didnot have antimicrobial or quorum quenching activity. Therefore the isolate is not very suitable to be used as probiotic.

Isolate YC3 which was isolated from Product Y was able tolerate the acidic pH, bile salts and showed 60.73% hydrophobicity.The isolate did not showed antimicrobial activity against two pathogens *S. typimurium* ATCC 14028 and *Streptococcus pyogenes* ATCC 19615. The

isolate showed pigment inhibition against *Serratia marcescens* which indicates quorum quenching potential. The isolate showed bile salt hydrolytic activity by forming a white opaque colony. The isolate did not showed amylase, protease and lipase enzyme activity. This isolate possess all the necessary properties of an ideal probiotics. It has very good acid tolerance, bile salt tolerance and cell surface hydrophobicity along with quorum quenching potential. Therefore the isolate is very suitable to be used as probiotics.

Isolate FI1, FI2 and FI3 which was isolated from Product F. Isolate FI1 and FI2 were capable to tolerate acidic pH, bile salt and showed 49.55% and 47.5% hydrophobicity respectively. Isolate FI3 was not capable to tolerate acidic pH but capable to tolerate bile salt and showed 26.80% hydrophobicity. Isolate FI1, FI2 and FI3 showed antimicrobial activity against *Streptococcus pyogenes* ATCC 19615. Isolate FI1 and FI2 showed pigment inhibition against *Serratia marcescens* which shows their quorum quenching potential. Isolate FI1 showed zone of clearance for protease enzyme and showed bile salt hydrolytic activity. Isolate FI1 and FI2 possess all properties of an ideal probiotics that was acid tolerance, bile salt tolerance, very good cell surface hydrophobicity. Apart from this both possess antimicrobial and quorum quenching potential against pathogens. In additionally FI1 possess protease activity for digestion therefore isolate FI1 and FI2 were very suitable to be use as a probiotics. Isolate FI3 cannot tolerate acidic conditions and don’t show very good cell surface hydrophobicity therefore not very suitable to be use as a probiotics.

Isolate EC2 and EC4 which was isolated from Product E. Both isolates EC2 and EC4 were capable to tolerate acidic pH, bile salt, showed 14.75% and 46.42% hydrophobicity respectively. Isolates EC2 and EC4 showed white opaque colonies indicating bile salt hydrolytic activity. The isolate did not showed antimicrobial activity, quorum quenching activity, amylase, protease and lipase enzyme activity. Since isolates don’t show

antimicrobial, quorum quenching potential and enzyme for digestion therefore not very suitable to be use as a probiotics.

Isolate PI1 which was isolated from Product P was able tolerate the acidic pH, bile salts and showed 41.66% hydrophobicity. Isolate showed bile salt hydrolytic activity and protease enzyme activity. The isolate did not showed antimicrobial activity, quorum quenching activity, amylase and lipase enzyme activity. Isolate PI1 show very good acid tolerance, bile salt tolerance and cell surface hydrophobicity along with protease enzyme for digestion which makes it a good candidate for probiotic use.

Isolate YI1 which was isolated from yakult was able tolerate the acidic pH, bile salts and showed 25.92% hydrophobicity. The isolate did not showed antimicrobial activity, quorum quenching activity, bile salt hydrolytic activity, amylase, protease and lipase enzyme activity. The isolate YI1 was not very suitable candidate for probiotic use since it do not show antimicrobial, quorum quenching and enzyme production and have very weak cell surface hydrophobicity.

After comparing the results FI1 which was isolated from Product F is the best isolate because the isolate possess all the properties of an ideal probiotics that is acid tolerance, bile salt tolerance, very good cell surface hydrophobicity. Apart from this the isolate possess antimicrobial and quorum quenching potential against pathogens. In addition it possess protease activity for digestion.

The bacterial isolate YC3 which was isolated from Product Y is the second best isolate because the isolate has very good acid tolerance, bile salt tolerance and cell surface hydrophobicity along with quorum quenching potential.

The isolate FI2 which was isolated from Product F is the third best isolate because the isolate can tolerate acid, bile salt tolerance and has very good cell surface hydrophobicity. The isolate possess antimicrobial and quorum quenching potential against pathogens.

The isolate PI1 which was isolated from Product P is the fourth best isolate because it shows very good bile salt tolerance, acid tolerance and cell surface hydrophobicity along with protease enzyme for digestion.

The isolates CC4, EC2, EC4,YI1 and FI3 which as isolated from curd, Product E , yakult and Product F respectively is not suitable to be used as probiotics because the isolates donot possess all the necessary properties of an ideal probiotics.

5. CONCLUSION

Conclusion

Nine isolates namely CC4, YC3, EC2, EC4, FI1, PI1, YI1, FI2, FI3 were isolated from the probiotic capsule and sachet of different brands (Product E, Product Y, Product P and Product F) and commercially available probiotic dairy products (curd and yakult). Probiotic screening test including acid tolerance, bile salt tolerance, cell surface hydrophobicity, antimicrobial activity, quorum quenching potential, bile salt hydrolytic activity, screening of digestive enzymes was carried out. Out of 9 isolates found that the isolate FI1 is the best because the isolate possess all the properties of an ideal probiotics that is survive at acidic pH , bile salt tolerance, very good cell surface hydrophobicity. Apart from this the isolate possess antimicrobial and quorum quenching potential against pathogens. In addition it possess protease activity for digestion. The bacterial isolate YC3 is the second best isolate because the isolate has very good bile salt tolerance, acid tolerance and cell surface hydrophobicity along with quorum quenching potential. The isolate FI2 is the third best isolate because the isolate can tolerate acid, bile salt tolerance and has very good cell surface hydrophobicity.The isolate possess antimicrobial and quorum quenching potential against pathogens. The isolate PI1 is the fourth best isolate because it shows very good bile salt tolerance, acid tolerance and cell surface hydrophobicity along with protease enzyme for digestion.

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Images references

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APPENDIX I

Media composition

1. Saline

Composition	g/litre
Sodium chloride	8.5 g
Distilled water	1000 ml

2. De Man-Rogosa-Sharpe agar (MRS agar)

Composition	g/litre
Protease peptone	10.0
Beef extract	10.0
Yeast extract	5.0
Dextrose	20.0
Polysorbate 80/tween 80	1.0
Ammonium citrate	2.0
Sodium acetate	5.0
Magnesium sulphate	0.1
Manganese sulphate	0.05
Dipotassium hydrogen phosphate	2.0
Agar	15.0
Distilled water	1000 ml
pH	6.5

Suspend 69.21 grams in 1000ml distilled water. Adjust the pH to 6.5 and then add agar. Boil to dissolve the medium completely. Sterilize by autoclaving at 15 lbs. pressure (121^0C) for 20 minutes. Cool to 45-50^0C. Mix well and pour into sterile petri plates.

3. Nutrient broth

Composition	g/litre

Beef extract	10 g
Sodium chloride	5 g
Peptone	10 g
Distilled water	1000 ml

4. Simmons citrate agar

Composition	**g/litre**
Magnesium sulphate	0.20
Ammonium dihydrogen phosphate	1.00
Dipotassium phosphate	1.00
Sodium citrate	2.00
Sodium chloride	5.00
Bromothymol blue	0.08
Agar	15.00

5. MRS broth

Composition	**g/litre**
Protease peptone	10.0
Beef extract	10.0
Yeast extract	5.0
Dextrose	20.0
Polysorbate 80/tween 80	1.0
Ammonium citrate	2.0
Sodium acetate	5.0
Magnesium sulphate	0.1
Manganese sulphate	0.05
Dipotassium phosphate	2.0
pH	6.5

APPENDIX II

Biochemical composition and reagents

1. Crystal violet

Composition	g/100ml
Crystal violet	1 g
Absolute alcohol (95%)	10 ml
1% ammonium oxalate	90 ml

2. Grams iodine

Composition	g/100 ml
Potassium iodide	0.66 g
Iodine	0.33 g
Distilled water	100 ml

3. Safranine

Composition	g/100 ml
Safranine	0.5 g
Ethanol (95%)	100 ml

4. Sugars

a) Glucose (1%)

Composition	g/100 ml
Glucose	1 g
Distilled water	100 ml

b) Fructose (1%)

Composition	g/100 ml
Fructose	1g
Distilled water	100ml

c) Maltose (1%)

Composition	g/100 ml
Maltose	1 g

Distilled water	100 ml

a) Sucrose (1%)

Composition	**g/100 ml**
Sucrose	1 g
Distilled water	100 ml

b) Lactose (1%)

Composition	**g/100 ml**
Lactose	1 g
Distilled water	100 ml

5. Phenol red (0.01 g/litre)

Composition	**g/litre**
Phenol red	0.01 g
Distilled water	1000ml

6. Catalase reagent

   3% hydrogen peroxide.

7. Malachite green stain (0. 5%)

Composition	**g/100 ml**
Malachite Green	0.5 g
Distilled water	100

8. NaOH (1 M)

Composition	**g/100 ml**
Sodium hydroxide	4 g
Distilled water	100 ml

9. HCl (1 M)

Composition	**g/100 ml**
Conc. HCl	8.58 ml
Distilled water	91.42 ml

10. Iodine solution

Composition	**g/100 ml**
Potassium iodide	0.2 g
Iodine crystals	0.1 g
Distilled water	100 ml

SUMMARY

Summary

Probiotics were defined as “living microorganisms when taken in suitable amounts provide a health benefit on host”. Many people take probiotics to help with a variety of health problems such as

1. **Lactose intolerance**: Lactose is the sugar in milk and milk products. Lactose intolerance is the inability to digest lactose due to inadequate lactase enzyme. Milk fermented with probiotic bacteria help to improve lactose digestion in lactose intolerant patient.
2. **Antibiotic associated diarrhoea** : Antibiotic associated diarrhoea happens after taking oral antibiotics. Antibiotics disturbs the normal bacterial flora of the digestive tract. Antibiotics produce diarrhoea. Probiotics are used to prevent antibiotic associated diarrhoea.
3. **Indigestion**: Probiotics help to alleviate indigestion by restoring the balance of healthy bacteria.

Probiotics organisms must be taken in viable form in order to benefit to the host. The microorganism is required to survive the passage through the intestinal tract, therefore have the ability to tolerate the pH of the gut (1.5-3.5), it should tolerate the bile salts and ability to adhere to intestinal epithelial cells.

The aim of the present study is to determine the efficacy of the commercially available probiotic products and to find out which is the best commercially available probiotic product available in the market. The objectives is to select different commercially available probiotic products, isolating probiotic microorganisms, screening of the isolates for their probiotic potential and finally to find out which is the best probiotic product available in the market.

The total four commercially available probiotic formulations (capsule and sachet) of different brands and probiotic dairy products (curd and yakult) were selected for the study. All the products were purchased from local supermarkets and pharmacies. The total nine isolates were isolated and named them as CC4, YC3, FI1, EC2, EC4, PI1, YI1, FI2 and FI3. Probiotic screening test such as acid tolerance, bile salt tolerance, cell surface hydrophobicity, bile salt hydrolytic activity, antimicrobial and quorum quenching potential of probiotics, screening of digestive enzymes were carried out and the results obtained are as follows:

Table 6: Compiled result for screening of probiotic properties of isolates obtained from commercially available probiotic formulation and probiotic dairy products

	Isolates								
	CC4	**YC3**	**FI1**	**EC2**	**EC4**	**PI1**	**YI1**	**FI2**	**FI3**
Acid tolerance	++	+++	++	++	++	+++	++	++	-
Bile salt tolerance	++	+++	++	++	++	+++	+++	++	++
Cell surface hydrophobicity	+	+++	++	+	++	++	+	++	+
Antimicrobial activity	-	-	+	-	-	-	-	+	+
Quorum quenching activity	-	+	+	-	-	-	-	+	-
Bile salt hydrolytic activity	+	+	+	+	+	+	-	-	-
Amylase enzyme activity	-	-	-	-	-	-	-	-	-
Protease enzyme activity	-	-	+	-	-	+	-	-	-
Lipase enzyme activity	-	-	-	-	-	-	-	-	-

+++ : excellent growth ++ : growth + : positive - : negetive

Isolate CC4 which was isolated from curd was able tolerate the acidic pH, bile salts and showed 32.69% hydrophobicity. The isolate did not showed antimicrobial activity, quorum quenching activity, bile salt hydrolytic activity, amylase, protease and lipase enzyme activity. This isolate possess few properties of an ideal probiotic didnot have antimicrobial or quorum quenching activity. Therefore the isolate was not very suitable to be used as probiotic.

Isolate YC3 which was isolated from Product Y was able tolerate the acidic pH, bile salts and showed 60.73% hydrophobicity. The isolate did not showed antimicrobial activity against two pathogens *S. typimurium* ATCC 14028 and *Streptococcus pyogenes* ATCC 19615. The isolate showed pigment inhibition against *Serratia marcescens* which indicates quorum quenching potential. The isolate showed bile salt hydrolytic activity by forming a white opaque colony. The isolate did not showed amylase, protease and lipase enzyme activity. This isolate possess all the necessary properties of an ideal probiotics. It had very good acid tolerance, bile salt tolerance and cell surface hydrophobicity along with quorum quenching potential. Therefore the isolate is very suitable to be used as probiotics.

Isolate FI1, FI2 and FI3 which was isolated from Product F. Isolate FI1 and FI2 were able to tolerate acidic pH, bile salt and showed 49.55% and 47.5% hydrophobicity respectively. Isolate FI3 was not able to tolerate acidic pH but able to tolerate bile salt and showed 26.80% hydrophobicity. Isolate FI1, FI2 and FI3 showed antimicrobial activity against *Streptococcus pyogenes* ATCC 19615. Isolate FI1 and FI2 showed pigment inhibition against *Serratia marcescens* which shows their quorum quenching potential. Isolate FI1 showed zone of clearance for protease enzyme and showed bile salt hydrolytic activity. Isolate FI1 and FI2 possess all properties of an ideal probiotics that was acid tolerance, bile salt tolerance, very good cell surface hydrophobicity. Apart from this both possess antimicrobial and quorum quenching potential against pathogens. In additionally FI1 possess protease activity for digestion therefore isolate FI1 and FI2 were very suitable to be used as a probiotics. Isolate FI3 cannot tolerate acidic conditions and don’t show very good cell surface hydrophobicity therefore not very suitable to be used as a probiotics.

Isolate EC2 and EC4 which was isolated from Product E. Both isolates EC2 and EC4 were able to tolerate acidic pH , bile salt, showed 14.75% and 46.42% hydrophobicity respectively. Isolates EC2 and EC4 showed white opaque colonies indicating bile salt hydrolytic activity.

The isolate did not showed antimicrobial activity, quorum quenching activity, amylase, protease and lipase enzyme activity. Since isolates didn't show antimicrobial, quorum quenching potential and enzyme for digestion therefore not very suitable to be used as a probiotics.

Isolate PI1 which was isolated from Product P was able tolerate the acidic pH, bile salts and showed 41.66% hydrophobicity. Isolate showed bile salt hydrolytic activity and protease enzyme activity. The isolate did not showed antimicrobial activity, quorum quenching activity, amylase and lipase enzyme activity. Isolate PI1 showed very good acid tolerance, bile salt tolerance and cell surface hydrophobicity along with protease enzyme for digestion which makes it a good candidate for probiotic use.

Isolate YI1 which was isolated from yakult was able tolerate the acidic pH, bile salts and showed 25.92% hydrophobicity. The isolate did not showed antimicrobial activity, quorum quenching activity, bile salt hydrolytic activity, amylase, protease and lipase enzyme activity. The isolate YI1 was not very suitable candidate for probiotic use since it do not show antimicrobial, quorum quenching and enzyme production and have very weak cell surface hydrophobicity.

After comparing the results FI1 which was isolated from Product F is the best isolate because the isolate possess all the properties of an ideal probiotics that was acid tolerance, bile salt tolerance, very good cell surface hydrophobicity. Apart from this the isolate possess antimicrobial and quorum quenching potential against pathogens. In addition it possess protease activity for digestion.

The bacterial isolate YC3 which was isolated from Product Y was the second best isolate because the isolate has very good acid tolerance, bile salt tolerance and cell surface hydrophobicity along with quorum quenching potential.

The isolate FI2 which was isolated from Product F was the third best isolate because the isolate can tolerate acid, bile salt tolerance and has very good cell surface hydrophobicity. The isolate possess antimicrobial and quorum quenching potential against pathogens.

The isolate PI1 which was isolated from Product P is the fourth best isolate because it shows very good acid tolerance, bile salt tolerance and cell surface hydrophobicity along with protease enzyme for digestion.

The isolates CC4, EC2, EC4,YI1 and FI3 which as isolated from curd, Product E, yakult and Product F respectively was not suitable to be used as probiotics because the isolates didnot possess all the necessary properties of an ideal probiotics.

The isolate FI1 is the best isolate, YC3 is the second best isolate, FI2 is the third best isolate, PI1 is the fourth best isolate. The best commercially available probiotic product is Product F, second best is the Product Y, third best product is Product P. The probiotic formulations are best than the probiotic dairy products.