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Recent Application of Lactic Acid Bacteria As Source of Industrially Important Compounds

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Lactic acid bacteria, in the form of starter cultures are essential for many industrial processes in the dairy and food industry, and can enhance the overall quality of the fermented food products. In this regard, the identification and application of strains delivering industrially important compounds is a fascinating field. This paper will discuss recent application of lactic acid bacteria as source of vitamins, low calorie sweeteners, organic acids, aroma compounds and exopolysaccharides and also discuss how the proper selection of starter cultures can be useful in developing modern food products.

Keywords: Lactic acid bacteria, Vitamin B, Polyol, Exopolysaccharides, Organic acid.

Lactic acid bacteria (LAB) are important for food industries, mainly for the dairy and food industries. LAB is gram-positive, nonsporulating, non-respiring, cocci or rods. LAB ferment carbohydrates and produce lactic acid as end product. LAB consist of *Lactobacillus*, *Leuconostoc*, *Pediococcus*, *streptococcus* and several new genera; *Aerococcus*, *Alloiococcus*, *Carnobacterium*, *Dolosigranulum*, *Enterococcus*, *Globicatella*, *Lactococcus*, *Oenococcus*, *Tetragenococcus*, *Vagococcus*, and *Weissell* [1]. LAB cannot synthesize cytochromes and porphyrins (components of respiratory chains) and therefore cannot generate ATP by creation of a proton gradient. Only by fermentation (usually of sugars) LAB can obtain ATP. Based upon the products produced from the fermentation of glucose, LAB can be divided into two groups. The first group, homofermentative LAB converts sugars almost quantitatively to lactic acid. The second group, hetrofermentative LAB produces not only lactic acid but also produce ethanol/acetic acid and carbon-dioxide. Habitats of LAB are rich in nutrients, such as various food products like milk, meat and vegetables, but some LAB are also present in mouth, intestine and vagina of mammals.

Milk products fermented by LAB, have been taken in the area from Europe to Asia and a part of Africa since prehistoric era. At the beginning of the 20th century Ellie Metchnikoff (1845-1916, winner of the 1908 Nobel Prize for physiology) advocated the health benefits of LAB. Strains of LAB are employed as probiotics [2]. Today the universal meaning of the term "Probiotic" was established by the World Health Organization and the Food and Agriculture Organization of the United States. These two organizations defined probiotics as "live microorganisms which when administered in adequate amounts, have a beneficial effect on health of the host organism".

LAB has great application in the fermented food industries. Their most important application is in the dairy industry, while next to that is the fermented meat and vegetable products industries. LAB has ability to produce industrially Important Compounds for different food

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applications. They produce various compounds such as vitamins, low calorie sweeteners, organic acids, aroma compounds, and exopolysaccharides [3, 4]. These ingredients are important for their effects on food flavor, texture and nutrition. Production of these compounds can be improved by metabolic engineering. In the food industry, it is known that there is variation among strains and species of starter culture bacteria with regard to their ability to produce industrially important compounds.

Lactic acid bacteria as source of vitamin B

B vitamins are a group of water-soluble vitamins, having important role in cell metabolism and antioxidant activities of human body. Human cannot synthesize all these vitamins. It is well known that some intestinal bacteria like LAB can produce some vitamin B (folate, vitamin B12 or cobalamin, riboflavin and thiamine) [5, 6]. Vitamin B is also reported as result of the LAB fermentation in yogurt, cheese and other fermented foods.

Folate

Folate is the term used to describe the folic acid derivatives, such as the folyl glutamates. They are naturally present in foods and used as nutritional supplements. Folate participates in many metabolic pathways like the biosynthesis of DNA and RNA and the inter-conversions of amino acids. Folate-producing probiotic LAB can be used to prevent the localized folate deficiency. The oral administration of probiotic LAB strains may confer protection against inflammation and cancer, both by delivering folate to colonic-rectal cells. Intestinal microbiota can produce folate and this folate will absorb across the large intestine and incorporated into the liver and kidneys [7, 8, 9.]. Different LAB has different ability to produce folate. Not only the yogurt starter cultures and *Lactococcus lactis* have the ability to produce folate but this important property also exists in other LAB species such as, *L. acidophilus, Leuconostoc lactis, Bifidobacterium longum* [11], and some strains of *Propionibacteria* [12, 13]. Majority of folate produced by *Leuconostoc lactis* is lesser bioavailable due to its intracellular production. Metabolic engineering can be used to increase folate levels in *Leuconostoc lactis* [14, 15], *Lactobacillus gasseri* [16] and *Lactobacillus reuteri* [17].

Cobalamin

For the metabolism of biochemical compounds of our body Cobalamins (Vitamin B12) are required [18]. Vitamin B12 cannot be synthesized by human body, so must be obtained from exogenous sources like the intestinal microbiota or foods [5]. Recently, a publication has described production potency of vitamin B12 by LAB Isolated from Japanese pickles [19]. In this study it has been reported that among the microorganisms some strains of the *Lactobacillus spp. (Lactobacillus sakei* CN-3, *Lactobacillus plantarum* CN-49, *Lactobacillus sakei* CN-2, *L. plantarum* CN-225, *Lactobacillus coryniformis* CN-22) have the ability to produce vitamin B12. *Lactobacillus reuteri* CRL1098 was able to metabolize glycerol in a cobalamin -free medium. It indicates that, LAB might be able to produce cobalamin.The intracellular bacterial extract of *Lactobacillus reuteri* CRL 1098 contains cobalamin- like compound [20]. To improve cobalamine yield, random mutagenesis and genetic engineering can be used [21, 22]. In *Propionibacterium freudenreichii* different metabolic engineering strategies have been applied to increase vitamin B12 production [23, 24.]

Riboflavin

Riboflavin (Vitamin B2) plays an important role in cellular metabolism. Riboflavin acts as the precursor of the coenzymes flavin mononucleotide (FMN) and flavin adenine dinucleotide (FAD) both acting as hydrogen carriers in biological redox reactions. Riboflavin is present in many foods such as dairy products, eggs, meat, green vegetables. It's deficiency cause damages in the liver, skin and change the brain glucose metabolism [6, 5], with symptoms like hyperaemia, sore throat and odema of oral and mucous membranes [25]. Recently, a publication has described the screening of riboflavin-producing strains from different fermented milk products obtained in the Vellore region of India [26]. Just a single strain *Lactobacillus fermentum* MTCC 8711 was identified as being an efficient riboflavin-producing strain. It produced 2.29 mg l^{-1} of riboflavin after 24 h of growth in the chemically defined media [26]. The toxic riboflavin analogue roseoflavin was used to isolate natural riboflavin-overproducing variants of the food-grade micro-organisms *Lactococcus lactis*[27], *Lactobacillus plantarum, Leuconosctoc mesenteroides* and *Propionibacterium freudenreichii* [28].

Thiamin

Thiamin (Vitamin B1) helps the body's cells to convert carbohydrates into energy. It is also essential for the functioning of the many organs of our body. All living organisms use thiamin, but it is synthesized only in bacteria, fungi, and plants. Recently, a publication has described production potency of thiamin by LAB isolated from Japanese Pickles [18]. In this study each bacterium was inoculated in thiamin free medium, after incubation thiamin concentration of supernatants (extracellular) and cells (intracellular) were determined. *Lactobacillus plantarum* L-82 produced Extracellular 1.1 µg/liter thiamine and Intracellular 9.8 µg/liter thiamine, total production was 10.9 µg /liter thiamine. The concentration of thiamine in milk can be 11% increased by 48 h of fermentation with *Bifidobacterium longum* [29]. Thiamine level in medium can be increase by Soy fermentation with *Streptococcus thermophilus* ST5 and *Lactobacillus helveticus* R0052 [30].

Lactic acid bacteria as source of low calorie sweeteners

Low calorie sweeteners produce by LAB are also known as polyols. Polyols are sugar alcohols largely used as sweeteners and they have several health-promoting effects. They have low-caloric, low-glycemic, low-insulinemic, anti-cariogenic, and prebiotic characteristics. Polyols successfully produce by LAB include mannitol, sorbitol, tagatose and trehalose. Their production can be enhancing with the help of metabolic engineering.

Mannitol

(D-)Mannitol is a naturally occurring six-carbon sugar alcohol or polyol. It is about 50% as sweet as sucrose. Mannitol has a low caloric content. Mannitol has been used safely around the world for over 60 years. We can add mannitol directly to foods, or the use of mannitol-producing microorganisms might direct lead to ''natural'' mannitol-containing foods. Mannitol is synthesized by many eukaryotes like fungi and yeasts. Few bacteria, mainly heterofermentative LAB, without the co-formation of sorbitol produce mannitol (Homofermentative LAB also produce mannitol but in very low levels). By using mannitol dehydrogenase (MDH) these LAB are known to convert fructose to mannitol.

By optimizing the mannitol fermentation of heterofermentative LAB, Increased mannitol yields have been achieved [31]. Recently, a publication has described Mannitol production by LAB grown in supplemented carob syrup [32]. *Leuconostoc fructosum* NRRL B-2041 produce 2.36 g/l mannitol per hour [33]. Choice of carbon sources and fermentation conditions are directly affect the mannitol yield by LAB. Several strategies have also been reported for enhancing mannitol production from *Lactococcus lactis* and *Lactobacillus plantarum*.

Sorbitol

Sorbitol is a six-carbon sugar alcohol. Based on its sweetness and its high solubility it is largely used as an ingredient in the food industry. It has 60% of the sweetness of sucrose. It leaves a sweet, cool and pleasant taste. It is an excellent humectant and texturizing agent, it may be helpful to people with diabetes. Sorbitol occurs naturally in various fruits and berries. In LAB, sorbitol production through metabolic engineering has been reported with *Lactobacillus plantarum* [34] and *Lactobacillus casei*. Nissen *et al*. [35] constructed a *Lactobacillus casei* strain in which the sorbitol-6-P-dehydrogenase gene (gutF) was integrated into the chromosomal lactose (lac) operon.

Trehalose

Trehalose is also known as mycose. It is a natural alpha-linked disaccharide. Trehalose sugar has ability to inhibit fat cell enlargement and progress of type 2 Diabetes. Study identifies the beneficial effects of trehalose in preventing metabolic syndrome. According to Studies trehalose is more stable than other sugars. Within the genus *Propionibacterium*, trehalose is widespread[36]. Due to stress condition trehalose accumulation in some *Propionibacterium like Propionibacterium acidipropionici* and *Propionibacterium freudenreichii subsp. shermanii* [37] has also been observed. In particular, *Propionibacterium freudenreichii subsp. shermanii* strain NIZO B365 trehalose content increases considerably in response to osmotic, oxidative and acid stress [38].

Tagatose

Tagatose is an isomer of fructose that occurs naturally in some dairy products. On blood glucose and insulin levels, It has a minimal effect. It also provides a prebiotic effect. Tagatose is mainly used as a flavor enhancer or as a low carbohydrate sweetener. L-arabinose isomerase catalyzes the conversion of D-galactose to D-tagatose [39, 40]. The LAB reported to contain L-arabinose isomerase are *Lactobacillus plantarum*, and *Bifidobacterium longum* [41].

Lactic acid bacteria as source of organic acids

The preservative effect of LAB during the manufacture and subsequent storage of fermented foods is mainly due to the acidic conditions that they create in the food during their development. This souring effect is primarily due to the fermentative conversion of carbohydrates to organic acids (lactic and acetic acid). Acid production directly makes the food pH acidic, an important characteristic that can be used to increase shelf-life and safety of the final product. Organic acids possess a long chain of carbons attached to a carboxyl group. Antimicrobial Potential of LAB is also mainly due to organic acids.

Lactic acid

Lactic acid is also known as milk acid. The natural presence of lactic acid in dairy products enhance dairy flavor. Antimicrobial action of lactic acid makes lactic acid an excellent acidification agent for many dairy products. It produces by natural fermentation in products. LAB have complex nutrient requirements and they ferment sugars via glycolytic pathway (Homofermentative metabolism), and phosphoketolase pathway (Hetrofermentative metabolism), resulting in homo-, hetero-, or mixed Lactic acid fermentation [42]. It is believed that most of the LAB used for commercial lactic acid production is belongs to the genus *Lactobacillus* [42, 43]. Raw materials, such as starchy and cellulosic materials can be used for lactic acid production [42]. Starchy and cellulosic materials are currently receiving a great deal of attention, because they are cheap and easily available[44-46].

Acetic acid

Acetic acid is also known as ethanoic acid. It is an organic acid having sour taste and pungent smell. Acetic acid produced naturally by fermentation can be called vinegar. In the food industry, acetic acid is used as an acidity regulator and as a condiment. Acetic acid can be used as preservative due to excellent bacteriostatic properties. Through heterofermentative pathways LAB strains produce acetic acid in small amount. Recently one publication has described varying quantities of organic acid production by lactic acid cocci[62]. In this study Lactic acid production was in large amounts and acetic acid was only 49.65 mg/ Liter by *Pediococcus sp*. G5 [62], but they are more antimicrobially effective than lactic acid because acetic acid has higher pKa values than lactic acid (lactic acid 3.08, acetic acid 4.75). In the comparison of lactic acid, acetic acid has higher antimicrobial activity towards *Listeria monocytogenes* [63, 64] and *Bacillus cereus* [65.] Antifungal activity of several LAB against *penicillium discolor* is due to acetic acid in the medium [66.] Recently one publication has described production of acetic acid by LAB from pure and biodiesel derived raw glycerol [60]. *Lactococcus lactis subsp.la* produced 2.33 gm/ Liter acetic acid from pure glycerol and 2.13 gm/ Liter acetic acid from Biodiesel derived raw glycerol [60].

Lactic acid bacteria as source of aroma compounds

The typical flavors of fermented milk are mainly due to acids and aroma compounds. Acids responsible for flavor are lactic, pyruvic, oxalic, succinic, acetic, propionic and formic acids. Compounds responsible for flavor are carbon compounds such as acetaldehyde, acetone, acetate and diacetyl and volatile sulfur compounds. Products from the thermal degradation of proteins, lipids or lactose are also responsible for flavors of fermented milk. Among all this diacetyl, acetaldehyde and volatile sulfur compounds are found in significant quantities and are responsible for the characteristic smell of dairy food.

Diacetyl

Diacetyl (2,3-butanedione) is a volatile product of citrate metabolism. It produced by certain bacteria, including *Lactococcus lactis* and *Leuconostoc citrovorum*. In the production of butter, buttermilk and several cheeses, citrate -utilizing LAB produces diacetyl during milk fermentation and diacetyl generates the typical butter aroma in these products[67]. Production of diacetyl from lactose rather than citrate has been the aim of several metabolic engineering strategies due to its value as aroma compound. Hefa Cheng showed that More than 100 volatiles are found in yogurt at low to trace concentrations[68]. To increase the levels of naturally occurring buttery aroma associated with fermentation, starter distillates (SDL) are used as ingredients in the formulation of many food products. According to Rincon-

Delgadillo *et al*., high amount of Diacetyl, ranging from 1.2 to 22,000µg/g was present in the SDL [69]. Most recently, a publication has described *Lactococcus lactis* strains producing diacetyl and acetoin isolated from diverse origins [70]. In this publication it has been reported that both domesticated and environmental strains produced diacetyl or acetoin.

Acetaldehyde

As the main aromatic compound in yoghurt, Acetaldehyde was firstly reported by Pette *et al*. [71]. Production of acetaldehyde by *Streptococcus thermophilus* and *Lactobacillus. bulgaricus* occurs during yoghurt fermentation. The final amount of acetaldehyde is dependent on enzymes, which are able to catalyse the formation of carbon compounds from the various milk constituents. Three metabolic pathways (from glucose in the glycolytic pathway, from the degradation of DNA, and from L-threonine with threonine aldolase) producing acetaldehyde were identified. From glucose 90% of acetaldehyde produced by *Lb. bulgaricus* and 100% produced by *Streptococcus. thermophilus* [72]. Over expression of glyA in *Streptococcus thermophillus* strains by Chaves *et al*. [73] resulted in overproduction of acetaldehyde. Acetaldehyde production through metabolic engineering for *Lactococcus lactis* was reported by Bongers *et al*. [74].

Volatile sulfur compounds (VSC)

Methanethiol, dimethyl disulfide and dimethyl trisulfide are important volatile sulfur compounds play an important role for Cheddar cheese flavor. Methionine is the aromatic and the branched-chain amino acid. Majority of sulphur aromatic compounds come from Methionine [61]. *Lactobacilli, Lactococci* and *Micrococci* produce lesser amounts of methanethiol from methionine [75, 76]. Methanethiol is easily oxidized to dimethyl disulphide and dimethyl trisulphide [77]. Recently, a publication has described Volatile sulphur compounds-forming abilities of LAB [78]. In this study LAB from different ecological origins were screened for their abilities to produce VSCs from L-methionine, *Streptococcus thermophilus* STY-31 was best for VSC production, therefore could be used as a starter culture in cheese manufacture.

Table 1. Lactic acid production by lactic acid bacteria using different raw materials

Raw material	Organism	lactic acid gm/Liter	Reference
Molasses	*Lactobacillus delbrueckii* NCIMB 8130	90.0	47
	Lactobacillus delbrueckii subsp. delbrueckii Mutant Uc-3	166.0	48
Wheat	*Lactococcus lactis ssp. lactis* ATCC 19435	106.0	49
Corn	*Lactobacillus amylovorus* ATCC 33620	10.1	50
Cassava	*Lactobacillus amylovorus ATCC 33620*	4.8	50
Potato	*Lactobacillus amylovorus ATCC 33620*	4.2	51
Rice	*Lactobacillus sp.* RKY2	129.0	51
Barley	*Lactobacillus casei* NRRL B-441	162.0	52
	Lactobacillus amylophilus GV6	27.3	53
Cellulose	*Lactobacillus coryniformis ssp. torquens* ATCC 25600	24.0	54
Waste paper	*Lactobacillus coryniformis ssp. torquens* ATCC 25600	23.1	55
Wood	*Lactobacillus delbrueckii* NRRL B-445	108.0	56
Whey	*Lactobacillus helveticus* R211	66.0	57
	Lactobacillus casei NRRL B-441	46.0	58
Corn starch	*L. amylophilus* GV6	76.2	59
	L. rhamnosus HG 09	57.6	59
Biodiesel derived	*Lactobacillus delbrueckii*	4.37	60
Raw glycerol	*Lactobacillus pentosus*	1.43	60
Pure glycerol	*Lactococcus lactis*	2.26	60
	Lactobacillus delbrueckii	1.68	60
	Lactobacillus pentosus	*1.12*	60
	Lactobacillus casei	0.99	61

Lactic acid can be produced by LAB in its L- or D-isomer form. L-lactic acid is important for food and pharmaceutical applications, while D-lactic acid is toxic for humans.

Lactic acid bacteria as source of exopolysaccharides

Exopolysaccharides (EPSs) are high-molecular-weight polymers that are composed of sugar residues and are secreted by a microorganism into the surrounding environment. For their ability to secrete extracellular polysaccharides, LAB has aroused interest [79, 80]. The EPSs produce by LAB have great role in rheology and texture of fermented dairy products [81, 82]. Some EPSs produced by LAB present potential health-beneficial Properties, such as immune stimulation[83], anti-ulcer and cholesterol-lowering activities[84]. EPS from microbial sources can be classified into two groups: homopolysaccharides and heteropolysaccharides based on their monosaccharide composition and biosynthetic pathway [85].

Homopolysaccharide (HoPS)

Homopolysaccharides are polysaccharides (polymers) composed of a single type of sugar monomer. LAB produce HoPS consist of identical monosaccharides, d-glucose or d-fructose. According to monosaccharides HoPS can be divided into two major groups: glucans and fructans [86]. LAB employs sucrase-type enzymes to convert sucrose into homopolysaccharides consisting of either glucosyl units (glucans) or fructosyl units (fructans). The enzymes involved are labeled glucansucrases (GS) & fructansucrases (FS), respectively. Glucansucrase produces glucans as dextran, alternan and reuteran. Similarly, fructansucrase produces levan and inulin- type of fructans.

Dextran

Dextran is a high molecular mass glucan that is synthesized from sucrose and composed of chains of D-glucose units. In food industry it is being used as thickener for jam and ice cream. It prevents crystallization of sugar. For the maintenance of flavor of various foodstuffs it is very important. Hucker and Pederson[87] was the first who reported the production of dextran by strains of *Leuconostoc* species from sucrose. The most commercially used strain of *Leuconostoc mesenteroides* is NRRL B- 512F [88]. Shah Ali [89] showed that *Leuconostoc mesenteroides* PCSIR-4 and PCSIR-9 produce dextran of different quality. Farwa Sarwat [90] showed that *Leuconostoc mesenteroides* CMG713 produce maximum dextran after 20 hours of incubation at 30°C with 15% sucrose at pH 7.0.

Alternan

Alternan is a glucan having alternate á-1, 6 and á-1, 3 linkages. This structure is responsible for its high solubility and low viscosity. Because of these characteristics glucan can be used as a low viscosity texturizer in foods. *Leuconostoc mesenteroides* NRRL B-1355 was first reported to be an alternan-producing strain [91]. Other strains producing alternansucrase are *Leuconostoc mesenteroides* NRRL B-1501 and NRRL B-1498.

Reuteran

Reuteran is a water soluble glucan produced by reuteransucrase. *Lactobacillus reuteri* strain LB 121, *Lactobacillus reuteri* strain ATCC 55730 and *Lactobacillus reuteri* strain 35–5 have been reported to produce reuteran. Because of water solubility, it can be used in bakery [92.]

Levan

Levan is a fructan composed of d-fructofuranosyl residues joined by β-2, 6 with multiple branches by β-2, 1 linkage. It can be used as a functional biopolymer in foods and cosmetics. This functional biopolymer has great application in pharmaceutical and chemical industries. Levan is also beneficial for health, because it is a polymer having antitumor properties [93]. LAB genera producing levan are *Steptococcus, Leuconostoc* and *Lactobacillus*. Levan from *Lactobacillus. sanfranciscensis* LTH 2590 has prebiotic effects [94].

Inulin-type

Inulin is a fructan composed mainly of fructose units, and also have a terminal glucose. It can be used to replace sugar. Inulin contains 25-35% of the food energy of starch and sugar (carbohydrates). Inulin acts as a prebiotic and promot the growth of intestinal bacteria. Due to its prebiotic property, it increases calcium absorption and magnesium absorption in our body [95, 96]. *Lactobacillus johnsonii* NCC 533 produces high molecular mass inulin from sucrose by using an inulosucrase enzyme[97]. *Streptococcus mutans* strain JC2, *Leuconostoc citreum* CW28 and *Lactobacillus reuteri* 121 [98] are some other LAB which produce inulins.

Heteropolysaccharide (HePS)

Polysaccharides consisting of molecules of more than one sugar or sugar derivative are called heteropolysaccharides (heteroglycans). HePS comprise gellan, xanthan and kefiran. Among all these HePS kefira is main HePS from LAB.

Kefiran

Kefir grains consist of a polysaccharide gel embedding LAB and yeasts [99, 100]. *Lactobacillus kefiranofaciens* is an important organism associated with kefir grains. This organism produces the kefiran polymer; this polymer forms the matrix of the kefir grains [101,102]. Other microorganisms associated with kefir are the homofermentative strains *Lactobacillus acidophilus* and *Lactobacillus kefirgranum*, the obligately heterofermentative strains *Lactobacillus kefir* and *Lactobacillus parakefir* [103]. Kefiran is reported to have antimicrobial and wound healing properties; it has ability to lower blood pressure and cholesterol in serum and it has capacity to retard tumor growth also [104]. It enhance IgA level at both the small and large intestine level and influence the systemic immunity through the release of cytokines into the blood [105].

CONCLUSION

LAB are very promising sources for industrially important compounds. It has great application, which can satisfy the consumer's demands for functional foods. They can be used in the diet of humans and animals, with particular health improving industrially important compounds. Despite recent advances, the study of LAB and their industrially important compounds are still an interesting field of research that needs further research.

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