Chapter 5
Microorganisms Involved in Spontaneous Fermentation and their Health Risk

Abdel Moneim Elhadi Sulieman

5.1 Introduction

Fermentation, the process of extracting energy from oxidation-reduction reactions of chemical compounds, including carbohydrates, and by using an intrinsic electron acceptor, which is often an organic compound. Through fermentation, the food becomes more nutritious, more digestible, safer for the consumer, and has a better flavor. Fermentation is also a highly efficient conservation process and a relatively lower energy cost compared to other conservation methods (https://ar.wikipedia.org/wiki/).

Consuming fermented food sources has been accounted for to bring improvements in a scope of health parameters. These beneficial outcomes can be applied by a blend of the live microorganisms that the fermented foods contain, as well as the bioactive components delivered into the food sources as by-products of the fermentation process. In numerous examples, and especially in dairy fermented foods, the microorganisms associated with in the fermentation process belong to the lactic acid group of bacteria (LAB) (Harsh et al. 2020).

The fermentation process plays an important role under anaerobic conditions, as there is no oxidative phosphorylation to maintain the production of ATP by the degradation process. Pyruvate is also represented by many different compounds during the fermentation process. Where the lactic fermentation process expresses the production of lactic acid from pyruvate; whereas, the alcoholic fermentation process expresses the conversion of pyruvate to ethanol and carbon dioxide; however, the heterogeneous lactic fermentation process is the production of lactic acid (lactic) in addition to other acids and alcohols. The fermentation process need not be carried

A. M. Elhadi Sulieman (✉)
Department of Biology, College of Science, University of Hail,
Hail, Kingdom of Saudi Arabia

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out or carried out in an anaerobic environment. For example, even with abundant oxygen, yeast cells largely prefer fermentation to oxidative phosphorylation.

Fermented foods and beverages accompanied and likely encouraged the progress from hunter-gatherer communities to sessile farming communities in the Neolithic revolution about 14,000 years ago (Arranz-Otaegui et al. 2018; Hayden et al. 2013). They have remained staples of human diets for quite a long time and are an undeniably famous food classification. However, their developing prominence in the previous 20 years has prompted various misunderstandings and questions.

Fermented food is a source of probiotics, which are beneficial bacteria, which forms a protective lining inside the intestine to protect the body from microbes that cause diseases such as Salmonella and Ecoli bacteria, and since 80% of the immune defenses are found in the digestive system, eating more fermented substances enhances whole body functions.

Fermented foods are foods that contain an amount of beneficial bacteria, as these bacteria are used in the manufacture of the product, such as using bacteria to convert milk into curd. It is worth noting that these bacteria are an important addition to the nutritional value of the product due to its effect on beneficial bacteria in the intestine and its multiple benefits. Including a few servings of fermented foods in your diet every week can have a huge impact on gut health, weight management, blood sugar levels, and more. Nevertheless, it can be harmful in some cases, such as when fermented milk becomes spoiled (https://ar.wikipedia.org/wiki/).

Fermentation processes in foods regularly lead to changes in nutritional and biochemical quality comparative with the beginning fixings. Fermented foods contain complex environments comprising of enzymes from raw ingredients that interact with the fermenting microorganisms' metabolic activities. Fermenting microorganisms give a remarkable methodology towards food stability via physical and biochemical changes in fermented foods. These fermented foods can profit consumers contrasted to simple foods in terms of antioxidants, production of peptides, organoleptic and probiotic properties, and antimicrobial activity. It likewise helps in the levels of anti-nutrients and toxins level (Ranjana et al. 2020).

Microorganisms important in food fermentation might be included as pure or blended cultures or, sometimes, the desired microorganisms might be available in adequate numbers in the original raw materials (Dirar 1992). Fermentation is powerful technique for food conservation. The high encompassing temperatures of the tropics heighten the requirement for low-cost preservation strategies, for example, drying and traditional fermentations. In fermentations, microbes preserve foods as aftereffect of serious development, products of their metabolism, for example, organic acids, and bacteriocin production (Stiles 1996).

The term industrial fermentation refers to the intended utilization of the microorganisms such, with the aim of making products beneficial to humanity. Where fermented products represent many applications, including food processing as well as in general industry. The use of microbiology and procedure innovation brought about enormous upgrades in the nature of the aged food items. The quality enhancements have been incredible to the point that today all huge creation of matured food is mechanical.

Fermentation can essentially be practiced by spontaneous fermentation, back-slopping or by expansion of starter culture. The application of microbiology and process technology brought about enormous improvements in the nature of the fermented food products. The quality enhancements have been incredible to the point that today all significant production of fermented food is industrial.

In back-slopping, a small portion of a previously successful fermentation is utilized to inoculate new substrate was utilized to create starter cultures for future fermentations. However, procedures tumbled from favor in the nineteenth century simultaneously with the ascent in favor in the nineteenth century simultaneously with the ascent in and governmental legislative guidelines concerning sanitation (Whittington et al. 2019; Josephsen and Jespersen 2004).

Industrial fermentations are the biological changes responsible for the transformation of carbohydrates and similar substances under aerobic or aerobic conditions by the action of the micro-organisms, which contain enzymes and organic acids as inhibitors or fatal to some microorganisms, as these substances give the taste and smell. The color and texture are special to make the product different from the raw material.

It has been realized that fermented foods are more nutritious than their unfermented partners (Hasan et al. 2014). The expanded dietary benefit in fermented foods sources is because of the fermenting microorganisms present in them. Microorganisms are both catabolic and anabolic, break down complex compounds, and synthesize complex vitamins and other growth factors (Kennedy 2016).

5.2 Stages of Microbial Growth

When an organism is placed within a specific growth medium, the medium is fertilized with that specific bone. Here, we note that the growth of the inoculator does not happen at once, by merging, but it takes some time for this to happen. It is the period of time required to adapt, which is called the lag phase. Next to this lag phase is the phase of the organism's growth rate increasing steadily, for a specified period - this period is called the log period or the exponential period. Whereas, after a certain period of exponential phase, the rate of growth begins to slow down, due to a continuous decrease in nutrient concentrations and /or a continuous increase in (aggregate) concentrations of toxic substances. This phase, at which the rate of growth slows down, is called the deceleration phase. After the slowdown has passed, growth stops and the microbial culture enters a static phase, or a stable or stable phase. The biomass remains constant, except when specific, combined chemicals on the culture analyze the cells (chemical analysis). And if other microbes do not pollute the culture, the chemical structure remains unchanged. In addition, the mutation and alteration of the farm organelles may represent a source of pollution as well, which is called internal pollution.

The study of the bacterial growth curve is useful in dealing with bacteria and knowing their activity and different growth stages, as this is useful in:

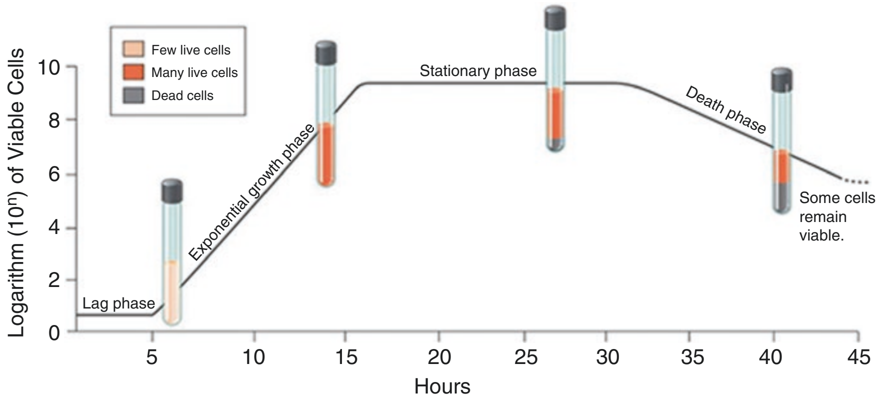

Fig. 5.1 Microbial growth curve

1. Areas of resistance and/or control
2. Pathological injuries
3. Microbial food spoilage and poisoning.

The general active phase is the most sensitive phase and is likely to die from heat or chemicals.

The number of bacteria cells can be calculated at any of the different stages of growth using the following equation:

Ns = (Ni) 2n
Ns = the total number of cells at a point on the curve.
Ni = the initial number of bacterial cells.
n = number of generations
2n = the number of cells per generation (Fig. 5.1).

5.3 Microflora of African Fermented Foods

Fermentation is caused by microbes such as bacteria, mold, and yeast. For example, fungi or yeasts are found on a mixture of sugar with mineral salts to produce penicillin. Yeast breaks down the sugar from the beans soaked in water into ethyl alcohol and carbon dioxide gas when making beer. The sugar in grape juice also decomposes in the same way as when making wine. Fermentation is also considered essential in the production of bread, cheese and curd. But it can be harmful in some cases, such as when fermented milk becomes spoiled. Fermented products that are beneficial for humans are made in large quantities. Although different types of materials are produced by the fermentation process.

Traditional fermented foods assume a significant function in the diet of various communities around the globe. Africa is maybe the landmass with the most extravagant assortment of fermented foods, where fermentation actually assumes a significant function in fighting food deterioration, foodborne illnesses and represents a significant postharvest value addition. In fact, fermentation is yet a generally locally established home-based process utilized all through the continent (Maria Diaz et al. 2019).

Many scientists are investigating the curative and preventive properties of consuming a fermented food that contains beneficial microbes. Studies and experiments confirmed that eating such a type of food gives the body, in addition to pathological microbial resistance, the longevity without aging and strength to the point that some of them believe that eating these bacteria enable a person to live in a healthy state characterized by a high degree of activity and vitality. Or the person lives without showing symptoms of aging.

A wide assortment of crude materials are traditionally fermented in various districts of Africa. Subsequently, fermented foods with various qualities are delivered and they have been classified in groups, for example, fermented non-alcoholic cereals (mostly created from sorghum, millet and maize), starchy root crops (fundamentally delivered from cassava), animal proteins (mainly dairy products), vegetable proteins (created from legumes and oilseeds) and alcoholic beverages (delivered from cereals, sap, honey or fruits, among other materials). Fermented products have been depicted to give health benefits, such as protection against gastrointestinal disorders, prevention of hypertension and heart disease or protection from diabetes and osteoporosis. Furthermore, traditional African fermented foods contain live microorganisms that can produce health-promoting compounds, such as antimicrobials, essential nutrients or molecules with antioxidant activity, and can go about as probiotic strains (Franz et al. 2014; Tamang et al. 2016).

Lactic acid bacteria (LAB) are the most ordinarily utilized microorganisms for protection of foods. Their significance is related fundamentally with their safe metabolic activity while developing in foods using accessible sugar for the creation of organic acids and different metabolites. Their normal occurrence in foods and feeds combined with their enduring use adds to their common acceptance as GRAS (Generally Recognized As Safe) for human consumption (Aguirre and Collins 1993). In an industrial scale a specific characterized starter culture, which has been created under controlled conditions, is of first inclination with the goal that the characteristics of the completed item could be reliably kept up for quite a while. Also, current strategies for quality innovation makes workable for the microbiologists to plan and create starter cultures with explicit characteristics.

Numerous microbiological studies manage identification of organisms isolated from different fermented foods. Lactic acid bacteria isolated from tomatoes that were naturally fermented under partial anaerobic conditions were found to be Leuconostoc mesenteroides, Lactobacillus brevis and Streptococcus sp. (Beltrán-Edeza and Hernández-Sánchez 1989).

In Sudan, Badi (1987) stated that Kasha fermentation was both acidic and alcoholic, she reported that the organisms involved members of the genera *Lactobacillus* and *Streptococcus* among bacteria and *Candida* and *Saccharomyces* among the yeasts. El Mahdi (1985) found lactic bacteria, proteolysis bacteria, yeast and molds present in counts of the order of billions of cells per ml of sour Ajin. Mohammed (1991) found that when Dabar sorghum flour in Sudan was fermented utilizing an inoculums from a previous batch of fermented dough, at the steady temperature of 30 °C, the microbial population was dominated by lactic acid bacteria, with yeast and mold counts staying low. The most prevelant microbial species that was *Pediococcuc confuses Lactobacillus confuses*, *L. brevis* and *Enterococcus francium*. The authors found that the yeasts *Candida intermedia* and *Debaryomyces hansenii* could be important in the fermentation of sorghum for making of *Kisra*. Hamad (1995) found that 99% of the bacteria isolated from different fermenting dough's were *Lactobacilli* namely, *Lactobacillus fermented*, *Lactobacillus refuter* and *Lactobacillus malodorous*.

Information about the microbial environment of natural food fermentations can be utilized to recognize biomarkers to evaluate the nature of fermented foods and would help in the design of optimum starter cultures. Overwhelming bacterial groups present in African fermented foods have been broadly analyzed utilizing culture-dependant methods however these techniques present a few constraints, for example, not having option to recognize not able to identify microorganisms in low numbers in complex environments with predominant populations (Cocolin et al. 2013). As an alternative, culture-independent methods, especially amplicon sequencing, are increasingly being used to study the bacterial populations of fermented foods, although to date, few studies have focused on African foods.

5.4 Important Microorganisms Dominating Fermented Foods

5.4.1 Lactic Acid Bacteria

Lactic acid bacteria are widely found in many nutrient-rich media such as milk, meat, drink, and vegetables, in addition to their presence in soil, lakes and the digestive system of animals and humans. Fermentation of the sugar has defined as that process results in production of lactic acid, which many bacteria carry out, has been around for a long time, and man has applied it. It contains numerous food products, and it plays an essential and important role in the fermentation industry, and dairy products. Tserovska (2002) studied bacteria have been extensively used for production a good starter culture, and to obtain fermentation products that have stable characteristics and are resistant to bacteriophage have been used throughout history in food production and preservation.

Many studies have shown the health effects of many strains of acid bacteria milk on humans, and these studies have tried to find out how these bacteria work in digestive system, and many benefits have been found, the most important of which is improving the digestion of lactose and treating diarrheal disorders (Gillilard 1990; Drouault and Cortheir 2001).

LAB-driven fermentations regularly yield by-products with bioactivity and a different scope of wellbeing advancing impacts, including protection against infectious agents, immunomodulatory impacts, hostile to allergenic impacts, anti-obesity impacts, hostile to oxidant impacts, enhancing the bioavailability of vitamins/minerals, anti-anxiety impacts, among others (Oguntoyinbo and Narbad 2015; Zhao et al. 2015; Linares et al. 2017).

The lactic acid bacteria implicated in Africa fermented foods comprise of species that belong to the genera Lactobacillus, Lactococcus, Leuconostoc and Pediococcus (Oyewole 1997). These microorganisms vary in their composition from one product to the other. As a result of changes in conditions, a great number of fermented milk types been developed (Sulieman et al. 2006). Variables include heat treatment of the milk, fermentation temperature, inoculum percentage and the concentrating of the milk. According to these conditions, different types of lactic acid bacteria become predominant e.g., producing various flavour components. Most types contain two to four types of bacteria.

Lactic acid bacteria produce additives and flavor compounds that have an important role in food, so called functional foods, and it increases the nutritional and health value of these products (Holzapfel et al. 2001; Rinkinen (2003). They also produce several antimicrobials of great importance in food preservation (Axelsson 2004). In addition, lactic acid precursors play an important role in giving the correct texture of fermented dairy products (Fryer and Rossi 2004).

LAB are the most important in dairy products in terms of their vital activity and in terms of their percentage of total flora, as they ferment lactose, producing a high percentage of lactic acid. Moreover, they produce flavoring substances, and they are Gram-positive bacteria, aerobic – anaerobic. They do not produce the enzyme catalase (this enzyme breaks down oxygenated water into water and oxygen). It is worth noting that lactic acid bacteria ferment sugars under anaerobic conditions.

LAB belong to the family Lactobacillaceae and are divided morphologically into two groups:

1. **Streptococcaceae bacteria**

It is a single or double bacteria or found in short chains, and it is one of the most important species related to it that is important in the dairy and cheese industry. The impotant genera and species of this group include: the genera: Strptococcus such as *Str.Lactis, Str.diacetylactis*:, *Str.cremoris, Str.thermophilu.* The genera Leucenostoc such as *Leu.dextranicum, Leu.citrovorum* (Fig. 5.2).

2. **Lactobacillus bacteria**

Lactobacillus bacteria is an important genera of LAB that convert lactose sugar and a number of other sugars into lactic acid, hence its name (Lactic acid bacteria) and the genus Lactobacillus spreads in many places, including the normal flora of humans. They are found in the mouth, intestine and vagina and have an important influence in maintaining the normal bacterial balance of these parts, while some researchers believe that these bacteria have an effect on the development of tooth

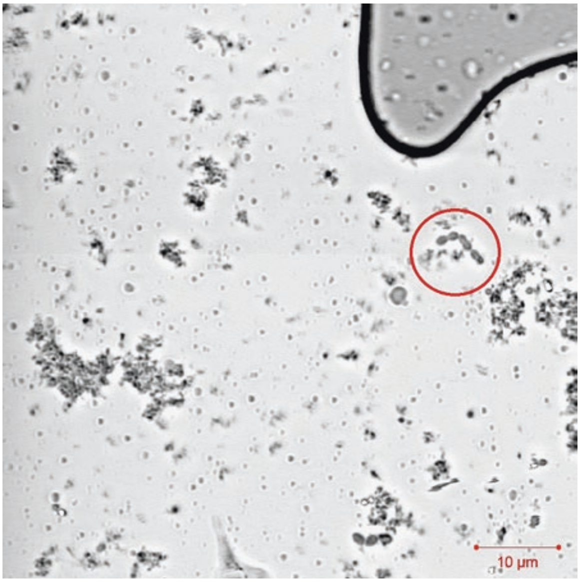

Fig. 5.2 Streptococcus thermophiles colonies. (Source: https://commons.wikimedia.org/)

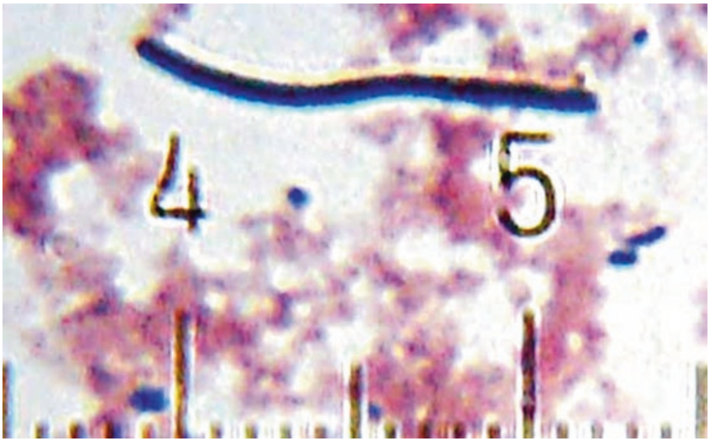

Fig. 5.3 Lactobacillus delbrueckii subspecies bulgaricus from a sample of Activia® brand yogurt. (Source: https://commons.wikimedia.org/)

decay, and are found in the gastrointestinal tract of many animals, and they are spread in nature (Fig. 5.3).

The important genera and species include:

(a) Thermobactrium:

Among the most important types of it:

Lactobacillus Lactis: Lactobacillus acidophilus: Lactobacillus bulgaricus Lactobacillus helveticus

(b) Streptobacterium, such as Lactobacillus plantarum, Lactobacillus Casei
(c) Batabacterium, one of the most important species to which it belongs: Lactobacillus brevis

Lactobacillus spp. can produce specific anti-microbial substances, which have been observed to inhibit the growth of some pathogenic microorganisms (Saad et al. 2001; Yost et al. 2002). These beneficial bacteria are most effective during periods or disease or stress and following antibiotic treatment (Table 5.1).

Table 5.1 Micro-organisms commonly found in fermenting fruit and vegetables

Organism	Type	Reaction
Acetobacter genus A. aceti A. pasteurianus A. peroxydans	Aerobic rods	Oxidise organic compounds (alcohol) to organic acids (acetic acid). Important in vinegar production
Streptococcaceae family	Gram positive cocci	
Streptococcus genus S. faecalis S. bovis S. thermophilus		Homofermentative. Most common in dairy fermentations, but S. Faecalis is common in vegetable products. Tolerate salt and can grow in high pH media.
Leuconostoc genus L. mesenteroides L. dextranicum L. paramesenteroides L. oenos	Gram positive cocci	Heterofermentative. Produce lactic acid, plus acetic acid, ethanol and carbon dioxide from glucose. Small bacteria, therefore have an important role in initiating fermentations.
Pediococcus genus P. cerevisiae P. acidilactici P. pentosaceus		Saprophytic organisms found in fermenting vegetables, mashes, beer and wort. Produce inactive lactic acid.
Lactobacillaceae Family	Gram positive rods. Nonmotile	Metabolise sugars to lactic acid, acetic acid, ethyl alcohol and carbon dioxide.
Lactobacillus genus		The genus is split into two types – Homo- and hetero-fermenters. Saprophytic organisms. Produce greater amounts of acid than the cocci
Homofermentative Lactobacillus spp. L. delbrueckii L. leichmannii L. plantarum L. lactis L. acidophilus		Produce only lactic acid. L. plantarum important in fruit and vegetable fermentation. Tolerates high salt concentration.
Heterofermentative Spp. L. brevis L. fermentum L. buchneri		Produce lactic acid (50%) plus acetic acid (25%), ethyl alcohol and carbon dioxide (25%). L. brevis is the most common. Widely distributed in plants and animals.
Yeasts	Saccharomyces Cerevisiae S. pombe Many aerobic, some anaerobes	S. cerevisiae can shift its metabolism from a fermentative to an oxidative pathway, depending on oxygen availability. Most yeasts produce alcohol and carbon dioxide from sugars.
Debaromyces Zygosaccharomyces rouxii Candida species Geotrichum candidum		Zygosaccharomyces rouxii Candida species Geotrichum candidum Tolerant of high salt concentrations tolerates high salt concentration and low aw

5.4.2 Yeasts

The occurrence of yeasts in indigenous African fermented food and beverage products has been studied for a scope of end products. Nonetheless, far less investigations have inspected have examined the yeast dynamics during the fermentations. The yeast species overwhelming indigenous fermented food and drinks are those that can able to adapt to the changing intrinsic conditions brought about by physicochemical changes, because of microbial activity (Navarrete-Bolaños 2012).

Species diversity is also affected by various extrinsic factors associated to the technological processing stages incorporating fermentation length and temperature, water quantity added, raw materials utilized, stirring, pasteurization as well as level of hygiene and sanitation (Jespersen 2003; Achi and Ukwuru 2015). Consequently, a complete comprehension, connecting intrinsic and extrinsic factors to microbial assorted variety and progressions is of outmost significance for overhauling indigenous sub-Saharan African fermented food and drinks.

A few functional properties of yeasts have been accounted for the preparing of indigenous sub-Saharan African fermented food and drinks. These incorporate fermentation of carbohydrates, flavor compound development, stimulation of LAB, degradation of cyanogenic glycosides, creation of tissue-degrading enzymes, binding and/or degradation of mycotoxins as well as probiotic properties (Pernille et al. 2019; Omemu et al. 2007; Padonou et al. 2010; Achi and Ukwuru 2015; Tamang et al. 2016). Nonetheless, in the majority of the contemplated indigenous sub-Saharan African fermented food and beverages, the functional properties of recognized yeasts have not yet been widely explained.

Genera of yeasts reported from fermented foods, alcoholic beverages and non-food blended amylolytic starters are Brettanomyces, Candida, Cryptococcus, Debaryomyces, Dekkera, Galactomyces, Geotrichum, Hansenula, Hanseniaspora, Hyphopichia, Issatchenkia, Kazachstania, Kluyveromyces, Metschnikowia, Pichia, Rhodotorula, Rhodosporidium, Saccharomyces, Saccharomycodes, Saccharomycopsis, Schizosaccharomyces, Sporobolomyces, Torulaspora, Torulopsis, Trichosporon, Yarrowia, and Zygosaccharomyces (Watanabe et al. 2008, Tamang and Fleet 2009; Kurtzman et al. 2001; Lv et al. 2013) (Table 5.2).

5.4.3 Yeast Interactions with LAB

Yeasts and LAB frequently exist together during spontaneous fermentations. While yeasts can develop in a moderately basic medium, LAB are more meticulous and require more supplements as, e.g., amino acids and vitamins (Viljoen 2006; Ponomarova et al. 2017). The cooperations among yeasts and LAB can be both synergistic and antagonistic, however are frequently of shared advantage. Mutualistic interactions between yeasts and LAB have been depicted for ogi, a non/low-alcoholic cereal-based beverage.

Table 5.2 Yeast species frequently found in dairy products

Identification according to Kreger-van Rij (1984)	Some equivalent names in earliest Literature
Debaryomyces hansenii	*D.subglobosus: Torulaspora hansenii*
Candida famata	*Torulopsis candida: T. famata*
Kluyveromyces marxianus	*Kluy.bulgaricus:Saccharomyces lactis: S.fragilis*
Candida Kefyr	*C.Pseudotropicalis:Torulopsis Kefyr: Torula cremoris.*
Candida stellata	*Torulopsis stellata*
Saccharmyces (Yarrovia) Lipolytica	Candida lipolytica
Candida holmii	*Torulopsis holmii*
Saccharomyces exigubs	
Pichia membranaefaciens	*Candida krusei*
Pichia fermentans	*Candida krusei*
Rhodotorula glutinis	
Rhodotorula rubra	

Source: Kreger-van-Rij (1984)

The yeasts will benefit by a diminishing in pH by the acidification encouraged by the activity of the LAB, noteworthy higher development of *Lactobacillus plantarum* has been demonstrated when co-cultured with either *S. cerevisiae* or particularly *P. kudriavzevii*, demonstrating that these yeast species give development factors for the LAB (Omemu et al. 2007), undoubtedly amino acids (Ponomarova et al. 2017).

Mutualistic associations have similarly been accounted for between species of LAB and yeasts originating from indigenous African fermented milk products (Sulieman et al. 2013; Pernille et al. 2019). At the point when co-culturing Lactococcus lactis subsp. lactis biovar diacetylactis with K. marxianus in milk, the viability of L. lactis subsp. lactis biovar diacetylactis was improved, while K. marxianus could benefit from galactose, arising from lactose degradation by L. lactis subsp. lactis biovar diacetylactis (Gadaga et al. 2001). Moreover, Lactobacillus paracasei subsp. paracasei arrived at fundamentally higher final counts when co-cultured in milk with especially K. marxianus, S. cerevisiae or Naumovozyma dairenensis (f. Saccharomyces dairenensis).

5.5 Functional Properties of Microorganims in Fermented Foods

The fermenting microorganism have numerous functional properties will be discussed in details in another chapter. These functional properties include production of antimicrobial compounds, probiotic microorganisms, antioxidant activity, production of vaccines, production of enzymes improvement of the immune system and degradation of anti- nutritive compounds.

(Xiangna et al. 2018; Grasson 2002; Asmahan 2010; Perdigon et al. 2001; Steinkrause 1995; MacDonald et al. 2012).

5.6 Conclusions

Fermented foods are prepared by the action of micro-organsims under controlled conditions, which bring about a physical change in these foods and cause a change in the biochemistry and the forms of the nutrients that make up these foods. These foods may be prepared from grains, legumes, roots, vegetables, fruits, edible parts of any plant, fish, milk or meat. Through fermentation, the food becomes more nutritious, more digestible, safer for the consumer, and has a better flavor. Fermentation is also a highly efficient conservation process and a relatively lower energy cost compared to other conservation method.

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