

Classification, Antimicrobial Potential, Industrial Applications and Probiotic Capability of Lactic Acid *Bacteria*: A Review Article

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Abstract: Lactic Acid *Bacteria* (LAB) are economically important organisms and existed in foods, soil, intestinal gut of animals, used as starter cultures for food fermentations such as yoghurt, cheese, pickles, sausages, fermented fish and fermented fruits. LAB are gram positive, catalase negative and non-spore forming rods or cocci. LAB include recently many genera such as *Lactobacillus*, *Lactococcus*, *Leuconostoc*, *Carnobacterium*, *Streptococcus*, *Enterococcus*, *Vagococcus*, *Pediococcus*, *Allojococcus*, *Tetragenococcus* and *Weissella*. A key for differentiation of LAB into genera is illustrated in this review and is based on easily determinable characteristics allowing a rapid assignment of a new isolate to any LAB genera. LAB produce organic acids, diacetyl, H₂O₂, ethanol, acetaldehyde, bacteriocins and antifungal substances. They are recently used as starter cultures for food fermentations with their use as probiotics, since, they were approved recently to tolerate acidic conditions of stomach, tolerate bile acids and bile salts of intestine and attach smooth human tissues. The medicinal uses of probiotics are discussed in the present review.

Key words: Lactic Acid *Bacteria* (LAB), probiotics, starter, culture, industrial importance, produce organic

INTRODUCTION

Lab are a group of gram-positive, non-spore forming, cocci or rods which produce lactic acid as the major end product from carbohydrate fermentation. They consisted of many genera including *Aerococcus*, *Allojococcus*, *Carnobacterium*, *Enterococcus*, *Lactobacillus*, *Lactococcus*, *Leuconostoc*, *Pediococcus*, *Streptococcus*, *Tetragenococcus*, *Vagococcus* and *Weissella* (Stiles and Holzappel, 1997). LAB produce several compounds such as bacteriocins, H₂O₂, diacetyl, acetaldehyde, ethanol, antifungal proteins and CO₂ that can inhibit pathogenic and food spoilage *Bacteria* and improve the smell, colour and texture throughout food fermentations (Caplice and Fitzgerald 1999; Ross *et al.*, 2002). The antifungal agents produced by LAB can reduce the problem of toxinogenic moulds (Batish *et al.*, 1997). These microorganisms have beneficial effects in human health such as treating allergy, increasing immune system, reducing urogenital infections, treating the ulcers, preventing the initiation of colon cancer, decreasing the cholesterol level in the blood, treating the cases of diarrhea and constipation and treating lactose-intolerance cases (Holzappel and Schillinger, 2002).

LAB and their classification: LAB are gram-positive catalase negative rods their primary metabolite is lactic acid. LAB are commonly found in foods including fermented vegetables, fruits, beverages and dairy products. Currently, LAB comprise the Genera *Lactobacillus*, *Leuconostoc*, *Pediococcus*, *Carnobacterium*, *Lactococcus*, *Enterococcus*, *Aerococcus*, *Vagococcus* and *Streptococcus* (Nettle and Barefoot, 1993).

Lactobacilli: Lactobacilli include 3 groups: group 1, the obligately homo-fermentative lactobacilli which degrade hexose but not pentose, group 2, the facultatively hetero-fermentative lactobacilli which ferment hexose almost exclusively to a lactic acid, acetic acid and ethanol, group 3, the obligately hetero-fermentative lactobacilli. They ferment both hexoses and pentoses to lactic acid and ethanol (Enan *et al.*, 1996). *Lactobacillus* (*L.*) *plantarum* is a versatile and industrially important lactic acid bacterium which can be found in fermented pickles (Zago *et al.*, 2011a, b). It shows probiotic capabilities (Hebert *et al.*, 2000). The probiotic properties are resistance to biological barriers, the antimicrobial activity and cholesterol-lowering effect (Hebert *et al.*, 2000). The antimicrobial activity enable this bacterium to protect

the produced pickles this antimicrobial activity of *L. plantarum* is due to its secretion of lactic acid and bacteriocin (Enan *et al.*, 1996; Enan, 2006a-d; Enan *et al.*, 2013a-c, 2014a-c).

Leuconostoc: Cells of Leuconostocs are spherical or lenticular and occur in pairs or chains. They are facultative anaerobes. Leuconostocs are morphologically different from Lactobacilli (Garvie, 1986). However, physiologically they are quite similar to the gas-producing hetero fermentative Lactobacilli (Holzapfel and Schiliinger, 1992). The classification and identification of the *Leuconostoc* spp. has therefore been equivocal. A polybasic approach for their classification and identification is necessary (Fantuzzi *et al.*, 1992). The studies of DNA: DNA homology and rRNA similarity (Martinez-Murica and Collins, 1990, 1991a, b) differentiated the *Leuconostoc* species from lactobacilli. These studies added two new genera: *Leuconostoc paramesenteroides* and *Leuconostoc oenes* to the Genus *Leuconostoc* (Martinez-Murica and Collins, 1991b). The Genus *Leuconostoc* comprises the following species: *Leuconostoc mesenteroides*, *Leuconostoc dextranicum* (Beijerinck, 1972); *Leuconostoc cremories* (Knudsen and Sorensen, 1929); *Leuconostoc lactis* (Garvie, 1967); *Leuconostoc mesenteroides* subsp. *amelibiosum* (Hoover, 2000; Kandler, 1970); *Leuconostoc gelidum* and *Leuconostoc carnosum* and *Leuconostoc fallax* (Martinez-Murica and Collins, 1991a). *Leuconostoc amelibiosum* (Schilling *et al.*, 1987) which was formerly *Leuconostoc mesenteroides* subsp. *amelibiosum*. Both *Leuconostoc dextranicum* and *Leuconostoc cremoris* are now considered to be subspecies of *Leuconostoc mesenteroides* (Pot *et al.*, 1994).

Carnobacterium: The Genus *Carnobacterium* was proposed by Collins *et al.* (1987) to accommodate species of non-aciduric *Lactobacillus piscicola* and *Lactobacillus divergens*. The latter 2 species (atypical lactobacilli) were differentiated from *Lactobacillus* spp. by their inability to grow on acetate agar, the growth at high pH values of 8.5-9.5 and the synthesis of oleic instead of cis-vaccenic acid produced by Lactobacilli (Collins *et al.*, 1987). Based on these biological characteristics these two atypical Lactobacilli were allocated in a new genus, *Carnobacterium* as *Carnobacterium divergens* (formerly called *Lactobacillus divergens*) and *Carnobacterium piscicola* (formerly called *Lactobacillus piscicola*). Collins *et al.* (1987) created two new species, *Carnobacterium gallinarum*. *Carnobacterium* vmobile. The above four species of *Carnobacteria* showed a high degree of rRNA sequence

similarity with each other and formed a phylogenetically coherent group, quite from all LAB (Wallbanks *et al.*, 1990). From the recommendations reported by Collins *et al.* (1990), it is obvious that *Carnobacterium piscicola*, *Lactobacillus piscicola* (Hiu *et al.*, 1984) should be replaced by *Carnobacterium maltaromicus* *Lactobacillus maltaromicus* (Miller *et al.*, 1974) as both species showed 100% rRNA sequence similarity and because maltaromicus species was described earlier than *Lactobacillus piscicola*.

Streptococcus: The genus *Streptococcus* is reserved for non-spore forming coccoid or coccobacillary chemo-organotrophic microorganisms, arranged in pairs or chains. The *Streptococcus* species ferment carbohydrates to lactic acid as a major end-product and are generally aerotolerant. The optimum temperature for growth of these organisms is usually about 37°C but maximum and minimum temperatures vary among species. The species of the genus *Streptococcus* can roughly be divided into hemolytic species and the oral (viridans) species (Pot *et al.*, 1994).

Enterococcus: The genus *Enterococcus* includes the Enterococcal group of Streptococci (formerly faecal streptococci), possessing the group D antigen (Schleifer and Kilpper-Balz, 1984). Numerous species of the genus *Streptococcus* have been classified as enterococcal organisms belonging to the enterococcal group (Jones *et al.*, 1972). DNA: DNA and DNA; rRNA hybridization experiments (Kilpper-Balz *et al.*, 1982). Proved the identification of two species of the genus *Enterococcus* as *Enterococcus faecalis* and *Enterococcus faecium*, respectively (Schleifer and Kilpper-Balz, 1984). Since, this renaming in 1984, 17 other species have been included in the genus *Enterococcus*, either by transfer or by new valid description (Pot *et al.*, 1994).

Vagococcus: Sequence analysis of 16 SrRNA performed on Lactococci, Enterococci and motile N strains (Collins *et al.* (1989) showed a separate position of the Enterococci which are somewhat closer to strains of *Listeria* and *Bacillus* than to the Streptococci. From this study, it was shown that a motile group N Streptococcal strain seemed to branch as one single lineage with the Enterococci. This strain was isolated from chicken faeces (Hashimoto *et al.*, 1974) and reacted with group N antiserum and was further allocated to the so-called new genus *Vagococcus* and designated *Vagococcus fluviialis* (Collins *et al.*, 1989a). A second species, *Vagococcus salmoninarum* isolated from Salmonid fish has been described (Wallbanks *et al.*, 1990).

Pediococcus, Aerococcus, Tetragenococcus and Alloiococcus: Cells of these genera are morphologically similar. The spherical, divide in 2 planes at right angles to form tetrads, gram positive, non-motile, facultatively anaerobic and require a rich medium, containing complex growth factors to grow. All species of these genera grow at 300°C but optimum temperatures range from 25-400°C (Garvie, 1986; Pot *et al.*, 1994). At present, the genus *Pediococcus* includes eight species. However, *Aerococcus* comprises only one species, *Aerococcus viridans* (Pot *et al.*, 1994). The differentiation between the members of both genera equivocal by simple morphological or physiological tests. *Aerococcus viridans* and *Pediococcus urinaequi*, for example, are phenotypically very similar and DNA: DNA hybridizations (Dellaglio *et al.*, 1981) showed intrageneric relationships. The placement of *Pediococcus halophilus* in the genus *Pediococcus* has also been controversial as it phenotypically resembles *Aerococcus viridans* more closely than the *Pediococci*. DNA: DNA hybridizations (Dellaglio *et al.*, 1981), however, showed no intrageneric relationships. Collins *et al.* (1990) clarified this taxonomic problem by comparison of 16S rRNA sequence for all species of *Aerococcus* and *Pediococcus* (except *Pediococcus inopinatus*) and *Pediococcus halophilus* was transferred to the new genus *Tetragenococcus* as *Tetragenococcus halophilus* (Pot *et al.*, 1994). The genus *Alloiococcus* is one of the most recently described genera of LAB, created for an unknown bacterium isolated from middle ear fluids of children with persistent otitis. Only one species of this genus was described *Alloiococcus otitis* (Aguirre and Collins, 1992).

Weisella: *Weisella* belongs to the group of *Bacteria* known as LAB. They are gram-positive, catalase-negative, non-endospore forming cells and with cocci or rod shaped. *Weisella* species are obligately heterofermentative they can ferment carbohydrate and produce end products such as lactic acid, acetic acid, ethanol and CO₂. *Weisella* species generally, difficult to be distinguished from other heterofermentative cocci (*Leuconostoc*) or rod (*Lactobacillus*) strains on the basis of phenotypic or biochemical properties alone, so, the accurate identification of *Weisella* strains occur by molecular biological methods such as sequencing of 16SrRNA or other house keeping genes, DNA: DNA hybridization and by rep-PCR or fAFLP. Methods. phylogenetically, the *Weisella* belong to frimicutes, class: Bacilli, Order: Lactobacillales and Family: Leuconostoceae. They require complex nutritional requirements and need peptides, amino acids, fermentable carbohydrates, nucleic acids, fatty acids and vitamins for growth. All species can grow at 15°C and some can grow upto 42-45°C. There are 19 species of *Weisella* they found in vareity rang of habitats such as on the skin in the milk

and feces of animals. They have been isolated from saliva, breast milk, vagina of humans from plants and vegetables such as European and African traditional fermented foods. *Weisella* act as probiotic *Bacteria* where *W. cibaria* have a high probiotic potential for controlling diseases. Moreover, *W. confusa* and *W. cibaria* strains are producing amount of polymers such as oligopolysaccharides and extracellular polysaccharides mainly dextran that play major role in industrial applications such as beverage fermentation. Other strains of *Weisella* species such as *W. viridescens*, *W. cibaria* and *W. confusa* are pathogenic *Bacteria* causing infections to human such as bacteremia, Endocarditis. While *W. cети* strains act as etiological agent of “Weissellosis” which is a disease affecting farmed rainbow trout (Fusco *et al.*, 2015).

Key for differentiation of the genera of LAB: The LAB can be characterized as gram-positive, non-sporing microaerophilic *Bacteria* whose main fermentation product from carbohydrates is lactate (Kandler, 1983). Currently, they are subdivided into the genera *Lactobacillus*, *Carnobacterium*, *Leuconostoc*, *Streptococcus*, *Lactococcus*, *Enterococcus*, *Vagococcus*, *Pediococcus*, *Aerococcus*, *Tetragenococcus* and *Alloiococcus* (Pot *et al.*, 1994). A key for differentiation of LAB into genera is illustrated in Fig. 1. It is based on easily determinable “characteristics allowing a rapid assignment of a new isolate to any of the genera of LAB. This key is a modification of the differentiation scheme for LAB which has been laid by Schillinger and Lucke (1987). It includes the recent LAB genera: *Carnobacterium*, *Vagococcus*, *Aerococcus*, *Tetragenococcus* and *Alloiococcus*. It is also inserted additional physiological criteria for differentiation of lactobacilli. Gas production from glucose is used as a first step in the differentiation of LAB. For a classification of the homofermentative lactobacilli producing no gas from glucose their growth temperature (Schillinger and Lucke, 1987) and their ability to ferment pentoses (Pot *et al.*, 1994) were used as taxonomic criteria. The facultatively homofermentative strains (formerly called *Streptobacterium*) grow at 15°C and ferment pentoses whereas the obligately homofermentative strains (formerly called *Thermobacterium*) can't do so. The *Carnobacterium* species (the atypical *Lactobacilli*) grow on acetate agar where as *Lactobacillus* species do not. The heterofermentative *Lactobacilli* (formerly called *Betabacterium*) can be differentiated from *Leuconostoc*s by producing Ammonia from arginine (exceptions, *Lactobacillus fructosus*, *Lactobacillus viridescens*,

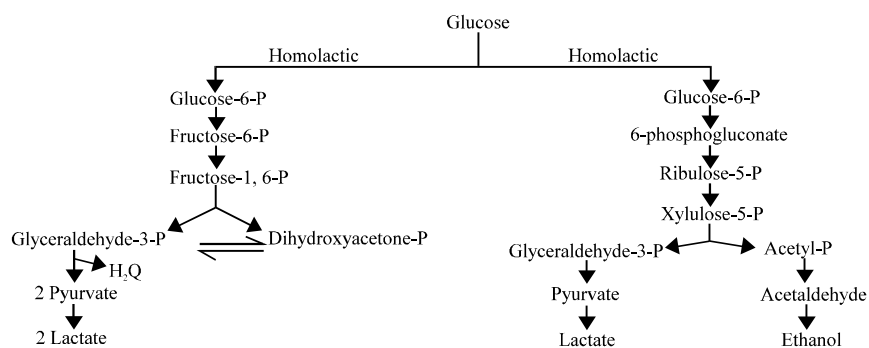


Fig. 1: Generalized scheme for the fermentation of glucose to lactic acid *Bacteria* (Caplice and Fitzgerald, 1999)

Lactobacillus sanfrancisco) and by forming DL lactic acid from glucose (exceptions, *Lactobacillus divergens*, *Lactobacillus carnis* both form L-lactic acid) whilst *Leuconostocs* don't hydrolyze arginine and form only D-lactic acid (Schillinger and Lucke, 1989). Growth at 45 or 10°C and on media supplemented with 6.5% NaCl are taxonomic criteria used for differentiation of Streptococcal genera (Fig. 1). Reaction with group N antiserum is used to allocate the new genus *Vagococcus* from the genus *Enterococcus*. An unknown strain, *Pediococci* like-strain is classified to the new genus *Alloioococcus*. The cells are further differentiated by 16S rRNA sequence analysis to the genera *Pediococcus*, *Aerococcus* and *Tetragenococcus*. The genus *Bifidobacterium* is not included in this key, since, its identification and its taxonomic position as a LAB are still unreliable (Pot *et al.*, 1994). However, few researchers recommended the classification of *Bifidobacterium* as LAB (Mitsuoka, 1984). Cultural characteristics and physiology of these *Bacteria* are similar to other *Bacteria* (*Lactobacillus*, *Corynebacterium* and *Actinomyces*). For more details about the taxonomic position of the *Bifidobacterium*, the reader is referred to the reports of many researchers viz. Biavati *et al.* (1991), Yaeshima *et al.* (1991) and Pot *et al.* (1994).

LAB AND THEIR USES IN FOODS

LAB are industrially important organisms recognized for their ability of fermentation due to their health and nutritional benefits (Gilliand, 1990). Species that belong to genera *Lactobacillus*, *Lactococcus*, *Streptococcus*, *Pediococcus*, *Leuconostoc* and newly recognized *Carnobacterium* are involved in fermentation processes. These organisms are isolated from dairy and meat

products, grains, plants, vegetables and the mucosal surfaces of animals (Lindgrea and Dobrogosz, 1990). They are used as starter cultures in the dairy, baking, meat, vegetables and alcoholic beverages industries. They produce compounds during lactic fermentations such as lactic acid, acetic acid, bacteriocins, diacetyl, ethanol and hydrogen peroxide (Lindgren and Dobrogosz, 1990). Not only this, compounds have beneficial effects for food taste, smell, color and texture but they also inhibit growth of pathogens and undesirable microflora (Rattanachaiakunsoon and Phumkhachorn, 2010).

Using LAB as starter cultures for food fermentations:

Starter cultures are defined as microbial preparation of one or more micro organisms that are supplied to a raw material to produce a fermented food (Ray, 1992; Caplice and Fitzgerald, 1999). Commercial starter cultures are used currently in industrial fermented foods and are available as frozen and freeze dried concentrates or lyophilized preparations (Sandine, 1996). These functional starter cultures that are used in food and beverage fermentation improve also the fermentation process, enhancing the quality of the end product (Leroy and De Vuyst, 2004). Selected starter cultures can remove formation of D-lactic acid or racemate of lactic acid (DL) or the formation of biogenic amines (Leroy and De Vuyst, 2004; Joosten *et al.*, 1995).

Starter cultures and functional starter in fermentation of foods:

LAB act as starter cultures in fermented foods and beverages because they can improve nutritional, organoleptic, technological and shelf-life characteristics of these foods (Wood and Holzappel 1995; Leroy and De Vuyst, 2004). LAB produce various organic acid from

carbohydrates by its initiation of rapid and adequate acidification of raw materials. The main acid produced by LAB are lactic acid followed by acetic acid; LAB can also produce ethanol, aroma compounds, Bacteriocins, exopolysaccharides and some enzymes (De Vuyst and Leroy, 2007). The production of fermented foods and

beverages was based on a spontaneous fermentation that occur by LAB. The addition of selected starter cultures to food matrix cause the high degree of fermentation process and the stability of the final product (Enan *et al.*, 2013b).

Starter cultures as probiotics: Successful starter cultures of LAB showed beneficial effects on human health through the consumption of yoghurts and fermented milk (Metchnikoff *et al.*, 1910). Yoghurt is manufactured using *Streptococcus thermophilus* and *Lactobacillus delbrueckii* ssp. *bulgaricus* as starter cultures (Shah, 2007). A manufacturer's selection of starter cultures strains occur by checking the following aspects (Tamine *et al.*, 2005; Enan *et al.*, 2014).

The ability of probiotic to show fast growth in medium to increase cell count. The ability of microorganisms to tolerance the freezing and drying stages of preparation. Tolerance of *Bacterial* stains to bile salts and the gastric acid during their passage through gastrointestinal tract, so, the probiotics must be stable for achieving health benefits (Tamine *et al.*, 2005). Resistance to antibiotics to allow growth in food preserved by either biocides or antibiotics and to resist antibiotics in vivo if they are ingested by humans as a probiotic capability and resistant to be lysed by bacteriophages.

Lab as starter cultures for making fermented dairy products: LAB are used as starter cultures in fermentation of dairy products. The main LAB that are used in dairy products fermentation are *Lactobacillus* spp. (*L. acidophilus*, *L. lactis*, *L. casei*, *L. plantarum*, *L. rhamnosus*, *L. reuteri*, *L. delbrueckii* ssp. *bulgaricus*) and the *Enterococcus* spp. (*E. faecalis*, *E. faecium*). *Carnobacterium* spp. and *Leuconostoc* spp. were also involved slowly in dairy products making (Shah, 2007; Tamine *et al.*, 2005). Dairy products are considered as ideal vehicles for delivering probiotics to the human gut such as Yoghurt, followed by cultured butter milk, cheeses, kefir, ice cream (Shah, 2007; Socal *et al.*, 2010; Tamine *et al.*, 2005) or frozen desserts as chocolate mousse (Aragon-Algero *et al.*, 2007). Moreover, proteolytic strains of LAB provide probiotics by releasing bioactive peptides called angiotensin and intum enzyme inhibitors which are examined for their hypotensive role

(Conlin *et al.*, 2000).

FUNCTIONAL STARTER CULTURES IN FERMENTED NON-DAIRY PRODUCTS

Fermented meat and meat products: The preservation of meat and meat products by fermentation were based on natural meat microorganisms. Pure strains of LAB meat starter cultures were used by Erkkila (2001) and are belong to *Pediococcus cerevisiae*. It was used for the immediate and rapid production of organic acids during fermentation process, resulting in shift of pH value towards acidic levels where pathogenic *Bacteria* will not grow (Ammor and Mayo, 2007). Therefore, the original characteristics of the produced foods and end-products are improved. The meat LAB starter cultures belong to the *Lactobacillus*, *Pediococcus* and *Carnobacterium* strains which isolated from dry sausages (Ammor and Mayo, 2007). Selected previous strains have the best survival activity under acidic conditions and high levels of bile salts. The starter cultures play an important roles by preservation of foods by inhibiting activity of pathogens and spoilage via. production of bacteriocin and biogenic amines (Ammor and Mayo, 2007).

Fermented vegetables: Fermentation of vegetables are commonly occurred by LAB strains that consume carbohydrates and fermenting them with production of organic acids. Usually fermented vegetable juices are produced from cabbage, red beet, carrot and tomato (Buruleanu *et al.*, 2010). LAB play an important role in pickles and table olives fermentation, affecting the final flavour and shelf-life (Fleming, 1984; Medina *et al.*, 2010).

Starter cultures in silages: Under anaerobic conditions, LAB can ferment carbohydrates into organic acids, mainly lactic acid or acetic and formic acids. LAB are added into silage to enhance lactic acid fermentation under anaerobic conditions. This can decrease pH to acidic levels where pathogenic *Bacteria* can't grow and can preserve the nutritional value and palatability of the forage (Broberg *et al.*, 2007). Among genera of LAB that are used in silage *Lactobacillus plantarum*, *Enterococcus faecium*, *Pediococcus acidilactici*, *Pediococcus pentoseceus* and *Lactobacillus acidophilus* (Weinberg *et al.*, 2004).

Effect of both pH value and temperature on growth of LAB: Growth of LAB at wide pH and temperature ranges is an interesting property for use of these *Bacteria* in food industry. A LAB growing at acidic, neutral and alkaline pH value is better than the one which can grow

only at acidic pH. This enables the LAB starter culture to ferment different food types (Adamberg *et al.*, 2003). In addition, growth of LAB in broth media and in fluid foods such as pickles brine, juices of fruits and even in solid food systems are occurred wherein they consume carbohydrates and ferment them, producing lactic acid and other organic acids. In such acidic conditions,

pathogenic *Bacteria* can't grow (Vandenberg, 1993). The

growth of LAB at wide temperatue range is interest to enable such LAB strain to be used in food fermentation at wide temperature range. For instance, *Lactococcus lactis* and *Streptococcus thermophilus* grew well at 20-40°C with optimum temperature at 40°C with making of yoghurt as a useful food product (Beal *et al.*, 1989). Thus, ability of certain LAB to grow at wide pH and temperature range is of interest in food fermentation. Most of LAB that were used on starter cultures for food fermentations were selected basically to full fill many criteria such as fast growth at wide pH and tempratue ranges. Of these LAB strains, *Lb. acidophilus* for milk fermentation at un-controlled pH, *Lactococcus lactis* and *Streptococcus thermophilus* for yoghurt making, *Lb. fermentum* and *Lb. plantarum* for sausage making, *Lb. plantarum* for pickles making, *Carnobacterium piscicola* for fermented beef meat and fish and heuconostoc mesentroides for fermented meat and vegetable products (Gupta *et al.*, 1996).

Acid and bile tolerance: Probiotic LAB affected by various environmental conditions upon ingestion by the host and during transit in the GIT. Firstly, to survive in stomach they need to survive acidic conditions of stomach and still alive to transit to small intestine. Bile tolerance is one of the essential criteria for surviving and selecting of a probiotic strain, it is necessary for any LAB to be probiotic to still alive in small intestine and in turn can flocculate lipids by its ability to secrete cholesterol oxidase enzyme. Bile acids are synthesized in the liver by cholesterol and are secreted from the gall-bladder then generating into duodenum. It plays an important role in emulsification of lipids die to the bile arrive duodenum from gall-bladder via. bile duct. Bile acids are surface active, amphipathic molecules (having hydrophilic and hydrophobic part) with potent antimicrobial activity and act as detergents, damaging biological membranes and disrupt cellular homeostasis. Therefore, the ability of LAB to tolerate bile is important for their surviving in the GIT (Begley *et al.*, 2005). It was observed that the *Bacterial* stress originated by low pH may be overcome after the subsequent treatment in presence of bile (Charteris *et al.*, 1998). The bile concentration of about 0.3% is usually used for screening bile tolerant strains and this is considered as an average intestinal bile concentration

of the human GIT (Gilliland *et al.*, 1984). For instance *L.rhamnosus* that was isolated from infant feces is Gram+ve catalase negative, non-motile and non-sporing. *Lb. rhamnosus* survives as probiotic strain by having the ability to grow in 0.4% phenol and still viable in 0.6% phenol, atoxic metabolite produced by intestinal

Bacteria during putrefaction in the GIT (Khedekar, 1988) and it possesses the ability to grow in 6% NaCl (Jacobsen *et al.*, 1999). The ability of *Lb. rhamnosus* to grow and survive in the presence of bile, Nacl and phenol can help them to grow, survive and effect beneficially to the host by flocculation of lipids in duodenum and secretion of protease, lipase and amylase, production of lactic acid and production of antimicrobial proteins (Pithva *et al.*, 2012).

Production of enzymes: LAB have been found to produce a diverse types of enzymes which may influence the compositional, processing and organoleptic properties and quality of foods and feeds, production of such enzymes into the gastrointestinal tract that occur potential synergistic effects on digestion, giving good digestion and decreasing malabsorption of intesting (Naidu *et al.*, 1999). These beneficial organisms may be used as an alternate source for the preparation of enzyme extracts that are able to do functions under the environmental conditions of fermentation (Tamang, 2011). The enzymatic activity has been studied mainly in LAB isolated from wine or other fermented foods as cheeses of yoghurt (Mtshali, 2007). Well defined species of the LAB *Lactobacillus*, *Lactococcus*, *Pedicoccus* and *Bifidobacterium* existed in fermented foods were found to produce carbohydrate degracling enzymes as amylases, xylanases and glucosideses and enable them to be used in improvement of bread texture, fermentation of fruit juices to beneficial products such as wine and beer they produce aroma and flavor for fermented products. Many strains of LAB were found also to produce certain peptides to improve quality of cheese and proteolysis and their lipolysis provide flavor of the produced cheese (Guldfeldt *et al.*, 2001; Gonzalz *et al.*, 2010). It was high level and other proteases help in digestion of proteins they were found to produce lactase enzyme which can prevent the lactose-intolerance case at old people. Enzymes of LAB play major role to produce by fermentation associated enzyme activities and wine flavor were improved. LAB grow in wine during malolactic fermentation following alcoholic fermentation, while abroad range of secondary modifications improve the taste and flavor of wine (Matshali, 2007).

Antimicrobial potential of LAB: LAB produce antimicrobial compounds such as hydrogen peroxide, carbon dioxide, diacetyl, lactic acid and other

organic acids, acetaldehyde, ethanol and bacteriocins (Mobolaji and Wuraola, 2011; Abdel-Shafi *et al.*, 2013). These are discussed in the following:

Lactic acid: The primary antimicrobial effect exerted by LAB is the production of lactic acid (Mobolaji and Wuraola, 2011; Enan *et al.*, 2012). Lactic acid is the major organic acid of LAB fermentation. Lactic acid exists in undissociated and dissociated forms and the extent of the dissociation depends on pH (Ammor *et al.*, 2006). Lactic acid can be produced in L- or D-isomer form. The L-lactic acid is more inhibitory than the D-lactic acid (Papagianni, 2012). Antimicrobial activity of lactic acids is occurred throughout maintenance of cell membrane potential, inhibiting active transport, reducing intracellular pH and inhibiting a variety of metabolic activity (Rattanachaikunsopon and Phumkhachorn, 2010). At low pH, lactic acid is in large amount and undissociated form. It was inhibitory towards spore forming *Bacteria* and is not effected against yeasts and moulds (Yang, 2000).

Diacetyl: Diacetyl is certain product of LAB fermentation that is important flavoring compound in dairy products. The strains of *Lactococcus lactis* ssp. *lactis biovar, diacetylactis* and some species belonging to *Leuconostoc* and *Weissella* these Genera are used as diacetyl producers. Diacetyl inhibits strains of *Listeria*, *Salmonella*, *Yersinia*, *Esherichia coli* and *Aeromonas* (Yang, 2000, Ammor *et al.*, 2006; Abdel-Shafi *et al.*, 2016).

Hydrogen peroxide: Hydrogen peroxide (H_2O_2) is used in foods, dental products and environmental products. H_2O_2 is involved in oxidation and biochemical processes (Abbas *et al.*, 2010). H_2O_2 is produced by LAB and acts as antimicrobial product that cause denaturation of enzymes leading to peroxidation of lipids membrane that increased permeability of membrane and production of bactericidal free radicals such as superoxide (O_2^-) and hydroxyl group (OH) which can damage DNA (Yang, 2000; Ammor *et al.*, 2006; Sunil and Narayana, 2008). H_2O_2 is produced by using enzymes as the flavor protein oxidoreductases, NADH peroxidase, NADH oxidase and α -glycerophosphate oxidase (Rattanachaikunsopon and Phumkhachorn, 2010). The synthesized H_2O_2 inhibits growth of pathogenic microorganisms that cause food spoilage at cold temperatures. Some food borne pathogens as *Aeromonas hydrophila*, *Listeria monocytogenes* and *Clostridium botulinum* type E can grow at thus, low temperatures up to 5°C (Ismail *et al.*, 2014).

Reuterin: Reuterin is antimicrobial compound produced by a number of lactobacilli, produced under anaerobic conditions and in presence of glycerol. It is able to inhibit the growth of *Aspergillus* and *Fusarium* and plays a role

in preventing formation of mycotoxins in fermented foods. It is active against (G+ve and G-ve) *Bacteria* as yeasts, protozoa, fungi and viruses (Nes *et al.*, 2012). Many spoilage organisms are sensitive to reuterin such as *Salmonella*, *Shigella*, *Clostridium*, *Staphylococcus*, *Listeria*, *Candida* and *Trypanosoma* (Yang, 2000).

Bacteriocins: Bacteriocins are antimicrobial proteins produced by *Bacteria* they are heat resistance and could be sensitive to protease enzyme and some bacteriocins contain lipid and/or carbohydrate moieties in their molecule. Bacteriocins are stable at acidic pH levels, however, few bacteriocins are active also at pH 7.0 and slightly alkaline levels. They inhibit (G+ve and G-ve) *Bacteria* as well as some fungi. Bacteriocin compounds are classified as follows:

Group 1: It included Lantibiotics that contain lantionine amino acid in its active molecules. The bacteriocin nisin acid is a typical example of this group and was produced by *Lactococcus lactis* ssp. *lactis* and referred the term GRAS (Generally Regarded As Safe) by WHO in 1988 and approved to be used as antilisterial and anti-clostridial for preservation of meat and dairy products (Brink *et al.*, 1994; Enan *et al.*, 1994a; Eckner, 1992; Nettles and Barefoot, 1993; Vuyst and Vandamme, 1994).

Group 2: It is a large group of small-heat stable proteins. That are subdivided into three groups: (Montville and Winkowski, 1997):

- Subgroup 1; Members of this group are bavaricin Mn, Curvacin
- Subgroup 2; Require for peptides for activity and member of this groups are lactococcins G and M and lactacin F
- Subgroup 3; Consists of lactacin B that require reducing cysteines for activity

Group 3: It was classified into larger heat labile proteins as helveticins J and V and lactacins A and B:

Group 4: Bacteriocins of this group have lipid or carbohydrate moieties in their molecules such as leuconocins, lactocin 27 and pedicin SJ-1 (Yang *et al.*, 2014). The bacteriocin nisin is commercially available and added to milk, cheese, dairy products and baby foods since 1988. Bacteriocins are recently considered as safe

food additives after intake by the gastrointestinal system (Yang *et al.*, 2014) due to their sensitivity to some proteases, so, their harmless are possibly digested. (Cleveland *et al.*, 2001; Bernbom *et al.*, 2006; Enan and Amri, 2006).

Antifungal agents: Some strains of LAB inhibited yeast, some Ascomycetes, Zygomycetes and some imperfect fungi. For instance, *L. lactis* subsp. *lactis biovar diacetylactis* and *Streptococcus thermophilus* produced antagonistic substances that inhibited *Aspergillus Fumigatus* and Rhizopus spp. (Batish *et al.*, 1989); *Leuconostoc oenes* showed inhibitory activity against *Saccharomyces cerevisiae L. plantarum* and *Pediococcus* strains were found to be inhibitory towards *Fusarium ssp.* (Niku-Paavola and Haikara, 1992). The inhibitory substance was purified by gel filtration and had a molecular mass of about 100-400 and contained amino groups and phenolic hydroxyl or aromatic ring structures (Enan *et al.*, 2016).

Non-identified agents: Several strains of LAB were described to produce a number of low molecular mass non-proteinaceous substances that exhibited broad spectrum of activity against Gram-positive and Gram-negative *Bacteria*. These substances were distinguishable from other inhibitory agents produced by LAB they are still non-identified. The reports on these substances have been published by many researchers (Hamden and Mikolajik, 1974; Branen *et al.*, 1975; Reddy and Ranganathan, 1983a, b, 1987; Lewus *et al.*, 1991; Skytta *et al.*, 1993).

Inhibition of fungal toxin biosynthesis: It was found recently that many strains of LAB inhibited fungal growth. The inhibitory activity was attributed mainly to organic acids such as lactic acid, diacetyl, ethanol and acetaldehyde. Also, some published results have showed that the antifungal activity of LAB was attributed to antimicrobial proteins bacteriocins (Sanchez *et al.*, 2017).

Inhibition of mycotoxins biosynthesis by LAB: Mycotoxins are secondary metabolites produced by a wide variety of fungi, including *Aspergillus*, *Fusarium* and *Penicillium*. They cause nutritional losses and represent unsafed to the food and feed chain. Humans have long been exposed to mycotoxins directly, via. foods of plant origin including cereals by air (both indoors and outdoors) or indirectly, via. food of animal origin. The most economically important mycotoxins occurring in food and feed are aflatoxins, ochratoxin A, patulin and the *Fusarium* toxins (Zearalenon, Trichothecenes, Fumonisin etc.) (Chassy, 2010). There are 3 possibilities to avoid the harmful effect of contamination of food and feed caused by mycotoxins:

- Prevention of contamination
- Decontamination of mycotoxin-containing food and feed
- Inhibition of absorption of mycotoxin content of consumed food into the digestive tract (Halasz *et al.*, 2009)

Inhibition of mycotoxin biosynthesis by LAB have focused on aflatoxins (Thyagaraja and Hosono, 1994). LAB can release molecules during cell lysis this molecules can inhibit mould growth and loss the accumulation of their mycotoxins (Gaurama and Bullerman, 1995). This “anti-mycotoxinogenic” metabolites that inhibits aflatoxins accumulation are found in *Lactobacillus* cell-free extracts (Gourama, 1991). This is due to a heat stable, low-molecular-weight inhibitory compound such as organic acids which make a strong acidic environment wherein fungi grow slowly or can't grow, antifungal substances, H₂O₂, ethanol and diacetyl (Luchese and Harrigan, 1990).

LAB as probiotics: The beneficial *Bacteria* are recognized as Probiotics. Probiotics (derived from Latin and Greek) means “for life” is defined according to FAO/WHO (2001) as: Live microorganisms which when administered in adequate amounts confer a health benefit on the host. Some substances such as oligosaccharides serve as substrates for Probiotics and they are important for their growth. These are called prebiotics. Prebiotics are those substances that aren't used or metabolized by non-probiotic *Bacteria* like *Bacteroides* spp. and *E. coli*. Breast milk and some vegetables are sources for prebiotics (Hamilton-Miller, 2004). There are different kinds of *Bacteria* that can be used as Probiotics, e.g. *Lactobacilli* and *BifidoBacteria* (Macfarlane *et al.*, 2004). These *Bacteria* show symbiotic relationship with human. They are found in the mucus membrane present on epithelial cells of the gut (Holzapfel and Schillinger, 2002) where they inhibit the growth and attachment of harmful *Bacteria* by producing bactericidal chemicals against these *Bacteria*. With the development of evidences regarding usefulness and safety of probiotics these *Bacteria* are replacing the traditional prophylactic and treatment regimes (Enan *et al.*, 2013).

Sources of probiotics: Yogurt is known to be the most popular source of probiotics. Yogurt is prepared from milk fermented by *Bacteria* that modify lactose into lactic acid. Lactic acid is responsible for giving yogurt its characteristics (sharp taste usually changed into good taste by using sweeteners and flavouring) and also denatures and precipitates casein, resulting in a semisolid consistency. “Bioyoghurts” are formed by a similar method but *Bacteria* used for fermentation are of different strains, usually *L. acidophilus*. Medically, probiotics can

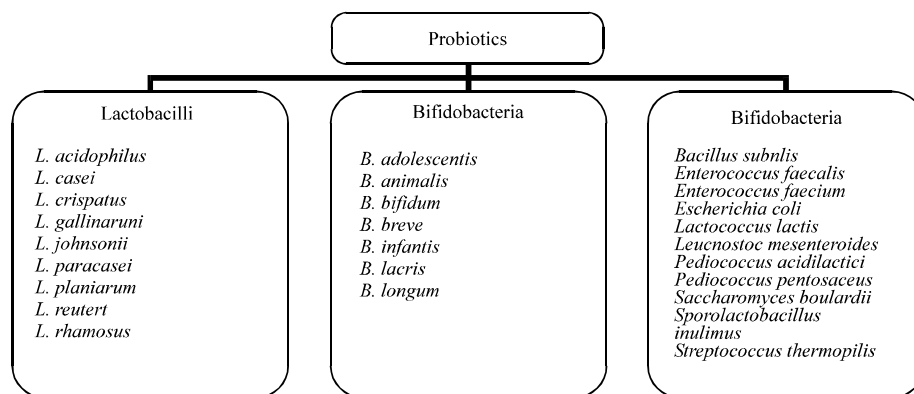


Fig. 2: Different types of *Bacteria* which are recognized as probiotics (Furrie, 2005)

be available in supplements consisting of freeze dried *Bacteria* in tablets, capsules and powders. Thousands strains of probiotics are easily obtainable now and all of them show different beneficial effects Many *Bacteria* used as probiotics are given in Fig. 2.

Mechanism of action of probiotics: The mechanisms by which probiotics exert biological effects are still poorly understood but the non specific terms such as colonization, resistance or competitive exclusion are often used to explain their mode of action (Elo *et al.*, 1991). Colonization resistance or competitive exclusion describes a phenomenon whereby the indigenous anaerobic flora limits the concentration of potentially pathogenic (mostly aerobic) flora in the digestive tract (Vollaard and Clasener, 1994). The concept of competitive exclusion was first developed during the early 1970s when it was discovered that the administration of mixed adult intestinal microorganisms conferred adult type resistance against Salmonella infection to newly hatched chicks (Nurmi *et al.*, 1992). Oelschlaeger (2010) reported that the effects of probiotics may be classified in three modes of action:

Probiotics might be able to modulate the host's defences including the innate as well as the acquired immune system. This method is most probably important for the prevention and therapy of infectious diseases and treatment of chronic inflammation of the digestive tract or parts thereof. This probiotic action could also be important for the eradication of neoplastic host cells.

Probiotics can also have a direct effect on other microorganisms, commensal and/or pathogenic ones. This mode of action is important for the prevention and therapy of infections and restoration of the microbial equilibrium in the gut.

Probiotic effects may be based on actions affecting microbial products like toxins and host products such as

bile salts and food ingredients. These actions may lead to inactivation of toxins and detoxification of host and food components in the gut. The type of actions a certain probiotic finalizes relies on its metabolic properties, the molecules presented at its surface or on the components secreted. DNA or peptidoglycan of the *Bacterial* cell might be important for its probiotic effectiveness. The individual combination of such properties in a certain probiotic strain determines a specific probiotic action and as a consequence its effective application for the prevention and/or treatment of a certain disease.

Safety considerations: Probiotics are live microorganisms and therefore, it is feasible that they could infect the host. First selection criteria mentioned that a probiotic supplement has to be Generally Regarded As Safe (GRAS) microorganisms (Reid *et al.*, 2003; Cabana *et al.*, 2006). Different species of Lactobacillus and Bifidobacterium are normal residents of the gastrointestinal and/or vaginal microbiota and do not exert infectivity or toxicity. The risk of infection with these microorganisms is lower (Gupta and Garg, 2009) Probiotics are safe for using in healthy people but should be used with caution in high risk cases such as: immunocompromised patients and premature infants. Current WHO/FAO guidelines (2001) recommend that, before using probiotic strains, a number of parameters should be assessed to prevent health damages, including antibiotic susceptibility patterns, toxin production, metabolic and haemolytic activities, infectivity in immunocompromised animal models, side-effects and adverse incidents in humans (Senok *et al.*, 2005).

Medicinal role of probiotics

Diabetes: Diabetes is a chronic disease that occurs when the pancreas doesn't produce enough insulin or when the body cannot effectively use the insulin it produces.

Hyperglycemia is a common effect of uncontrolled diabetes and over time leads to serious damage to many organs and systems. Recent researches illustrated that there is a connection between *Bacterial* population in gut and metabolic diseases in human (especially, diabetes). These studies showed that there is a relationship between the composition of the intestinal microbiota and metabolic diseases like obesity and diabetes (Larsen *et al.*, 2010). Probiotics are supposed to treat diabetics via balancing microbial gut flora. It is also suggested that the use of probiotics, e.g. *Bifidobacterium* spp can decrease the insulin resistance and can also lower the incident hypertensive conditions that are closely related to diabetes (Cani and Delzenne, 2011).

Inflammation: Some strains of LAB may decrease the inflammation by modulating inflammatory and hypersensitivity responses. This effect may be accomplished by regulation of inflammatory mediator called cytokines (Reid *et al.*, 2003).

Peptic ulcer: Some strains of LAB may control *Helicobacter pylori* infections (cause of peptic ulcers) in adults when given concomitantly with standard medical treatments. *Lactobacillus*, *Bifidobacterium* and *Saccharomyces* are suggested to be adjuncts to antibiotics for the treatment of *H. pylori* infections.

Hypertension: The incidence of increased blood cholesterol has been increased in adults, children and adolescence. Hypertension can occur due to lipid abnormality, hypercholesterolemia and obesity (Yekeen *et al.*, 2003). Mann and Spoer (1974), illustrated that *Lactobacillus*-fermented milk has hypocholesterolemic effects. *Bifidobacteria* can also cause a significant reduction in serum cholesterol when it elevates. Most of the cholesterol is synthesized and absorbed in the intestine so intestinal micro flora has shown to affect on cholesterol level in blood. Probiotics have been proved to be beneficial in lowering hypertension by decreasing blood cholesterol level and increasing resistance of LDL to oxidation (Goel *et al.*, 2006).

Halitosis: Halitosis is the unpleasant odour exhaled in breathing. It is not considered a disease but is caused by some other diseases like periodontitis. Probiotics can be used in treatment both GIT and mouth mediated halitosis (Delanghe *et al.*, 1997).

Oral candidiasis: Some strains of probiotics as *L. rhamnosus* strain and *propionibacterium freudereichii* are effective in reducing the *Candida* yeast count (Haukioja, 2010).

Viral infection: Probiotics are useful against many viral diseases. It is known that probiotics do not exert antiviral effect by direct action on viruses but do so by the mechanism of immunostimulation (De Vrese and Schrezenmeir, 2002). Such probiotics which exercise immunostimulation also exhibit potential antiviral effect. Species that thrive at relatively high temperatures have some anti-influenza effect and can also exhibit anti-herpetic effect when administered to guinea-pigs (Liaskovs *et al.*, 2007).

Lactose intolerance: Lactose intolerance means the inability of adults to digest lactose due to lack of lactose metabolizing enzyme lactase. Approximately most people deficient in lactase generally tolerate lactose better from yogurt than from milk. Lactose is the sugar found in milk products. Kim and Gilliland (1983) found that feeding fermented milk to lactose intolerant subjects resulted in a significantly lower level of hydrogen in the breath when compared to the hydrogen level for subjects fed unfermented milk. Hydrogen in the breath is a marker for *Bacterial* metabolism of lactose in the large bowel. A lower hydrogen level indicates that lactose has been metabolized prior to entering the large intestine.

Atopic diseases: Atopic dermatitis is the first symptom of atopic disease and it is a chronic skin condition associated with inflammation and pruritis (Simpson, 2010), eczematous papules, itch and plaques. It is one of the most prevalent skin diseases. There is a high risk of developing atopic disease in children whose mother is atopic. Probiotics may play a considerable role in reducing occurrence of atopic diseases. The risk of occurrence of eczema during first 2 years of infant life was reduced significantly in those whose mother received probiotics as compared to those whose mother takes placebo (Rautava *et al.*, 2002).

CONCLUSION

The prime objectives of this review was to give an overview about classification of LAB their antimicrobial potential and their use in food industry and their probiotic capability.

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