



# Modern exploration of microbial diversity in traditional fermentation practice: A Review

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**Abstract:** Fermentation is an age-old chemical process which plays a vital role in various aspects of food processing all over the world and helps in enhancing flavor, value addition and provides many health benefits to the consumer. Microbial diversity is magnificent and essential to almost all life on Earth. The microorganisms that participate in the fermentation process convert mainly sugars and other carbohydrates to organic acids, carbon dioxide and alcohols that alter the texture, appearance, flavor, nutritional value and safety status of the original grain-based substrate. Traditional fermented foods often rely on spontaneous fermentation from mixtures of microorganisms, without close control of the species or strains present. Understanding the microbial profiles, population structures, dynamics and functioning during fermentation in order to establish controls and achieve a fermented product with high consistency is critical for food scientist. Culturalable microorganisms naturally ferment majority of global fermented foods. The application of culture-independent technique has thrown new light on the diversity of a number of unknown and non-cultural microorganisms in naturally fermented foods. The use of High-throughput and 'next-generation' sequencing tools offer many exciting new possibilities for food research for investigating microbial dynamics within food fermentations to the ecosystem of the food-processing. Other technique such as amplicon sequencing, metagenomics and transcriptomics present novel applications for exploring microbial communities in, on and around our foods. This review discussed many uses of these tools for food-related and food facility-related research and highlights where they may yield nuanced insight into the microbial world of food production systems.

**Keywords:** Diversity; Exploration; Fermentation; Microbes.

## 1 INTRODUCTION

Fermentation is a form of energy-yielding microbial metabolism in which an organic substrate, typically a carbohydrate, is incompletely oxidized [1]. This bioprocess leads to biomass formation and metabolite production, generally driven by microorganisms. Over centuries, food fermentation techniques have developed through experience and now account for about one-third of global food consumption, particularly within rural households and village communities [2]. Fermented products such as alcoholic beverages, vinegar, sauerkraut, sausages, cheeses, yogurts, and breads occupy a significant share of the food market.

As one of the oldest biotechnologies, fermentation enables the production of foods with extended shelf life and desirable sensory qualities [3]. Fermented foods typically exhibit enhanced microbial safety and stability, with some remaining shelf-stable even at room temperature. Beyond preservation, fermentation enhances palatability, nutritional value, and food safety while reducing cooking time and fuel requirements [4, 5, 6 and 7].

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## 2. HISTORY OF FERMENTATION AND FERMENTED FOODS

The origins of fermentation as a food-processing method date back thousands of years. Historical records suggest that fermentation practices began in the Indian subcontinent before the Indus Valley civilization [8]. Cheese making was established nearly 8000 years ago in the Fertile Crescent between the Tigris and Euphrates Rivers, coinciding with early domestication of plants and animals [9]. Alcoholic fermentations used in brewing and winemaking were likely pioneered between 2000–4000 BCE by the Egyptians and Sumerians [8].

Scientific understanding of fermentation emerged with Antonie van Leeuwenhoek's discovery of microorganisms in 1665 [10]. Louis Pasteur later disproved the theory of spontaneous generation in 1859 through carefully designed experiments [11]. In 1877, Sir John Lister demonstrated the role of *Lactococcus lactis* in fermented milk [12]. Pasteur famously described fermentation as "la vie sans l'air" (life without air) [13].

Modern industrial fermentation largely depends on defined microbial starter cultures, replacing the traditional use of undefined mixtures. This shift has improved culture performance, product quality, and consistency, though it also reduced microbial diversity in industrial production. In 2002, the International Dairy Federation (IDF) published a comprehensive list of microorganisms recognized as safe for use in dairy fermentation [14].

## 3. MECHANISMS OF FERMENTATION: MICROBIAL METABOLISM

Microorganisms are central to fermentation, breaking down sugars and organic molecules into simpler compounds while producing valuable by-products. Glycolysis, for instance, converts glucose into pyruvate, yielding ATP and NADH an essential pathway in fermentation. Other key metabolic routes include the tricarboxylic acid (TCA) cycle and the pentose phosphate pathway. *Saccharomyces cerevisiae* (baker's yeast) is particularly important in ethanol production, playing a central role in the bioethanol industry [15]. Under oxygen-limited conditions, microorganisms switch from oxidative phosphorylation to fermentation as an adaptive survival strategy. This anaerobic metabolism has applications in food, energy, and pharmaceuticals [16].

In alcoholic fermentation, *S. cerevisiae* converts carbohydrates into ethanol and carbon dioxide, contributing to both ethanol production and flavor development in beer, wine, and other beverages [17]. Lactic acid fermentation, on the other hand, is driven by lactic acid bacteria such as *Lactobacillus*. This process gives yogurt, sauerkraut, and kimchi their characteristic tang and also preserves vegetables by creating an acidic environment that inhibits spoilage organisms [18,19].

### 3.1 Stages Involved in the Fermentation Process

#### **Stage 1:** Introduction of Microbes:

Fermentation begins with the inoculation of specific microorganisms into a suitable substrate, commonly sugars, which serve as their primary energy source. The type of microbe used depends on the desired end product for example, *Saccharomyces cerevisiae* in bread production or *Lactobacillus* in yogurt [18].

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**Stage 2: Sugar Breakdown:**

Once introduced, the microbes initiate the degradation of complex carbohydrates into simpler compounds such as ethanol, lactic acid, or carbon dioxide. This metabolic conversion is crucial for the development of flavor, texture, and preservation of the final product [20].

**Stage 3: Flavor Development:**

One of the most notable contributions of fermentation is the enhancement of flavor. The distinct tastes and aromas of fermented foods arise from a wide variety of volatile compounds esters, aldehydes, and organic acids produced during microbial metabolism [21]

**Stage 4: pH Regulation:**

Maintaining optimal acidity during fermentation is critical for microbial growth and activity. Microorganisms regulate pH by producing acidic or alkaline metabolites, thereby creating an environment conducive to their survival and metabolic processes [20, 22]

**Stage 5: Control and Monitoring:**

Precise regulation of environmental parameters such as temperature, humidity, and oxygen availability is vital for successful fermentation. Even minor fluctuations in these variables can significantly impact microbial activity and, consequently, product quality.

**Stage 6: Time Frame:**

Fermentation duration varies widely, from a few hours in bread dough preparation to several weeks or months for products such as matured cheeses. The length of the process is influenced by microbial species used and the desired characteristics of the end product. Fermentation not only enhances flavor but also acts as a natural preservation method [23].

**3.2 Microorganisms Involved in Food Fermentation**

As one of the oldest preservation methods, fermentation has long been central to the diets of many indigenous populations. The outcome and quality of fermented foods largely depend on the diversity of microorganisms involved. The three main groups contributing to food fermentations are bacteria, yeasts, and molds.

**3.2.1 Bacterial Fermentation**

Lactic acid bacteria (LAB) and *Bacillus* species are the predominant groups in many traditional fermented foods. Other bacteria such as *Corynebacterium*, *Micrococcaceae*, and *Acetobacter* are also frequently associated with food fermentations.

**Table 1:** *Bacillus* strains Identified in Foods.

Microorganisms	Fermented Food Products	References
<i>Bacillus subtilis</i>	Fermented African locust bean, Fermented soybean dawadawa, Fermented soybean food (sufu),	[24]
<i>Bacillus licheniformis</i>	Mongolia of China, fermented soybean (Chunkook-Jang, Meju), fermented	[25]
<i>Bacillus cereus</i>	Food pathogen	[26]

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### 3.2.2 Yeast Fermentation

Yeasts are indispensable in the production of numerous fermented foods. They ferment sugars, produce secondary metabolites, and inhibit undesirable molds. The most widely used yeast is *Saccharomyces cerevisiae*, essential in bread, beer, and wine production, and available commercially as liquid yeast, compressed yeast, active dry yeast, and instant active dry yeast [27]. Other species such as *Torulaspora delbrueckii* and *Kluyveromyces thermotolerans* are also applied in frozen dough production due to their tolerance to low temperatures [28]

### 3.2.3 Mold (Fungal) Fermentation

Fungi are central to the manufacture of foods like beer, wine, bread, and cheese. They are also exploited for the industrial production of essential nutrients including methionine, glutamic acid, riboflavin, and vitamin B12 [29]. In sausage ripening, molds play a key role alongside LAB, improving surface appearance, flavor, and aroma by breaking down lipids and proteins. Toward the end of the drying process, molds often dominate the microflora [30, 31, 32 and 33]

## 3.3 Molecular Techniques for Microbial Community Profiling of Fermented Foods

Over the past decade, molecular-based approaches, particularly advanced sequencing platforms, have transformed the study of fermented food microbiomes. By generating phylobiomes, metagenomes, and metatranscriptomes, these methods complement traditional culture techniques and provide deeper insights into fermentation ecosystems and the identity of participating microorganisms [34].

### 3.3.1 Microbial Community Profiling

Early applications of molecular profiling in fermented foods primarily relied on PCR amplification of 16S rRNA gene fragments, followed by denaturing gradient gel electrophoresis (DGGE) for visualization. This approach was first used in products such as pozol (fermented maize dough) and artisanal cheeses [34]. With technological advancement, sequencing of PCR-derived rDNA regions has become a more widely adopted strategy for microbial characterization. Although this is often termed metagenomic sequencing or pyrosequencing, the concept of a phylobiome more accurately describes the taxonomic information obtained.

These techniques have since been applied to a growing range of fermented foods, especially traditional Asian products, including pulque (fermented agave beverage) [35], pozol, kimchi (fermented vegetables) [36], doenjang (soybean paste), kochujang (chili-rice-soybean paste) [37], and rice bran fermentations [34].

### 3.3.2 Genomics of Microbes from Traditional Fermentations

Metatranscriptomics has emerged as a valuable tool for understanding microbial interactions and metabolic activity during traditional fermentations. In kimchi, microarray-based transcriptome studies revealed that alongside dominant organisms such as *Leuconostoc mesenteroides* and *Lactobacillus sakei*, less abundant microbes (*L. graminis*, *L. curvatus*, *Weissella viridescens*, and *W. minor*) displayed significant transcriptional activity, indicating functional contributions to fermentation [37]. Similar work on sourdough ecosystems

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highlighted *L. plantarum* and *L. fermentum* as key transcriptionally active members in stable fermentations [38].

### **3.4 New Tools for Identification of Microorganisms in Fermented Foods**

The introduction of next-generation sequencing (NGS) has revolutionized microbial ecology research. Technologies such as Illumina, nanopore, and ion semiconductor sequencing detect nucleotide incorporation at single-molecule resolution, enabling unprecedented analysis of microbial diversity [39].

Traditional culture-based methods often overlook unculturable microorganisms that may influence product quality. To address this, culture-independent approaches—based on direct DNA extraction—are increasingly applied to reveal both culturable and non-culturable populations [40, 41 and 42]. These methods provide a broader understanding of species richness, diversity within genera, and functional roles in fermentation [43, 44 and 45].

A wide range of molecular tools has been developed for strain-level identification, including species-specific PCR [46], quantitative PCR [47], Random Amplification of Polymorphic DNA (RAPD) [48], repetitive element-based PCR (rep-PCR) [49], amplified fragment length polymorphism (AFLP) [50], and multilocus sequence analysis (MLSA) targeting housekeeping genes [51]. Although DGGE and TGGE have been widely used for microbial profiling, they present limitations such as time demands, difficulty in quantifying abundance, and challenges in resolving complex banding patterns [52, 37].

Proteomic technologies, notably matrix-assisted laser desorption ionization–time-of-flight mass spectrometry (MALDI-TOF MS), have further improved microbial identification, successfully detecting *Bacillus* spp. in African fermented foods and lactic acid bacteria in various global products [53, 54, 55, 56 and 57].

### **3.5 Application of Microbial System-Level Tools in Food Production**

There are different Microbial System -level tools being employed in food production, which includes: Amplicon Sequencing, Metagenome Sequencing, Metatranscriptomics etc., which have additional advantages over the Traditional Fermentation Practices.

#### **3.5.1 Microbial Community Dynamics**

Microorganisms determine the safety, stability, and nutritional attributes of foods, functioning as both beneficial fermenters and spoilage agents. Amplicon sequencing has been widely applied to pearl millet [58], Asian vegetable fermentations, seafood, rice beer, olives, sourdough, kombucha, kefir, wine, and spontaneously fermented beers [59, 60]. These fermentations often involve undefined or mixed microbial consortia, and describing their dynamics helps identify biomarkers of quality, diagnose spoilage events, and optimize processing. Amplicon sequencing also aids in biosurveillance of rare or novel organisms that standard culture methods might overlook [59].

Metagenomic sequencing offers additional advantages by avoiding PCR primer bias, enabling full-length gene recovery, and improving species-level resolution. It also permits functional analysis of entire microbial communities, including viruses and non-culturable taxa. For

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example, metagenome-based reconstructions of cheese-rind communities revealed enrichment of amino acid metabolism pathways associated with characteristic cheese aromas, and identified the bacterial enzyme responsible for converting methionine to methanethiol [61, 62]. Metatranscriptomics, although less frequently applied, provides complementary insights by revealing the transcriptionally active microbial fraction. In kimchi fermentations, *Leuconostoc mesenteroides* dominated early stages, while *Lactobacillus sakei* and *Weissella koreensis* became more active later. Interestingly, the transcriptionally active community differed somewhat from profiles generated by amplicon sequencing [63].

### 3.5.2 Food Building Ecosystems

The microbiota associated with man-made structures has gained increasing attention in recent years due to its potential impact on human health [64]. Since much of the modern diet in developed nations is processed, packaged, stored, and transported indoors under artificial conditions, the microbial ecosystems within food-processing environments play a crucial role in determining food quality and safety. Over the past decade, methods for integrating microbial data with metadata have advanced significantly, offering promising opportunities to address research questions that were once unattainable [65].

Currently, no metagenomic or metatranscriptomic studies have been conducted specifically on food systems in built environments. The relatively low sample throughput of these techniques still limits their application in visualizing comprehensive microbial landscapes. However, the development of new bioinformatics tools is improving opportunities for investigating the microbes and genetic features present in food facilities [66]. For example, [59] applied SourceTracker to analyze contamination routes within a brewery by comparing brewery substrates with publicly available human-associated microbiomes. Similar tracking approaches have also been used to study the sources of hospital-acquired infections [66] and to complement conventional methods of monitoring target genes in clinical environments, such as tracking carbapenem resistance in hospitals using whole-genome isolate sequencing [67].

### 3.5.3 A New Concept of Microbial Terroir

Microbial Terroir refers to the unique combination of Microorganisms present in a specific environment such as Regions of soil, water or traditional food production.

Microorganisms play essential roles in food safety and quality during both pre-harvest and post-harvest stages. Understanding the factors that shape microbial consortia in agricultural products could therefore provide significant benefits. Soil acts as a reservoir for many bacteria that colonize grapevine surfaces [68], and vineyard soils often display consistent microbial biogeographic patterns, suggesting that edaphic (soil-related) factors influence these microbial communities [23, 59].

Different strains of *Saccharomyces cerevisiae* have been shown to generate distinct metabolic profiles in wine, demonstrating a direct link between regional microbiota and the chemical properties of finished wines [23]. A similar regional character has been observed in some dairy fermentations. As reported by [59] that both the production region and the type of milk significantly affect the microbial composition of fermented matsoni, a traditional Caucasian

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fermented milk product. These findings suggest that traditional production methods contribute to the preservation and transfer of unique regional microbiota from one batch to another. Bridging the gap between agricultural practices and food product characteristics holds great potential for improving food supply, security, consumer acceptance, and economic value in a world with a rapidly growing population.

#### 4. FUTURE PROSPECTS

We are now in the post-genomic era of microbiology, where many microorganisms involved in food fermentation have already been sequenced. This genomic wealth enables a knowledge-driven approach to harnessing microbes for food production, ranging from metabolic engineering to molecular mining of microbial traits for nutritional and technological benefits. The central challenge in applying genomics and proteomics to food systems lies in translating this vast information into improved microbial performance, enhanced safety and better food quality. Ongoing research continues to explore the microflora of traditional fermented foods and their specific functional roles in shaping product uniqueness. As sequencing costs decline and analytical speed increases, microbial monitoring of food systems is becoming more feasible and cost-effective. These advancements are expected to uncover new insights into the microbial dynamics that shape food ecosystems.

#### 5. CONCLUSION

This review highlights the pivotal role of fermentation in improving flavor, nutritional value, and economic significance of food products. Fermentation contributes to global food security and value addition, while also offering health benefits. The modernization of indigenous fermented foods through emerging food technologies further enhances their value. As next-generation sequencing technologies continue to advance, they are expected to provide deeper understanding of the microbial complexity in food systems, ultimately contributing to safer, higher-quality, and more sustainable food production.

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