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Biosynthesis of vitamins and enzymes in fermented foods by lactic acid bacteria and related genera - A promising approach

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review

Summary

Lactic acid bacteria (LAB) are widely employed in food fermentation processes for the biosynthesis of certain important products or metabolites. Fermented food provides plenty of vital nutrients and bioactive components that affect a number of functions of human body in a positive way. Fermented milks can be made more functional by incorporating probiotic strains and furthermore, if they are capable of synthesizing essential biomolecules such as vitamins, enzymes, exopolysaccharides, bacteriocins or bioactive peptides serve into the functional and technological properties of the products. Current paper reviews recent advances associated with biosynthesis of vitamins and enzymes by virtue of LAB and related genera. The outcomes of several studies indicate promising applications at commercial level; however adequate selection of strain is vital to increase the concentration and bioavailability of such biomolecules in fermented foods.

Keywords: probiotic, folic acid, riboflavin, menaquinone, beta-galactosidase

Introduction

In dairy and food industries, lactic acid bacteria (LAB) and related organisms have been used as starter culture and they plays very imperative role in fermentation. They may improve nutritional, organoleptic, technological and shelf-life characteristics in diverse fermented foods and beverages (Shah and Prajapati, 2013; Capozzi et al., 2012). The LAB initiate rapid and adequate acidification in the raw materials through the production of various organic acids from carbohydrates. Among that lactic acid is the most abundant, followed by acetic acid, ethanol, bacteriocins, aroma compounds, exopolysaccharides and some enzymes within the food matrices. Some of these metabolites exert antimicrobial activity and are found to inhibit spoilage causing and/or disease causing bacteria and thus help to maintain and preserve the nutritive qualities of foods for an extended shelf life (O'Sullivan et al., 2002). Because of GRAS status, the use of LAB or their metabolites, as a natural preservative in food has gained much importance in recent years.

The LAB also have a huge market as probiotics, bacteria which confer health benefits in humans upon ingestion (FAO/WHO, 2006). Firstly, Metchnikoff discovered the beneficial effects of LAB on human health in the population who were regularly consumed yoghurts and fermented milks. This innovative discovery opened new door for health and pharmaceutical industries to explore the beneficial effects of LAB. Afterwards many probiotics have been claimed and efficiently proven to cure or reduce various health problems including lactose intolerance, cholesterol reduction, immunomodulation in gastric illnesses and diarrhoea, food allergy, antimutagenic and antimicrobial activities. In market, probiotic cultures are used for the manufacturing of a number of products such as yoghurt, cheese, ice cream, chocolates pharmaceutical tablets, infant formulas and dietary supplements (Tamine et al., 2005). Intestinal microbiota has also been shown to produce short chain fatty acids (SCFA), conjugated linoleic acid (CLA), essential amino acids, group B vitamins and vitamin K, contributing to the well-being of a host (Marques et al., 2010). It can be revealed from Fig. 1 that LAB find enormous applications at industrial level for the biosynthesis of a number of compounds as metabolic end products or secondary metabolites such as organic acids; exopolysaccharides (EPS), bacteriocins, enzymes and vitamins.

Adequate information is available regarding application of LAB in the biosynthesis of organic acids, especially lactic acid. Similarly, plenty of literature mentioned importance and potential appliance of exopolysaccharides and bacteriocins like nisin from LAB (Patel et al., 2012; O'Connor et al., 2005). Though it is well established that certain

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LAB have capability to synthesize water-soluble vitamins specifically those included in the B-group (folic acid, riboflavin, cobalamin and biotin), since they are produced in minute quantity not much information is available regarding their amount of production. Current article gives an overview of research being conducted so far with regard to biosynthesis of some vitamins and enzymes from these industrially important bugs and discusses the future possibilities.

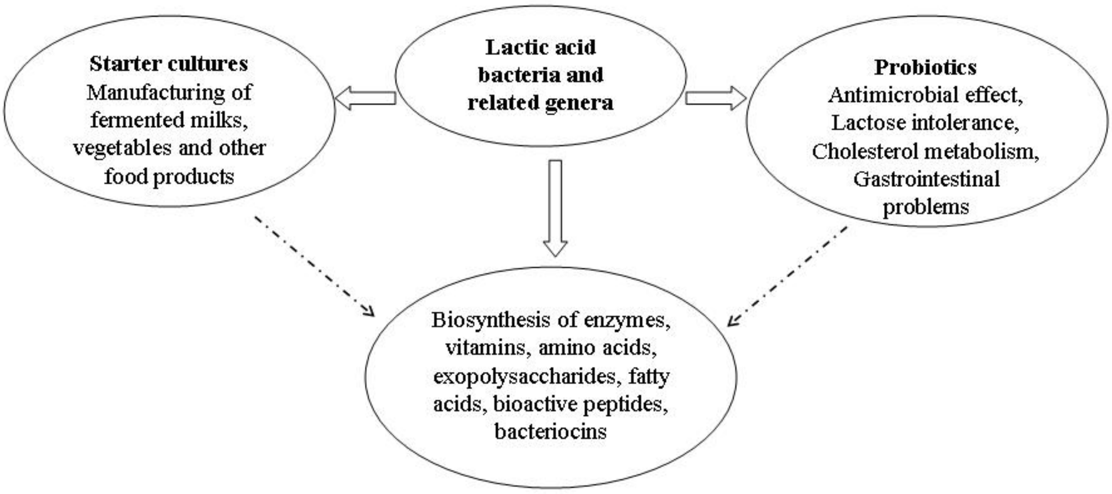

Fig. 1. Biosynthesis of various biomolecules by LAB and related genera

Vitamin biosynthesis by Lactic acid bacteria and Bifidobacterium

Vitamins are involved in many essential functions of the body like cell metabolism, synthesis of nucleic acids and antioxidant activities. Most of the vitamins cannot be synthesized by humans and animals; however several species of bacteria, yeasts, fungi and algae may serve to produce folic acid, vitamin B12 or cobalamin, vitamin K2 or menaguino, riboflavin, thiamine, and other essential vitamins (LeBlanc et al., 2011). In fermented milk products like curd, yogurt, cultured butter milk, cheeses and other milk and cereal based fermented foods biosynthesis and liberation of vitamins have also been reported by the aid of LAB fermentation. Furthermore, inherent beneficial microbes present in the gastrointestinal tract have been recognized as a source of some water soluble vitamins. The possible strategies to increase B-group vitamin content in cereals-based products may lead to the elaboration of novel functional fermented foods (Capozzi et al. 2012). In addition, the use of genetic strategies to increase vitamin production or to create novel vitamin-producing strains is more fascinating.

1. *Folic acid (or Vitamin B9)*

Folic acid, which belongs to the B-group of vitamins is also called vitamin M. It is important for many metabolic reactions, in the biosynthesis of DNA and RNA and the inter-conversions of amino acids. Also, folate possesses antioxidant competence that protects the genome by preventing free radical attack (LeBlanc et al., 2011). The term folate is used to describe folic acid derivatives such as the folyl glutamates which are naturally present in foods while folic acid is the chemically synthesized form of folate, commonly used for food fortification and nutritional supplements. Dietary folate is essential for humans, since it cannot be synthesized by mammalian cells. Folate may found in legumes, leafy greens, some fruits and vegetables, in liver and fermented dairy products. As suggested by Wouters et al. (2002), in yogurts the amount of folate may be increased depending on the starter cultures used and the storage condition to values above 200 µg / litre. Folate deficiencies are associated with a variety of disorders like osteoporosis, Alzheimer’s disease, coronary heart disease and increased risk of breast and colorectal cancer as indicated from epidemiological studies (Rossi et al., 2011).

In the current time, food industries focuses on the strategy to select and employ folate producing probiotic strains, to produce fermented products with elevated amount of “natural” folate without increasing production cost and inherent tendency to provide desire health benefits (Rossi et al., 2011). Among LAB, many *Lactobacillus* spp. and lactococci spp. including *L. Plantarum, L. bulgaricus, Lc. lactis,* *Streptococcus thermophilus* and *Enterococcus* spp. have the ability to produce folate. On the other hand, some lactobacilli (*L. gasseri, L. salivarius, L. acidophilus* and *L. johnsonii*) used as both starter cultures and probiotics, cannot synthesize folate because they lack few specific genes involved in folate biosynthesis (LeBlanc et al. 2007).

Sybesma et al. (2003) reported production of folate from *Lc. lactis*, *Stre. thermophilus*, and *Leuconostoc* spp. and most *Lactobacillus* spp., except of *L. plantarum*. Authors described that several environmental parameters could influence folate production; high external pH and the addition of *p*-aminobenzoic acid stimulated folate production, while high tyrosine concentrations led to decreased folate biosynthesis. On the other hand, some starter cultures and probiotic lactobacillus strains in non-dairy foods utilize more folate than they produce as reported by O'Connor et al. (2005).

Recently, Goswami (2012) evaluated production of folic acid and biotin from *L. helveticus* MTCC 5463, a probiotic strain and *L. rhamnosus* MTCC 5462, a normal lactobacilli. Both the strains increased contents of folic acid in fermented milks either used singly and in combination; however, biotin concentration increased only with probiotic strain. Similar to this, in another *in vitro* investigation increased production of folic acid was observed in human subjects after administration of probiotic *Bifidobacterium* strains (Strozzi and Mogna, 2008).

Bifidobacterium species are generally believed to produce group B vitamins including folate, cobalamin, pyridoxine, riboflavin and thiamin. The ability of *Bifidobacteria* to produce folate is dynamic though it is strain specific and depends on the medium. Optimization and selection of suitable growth conditions can result in high levels of folate per cell unit biomass. Recently, 19 strains of bifidobacteria were screened to find main folate forms composition in synthetic folate-free and complex folate-containing media (D'Aimmo et al., 2012). In another study, 76 strains of 15 *Bifidobacterium* species of human and animal origin were tested for their ability to produce folate (Pompei et al., 2007). Total 17 strains belonging to 9 species were capable of folate production, with two strains of *B. adolescentis* and one strain of *B. pseudocatenulanum* being the top-three producers. Earlier 24 strains belonging to species of *B. bifidum*, *B. breve*, *B. longum longum*, *B. longum infantis* and *B. adolescentis* were tested for their ability to produce B vitamins (Deguchi et al., 1985). In a separate experimental approach, all tested strains were able to produce pyridoxine (vitamin B6) and small amounts of cobalamin, which is very important in early days of life as it is responsible for the normal growth and development of the nervous system (Marques et al., 2010).

Production of thiamin and nicotinic acid (vitamin B3) found to be strain-dependant as all tested strains of *B. bifidum*, *B. longum, B. infantis*, and some of *B. breve* were high producers, while *B. adolescentis, B. longum* and some strains lacked this ability (Deguchi et al., 1985). Despite of that however, it remains unclear whether the amount of vitamins produced by bifidobacteria makes any contribution to the vitamin status of the host (Rossi and Amaretti, 2010).

Crittenden et al. (2003) carried out mixed culture fermentations of reconstituted skim milk using folate-producing strains of *Bifidobacterium* in conjunction with *Streptococcus thermophilus* and/or *Lactobacillus delbrueckii* subsp. *bulgaricus* from conventional yogurt, demonstrating that it is possible to increase folate levels in fermented milk products through appropriate selection of bacterial strains.

Tempeh, a traditional Indonesian soybean based fermented food shown elevated levels of B group vitamin from *streptococcus* and e*nterococcus* species as reported by Keuth and Bisping (1993).

2. *Vitamin B12 (or Cobalamin)*

One of the very important vitamins, vitamin B12 popularly known as cobalamin is required for the metabolism of fatty acids, amino acids, nucleic acids and carbohydrates (Quesada-Chanto et al., 1994). It cannot be synthesized by mammals and must be obtained from exogenous sources like foods or the intestinal microbiota. This vitamin is the largest and most structurally complicated vitamin and can be produced industrially only through bacterial fermentation-biosynthesis. Some members of the genus *Lactobacillus* have the ability to produce vitamin B12, in particular a probiotic strain of *L. reuteri* which exhibits hypocholesterolaemic activity in animals can produce B12 (Taranto et al., 2003). A few species of Propionibacteria such as *Propionibacterium shermani* is also responsible for producing vitamin B12 in certain cheese varieties (Burgess et al., 2004).

Deficiency of vitamin B12 can potentially cause severe and irreversible damage, especially to the brain and nervous system and haematopoietic (pernicious anaemia). At levels only slightly lower than normal, a range of symptoms such as fatigue, depression, and poor memory may be experienced (Anon, 2011). Furthermore, this deficiency in male animal models influenced the number of offspring which showed growth retardation and decrease in some blood parameters (Molina et al., 2008).

Beitane and Ciprovica (2012) assessed the effect of prebiotic compounds lactulose and inulin at different concentrations during milk fermentation on production of B group vitamins such as thiamin, riboflavin, pyridoxine and cobalamin using *Bifidobacterium lactis*. The concentration of vitamin B6 in fermented milk was possible to increase ($p > 0.05$) by adding lactulose in concentrations to 3 % and inulin to 4 %. Authors concluded that the concentration of prebiotics has the significant influence on vitamin B12 content in fermented milk ($p < 0.05$).

3. *Vitamin B2 (or riboflavin)*

Riboflavin functions as a vital element in cellular metabolism being the precursor of coenzymes acting as hydrogen carriers in biological redox reactions. Riboflavin is present in many foods such as green vegetables, dairy products, eggs and meat. Riboflavin deficiency can lead to damages in liver, skin and changes in the brain glucose metabolism (LeBlanc et al., 2011), with symptoms like hyperaemia, sore throat, odema of oral and mucous membranes, cheilosis and glossitis (Wilson, 1983).

Recent article mentioned screening of riboflavin producing LAB from Vellore region of India (Jayashree et al., 2010). As described by author efficient riboflavin producing bacterium *L. fermentum* MTCC 8711 showed 2.29 mgl^{-1} of riboflavin in chemical defined media after 24 h. Riboflavin-producing LAB strains were isolated and used as a convenient biotechnological application for the preparation of bread (fermented sourdough) and pasta to enrich them with vitamin B2 (Spano, 2011). Burgess et al. (2004) carried out genetic analysis of the riboflavin biosynthetic (*rib*) operon in *Lc. Lactis* subsp. *cremoris* strain NZ9000. The strain showed enhanced riboflavin synthesis because of simultaneous overexpression of riboflavin biosynthetic genes (ribG, ribH, ribB and ribA) in *L. lactis*. While through by-directed mutagenesis followed by metabolic engineering, Sybesma et al. (2004) modified two complicated biosynthetic pathways in *L. lactis* that resulted in simultaneous overproduction of both folate and riboflavin.

4. *Vitamin K*

Vitamin K plays significant role in blood clotting, bones and kidneys function, tissue calcification and atherosclerotic plaque (Olson, 1984; Brown, 2010). Normally, vitamin K is present as phylloquinone (Vitamin K1) in green plants and as menaquinone (K2) produced by some intestinal bacteria like LAB, especially by various strains of the genera *Lactococcus*, *Lactobacillus*, *Enterococcus*, *Leuconostoc* and *Streptococcus* (Cooke et al., 2006; O'Connor et al., 2005). Cooke et al. (2006) isolated *Enterobacter agglomerans*, *Serratia marcescens* and *Enterococcus faecium* from neonatal feacal flora that were able to produce various forms of menaquinone as analyzed in liquid chromatography-mass spectrometry. Vitamin K deficiency is found to be associated with some clinical disorders like intracranial haemorrhage in newborn infants and possible bone fracture resulting from osteoporosis (LeBlanc et al., 2011). In this regards, LAB producing menoquinone could be useful to supplement vitamin K in humans (Morishita et al., 1999).

Apart from such naturally available microbiota, the strain improvement methods through genetic manipulations also showed promising outcomes. In one of the experimental approach Burgess et al. (2004) carried out over-expression of riboflavin biosynthesis genes in *L. lactis* ssp. *cremoris* NZ9000 that led to conversion of riboflavin utilizing strain in to vitamin B2 producing factory (24 mg l^{-1} of riboflavin). Similarly, metabolic engineering also showed to improve folate production in other LAB strains such as *L. gasseri*, *L. reuteri*, (Wegkamp et al., 2004, Santos et al., 2008). In *L. lactis,* by directed mutagenesis followed by selection and metabolic engineering resulted in simultaneous overproduction of folate and riboflavin (Sybesma et al., 2004). Random mutagenesis and genetic engineering lead to improved production of Vitamin B12 by *Propionibacterium freudenreichii* (Martens et al., 2002; Burgess et al., 2004). Moreover, the biosafety assessment of genetically modified LAB (*fol*C, *fol*KE, *fol*C+*fol*KE) demonstrated that they were safe as native strains from which they were derived (Le-Blanc et al., 2010).

Enzyme biosynthesis

LAB have been found to synthesize a diverse type of enzymes which may influence the compositional, processing and organoleptic properties plus overall quality of foods and feeds. They release various hydrolytic enzymes into the gastrointestinal tract that may exert potential synergistic effects on digestion and alleviate symptoms of intestinal malabsorption (Naidu et al., 1999). In other words, these beneficial organisms may serve as an alternate source for the preparation of enzyme extracts that are able to function under the environmental conditions of fermentation (Tamang, 2011). The enzymatic activity has been studied mainly in LAB isolated from wine or other fermented foods like cheeses and yoghurt

(Mtshali, 2007; Matthews et al., 2004). Distinct species of *Lactobacillus*, *Lactococcus, Pediococcus* and *Bifidobacterium* connected with fermented foods have been found to produce carbohydrate degrading enzymes like glucosidases, amylases and xylanases (Patel et al., 2012; Novik et al., 2007). In general, LAB have been found to produce higher alpha/ beta-galactosidase than Bifidobacteria (Alazzeh et al., 2009). Novik et al. (2007) acknowledged synthesis of hydrolases from LAB and Bifidobacteria. After six months of storage, bacteria retained about 60-70 % of beta-galactosidase and alpha-amylase activities. LAB produced amylases which are the most stable and find application to be used during sourdough technology for the natural improvement of bread texture (Mogensen, 1993).

LAB contribute to the aroma and flavor of fermented products when employed as starter cultures. Certain peptidases produced by *Lc. lactis* subsp. *cremoris* improved the sensory quality of cheese and furthermore, proteolysis and lipolysis could enhance the flavour of most varieties of cheese (Guldfeldt et al., 2001; Gonzalez et al., 2010). LAB strains isolated from a traditional Spanish Genestoso cheese were evaluated for the enzymatic activity and it was reported that dipeptidase activity of high level was associated with *Lactococcus* spp, enterolytic activity was detected for *Enterococcus* spp., while carboxypeptidase activity was very less or undetectable (Gonzalez et al., 2010).

Enzymes of various LAB and related species play an important role in winemaking. As a result of fermentation and associated enzymatic activities, wine flavor and aroma develops. These bacteria grow in wine during malolactic fermentation, following alcoholic fermentation, while a broad range of secondary modifications improve the taste and flavor of wine (Mtshali, 2007).

Table 1. Vitamin biosynthesis by LAB and related genera

Vitamin	Producing bacterium/strain	Product/subject	Reference(s)
Folic acid	*Streptococcus thermophiles, Lactobacillus bulgaricus*	Yoghurt	Wouters et al., 2002
	Bifidobacterium spp., *Stre. thermophilus* and/or *Lactobacillus delbrueckii* subsp. *bulgaricus*	Reconstituted Skim milk	Crittenden et al. 2003
	Lc. lactis, Stre. thermophilus, *Leuconostoc* spp. *Lactobacillus* spp.		Sybesma et al., 2003
Folic acid and biotin	*Bifidobacterium* spp.	Human volunteers	Strozzi and Mogna, 2008
	Bifidobacterium spp.		Pompei et al., 2007
	Bifidobacterium spp.	Synthetic media	D'Aimmo et al., 2012
	L. helveticus MTCC 5463, *L. rhamnosus* MTCC 5462	Fermented milk	Goswami, 2012
Folic acid, thiamin and Vitamin B3 (nicotinic acid)	*B. bifidum*, *B. breve*, *B. longum longum*, *B. longum infantis, B. adolescentis*		Deguchi et al., 1985
Riboflavin	*L. fermentum* MTCC 8711	chemical defined media	Jayashree et al., 2010
	Lactic acid bacteria	sourdough	Spano, 2011
Vitamin B6 (pyridoxine) and Vitamin B12 (cobalamin)	*B. bifidum*, *B. breve*, *B. longum longum*, B. *infantis*		Marques et al., 2010
Vitamin B12 (cobalamin	*L. reuteri* and other lactobacilli		Taranto et al., 2003
	Propionibacterium shermani		Burgess et al., 2004
Vitamin B complex	*Enterococcus* spp.	Tempeh	Keuth and Bisping, 1993
	Bifidobacterium lactis	Fermented milk	Beitane and Ciprovica, 2012
Vitamin K	*Lactococcus*, *Lactobacillus*, *Enterococcus*, *Leuconostoc* and *Streptococcus*		O'Connor et al., 2005; Cooke et al., 2006

Conclusions

Lactic acid bacteria served as inimitable source for developing novel products and applications, especially those that can satisfy the increasing consumer's demands for natural products and health benefits. The identification and application of LAB and related strains delivering health-promoting compounds is very promising field and furthermore, their ability to enrich the food matrix or human body with the aid of producing vitamins and biocatalysts further enhance the scope of utilizing them for medicinal and health applications. Vitamin producing LAB could be a worthwhile alternative to fortification programmes and be useful in the elaboration of novel vitamin-enriched food products. Recent advances dealing with LAB and their functional ingredients suggest that we have yet to realize their full potential.

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