World Journal of Food Science and Technology
2018; 2(1): 19-24
http://www.sciencepublishinggroup.com/j/wjfst
doi: 10.11648/j.wjfst.20180201.13

A Review on Food Fermentation and the Biotechnology of Lactic Acid Bacteria

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To cite this article:
Mesele Admassie. A Review on Food Fermentation and the Biotechnology of Lactic Acid Bacteria. *World Journal of Food Science and Technology*. Vol. 2, No. 1, 2018, pp. 19-24. doi: 10.11648/j.wjfst.20180201.13

Received: March 23, 2018; **Accepted**: April 16, 2018; **Published**: May 19, 2018

Abstract: Fermented foods are among the first processed food products consumed since the development of human civilizations. Food fermentation processes can be categorized by the primary metabolites and microorganisms involved. Lactic acid bacteria (LAB) are a large group of closely related bacteria that have similar properties such as lactic acid production, which is an end product of the fermentation. Biotechnology is the use of living systems and organisms to develop or make useful products, or any technological applications that uses biological systems, living organisms or derivatives, to make or modify products or processes for specific use. Depending on the tools and applications, it often overlaps with the fields of bioengineering and biomedical engineering. Some of the applications were identified in this paper to include biotechnology in food fermentation to enhance properties such as the taste, aroma, shelf-life, texture and nutritional value of food. When used regularly, LAB fermented foods boost the immune system and strengthen the body in the fight against pathogenic bacterial infections, bacteriocins produced by LAB may become a potential drug candidate for replacing antibiotics in order to treat multiple drugs resistance pathogens in the future. The modern extension of food/feed fermentation technology consists of processes designed to obtain particular compounds using microbial metabolism as the chemical machinery. Thus, LAB fermentation is not only of a major economic importance, and the starting food materials, but it also promotes human health and increase food/feed production in the world.

Keywords: Lactic Acid Bacteria, Fermentation, Pathogenic, Bacteriocins

1. Introduction

Fermented foods are staples of the human diet and have been produced and consumed since the development of human civilizations [1]. The process whereby microorganisms and their enzymes bring about these desirable changes in food materials is known as fermentation. Food fermentation processes can be categorized by the primary metabolites and microorganisms involved:lactic acid bacteria (LAB) belonging to genera such as *Leuconostoc*, *Lactobacillus*, and *Streptococcus*. Fermentations can also be described based on the food substrates, which include meats and fish, dairy, vegetables, soy beans and other legumes, cereals, starchy roots, and grapes and other fruits. Raw materials that contain high concentrations of monosaccharide's and disaccharides, or in some cases starch, are fermented by lactic acid bacteria [1]. Lactic acid bacteria (LAB) are a group of gram-positive, non-sporulating, anaerobic or facultative aerobic *cocci* or rods, fastidious on artificial media, but they grow readily in most food substrates and lower the pH rapidly to a point where competing organism are no longer able to grow, and it is one of the main fermentation products of the metabolism of carbohydrates [2].

Biotechnology is defined as any technological application that uses biological systems, living organisms or derivatives thereof to make or modify products or processes for specific use Biotechnology as applied to food processing in most developing countries makes use of microbial inoculants to enhance properties such as the taste, aroma, shelf-life, texture and nutritional value of foods [3].

Spontaneous fermentation may fail, because it is not possible to ensure the same quality of the end product. However, spontaneously fermented products represent a source of LAB with potential interesting functional and technological properties, and the choice of the starter cultures has a critical impact on the palatability, processability, nutritional attributes, sustainability, and safety of food and feed [4]. This review will address how biotechnology of fermented food/feed of Lactic Acid Bacteria currently supports human health, and other possible effects.

2. Biotechnology in Food Fermentation

Microorganisms are integral part of the processing system during the production of fermented foods. Microbial cultures can be genetically improved using both traditional and molecular approaches, and improvement of bacteria is the subject of much academic and industrial research. Traits which have been considered for commercial food applications in both developed and developing countries include sensory quality (flavor, aroma, visual appearance, texture, consistency and general acceptability) [3].

3. The Role of Fermentation to Food

3.1. Flavor Enhancement

Fermentation makes the food palatable by enhancing its aroma and flavor. These organoleptic properties make fermented food more popular than the unfermented one in terms of consumer acceptance [5].

3.2. Nutritional Quality

A number of foods especially cereals are poor in nutritional value, and they constitute the main staple diet of the low-income populations. However, LAB fermentation has been shown to improve the nutritional value and digestibility of these foods. The acidic nature of the fermentation products enhances the activity of microbial enzymes at a temperature range of 22-25ºC[6]. The enzymes, which include amylases, proteases, phytases and lipases, modify the primary food products through hydrolysis of polysaccharides, proteins, phytates and lipids respectively. Thus, in addition to enhancing the activity of enzymes, LAB fermentation also reduces the levels of anti-nutrients such as phytic acid and tannins in food leading to increased bioavailability of minerals such as iron, protein and simple sugars. The number of vitamins is also increased in the ferment [7].

3.3. Preservative Properties

The preservative activity of LAB has been observed in some fermented products such as cereals, and yogurt. The lowering the pH to below 4 through acid production, inhibits the growth of pathogenic microorganisms which can cause food spoilage, food poisoning and disease [8]. For example, LAB has antifungal activities [9]. By doing this, the shelf life of fermented food is prolonged.

3.4. Detoxification

Detoxification of mycotoxins in food through LAB fermentation has been demonstrated over the years [10]. Using LAB fermentation for detoxification is more advantageous in that it is a milder method which preserves the nutritive value and flavor of decontaminated food. In addition to this, LAB fermentation irreversibly degrades mycotoxins without leaving any toxic residues. The detoxifying effect is believed to be through toxin binding effect [10].

3.5. Antibiotic Activities

LAB is applied as a hurdle against non-acid tolerant bacteria, which are ecologically eliminated from the medium due to their sensitivity to acidic environment [8]. Also, fermentation has been demonstrated to be more effective in the removal of gram negative than the gram-positive bacteria, which are more resistant to fermentation processing [11]. As such, fermented food can control diarrheal diseases in children [11]. Moreover, LAB is also known to produce protein antimicrobial agents such as bacteriocins. Bacteriocins are peptides that elicit antimicrobial activity against food spoilage organisms and food borne pathogens but do not affect the producing organisms. LAB also synthesizes other anti-microbial compounds such as, hydrogen peroxide, reuterin, and reutericyclin [12]. Other applications of LAB include their use as probiotics that restore the gut flora in patients suffering from diarrhea, following usage of antibiotics that destroy the normal flora [8].

4. Sources of LAB

Some LAB is associated with the mouth flora, intestine and vagina of mammals, while others are present in fermented seafood, such as *Lactobacillus plantarum* and *Lactobacillus reuteri*, which are reported to be associated with plaa-som fermented Thai fish [13]. LAB are the most important bacteria used in the fermentation industry of dairy products, such as yogurt, cheese, sour milk and butter, and in combination with yeast are commonly used to ferment cereal products such as dough [14].

5. Role of Lactic Acid Bacteria for Food Industry (Biotechnology)

Fermentation is one of the oldest ways of food preservation. This is made possible by the use of Lactic Acid Bacteria or LAB that plays the major role in this process. Even with the absence of oxygen, it drives the reactions of converting the carbohydrate to lactic acid with addition of carbon dioxide and other organic compounds such as mannitol and dextran. It also contributes to smell, taste, texture and color of the foods.

Due to its many uses in the food industry, a study by [15] highlighted the impact of the potential biotechnological applications LAB in food-making process. Genetically engineered tactics with better fermentation efficiency better shelf life, nutritional and sensory properties for the product.

When food products are made, undesirable contaminants can lead to poor flavor, low yield and food poisoning. LAB can be genetically engineered to grow faster than contaminants, as well as inhibit and destroy the growth of contaminants including pathogens by producing antimicrobial agents [16].

Because LAB has many uses, cultures are being kept. In order to preserve LAB for future uses, freezing is frequently used. Nonetheless, its analytical way of preservation decreases the acidifying and viability ability when thawed. Genetically modified (GM) LAB is being created in order to increase the performance of the said bacteria even subjected to such condition. However, the European Union and also Ethiopia doesn't widely accept GM products. Therefore, the legislation is strict in the introduction of GMO functional foods even it has clear benefits to consumers. Even up to these days, methods and test are being done to products in order to assure that there are no strains of genetically modified LAB or even GM foods enter the market without notice. The gap between GM products producers, legislation and consumers are not tied into united point of view.

5.1. LAB as Starter-Cultures in Cheese Industry

Milk is a highly perishable food raw material, therefore, its transformation in cheese or other form of fermented dairy product cheese-making is based on application of LAB in the form of defined or undefined starter cultures that are expected to cause a rapid acidification of milk through the production of lactic acid, with the consequent decrease in pH, thus affecting a number of aspects of the cheese manufacturing process and ultimately cheese composition and quality [17]. The starter-culture applied in this, so-called, natural fermentation, is usually a poorly known micro flora mix that although having a predominance of LAB, may also contain non-LAB microorganisms, and its microbial diversity and load is usually variable over time. In fact, studies directed to characterize traditional cheeses show that those made from raw milk harbor a diversity of LAB [18] depending on geographical region, where a few may show particular interesting technological features that upon optimization may have industrial applications. For example, because wild strains need to withstand the competition of other microorganisms to survive in their hostile natural environment, they often produce antimicrobials substances called bacteriocins, which are natural antibacterial proteins that can be incorporated directly into fermented foods or indirectly as starter culture [18].

5.2. Yoghurt Culture Bacteria

The thermophilic LAB, *Streptococcus thermophilus* and *Lactobacillus delbrueckii* subsp. *Bulgaricus* are used together as important starter microorganisms in the production of yoghurt and some kind of cheeses. Because both bacteria are able to grow in milk, this indirect positive interaction is called proto-cooperation. This positive relationship often has a beneficial effect on bacterial growth and on the production of lactic acid and aromatic compounds. Lactic acid production results in the lowering of pH and this makes it unsuitable for growth of spoilage or pathogenic microorganisms [19].

5.3. Lactic Acid Bacteria for Bread Production

Sourdough fermentation is the most natural and best-performing process to ensure optimal sensory and functional characteristics of bread, also, it should be noted, that the selection of LAB, having peculiar characteristics, can reduce the mold growth [20] and acrylamide formation in bread [21]. The acrylamide content in mixed rye - wheat bread could be lowered by using LAB with lower amylolytic activity [22]. Thus, the significant effect of LAB protease activity on the fermentation process as well as on acrylamide formation in bread can be related. Aspergillus niger glucoamylase in conjunction with an appropriate LAB strain could be used as one of the factors regulating acidification in dough, herewith decreasing the acrylamide content in bread.

Different tendencies were found in bread produced with Helianthus tuberosus L. (JA) tubers, which is a good source of prebiotic inulin [22]. The proteolytic activity of LAB does not correlate with acrylamide content in wheat bread made with JA (Jerusalem artichoke) additives but there was a strong correlation between acrylamide and the sourdough and with LAB amylase activity excreted in JA material. Also, a number of fermented products utilize cereals in combination with legumes, thus improving the overall protein quality of the fermented product. Cereals are deficient in lysine, but are rich in cystine and methionine, legumes on the other hand are rich in lysine but deficient in Sulphur-containing amino acids [23]. Hereby, by combining cereals with legumes, the overall protein quality of bread could be improved. Fermentation of lupine by pure LAB significantly improved the nutritive value of lupine eliminating the trypsin inhibitors (reduction of 21.8%) and increasing the soluble protein content up to 18.9% thus improving protein digestibility by 17.1% [23]. prevent the growth of fungi and enhance the flavor of bread

(i) Shelf life: Since LAB isolates are safe for use in foods, they are a significant alternative to chemical preservatives. Several researchers in the area of the bakery industry have successfully added LAB to dough and these strains grew well, producing the desired antifungal compounds in the dough.

(ii) Flavor: fungi are responsible for the formation of off-flavor and the production of mycotoxins; adding LAB to dough can prevent the growth of fungi and enhance the flavor of bread. The addition of *L. paracasi*D5 and *L. fermentum*Te007 in the production of white bread (di-acetyl) resulted in an improved aroma and a pleasant caramel like flavor in the baked bread itself.

(iii) Quality and acceptability: The key role in achieving the optimum growth and activity of the bakery yeast is played by selecting a LAB that does not exhibit inhibition activity against the bakery yeast. In a test tube mix of 10 ml water, 5 g of white flour, the LAB strain and baking yeast, incubate and observe the production of gas at the top of the tube, which is a good indicator of the yeast activity the use of LAB in bread in terms of improving the quality of wheat bread, bread volume and crumb structure has been reported[24].

5.4. LAB Biotechnological Potential in Pasta-Making

The potential industrial exploitation is to define a LAB employ in pasta manufacturing which is coherent with the required final quality of pasta products. In current trends, an important field of application of LAB-based biotechnology is the huge growing gluten-free (GF) market. This interesting niche requires high innovative contributes to solve the celiac disease issue, also in the pasta segment. There are two main routes to achieve this purpose: (i) the use of selected LAB to degrade gluten and (ii) the employ of particular LAB to increase the quality GF products. The first strategy has been already applied to pasta manufacture recent biotechnological evolution couple lactobacilli and fungal proteases to improve durum wheat semolina gluten degradation, maintaining cooking, and sensory properties higher than those of conventional gluten-free pasta The second approach consider LAB "as 'burgeoning 'cell factories for the delivering of functional bio-molecules and food ingredients for the production of high quality GF cereal products" with the aim to improve texture, aroma, nutritional properties, health benefits and shelf life [25]. Lactic acid bacteria (LAB) were obtained from durum wheat flour samples and screened for roseoflavin-resistant variants to isolate natural riboflavin-overproducing strains. Two riboflavin over producing strains of *Lactobacillus plantarum* isolated as described above were used for the preparation of pasta (using a pre fermentation step) to enhance their vitamin B2 content. Pasta was produced from monovarietal semolina obtained from the durum wheat cultivar PR22D89 and, for experimental purposes, from commercial re-milled semolina. Several samples were collected during the pasta-making process (dough, extruded, dried, and cooked pasta) and tested for their riboflavin content by a high-performance liquid chromatography method. The applied approaches resulted in a considerable increase of vitamin B2 content (about 2-fold increases in pasta) [26].

5.5.Lactic Acid Bacteria for Meat Products Value and Safety Increasing

The nutritional strategies to improve the quality of food products of animal origin are relatively new approach. According to researchers, fermented with selected LAB tomato powder can be recommended as both a coloring agent and a source of lycopene in the preparation of ready-to-cook minced pork meat [27]. Saturejamontana L. plants fermented with BLIS (bacteriocin-like inhibitory substances) producing LAB could be a good alternative for ready-to-cook minced pork processing to prevent meat discoloration and microbial spoilage, thus increasing acceptability and shelf-life of meat products [27]. The cheap natural marinades, based on potato juice fermented with selected LAB, could be recommended for pork meat treatment to improve its tenderness and color, thus increasing the overall acceptability [27]. Furthermore, the influence of surface treatment selected LAB, previously cultivated in a potato juice, and on the formation of polycyclic aromatic hydrocarbons (PAHs) and biogenic amines (BAs) in cold smoked pork meat sausages was investigated. Moreover, sausages treatment with LAB after the smoking on PAHs content changes was evaluated. Additionally, the inhibitory effects of LAB metabolites on food spoilage bacteria was determined [4]. The results confirm that potato juice could be used as an alternative substrate for LAB cultivation and obtained fermented bio products could be applied for surface treatment of cold smoked pork sausages in case to reduce microbial contamination, and PAHs content in final product.

5.6. Lactic Acid Bacteria Increasing Nutritional Value of Tomatoes

Processing of tomatoes with selected LAB strains resulted in several important changes in carotenoid concentration and lycopene isomer profile. Treatment with LAB breaks down the tomato cell matrix and makes the carotenoids more available, which resulted in higher level of total carotenoids. Moreover, lactic acid fermentation of tomato resulted in a large lycopene bioavailability accompanied by increased cis-lycopene content. According to selected LAB may be useful for preservation of tomatoes, which could be recommended as a way of obtaining more biologically accessible products with functional value [28].

5.7. Importance of the Metabolites of LAB

LAB is well known for their antifungal activity, which is related to the production of a variety of compounds including acids, alcohols, carbon dioxide, diacetyl, hydrogen peroxide, phenyl acetic acid, bacteriocins and cycle peptides [29]. These compounds were added to several foods in order to conserve them from food-borne and spoilage microorganisms.

Organic acids are the main product of LAB in the fermentation systems of the raw materials. The main acids produced by LAB are lactic acid and acetic acid, besides certain other acids depending upon the strain of LAB. These acids will be diffused through the membrane of the target organisms in their hydrophobic un-dissociated form and then used to reduce the cytoplasmic pH and stop metabolic activities. Other factors that contribute to the preservative action of the acids are the sole effect of pH, the extent of the dissociation of the acid and the specific effect of the molecule itself on the microorganisms. Bacteriocins exhibit good potential for use in the food industry and as bio-preservation agents. Bacteriocins are small, ribosomally synthesized, antimicrobial peptides or proteins that display inhibition activity toward related species, with no reports about fungal inhibition. The notable property of LAB supernatant is the heat stability of the antifungal compounds present in it. This will promote the use of LAB supernatant and/or antifungal compounds in heat-treated foods. The supernatant of certain LAB observed to be active within a wide range of pH, starting from as low as three and up to nine depending upon the strain [30]. This could be considered as a major factor whereby LAB is used in food preservation when compared with the chemical preservative which are usually active at low pH between 3 and 4.5. Additionally, LAB have a broad spectrum of anti fungal activity against several food spoilage and mycotoxin-producing fungi while commercial preservatives are usually used to control only one or few fungi.

5.8. Lactic Acid Bacteria for Higher Value Feed Production

The 50ml of with selected LAB fermented potato juice containing 9.6 log10 CFU/ml of LAB per day, administered for 14days, reduced the risk of developing acidosis (it stabilized blood pH), reduced lactate and CO2 concentrations, and the risk of liver lesions in blood and E. coli in the faeces of calves [31].

As well as the isolated *Pediococcusacidilactici* BaltBio01 and *Pediococcuspentosaceus* BaltBio02 strains were cultivated in barley and wheat bran substrate, and the developed fermented feed stock, with high content of valuable *Pediococcus*, was used for Lithuanian black and white dairy cattle feeding [32]. In addition, the influence of fermented feed stock on milk production and composition was determined. Isolated strains demonstrated versatile carbohydrate metabolism, grown at 30 °C and 37 °C temperatures, acidic tolerance, showed to be non-resistant to antibiotics and have antimicrobial activity. Cereal by-products (barley and wheat bran) bioconversion using isolated microorganisms allows to produce safer (reduced Enterobacteria, aerobic bacteria, yeast and fungi count) fermented feed stock with high content of Pediococcus.

5.9. Food Fermented Lactic Acid Bacteria Used as Probiotic

Live microbes associated with food fermentations can provide beneficial functions in the gastro intestinal tract is consistent with the emerging view that core health benefits of probiotic cultures can be assigned to a species, rather than to specific strains of a species [33]. At least this is the case for some species of LAB for which certain strains have long been applied as probiotics. Most human trials have found that the LAB strains tested may exert anti-carcinogenic effects by decreasing the activity of an enzyme called β-glucuronidase which can generate carcinogens (Heterocyclic amines) in the digestive system. Lactobacilli de- conjugate the bile salts in the intestine to form bile acids and thereby inhibit micelle formation. This leads to decreased absorption of cholesterol [34]. Several small clinical trials have indicated that consumption of milk fermented with various strains of LAB may result in modest reductions in blood pressure. It is thought that this is due to the ACE (Angiotensin-converting-enzyme) inhibitor-like peptides produced during fermentation. Increasing or improving phagocytosis as well as increasing the proportion of T- lymphocytes and natural killer cells. Probiotics are also known to reduce dental carries in children as well as aid in the treatment of Helicobacter pylori infection [35].

Therefore, a reasonable argument could be made that these foods should be considered to have similar health benefits as those conferred by probiotic lactobacilli of the same species. It is worth noting that some countries (e.g. Italy and Canada) incorporate a list of species considered as probiotics in their regulatory guidelines. In contrast, in most of Europe, and also in Ethiopia, foods are not permitted to use or mention ‘probiotics’ or ‘contain probiotics’ on labels.

6. Conclusion

The value of fermented food plays a vital role in diet, human health and nutrition. The benefits which are associated with fermentation are increased shelf life and sustainability, and nutritional value, Bacteriocins produced by LAB may become a potential drug candidate for replacing antibiotics in order to treat multiple drugs resistance pathogens in the future human health. Application of lactic acid bacteria for the food/ feed biotechnology industry look potentials and warrant further research especially in Africa, and particularly in Ethiopia, in order to increase the performance of LAB genetically modified (GM) LAB is being generated. This review will clarify the significance, and probably the requirement, of certain fermented foods in the human diet and justification for inclusion into Ethiopian national dietary guidelines.

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