

Review Article

# Review on Microbiology of Cereal-Based Spontaneously Fermented Foods and Beverages

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## Abstract

Spontaneously fermented cereal-based foods and beverages are fermented by diverse arrays of microorganisms which play significant roles at different stages of fermentation. The aim of this review is to summarize the scientific data on the microbiology of cereal-based spontaneously fermented foods and beverages. Yeasts are a large group of beneficial bacteria in food fermentation followed by lactic acid bacteria. Molds also play an important role in the production of various foods and non-food products. The possible functions of yeast in the fermentation of carbohydrates are aroma production, stimulation of lactic acid bacteria and degradation of mycotoxin. However, this review results reveal that all yeasts and molds are not beneficial microbes. According to the reports of many researchers, *Enterobacteriaceae* and total coliforms are not persisted to the end of fermentation. However, aerobic mesophilic bacteria, staphylococcus spp., and aerobic spore-forming bacteria are persisted to the end of some fermented food and beverage products. In spontaneous fermentation, aerobic spore-forming bacteria play crucial role in the begging stage of fermentation to breakdown of complex structure of food composition which facilitates the environment for the yeast and lactic acid bacteria. This review concluded that spontaneous fermentation process reduces both pathogenic and spoilage microorganisms to non-significant level thus make the end product safe for consumption.

## Keywords

Fermented Foods and Beverage, Microorganisms, Spontaneous Fermentation

## 1. Introduction

Spontaneously fermented cereal-based foods and beverages are fermented by diverse arrays of microorganisms (Figure 1) such as lactic acid bacteria, yeasts, molds, and different *Bacillus* spp. [1-3]. These different groups of microorganisms play significant roles at different stages of fermentation, thus enhancing their organoleptic and preservative properties while improving their nutritional quality [4, 5].

On the other hand, the establishment of a particular microflora in the substrate depends on water activities, pH, food

matrix composition, salt concentration, and method of preparation [2]. During spontaneous fermentation, microbes either occur in succession or co-exist with other microbial groups in a synergistic effect. For example, yeast multiplication is favored by an acidic environment developed due to the metabolic activities of LAB, while bacterial growth is favored by the yeast's activities as it provides several growth factors such as vitamins, minerals, and nitrogen compounds during fermentation [6].

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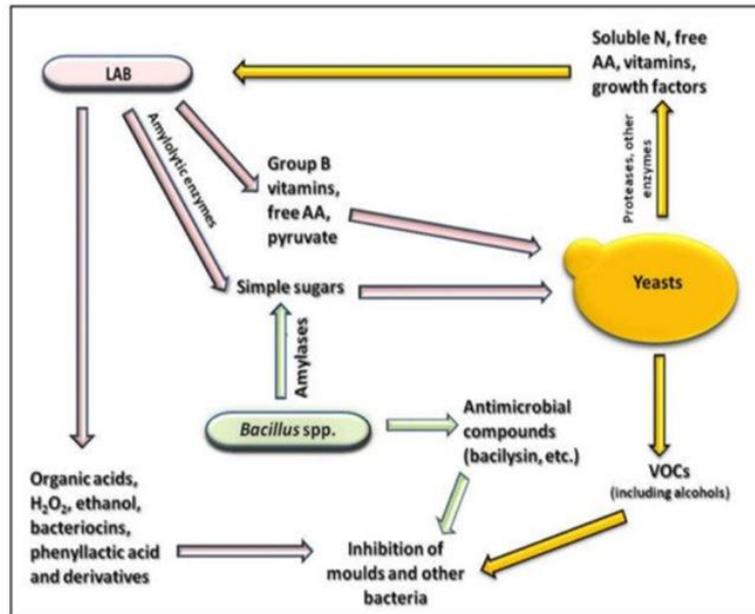


Figure 1. The interactions among the principal microbial groups during spontaneous fermentation [3].

## 2. Microorganisms Participated in Cereal-Based Spontaneous Fermentation

### 2.1. Lactic Acid Bacteria (LAB)

Lactic acid bacteria are a group of gram-positive, non-respiring, non-spore-forming, non-pigmented, and non-motile cocci or rods that produce lactic acid as the major end product of the fermentation of carbohydrates [7, 8]. They are catalase- and oxidase-negative due to a lack of cytochromes and porphyrins. Some of them are producing hydrogen peroxide, thus destroying pathogenic microorganisms in fermented products [9]. Regarding nutrients requirement during their growth time, they need special nutritional supplements such as amino acids and B-vitamins; therefore, they are categorized as fastidious microorganisms [10].

Lactic acid bacteria (LAB) are a large group of beneficial bacteria in food fermentation for the production of alcoholic and non-alcoholic beverages, porridges, sausage, gruels, snacks, and dairy products [11-16]. During fermentation, LAB improves keeping quality and enhances the palatability of food and beverage products [17-19]. Hu and others [20] reported that the associations of two or three strains of LAB and yeasts during the fermentation of dough contribute metabolites that impart taste and flavor to food products. The strains of LAB are capable of lowering the pH of foods below 4.0. As a result, both spoilage and pathogenic microorganisms are eliminated, making lactic acid bacteria predominant in most fermentation processes [21].

Among LAB, the genera *Lactobacillus* is highly hetero-

geneous and commonly found in fermented milk, meat, vegetables, and cereals [22, 23]. Among them, *Lactobacillus acidophilus* strains are added to dairy foods due to their physiological benefits [10]. It has been reported that during dough fermentation of maize, *Pediococcus* species and *Lactobacillus brevis* dominate the latter stage of fermentation [24, 25]. Also, the lactic acid bacteria isolated from *Ogi*, *Azo*, and *Borde* were mostly the *Lactobacillus* species [22, 23] which prefer acidic conditions to all other lactic acid bacteria [26, 27]. Olympia and others [28] reported that the strain of *Lactobacillus plantarum* hydrolyses soluble starch, while they do no effect on dextran and cyclodextrins. The breakdown of the starch might be due to the presence of enzymes such as  $\alpha$ - and  $\beta$ -galactosidase produced by this strain [29]. Nigatu and other [30] have demonstrated that the genera of lactic acid bacteria such as *Pediococcus*, *Lactobacillus*, *Streptococcus*, and *Leuconostoc* were responsible for the acidic characteristics of dough of teff and Kocho fermentation.

The first biochemical and physiological characterization trial of lactic acid bacteria was carried out in the nineteenth century by Orla-Jensen scientists [31]. According to this classification, four genera of lactic acid bacteria such as *Lactobacillus*, *Pediococcus*, *Leuconostoc*, and *Streptococcus* were identified [32]; however, recently lactic acid bacteria were re-classified by scientists into 25 genera [33]. LABs are divided into two distinct phyla according to taxonomical classification: Firmicutes and Actinobacteria. Phylum Firmicutes contains such genera as *Lactobacillus*, *Lactococcus*, *Leuconostoc*, *Oenococcus*, *Pediococcus*, *Streptococcus*, *Enterococcus*, *Tetragenococcus*, *Aerococcus*, *Carnobacterium*, *Weissella*, *Alloiooccus*, *Symbiobacterium*, and *Vagococcus*. While within the Actinobacteria phylum, lactic acid bacteria belong to the *Atopobium* and *Bifidobacterium* genera [34].

The genus *Lactobacillus* is recognized as being phylogenetically highly heterogeneous consisting of over 152 validly described species. They are gram-positive, microaerophilic, and non-flagellated rods or coccobacilli. *Lactobacillus* is present either as pairs or in chains of different lengths. Lactobacilli are known to resist acids and ferment a diversity of food products than other genera of LAB [35, 36]. Based on their carbohydrate fermentation, members of the genus *Lactobacillus* are subdivided into three groups: obligate homo-fermentative, facultative hetero-fermentative, and obligate hetero-fermentative.

*Lactococcus* is a genus of lactic acid bacteria formerly included in the genus *Streptococcus*. The first studies of this genus were made by Lister, [37], as a microorganism responsible for milk fermentation. Morphologically they are ovoid or spherical cells that occur either singly, in pairs, or in chains under a microscope. A *Lactococcus* genus was distinguished from *Streptococcus* and *Enterococcus* by growth at 10 °C but not at 45 °C [38]. They are mostly used in dairy fermentation although some of them are still suitable for cereal fermentations [39].

The name *Streptococcus* was derived from two Greek words 'streptos' meaning easily twisted and 'kokkos' meaning grain which was first used in 1874 by Billroth, [40]. The organism does not grow at 15 °C, but most strains can grow between 40 °C and 50 °C. *Streptococcus* species could proliferate at low pH following *Lactobacillus* [41]. Many species of this genus are harmful to human beings, but *Streptococcus thermophiles* variant *salivarius* is widely used as a starter in the manufacture of dairy products as they have GRAS (Generally recognized as safe) status [42]. The end products of lactose fermentation by the aforementioned species are lactate, acetaldehyde, and diacetyl which contribute to the flavor and taste of dairy end products [43].

The genera *Pediococcus* is recognized as gram-positive, catalase-negative, oxidase-negative, and homo-fermentative. They grow under facultative aerobic to microaerophilic conditions. The cells are uniformly spherical and they differ from other lactic acid bacteria by alternate division in two perpendicular directions resulting in tetrad formation, though they sometimes occur in pairs and in chains [44]. Members of the genus *Pediococcus* can grow at 45 °C; however, the optimum growth temperature is 32 °C. Some species of the same genus can grow in salt-concentrated foods like brined vegetables (up to 18% NaCl) [38].

Enterococci are facultatively anaerobic, non-spore-forming, and homo-fermentative cocci that tolerate harsh conditions such as extreme temperature (10-45 °C), pH (4.5-10.0), and high sodium chloride concentrations [45]. Enterococcus represents 6% of the fecal microbiota and 23% of the gut microbiota [46]. They play role in ripening and aroma development in fermented foods [47, 48]. Some *Enterococcus* strains have been used as human probiotics, while few strains are important nosocomial pathogens causing bacteremia, endocarditis, and other infections [49].

Members of the genus *Leuconostoc* are mesophilic, diplococci in the oval chain, and hetero-fermentative commonly used as adjunct cultures in the dairy industry [12]. However, they are isolated from different fermented and unfermented foods [44, 50]. They are distinguished from hetero-fermentative *Lactobacillus* by their inability to hydrolyze arginine [38] besides their difference in shape.

## 2.2. Yeast

Like lactic acid bacteria, yeasts are also responsible for the fermentation of different food products [51-53]. They have a role in the fermentation of cereal-based fermented foods, beverages, and condiments [54-56]. The predominant strains reported from fermented cereals were *Saccharomyces cerevisiae*, *Geotrichum candidum*, *Candida krusei* and *Candida tropicalis* [57]. Yeasts are intimately involved in the production of alcoholic beverages due to their ability to ferment sugar into ethanol efficiently and tolerate ethanol concentrations of 15-20% v/v [58]. The genus *Saccharomyces*, in particular, *Saccharomyces cerevisiae* is strongly associated with the production of fermented cereal products made for human consumption, especially in alcoholic fermentation [7]. Hammes and others [59] reported that *Saccharomyces cerevisiae* is the principal yeast of most bread fermentation which causes expansion and leavening of dough, which ultimately give palatability to bread. *Torulopsis delbrueckii* and *Kluyveromyces thermotolerans* are used to prepare frozen dough bread due to their freeze-tolerant ability than *S. cerevisiae* [60].

The possible functions of yeast in the fermentation of carbohydrates are aroma production, stimulation of lactic acid bacteria, providing essential metabolites, inhibition, and degradation of mycotoxin, and degradation of cyanogenic glucosides [57, 61, 62]. Their strains contribute significantly to the structural quality and organoleptic characteristics of the fermented products [7, 63]. Yeast exhibits a wide range of enzymatic activities that produce flavor compounds such as alcohols, acids, esters terpenes, and lactones during cereal fermentation. Walker and Stewart, [64] reported that *Saccharomyces cerevisiae* produces numerous secondary metabolites which dictate the final flavor and aroma characteristics of beverages. In addition to organoleptic properties, yeasts in cereal fermentation increase the protein content of fermented food products [7, 65].

On the other hand, some species of yeasts are common contaminants of cereal, dairy, meat, and fruit and vegetable products that provide a favorable niche for their growth [66-69]. They are responsible for significant economic losses due to visible and/or invisible defects that lead to significant food losses. In addition, they produce mycotoxins as secondary metabolites that pose health risks to human beings after the consumption of food products [70]. The control of fungal spoilage is a major concern for scientists that are looking for efficient solutions to prevent fungal spoilage and toxicity in

fermented foods [66]. Fermentation is one of the robust solutions to mitigate fungal spoilage in cereal crops and eliminate its toxicity [70-72].

### 2.3. Molds

Molds play an important role in the production of various pigments, foods, beverages, antibiotics, pharmaceuticals, and enzymes [73, 74]. On the other hand, some species of molds cause a negative effect through the biodegradation of natural materials; especially in products with high sugar content and low pH [75]. These include species of Zygomycetes, Penicillium, Aspergillus, Rhizopus, Mucor, Geotrichum, Fusarium, Alternaria, Cladosporium, Eurotium, and Byssoschlamys which causes food spoilage and some of them produce toxins [16, 76]. It has been reported that species of Actinomucor, Amylomyces, Aspergillus, Monascus, Mucor, Penicillium, Rhizopus, and Ustilago are found in many fermented foods, Asian non-food amylolytic starters, and alcoholic beverages [77, 78].

### 2.4. Aerobic Mesophilic Bacteria (AMB)

Aerobic mesophilic bacteria are the total number of bacteria that can grow in an aerobic environment under moderate incubation temperature. They are either gram-positive or gram-negative. Aerobic mesophilic bacteria are indicators of quality providing useful information about the shelf life of storage foods and beverages [79]. In many fermented foods, beverages, and condiments, AMBs are observed at the beginning and even at the final stage of fermentation [54, 27, 56, 80]. However, Anumudu and others [81] reported that members of AMB that started the fermentation did not persist until the end of the fermentation stage during *Ogi* fermentation.

### 2.5. Enterobacteriaceae

Enterobacteriaceae are gram-negative, facultatively anaerobic, fermentative, mesophilic, non-spore-forming bacteria that include both pathogenic and spoilage bacteria [80]. They are incapable of growth below water activity 0.95. These organisms have the potential to spoil foods [79, 82, 83]. Enterobacteriaceae are strongly affected by high temperature, salt concentration, and low pH. Nevertheless, they secrete acyl homo-serine lactones to regulate proteolytic enzyme production and iron chelation during the spoilage of foods [84]. Some members of Enterobacteriaceae produce off-odors and off-colors in beer and spoil most of the fermented food products [84]. Representative spoilage genera of Enterobacteriaceae include *Escherichia*, *Erwinia*, *Enterobacter*, *Citrobacter*, *Serratia*, and *Proteus*.

The members of Enterobacteriaceae present in cereal-based fermented foods are *Salmonella* spp., *Enterobacter* spp., *Klebsiella* spp., *E. coli*, and *Shigella* spp. [85-88]. The occurrences of these microbes are due to poor hygienic prac-

tices during the processing, handling, transportation, and utilization of contaminated water [89, 90]. Even during fermentation, some of these organisms produce stress responses that facilitate their adaptation to an acidic environment [91-93]. However, many researchers reported that Enterobacteriaceae could not persist until the final stage of food fermentation [54, 88, 94]. This could indicate that tolerance to very stressful environments by members of the family Enterobacteriaceae is minimal and their tolerance fades with concentration and time of exposure.

### 2.6. Staphylococci

Staphylococci are involved in the spoilage of both fresh produce and processed foods and beverages [76]. It has been reported that the species of staphylococci occur in many different fermented foods and beverages [87, 88, 95]. In some fermented foods, their colony counts are below the threshold at the final stage of fermentation [88, 96]; however, above the threshold in other fermented beverages at the same fermentation stages [54, 97]. One of the most common vehicles for the transmission of these microbes to food and beverage products are the contact between food and human being as they are found on the human skins and some parts of human respiratory organs [98].

### 2.7. Aerobic Spore-Forming Bacteria (ASFB)

Bacillus species are known to contaminate raw and processed foods [76, 99, 100]. They are often counted as spoilage microorganisms in dairy industries [101]. Among the most important species of the genus Bacillus for food safety concerns is *Bacillus cereus*. According to the food safety authority of different countries, the borderlines for *Bacillus cereus* in processed foods are  $10^3$ - $\leq 10^5$  cfu/g [102, 103]. The big problems with these microbes are invisibility of their toxins and spores until there is a foodborne outbreak [104]. On the other hand, species of the genus Bacillus secrete different types of enzymes that could use in spontaneous fermentation to start catabolic activities and facilitate conditions for other microorganisms [105].

### 2.8. Total Coliform Bacteria (TC)

Total coliforms are a large group of gram-negative, rod-shaped, and thermotolerant microorganisms. They are known to produce acid and gas during the fermentation of lactose. The bacteria encompass thermotolerant and faecal coliforms. The presences of total coliforms in foods are not only faecal contamination but also *Citrobacter*, *Enterobacter*, *Hafnia*, and *Klebsiella*. However, the presence of faecal streptococci is evidence of faecal contamination. Faecal streptococci are total coliforms those highly resistant to dry environments [106]. Most of the time total coliforms are from contaminated water [106, 107].

### 3. Conclusion

This review concluded that in spontaneous fermentation as beneficial microorganisms start to produce secondary metabolites both pathogenic and spoilage microorganisms start to eliminate the system. However, not almost all of the pathogenic and spoilage microorganisms eliminate from fermented products. Yeasts stimulate the growth of lactic acid bacteria through providing essential metabolites such as vitamins, while lactic acid bacteria produce organic acids. Thus, most of the spontaneously fermented foods and beverages are dominated by lactic acid bacteria and yeasts.

### Abbreviations

AMB	Aerobic Mesophilic Bacteria
ASFB	Aerobic Spore-Forming Bacteria
LAB	Lactic Acid Bacteria
TC	Total Coliform Bacteria

### Author Contributions

Daniel Asfaw Kitessa is the sole author. The author read and approved the final manuscript.

### Conflicts of Interest

The author declares no conflicts of interest.

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