

LACTIC ACID BACTERIA: A REVIEW

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ABSTRACT

Lactic Acid Bacteria are a group of Gram positive bacteria, non respiring, non spore- forming cocci or rods, which produces lactic acid as the main end product of the fermentation of carbohydrates. Two major are recognized, homofermentative and heterofermentative LAB. Most representatives of these groups have been consumed for thousands of years and do not pose any health risk or hazard and are designated as GRAS organisms. Lactic Acid Bacteria posses several applications that include in the food/dairy industries, as a probiotic in the improvement of human and animal health used as biofertilizer and the use of its exopolysaccharide in various industries.

Keywords: Lactic acid bacteria, Dairy products, probiotics, exopolysacchiride.

INTRODUCTION

Lactic Acid Bacteria

The term lactic acid bacteria cover a large group of micro-organisms. The first pure culture of lactic acid bacterium was obtained in 1873 and the similarity between milk souring bacteria and other lactic acid-producing bacteria of other habitats was recognized in the early 1900s. The basis of systematic classification of LAB was elaborated and published in 1919 by Orla-Jensen. Although revised to a considerable extent, the main characteristics of classification have remained unchanged (Hayek and Ibrahim, 2013).

Lactic Acid Bacteria (LAB) are a group of Gram-positive, non – sporulating, anaerobic or facultative aerobic rods or cocci, which produce Lactic acid as one of the main fermentation products of the metabolism of carbohydrates (Hayek and Ibrahim, 2013). Present classification of Lactic Acid Bacteria (LAB) using cellular morphology, mode of glucose fermentation, growth temperature range and sugar utilization pattern, four genera were recognized; *Lacto bacillus*, *Leuconostoc*, *Pediococcus* and *Streptococcus*. Molecular biological methods have increased the number of the genera included in the group (Vonwright and Axelsson, 2012). The current taxonomic classification includes LAB group in the phylum firmicutes, class *bacilli*, and order Lactobacillales (Parada *et al.*, 2007).

LAB can be divided into 2 groups based on glucose fermentation into homo and heterofermentative LAB. Homofermentative LAB convert sugars almost quantitatively to lactic acid. The second group heterofermentative LAB produce not only lactic acid but ethanol/acetic acid and carbon dioxide.

LAB are widely distributed in nature they have been isolated from grains, green plants, dairy and meat products, fermenting vegetables and mucosal surface of animals (Lindgren and Dobrogosz, 1990); vacuum – packaged refrigerated beef (Sakala *et al.*, 2002); Sourdoughs (Muhammad *et al.*, 2018) and traditional Indian fermented foods such as Appam batter and vegetable pickles (Jamuna and Jeevaratnam, 2004).

Most representatives of these groups have been consumed for thousands of years, and do not pose any health risk or hazard to humans and are designated as GRAS organisms (generally recognized as safe). They are known to inhibit bacterial pathogens which disturb the normal microbial flora of the gastrointestinal tract.

Lactic Acid Bacteria (LAB) are widely used in the food industry as starter culture. LAB exerts a strong antagonistic activity against many food specie organisms and foodborne, pathogens (Sridhar Rao, 2005). *Lactobacilli* have been used since decades against infectious diseases (Bernet *et al.*, 1994) and have been extensively studied for their ability to protect against pathogens. Its use in fermented food has been essential for millennia, LAB are generally employed because they significantly contribute to the flavor, texture, and in many cases to the

nutritional value of the food products (Walsh *et al.*, 2008). LAB play a defining role in the biopreservation and microbial safety of fermented foods (Caplice and Fitz Gerald, 1999), thus promoting the microbial stability of the final products of fermentation (Massa 2014). Protection of food is due to the production of organic acids, carbon dioxide (CO₂), ethanol, hydrogen peroxide and diacetyl (Atrih *et al.*, 2001). Several antifungal compounds are also produced, that include fatty acids (Corsetti *et al.*, 1998) or phenyllactic acid (Lavermicocca *et al.*, 2000), bacteriocins and antibiotic such as reutericyclin (DeVuyst and Vandamme, 1994). Growth of LAB also displays promising positive roles in non-fermented foods as seen in vacuum packaged meat products (Castellano *et al.*, 2004). In these cases, LAB such as *Carnobacterium sp.*, *Lactobacillus sp.* and *Leuconostoc sp.* are the main specie organisms (Bello *et al.*, 2001), but a selective growth promotion of LAB capitalizing on their ability to control meat-borne pathogens with a preferential growth of benign strains would minimize their specie effects.

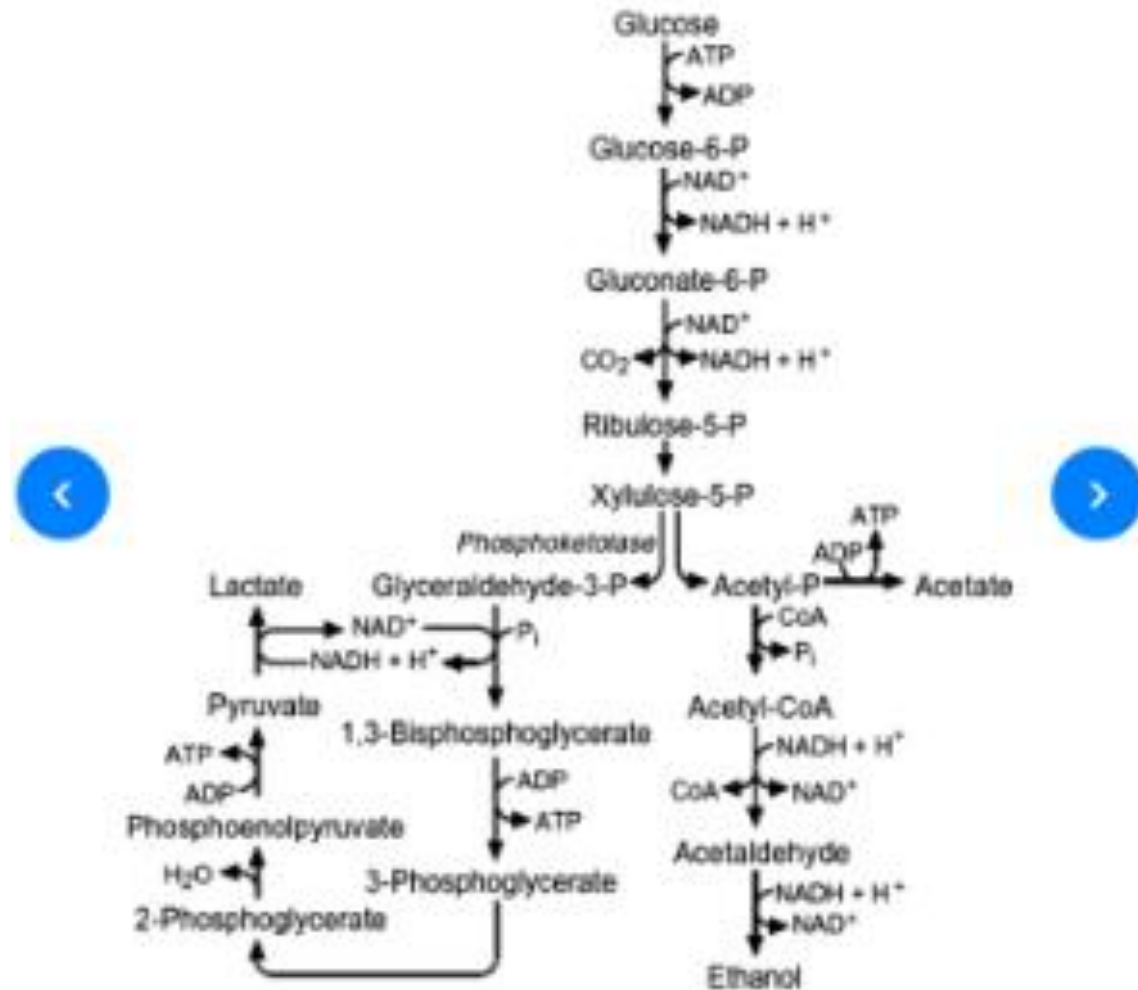


Figure 1: Hetero-Fermentative pathway showing the production of lactic acid, carbon-dioxide and ethanol.

Source: Wackett, (2016)

Application/Uses of Lactic Acid Bacteria (LAB)

Preservation of milk by fermentation was used early in human history. Sumerian writings about dairying go back to about 6000 BC. Procedures for the fermentation of meat were developed as early as the fifteenth century BC in Babylon and China. Methods for the fermentation of vegetables were known in China in the third century BC. Since these times, many different cultures in various parts of the world have used LAB to improve storage qualities, palatability, and nutritive value of perishable foods

such as milk, meat, fish, and vegetables. Today in the developed world, Lactic acid bacteria are mainly associated with fermented dairy product (Wackett, 2016).

Lactic Acid Bacteria in Dairy Industry

Fermented milks and cheese are dairy products preserved partly by acid produced by bacterial activity. Fermented milks include a lot of products such as yoghurt, acidophilus milk, cultured butter milk, kefir, taette and various others. Cultured butter milk is obtained from pasteurized skim milk or part skim milk cultured with lactic acid and some aroma compound bacteria such as *Streptococcus lactis* and *Streptococcus cremoris*. Sour cream is manufactured by ripening pasteurized cream with lactic acid and aroma producing bacteria. Yoghurt is made from, depending on the type of product, milk with fat content ranging from 1 to 5%. Generally, stabilizer is also used in order to produce smoothness, in body and texture, impact gel structure, and reduce wheying or syneresis. Plain yoghurt normally contains no added sugar or flavor. Different types of fruit yoghurts are also produced using fruit products as ingredients. *Lactobacillus bulgaricus* and *Streptococcus thermophilus* are the LAB used in such production. Kefir belongs to the class of acid with low alcohol fermented dairy products (Torodov, 2008). It is produced by fermentation with mixed LAB-yeast culture. Lactic acid fermentation is involved in the making of most kinds of cheese. Cottage cheese is made from pasteurized milk. Coagulation is accomplished with lactic *Streptococci* and rennet. Cottage cheese and other unripen cheese must be chilled and kept cold until consumed. They have a comparatively short keeping time. The Swiss type cheeses are ripened. During this process, due to activity of lactic acid bacteria (in some types activity of molds) the characteristic texture and aroma is developed. The most typical changes during ripening of Swiss-type cheeses are connected with activity of proteolytic enzymes. Different peptides, amino acids and, depending on conditions of ripening, amino acids are formed, contributing to the characteristic flavor of cheese. The flavor of cheese is not the result of proteolytic activity only. Lipolysis (primarily in mold-ripened cheese) may also occur and many other compounds connected with the side pathways of lactic acid fermentation are also present (Trias, 2008).

Lactic Acid Bacteria as a Probiotic

The word “probiotic” is derived from Greek “for life” and had over the years several different meanings. The use of the term “probiotics” in the context in which it’s used today dated 1974 when Parker defined “probiotics” as organisms and substances contributing to intestinal microbial balance”. Fuller in 1989 redefined probiotics as “A live microbial feed supplement which beneficially affect animal host, improving its intestinal microbial balances (Fuller, 1992). Probiotics are “live microorganisms that, when administered in adequate amount, confer a health benefit on the host” (WHO, 2016). It generally refers to viable bacteria, cultured dairy products or food supplement containing viable LAB. Most probiotic strains belong to the genera *Lactobacillus* and *Bifidobacterium*. The beneficial effects of probiotics include immunological stimulation, improvement of digestion and absorption, synthesis of vitamins, inhibition of potential pathogens, decrease of cholesterol level, lowering of gas distension, and restoration of

the normal flora, after antibiotic therapy (Yang *et al.*, 2014). The belief in the beneficial effect of probiotics approach is based on the knowledge that intestinal microflora provides protection against various diseases. Although there seems no doubt that gut microflora is protective, in order to produce an effective probiotic, one must know which micro-organisms are responsible for induced effect.

Several basic properties are required to promote an effective probiotic. LAB are required to promote an effective probiotic LAB strains. Among the most important properties is ability to survive passage through the mouth, stomach, small intestine and large intestine. Thus, the strain must be stable in gastric conditions. In order to be able to influence functions of human intestinal tract, probiotics strain must have the capacity to adhere to intestinal mucosal cells and to grow in intestinal conditions.

Other requirements of an effective probiotic strain include antagonism against pathogenic bacteria, production of antimicrobial substances and proven safety in human use, besides all mentioned properties proposed probiotic strain needs to be suitable for industrial application in common dairy processes or in production of pharmaceutical preparation. The table below summarizes the characteristics of a good probiotic strain (Farjardo *et al.*, 2012).

Some properties of a good probiotic strain

1. Acid stability (especially gastric acid) bile stability
2. Human origin (specie – specific properties)
3. Adherence to human intestinal cells
4. Colonization of human intestinal tract
5. Production of antimicrobial substances
6. Antagonisms against pathogenic bacteria
7. Good growth in vitro
8. Safety in human use

Lab culture can also be given to most livestock specie to consume through their feed and/ or water as a probiotic to wholly foster a healthy gut flora, enhances their improved system, and acid in digestion (Farjardo *et al.*, 2012).

Lab culture can transform a malodorous, anaerobic livestock pen, into an odorless system when used in conjunction with an IMO – inoculated deep litter system (Duponte and Fischer, 2012).

Lactic Acid Bacterial Exopolysaccharide (EPS)

A variety of microbial and plant derived biopolymers exists with a variety of food applications. Dextran is the first industrial polysaccharide produced by LAB like *Leuconostoc mesenteroides*. It was discovered in 1880 in sugar cane or beet syrups where dextran was found to be responsible for the thickening and gelation of the syrups (Crescenzi, 1995). Due to their structural differences, some dextrans are water soluble and others are insoluble. Dextran can be

used in confectionary to improve moisture retention, viscosity and inhibit sugar crystallization. In gum and jelly candies, it acts as a jelling agent. In ice cream it acts as a crystallization inhibitor, and in pudding mixes it provides the desirable body-texture and mouth feel (Whistler and Danie, 1990). In addition, dextran has also been used as blood plasma extenders (Kulicke and Heinze, 2005) and as the basic component of many chromatographic stationary phases (Franz, 1986).

Dextran from *Leu. mesenteroides* protects the producer strain during starvation and helps in survival at alkaline and acidic conditions (Kim *et al.*, 2000). Xanthan gum, produced by the plant-pathogen, *Xanthomonas campestris* is the second microbial EPS which was approved for use in foods in 1969. Xanthan has been described as "benchmark" product with respect to its importance in both food and non-food applications (Sutherland, 1998), which include dairy products, drinks, confectionary dressing, bakery products, syrups and pet foods, as well as the oil, pharmaceutical, cosmetic, paper, paint and textile industries. The production of xanthan is relatively inexpensive because of the high conversion of substrate (glucose) to polymer (60-70%) (Sutherland, 1998). This biopolymer exhibits a high viscosity at low concentrations in solution and strong pseudoplasticity, and stable over a wide range of pH, temperature and ionic strength. Microbial polysaccharides have rheological properties that match the industrial demands and can be produced in large amounts and high Purity. Since 1940, dextran and levan have found numerous pharmaceutical and food applications. Fructose-oligosaccharides (FOS) have interesting properties for food applications as they have a low sweetness compared to sucrose, are essentially calorie-free, and noncariogenic (Yun, 1996). Food applications of inulin and FOS are based mainly on their prebiotic properties. Remarkably, fructose based polymers have ability to be fermented by gut microflora which leads to improvement of the intestinal flora and increases mineral absorption (Yamamoto *et al.*, 1999; Xug *et al.*, 2006). The levan from *L. sanfranciscensis* LTH 2590 also exhibits prebiotic effects as demonstrated *in vitro* by different experimental approaches (Bello *et al.*, 2001; Koraki, *et al.*, 2002). Fructans play a role in the cellular stress tolerance of plants through stabilization of membranes (Oliver *et al.*, 2001). The property of FOS and EPS to protect bacteria against various stresses enable their application to improve the survival and activity of LAB during the preparation and storage of fermented milk products. EPS produced by LAB, are widely used to improve the body and texture of yoghurt and other fermented milk products like dahi (Broadbent *et al.*, 2003; Faber *et al.*, 2001). Dahi is a popular fermented milk product of India having 3.5-8% fat, similar to yoghurt, consumed in almost every household (Prajapati and Nair, 2003). Health awareness among consumers generated more demands for low-caloric, especially low-fat or fat-free dairy products in the market. However, since milk fat contributes to the flavor, body and texture development of the dairy products, removal leads to textural and functional defects in low fat fermented milk products. In case of low fat yoghurt and dahi, a lack of flavour, weak body and poor texture are the major problems (Güven *et al.*, 2005). In this perspective, EPS producing LAB as 'biothickeners' offer natural, more acceptable and preferred approach to many additives. These cultures meet the consumer requirement for products with low levels of chemical additives

(DeVuyst *et al.*, 2001; Jolly *et al.*, 2002), reduce the amount of total solids required without affecting the textural attributes (DeVuyst *et al.*, 2001; Wachter – Roderte *et al.*, 1993) and improves sensory properties (Folkengberg *et al.*, 1993). Low fat dahi made using different EPS producing cultures of *L. Lactis* subspecies, *Lactis* PM23, *S. thermophilus* ST and *L. Lactis* NCDC 191 found to be more acceptable in terms of body, texture and flavour as compared to dahi made with EPS negative culture NCDC 167. The microstructural studies showed that dahi made with EPS-producing strains had more open structure and pores with discontinuous case in matrix than the controlled dahi, which had relatively compact linear structure (Praveen, 2000). To overcome the defects of low fat cheeses, manufacturers have used texture promoting or ropy cultures for many years particularly where addition of stabilizer is prohibited. Such ropy strains may impart higher flavour intensity in the fermented milk due to the carbohydrate masking the flavour, mouth feel and other attributes may also be affected. The apparent viscosity of skim milk gel made by two ropy cultures, namely B-6 and KT-24 (later identified as *Lc. Lactis* subspecies *Lactis* strains) was increased as compared to that made by non-ropy cultures (Bahare *et al.*, 2009; Dabour *et al.*, 2005). Recently, Florencia (Florencia, 2013) studied rheology of speciereadable goat cheese made with autochthonous lactic cultures differing in their ability to produce EPS and concluded that cheese made with EPS producing strain showed smaller elastic or storage module (G'), viscous or loss module (G''), and complex viscosity (η^*) values over the range of frequencies studied and smaller critic stress values than the cheese without EPS producing strain. EPS-producing *L. rhamnosus* JAAS8 in combination with non EPS-producing yogurt strains increased both the water holding capacity (WHC) and viscosity of the fermented products when compared with the control made with non-EPS-producing yogurt starters (Yang *et al.*, 2010). Partial or total replacement of non-EPS-producing *L. delbrueckii* subspecies *bulgaricus* with EPS-producing *L. rhamnosus* JAAS8 resulted in significant increases of about 16% and 21%, respectively, in apparent viscosity and an increase of about 2% in the WHC of the fermented products. To reduce the amount of added milk solids, to improve yoghurt viscosity, to enhance texture and mouth feel and to avoid syneresis during fermentation or upon storage of the fermented milk products, EPS producing functional starters are interesting. Yogurt made with EPS producing cultures has better water binding capacity, which decreases the product's susceptibility to syneresis (Hassan *et al.*, 1995; Amatayakel *et al.*, 2005). However, no simple correlation has been established between viscosity and quantity of EPS produced. It is opined that it may be more beneficial to use a combination of ropy and non-ropy starter cultures than using only ropy strains (Laws and Marshall, 2001). Besides dahi, yoghurt and cheeses, the other fermented milk products in which EPS cultures have been shown to affect product rheology are sour cream, kefir, and European cultured dairy products.

Use of slime producing *S. thermophilus* strains greatly improved rheological properties of cream turo and number of other Hungarian cultured milk and cultured cream products (Obert, 1984).

Kefir is a traditional self-carbonated, slightly alcoholic fermented milk from Eastern Europe (Tamime *et al.*, 2005). Kefir is prepared using kefir grains which consists of homofermentative

and heterofermentative LAB, yeasts and acetic acid bacteria. These cells are embedded in kefran, a slimy polysaccharide, which is also found to affect texture of kefr (Duboc and Mollet, 2001). Furthermore, some associated LAB strains also produces EPS within the matrix acting as natural viscosifying agent.

Dextran from *Leu. mesenteroides* finds commercial application in baking improvers. A study performed by Brandt *et al.* (2003) provided evidence that EPS effectively improve dough rheological parameters and bread quality. Remarkably, EPS produced *in situ* was more effective when compared to externally added levan and addition of 1% (flourbase) sucrose to wheat doughs sufficed to induce polymer formation by *Lactobacilli* to effective concentrations. The *in situ* formation of EPS from sucrose resulted in further metabolites such as mannitol, glucose, and acetate that may contribute to the improved bread quality (Koraki *et al.*, 2001).

The EPS producing strains of *Weissella* in sourdoughs improved the textural properties and quality of bread (Galle *et al.*, 2010; DiCagno, 2006). Polymers produced from *Lactobacilli* thus may be expected to beneficially affect one or more of the following technological properties of dough and bread: (i) water absorption of the dough, (ii) dough rheology and machinability, (iii) dough stability during frozen storage, (iv) loaf volume and (v) bread staling.

During prolonged fermentation and storage, idli batters start collapsing and whey is separating that leaves idli with a hard, unwanted texture. Addition of different hydrocolloids in the batter may help to improve the texture of the final product (Nisha *et al.*, 2005). Xanthan at a concentration of 0.1% improved the textural properties resulting in sensory analysis scores that are higher compared to idli without additives (Thakur *et al.*, 1995). Nisha *et al.*, (2005) added different hydrocolloids to idli batter where all gave an increased viscosity. The decrease in batter volume during storage was less compared to the control and less whey separation was reported.

However, only agar and guar gum gave idli that were acceptable from a sensory point of view with the right mouth feel and texture. Starter cultures producing EPS may be used to provide natural stabilizing agents *in situ*. EPS may work as natural thickening agents, giving the product a higher viscosity and reducing syneresis (DeVuyst and Degees, 1999) thus giving an improved product without the use of additives.

Lactic Acid Bacteria as Biofertilizers

Biofertilizers or microbial inoculants are preparations of live or latent cells of efficient strains of nitrogen fixing bacteria strains, lactic acid bacteria, phosphatase solubilising or cellulolytic microorganism used for application to seeds, soils or composting areas with the objective of increasing the number of such microorganisms and accelerate those microbial process which augment the availability of nutrients that can be easily assimilated by plants.

Lactic acid bacteria have been used for decades in agricultural system to improve soil, control disease and promote plant growth. An understanding of the symbiotic relationship between plant

and lactic acid bacteria could be exploited to improve agricultural plant production (Ikeda *et al.*, 2013).

Lactic acid bacteria used as biofertilizer increases nutrients use efficiency and open new routes of nutrient acquisition by plant, thus micro-organisms applied to plants can have a dual function of biocontrol agent and biostimulant (Boraste *et al.*, 2009).

It has been discovered that synergistic growth of LAB and photosynthetic bacteria makes it possible to be used as biofertilizer which helps to promote the growth of photosynthetic bacteria (PB) and used in organ saline paddy field (Abdel-Ghany *et al.*, 2013).

LAB are very effective in improving air ventilation in soil and are highly effective at promoting the growth of fruit trees and leafy vegetables. Lab absorbs carbohydrate as material base from yeast of phototrophic bacteria to produce lactic acid. Lactic acid has a strong bactericidal ability that can effectively inhibit the activity of harmful microorganisms.

Lactic acid biofertilizers can provide an economically viable support to small and marginal farmers for realizing the ultimate goal of increasing productivity.

Lactic acid bacterial culture is used with waters in a ratio of 1:1000 and mixed with plant nutrient solution such as Fermented Plant Juice (FPJ) (Miller *et al.*, 2013) and applied as a foliar specie ray to leaf surfaces or fruit crops. LAB culture can also be used in conjunction with either nutrient solution to treat seed before planting, thereby improving seed germination, inoculate the seed with beneficial microbes and deters fungal problem, such as damping off (Hamed *et al.*, 2011).

LAB is used with IMO (indigenous microorganisms) in natural farming, in making composts or compost teas for soil preparation prior to planting (Park and Duponte, 2008). Application of LAB culture can accelerate the decomposition of organic amendments in soil and enhance the release of plant nutrients for absorption (Higa and Kinjo, 1989).

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