INTRODUCTION

Each year gastrointestinal infections are responsible for significant morbidity and mortality worldwide. The World Health Organization estimates, more than four billion cases of diarrheal disease annually, another fact is 2.2 million deaths contributed by diarrheal disease in 2004, making it the fifth leading cause of death at all ages worldwide (WHO, 2008).

The incidence and prevalence rates of Inflammatory bowel disease in Asia-Pacific region are low as compared to Europe (2.2 million) and North America (1.4 million) (Culligan et al., 2009), however the rate is increasing...
rapidly. There are considerable variations in the incidence and prevalence rates of IBD in various ethnic groups in Asia. The highest incidence rates are recorded in India, Japan and the Middle East and existence of genetic predisposition of South Asians (Indians, Pakistanis and Bangladeshis) to ulcerative colitis (UC). The development of IBD is more prone in certain racial groups e.g. Indians in South-East Asia have higher rates than Chinese and Malays; host genetic predisposition and environmental factors may be responsible for this difference. There is a male predominance in Crohn’s disease in the Asian population (Ahuja and Tandon, 2010).

Necrotizing enterocolitis (NEC) is affecting about 5% of all very preterm or very low birth weight infants (VLBW: <1500 g) and about 10% of all extremely preterm or extremely low birth weight (ELBW: <1000 g) infants. The rate of NEC-associated acute mortality is generally reported to be greater than 10% overall and more than 25% for infants with NEC. In India the incidence of NEC in babies less than 32 weeks gestation was 5.2% and mortality rates vary from 10 to 40% depending on gestational age of the baby (Patel and Shah, 2012). According to a study by ICMR, in India, hypercholesterolemia (13.9%), hypertriglyceridemia (29.5%), low HDL-C (72.3%), high LDL-C (11.8%) levels had been reported and abnormalities in one of the lipid parameters as 76.9%, 77%, 80% and 82.9%, which relates to 35.9 million, 55.5 million, 14.5 million and 7.6 million individuals with dyslipidemia in Tamil Nadu, Maharashtra, Jharkhand and Chandigarh respectively. Highest rates of hypercholesterolemia in Tamil Nadu (18.3%), highest rates of hypertriglyceridemia in Chandigarh (38.6%), highest rates of low HDL-C in Jharkhand (76.8%) and highest rates of high LDL-C in Tamil Nadu (15.8%) shows regional disparity. Urban residents had the highest prevalence of lipid abnormalities compared to rural residents, exception low HDL-C and Maharashtra (Mohan et al., 2014: ICMR' INDIAB Collaborative Study Group). Probiotics are being considered in the disease control strategies to overcome disorders such as inflammatory bowel disease, diarrhea, irritable bowel syndrome, hypercholesterolemia and these could be due to deficient or compromised intestinal microflora (Dunne et al., 2001). Lactic acid bacteria, especially Lactobacillus, are the most commonly used microorganisms as probiotics because of the perception that they are desirable members of the intestinal microflora and because these bacteria have 'Generally Recognized As Safe' (GRAS) status (Shokryazdan et al., 2014). Lactobacillus acidophilus, Lactobacillus rhamnosus GR-1, Lactobacillus GG, Lactobacillus reuteri RC-14, Lactobacillus rhamnosus E/N, Lactobacillus rhamnosus Oxy, Lactobacillus rhamnosus Pen, VSL#3 formulation and others are being used as antimicrobial agents (details mentioned on page no. 32 ) due to their probiotic properties. Few examples of commercially available are VSL#3 (Bifidobacterium breve, Bifidobacterium infantis, Bifidobacterium longum, Lactobacillus acidophilus, Lactobacillus plantarum, Lactobacillus bulgaricus, Lactobacillus paracasei and Streptococcus thermophilus) by Sigma Tau Pharmaceuticals, Probioplus DDS (Lactobacillus acidophilus, Bifidobacterium longum, Bifidobacterium bifidum, Bifidobacterium lactis) by UAS Laboratories and many others. There are barriers in the human body which prevent the growth/ entry of external organisms/particles such as lysozyme, present in saliva (mouth) has antimicrobial activity which targets gram positive bacteria as it breaks the cell wall made of peptidoglycans, extreme acidic pH of the stomach for the microorganisms, bile salts secreted in human gut reduce bacterial (gram positive) population. Therefore for a probiotic isolate to deliver health benefits should reach intestine in viable number thus should be tolerant to lysozyme, acidic pH and bile salts. After reaching intestine in viable numbers it should have the ability to adhere to the mucosal membranes for its proliferation and effective health benefits. Keeping above mentioned points in mind the work was designed to isolate lactobacilli from the dairy products and screen them for potential use for commercialization. AIM AND OBJECTIVES AIM AND OBJECTIVES Aim: Isolation of probiotic bacteria for potential use of humans. Objectives: ' Isolation of acid producing bacteria ' Identification of Lactobacillus ' Morphology ' Biochemical ' Molecular (PCR based) ' Probiotic attributes of Lactobacillus ' Acid, Bile and Lysozyme tolerance ' Hydrophobicity ' Antimicrobial activity ' Comparison with commercially available probiotics (BIFILAC, BIGLAC, YAKULT ) REVIEW OF LITREATURE REVIEW OF LITREATURE HUMAN MICROBIOME Microbial cells are 10 times more than human cells in human body. This population is responsible for certain metabolic reactions which are helpful to humans. Human Microbiota refers to the set of microorganisms that survives in human body (on surface tissue; skin, mucosal membranes) (Blaser and Musser, 2001). The microflora of the intestine is considered to be post-natally acquired organ having large diversity of bacteria which can perform different functions for the host and this organ is exposed to environmental influences and thus gets modulated in its composition and functions by such external factors such as nutrition. The human intestinal tract is inhabited
by more than 1057 cultivable microbial species (Mirjana et al., 2014). An increased awareness of the role that this normal microbiota play in stimulating the host immune response can be gained through protection against potential pathogens (Round and Mazmanian, 2009). The bacteria present in the human gastrointestinal tract consist of complex ecosystem including both facultative anaerobic and aerobic microorganisms. Distribution of microbiota in gut depends upon environmental factors, physiological interactions, and dietary patterns. Diet is an important factor that regulates the number and occurrence of particular microbial species colonizing the human gut (Naidu et al., 2010). The immune system has the ability to counter infectious agents, however large number of microbes exist in synergy with the human host. Lactobacilli and Bifidobacteria are associated with beneficial effects on the host, (e.g. promotion of gut maturation and integrity, antagonisms against pathogens and immune modulation, maintenance of intestinal immune homeostasis and prevention of inflammation) (Schiffrin et al., 2002). Commensal colonization of the gut affects nutritional and defensive functions of the intestine by modulating gene expression (Hooper et al, 2001). Different bacteria may induce different gene activation. Thus the manipulation of the host microflora may represent a new possibility in the prevention or management of GI pathophysiological disorders. Mucosal surfaces are exposed to commensal and pathogenic microbes. Although the real magnitude of the host exposure to pathogens is difficult to assess, it appears that these encounters rarely result in infectious diseases. Mucosal mechanisms of defense are responsible for protection, however the control of inflammatory reactivity is an important condition in preserving the integrity of the mucosal interface with the external world. (Source: Fujimura et al., 2010) Figure 3.1: Factors that influence the infant gut microbiome and early immune development. Exposure to farm animals and pets, vaginal birth and breast milk, all of which have a potential microbial link, are putatively associated with a beneficial effect on the developing gut microbiome and host immune response. Factors such as urbanization (lack of microbial exposures), formula-only diet and antibiotic administration during the neonatal stage are associated with the development of subsequent chronic diseases such as asthma and atopy, putatively through the development of aberrant gut microbiomes (Fujimura et al., 2010). Well-established Normal flora/Probiotic effects are: (de Vrese and Schrezenmeir, 2008). 1. Prevention and/or reduction of duration and complaints of rotavirus-induced or antibiotic-associated diarrhea as well as alleviation of complaints due to lactose intolerance. 2. Reduction of the concentration of cancer-promoting enzymes and/or putrefactive (bacterial) metabolites in the gut. 3. Prevention and alleviation of unsppecific and irregular complaints of the gastrointestinal tracts in healthy people. 4. Beneficial effects on microbial aberrancies, inflammation and other complaints in connection with: inflammatory diseases of the gastrointestinal tract, Helicobacter pylori infection or bacterial over growth. 5. Normalization of passing stool and stool consistency in subjects suffering from constipation or an irritable colon. 6. Prevention or alleviation of allergies and atopic diseases in infants. 7. Prevention of respiratory tract infections (common cold, influenza) and other infectious diseases as well as treatment of urogenital infections. Insufficient or at most preliminary evidence exists with respect to cancer prevention, hypocholesterolemic effect, improvement of the mouth flora and caries prevention or prevention or therapy of ischemic heart diseases or amelioration of autoimmune diseases (e.g. arthritis) (de Vrese and Schrezenmeir, 2008). (Source: https://unlockinglifescode.org/explore/genomic-medicine/microbiome

Figure 3.2: Representing different organisms present on/in different parts of the body.

The microbiota of the stomach is predominantly composed of Gram-positive aerobes with concentration of <103 colony forming units (cfu)/ml. On the other hand, highest concentration of microorganisms colonize small and large intestine (Mitsuoka et al., 1992). Human microbiome represents a set of microorganisms belonging to various taxonomic groups, a list of microbes defining taxonomic groups is mentioned in a table in appendix and the figure below represents different microbes present in or on the human body parts. LACTIC ACID BACTERIA AS NATURAL COLONIZER OF HUMAN GUT There was a general assumption that Lactobacillus species (large number) form stable and numerically significant populations in the human intestinal tract having epithelial associations (Velez et al., 2007). But the most lactobacilli present are allochthonous members coming from food, oral cavity and other parts of GIT: only a small number of Lactobacillus species are true inhabitants of the mammalian intestinal tract. This gives us information for selecting strains for use as probiotics having applications in food and pharmaceutical industry (Walter, 2008). It is somehow intriguing how lactobacilli could maintain a reputation as numerically important intestinal inhabitants, given that the vast majority of experimental studies after 1960 showed that they form only
marginal populations in the human gut. With the use of total anaerobic culturing techniques it was found that lactobacilli form a very small proportion of the cultivable human fecal microbiota and can rarely be cultured at population levels exceeding 10^8 cfu per gram, reported averages of around 10^6 cfu per gram (Dal Bello et al., 2006; Dal Bello et al., 2003). This accounts for only about 0.01% of the total cultivable counts. Subject-to-subject variation is significant, and lactobacilli are not detectable in around 25% of human fecal samples (Tannock et al., 2000).

**BREAST-FED INFANTS ACQUIRING MICROBIOTA**

Human milk consist of numerous different kind of oligosaccharides derived from lactose such as Lacto-N-Tetraose and Lacto-N-Fucopentaose (I and II) which are present in high concentration up to 1-2 g/l (Rasic et al., 1983). Some of the complex oligosaccharides are even found to be present in human milk at concentration between 3-6 g/l. Such complex oligosaccharides act as growth factors stimulating growth of lactic acid bacteria (Zilliken et al., 1954). This suggests that Bifidobacterium and Lactobacillus are predominant microorganisms in the feces of breast-fed infants compared to bottle fed infants (Tissier, 1905). Moreover, these oligosaccharides inhibit bacterial adhesion to epithelial surface, which is an initial stage for any gastro infection process. These oligosaccharides are generally analogues of epithelial cell surfaces and are involved in the development of non-immunological defense system prevailing in human milk-fed infants. This gives the human milk unique capability for lactic acid bacteria predominant in the bowel of infants. The “bifidogenic effect” of human milk is not related to a single growth-promoting substance, but rather to a complex of interacting factors. In particular the prebiotic effect has been discribed to the low concentration of proteins and phosphates, the presence of lactoferrin, lactose, nucleotides and oligosaccharides. (Naidu et al., 2010; Coppa et al., 2006).

Symbiotic relation of microbes in intestine develops within two days of birth. Bifidobacteria, lactobacilli and bacteroids count increases rapidly in breast fed infants in comparison to bottle fed infants (Coppa et al., 2006). The Lactobacillus species distribution in the OSF fed (standard formula supplemented with galacto- and fructo-oligosaccharides in a 9:1 ratio) group was comparable to breast-fed infants, with relatively high levels of L. acidophilus, L. paracasei, and L. casei. The Standard formula fed infants, on the other hand, contained more L. delbrueckii and less L. paracasei compared to breast-fed infants and OSF-fed infants. An infant milk formula containing a specific mixture of prebiotics is able to induce a microbiota that closely resembles the microbiota of breast fed infants (Knol et al., 2005). Predominance of Lactobacillus in the intestine in breast-fed infants is explained by the presence of high acidity in infants gut leading to reduced counts of putrefactive bacteria. The higher levels protein and phosphate of infant formula leads to greater buffering capacity, which results in higher pH, allowing other bacteria to grow along with bifidobacteria. Whereas, in breast-fed infants in contrast to the formula fed infants Enterobacterium and Enterococci counts remain stable while there is increase in Bifidobacteria, Lactobacillus and Bacteroides count (Balmer et al., 1994; Naidu et al., 2010) When compared on an equivalent protein basis, the growth promotion activity of human milk was greater than that of cow milk for various lactic acid bacteria (Bifidobacterium for example B. bifidum serovar pennsylvanicus and B. longum but comparable for B. bifidum, B. infantis, and B. breve (Oda et al., 2013; Petschow and Talbott, 1991).

**IMMUNE ROLE OF MICROBIOTA**

Mucous membranes are the unique environment where different bacterial species are able to survive and express their effects. Microflora of the gastrointestinal tract plays a crucial role in the anatomical, physiological, and immunological development of the host. Commensals play essential role in immune-modulation and host function homeostasis by modulating the gene expression, but do not elicit strong immune response (Hooper et al., 2001; Schiffrin et al., 2002). The microbiota of intestine has a role in the development of innate and adaptive immune responses in host (Cebra, 1999). If immunological tolerance to commensals is disturbed it may lead to chronic inflammatory bowel disease (Duchmann et al., 1995). Commensal bacteria lead to the stimulation of mucosal mechanisms of defense and the maintenance of the homeostasis of the immune response (Gomez-Llorente et al., 2010). The ability to discriminate between pathogens and commensals is crucial for the intestinal mucosa. The discrimination may depend on bacterial feature recognition by PRR sensing bacterial presence and inducting response against pathogenic bacteria (Belkaid and Hand, 2014). Before induction of tissue damage in the lumen of the gut the recognition of pathogens have theoretical approaches, but commensal bacterial stimulation of intestinal epithelia result in the production of defensins (bactericidal proteins). Epithelial expression of defensins depends on the recognition of bacterial molecules by PRR. In the absence of invasion the innate host response by epithelial discriminates commensals and pathogens. Difference in the response to non pathogenic bacteria is observed by different chemokine profiles (Gomez-
Llorente et al., 2010). In addition, commensal bacteria can even elicit systemic antibody responses such as secretary IgA that protects host from invading enteropathogens (Rougier et al., 2014). It is evident in many studies that gut microflora contributes to the anti-inflammatory effect in gut during gastrointestinal infections (Groux et al., 1997; Newberry et al., 1999). Commensals activating an important lymphocyte subset for the balance of protective and pathogenic immune responses by down regulating inflammatory process by stimulation of IL-10 (restrains immune response to pathogens and microflora preventing pathogenesis) or TGFb. (Schiffrin et al., 2002; Hamad et al., 2014; Groux et al., 1997). Innate immunity stimulated by commensals like Bifidobacterium and Lactobacillus has role in regulation of intestinal homeostasis that controls the inflammatory reaction at the lamina propria (Plantinga et al., 2010). In response to gut microflora the intestinal epithelial cells and macrophages expresses COX-2 (cyclooxygenase-2; essential modulator of intestinal responses to dietary antigens) which results in production of metabolites such as PGE2 (Prostaglandin E 2) having immune-modulatory activities such as resulting in down regulation of MHC-class II antigens that leads to pro-inflammation and induction of IL-10 production that stimulates anti-inflammatory effect (Schiffrin et al., 2002). (Source: Round and Mazmanian, 2009) Figure3.3: Model for Bacteroides fragilis-mediated protection from disease induced by Helicobacter hepaticus B. fragilis produces an immunomodulatory polysaccharide (PSA) that induces an immunoregulatory programme that provides protection from inflammation induced by H. hepaticus. PSA is taken up by intestinal dendritic cells (DCs), which presumably migrate to the local mesenteric lymph nodes (MLNs) where they initiate T-cell responses by presenting PSA on MHC class II molecules to CD4+ T cells. This process helps to restore a balanced T helper (TH) and regulatory T (TReg) cell profile. Subsequently, naive TH cells adopt anti-inflammatory functions that include expression of interleukin-10 (IL-10). IL-10 is required to suppress the production of pro-inflammatory cytokines (such as IL-17, IL-23 and tumour-necrosis factor (TNF) induced by H. hepaticus during experimental colitis. It is this balance of the pro-inflammatory responses to H. hepaticus by regulation induced by B. fragilis that results in the control of intestinal inflammation (Round and Mazmanian, 2009). HISTORY OF PROBIOTICS Elie Metchnikoff regarded as grand father of modern probiotics and a Nobel laureate (Nobel Prize for Medicine in 1908), has pioneered research in immunology leading to the discovery of intercellular digestion in a flatworm and discovered the process of phagocytosis demonstrating the specificity of white blood cells to break down harmful bacteria in the body. He observed that regular consumption of lactic acid bacteria in fermented dairy product, was associated with enhanced health and longevity in Bulgarian peasant populations and demonstrated how healthy bacteria helped digestion and improved the immune system. He compiled his work on probiotics in a book entitled 'The prolongation of life' published in 1907. He suggested that "The dependence of the intestinal microbes on the food makes it possible to adopt measures to modify the flora in our bodies and to replace the harmful microbes by useful microbes" (Kingsley et al., 2007; Metchnikoff, 1908). Henry Tissier, a French paediatrician, observed that children with diarrhea had low bacterial numbers in their stools having a peculiar, Y shaped morphology. These 'bifid' bacteria on other hand were abundant in healthy children (Tissier, 1906). He suggested that these bacteria could be administered to patients with diarrhea to help restore a healthy gut flora (Kingsley et al., 2007). Between 1908 and 1964, little or nothing was heard of microbial therapy. ' The launch of Yakult in Japan in the 1930s. ' In 1965, the term 'probiotics' was first used by Lilly and Stillwell (Lilly and Stillwell, 1965) in a different context to represent 'substances secreted by one organism which stimulate the growth of another'. ' Parker (Parker, 1974) described probiotics as 'organisms and substances which contribute to intestinal microbial balance'. ' Fuller (Fuller, 1989) proposed that probiotics were 'live microbial supplements which beneficially affects the host animal by improving its microbial balance. ' S. Salminen and colleagues, (Salminen et al., 1998) defined probiotics as 'foods containing live bacteria which are beneficial to health'. The United Nations Food and Agriculture Organization and the World Health Organization (FAO/WHO) in 2001, sponsored an Expert Consultation, during the consultation, chaired by Dr. Gregor Reid, the Director of the Canadian Research and Development Centre for Probiotics, a definition of probiotics was adopted as 'live microorganisms which when administered in adequate amounts confer a health benefit on the host' (Joint report by FAO/WHO, 2001). The definition is widely used and accepted as it includes all applications of live microbes and not just the intestinal role. Probiotics beyond fermented dairy products: Probiotics are mostly incorporated in yogurt, fermented milk and other dairy products (ice cream, curd), but a number of other products are sold as tablet, capsule, powder forms and even probiotic chocolates(Ombar, Attune, Sun Biotics, galaxy probiotic). The dairy
been studied the most is TLR-4, whose primary ligand is gram-negative lipopolysaccharide (LPS) (i.e.,
with molecular patterns on both pathogens and commensal bacteria (Akira et al., 2001). The TLR that has
us about toll-like receptors (TLRs) on eukaryotic epithelial, endothelial, and lymphoid cells which can interact
this interaction stimulates host defenses in the gut (Shi and Walker, 2004). Understanding at cellular level tells
gastrointestinal mucosa, communicates with the underlying epithelial and mucosal lymphoid elements and
regulates mucosal immunity. Bacterial-epithelial interaction: Colonizing bacteria that interact with the
gastrointestinal tract. Bacterial-Epithelial interaction: Colonizing bacteria that interacting with the
gastrointestinal mucosa, communicates with the underlying epithelial and mucosal lymphoid elements and
this interaction stimulates host defenses in the gut (Shi and Walker, 2004). Understanding at cellular level tells
us about toll-like receptors (TLRs) on eukaryotic epithelial, endothelial, and lymphoid cells which can interact
with molecular patterns on both pathogens and commensal bacteria (Akira et al., 2001). The TLR that has
been studied the most is TLR-4, whose primary ligand is gram-negative lipopolysaccharide (LPS) (i.e.,

Probiotics and prebiotics are a promising tool in the prevention of antibiotic-associated diarrhea. Probiotics
are live microorganisms that have been shown to provide health benefits when administered in adequate
amounts confer a health benefit on the host. Prebiotics are defined as non-digestible but fermentable food
having beneficial effect on host, by selecting the growth and activity of one
organism over others in the gut. Example oligosaccharides in human breast milk (Quigley, 2012) and
particularly inulin, its hydrolysis product oligofructose, and (trans) galactooligosaccharides. They are dietary
fibers with a well-established positive impact on the intestinal microflora. Other health effects of probiotics
(prevention of diarrhea or obstipation, modulation of the metabolism of the intestinal flora, cancer
prevention, positive effects on lipid metabolism, stimulation of mineral adsorption and immunomodulatory
properties) (de Vrese and Schrezenmeir, 2008). Table 3.1: Difference and similarities between probiotics and
prebiotics. PROBIOTICS PREBIOTICS Live organisms Fermentable food Exogenous origin Stimulate indigenous
microflora Provide health benefits Stimulation of gut microflora Synbiotics are defined as the combination of
probiotics and prebiotics aiming to increase the survival and activity of probiotic in vivo and stimulating
indigenous bacteria (Quigley, 2012). List of probiotic Bacteria: - ' Bacteriodes fragilis ' Bacteriodes
thetaiotaomicron ' Bifidobacteria infantis ' Bifidobacterium lactis ' Bifidobacterium bifidum ' Bifidobacterium
longum ' E. coli nissle 1917 ' Faecalibacterium prausnitzii ' Lactobacillus bulgaricus, ' Lactobacillus casei ' Lactobacillus fermentum ' Lactobacillus GG ' Lactobacillus paracasei # ' Lactobacillus plantarum ' Lactobacillus reuteri ' Lactobacillus rhamnosus ' Lactobacillus salivarius ' Pediococcus parvulus # ' Oenococcus oeni # ' Saccharomyces boulardii ' Streptococcus thermophilus* (Round and Mazmanian, 2009; #Munoz-Quezada et al., 2013; *University of Maryland Medical Center) Table 3.2: Lactobacillus species commonly
detected in human feces, saliva and food (Walter, 2008) Species Feces Oral Cavity Food L. acidophilus + + + - L.
crispatus + (P) - + L. gasseri + (P) + - L. johnsonii + + + + L. salivarius + (P) + + L. ruminis + (P) + - L. casei + + + L.
paracasei + + + + L. rhamnosus + + + L. plantarum + + + L. reuteri + (P) + + + b L. fermentum + + L. brevis + + + + L. delbrueckii + + + + L. sakei + + + + L. vaginalis + + + + L. curvatus + + + a P indicates species that were
reported to persist in some human subjects ' b Lactobacillus reuteri can be found regularly only in sourdough
and in other fermented cereals such as fermented oatmeal. Fecal isolates of these species are therefore
unlikely to originate from food. ' (+) presence; (-) absence LACTOBACILLUS AS PROBIOTIC ' Lactobacillus-
containing probiotics are used to restore commensal vaginal flora has been proposed for the treatment and
prophylaxis of bacterial urogenital infections (Barrons and Tassone, 2008). ' The evidence for using
Lactobacillus to prevent diarrhea is mixed. Some clinical research suggests Lactobacillus acidophilus may be
effective when used to prevent traveler’s diarrhea (caused by eating contaminated food) (McFarland, 2007)
and can be used to prevent and reduce incidence of necrotizing enterocolitis (Bin-Nun et al., 2005; Lin et al.,
2005). Other studies have found that Lactobacillus GG was effective (Szajewska et al., 2001). ' A mix of
probiotics (Saccharomyces boulardii and a mixture of Lactobacillus acidophilus and Bifidobacterium bifidum)
helped treat traveler’s diarrhea in one study. (Source: University of Maryland Medical Center) ' Lactobacillus
rhamnosus GR-1 and Lactobacillus reuteri RC-14 are the world’s most documented probiotic strains for
women’s health. They were selected for their ability to interfere with infectivity of a range of bacteria and
yeast in the vagina, as well as confer benefits to the intestine and reduce the risk of bladder infections.
(Anukum and Reid, 2008). ' L. rhamnosus E/N, L. rhamnosus Oxy and L. rhamnosus Pen can be used for
prevention of antibiotic associated diarrhea (Rusczynski et al., 2008). ' VSL#3 formulation significantly reduce
stool frequency and requirement for oral rehydration salts (ORS), resulting in reduced recovery time (Dubey et
al., 2008). ' Lactobacillus rhamnosus NBHK007, reduced the tumor growth secretion by 100% after 48'h of
incubation (Tsai et al., 2014). ' MECHANISMS OF ACTION OF PROBIOTICS (Walker, 2008) In the past decade,
scientists have studied the mechanism, by which commensal bacteria improve mucosal defenses of the
gastrointestinal tract.
endotoxin). This receptor interacts with LPS as an LPS-binding protein complex after being anchored to the cell surface by a surface molecule, CD14. This interaction activates a series of signaling molecules in the cell to release the transcription factor nuclear factor ??B (NF??B) into the nucleus, which in turn transcribes inflammatory cytokines (IL-8 and IL-6) involved in acute innate inflammatory response to an invading pathogen and commensals (Kawai and Akira, 2006). This plays role in both innate and adaptive mucosal immunity. Development of Host Defense: The nature and composition of the colonizing bacteria (colonizing germ free neonatal gastrointestinal tract at birth) are important contributors to the development of gastrointestinal host defenses against infection and allergic reaction. Figure 3.4: Probiotic effects on the development of host defense and its components affecting the initial colonization (Walker, 2008). The full-term neonate at birth has the capacity to mount an effective host defense. The gut lymphoid tissue must first be stimulated by colonizing bacteria to produce active components of the defense (Cheravil and Walker, 2008). This includes expression of follicular epithelial cells over Peyer’s patches in the ileum and colon. These cells facilitate interaction between microbes and lymphoid cells (Kerneis et al., 1997). Intraepithelial and lamina propria sites are activated to produce protective cytokines, and mesenteric lymph nodes secrete polymeric Immunoglobulin A (pIgA). Dendritic cells in the lamina propria extend their appendices between epithelial cells and through TLR-2 and TLR-4 (on surface of dendritic cells) they can recognize the presence commensal-bacterial molecular patterns. The interaction leads to maturation of the dendritic cells and to the release of cytokines, which manages the conversion of naive T-helper cells (Th0) into a mature, balanced response of T-helper cells (Th1, Th2, and Th3/Tr1), an important component in the prevention of disease (Walter, 2008). Commensal bacteria cross epithelial cells and interact with antigen-presenting cells in mesenteric lymph nodes activating the naive plasma cells to become pIgA-producing B cells (Macpherson and Uhr, 2004), pIgA coats the mucosal surface controlling the subsequent microbial and antigen penetration. Mechanism to control chronic inflammation of the gut, the interaction between CpG DNA of bacteria and the intracellular receptor TLR-9, to which the bacteria bind and thus activate T regulatory cells by the production of anti-inflammatory cytokines (e.g., IL-10) (Rachmilewitz et al., 2004). Figure 3.5: The interaction of commensal bacteria CpG DNA with the intracellular toll-like receptor 9 (TLR-9) in dendritic cells, which stimulate the production of Th3/Tr1 cells. Commensal bacterial penetrate micro fold cells to enter Peyer’s patches and interact with dendritic cells, or they interact with dendritic cells directly. CpG DNA from these bacteria interacts with the TLR-9 intracellular receptor in dendritic cells and triggers a cytokine response that facilitates the maturation of T regulatory (T>REG) cells that down-regulate acute inflammatory responses to prevent chronic intestinal inflammation. TGF, transforming growth factor (Walter, 2008).

Commensal bacteria can also secrete small molecules that enter intestinal epithelial cells to inhibit activation of NF??B (Neish et al., 2000). In addition, it has been reported recently that prolonged exposure to molecular patterns can activate cell surface and intracellular negative regulators which in turn, turn off transcription factors leading to a reduction in the production of inflammatory cytokines and chemokines (Otte et al., 2004). Specific examples of probiotics:-

Saccharomyces boulardii, enhance the specific IgG and IgA antibody response to Clostridium difficile toxin A after Clostridium infection (Qamar, 2001). Lactobacillus rhamnosus strain GG increases IgA response to the rotavirus in infants having gastroenteritis (Isolauri et al., 1991). A study involving healthy adults, receiving typhoid vaccine for foreign travel showed that those given Lactobacillus GG for 10 days before vaccination had significantly higher levels of anti-typhoid antibodies (Teitelbuan and Walker, 2002).

The use of probiotics is effective in enhancing the mucosal barrier to pathogens and antigen presentation. Probiotic, Lactobacillus strains, stimulate up-regulation of mucous genes in intestinal goblet cells. The effect of these probiotics on the activation and secretion of mucus in the intestine is directly correlated to the inhibition of pathogenic Escherichia coli attachment and of damage to the intestinal tract (Mack, 1999).

COMMERCIALIZATION

Existing players in the domestic probiotic category include Japan’s Yakult Danone, Nestle, NDBB’s Mother Dairy and GCMMF’s Amul, in addition to the various smaller ones. Guidelines for probiotics have been given in 2011 by Indian Council of Medical Research (ICMR), Department of Health Research Ministry of Health & Family Welfare, New Delhi and Department of Biotechnology (DBT), Ministry of Science and Technology, New Delhi.
Isolated in 1990 and tested in the last 20 years, the first two indigenous probiotic cultures from India—Lactobacillus helveticus MTCC 5463 and Lactobacillus rhamnosus MTCC 5462 are now available to the industry for probiotic products manufacture. These cultures were developed by the Seth Mansukhlal Chhaganlal (SMC) College of Dairy Science, Anand Agriculture University in Gujarat for supplements and dairy products, have now been made available for commercial use. Before this probiotic cultures are largely imported from Denmark by the dairy and other food industry of India to manufacture probiotic products. Its estimated market size is around $20 million in the country. Indian patent for a process to manufacture a herbal probiotic fermented milk product ‘lassi’ also having herbal content—safed Musli have been filed by department of the college. Lassi is further being tested by the SMC to check its potential in reducing the cholesterol level in humans (THE ECONOMICS TIMES, PTI May 7, 2012).

INDIAN SCENARIO (http://www.probioticindia.com/)
In probiotic revolution, being the largest producer of milk and having world’s highest cattle population India can play an important role. Indian probiotic industry is in its infancy stage and presently accounts for only a small fraction i.e. less than 1% of the total world market turnover in the probiotic industry. Probiotic industry is developing at a steady pace. India is emerging as a major probiotic market of the future with annual growth rate of 22.6% until 2015 with a few players in Indian probiotic industry such as Amul, Mother Dairy, Yakult Danone and Nestle along with other minor players. The Indian probiotic market turnover is expected to reach $8 million by the year 2015. Probiotics in India generally comes in two forms, milk and fermented milk products with the former occupying 62% of the market share and the latter having 38% market share (Indian consumer survey, 2010).

Table 3.3: Probiotic food products and companies in India (http://www.probioticindia.com/).

<table>
<thead>
<tr>
<th>Company Name Products</th>
<th>Market</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amul Probiotic ice cream</td>
<td>10% of ice cream and 25% of dahi sales</td>
</tr>
<tr>
<td>Probiotic lassi</td>
<td></td>
</tr>
<tr>
<td>Mother Dairy b-Activ Probiotic Dahi</td>
<td></td>
</tr>
<tr>
<td>b-Activ Probiotic Lassi</td>
<td></td>
</tr>
<tr>
<td>b-Activ Curd</td>
<td></td>
</tr>
<tr>
<td>Nutrifit (Strawberry and Mango)</td>
<td>15% of the turnover of their fresh dairy products</td>
</tr>
<tr>
<td>Nestle ActiPlus Probiotic Dahi</td>
<td></td>
</tr>
<tr>
<td>Yakult Danone India Pvt.Ltd.(YDIPL) Yakult 50:50 joint venture between Japan’s Yakult Honsha and The French- Danone Group Major players in the probiotics drug market in India, Ranbaxy (Binifit), Dr. Reddy’s Laboratories, which has four probiotic brands, Zyduz Cadila, Unichem, JB Chem, and Glaxo SmithKline. While probiotics in the form of drugs are widely accepted, probiotic foods are still not accepted much, acceptance is growing but slowly, it will take a time for the change in mindset of Indian consumers.</td>
<td></td>
</tr>
</tbody>
</table>

GLOBAL SCENARIO OF PROBIOTICS (http://www.probioticindia.com/)
According to a new market research report, ‘Probiotics Market’ (2009-2014), published by Markets and Markets, the global probiotics market is expected to be worth US $31.1 billion by 2015 with the Europe and Asia accounting for nearly 42 and 30% of the total revenues respectively. Europe forms the largest market for probiotics with an estimated $13.5 billion by 2014. Asia is the second largest segment, growing at with an estimated CAGR of 11.2% to reach $9.0 billion by 2014. Probiotics, with a market of $3.23 billion is a large part of Japanese functional foods. The Japanese spent $126 per person per year on functional foods and it is higher than other countries including US ($67.9), Europe ($51.2) and Asia ($3.20).

According to Euromonitor International’s packaged food data, in 2000, pro/prebiotic yogurt (both drinking and spoonable combined) accounted for one-quarter of global yogurt sales by value after a decade in 2010, it accounted for one-third global value for sales of probiotic dietary supplements almost tripled, amounting to US$2.2 billion in 2010, thereby, further suggesting that probiotics are spreading like wildfire around the globe. It is now well recognized that probiotics hold great promise and have the prospects to serve as candidate biotherapeutics in the management of inflammatory metabolic disorders including cardiovascular diseases such as atherosclerosis, hypertension and stroke etc. So many food companies are expanding their market profile to the most promising growth markets for probiotics.

Table 3.4: Probiotic products available worldwide.
Country Product Name
Japan Yakult
Meiji Bulgaria Yoghurt and Yoghurt drinks
Morinaga Bifidus Yoghurt
Calpis Ameal S120, a probiotic yogurt drink
Europe Life way Kefir
Culturelle Probiotic Infant Formula
France, Bravo Friscus
Yoplait Yoghurt
Danone Activia
Actimel Probiotic Yoghurt
USA Activia Creamy Yoghurt
Danone, GYoPlus
Blue Bunny-Sedona Yoghurt Ice Cream, Choclate Sweet Scoops- Frozen Yoghurt
Yovation- Pierre’s Probiotic Ice Cream
UK Vita-Yo Creamy Probiotic Yoghurt
Yeo Valley- Biolive yoghurt and natural Fat free Yoghurt
Unilever’s Flora Pro-Activcholesterol
Finland Valio Gefilus?? and Valio Kidius Gelfilus?? and Evolus?? Milk drink and Yoghurt (LLG)
Yosa Yoghurt oat product
Bioferme, Valio Vilis
Canada BioBest Plant Sterols probiotic Yoghurt
Kraft LiveActive cheddar cheese and Chocolate Raspberrt Bars
Libert?? Yoghurt
Olympic Natural No Fat Probiotic yogurt
Brazil Chamyto probiotic drink Nestle
Actimel L. casei Defensis
Danito L. casei, Danone
Sofyl Yakult
Sweden ProViva yogurt
Yogenfr??z frozen yoghurt
BioGaia products
Live Active probiotic products
Denmark Klover drinkable yoghurt
Danimal Lactobacillus GG
ProBioArlw Cultura
Italy GanedenBC30?? probiotic low-fat yogurt
Probiotic dairy drinks Latteria Sociale Merano
Danacol fermented dairy beverage
Yolive frozen Yoghurt
Czech Republic Olma Revital Active Yoghurt and drink yogurt
Germany Probiotic Vitality Yogurt
Soyogurt
France b. Aktiv LGG Dukat Yoghurt and drinks
New Zealand BioFarm Acidophilus Yoghurt
Spain Kaiku Vita, a functional dairy drink from Valio
Bio Herbal bifidus active green tea yoghurt Danone
Australia F & N’s aLIVE fruit-chunk yoghurt
Yo-Plus digestive Yogurt
Wallaby Organic Yogurt
Bio-Life Probiotic Yoghurt
Vallia probiotic yoghurt
MATERIALS AND METHODS

Table 4.1: Materials used in study and there source.

Source | Materials Used
--- | ---
Borosil Glass Works Ltd., India | Glassware
HiMedia Laboratories, India | Agar powder, bacteriological Agarose special, low EEO
Bromocresol purple, certified | Bromophenol blue, certified
DeMann Roger Sharpe (MRS) medium | Ethidium bromide solution (10mg/ml)
EDTA | Glass slides
Glass, anhydrous, pure | Glacial acetic acid
Gram staining kit | Hugh Leifson medium
Lysozyme | Nutrient broth
MgCl2 | Ox bile, bacteriological Oxidase disc
PPA | Peptone, bacteriological Phenol red
Potassium chloride | Potassium dihydrogen phosphate
Simmon citrate agar | Sodium Chloride
Sugar disc [Arabinose (Ar), cellobiose (Ce), xylose (Xy), inulin (In), lactose (La), mannose (Mo), raffinose (Rf), rhamnose (Rh) and trehalose (Te)]
Triple Sugar Iron agar medium | Tris base
Tris HCl, AR | Xylene, AR
Xylene cyanol FF | Urea
Cosmos Pharmacls Ltd., Solan, H.P., India | Biglac
Rankem RFCL Ltd., India | Hydrogen peroxide (30%)
Sigma-Aldrich Corp., USA | Primers
Solis BioDyne, Biomax Corporation, India | 100 base pair ladder
Tablets (India) Limited, Puducerry, India | Bifilac
Tarsons products Pvt. Ltd., India and Ess & Ess Polylab Pvt. Ltd., India | Plastic ware
ThermoScientific, USA | Auto-piptes
dNTPs | Taq DNA polymerase
Taq buffer | Yakult
Table 4.2: List of instruments used.
4.1. Isolation of Lactobacillus from dairy products.

4.1.1 Sample collection
Fresh curd sample was collected in sterile vials from curd prepared for domestic need. Other curd samples used were Verka curd, Metro curd, Nestle Mango yogurt and Nestle probiotic curd. Fresh milk samples were collected in sterile vials from domesticated cow, goat and sheep. The samples were immediately brought to laboratory and processed for isolation of bacteria. Commercial products (Yakult, Bifilac and Biglac) were also collected and similarly processed.

4.1.2 Isolation
DeMann Roger Sharpe (MRS) medium is used for isolation of Lactobacillus as it is a selective medium for the same. It consists of Polysorbate 80 as selective agents for the luxurious growth of Lactobacillus and ammonium citrate and sodium acetate as inhibitors for Streptococcus.

Table 4.3: Composition of MRS media

<table>
<thead>
<tr>
<th>Components</th>
<th>Amount (g/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Protease peptone</td>
<td>10</td>
</tr>
<tr>
<td>Beef extract</td>
<td>10</td>
</tr>
<tr>
<td>Yeast extract</td>
<td>5</td>
</tr>
<tr>
<td>Dextrose</td>
<td>20</td>
</tr>
<tr>
<td>Polysorbate 80</td>
<td>1</td>
</tr>
<tr>
<td>Ammonium citrate</td>
<td>2</td>
</tr>
<tr>
<td>Sodium acetate</td>
<td>5</td>
</tr>
<tr>
<td>Magnesium sulphate</td>
<td>0.100</td>
</tr>
<tr>
<td>Manganese sulphate</td>
<td>0.050</td>
</tr>
<tr>
<td>Dipotassium phosphate</td>
<td>2</td>
</tr>
<tr>
<td>Final pH 6.8±0.2 at 25oC</td>
<td></td>
</tr>
</tbody>
</table>

The media powder (55.515g) from HiMedia was dissolved in water and volume made to 1000ml having final pH of 6.57. The media was prepared by adding 2% agar and 0.004% bromocresol purple dye and autoclaved at 15psi for 15 minutes.

The samples were serially diluted with peptone water and 100 μl of each dilution was pour plated with molten MRS-dye agar. After solidification of the medium the plates were incubated at 37oC for 24 hours.

4.2. Identification of acid producing bacteria as Lactobacillus

Lactobacillus belongs to group of lactic acid producing bacteria, which converts the purple color of MRS-dye plates to yellow. The change in color of the MRS-dye plates indicates the presence of lactic acid producing bacteria. The acid producing isolates were further subjected to physiological, biochemical and polymerase chain reaction based (PCR) identification.

4.2.1 Physiological
Identification was based on colony characteristics, gram character, morphology and arrangement of bacterial cells.

4.2.1.1 Colony
Colony Characteristics such as color, texture, margin and elevation were observed for each isolate streaked on MRS plates isolated from samples collected.

4.2.1.2 Gram staining
Hans Christian Gram was the first one to introduce the technique of staining for identification purpose (Gram, 1884). The staining procedure differentiates bacteria into two large groups (i.e. gram positive and gram negative) on the basis of cell wall structure. Bacteria having thick peptidoglycan layer are stained blue to purple and termed as Gram positive organisms and bacteria having thin peptidoglycan layer (high lipid content) are stained red to pink and termed as Gram negative. It requires four basic steps; include applying a primary stain (crystal violet) to a heat fixed smear, followed by the addition of a mordant (Gram’s Iodine), rapid decolorization and lastly, counter staining with safranin.

Chemical mechanism of the Gram stains (Davies et al., 1983). In aqueous solutions crystal violet dissociates into CV+ and Cl− ions that penetrate through the wall and membrane of both gram positive and gram negative cells. The CV+ interacts with negatively charged components of bacterial cells, staining the cells purple. When added, iodine (I− or I3−) interacts with CV+ to form large CVI complexes within the cytoplasm and outer layers of the cell. The decolorizing agents, interacts with the lipids of the membranes of both gram positive and gram negative bacteria. The outer membrane of the gram negative cell is lost from the cell, leaving the peptidoglycan layer exposed. Gram negative cells have thin layers of peptidoglycan, one to three layers deep with a slightly different structure than the peptidoglycan of gram positive cells. With ethanol treatment, gram negative cell walls become leaky and allow the large CVI complexes to be washed from the cell. The highly cross linked and multilayered peptidoglycan of the gram positive cell is dehydrated by the addition of ethanol. The multilayered nature of the peptidoglycan along with the dehydration from the ethanol treatment traps the large CVI complexes within the cell. After decolorization, the gram positive cell remains purple in color, whereas the gram negative cell loses the purple color and is only revealed when the counter stain, the positively charged dye safranin, is added.

Table 4.4: Components of Gram staining kit by HiMedia.

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>Amount</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Gram’s Crystal Violet</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Solution A</td>
<td></td>
<td></td>
</tr>
<tr>
<td>‘ Crystal violet</td>
<td>2.000 g</td>
<td>20.000 ml</td>
</tr>
<tr>
<td>‘ Ethyl alcohol (95%)</td>
<td>0.800 g</td>
<td>80.000 ml</td>
</tr>
<tr>
<td>Solution B</td>
<td></td>
<td></td>
</tr>
<tr>
<td>‘ Ammonium oxalate</td>
<td>1.000 g</td>
<td>2.000 g</td>
</tr>
<tr>
<td>Distilled water</td>
<td></td>
<td></td>
</tr>
<tr>
<td>‘ Solution A and solution B are mixed and stored for 24 hours to obtain a stable stain.</td>
<td>300.000 ml</td>
<td>1.000 g</td>
</tr>
<tr>
<td>Gram's Iodine</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Iodine</td>
<td>2.000 g</td>
<td></td>
</tr>
<tr>
<td>Potassium iodine</td>
<td>300.000 ml</td>
<td></td>
</tr>
<tr>
<td>Distilled water</td>
<td>1.000 g</td>
<td></td>
</tr>
<tr>
<td>‘ Gram's Decolorizer</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ethyl alcohol (95%)</td>
<td>2.000 g</td>
<td></td>
</tr>
<tr>
<td>Acetone</td>
<td>50.0 ml</td>
<td></td>
</tr>
</tbody>
</table>
50.0 ml
Safranin, 0.5% (w/v)
Safranin O
Ethyl alcohol (95%)
0.500g
100.000ml

Procedure: Clean glass slides were taken and uniform thin smear was made using the inoculums from the culture (24 hours old) on MRS agar plates. The smear was air dried and heat fixed over the flame. Slides were kept on staining tray and crystal violet is added and kept for 1 minute, followed by washing of slide with slow running water. Next iodine was added and kept for 1 minute, followed by washing with running water. Smear was decolorized, decolorizer was added and kept for 30 seconds, followed by washing with running water. Next safranin was added and kept for 1 minute, followed by washing with running water. The slides were air dried and examined under microscope at 100 X magnification. Bacterial cell shape and gram character were observed.

4.2.2 Biochemical

Microorganisms use certain biochemicals for metabolism and this property is used for identification of groups of bacteria belonging to same genera and bacteria from same genus can be differentiated on their ability to utilize sugars and amino acids. Biochemicals used for identification of Lactobacillus are mentioned here and sugar utilization further used for identification of bacteria.

4.2.2.1 Catalase test

Enzyme-based tests play a crucial part in the identification of bacteria. Sherman, 1925 developed the first bacterial classification system based on catalase production and reactions. The catalase enzyme by the bacteria is used to neutralize the hydrogen peroxide having bactericidal effects. Catalase catalyses the breakdown of hydrogen peroxide (H2O2) to water and oxygen. This reaction is evident by the rapid formation of bubbles.

2H2O2 + Catalase ' 2H2O + O2

Procedure: Clean glass slides were used. A drop of hydrogen peroxide was placed on it. Using a loop/toothpick a culture was picked from a 24 hrs old culture and mixed with the drop of H2O2 and noted for the appearance of bubbles.

4.2.2.2 TSI

In 1940, Difco Laboratories, Sulkin and Willet, and Hajna described a triple sugar ferrous sulfate medium for the identification of enteric bacilli. (Difco manual, 11th edition)

Beef Extract, Yeast Extract, Bacto Peptone, and Protease Peptone provide nitrogen, vitamins, and minerals. Triple Sugar Iron Agar contains three carbohydrates (dextrose, lactose and sucrose). When these carbohydrates are fermented, the resulting production of acid is detected by the phenol red indicator. The color changes that result are yellow for acid production and red for alkalinization. Sodium thiosulfate is reduced to hydrogen sulfide. Hydrogen sulfide then reacts with an iron salt yielding the typical black iron sulfide. Sodium chloride maintains the osmotic balance of the medium. Bacto Agar is a solidifying agent.

Table 4.5: Composition of Triple Sugar Iron Agar

<table>
<thead>
<tr>
<th>Components</th>
<th>Amount (g/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Beef extract</td>
<td>3.000</td>
</tr>
<tr>
<td>Peptone</td>
<td>20.000</td>
</tr>
<tr>
<td>Yeast extract</td>
<td>3.000</td>
</tr>
<tr>
<td>Lactose</td>
<td>10.000</td>
</tr>
<tr>
<td>Sucrose</td>
<td>10.000</td>
</tr>
<tr>
<td>Dextrose monohydrate</td>
<td>1.000</td>
</tr>
<tr>
<td>Ferrous sulphate</td>
<td>0.200</td>
</tr>
<tr>
<td>Sodium chloride</td>
<td>5.000</td>
</tr>
<tr>
<td>Sodium thiosulphate</td>
<td>0.300</td>
</tr>
<tr>
<td>Phenol red</td>
<td>0.024</td>
</tr>
<tr>
<td>Agar</td>
<td>12.000</td>
</tr>
</tbody>
</table>

Final pH 7.4??0.2 at 25oC
Procedure: The media powder 64.42g from HiMedia was dissolved in water and made the volume up to 1L. It was heated to melt the agar and dispensed in sugar tubes and autoclaved at 15psi for 15 minutes. The media was made to settle in slope. The media was inoculated with 24 hours old culture by stabling the butt and streaking the slant. The tubes were incubated at 37°C for 24 hours and the change was noted.

4.2.2.3 Oxidase test

The oxidase test assays for the presence of cytochrome oxidase. The organism producing the cytochrome oxidase enzyme, changes the reduced colorless reagent to oxidized colored product. Gordon and McLeod, 1928 introduced the use of a dimethyl-p-phenylenediamine dihydrochloride solution to test for the presence of oxidase systems. The sensitivity of the oxidase test is increased by the use of tetra-methyl-p-phenylenediamine dihydrochloride solution giving a quicker reaction.

The final stage of bacterial respiration that is the electron transport chain involves a series of membrane-embedded components. The final step in the chain involves the use of the enzyme cytochrome oxidase, which catalyzes the oxidation of cytochrome c while reducing oxygen to form water. The oxidase test often uses a reagent, tetra-methyl-p-phenylenediamine dihydrochloride, as an artificial electron donor for cytochrome c. When the reagent is oxidized by cytochrome c, it changes from colorless to a dark blue or purple compound, indophenol blue.

Oxidase discs are sterile filter paper discs impregnated with N, N-dimethyl-p-phenylenediamine oxalate, ascorbic acid and ??-naphthol.

Procedure: The oxidase disc were placed on clean glass slide and inoculated with 24 hour old culture and noted for the change in color.

4.2.2.4 HL test

The oxidative-fermentative test is used to determine if bacteria can metabolize carbohydrates oxidatively, by fermentation, or are nonsaccharolytic and therefore have no ability to use the carbohydrate in the media. The oxidative-fermentative (OF) test was developed by Hugh and Leifson (Hugh and Leifson, 1953). Hugh and Leifson were the first to refer to the production of acid from carbohydrates under aerobic conditions only, as oxidative. It was noted that the amount of acid produced by bacteria using carbohydrates under aerobic conditions was less than the amount of acid produced during fermentative metabolism.

The oxidative-fermentative test determines if certain bacteria can metabolize glucose by fermentation or aerobic respiration (oxidatively). During the anaerobic process of fermentation, pyruvate is converted to a variety of mixed acids depending on the type of fermentation. The high concentration of acid produced during fermentation will turn the bromthymol blue indicator in the media from green to yellow in the presence or absence of oxygen.

Certain non-fermenting bacteria metabolize glucose using aerobic respiration and therefore only produce a small amount of weak acids during the Krebs cycle and Entner Doudoroff Pathway. The increased concentration of glucose in the medium enhances the production of these weak acids to a level that can be detected by bromthymol blue indicator. To further enhance detection of these weak acids, this medium contains a reduced concentration of peptones. This reduces the production of amines from the metabolism of amino acids, therefore reducing the neutralizing effect of these products. Dipotassium phosphate buffer is added to further promote acid detection. Bacteria giving this reaction in OF media are oxidative.

Table 4.6: Composition of Hugh Leifson media.

<table>
<thead>
<tr>
<th>Components</th>
<th>Amount (g/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peptic digest of animal tissue</td>
<td>2.000</td>
</tr>
<tr>
<td>Sodium chloride</td>
<td>5.000</td>
</tr>
<tr>
<td>Dipotassium phosphate</td>
<td>0.300</td>
</tr>
<tr>
<td>Glucose</td>
<td>10.000</td>
</tr>
<tr>
<td>Bromothymol blue</td>
<td>0.030</td>
</tr>
<tr>
<td>Agar</td>
<td>3.000</td>
</tr>
</tbody>
</table>

Final pH 7.1??0.2 at 25??C

Procedure: The media powder 23.33gm was dissolved in water and volume made up to 1L and was boiled. It was dispensed in sugar tubes and autoclaved at 15psi for 15 minutes. The tubes were inoculated with 24 hours old culture by stabbing the half way the media. The tubes were incubated at 37°C for 24 hours and observed for color change.
4.2.2.5 PPA

Phenylalanine medium tests the ability of an organism to produce deaminase enzyme which removes the amine group from phenylalanine (amino acid) and it is released as free ammonia. This reaction leads to the production of phenylpyruvic acid in media; this acid reacts with ferric chloride and produce dark green color. Ferric chloride acts as an indicator and added after the incubation period.

Table 4.7: Composition of Phenylalanine agar.

<table>
<thead>
<tr>
<th>Components</th>
<th>Amount (g/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Yeast extract</td>
<td>3.000</td>
</tr>
<tr>
<td>Sodium chloride</td>
<td>5.000</td>
</tr>
<tr>
<td>DL-Phenylalanine</td>
<td>2.000</td>
</tr>
<tr>
<td>Disodium phosphate</td>
<td>1.000</td>
</tr>
<tr>
<td>Agar</td>
<td>15.000</td>
</tr>
</tbody>
</table>

Final pH (at 25°C) 7.3??0.2

Procedure: The media powder 26 gm was dissolved in water and volume made up to 1L and boiled. It was dispensed in sugar tubes and autoclaved at 15psi for 15 minutes. The media was allowed to settle as slants. The tubes were inoculated with 24 hours old culture by streaking the slants. The tubes were incubated at 37°C for 24 hours. Three to four drops of 10% ferric chloride was added to the tubes and noted for change in color.

4.2.2.6 Urease (Bailey and Scott, 1974)

The alkaline fermentation of urine (urea) with production of ammonia occurs due to the action of microorganisms by Reoch in 1875. The urease test identifies those organisms that are capable of hydrolyzing urea to produce ammonia and carbon dioxide. Urease is the enzyme which hydrolyzes urea to carbon dioxide and ammonia.

\[(NH_2)_2CO + H_2O \rightarrow CO_2 + 2NH_3\]

Urease test media contain 2% urea and phenol red as a pH indicator. An increase in pH due to the production of ammonia, results in a color change from yellow (pH 6.8) to bright pink (pH 8.2). Urea agar has reduced buffer content and contains peptones and glucose. This medium supports the growth of many bacteria allowing for the observation of urease activity.

Table 4.8: Composition of media used for urease test.

<table>
<thead>
<tr>
<th>Components</th>
<th>Amount (g/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peptone</td>
<td>10</td>
</tr>
<tr>
<td>Urea</td>
<td>20</td>
</tr>
<tr>
<td>Phenol red</td>
<td>1</td>
</tr>
<tr>
<td>Agar</td>
<td>20</td>
</tr>
</tbody>
</table>

Procedure: Peptone and agar was dissolved in water and volume made up to 1L. This was autoclaved at 15 psi for 15 minutes; to the molten media added urea and phenol red mixed it. Dispensed in sugar tubes and allowed to settle media as slants. The tubes were inoculated with 24 hours old culture on MRS plate by streaking on the slant. The tubes were incubated at 37°C for 24 hours and change in color was noted.

4.2.2.7 Citrate (Koser, 1924)

The citrate test screens a bacterial isolate for the ability to utilize citrate as its carbon and energy source. A positive diagnostic test rests on the generation of alkaline by-products of citrate metabolism. The subsequent increase in the pH of the medium is demonstrated by the color change of a pH indicator.

The citrate test is based on the ability of the organism to produce their metabolic by-products. Citrate is the sole source of carbon in the Simmons citrate medium while inorganic ammonium salt (NH4H2PO4) is the sole fixed nitrogen source. When an organic acid such as citrate is used as a carbon and energy source, alkaline carbonates and bicarbonates ultimately are produced. The visible presence of growth on the medium and the change in pH indicator color due to the increased pH are the signs that an organism can import citrate and use it as a sole carbon and energy source; such organisms are considered to be citrate positive.

Citrate, a Krebs cycle intermediate, is generated by many bacteria but utilization of exogenous citrate requires the presence of citrate transport proteins (permeases). Upon uptake by the cell, citrate is cleaved by citrate lyase to oxaloacetate and acetate. The oxaloacetate is then metabolized to pyruvate and CO₂.
\[
\text{citrate} = \text{oxaloacetate} + \text{acetate} \\
\text{oxaloacetate} = \text{pyruvate} + \text{CO}_2 \\
\text{pyruvate} = \text{acetate} + \text{formate (Alkaline condition)} \\
\text{pyruvate} = \text{acetate} + \text{lactate} + \text{CO}_2 \text{ (Neutral to acidic conditions)} \\
\text{pyruvate} = \text{acetoin} + \text{CO}_2 \text{ (Neutral to acidic conditions)}
\]

The carbon dioxide, released will react with water and the sodium ion in the medium to produce sodium carbonate, an alkaline compound that will raise the pH. In addition, ammonium hydroxide is produced when the ammonium salts in the medium are used as the sole nitrogen source. The bromothymol blue pH indicator is a deep forest green at neutral pH. With an increase in medium pH (to above 7.6), bromothymol blue changes to blue.

Table 4.9: Composition of Simmons citrate media.

<table>
<thead>
<tr>
<th>Components</th>
<th>Amount (g/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Magnesium sulphate</td>
<td>0.200</td>
</tr>
<tr>
<td>Ammonium dihydrogen phosphate</td>
<td>1.000</td>
</tr>
<tr>
<td>Dipotassium phosphate</td>
<td>1.000</td>
</tr>
<tr>
<td>Sodium citrate</td>
<td>2.000</td>
</tr>
<tr>
<td>Sodium chloride</td>
<td>5.000</td>
</tr>
<tr>
<td>Bromothymol blue</td>
<td>0.080</td>
</tr>
<tr>
<td>Agar</td>
<td>15.000</td>
</tr>
</tbody>
</table>

**Final pH (at 25°C) 6.8±0.2**

Procedure: The media powder 24.28 gm was dissolved in water and volume made up to 1L and boiled. It was dispersed in sugar tubes and autoclaved at 15psi for 15 minutes. The media was allowed to settle as slants. The tubes were streaked using a loop with 24 hours old culture on MRS plate. The tubes were incubated at 37°C for 24 hours and observed for color change.

4.2.2.8 Sugar fermentation (Barker, 1956)

Bacteria can utilize different Carbohydrates as their energy source depending upon the presence of the specific enzymes involved in metabolism. The presence of such enzymes enables bacteria to metabolize different carbohydrates which help us in identification.

Table 4.10: Composition of minimal media used sugar utilization.

<table>
<thead>
<tr>
<th>Components</th>
<th>Amount (g/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peptone</td>
<td>10</td>
</tr>
<tr>
<td>Sodium chloride</td>
<td>5</td>
</tr>
<tr>
<td>Phenol red</td>
<td>0.1</td>
</tr>
<tr>
<td>Agar</td>
<td>20</td>
</tr>
</tbody>
</table>

Sugar disc used are Arabinose (Ar), cellobiose (Ce), xylose (Xy), inulin (In), lactose (La), mannose (Mo), raffinose (Rf), rhamnose (Rh) and trehalose (Te)

Procedure: All the components were dissolved in water and volume made up to 1000 ml and autoclaved at 15 psi for 15 minutes. Agar plates were prepared. 100 ??l of inoculums was spread on the agar plates from 24 hours old culture in MRS-broth and incubated for 20 minutes at 37oC. Sugar discs were placed on the agar plates using forceps and incubated at 37oC for 24 hours. The change in color is noted.

4.2.3 Genus specific PCR

PCR technology is a time effective and recent approach used for identification of bacteria in comparison with physiological and biochemical methods. This helps us to identify bacterial genus by targeting intergenic fragment between 16S rRNA and 23S rRNA (highly conserved gene fragment) (Gaudana et al., 2010). The intergenic fragment was targeted by primer set

**LB 1 (forward primer)** 5'??-TGGAAAACAGGTGCTAATA-3'??
**LB 2 (reverse Primer)** 5'??-GTCCATTGTGGAAGATTC-3'??

Colony PCR: The LB 1 was supplied at quantity of 56.6 nmol and LB 2 at 60.4 nmol. The primer concentration was made to 100 ??M by using 566 ??l and 604 ??l of sterile water. Primer concentration was diluted by using 90??l sterile water and 10??l of Primer and used as working stock solution having concentration of 10 ??M. Colony PCR was performed by inoculating single colony in 25 ??l sterile water and incubated at 95oC for 20 minutes.
Table 4.11: PCR reaction mixture.

<table>
<thead>
<tr>
<th>Components</th>
<th>Amount (??l)</th>
</tr>
</thead>
<tbody>
<tr>
<td>DNA template (colony suspension)</td>
<td>5.0</td>
</tr>
<tr>
<td>Taq Buffer (10X)</td>
<td>2.5</td>
</tr>
<tr>
<td>MgCl2 (10mM)</td>
<td>2.5</td>
</tr>
<tr>
<td>Forward primer (10mM)</td>
<td>0.5</td>
</tr>
<tr>
<td>Reverse primer (10mM)</td>
<td>0.5</td>
</tr>
<tr>
<td>dNTPs (10mM)</td>
<td>0.5</td>
</tr>
<tr>
<td>Taq polymerase (1U/??l)</td>
<td>0.625</td>
</tr>
<tr>
<td>Water</td>
<td>12.875</td>
</tr>
<tr>
<td>TOTAL VOLUME</td>
<td>25</td>
</tr>
</tbody>
</table>

The PCR reactions were done in thermo cycler (Mastercycler nexus gradient, Eppendorf, Germany), which was programmed as

<table>
<thead>
<tr>
<th>Reaction Temperature (oC)</th>
<th>Time (seconds)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial denaturation</td>
<td>95</td>
</tr>
<tr>
<td>Denaturation</td>
<td>95 45</td>
</tr>
<tr>
<td>45</td>
<td>45</td>
</tr>
<tr>
<td>45</td>
<td></td>
</tr>
<tr>
<td>Annealing</td>
<td>57</td>
</tr>
<tr>
<td>Extension</td>
<td>72</td>
</tr>
<tr>
<td>Final extension</td>
<td>72 420</td>
</tr>
<tr>
<td>Hold</td>
<td>4</td>
</tr>
</tbody>
</table>

Table 4.12: Composition of buffers and solutions used.

| EDTA (0.5M, pH 8)                           | 186.1 g       |
|                                            | Water 800.0 ml|
| pH was adjusted to 8 using sodium hydroxide and volume made up to 1000 ml with water. |
| TAE Buffer (50X, pH 8)                     | 242.0 g       |
| Tris base                                 | 57.1 ml       |
| Glacial acetic acid                       | 80.0 ml       |
| EDTA (0.5 M, pH 8)                         | 100.0 ml      |
| Volume made up to 1000 ml with water.     |
| Agarose gel loading buffer (6X)            |               |
| Bromophenol blue                          | 0.03 % (w/v)  |
| Xylene cyanol FF                          | 0.03% (w/v)   |
| Glycerol 60 % (w/v)                       |               |
| Tris HCl 10 mM                            |               |
| EDTA 60 mM                                 |               |

Agarose gel electrophoresis: Agarose (1.0%, w/v) was dissolved in TAE buffer and was heated till it was completely dissolved. Once cooled to 45oC ethidium bromide was added to make concentration of 0.5 ?? g/ml, mixed properly and dispensed onto gel casting tray containing comb. On solidification the gel is placed in electrophoresis chamber containing 1X TAE buffer and was loaded with PCR products mixed with 1X loading dye (dye: sample; 5:1). A 100 base pair ladder was used as a standard marker. Agarose gel run was at 100V. After this, the gel was observed under UV light and documented using gel documentation system (Biorad, USA).

4.3. Maintenance of Lactobacillus cultures

Maintenance of bacterial cultures is an important part of the study. Long term storage under proper conditions helps in preservation of bacterial cultures for future use. Bacterial cultures were grown in MRS broth for overnight. 500 ??l of culture was mixed with 500 ??l of sterile glycerol (50%, v/v) in autoclaved cryovials. These vials were vortixed, labeled properly and stored at -80oC (80oC Ultra low temperature freezer, New Brunswick Scientific, USA)

4.4. Probiotic attributes
4.4.1 Lysozyme tolerance (Saran et al., 2012)
Lysozyme (present in saliva) has antimicrobial activity which targets gram positive bacteria as they break the cell walls made of peptidoglycans. The probiotic organism should have the ability to tolerate the lysozyme for its effective health benefits. The tolerance is checked by supplementing the MRS broth with 1% lysozyme.
Procedure: MRS broth containing 1% lysozyme was inoculated with Lactobacillus isolate (grown for 16-18 hours) and simultaneously MRS broth was also inoculated (control). Inoculated media is incubated at 37°C for 4 hours under static condition. Samples were taken at the interval of one hour and were serially diluted and plated on MRS-agar plates. The plates were incubated at 37°C for 24 hours and the numbers of viable cells [colony forming units (cfu/ml)] were enumerated. The tolerance to lysozyme was calculated in terms of percentage survivability, the formula used:-
\[
\text{Percentage survivability} = \frac{\log_{10} \text{viable cells after 4 hours of incubation}}{\log_{10} \text{viable cells inoculated initially}} \times 100
\]

4.4.2 Low pH tolerance (Gaudana et al., 2010)
Another barrier is the extreme acidic pH of the stomach for the microorganisms. For Lactobacillus to confer health benefits, it should be acid tolerant at low pH (2.5) and this tolerance is checked by growing the Lactobacillus in MRS broth (pH of 2.5). The pH of the media is adjusted using 1N HCl.
Procedure: MRS broth at pH 2.5 was inoculated with Lactobacillus isolate (grown for 16-18 hours) and simultaneously MRS broth at pH 7.0 was also inoculated (control). Inoculated media is incubated at 37°C for 4 hours under static condition. Samples were taken at the interval of one hour and were serially diluted and plated on MRS-agar plates. The plates were incubated at 37°C for 24 hours and the numbers of viable cells [colony forming units (cfu/ml)] were enumerated. The tolerance to low pH was calculated in terms of percentage survivability, the formula used:-
\[
\text{Percentage survivability} = \frac{\log_{10} \text{viable cells after 4 hours of incubation}}{\log_{10} \text{viable cells inoculated initially}} \times 100
\]

4.4.3 Bile tolerance (Gaudana et al., 2010)
The bile salts secreted in human gut is yet another way of reducing bacterial (gram positive) population. Therefore for Lactobacillus isolate to deliver health benefits should reach intestine in viable number and also should be tolerant to bile salts. This tolerance is checked by growing the Lactobacillus in MRS broth containing 0.5% Ox bile.
Procedure: MRS broth containing 0.5% Ox bile was inoculated with Lactobacillus isolate (grown for 16-18 hours) and simultaneously MRS broth was also inoculated (control). Inoculated media is incubated at 37°C for 4 hours under static condition. Samples were taken at the interval of one hour and were serially diluted and plated on MRS-agar plates. The plates were incubated at 37°C for 24 hours and the numbers of viable cells [colony forming units (cfu/ml)] were enumerated. The tolerance to bile salts was calculated in terms of percentage survivability, the formula used:-
\[
\text{Percentage survivability} = \frac{\log_{10} \text{viable cells after 4 hours of incubation}}{\log_{10} \text{viable cells inoculated initially}} \times 100
\]

4.4.4 Bacterial surface hydrophobicity (Shimoni et al., 2010)
Specific and nonspecific interactions are involved in the adhesion, hydrophobic interactions (nonspecific) play a role in adherence can be tested using a xylene-water system (BATH-bacterial adherence to hydrocarbons). Hydrophobicity is an important attribute for probiotic organisms (Das and Goyal, 2014). It tests the ability to adhere and colonize ileum and colon so that it confers health benefits. More the cell surface hydrophobicity more is their ability to adhere on intestinal epithelial cells.
Table 4.13: Composition of Phosphate buffer saline (10mM, pH7.0).

<table>
<thead>
<tr>
<th>Components</th>
<th>Amount (g/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sodium chloride</td>
<td>8.0</td>
</tr>
<tr>
<td>Dipotassium hydrogen phosphate</td>
<td>1.16</td>
</tr>
<tr>
<td>Dihydrogen potassium phosphate</td>
<td>0.25</td>
</tr>
<tr>
<td>Potassium chloride</td>
<td>1.24</td>
</tr>
</tbody>
</table>

The volume was made up to 1000ml with water and ph adjusted to 7.0 with the help of 1N HCl.
Procedure: The Lactobacillus isolates were grown in MRS broth at 37°C for 16-18 hours and then centrifuged at 10000 X g for 10 minutes to obtain cell pellet. The pellet was washed using PBS buffer and resuspended...
the pellet in PBS buffer and adjusted the absorbance to 1.0 at 560nm (A0) using PBS buffer blank. Three ml of resuspended cells (at A0=1.0) was added to a test tube and 600 ?l of xylene was added to it. These tubes were vortexed for 90 seconds and the tubes at 37oC for 20 minutes. After this incubation period, the aqueous layer is carefully taken and absorbance at 560 nm is noted. The Bacterial Cell Surface hydrophobicity was calculated using the formula:-
Hydrophobicity (%) = \( \frac{A1}{(A0-A1)} \times 100 \)

4.4.5 Antimicrobial activity (Saran et al., 2012)
Common enter pathogens of humans are Esherichia coli ATCC 25922, Acinetobacter baumannii ATCC 19606, Shigella flexneri (clinical isolate), Staphylococcus aureus ATCC 25923, Klebsiella pneumoniae (clinical isolate), Enterococcus faecalis MTCC 439, Pseudomonas aeruginosa ATCC 27853, Proteus vulgaris (clinical isolate). The Lactobacillus should have antimicrobial activity against such organisms preventing humans for specific diseases. The agar well method was used to test antimicrobial activity against enter pathogens.

Table 4.14: Composition of Nutrient agar.

<table>
<thead>
<tr>
<th>Composition</th>
<th>Amount (g/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peptic digest of animal tissue</td>
<td>5.000</td>
</tr>
<tr>
<td>Sodium chloride</td>
<td>5.000</td>
</tr>
<tr>
<td>Beef extract</td>
<td>1.500</td>
</tr>
<tr>
<td>Yeast extract</td>
<td>1.500</td>
</tr>
<tr>
<td>Agar</td>
<td>15.000</td>
</tr>
<tr>
<td>Final pH (at 25??C)</td>
<td>7.4??0.2</td>
</tr>
</tbody>
</table>

Procedure: The Lactobacillus isolates were grown in MRS broth at 37oC for 16-18 hours and then centrifuged at 10000 X g for 10 minutes to obtain cell free supernant (CFS). The CFS was collected in fresh MCT's. The NA plates were spread plated with diluted 16-18 hours old cultures (100??l culture + 900??l PBS) of enter pathogens. The wells of 9 mm diameter were punctured in the agar plates. 150??l of CFS was added to the wells and plates were incubated at 37oC for 12 hours and were examined for inhibition zone diameter (clear zone around the well).

4.4.6 Biocompatibility test
Lactobacillus isolates to be used in the form of cocktails should have biocompatibility with one another. Biocompatibility amongst the selected strains is tested on MRS agar plates.

Procedure: The isolates were streaked perpendicular to each other on MRS agar plates and incubated at 37oC for 24 hours. After incubation the plates were checked for the growth of isolates.

RESULTS
5.1. Isolation of Lactobacillus from dairy products.
Dairy (curd and milk) samples were used for isolating the Lactobacillus with help of MRS media. MRS agar containing bromocersol purple dye (BCP) (as indicator) was used to isolate acid producing colonies; the dye changes its color from purple to yellow in the presence of acid producing colonies as represented in the figure 5.1.

Figure 5.1: Photographic images representing growth on MRS-BCP agar of acid producing lactobacilli. (A) plate showing initial color of bromocresol purple dye (B) plate showing acid producing colonies (color change to yellow) 24 hours of incubation at 37oC.

5.2. Identification of acid producing bacteria as Lactobacillus
5.2.1. Physiological identification
6 curd samples and 3 milk samples were processed obtaining 142 acid producing colonies which were streaked on MRS agar plates and subjected to Gram staining for Gram character and morphology. After 24 hours of incubation of streaked MRS agar plates, the Lactobacillus grows as very small (pin point) sized, elevated, having entire margins and white to creamish white color (figure5.2).

Figure 5.2: Photographic images representing growth of acid producing colonies (C2.9 isolate) on MRS agar plates (A) growth after 24 hours of incubation (B) growth after 48 hours of incubation.

These 142 isolates were subjected to Gram staining. Isolates with Gram positive character and rod like morphology selected for further test as this criterion makes them putative Lactobacillus isolates. 120 isolates out of 142 qualified as potential lactobacilli with Gram positive character and rod like morphology (Figure 5.3).
Gram character and morphology of the isolates selected for study is given in the table 5.1.

Table 5.1: Gram character and morphology of the lactobacilli isolates.

<table>
<thead>
<tr>
<th>Isolate</th>
<th>Gram Character</th>
<th>Morphology</th>
</tr>
</thead>
<tbody>
<tr>
<td>C1.2</td>
<td>Positive</td>
<td>Long thin singular rods</td>
</tr>
<tr>
<td>C1.3</td>
<td>Positive</td>
<td>Small rods in chains</td>
</tr>
<tr>
<td>C1.6</td>
<td>Positive</td>
<td>Singular coccobacillus</td>
</tr>
<tr>
<td>C1.11</td>
<td>Positive</td>
<td>Thick singular rods</td>
</tr>
<tr>
<td>C1.12</td>
<td>Positive</td>
<td>Small rods in branched chains</td>
</tr>
<tr>
<td>C1.17</td>
<td>Positive</td>
<td>Thick rods in Chains</td>
</tr>
<tr>
<td>C1.25</td>
<td>Positive</td>
<td>Long thin rods in bunches</td>
</tr>
<tr>
<td>C2.8</td>
<td>Positive</td>
<td>Long thin rods</td>
</tr>
<tr>
<td>C2.9</td>
<td>Positive</td>
<td>Small rods</td>
</tr>
<tr>
<td>C2.19</td>
<td>Positive</td>
<td>Thick coccobacilli</td>
</tr>
<tr>
<td>C2.23</td>
<td>Positive</td>
<td>Small thick rods</td>
</tr>
<tr>
<td>C2.32</td>
<td>Positive</td>
<td>Long thin singular rods</td>
</tr>
<tr>
<td>C3.1</td>
<td>Positive</td>
<td>Small rods</td>
</tr>
</tbody>
</table>

Figure 5.3: Microscopic image (100X magnification) of C2.19 isolate.

5.2.2. Biochemical identification

These putative lactobacilli isolates were further subjected to various biochemicals for identification purpose. 38 isolates out of 60 isolates were catalase negative, which were further subjected to other biochemical test such as utilization of sugars based on triple sugar iron agar, ability to ferment glucose anaerobically in HL media and urea producing ammonia, utilization of citrate as carbon and energy source, ability to produce cytochrome oxidase and deaminase enzymes and utilization of various sugars as energy source. Total 19 isolates were identified as Lactobacillus. Isolates showing following biochemical characteristics mentioned the table 5.2 and figure 5.4 were identified as Lactobacillus. Table 5.2 also shows biochemicals for Lactobacillus plantarum and Lactobacillus acidophilus.

Table 5.2: Biochemical characteristics of the lactobacilli isolates.

<table>
<thead>
<tr>
<th></th>
<th>Ca</th>
<th>Ci</th>
<th>HL</th>
<th>Ox</th>
<th>PPA</th>
<th>TSI</th>
<th>Ur</th>
<th>Ar</th>
<th>Ce</th>
<th>In</th>
<th>La</th>
<th>Mo</th>
<th>Rf</th>
<th>Rh</th>
<th>Te</th>
<th>Xy</th>
</tr>
</thead>
<tbody>
<tr>
<td>C1.2</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>C1.3</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>C1.6</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>C1.11</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>C1.12</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>C1.17</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>C1.25</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>C2.8</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>C2.9</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>C2.19</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>C2.23</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>C2.32</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>C3.1</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>S1</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>S2</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>S3</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

’ (positive); – (negative)

Ca: catalase; Ci: citrate; HL: Hugh Leifson- anaerobic fermentation of glucose; Ox: oxidase; PPA: paraPhenlyalanine agar; TSI: triple sugar iron agar (A/A- acidic slant acidic butt as positive); Ur: urease agar; Ar: arabinose; Ce: cellobiose; In: inulin; La: lactose; Mo: mannose; Rf: raffinose; Rh: rhamnose; Te: trehalose; Xy: xylose

S1: Lactobacillus sporogenes; S2: Lactobacillus acidophilus; S3: Lactobacillus casei shirota

Figure 5.4: Photographic images of test tubes of biochemicals and plates for sugar utilization. Positive control (+C), negative control (-C) and test (T). (1) HL media-anaerobic fermentation of glucose, (2) TSI agar slants-
utilization of sugars, (3) Simmon citrate agar slants-citrate utilization, (4) Urease agar slants-conversion of
urea to ammonia, (5) PPA slants- production of deaminase, (6) sugar utilization.

5.2.3. PCR based identification (isolates belonging to Lactobacillus genus)

13 isolates out of 19 (biochemically recognized) were subjected to Lactobacillus genus specific PCR based
identification for confirmation. These 13 isolates amplified 250 bp intergenic gene fragment (16S rRNA- 23S
rRNA) specific for Lactobacillus genus. Remaining 6 were considered as false positives. Figure 5.5 shows the
amplified bands (250 bp) on 1% agarose gel electrophoresis.

Figure 5.5: PCR DNA product analysis on 1% agarose gel; Lane 1: 100bp marker; Lane 2: positive control (L.
sporogenes’ BIFILAC); Lane 3: positive control (L. acidophilus – BIGLAC); Lane 4: positive control (L. casei
shirota – YAKULT); Lane 5: negative control (Escherchia coli); Lane 6: C1.2 isolate; Lane 7: C2.8 isolate; Lane 8:
C3.1 isolate.

5.3 Probiotic attributes of lactobacilli isolates

All 13 isolates and 3 standard isolates (Lactobacillus sporogenes, Lactobacillus acidophilus and Lactobacillus
casei shirota) belonging to Lactobacillus genus were tested for probiotics characteristics such as tolerance to
acid, bile and lysozyme; cell surface hydrophobicity and antimicrobial activity.

5.3.1. Lysozyme tolerance

All 13 isolates and 3 standard isolates were test for tolerance against 1% lysozyme in MRS growth media for 4
hours and all isolates (C1.2, C1.3, C1.6, C1.11, C1.12, C1.17, C1.25, C2.8, C2.9, C2.19, C2.23, C2.32, C3.1)
showed complete tolerance to lysozyme with negligible log reduction as compared to control. No isolate was
inhibited by 1% lysozyme in the growth medium. Whereas, L. sporogenes, L. acidophilus and L. casei shirota
also exhibit 100% survivability (Table 5.3). The growth curve for 4 hours is represented in the figure 5.6.

5.3.2. Acid tolerance

All 13 isolates and 3 standard isolates were tested for tolerance against MRS growth media at pH 2.5 for 4 hours
and 7 (C1.2, C1.3, C1.11, C2.8, C2.9, C2.23, C3.1) isolates showed complete tolerance to acidic conditions
exhibiting more than 100% survivability with negligible log reduction and 3 isolates (C1.6, C2.19, C2.32) having
survivability in range of 90% – 100% with comparable log reduction to control. 3 isolates (C1.12, C1.17,
C1.25) was inhibited by MRS growth medium at pH 2.5. Whereas, L. sporogenes, L. acidophilus and L. casei
shirota exhibit 100% survivability (Table 5.3). The growth curve for 4 hours is represented in the figure 5.6.

5.3.3. Bile tolerance

All 13 isolates and 3 standard isolates were tested for tolerance against 0.5% Ox bile in MRS growth media for 4
hours. 10 isolates (C1.3, C1.6, C1.11, C1.12, C1.17, C1.25, C2.9, C2.23, C2.32, and C3.1) showed complete
tolerance to bile with negligible log reduction as compared to control having more than 100% survivability.
C1.2 isolate having 93.44% survivability comparable log reduction to control and 2 isolates (C2.8 and C2.19)
are having 76.33% and 76.11% survivability respectively. Whereas, L. sporogenes, L. acidophilus and L. casei
shirota exhibit 100% survivability (Table 5.3). The growth curve for 4 hours is represented in the figure 5.6.

5.3.4. Cell surface hydrophobicity

All 13 isolates and 3 standard isolates were subjected to BATH test to determine the hydrophobicity of
lactobacilli isolates cell surface. Out of 13, 7 isolates (C1.2, C1.6, C1.12, C1.25, C2.9, C2.19, C2.23, C2.32)
showed hydrophobicity of more than 60%, 3 isolates (C1.3, C1.11, C1.17) having hydrophobicity in range of
50% – 60% and 2 isolates (C2.8 and C3.1) having hydrophobicity of 35.47% and 11.32% respectively. Whereas,
L. sporogenes showed 86.64%, L. acidophilus 8.93% and L. casei shirota 72.27% hydrophobicity (Table 5.3).

Table 5.3: Probiotic attributes of the lactobacilli isolates.

<table>
<thead>
<tr>
<th>Sr. no</th>
<th>Isolates</th>
<th>Acid tolerance (%)</th>
<th>Bile tolerance (%)</th>
<th>Hydrophobicity (%)</th>
<th>Lysozyme tolerance (%)</th>
<th>Rank</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>S1</td>
<td>100</td>
<td>100</td>
<td>86.64</td>
<td>100</td>
<td>1</td>
</tr>
<tr>
<td>2</td>
<td>S2</td>
<td>100</td>
<td>100</td>
<td>8.93</td>
<td>100</td>
<td>2</td>
</tr>
<tr>
<td>3</td>
<td>S3</td>
<td>100</td>
<td>100</td>
<td>72.27</td>
<td>100</td>
<td>3</td>
</tr>
<tr>
<td>4</td>
<td>C2.9</td>
<td>100</td>
<td>100</td>
<td>87.13</td>
<td>100</td>
<td>4</td>
</tr>
<tr>
<td>5</td>
<td>C2.23</td>
<td>100</td>
<td>100</td>
<td>66.01</td>
<td>100</td>
<td>5</td>
</tr>
<tr>
<td>6</td>
<td>C1.2</td>
<td>100</td>
<td>93.44</td>
<td>88.57</td>
<td>100</td>
<td>6</td>
</tr>
<tr>
<td>7</td>
<td>C1.6</td>
<td>90.53</td>
<td>100</td>
<td>74.03</td>
<td>100</td>
<td>7</td>
</tr>
<tr>
<td>8</td>
<td>C2.32</td>
<td>91.15</td>
<td>100</td>
<td>64.59</td>
<td>100</td>
<td>8</td>
</tr>
<tr>
<td>9</td>
<td>C1.11</td>
<td>100</td>
<td>55.44</td>
<td>100</td>
<td></td>
<td>9</td>
</tr>
</tbody>
</table>
5.3.5. Antimicrobial activity

All 13 isolates and 3 standard isolates were subjected to agar well diffusion assay to demonstrate antimicrobial activity of cell free supernatant against common enteropathogens (Acinetobacter baumannii, Escherchia coli, Pseudomonas aeruginosa, Proteus vulgaris, Shigella flexneri, Klebsiela pneumoniae, Enterococcus faecalis, Staphylococcus aureus). None of the isolates showed activity against P. vulgaris and L. casei shirota did exhibit antimicrobial activity against any of the tested enteropathogen. All 13 isolates exhibit anti Enterococcus activity and 12 isolates (C1.2, C1.3, C1.6, C1.11, C1.12, C1.17, C1.25, C2.8, C2.9, C2.19, C2.23, C2.32) exhibit anti Shigella activity (with highest by C1.6 of 12 mm inhibition zone); 10 isolates (C1.3, C1.6, C1.11, C1.12, C1.17, C1.25, C2.8, C2.9, C2.23, C2.32) exhibit anti Staphylococcus activity with highest of 14 mm inhibition zone and 10 isolates which showed anti Pseudomonas activity are C1.2, C1.3, C1.6, C1.11, C1.12, C1.17, C1.25, C2.8, C2.9 and only 3 isolates (C2.19, C2.23, C2.32) exhibit anti Acinetobacter activity. L. sporogenes showed anti Enterococcus activity with 4 mm inhibition zone and L. acidophilus exhibit anti microbial activity against S. flexneri, K. pneumoniae, E. faecalis, P. aeruginosa, A. baumannii. 6 isolates (C1.3, C1.6, C1.11, C1.12, C1.17, C1.25) exhibit broad spectrum antimicrobial activity (against 6 enteropathogens tested) and 3 isolates exhibit anti microbial activity against 5 enteropathogens. Whereas only L. acidophilus exhibit anti microbial activity against 5 enteropathogens and L. sporogenes exhibit antimicrobial against only 1 enteropathogen. Details mentioned in the table 5.4 and zone of inhibition depicted in the figure 5.7.

Figure 5.7: Photographic images of agar plates representing zone of inhibition. (13:C2.23; 14:C2.32; 15:C3.1)

Table 5.4: Antimicrobial activity of the lactobacilli isolates (zone diameter in mm).

<table>
<thead>
<tr>
<th>Isolates</th>
<th>A.b</th>
<th>E.c</th>
<th>P.a</th>
<th>P.v</th>
<th>S.f</th>
<th>K.p</th>
<th>E.f</th>
<th>S.a</th>
</tr>
</thead>
<tbody>
<tr>
<td>C1.2</td>
<td>5</td>
<td>2</td>
<td>10</td>
<td>4</td>
<td>5</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C1.3</td>
<td>4</td>
<td>6</td>
<td>9</td>
<td>5</td>
<td>4</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C1.6</td>
<td>5</td>
<td>2</td>
<td>12</td>
<td>6</td>
<td>3</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C1.11</td>
<td>4</td>
<td>4</td>
<td>7</td>
<td>5</td>
<td>6</td>
<td>4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>C1.12</td>
<td>3</td>
<td>3</td>
<td>8</td>
<td>4</td>
<td>6</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C1.17</td>
<td>3</td>
<td>5</td>
<td>6</td>
<td>4</td>
<td>3</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C1.25</td>
<td>4</td>
<td>5</td>
<td>9</td>
<td>4</td>
<td>3</td>
<td>4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>C2.8</td>
<td>3</td>
<td>--</td>
<td>4</td>
<td>--</td>
<td>4</td>
<td>2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>C2.9</td>
<td>4</td>
<td>--</td>
<td>9</td>
<td>5</td>
<td>2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C2.19</td>
<td>4</td>
<td>4</td>
<td>5</td>
<td>9</td>
<td>5</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C2.23</td>
<td>4</td>
<td>5</td>
<td>6</td>
<td>5</td>
<td>14</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C2.32</td>
<td>7</td>
<td>4</td>
<td>6</td>
<td>4</td>
<td>7</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C3.1</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>3</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>S1</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>4</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>S2</td>
<td>5</td>
<td>5</td>
<td>6</td>
<td>7</td>
<td>6</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>S3</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

A.b: Acinetobacter baumannii; E.c: Escherchia coli; P.a: Pseudomonas aeruginosa; P.v: Proteus vulgaris; S.f: Shigella flexneri; K.p: Klebsiela pneumoniae; E.f: Enterococcus faecalis; S.a: Staphylococcus aureus
5.3.6. Biocompatibility test

Biocompatibility of isolates was tested to make sure that they do not inhibit each other and could be used in combination for combined probiotic effects. All 13 isolates were tested with one another on MRS agar plates as shown in figure 5.8 and all isolates were biocompatible with each other and can be used in various combinations.

Figure 5.8: Photographic images of agar plates representing biocompatibility of lactobacilli isolate (C2.9) with other isolates.

DISCUSSION

Probiotics as stated by a report by FAO and WHO in 2001 are ‘live microorganisms which when administered in adequate amounts confer a health benefit on the host’. They play a very important role in human health as they are used for treatment and prevention of any diseases in humans such as diarrhea (Dubey et al., 2008; Szajewska et al., 2001; McFarland, 2007; Ooi et al., 2009), IBD (Sokol et al., 2008), NEC (Bin-Nun et al., 2005; Lin et al., 2005), high lipid content (Tsai et al., 2014), Candidal vaginitis (Alvarez-Olmos and Oberhelman, 2001), antibiotic associated diarrhea (Ruszczynski et al., 2008). Use of probiotics as mucosal vaccines and immunomodulation because of their safety, ability to persist with indigenous flora, adjuvant properties and low intrinsic immunogenicity; Prevention of transmission of AIDS and STDs – the production of H2O2 by particular species of Lactobacillus is essential in the regulation of the vaginal flora as it is the most potent microbicide present in the human vagina and lactobacilli given at high concentrations is viricidal for HIV-1 (Hillier, 1998); Infection control programs and eradication of multidrug-resistant microorganisms, a case report describes a 68-year-old woman from Japan with a decubitus colonized by methicillin-resistant Staphylococcus aureus was successfully treated with a Lactobacillus preparation (Kimura et al., 1997);

Antibacterial effects, probiotic agents have specific activity against several pathogens, including Listeria monocytogenes, Salmonella thymurium, E. coli, and Helicobacter pylori. Therefore, probiotic agents may provide prototype antimicrobial substances that will be useful for pharmaceutical companies in the development of new antibiotics (Alvarez-Olmos and Oberhelman, 2001).

Lactobacillus produces lactic acid as a part of its metabolism which has antimicrobial activity (Neal-McKinney, 2012), this lactic acid produced converts purple color of the bromocresol purple dye to yellow (Lee and Lee, 2008), which acts as pH indicator and the presence of dye makes the media selective for acid producing colonies such as Lactobacillus (Ashraf and Shah, 2011). Bergey’s Manual of Systematic Bacteriology (Volume 3, 2009) states that Lactobacillus cells are Gram stain positive; vary from long, slender to bent rods, short, often coryneform coccobacilli and chain formation common. Here, following this criterion 120 isolates out of 142 qualified as potential lactobacilli (Figure 5.3; Table 5.1) and we concluded that, isolates showing Catalase, Oxidase, Urease, Citrate, paraPhenlyalanine negative; Hugh Leifson aerobic and anaerobic fermentation of glucose; Triple Sugar Iron as acidic slant acidic butt and sugar fermentation as arabinose, cellobiose, inulin, raffinose and rhamnose as variable; lactose, mannose, trehalose and xylose as positive fermentation were identified to belong to Lactobacillus genus (Bergey’s Manual of Systematic Bacteriology (Volume 3, 2009); Difco manual 11th edition, 1998). Total 19 isolates were identified as Lactobacillus, showing biochemical characteristics mentioned the table 5.2 and figure 5.4.

Dubernet et al., 2002 have developed a PCR based method for the identification of lactobacilli at the genus level by amplifying 16S rRNA and 23S rRNA intergenic fragment. This identification method has been used by Gaudana et al., 2010 and many others. For conformation if isolates belong to Lactobacillus genus they were tested for the presence of amplified 16S rRNA and 23S rRNA intergenic fragment (250bp) by using LB 1 (forward) and LB2 (reverse) primers (Byun et al., 2004) 13 isolates out of 19 amplified this fragment (250bp) other 6 were considered as false positives.

These 13 isolates identified as Lactobacillus were tested for their probiotic attributes such as acid, bile and lysozyme tolerance; cellular hydrobhobicity and antimicrobial activity to know there potential for use by humans (Gaudana et al., 2010; Das and Goyal, 2014; Saran et al., 2012).

Out of 7 isolates 10 isolates show 100% tolerance to acidic conditions (pH 2) namely C2.9, C2.23, C1.2, C1.11, C1.3, C3.1 and C2.8; 3 isolates (C1.6, C2.32 and C2.19) show tolerance less than 100% (>90%) and isolates namely C1.25, C1.12, C1.17 are not tolerant to acidic conditions. For an organism to exhibit probiotic effect it should be tolerant to acidic conditions in the stomach (Gaudana et al., 2010; Tokatl?? et al., 2015; Kaushik et
al., 2009), this eliminates C1.25, C1.12 and C1.17 from being used as probiotics. Tokatl?? et al., 2015 reported acid tolerance for various Lactic acid bacteria and highest reported is 85% by L. plantarum MF303 and also states that Lactobacillus has higher resistance to low pH conditions. Kaushik et al., 2009 reported a strain of Lactobacillus Lp9 has reported tolerance of 94% after 2 hours and to be a good candidate for use as probiotic, these two works stand in support.

Tolerance to bile salts is also an essential requirement to qualify as a probiotic organism (Gaudana et al., 2010; da Cunha et al., 2012; Tokatl?? et al., 2015). 10 out of 13 isolates show 100% tolerance to 0.5% Oxbile with (C2.9, C2.23, C1.6, C2.32, C1.11, C1.3, C3.1, C1.25, C1.12 and C1.17) with 1 isolate (C1.2) having 93.44% bile tolerance and 2 isolates C2.8 and C2.19 with 76% tolerance. Tokatl?? et al., 2015 reports L. brevis isolates with more than 90% tolerance and few L. plantarum isolates with tolerance as high as 99% and da Cunha et al., 2012 reports the bile tolerance of L. gasseri isolates, in the presence of 0.5% oxgall and found that most of the strains were sensitive to bile but the strain UFVCC 1091 showed the highest growth of 66% as compared to control.

Saran et al., 2012 states that resistance to lysozyme is important as it has antimicrobial activity against Gram positive bacteria and thus the isolates were tested for lysozyme tolerance and results showed 100 % tolerance by all the isolates. Garc??a-Ruiz et al., 2014 states that tested isolates of Pediococcus spp. showed more than 80%, Lactobacillus more than 60% tolerance to lysozyme whereas Oenococcus oeni showed low tolerance of 30% – 60%. Yadav et al., 2015 Cellular hydrophobicity plays an important role in functions of probiotic organisms as it determines the adherence of the bacterial (probiotic) cells to the intestinal cells (Das and Goyal, 2014). The isolates test showed hydrophobicity in range from 11.32% to 87.13%. Kumar et al., 2012 reported that four Lactobacillus spp., isolated from fermented food exhibit more than 60% cell surface hydrophobicity and Tokatl?? et al., 2015 found hydrophobicity values ranging from 0.66% to 82.41% for L. plantarum and from 0.17 to 97.96% for L. brevis. Kaushik et al., 2009 reports cell surface hydrophobicity of L. plantarum Lp9 strain to be 37.1%.

All 13 isolates were subjected to agar well diffusion assay to demonstrate antimicrobial activity of cell free supernatant against common enteropathogens (A. baumannii, E. coli, P. aeruginosa, P. vulgaris, S. flexneri, K. pneumoniae, E. faecalis, S. aureus) (Gaudana et al., 2010; de Cunhna et al., 2012; Kaushik et al., 2009; Munoz-Quezada et al., 2013). These 6 isolates C1.25, C1.3, C1.6, C1.11, C1.12 and C1.17 exhibit broad range of antimicrobial activity against 6 enteropathogens (E. coli, P. aeruginosa, S. flexneri, K. pneumoniae, E. faecalis, S. aureus); whereas C2.32 also show antimicrobial activity against 6 different set of pathogens listed in table 5.4. Isolates C2.9, C1.2 and C2.23 exhibit antimicrobial activity 5 enteropathogens, each against different set of organisms (Table 5.4) whereas C3.1 has activity against only E. faecalis and C2.8 had anti microbial activity against only 4 enteropathogens (Table 5.4). Lactobacillus paracasei CNCM I- 4034, Bifidobacterium breve CNCM I- 4035 and Lactobacillus rhamnosus CNCM I- 4036 probiotic strains tested by Munoz-Quezada et al. have antimicrobial activity against Listeria monocytogenus and rotavirus reported by (Munoz-Quezada et al., 2013); L. plantarum Lp9 reported by Kaushik et al. have antimicrobial activity against 5 enteropathogen (E. coli, L. monocytogenus, S. aureus, Salmonella typhi and Bifidobacterium cereus) and Lactobacillus gasseri is reported for antimicrobial activity against E. coli, L. monocytogenus, S. aureus and Salmonella typhimurium. (de Cunhna et al., 2012).

It is a task to find a strain, having all desirable probiotic attributes and the selection of the potential probiotic candidate is important as it depends on the purpose of the product to be obtained from it. Isolates such as C1.25, C1.12 and C1.17 exhibit 100% lysozyme, bile tolerance and 70.03%, 63.30% and 51.58% hydrophobicity respectively but these isolates are sensitive to acidic conditions and thus these cannot be used as probiotics and are marked red in the table 5.3 (Gaudana et al., 2010; Das and Goyal, 2014; Saran et al., 2012) where as Munoz-Quezada et al. in 2013 reported the use of 2 strains namely Lactobacillus paracasei CNCM I-4034 and Bifidobacterium breve CNCM I-4035 which are sensitive to acidic condition as probiotics (Munoz-Quezada et al., 2013). The isolates C2.9 and C2.23 exhibit 100% acid, bile and lysozyme tolerance and hydrophobicity of 87.13% and 66.01% respectively, higher than L. sporogenes (86.64%) component of BIFILAC, Tablets (India) Limited and L. casei shirota (72.27%) part of Yakult and L. acidophilus (8.93%) component of BIGLAC, Foregen Healthcare Limited, in case of C2.9 and C2.23 ranked 2 for their probiotic potential (hydrophobicity lower than L. sporogenes and L. casei shirota but higher than L. acidophilus) and thus these isolates hold very good potential for further investigation in vivo studies to evaluate their potential health benefits and their...
There are reports which cite even 60% of hydrophobicity for potential probiotic candidates such as Tokatl?? et al. in 2015, namely L. brevis MF 494 and L. brevis MF493 having 67.29% and 62.36% hydrophobicity respectively. Followed by these 2 isolates C1.2 with 100% acid and lysozyme, 93.44% bile tolerance and 88.57% hydrophobicity next are C1.6 and C2.32 with 100 bile and lysozyme tolerance, 90.53% and 91.15% acid tolerance respectively and 74.03% and 64.59% hydrophobicity respectively and then C1.11, C1.3 and C3.1 with 100% acid bile and lysozyme tolerance and 55.44%, 53.96% and 11.32% hydrophobicity respectively followed by C2.8 and C2.19 with 100% lysozyme tolerance, 100% and 95.44% acid tolerance, 76.33% and 76.11% bile tolerance respectively and 35.47% and 85.02% hydrophobicity respectively.

These 8 isolates also hold a potential to be used for their probiotic properties as these have better or comparable probiotic attributes with the potential strains cited in the literature such as L. plantarum MF 143 and L. plantarum MF 169 with 70% and 76% respectively (Tokatl?? et al., 2015); L. rhamnosus CNCM I- 4036 with 76.2% (Munoz-Quezada et al., 2013); and Pediococcus parvulus CIAL-16 and Pediococcus parvulus CIAL-85 with 86.57% and 86.97% respectively (Garcia-Ruiz et al., 2014) acid tolerance and the lactobacilli isolates from curd exhibit acid tolerance in the range of 90.53% to 100%; bile tolerance range is 76.11% to 100% whereas Tokatl?? et al. in 2015 reported 2 strains of L. plantarum MF 556 and MF 33 with 45% and 69% bile tolerance; 11 lactobacilli isolates out of 13 have hydrophobicity above 50% and 2 isolates C3.1 and C2.8 with 11.32% and 35.47% hydrophobicity whereas literature cites as low as 7.5% and 11.6% hydrophobicity by L. paracasei CNCM I-4034 and L. rhamnosus CNCM I-4036 respectively (Munoz-Quezada et al., 2013); 39.62% by L. plantarum MF 143 (Tokatl?? et al., 2015) and 50.8% by L. plantarum CIAL-121 (Garcia-Ruiz et al., 2014).

CONCLUSION

In this study, 13 isolates were identified as Lactobacillus from the used dairy products especially curd and subjected to acid, bile and lysozyme tolerance; and tested for cell surface hydrophobicity and antimicrobial activity. C2.9 Lactobacillus isolate exhibit higher probiotic attributes than the standards used (commercially available Lactobacillus) and 9 other isolates having comparable properties with the standards which holds a very good potential for further investigation to evaluate their potential health benefits and their application in the food industry.

REFERENCES

Barker, H.A. Bacterial fermentation. CIBA Lectures in Microbial Biochemistry. 1956.


Gomez-Llortente, C., A. Gil, M. Bermudez-Brito, J. Plaza-Diaz, S. Munoz-Quezada. Probiotic mechanisms of...


' https://unlockinglifescode.org/explore/genomic-medicine/microbiome


' Koser, S. A. Correlation of citrate utilization by members of the colon-aerogenes group with other differential characteristics and with habitat. J. Bacteriol. 1924; 9:59‘77.


' Lactobacillus acidophilus | University of Maryland Medical Center http://umm.edu/health/medical/altmed/supplement/lactobacillus-acidophilus#ixzz3auKUHZNn


' Metchnikoff, E., Optimistic studies New York: Putnam’s Sons. 1908; 161-183.


PTI May 7, 2012, 11.15AM IST THE ECONOMICS TIMES


Salminen, S., A.von Wright, L. Morelli, P. Marteau, D. Brassart, W.M. de Vos, R. Fonden, M. Saxelin, K. Collins,


APPENDIX

Table 1: Bacterial members of human gut microbiota.
Phylum Family Genus and Species Reference
Actinobacteria Actinomycetaceae Actinomyces oris Lagier et al., 2012
Actinomyces urogenitalis Favier et al., 2002
Arconobacterium pyogenes Taylor et al., 1985
Trueperella bernardiae Yassin et al., 2011; Lagier et al., 2012
Bogoriellaceae Georgenia muralis Lagier et al., 2012
Brevibacteriaceae Brevibacterium casei Lagier et al., 2012
Brevibacterium epidermidis Lagier et al., 2012
Brevibacterium massiliense Pfleiderer et al., 2013
Brevibacterium senegalense Lagier et al., 2012
Cellulomonadaceae Cellulomonas compositi Lagier et al., 2012
Cellulomonas denverensis Lagier et al., 2012
Cellulomonas parahominis Lagier et al., 2012
Cellulomonas massiliensis Lagier et al., 2012
Corynebacteriaceae Corynebacterium appendicis Lagier et al., 2012
Corynebacterium glucuronolyticum Lagier et al., 2012
Corynebacterium ulcerans Drasar & Hill, 1974
Corynebacterium xerosis Drasar & Hill, 1974
Dermabacteraceae Brachybacterium paraconglomeratum Manichanh et al., 2008
Dermabacter hominis Lagier et al., 2012
Dermacoccaceae Dermacoccus nishinomiyaensis Lagier et al., 2012
Kytoococcus Schroeteri Lagier et al., 2012
Kytoococcus sedentarius Pfleiderer et al., 2013
Dietziaceae Dietzia cinnamomea Lagier et al., 2012
Dietzia maris Lagier et al., 2012
Dietzia natronolimniae Lagier et al., 2012
Geodermatophilaceae Blastococcus massiliensis Pfleiderer et al., 2013
Gordoniaceae Gordonia rubripertincta Lagier et al., 2012
Gordonia terrae McLaughlin et al., 2010
Intra sporangiaceae Janibacter limosus Hoyles et al., 2012
Janibacter terrae McLaughlin et al., 2010
Microbacteriaceae Microbacterium foliorum Lagier et al., 2012
Pseudoclavibacter massiliense Lagier et al., 2012
Microbacterium lacticum Lagier et al., 2012
Agrococcus jejuensis Lagier et al., 2012
Micrococciaceae Micrococcus luteus Finegold et al., 1974
Arthrobacker albus Lagier et al., 2012
Arthrobacker oxydans Lagier et al., 2012
Micrococcus lylae Pfleiderer et al., 2013
Micromonoaporaceae Micromonospora aurantiaca Lagier et al., 2012
Mycobacteriaceae Mycobacterium avium Thorel et al., 1990
Mycobacterium abscessus Lagier et al., 2012
Mycobacterium florentinum Tortoli et al., 2005
Mycobacterium fortuitum Lagier et al., 2012
Norcardiaceae Rhodococcus equi Lagier et al., 2012
Rhodococcus erythropolis Walker et al., 2011
Rhodococcus rhodochrous Lagier et al., 2012
Promicrocmonosporaceae Promicrocmonospora flava Lagier et al., 2012
Cellulosimicrobiun cellulans Lagier et al., 2012
Propionibacteriineae Propionibacterium acidipropioni Ramotar et al., 1984
Aeromicrobiun massiliense Lagier et al., 2012
Propionibacterium jensenii Taylor et al., 1985
Propionibacterium avidum Eggerth, 1935
Streptomycetaceae Streptomyces massiliensis Pfleiderer et al., 2013
Streptomyces misionensis Lagier et al., 2012
Streptomyces thermovulgaris Dubourg et al., 2013
Streptomyces thermoviolaceus Hoyles et al., 2012
Micrococcineae Tropheryma whippeli Raoult et al., 2010
Timonella senegalenesis Lagier et al., 2012
Bifidobacteriaceae Bifidobacterium adolescentis Reuter, 1963
Bifidobacterium bifidum Tissier, 1900
Bifidobacterium breve Reuter, 1963
Bifidobacterium dentium Scardovi & Crociani, 1974
Bifidobacterium longum Reuter, 1963
Bifidobacterium pseudolongum Moore & Holdeman, 1974
Bifidobacterium ruminantium Lagier et al., 2012
Bifidobacterium scardovii Favier et al., 2002
Coribacteriaceae Collinsella intestinalis Kageyama & Benno, 2000
Gordonibacter pamelaeae W?rdenmann et al., 2009
Olsenella uli Lagier et al., 2012
Senegalemassilia anaerobia Lagier et al., 2012
Bacteroidetes Bacteroidaceae Bacteroides coprophilus Hayashi et al., 2007
Bacteroides intestinalis Bakir et al., 2005
Bacteroides uniformis Eggerth & Gagnon, 1933
Bacteroides vulgates Eggerth & Gagnon, 1933
Porphyromonadaceae Tannerella forsythia Macfarlane et al., 2004
Porphyromonas gingivalis Bik et al., 2006
Parabacteroides johnsonii Sakamoto et al., 2007
Barnesiella intestinhominis Morotomi et al., 2008
Prevotellaceae Prevotella copri Hayashi et al., 2007
Prevotella denticola Bik et al., 2006
Prevotella melaninogenica Shah & Collins, 1990; Mitsuoka, 1992
Prevotella ruminicola Holdeman et al., 1976; Avgustin et al., 1997
Rikenellaceae Alistipes onderdonkii Song et al., 2006
Alistipes indistinctus Nagai et al., 2009
Alistipes timonensis Lagier et al., 2012
Alistipes obesi Lagier et al., 2012
Cytophagaceae Dyadobacter beijingensis McLaughlin et al., 2010
Dyadobacter fermentans Frank et al., 2007
Hymenobacter rigui Frank et al., 2007
Rudanella lutea Frank et al., 2007
Flavobacteriaceae Capnocytophaga granulose Bik et al., 2006
Chryseobacterium hominis McLaughlin et al., 2010
Flavobacterium banpakuense Frank et al., 2007
Flavobacterium cheniæ Frank et al., 2007
Chitinophagaceae Bifissio spartinae McLaughlin et al., 2010
Sphingobacteriaceae Sphingobacterium multivorum Lagier et al., 2012
Pedobacter daejeonensis McLaughlin et al., 2010
Deinococcus- Thermus Deinococcaceae Deinococcus aquaticus Lagier et al., 2012
Firmicutes Halobacteroidaceae Halanaerobaculum tunisiense Lagier et al., 2012
Alicyclobacillaceae Tumebacillus permanentifrigoris Lagier et al., 2012
Bacillaceae Aeribacillus pallidus Lagier et al., 2012
Bacillus infantis Lagier et al., 2012
Bacillus subtilis Batchelor, 1919
Virgibacillus proomii Hoyles et al., 2012
Bacillales Incerta Sedis XI Exiguobacterium aurantiacum Lagier et al., 2012
Gemella haemolysans McLaughlin et al., 2010
Gemella morbillorum Holdeman & Moore, 1974
Gemella sanguinis Lagier et al., 2012
Listeriaceae Brochothrix thermosphacta Walker et al., 2011
Paenibacillaceae Brevibacillus brevis Taylor et al., 1985; Shida et al., 1996
Paenibacillus antibioticophila Dubourg et al., 2013
Paenibacillus illinoisensis Lagier et al., 2012
Paenibacillus lactis Lagier et al., 2012
Planococcaceae Kurthia timonensis Lagier et al., 2012
Lysinibacillus fusiformis Pfleiderer et al., 2013
Planococcus rifietoensis Lagier et al., 2012
Ureibacillus suwonensis Lagier et al., 2012
Staphylococcaceae Staphylococcus cohnii Lagier et al., 2012
Staphylococcus epidermidis Moore & Holdeman, 1974
Staphylococcus hominis Lagier et al., 2012
Staphylococcus intermedium Dubourg et al., 2013
Aerococcaceae Abiotrophia defective Lagier et al., 2012
Abiotrophia para-adiacens Bik et al., 2006
Aerococcus viridians Lagier et al., 2012
Facklamia tabacinasalis Pfleiderer et al., 2013
Carnobacteriaceae Granulicatella adiacens Lagier et al., 2012
Granulicatella elegans Walker et al., 2011
Enterococcaceae Enterococcus caccae Carvalho et al., 2006
Enterococcus durans Alston, 1928
Enterococcus faecium Finegold et al., 1974
Enterococcus hirae Robredo et al., 2000
Lactobacillaceae Lactobacillus acidophilus Moro, 1900
Lactobacillus brevis Benno et al., 1989
Lactobacillus casei Moore & Holdeman, 1974
Lactobacillus delbrueckii Finegold et al., 1974
Lactobacillus johnsonii Reuter, 2001
Lactobacillus oris Reuter, 2001
Lactobacillus plantarum Finegold et al., 1974
Lactobacillus vaginalis Dal Bello & Hertel, 2006
Leuconostocaceae Leuconostoc argentinium Heilig et al., 2002
Leuconostoc gelidum Lagier et al., 2012
Leuconostoc mesenteroides Dal Bello & Hertel, 2006
Weissella cibaria Bjorkroth et al., 2002
Streptococcaceae Lactococcus lactis Finegold et al., 1974
Streptococcus bovis Finegold et al., 1974
Streptococcus infantis Lagier et al., 2012
Streptococcus oralis Baker et al., 2000
Christensenellaceae Christensenella minuta Morotomi et al., 2012
Catabacter hongkongensis Lau et al., 2007; Pfleiderer et al., 2013
Clostridiaceae Clostridium fallax Finegold et al., 1974
Clostridium neonatal Li et al., 2008
Clostridium beijerinckii Finegold et al., 1977
Eubacterium multiforme Distaso, 1911
Ruminococcaceae Butyrivibrio fibrisolvens Eeckhaut et al., 2012
Clostridium viride Zupanic et al., 2012
Faecalibacterium prausnitzii Moore & Holdeman, 1974; Duncan et al., 2002
Ruminococcus flavefaciens Finegold et al., 1974
Family XIII Incerta Sedis Eubacterium brachy Woodmansey et al., 2004
Eubacterium sulci Lagier et al., 2012
Mogibacterium neglectum Bik et al., 2006
Mogibacterium vesum Wang et al., 2005
Peptostreptococcaceae Anoxynatronum sibiricum Peris-Bondia et al., 2011
Clostridium difficile Finegold et al., 1974
Filifactor villosus Lagier et al., 2012
Peptostreptococcus stomatis Lagier et al., 2010
Family XI Incerta Sedis Anaerococcus obesiensis Lagier et al., 2012
Anaerococcus vaginalis Mangin et al., 2004; Eckburg et al., 2005
Peptoniphilus grossensis Lagier et al., 2012
Peptoniphilus obesiensis Lagier et al., 2012
Lacnospiraceae Blautia faecis Park et al., 2012
Butyrivibrio crosstus Moore et al., 1976
Clostridium lactatifermentans Song et al., 2002
Clostridium lactatifermentans Song et al., 2002
Eubactaeae Anaerofustis stercorihominis Finegold et al., 2004
Eubacterium Barkeri Finegold et al., 1974
Eubacterium calnderi Lagier et al., 2012
Eubacterium limosum Moore & Holdeman, 1974
Erysipelotrichaceae Turicibacter sanguinis Cu??v et al., 2011
Erysipelotrichaceae XVI Clostridium innocuum Finegold et al., 1974
Eubacterium biforme Holdeman & Moore, 1974
Eubacterium cylindroids Cato et al., 1974
Streptococcus pleomorphus Ley et al., 2006
Erysipelotrichaceae XVII Coprobacillus cateniformis Kageyama & Benno, 2000
Eggerthia catenaformis Eggerth, 1935; Salvetti et al., 2011
Kandleria vitulina Mitsuoka, 1992; Salvetti et al., 2011
Stoquefichus massiliensis Pfleiderer et al., 2013
Erysipelotrichaceae XVIII Anaerorhabdus furcosa Moore & Holdeman, 1974; Shah & Collins, 1986
Clostridium ramosum Moore & Holdeman, 1974
Holdemania filiformis Willems et al., 1998
Solobacterium moorei Kageyama & Benno, 2000
Acidomonococcaceae Acidaminococcus fermentans Moore & Holdeman, 1974
Acidaminococcus intestine Jumas-Bilak et al., 2007
Phascolarctobacterium faecium Hayashi et al., 2002
Phascolarctobacterium succinatutens Watanabe et al., 2011
Peptococcaceae Peptococcus niger Van Eldere et al., 1987
Desulfotobacterium frappieri Van de Pas et al., 2001
Veillonellaceae Allisonella histaminiformans Hayashi et al., 2002
Mitsuokella jalaludinii Ley et al., 2006
Selenomonas ruminantium Benno et al., 1986
Veillonella rogosae Pfleiderer et al., 2013
Fusobacteria Fusobacteriaceae Clostridium rectum Benno et al., 1984
Fusobacterium naviforme Holdeman et al., 1976
Fusobacterium periodonticum Wang et al., 2005
Fusobacterium varium Benno et al., 1989
Letotrichiaceae Leptotrichia amnionii Bik et al., 2006; Gill et al., 2006
Leptotrichia buccalis Drasar & Hill, 1974
Lentisphaerae Victivallaceae Victivallis vadensis Zoetendal et al., 2003
Planctomycetes Planctomycetaceae Schlesneria paludicola Lagier et al., 2012
Proteobacteria Caulobacteraceae Brevundimonas bacteroides Bik et al., 2006
Brevundimonas diminuta Walker et al., 2011
Brevundimonas terrae Ley et al., 2006
Brevundimonas vesicularis McLaughlin et al., 2010
Aurantimonadaceae Aurantimonas altamirensis McLaughlin et al., 2010
Bradyrhizobiaceae Bradyrhizobium denitrificans Bik et al., 2006
Bradyrhizobium elkanii Walker et al., 2011
Bradyrhizobium japonicum Lepage et al., 2011
Afpia birgiae McLaughlin et al., 2010
Brucellaceae Ochrobactrum anthrophi Holmes et al., 1988
Ochrobactrum intermedium M??ller et al., 1999
Hyphomicrobiaceae Pedomicrobium ferrugineum Bik et al., 2006
Methyllobacteriaceae Methyllobacterium adhaesivum McLaughlin et al., 2010
Methyllobacterium jeotgali McLaughlin et al., 2010
Methyllobacterium populi Turnbaugh et al., 2009
Microvirga massiliensis Lagier et al., 2012
Phyllobacteriaceae Mesorhizobium loti McLaughlin et al., 2010
Phyllobacterium myrsinacearum Mai et al., 2013
Rhizobiaceae Agrobacterium tumefaciens Walker et al., 2011
Xanthobacteraceae Ancylobacter polymorphus Lagier et al., 2012
Rhodobacteraceae Paracoccus carotinifaciens McLaughlin et al., 2010
Paracoccus marinus McLaughlin et al., 2010
Paracoccus yeei McLaughlin et al., 2010
Amaricoccus kaplicensis McLaughlin et al., 2010
Acetobacteraceae Roseomonas mucosa McLaughlin et al., 2010
Rhodospirillaceae Skermanella aerolata McLaughlin et al., 2010
Sphinogomonadaceae Blastomonas natatoria Dubourg et al., 2013
Sphingomonas panni Ley et al., 2006
Sphingomonas pseudosanguinis Lagier et al., 2012
Sphingomonas adhaesiva McLaughlin et al., 2010
Achromigenaceae Achromobacter denitrificans Finegold et al., 1974
Achromobacter xylosoxidans Lagier et al., 2012
Achromigenes faecalis Petruschky, 1896
Bordetella hinzii Walker et al., 2011
Burkholderiaceae Burkholderia cepacia Wang et al., 2003
Lautropia mirabilis Bik et al., 2006
Limnobacter thiooxidans McLaughlin et al., 2010
Ralstonia mannitolilytica Zoetendal et al., 2002
Comamonadaeae Acidovorax facilis McLaughlin et al., 2010
Comamonas testosterone McLaughlin et al., 2010
Delftia acidovorans Bik et al., 2006
Variovorax boronicumulans McLaughlin et al., 2010
Oxalobacteraceae Herbaspirillum massiliense Lagier et al., 2012
Massilia aurea McLaughlin et al., 2010
Oxalobacter formigenes Allison et al., 1985
Sutterellaceae Parasutterella secunda Morotomi et al., 2011
Sutterella parvirubra Sakon et al., 2008; Williams et al., 2012
Sutterella stercoricanis Williams et al., 2012
Sutterella wadsworthensis Wexler et al., 1996
Neisseriaceae Kingella oralis Bik et al., 2006
Neisseria elongate Bik et al., 2006
Neisseria flavescens Lagier et al., 2012
Neisseria mucosa Lagier et al., 2012
Rhodocyclaceae Methyloversatilis universalis McLaughlin et al., 2010
Desulfovibrionaceae Desulfovibrio desulfuricans Gibson et al., 1991
Desulfovibrio fairfieldensis Loubinoux et al., 2002
Desulfovibrio piger Moore et al., 1976
Bilophila wadsworthia Baron, 1997
Campylobacteraceae Bacteroides ureolyticus Benno et al., 1989
Campylobacter faecalis Kachler et al., 2000
Campylobacter fetus Veron & Chatelain, 1973
Campylobacter hominis Lawson et al., 2001
Helicobacteraceae Flexispira rappini Archer et al., 1988
Helicobacter Canadensis Fox et al., 2000
Helicobacter cinaedi Engberg et al., 2000
Helicobacter pullorum Stanley et al., 1994
Aeromonadaceae Aeromonas allosaccharophila Janda & Abbott, 1998
Aeromonas caviae Kirov et al., 1999
Aeromonas jandaei Janda & Abbott, 1998
Aeromonas tecta Demarta et al., 2008
Succinivibrionaceae Anaerobiospirillum thomasii Malnick, 1997
Anaerobiospirillum succiniciproducens Malnick, 1997
Succinatimonas hippie Morotomi et al., 2009
Succinivibrio dextrinosolvens Lagier et al., 2012
Enterobacteriaceae Citrobacter freundii Finegold et al., 1974
Escherichia coli Moore & Holdeman, 1974
Klebsiella pneumoniae Moore & Holdeman, 1974
Proteus vulgaris M??ller, 1986
Pasteurellaceae Actinobacillus pleuropneumoniae Bik et al., 2006
Aggregatibacter aphrophilus Bik et al., 2006; N??rskov-Lauritsen & Kilian, 2006
Haemophilus parainfluenzae Lagier et al., 2012
Haemophilus sputorum Li et al., 2008
Moraxellaceae Acinetobacter baumannii Pandey et al., 2012
Acinetobacter baumannii Pandey et al., 2012
Moraxella catarrhalis Drasar & Hill, 1974
Moraxella osloensis Lagier et al., 2012
Pseudomonadaceae Pseudomonas alcaliphila Ley et al., 2006
Pseudomonas fluorescens Lambert-Zechovsky et al., 1985
Pseudomonas oleovorans Lagier et al., 2012
Pseudomonas stutzeri Lagier et al., 2012
Vibrionaceae Vibrio fluvialis Lee et al., 1981
Vibrio furnissii Brenner et al., 1983
Vibrio mimicus Chowdhury et al., 1987
Vibrio parahaemolyticus Molenda et al., 1972
Sinobacteraceae Nevskia ramose Walker et al., 2011
Xanthomonadaceae Lysobacter soli Lagier et al., 2012
Rhodanobacter ginsenosidimutans Wang et al., 2003
Stenotrophomonas maltophilia Apisarnthanarak et al., 2003
Stenotrophomonas rhizophila Lagier et al., 2012
Spirochaetes Brachyspiraceae Brachyspira aalborgi Mikosza et al., 2001
Brachyspira pilosicoli Mikosza et al., 2001
Spirochaetaceae Treponema berlinense Tito et al., 2012
Synergistetes Synergistaceae Cloacibacillus evryensis Ganesan et al., 2008
Pyramidobacter piscolens Lagier et al., 2012
Tenericutes Mycoplasmataceae Mycoplasma pneumoniae Chen et al., 2001
### Table 2: Fungal members of human gut microbiota.

<table>
<thead>
<tr>
<th>Phylum Family</th>
<th>Genus and Species</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Amoebozoa</strong></td>
<td>Entamoebidae</td>
<td>Entamoeba hartmanni Fotedar et al., 2007; Hamad et al., 2013</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Entamoeba moshkovskii Fotedar et al., 2007</td>
</tr>
<tr>
<td><strong>Apicomplexa</strong></td>
<td>Cryptosporidiidae</td>
<td>Cryptosporidium hominis Ortega et al., 1994</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Cryptosporidium viatorum Elwin et al., 2012</td>
</tr>
<tr>
<td></td>
<td>Eimeriidae</td>
<td>Cyclospora cayetanensis Ortega et al., 1994</td>
</tr>
<tr>
<td></td>
<td>Sarcocystidae</td>
<td>Cystoisospora belli Modigliani et al., 1985</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Sarcocystis hominis Modigliani et al., 1985</td>
</tr>
<tr>
<td><strong>Ciliophora</strong></td>
<td>Balantidiidae</td>
<td>Neobalantidium coli Dobell &amp; O'Connor, 1921</td>
</tr>
<tr>
<td></td>
<td>Fungi-Ascomycota</td>
<td>mitosporic Davidiellaceae Cladosporium sphaerospermum Taylor et al., 1985</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Aureobasidium pullulans Chen et al., 2010</td>
</tr>
<tr>
<td></td>
<td></td>
<td>mitosporic Trichocomaceae Penicillium brevicompactum Gouba et al., 2013</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Penicillium chrysogenum Taylor et al., 1985</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Trichocomaceae Aspergillus vericolor Taylor et al., 1985</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Aspergillus flavipes Gouba et al., 2013</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Ajellomycetaceae Ajellomyces capsulatus Li et al., 2012</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Ajellomyces dermatitidis Li et al., 2012</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Sclerotiniaceae Botryotinia fuckeliana Ott et al., 2008</td>
</tr>
<tr>
<td></td>
<td>Diplodasaceae</td>
<td>Galactomyces geotrichum Hamad et al., 2013</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Geotrichum silvicola Lagier et al., 2012</td>
</tr>
<tr>
<td></td>
<td></td>
<td>mitosporic Saccharomycetales Candida kruisii Anderson, 1917; Biasoli et al., 2002</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Candida intermedia Chen et al., 2010</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Saccharomycetaceae Zygosachcharomycys bisporus Anderson, 1917</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Saccharomyces cerevisiae Nam et al., 2008</td>
</tr>
<tr>
<td></td>
<td></td>
<td>mitosporic Cordycipitaceae Isaria farinosa Gouba et al., 2013</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Beauveria bassiana Gouba et al., 2013</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Plectosphaerellaceae Verticillium leptobactrum Scanlan &amp; Marchesi, 2008</td>
</tr>
<tr>
<td></td>
<td>Fungi-Basidiomycota</td>
<td>Lyophyllaceae Asterophora parasite Hamad et al., 2013</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Psathyrellaceae Psathyrella candelleana Scanlan &amp; Marchesi, 2008</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Corticiaceae Phanerochaete stereoides Hamad et al., 2013</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Meruliaceae Bjerkandera adusta Hamad et al., 2013</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Agaricostilbaceae Sterigmatomyces elviae Hamad et al., 2013</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Malasseziaceae Malassezia pachydermatis Hamad et al., 2013</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Malassezia globosa Hamad et al., 2013</td>
</tr>
<tr>
<td></td>
<td></td>
<td>mitosporic Sporidiobolales Rhodotorula mucilaginosa Scanlan &amp; Marchesi, 2008</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Tremellaceae Cryptococcus neoformans Li et al., 2012</td>
</tr>
<tr>
<td></td>
<td>Cryptococcus</td>
<td>fragicola Chen et al., 2010</td>
</tr>
<tr>
<td></td>
<td></td>
<td>mitosporic Tremellales Trichosporon cutaneum Hamad et al., 2013</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Trichosporon asahii Hamad et al., 2013</td>
</tr>
<tr>
<td></td>
<td>Metamonada</td>
<td>Hexamitidae Giardia intestinalis Dobell &amp; O’Connor, 1921</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Enteromonadidae Enteromonas hominis Spriegl et al., 1989</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Retortamonadidae Retortamonas intestinalis Jones-Engel et al., 2004</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Microsporidia Enterocytozooidae Enterocytozoon bieneusi Desportes et al., 1985</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Unikaryonidae Encephalitozoon intestinalis Weber et al., 1994; Hartskeerl et al., 1995</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Parabasalia Dientamoebidae Dientamoeba fragilis Jepps &amp; Dobell, 1918</td>
</tr>
</tbody>
</table>
Trichomonadidae Pentatrichomonas hominis Meloni et al., 2011
Stramenopiles Blastocystis Blastocystis hominis Raso et al., 2005
Table 3: Archaeal members of human gut microbiota.
Phyllum Family Genus and Speces Reference
Thaumarchaeota Nitrososphaeraceae Nitrososphaera gargensis Rieu-Lesme et al., 2005
Crenarchaeota Sulfolobaceae Sulfolobus solfataricus Rieu-Lesme et al., 2005
Euryarchaeota Halobacteriaceae Halorubrum koreense Nam et al., 2008
Halococcus morrhuae Nam et al., 2008
Methanobacteriaceae Methanobrevibacter ruminantium Nottingham & Hungate, 1968
Methanosphaera stadtmannae Miller & Wolin, 1985
Methanobrevibacter smithii Miller & Wolin, 1985
unclassified Methanomicrobia Methanomassiliicoccus Dridi et al., 2012

About Essay Sauce

EssaySauce.com is a completely free resource to help students research their academic work and learn from great essays!

View all posts by Essay Sauce

...(download the rest of the essay above)
Latest reviews:

- Health essays
- Dissertation example
- Arabicized business terms
- Automotive Industry

Search for student essays:

Search ...

About EssaySauce, the student essay site:

EssaySauce.com is a free resource for students, providing thousands of example essays to help them complete their college and university coursework. Students can use our free essays as examples to write their own.
Latest student essays:

- Ocular disease
- HUMAN action recognition
- Analysing data production
- Desorption study
- Surfactants (surface active agents)
- Islamic Finance and Its Impact on Customer Satisfaction
- Persian gulf
- Feminist approach (Bhumika) (notes)
- What does it mean to be a Muslim woman in 21st century? (Shari’ah)
- Appellate Body's analysis under section XIV(c)

Student essay categories:

- Accounting essays
- Architecture essays
- Business essays
Computer science essays
Criminology essays
Economics essays
Education essays
Engineering essays
English language essays
English literature essays
Environmental studies essays
Finance essays
Geography essays
Health essays
History essays
Hospitality and tourism essays
Human rights essays
Information technology essays
International Relations
Law essays
Leadership essays
Linguistics essays
Literature essays
Management essays
Marketing essays
Media essays
Medicine essays
Miscellaneous essays
Music Essays
Philosophy essays
Photography and arts essays
Politics essays
Project management essays
Psychology essays
Religious studies and Theology essays
Science essays
Social work essays
Sociology essays
Uncategorized
Zoology essays

Average review:

Overall rating: 0 out of 5 based on 0 reviews.
Q: Is EssaySauce.com free?

Yes! EssaySauce.com is a completely free resource for students. You can view our terms of use here.

Why use Essay Sauce?

The brightest students know that the best way to learn is by example! EssaySauce.com has thousands of great essay examples for students to use as inspiration when writing their own essays.

Is Essay Sauce completely free?

Yes! EssaySauce.com is a completely free resource for students. You can view our terms of use here.

Info:

About
Content policy
Essay removal request
Privacy
Terms of use