Intl. J. Food. Ferment. Technol. 4(2): 79-85, December, 2014

DOI Number: 10.5958/2321-7111.2015.00001.3

Review Paper

Recent advances in biosynthesis of vitamin and enzyme from food grade bacteria

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Paper No. 81 Received: 23 June, 2014 Accepted: 14 September, 2014 Published: 28 December 2014

Abstract

Fermented food provides plenty of vital nutrients and bioactive components that affect a number of functions of human body in a positive way. Fermented dairy and food products can be made more functional by incorporating probiotic bacteria such as strains belonging to genera *Lactobacillus* and *Bifidobacterium*. A few strains of such food grade bacteria are capable of synthesizing essential biomolecules such as vitamins, enzymes, exopolysaccharides, bacteriocins or bioactive peptides during the fermentation that aid into the functional and technological properties of the products. Present paper summarises the recent advances found in the field of biosynthesis of vitamins and enzymes. The outcomes of several studies are indicative of promising applications at commercial level.

Keywords: beta-galactosidase; folic acid, probiotics, riboflavin, vitamin K

In dairy and food industries, lactic acid bacteria (LAB) and related organisms play important role as starter culture in food fermentation (Joshi and Pandey, 1999). They may also lead to 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 (Anno., 2006).

The LAB also have a huge market as probiotics, bacteria which confer health benefits in humans upon ingestion (Annon., 2006). Firstly, Metchnikoff discovered the beneficial effects of LAB on human health in the population who regularly consume 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 manufacture of a number of products such as yoghurt, cheese, ice cream, chocolates, pharmaceutical tablets, infant formulas, dietary supplements, etc (Tamine *et al.* 2005). It can be revealed from Figure 1 that LAB finds numerous applications at industrial level for the biosynthesis of important compounds as metabolic end products such as organic acids; exopolysaccharides (EPS), bacteriocins, enzymes and vitamins.

A large body of niformation is available regarding application of LAB in the biosynthesis of organic acids, especially lactic acid (Yadav *et al.* 1993). Similarly, a 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 LAB have the capability to synthesize water-soluble vitamins specifically those included in the B-group (folic acid, riboflavin, cobalamine and biotin), but has not been documented in a comprehensive manner. The current article gives an overview of research being conducted on biosynthesis of vitamins as well as enzymes from these industrially important bugs and discusses the future possibilities.

Vitamin biosynthesis

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 B_{12} or cobalamin, vitamin K_2 or menaguino, riboflavin, thiamine, and other essential vitamins (LeBlanc *et al.* 2011). In table 1, the vitamins and their producer microorganism alongwith the names of researchers have been summarizes. In fermented milk products like curd, yogurt, cultured butter milks, cheeses and other milk and cereal based fermented foods, biosynthesis and liberation of vitamins have also been reported the aid of LAB fermentation (Prajapati, 1995; LeBlanc *et al.* 2007). Furthermore, inherent beneficial microbes present in the gastrointestinal tract have been recognized as a source of some water soluble vitamins (LeBlanc *et al.* 2007). 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 fascinating.

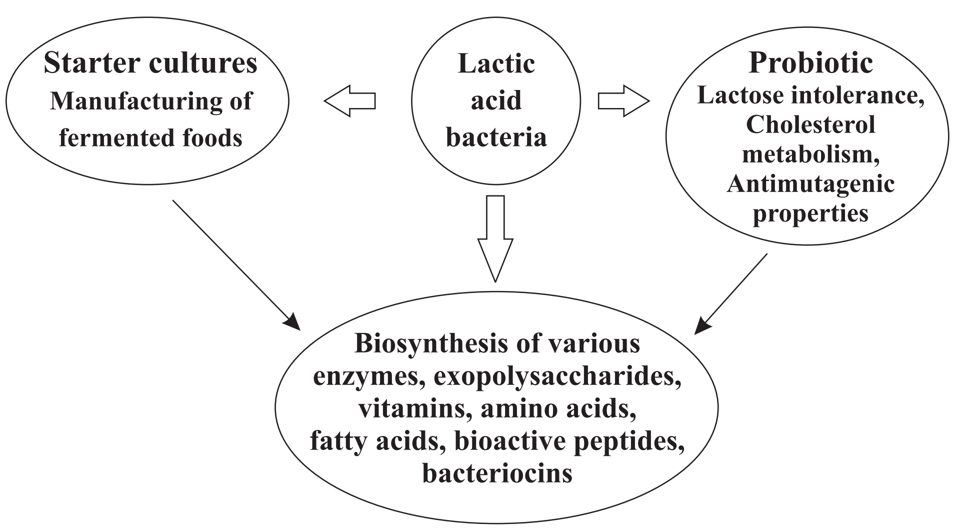

Figure 1. Diverse applications of lactic acid bacteria

1. Folic acid (or Vitamin B_9): 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). In fact, the term folate is used to describe folic acid derivatives such as the folyl glutamates which are naturally present in foods while folic acid that 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 be found in legumes, leafy greens, some fruits and vegetables, in liver and fermented dairy products (Eitenmiller and Landen, 1999). 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 / L. Folate deficiencies are associated with a variety of disorders like osteoporosis, Alzheimer's disease, coronary heart diseases and increased risk of breast and colorectal cancer as indicated by the epidemiological studies (Rossi *et al.* 2011).

Table 1. Vitamin biosynthesis by LAB and related genera

Vitamin	Producing bacterium/strain	Reference(s)
Folic acid and biotin	*Streptococcus thermophiles*, *Lactobacillus bulgaricus*	
	Bifidobacterium spp., *Stre. thermophilus* and/or *Lactobacillus delbrueckii* subsp. *Bulgaricus*	
	Lc. lactis, *Stre. thermophilus*, *Leuconostoc* spp. *Lactobacillus* spp.	
Bifidobacterium spp.		
	L. helveticus MTCC 5463, *L. rhamnosus* MTCC 5462	Wouters *et al.*, 2002
		Crittenden *et al.* 2003; Strozzi and Mogna, 2008
		Sybesma *et al.*, 2003
		Pompei *et al.*, 2007, D'Aimmo *et al.*, 2012
		Goswami, 2012
Folic acid, thiamin and Vitamin B_3-nicotinic acid	*Bifidobacterium* spp. like B. *bifidum*, B. *breve*, B. longum infantis is B. *adolescentis*	Deguchi *et al.*, 1985
Riboflavin	L. *fermentum* MTCC 8711	
Lactic acid bacteria		*Jayashree et al.*, 2010
		Spano, 2011
Vitamin B_6 (pyridoxine) and Vitamin B_{12} (cobalamin)	B. *bifidum*, B. *breve*, B. *longum*, B. *infantis*	Marques *et al.*, 2010
Vitamin B_{12} (cobalamin)	L. *reuteri* and other lactobacilli	
Propionibacterium shermani		*Taranto et al.*, 2003
		Burgess *et al.*, 2004
Vitamin B complex	Enterococcus spp.	
Bifidobacterium lactis		Keuth and Bisping, 1993
		Beitane and Ciprovica, 2012
Vitamin K	*Lactococcus*, *Lactobacillus*, *Enterococcus*, *Leuconostoc* and *Streptococcus*	O'Connor *et al.*, 2005; Cooke *et al.*, 2006

Nowadays the food industry focuses on the strategy to select and employ folate producing probiotic strains, to produce fermented products with elevated amount of “natural” folate without increasing the production cost and inherent tendency to provide desired health benefits (Rossi *et al.* 2011). Among LAB, many *Lactobacillus* spp. including *Lactococcus lactis, L. plantarum, L. bulgaricus, Streptococcus thermophilus* and *Enterococcus* spp. have the ability to produce folate. On the other hand, some lactobacilli strains (*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 stated 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 stated by O’Connor *et al.* (2005). In previous studies also, fermented milk products showed an increase in folic acid content (ropy milk exhibited a twofold increase) and a slight decrease in concentration of vitamin B_{12}. While there was a negligible change in the content of other vitamins (Alm, 1984)

Recently, Goswami and Prajapati (2013) 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 the contents of folic acid in fermented milks either using single or in combination; however, biotin concentration increased only with probiotic strain. Similar to this, in another i*n vitro* investigation, an increased production of folic acid was observed in human subjects after administration of probiotic *Bifidobacterium* trains (Strozzi and Mogna, 2008).

2. Vitamin B_{12} (or Cobalamine): One of the very important vitamins, vitamin B_{12} 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 B_{12}, in particular a probiotic strain of *L. reuteri* which exhibits hypocholesterolaemic activity in animals can produce B_{12} (Taranto *et al.* 2003). A few Propionibacteria such as *Propionibacterium shermani* is also responsible for producing vitamin B12 in certain cheese varieties (Prajapati, 1995).

Deficiency of vitamin B_{12} 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).

3. Vitamin B_2 (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).

Recently, 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 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.

5. Vitamin K: Vitamin K plays a 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 K_1) in green plants and as menaquinone (K_2) 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).

Enzyme biosynthesis

LAB have been found to synthesize diverse kind of enzymes which may influence the compositional, processing and organoleptic properties as well as 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 cheese and yoghurt (Mtshali 2007; Matthews *et al.* 2004). Distinct species of *Lactobacillus*, *Lactococcus*, *Pediococcus* and Bifidobacteria 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, the bacteria retained about 60-70% of beta-galactosidase and alpha-amylase activities. The 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).

Conclusion

Lactic acid bacteria served as unique 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 a 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 can 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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