# Beneficial microorganisms in food- a technological challenge

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**Abstract**

Considering the current state of microorganisms related to food, microbial system can bring about considerable changes by organizing products with desired functional characteristics, starter cultures and foods containing probiotics. Productivity and viability of probiotics strains during the processing of functional food is the most challenging aspect for the food technologist and microbiologist. This review aims to offer a panoramic overview of the technological challenges involved in viability and productivity of probiotics strains during processing of food that include high initial productivity and viability as a starter cultures.

**Key words** Microorganisms, probiotics, productivity, technological challenges, and viability

## 1. Introduction

A new trend in food microbiology is the use of probiotics (microorganisms that are beneficial for human health) and microorganisms (Florowska et al., 2016) that can be associated with the manufacturing of food constituents or functional food. These are usually applied as adjuncts cultures (microbial preparation of large number of cells administrated to raw material to produce a ferment food) (Champagne and Mollgaard, 2008). These features resulted into assorted and intricate technological aspects related to microorganisms. Various factors such as viability and productivity of starter cultures applied, processing and composition narration of raw material applied as a substrate and storage conditions of the final products can influence the activities of microorganisms in food system (Figure 1). Food preservation relies on the concept of inactivation of undesirable microorganisms. Competitive microbiota can be introduced into the human gut to inactivate the microbes. However, fermentation on the other hand can be counter-productive to the feasibility of microbes as it requires greater efficiency of microbial system. Fermentation can be considered as a process to produce probiotics and functional foods but this can also resulted into reduced microbial population in the final food products. Therefore, the technological aspect is to maintain and look after microbial viability in order to improve the productivity of beneficial microbiota.

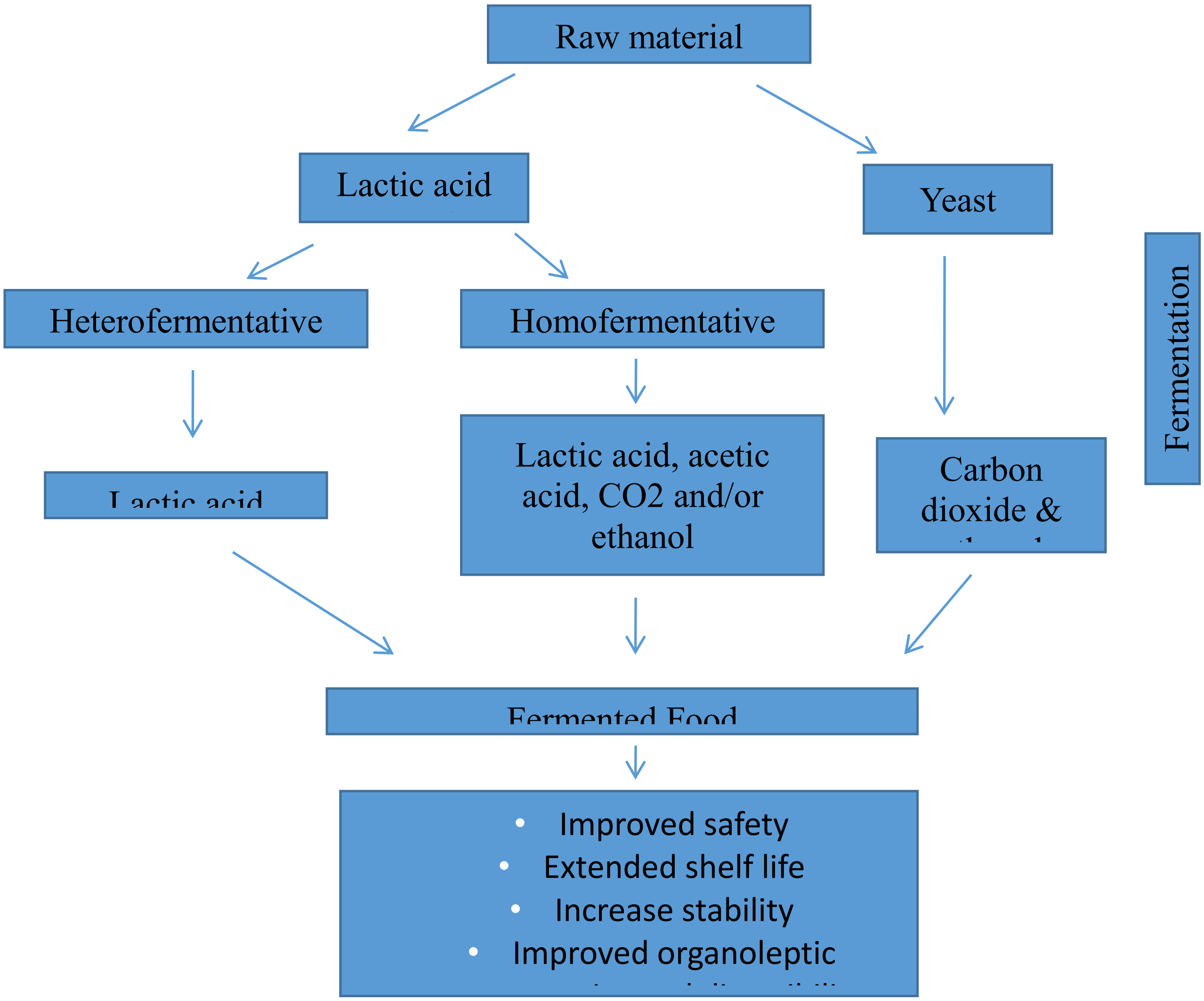


**Figure 1**. Schematic representation of fermentations in foods

**2. Technologies used to minimize the microbial population**

Conventional food processing technologies are designed to inactivate or reduce the microbial population. Processes include are light, electrical, stream or microwave energy applications in addition to ultrasound or hot air as a source of heat transfer (Sevenich et al., 2013). Processes that can be applied at room temperature include high hydrostatic pressure treatment, irradiations, anti-microbial and high intensity electric fields in addition to the combination of processes i.e. hurdle technology (Grauwet et al., 2010). Furthermore, cold or frozen storage and modified atmosphere packaging can be helpful to control or reduce the microbial growth.

To sustain raw material quality, in terms of functionality and physico-chemical properties, the novel non-thermal technology is intend for minimum, gentle and smooth processing which ultimately helps to maintain the safety of microorganisms and plummeting the ecological hazards that may occasionally encountered with the conventional mechanisms i:e blanching with hot water (Hui and Khachatourians, 1993; Lee et al., 2016; Botero-Uribe et al., 2017; Kumari and Farid, 2020). The most booming commercial and industrial application of non-thermal technology is to inactivate the vegetative microorganisms without compromising the functional, nutritional and sensory properties of food. This can be achieved by promoting non-thermal pasteurization using non-thermal technology in order to extend the shelf life of the food product. Furthermore, new fascinating applications of non-thermal techniques have been emerging in the last decade such as assisted freeze-thaw processes, accelerated solute diffusion processing and modification of functional characteristics of proteins and other macromolecules (Mota et al., 2013).

Figure 2 illustrates the model of inactivation under high pressure conditions that maintained during the isobaric state and during this practice, the microbes experience a rickety condition (i:e membrane permeabilization, subsequent damage of enzyme system, stress response to pH change) where reactivation can occur. Interestingly, similar kinds of sigmoid inactivation kinetics have also been observed during high intensity electric field pulses treatment (O'Bryan et al., 2015).

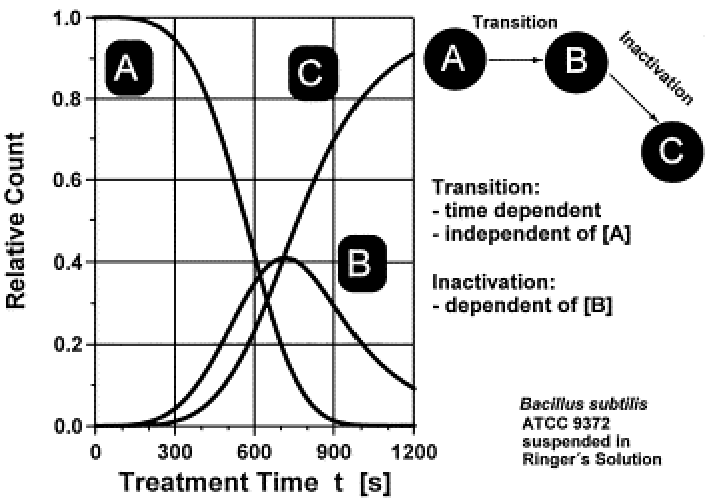


**Figure 2.** Three stage inactivation model of vegetative Bacillus subtilis cells under isobaric conditions during high pressure treatment: (A) stable state; balanced pH during pressure treatment; completely recoverable; (B) metastable state; lower pH after a certain time of treatment; recoverable with delay; (C) inactivate state; acidified cell; morphological changes; impossible to recover. Reprinted from (Knorr D. 1998) (with permission from Elsevier).

Alternatively, fermentation works best to maintain and optimize the production of microbes. Fermented-based products account for most of our food supply, as shown in Table 1. Dairy *Lactobacilli*, *Lactococci* and *Leuconostic* are the most significant microbes associated with food while Staphylococci are used for fermented foods of animal and plant sources. Similarly, bread and alcohol can be produced from Yeast and *Saccharomyces*, respectively (Hui and Khachatourians, 1993). Fermentation has been shown to aid in modification of physico-chemical characteristics of foods. Additionally, it also provides considerable effect on the functional performance and nutritional quality of the raw material. Lactic acid bacteria are the most commonly used microbial group in food industry. Fermented food characteristics such as shelf life, sensory attributes and safety depend upon the metabolic activities of these microbes. Genomic studies of lactic acid bacteria have illuminated several novel metabolic pathways that significantly improved the overall quality of food. Progress and versatility in the starter cultures can be imagined in future based on the growing scientific knowledge (Gobbetti, 2015).

Food ingredients i:e vitamins, fats, acids, amino acids, flavors, polysaccharides, pigments and other microbials such as bacteriocins can be produced by microorganisms along with food processing aids such as enzymes (Hui and Khachatourians, 1993).

In order to reduce the risk of spoilage and increase the rate of fermentation, *Lactobacilli* bacteria are commonly applied as silage inoculates. *Lactobacilli* have been known for centuries for their prominent role in the food especially in dairy technology. Recently, sub-generic division of *Lactobacillus* has been identified by application of advanced molecular methods. Pulsed Field Gel Electrophoresis (PFGE) has been used as a promising method to differentiate *Lactobacillus* strains while applying to the strains of dairy origin. It has been hypothesized that ingestion of *Lactobacillus* is not harmful since *lactobacillemia* that is induced by food is very rare and only takes place in predisposed patients (Bernardeau, 2008).

**Table 1** Example of fermented foods (Hui and Khachatourians, 1993; O'Bryan et al., 2015).

| Products | Ingredients | Pediococci and role |
|---|---|---|
| Pickled cucumbers | Cucumber + salt | *P.pentosaceus*, *P.acidilacticti*: lactic acid |
| Olive | Olive + salt | *P. pentosaceus*: lactic acid |
| Sauerkraut | Shredded cabbage + salt (2-5 %) | *P. pentosaceus*: lactic acid |
| Puto | Rice + sugar | *Pediococcus* spp.: lactic acid |
| Koko | Maize ( + sorghum) | *P. acidilactici*: lactic acid |
| Miso | Soyabean + rice + salt | *P. halophilus*, *P. pentosaceus* and *P. acidilactici*: lactic |
| Soy sauce | Soybean + salt | *P. halophilus*: lactic acid |
| Pearl wine | Grapes | *P. acidilactici*: malic acid to lactic acid |
| German wine | Desired fruits | *P. acidilactici*: malic acid to lactic acid |

The probiotics strains should fulfill the following criteria in order to be effective (Cota and Alvim, 2018); common characteristics (origin, safety, acid and bile resistance etc), functional characteristics (intrinsic microbiological properties) and technological characteristics (production and processing characteristics).

One of the important starter culture characteristics is the swift fabrication of great amount of lactic acid at the time of fermentation of plant origin. Due to high tolerance in low pH conditions and accumulation of acids, *L. plantarum* will normally end the process of lactic fermentation. While, the natural fermentation (no inoculums added) will result from the activities of lactic microflora that comes from a large and diverse epiphytic flora. Maintenance of conditions that eventually encourage the growth of desired microbes and restrain the development of spoilage and pathogenic microbes is the main theme of flourishing usual lactic acid fermentation (O'Bryan et al., 2015). The physicochemical characteristics of dry cued foal sausage were not affected by starter cultures except the pH values as the inoculation of the starter culture resulted in strong acidification in the beginning (Lorenzo et al., 2014). Furthermore, inoculation of *L. acidophilus* in the starter culture could also improve the sensory quality of dacia sausage (Simion et al., 2014).

## 3. Sources of material

### *3.1. Probiotics*

Different strains and species of microorganisms are currently used in European products (Guarner, 2007; Giusti, 2009; Tamang et al., 2016; Perin et al., 2019) including *acidophilis*, *Lactobacillus*, *L. reuteri*, *L. johnsonii*, *L. casei*, *Bifidobacterium bifidum*, *B. brevis*, *B. longum*, *B. animalis*, *Streptococcus thermophilus* and these microorganisms are associated with beneficial effects when inoculated in the human diet (Florowska et al., 2016). Probiotics are viable microorganisms, generally bacteria or yeast that has a beneficial effect on the human health when ingested in sufficient amount. Exploitation of gut flora to increase its beneficial and protective role symbolizes a new promising field for new strategies to control the inflammatory bowl diseases (Anadon et al., 2015).

### *3.2. Synbiotics*

Yogurt is the product of milk fermentation by the starters *Streptococcus thermophilus* and *Lactobacillus bulgaricus*. It represents a classical example of commercially available synbiotic (mixture of prebiotics combining the effects of the simulation of body's own bacteria and new ones) (Ouwehand and Roytio, 2015) that consists of inulin and three probiotic strains; enriched vitamins, micronutrients and skimmed milk in addition to soluble fiber and a product containing the probiotic oligofructose and two probiotic strains (Giusti, 2009).

### *3.3. Anti-microbials of microbial origin*

Examples of bacteriocins (antimicrobials proteins and peptides produced by bacteria) produced by *Lactobacillus* and other low molecular mass antimicrobials products are presented in Table 2 and Table 3, respectively.

**Table 2** Examples of bacteriocins of *Lactobacillus* (Hoover and Steenson, 1993)

| Producer strain | Bacteriocin | Spectrum |
|---|---|---|
| *L. helveticus* | Helveticin J | *L. helveticus*<br>*L. bulgaricus*<br>*L. lactis* |
| *L. sake* | Sakacin P | *Lactobacillus* spp. |
| *L. sake* | Lactocin S | *Lactobacillus* spp.<br>*Leuconostoc* spp. |
| *L. bavaricus* | Bavaricin A | *Lactobacillus* spp.<br>*Lactococcus* spp. |
| *L. acidophilus* | Lactacin F | *Lactobacillus* spp.<br>*Enterococcus faecalis* |
| *L. curvatus* | Curvacin A | *Lactobacillus* spp.<br>*Listeria monocytogenes* |

**Table 3.** Antimicrobial products of low molecular mass of microbial origin (Helander et al., 1997)

| Compound | Producer microorganisms | Target microorganisms |
|---|---|---|
| Acetic acid | Heterofermentative LAB | All microorganisms, pH dependent |
| Alcohols | Yeast heterofermentative LAB | All microorganisms |
| Lactic acid | All LAB | All microorganisms |
| Ruterin | *Lactobacillius reuteri* | Broad spectrum: gram-positive bacteria, gram-negative bacteria, fungi |
| Hydrogen peroxide | All LAB | All microorganisms |
| Carbon dioxide | Heterofermentative LAB | Most microorganisms |

D) Molecular Strategies

Based on the strategies and applications of molecular tools (Bremer et al., 2009), it is possible to discover and then unite the most enviable sensory and/or beneficial characteristics to create the better-quality *Lactobacillus* microbes for dairy applications.

**Table 4.** Potential targets for the application of genetically modified *Lactobacillus* (Bremer et al., 2009)

| Targets | Applications |
|---|---|
| **Fermentation efficiency** | Lactose metabolism, proteolytic systems |
| **Shelf life, safety** | Biopreservatives |
| **Nutritional properties** | Vitamin synthesis |
| **Phenotype stabilization** | Chromosomal integration |
| **Sensory properties** | Flavor, texture, appearance |
| **Therapeutic effects** | Cholesterol reduction |
| **New traits** | Enzyme production |

#### 4. Technological aspects of microorganisms

The technological aspects based mainly on the concept of viability of microorganisms in functional food and the fermentation performance of the related microbes for food material. Keeping cell viability and impact of processing in mind, some factors that may compromise over the efficacy of bacteriocins and related bacteriocinogenic lactic acid bacteria including the aspects of processing i:e shearing and freeze-thaw has been explained. These factors included emergence of bacteriocins-resistant pathogens or spoilage bacteria, conditions that may weaken biological activity of protein (bacteriocins), non-specific proteolytic enzymes, heavy metals, excessive agitation, foaming, binding to food components and oxidation (O'Bryan et al., 2015).

Cellular injury can be resulted from freeze drying of microbial starter cultures causing reduction in the viable counts in the dried cultures. Sucrose, maltose and sorbitol can be applied as cryoprotectants to improve the viability. Several strains of lactic acid bacteria (*L. plantarum*, *L. bulgaricus*) can be protected from drying out by accumulating compatible solutes such as carnitine and betaine. Preservation of starter culture is mainly achieved through freezing and freeze drying. Microencapsulation by spray freeze drying (SFD) is an efficient technique to produce microcapsules of *L. plantarum* that exhibit prominent characteristics such as good encapsulation efficiency and physical properties with effective retention time period of cell viability during storage (Rajam and Anandharamakrishnan, 2015). The application of cryoprotectants and immobilization support the idea of protection of lactic acid bacteria from cell damages by freezing. Nanocellulose, a kind of polysaccharide, in combination with other cryoprotective agents can act as an efficient protective agent against the cell membrane damages. This polysaccharide adsorbed on the surface of microorganism forming a protective layer that provides higher cell viability by keeping the glassy structure of ice near the cells (Nahr et al., 2015).

#### 5. Technological options modulating functionality

For functional foods, microbial system can be processed in three ways (Knorr, 1998);

a) Processing of starter cultures.
b) Processing of products with desired functional characteristics.
c) Processing of foods containing probiotics.

Processing effects on the starter cultures have to be taken into account for the processing of probiotics during fermentation, growth and subsequent handling such as homogenization, heating and packaging. Furthermore, composition and processing history of the raw material also play an important role in this aspect. Impact of the processing step such as freeze drying on the viability of starter culture has been discussed above. Applications of *Bifidobacteria* in wide range of dairy products are also very important in context of beneficial microorganisms in functional foods. Some *Bifidobacteria spp*. has been isolated and characterized in some commercial yogurt species sold in Europe and established the fact that besides other *Bifidobacteria*, many of these products contained *Bifidobacterium animalis* (the organism is not of human origin) and in some cases, the viable counts of *Bifidobacteria* were $< 10^2$ cfu/ml which makes the probiotic efficacy of such products less efficient (Perin et al., 2019). Eight different strains of *Lactobacillus* isolated from fermented food and human intestine. They reported that all the strains tolerated both pH 2 for 3 hours and 1% bile salts for 24 hours. A large variation was observed among the eight strains regarding their ability to degrade and diminish nitrite and cholesterol, scavenge free radicals and antagonize the pathogens. Strains CICC 23174 and CGMCC 1.557 showed the best probiotic characteristics and can be used as a potential source to produce probiotic fermented food (Ren et al., 2014).

#### 6. Process monitoring for function

This section cast light on interaction of raw materials, food processing, microbial systems and other food components. This mechanism is very important in context of productivity regarding metabolites of microbes, in order to identify and manage their structure and functions association. Novel technological techniques such as high hydrostatic pressure can improve the production of desirable metabolites compared to the conventional methods of strain selection and substrate improvement. High lactic acid production can be noticed for *Lactobacillus plantarum* when treated with high hydrostatic pressure compared with untreated cultures. Other physical and biochemical techniques can also be used for creating such stress like high intensity electric field pulses and enzymes, respectively. Bio-preservation purposes can also be achieved by implementing these techniques in combination. High bactericidal efficiency can be resulted by applying hydrostatic pressure or high electric field pulses in combination with bactriocins. Combination of two or more non-thermal technologies with conventional preservation techniques can be proved helpful to increase their anti-microbial effect (Ross et al., 2003). High hydrostatic pressure (HHP) technology has been recognized recently as a promising technique to inactivate the vegetative microbes in order to acquire the non-thermal pasteurization and extend the shelf life of food products. HHP can also be used to adapt the microbial biosynthesis pathway which may lead to the formation of new products with novel functional properties (Mota et al., 2013). The pressure resistant characteristics of microbes help in the selection of target microorganisms which ultimately proves helpful to provide settings for pasteurization conditions during HHP and reduce the likelihood of food safety issues after the process of HHP (Huang et al., 2014).

**7. Safety implications of materials and processes**

The application of lactic acid bacteria in fermented food appears to be safe if we increase the consumption of prebiotics and probiotics in human diet. Apart from rare instances, no such ill effects have been observed (Guarner, 2007). Safety probiotics cultures recommendations for novel and traditional food has been provided (Florowska et al., 2016). Literature seems to support the evidence of the lactic acid bacteria role in the food to be neither pathogenic nor toxigenic. Nonetheless, the same author also pointed out the role of bioactive proteins that can be proved as strong potentiators in a biological system in a very small amount i:e the action of Staphylococcal and botulinum toxins. Consequently, precautions should be taken while considering bacteriocinogenic lactic acid bacteria and fermentation supernatants for food applications apart from purified bacteriocins preparations (O'Bryan et al., 2015). Currently, there have been no reports of disease caused by probiotics which are available in the market. Moreover, some of the strains were studied for their safety without any reported problems and are known to have been in the market for more than 20 years (Franz et al., 2011). Additionally, probiotics have been found to reduce the problems of obesity and related metabolic diseases (Arora et al., 2013). The benefits that lactic acid bacteria provided need to be rejuvenated through development, research and application. The probiotic market is spreading quickly day by day and its application to dairy products has the potential to improve the lives of millions of people worldwide (Reid, 2015).

**8. Technological challenges and research needs**

Current technological techniques and processes emphasize on the production of lactic acid that may result during the process of fermentation in low microbial viability conditions. In order to further ensure the viability of microorganisms during food processing and storage, maintenance of process options and microbial strain generation are inevitable mechanisms to be monitored. Furthermore, microorganisms also need to maintain their viability through human intestinal tract throughout their passage. To meet these challenges, preparation of starter cultures as functional foods can be adopted as process modifications.

Future research activities needed to be carried out considering the integration of microbial system, storage, raw material, processing, distribution and consumption in order to better understand the product functionality. Highly selective and specific organisms can be developed as probiotics regarding the genetic modification of microorganisms. Limited information is available on the interaction between microbes and wide variety of food components. Since our current food supply is based on traditional thermal processing, application of novel non-thermal emerging technologies may also result in unique functional food.

**Conflict of interest**

Authors have no any conflict of interest.

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