

Cultured Complexity of Microbes and Attributes of Indian Fermented Beverages

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Abstract

The review unveils the microbial intricacies behind traditional Indian fermented beverages, elucidating the metabolic pathways shaping their unique flavors, aromas, and health benefits. Amino acid and fatty acid metabolisms contribute to diverse aroma compounds, while organic acid production influences acidity and tanginess. Microbial diversity, strain-level variations, and synergistic interactions add layers to the fermentation process. Ingredient influences in beverages like Lassi, Chhang, Kanji, Toddy, Feni, Apong, Kodo Ko Jaanr, and Handia are dissected. The role of yeast such as *Saccharomyces cerevisiae*, *Torulaspora delbrueckii*, *Pichia anomala* in fermentation and health aspects of probiotic microorganisms, such as *Lactobacillus* and *Bifidobacterium*, are highlighted. The article emphasizes the need for careful handling to maintain microbial viability and stability. It also explores the potential for innovation in commercial production while preserving the authenticity of traditional beverages. The article provides a concise overview of the intricate microbial tapestry that defines Indian fermented beverages.

Keywords

Metabolism, Probiotics, *Lactobacillus*, *Saccharomyces*

1. Introduction

Traditional Indian fermented drinks have a long and varied history that is strongly engrained in the country's cultural landscape. These beverages have considerable cultural, religious, and historical significance in addition to being a source of refreshment. Since ancient times, fermentation has been used in India to produce a wide range of beverages that are enjoyed throughout the country. This process involves microorganisms converting carbohydrates into alcohol and other substances [1]. Indian cultures are closely associated with the consumption of fermented beverages. They are widely used during rituals, events, and social gatherings and often serve as an essential component of ceremonies. Wastage of food due to quality degradation is a major concern in a tropical climate like India, thus fermentation has historically been an effective method to preserve food while utilizing any surplus product [2-4]. Communities were able to preserve and consume surplus foods like grains, fruits, and milk for a long time after they were harvested because of fermented beverages. This preservation-related consideration encouraged the development of distinctive fermentation methods and recipes that have been passed down from generation to generation [5].

The wide range of fermented beverages that can be found throughout India is a result of the country's diverse geography and climate. Every region has its own unique conventional methods of cooking and ingredients, frequently influenced by what is readily accessible nearby and norms of culture. In areas with a lot of palm trees, drinks like "toddy" or "palm wine" are popular since they are made from the sap of different palm trees. In these beverages, the yeast and bacteria that are

present naturally perform the majority of the fermentation process. Sugars are converted into alcohol by yeast, and flavor, aroma, and acidity are produced by bacteria [6, 7]. The specific strains of microorganisms found in various geographical locations add to the distinctive qualities of each beverage. Traditional fermented beverages still maintain a special place in the hearts of many despite the fact that India continues to modernize and expand global. These beverages are being promoted and preserved as a part of India's cultural heritage. To better comprehend these beverages' production methods and distinctive flavors, researchers are studying the microbiology and biochemistry that governs them.

2. Metabolic Pathways and Flavor Compounds

Metabolic pathways and flavor compounds are intricately linked, especially in the context of food and beverage production. Metabolic pathways, which involve a series of chemical reactions within a cell, contribute to the synthesis of intermediate compounds that act as precursors for secondary metabolism. These secondary metabolites often include volatile aroma compounds that give food its distinctive taste.

2.1 Glycolysis and Ethanol Formation in Fermentation

Glycolysis is a metabolic pathway that breaks down glucose into pyruvate, producing energy (ATP) and reducing power (NADH) [8]. Glucose is phosphorylated by the enzyme hexokinase, creating glucose-6-phosphate. It is converted to fructose-6-phosphate, which is further phosphorylated to fructose-1,6-bisphosphate. It is split into two three-carbon compounds: dihydroxyacetone phosphate (DHAP) and glyceraldehyde-3-phosphate (G3P) [10-12]. It is oxidized and phosphorylated, producing NADH and 1,3-bisphosphoglycerate. It donates a phosphate group to ADP, forming ATP and 3-phosphoglycerate. It is rearranged and dehydrated to form 2-phosphoglycerate, which is then converted to phosphoenolpyruvate (PEP). It transfers its phosphate group to ADP, producing ATP and forming pyruvate. In yeast, pyruvate is further processed in a process called alcoholic fermentation [9]. Pyruvate is decarboxylated by pyruvate decarboxylase to form acetaldehyde and CO₂. Acetaldehyde is then reduced by alcohol dehydrogenase using NADH, producing ethanol and regenerating NAD⁺ for use in glycolysis. This is how yeast ferments sugars to produce alcohol and carbon dioxide [11].

2.2 Amino Acid Metabolism

Amino acid metabolism during fermentation is a complex process in which microorganisms utilize amino acids not only to produce energy but also to synthesize a wide range of flavor compounds. Aromatic amino acids, in particular, act as precursors to the formation of aromatic compounds, which contribute to the unique aromas of fermented beverages [13]. Microorganisms absorb amino acids from their surroundings and utilize them as building blocks for cellular processes as well as substrates for flavor compound synthesis. Transamination occurs when the amino group (-NH₂) of one amino acid is transferred to a keto acid, resulting in the formation of a new amino acid and a new keto acid. This process helps in the interconversion of amino acids and contributes to the amount of amino acids available for metabolism [14].

Aromatic amino acids, such as phenylalanine, tyrosine, and tryptophan, can undergo decarboxylation reactions facilitated by enzymes. Phenylalanine decarboxylase converts phenylalanine to phenethylamine, contributing to floral aromas [16, 17]. Tyrosine and tryptophan undergo decarboxylation to produce tyramine and tryptamine, known for their distinct aroma profiles [15]. Transamination of aromatic amino acids further contributes to flavor diversity. Tyrosine transamination yields 4-hydroxyphenylacetaldehyde, imparting almond-like aromas [18], while tryptophan transamination produces indole and derivatives, contributing earthy and flowery aromas [19]. Amino acid metabolism during fermentation results in the synthesis of various derivatives, including aldehydes, alcohols, and sulfur compounds, enhancing the complexity of flavor [18]. Microorganisms possess specific enzymes, such as decarboxylases and transaminases, crucial for converting amino acids into flavor compounds, adding uniqueness to fermented beverages [20].

2.3 Fatty Acid Metabolism

During fermentation, fatty acids undergo breakdown and modification, forming derivatives that enhance the mouthfeel and flavor of beverages [21]. Microorganisms absorb or produce fatty acids, activated by Coenzyme A (CoA), leading to the formation of fatty acyl-CoA [22, 23]. Beta-oxidation then occurs, generating acetyl-CoA and providing energy while shortening the fatty acid chain. Acetyl-CoA is utilized in metabolic processes, including the synthesis of flavorful derivatives like aldehydes, alcohols, and esters. Enzymes such as acyl-CoA: alcohol acyltransferase convert acyl-CoA into esters, contributing fruity, floral, and aromatic notes. Microbial enzymes, including beta-oxidation enzymes, alcohol dehydrogenases, and acyltransferases, play a role in metabolizing fatty acids into these flavor compounds, enriching the overall sensory

experience of beverages [24-26].

2.4 Organic Acid Production

Microbial metabolism in fermentation plays a crucial role in organic acid production, including lactic acid, acetic acid, and citric acid [27]. Fermentative microorganisms utilize sugars and undergo glycolysis to form pyruvate, which enters the citric acid cycle, leading to the synthesis of organic acids [28]. Lactic acid is produced through lactic acid fermentation, involving the reduction of pyruvate using electrons and protons from NADH. Pyruvate can also be metabolized to produce citric acid [29]. Acetic acid is formed via alcoholic fermentation, and under oxygen-rich conditions, certain bacteria oxidize ethanol to generate acetic acid [30]. Citric acid is produced by specific yeast and bacteria through the citric acid cycle [31, 32]. The types and amounts of these organic acids influence acidity, tanginess, and flavor balance in beverages, altering the pH of the fermentation environment. Microorganisms regulate pH to optimize growth and metabolism [33].

3. Microbial Diversity in Traditional Indian Fermented Beverages

3.1 Strain-Level Diversity Within Microbial Species

Microbial species, such as *Saccharomyces cerevisiae* and *Lactobacillus plantarum*, exhibit diverse strains with distinct metabolic abilities, leading to genetic variations within a species [34, 35]. This diversity impacts fermentation, influencing biochemical and physiological traits, metabolic pathways, substrate processing, flavor creation, and growth rates [36]. Strains also differ in their ability to tolerate environmental stresses, affecting performance under varying conditions [37]. Yeast and bacterial strains contribute to diverse aroma and taste profiles in products like cheese and wine [38, 39]. Variations in esters, acids, and alcohols produced by different yeast strains influence beverage texture, mouthfeel, viscosity, and perceived smoothness [40]. Table 1 outlines microorganisms associated with various types of fermented beverages.

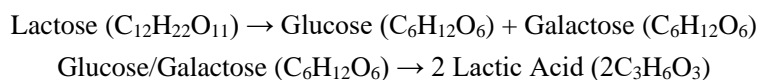
3.2 Synergistic Interactions Among Microbial Strains

Microbial communities are not isolated individuals but interact within the fermentation environment. The presence of diverse strains within a microbial community can lead to synergistic interactions [41]. Different strains can work together to break down complex substrates into simpler compounds [42]. One strain might produce enzymes that release nutrients for other strains. Some strains may produce metabolites that other strains can utilize as substrates [43]. This creates a symbiotic relationship, enhancing the overall efficiency of fermentation. Interactions among strains can result in the modification of flavor compounds [44], in milk and lupin [45], sunflower seed milk [46], and mixed dairy & plant-based yogurt [47]. Bacterial strains i.e., *Lactococcus lactis*, *Enterococcus faecalis*, *Lactiplantibacillus plantarum*, *Lactobacillus acidophilus*, *Lacticaseibacillus rhamnosus*, and *Pediococcus acidilactici* can modulate the pH of the environment through the production of lactic acid, influencing the growth of other strains and promoting a suitable fermentation environment. Synergistic interactions can promote community stability by preventing the dominance of a single strain. These interactions can also limit the growth of potential spoilage organisms through competition.

3.3 Ingredient Influence

3.3.1 Lassi

Yogurt, also known as curd, is both an ingredient and a source of beneficial microbes. Fermentation starts with a small amount of yogurt as a starter culture, which contains live bacteria, mainly *Lactobacillus delbrueckii subsp. bulgaricus* and *Streptococcus thermophilus* [48]. Starter cultures trigger lactic acid fermentation, where bacteria convert lactose into lactic acid. This involves breaking down lactose into glucose and galactose, and then converting them into lactic acid. This process gives lassi its tangy flavor and helps preserve it by lowering the pH.



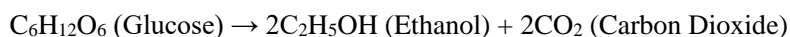
The primary starter cultures are *Lactobacillus delbrueckii subsp. bulgaricus*, *Lactobacillus acidophilus*, *Lactobacillus rhamnosus*, and *Streptococcus thermophilus*. Variations can include strains like *Lactobacillus rhamnosus* and *Bifidobacterium lactis*, known for health benefits like lowering blood pressure [49], increase in calcium content [50], improvement in blood sugar, total cholesterol and triglyceride levels [51]. Their incorporation into lassi could enhance its probiotic content [52, 53].

Table 1. Microbial Diversity Based on Beverage Types

Beverage	Origin	Fermentation Temperature	pH	Starter Cultures	Flavor Profile	Shelf Life
Lassi	North India	25-30°C	4.0-4.5	<i>Lactobacillus delbrueckii subsp. bulgaricus</i>	Creamy, tangy, often flavored with fruits	1-2 days (refrigerated)
Chhaang	Himalayan Region (Sikkim, Ladakh)	20-25°C	4.0-4.5	Various wild yeast and bacteria	Mildly alcoholic, slightly sour	2-3 days
Kanji	North India	Room temperature	3.5-4.5	Lactic acid bacteria, mustard seeds	Tangy, spicy, fermented mustard flavor	7-10 days
Toddy (Palm Wine)	South India (Kerala, Tamil Nadu)	25-35°C	3.0-4.0	Wild yeast, various bacteria	Mildly alcoholic, sweet, subtle fruity notes	24-48 hours
Feni	Goa	Ambient	4.0-4.5	Wild yeast, various bacteria	Distinctly fruity, cashew or coconut flavor	6 months to years
Apong	Assam	20-25°C	4.0-4.5	Wild yeast, various bacteria	Mildly alcoholic, rice-based, slightly sour	1-2 days
Kodo Ko Jaanr	Sikkim	Room temperature	3.5-4.5	Various wild yeast and bacteria	Tangy, millet-based, often flavored with herbs	2-3 days
Kallu (Toddy)	Andhra Pradesh, Telangana	25-35°C	4.0-4.5	Wild yeast, various bacteria	Mildly alcoholic, sweet, coconut undertones	24-48 hours
Handia	Jharkhand	Room temperature	4.0-4.5	Various wild yeast and bacteria	Earthy, rice-based, fermented taste	2-3 days

3.3.2 Chhang

Chhang is a traditional fermented beverage consumed in the Himalayan regions like Sikkim, Ladakh, and Himachal Pradesh. It is made from barley grains and water and sometimes includes herbs and flavoring agents. The fermentation process involves wild yeast and bacteria. Barley contains complex carbohydrates, primarily starch [54]. In the malting process, germinating barley activates enzymes (α -amylases, β -amylase) that convert starches into simpler sugars (maltose, glucose) for fermentation. The malted barley is mashed with hot water, activating the amylases and initiating the breakdown of starches into fermentable sugars, releasing maltose, glucose, and maltotriose [55]. Chhang fermentation uses wild yeast and lactic acid bacteria (LAB) from the environment. LAB like *Lactobacillus delbrueckii*, *Lactobacillus plantarum*, *Lactobacillus brevis*, *Leuconostoc mesenteroides*, and *Pediococcus* species give it a sour taste. Wild yeasts, such as *Saccharomyces cerevisiae*, initiate alcoholic fermentation [56]. *Saccharomyces cerevisiae* yeast ferment maltose and other sugars into ethanol through alcoholic fermentation. The chemical equation for alcoholic fermentation is:



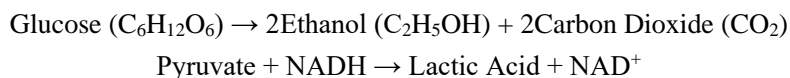
Yeast in Chhang fermentation converts sugars to ethanol and CO_2 , producing carbonation. LAB turns sugars into lactic acid, adding sourness and preserving the drink by lowering the pH to 2-3. Flavor compounds like esters, aldehydes, and phenols are produced during fermentation, contributing to the unique aroma and taste of Chhang. Esters add fruity/floral notes, and phenols can give spicy/smoky flavors. The CO_2 produced during fermentation causes effervescence and bubbles.

3.3.3 Kanji

Kanji is made primarily from black carrots and mustard seeds. The interaction between these ingredients, microorganisms, and the fermentation process contributes to the unique taste, aroma, and health benefits of kanji. Black carrots contain carbohydrates, particularly sugars like sucrose and glucose [57]. These sugars serve as the substrates for fermentation by lactic acid bacteria (LAB). LAB plays a pivotal role in kanji fermentation. LAB, particularly *Pediococcus acidilactici*, dominates the fermentation process [58]. The yeast used for fermentation is *Saccharomyces cerevisiae*, *Candida milleri* (*Saccharomyces bayanus*), and *Kloeckera apiculata* [59]. Black carrots have enzymes like amylases and invertases that simplify complex carbohydrates into sugars, aiding microbial fermentation. Mustard seeds have antimicrobial glucosinolates [60]. During the fermentation process, enzymes released by the LAB break down glucosinolates into pungent compounds known as isothiocyanates [61]. These compounds not only contribute to the spiciness of kanji but also exhibit antimicrobial properties that help preserve the beverage.

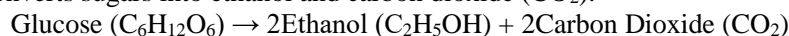
3.3.4 Toddy

Toddy is made from the sap of palm trees like coconut or date palms. The sap, rich in sugars such as sucrose, glucose, and fructose, undergoes fermentation. It contains microorganisms including yeasts i.e., *Saccharomyces chevalieri*, *Saccharomyces cerevisiae*, *Naumannella halotolerans*, and bacteria i.e., *Staphylococcus aureus* and *Pseudomonas aeruginosa* [62, 63]. Microorganisms in toddy convert sugars into alcohol, enhancing its flavor and aroma. Yeasts break down sugars via glycolysis to produce ethanol and CO_2 . Lactic Acid Bacteria (LAB) convert sugars into lactic acid through lactic acid fermentation.



3.3.5 Feni

Cashew apples, the swollen stalks of the cashew tree, are rich in sugars like glucose and fructose. These sugars are the substrate for microbial fermentation [64]. Wild yeast like *Saccharomyces cerevisiae*, *Torulasporea delbrueckii*, and *Hanseniaspora opuntiae* [65] and bacteria like *Lactobacillus delbrueckii subsp bulgaricus* and *Streptococcus salivarius subsp thermophilus* [66] present on the fruit initiate the fermentation process. Yeast metabolizes sugars through alcoholic fermentation. This process converts sugars into ethanol and carbon dioxide (CO_2).



Fermented cashew apple juice is distilled to extract alcohol. This process, based on boiling point differences, yields an alcohol-rich vapor that's condensed into feni. The distillation complexity arises from azeotropes, mixtures that boil at a constant temperature [67]. This led to challenges in obtaining high-purity alcohol.

3.3.6 Apong

Apong is a beverage made from rice, water, and sometimes herbs or flavors. The production process involves enzymatic

and microbial activities. Starch in rice undergoes amylolysis, breaking down into glucose and maltose [68]. Apong fermentation is carried out by a microbial consortium including wild yeast (*Bacillus velezensis*, *Pichia anomala*) [69, 70] and lactic acid bacteria (*Lactobacillus plantarum*, *Lactobacillus brevis*) [71, 72]. Wild yeast converts sugars to ethanol, while LAB produces lactic acid, contributing to the unique taste. Fermentation also produces flavor compounds like 3-methyl-1-butanol, 6-methylheptyl ester-2-propenoic acid, and various esters and organic acids, enhancing the aroma and flavor profile of Apong [71, 73].

3.3.7 Kodo Ko Jaanr

Kodo Ko Jaanr is a traditional fermented beverage from Sikkim, India, made from millets and rice. It undergoes a natural fermentation process. It is typically made from kodo millet (*Paspalum scrobiculatum*) and rice. The choice of ingredients significantly influences the fermentation process, microbial communities, and final characteristics of the beverage. These grains contain carbohydrates (starches, amylose, and amylopectin), proteins, and other nutrients like Phytic acid, polyphenols, tannins that serve as the energy and nutrient sources for the microorganisms during fermentation [74, 75]. LAB, such as *Lactobacillus plantarum*, *Pediococcus pentosaceus* and *Lactobacillus bifementans* are common inhabitants of Kodo Ko Jaanr fermentation [76]. These bacteria utilize sugars from the grains to produce lactic acid through a process known as heterofermentative lactic acid fermentation. Yeasts, including *Saccharomycopsis fibuligera*, *Pichia anomala* and *Saccharomyces cerevisiae* and *Candida glabrata* convert sugars into ethanol and carbon dioxide through alcoholic fermentation [77, 78]. The fermentation process breaks down complex carbohydrates and proteins into simpler forms, enhancing the availability of nutrients and their digestibility. This is facilitated by the enzymatic activities of microorganisms.

3.3.8 Handia

Handia, a traditional beverage from Jharkhand and Eastern India, is made from rice and indigenous herbs and roots. Rice starch is hydrolyzed into simpler sugars during mashing, aided by enzymes from rice and added herbs. These enzymes, including amylases, convert complex starch into fermentable sugars. Bakhar (ranu) is the source of fermentation organisms in handia production. Raw rice is pounded to a powder and used as the base ingredient for bakhar. The roots and barks of chosen plants, such as *Clerodendrum serratum*, *Dipteracanthus suffruticosus*, *Holarrhena pubescens*, *Madhuca indica*, *Smilax macrophylla*, *Woodfordia fruticosa*, *Xantolis tomentosa*, *Rauvolfia serpentine*, *Orthosiphon rubicundus* are added. The presence of herbs also adds unique flavors and bioactive compounds to the beverage. LAB includes different species of *Lactobacillus brevis* [79] and yeast includes *Saccharomyces cerevisiae* and *Saccharomyces pombe*, *Pichia kudriavzevii*, *Hanseniaspora guilliermondii* [80, 81].

4. Health and Functional Aspects of Microbes in Fermented Beverages

Many traditional Indian fermented beverages contain live beneficial microorganisms, such as lactic acid bacteria (LAB) and yeast. Specific strains of beneficial bacteria, such as *Lactobacillus* and *Bifidobacterium* species, are commonly found in fermented beverages. These strains include *Lactobacillus acidophilus*, *Lactobacillus plantarum*, *Lactobacillus rhamnosus*, *Bifidobacterium bifidum*, etc. These microorganisms can act as probiotics, promoting gut health by maintaining a balanced microbiota and supporting digestion [82]. Probiotics can enhance the expression of tight junction proteins (e.g., occludin, claudins), helping to strengthen the integrity of the intestinal barrier [83]. This prevents the passage of harmful substances and pathogens into the bloodstream. Probiotic bacteria can produce short-chain fatty acids (SCFAs) through the fermentation of dietary fiber and carbohydrates. SCFAs, including acetate ($C_2H_4O_2$), propionate ($C_3H_6O_2$), and butyrate ($C_4H_8O_2$), serve as an energy source for colonocytes and have anti-inflammatory effects [84]. Fermentation leads to the synthesis of bioactive compounds such as vitamins, antioxidants, and peptides that contribute to the health-promoting properties of the beverages [85]. Compounds like Vitamin B complex (example- B_{12} , folate), antimicrobial peptides, and antioxidants are synthesized by these microorganisms. These compounds can have anti-inflammatory, antimicrobial, and immune-modulating effects [86]. Fermentation can enhance the bioavailability of nutrients, making minerals and vitamins more accessible for absorption by the body. This can contribute to improved nutrient intake and overall health [87].

5. Conclusion

The complex microbial dynamics discovered in the research of Indian fermented beverages highlight the importance of microorganisms in influencing the flavours, fragrances, and health benefits of these traditional drinks. Amino acid metabolism, fatty acid metabolism, and organic acid synthesis all play important roles in the sensory complexity of beverages including Lassi, Chhang, Kanji, Toddy, Feni, Apong, Kodo Ko Jaanr, and Handia. Strain-level variability within microbial

species, as shown by *Saccharomyces cerevisiae*, *Lactobacillus* species, and others, provides broad metabolic capabilities, influencing the complex profiles of flavour components and textures. Synergistic interactions between microbial strains improve fermentation efficiency and contribute to community stability. Lassi incorporates specific *Lactobacillus* strains for probiotic benefits, Chhang relies on wild yeast and lactic acid bacteria for sourness, and Kanji uses a nuanced fermentation process involving black carrots, mustard seeds, and a diverse microbial consortium. The physiological and functional characteristics of microorganisms in these beverages demonstrate probiotics' ability to improve gut health, strengthen the intestinal barrier, and produce bioactive compounds such as short-chain fatty acids, antimicrobial peptides, and antioxidants. It becomes apparent that, while traditional methods provide a wealth of microbial variation, scaling up for industrial production requires rigorous optimization. It is critical to find a balance between maintaining microbial viability and implementing quality control methods. The combination of ancient knowledge and modern technologies offers an exciting opportunity for the creation of consistent and functional fermented beverages.

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