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Microbial Succession and Biochemical Interactions During Kombucha Fermentation

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Abstract:

Kombucha is a traditional fermented beverage obtained by fermenting sweetened tea using a symbiotic culture of bacteria and yeasts (SCOBY). The present study investigated the microbial succession pattern and major biochemical changes occurring during kombucha fermentation under laboratory conditions. The work focused on identifying dominant microbial groups at different fermentation stages, monitoring physicochemical parameters, and evaluating basic biochemical and antioxidant-related characteristics of the fermented beverage. Kombucha was produced using black tea, sucrose and a starter SCOBY, and fermentation was conducted for 7–14 days at ambient temperature. Microbial isolation was carried out using selective and non-selective culture media, followed by microscopic and staining techniques. Biochemical tests, pH variation, titratable acidity, and qualitative detection of proteins and carbohydrates were performed. The results confirmed the presence of acetic acid bacteria, yeasts and Gram-positive bacteria during fermentation, accompanied by a progressive decline in pH and development of a cellulose pellicle. The study highlights the dynamic microbial interactions responsible for acid production, biofilm formation and biochemical transformations, supporting the potential of kombucha as a functional fermented beverage.

Keywords: Kombucha, SCOBY, microbial succession, biochemical interactions, acetic acid bacteria, antioxidant activity

Introduction:

Kombucha is a slightly sweet and acidic fermented tea beverage produced through the metabolic activity of a symbiotic consortium of yeasts and bacteria, commonly known as SCOBY. During fermentation, a floating gelatinous biofilm composed mainly of microbial cellulose develops on the liquid surface. Traditionally, kombucha fermentation proceeds under aerobic conditions and involves a complex and dynamic microbial ecosystem. Historically, kombucha is believed to have originated in northeastern regions of **China** around 220 B.C., where it was consumed for its perceived health-promoting properties. Later, the beverage spread to East Asia and Europe through cultural exchange and trade. Renewed global interest in kombucha has emerged in recent decades due to its association with functional foods and natural antioxidant sources.

The SCOBY is frequently described as a “tea fungus” or “mushroom”; however, it is not a true fungus. It is a structured biofilm formed by acetic acid bacteria and yeasts embedded in extracellular cellulose. The microbial composition of the SCOBY is not constant and varies according to geographical origin, fermentation practices and substrate composition. Typically, yeasts initiate fermentation by converting sugars into ethanol and carbon dioxide, while acetic acid bacteria oxidise ethanol and sugars into organic acids and synthesize the characteristic cellulose pellicle. Kombucha fermentation represents a combined alcoholic, acetic and, in some cases, lactic fermentation process. This mixed metabolic activity leads to the formation of a broad range of metabolites including acetic acid, gluconic acid, glucuronic acid, ethanol, organic acids and secondary metabolites derived from tea polyphenols. Despite increasing consumer interest, limited experimental information is available regarding the temporal distribution of microorganisms and their biochemical roles during fermentation. Understanding microbial succession and biochemical interactions is essential for controlling fermentation kinetics, product consistency and safety, and for designing kombucha beverages with defined functional properties.

Chemical Composition of Kombucha:

The chemical composition of kombucha is influenced by several factors, including the origin of the inoculum, tea type, sugar concentration, fermentation time and temperature. Organic acids represent the main fermentation products, with acetic acid generally being the dominant acid formed during later stages of fermentation. Gluconic acid and glucuronic acid are also produced through the oxidative metabolism of sugars by acetic acid bacteria. Previous studies have demonstrated that the concentration of individual metabolites varies throughout fermentation and differs substantially between production batches. In addition to organic acids, kombucha contains residual sugars, ethanol, minerals, vitamins and modified tea polyphenols. Among these compounds, glucuronic acid is frequently highlighted due to its potential role in detoxification processes and health-related claims.

Microbial Composition of Kombucha:

Kombucha fermentation is considered a natural or back-slopped fermentation, as a portion of a previous successful fermentation is used to initiate a new batch. The dominant microbial groups involved include:

Yeasts, responsible for sugar hydrolysis and ethanol production,

Acetic acid bacteria (AAB), responsible for oxidation of ethanol and sugars and for cellulose synthesis,

Lactic acid bacteria (LAB), which may be present in some fermentations but are not consistently detected.

Filamentous fungi may occasionally appear but are regarded as spoilage organisms and are undesirable due to potential mycotoxin production. At the beginning of fermentation, the medium is aerobic and rich in sucrose. As fermentation progresses, oxygen availability gradually decreases due to microbial respiration and the formation of a thick cellulose pellicle. Accumulation of organic acids results in a continuous decrease in pH. Towards the later stages of fermentation, a highly acidic and nutritionally depleted environment is established, which strongly influences microbial survival and activity.

Yeasts in Kombucha Fermentation:

Yeasts play a key role in initiating kombucha fermentation. They hydrolyse sucrose into glucose and fructose and convert these monosaccharides into ethanol and carbon dioxide. Yeasts are facultative anaerobes and remain metabolically active under both aerobic and oxygen-limited conditions. In addition to ethanol, yeasts may produce glycerol and small amounts of acetic acid as part of redox balancing mechanisms.Their metabolic activity provides substrates such as ethanol and monosaccharides that are subsequently utilised by acetic acid bacteria, establishing a tightly linked metabolic network.tuning strategies on the EmpatheticDialogues dataset further enhance empathetic response modeling (Vu et al., 2025).

Acetic Acid Bacteria in Kombucha Fermentation:

Acetic acid bacteria are Gram-negative, obligately aerobic microorganisms belonging to the family *Acetobacteraceae*. They oxidise ethanol to acetic acid and convert glucose into gluconic and glucuronic acids via membrane-bound dehydrogenases. A distinctive feature of several kombucha-associated acetic acid bacteria is their ability to synthesise extracellular cellulose from sugars and alcohols. This cellulose forms the characteristic pellicle observed during fermentation. The production of microbial cellulose is affected by substrate type, sugar concentration and environmental pH. Furthermore, some species produce other extracellular polysaccharides such as dextrans, levans and acetan-type polymers, which contribute to biofilm architecture and microbial stability.

Lactic Acid Bacteria:

Lactic acid bacteria are inconsistently detected in kombucha fermentations. When present, they are usually acid-tolerant species and occur in relatively low abundance. LAB are Gram-positive, non-sporulating bacteria and are generally recognised as safe. Their contribution to kombucha fermentation is minor compared to yeasts and acetic acid bacteria, although they may influence flavour development and microbial stability in certain fermentations.

Microbial Interactions During Kombucha Fermentation:

Kombucha fermentation is governed by complex microbial interactions. Yeasts provide fermentable substrates and ethanol for acetic acid bacteria, while bacteria create an acidic environment that shapes yeast survival and metabolism. Autolysis of yeast cells releases vitamins and growth factors that stimulate bacterial growth. Microbial interactions can be commensalistic or amensalistic. Metabolic products of one group may stimulate or inhibit the growth of others. Sucrose hydrolysis, ethanol production, acetic acid formation and synthesis of gluconic and glucuronic acids occur in parallel, leading to two major fermentation outputs: the fermented tea and the cellulose biofilm. Understanding the timing and metabolic roles of individual microorganisms is essential for developing controlled or “designer” kombucha fermentations.

Factors Affecting Kombucha Fermentation:

Substrate: Kombucha is traditionally produced from sweetened black or green tea. Alternative substrates have also been explored, leading to variations in fermentation behaviour and metabolite profiles.

Fermentation time: Fermentation typically lasts between 7 and 21 days. Antioxidant activity and organic acid concentration generally increase with fermentation time. However, prolonged fermentation can result in excessive acidity and undesirable sensory characteristics. Regulatory guidance for home and commercial brewing is often aligned with food safety recommendations issued by organisations such as the **Food and Drug Administration**.

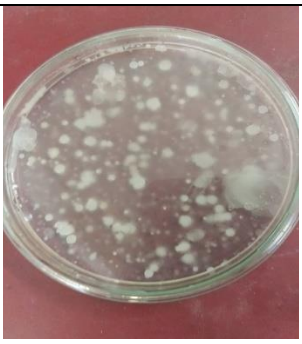
Temperature: Kombucha fermentation commonly occurs between 22 °C and 30 °C. Stable temperature supports optimal microbial growth and enzyme activity and influences polyphenol transformation and antioxidant capacity.

pH is a critical parameter controlling microbial growth and biochemical reactions. During fermentation, pH typically decreases to values between 3 and 4. Excessively low pH may negatively affect sensory quality and safety.

Kombucha as a Source of Antioxidants: Kombucha contains tea-derived polyphenols, ascorbic acid and fermentation-derived metabolites that contribute to its antioxidant activity. Fermentation enhances free radical scavenging capacity by modifying the structure of tea polyphenols and generating low-molecular-weight antioxidant compounds. Several experimental studies have demonstrated increased antioxidant activity during fermentation when compared to unfermented tea.

Detection of proteins and carbohydrate: Qualitative chemical tests were performed to detect the presence of proteins and reducing carbohydrates in fermented tea.

Sugar fermentation test: Isolates were inoculated into peptone water containing individual sugars (glucose, sucrose, lactose, fructose and mannitol) with phenol red indicator and Durham tubes. Acid and gas production were recorded after incubation at 37 °C.

	
Fig 1 : Sugar fermentation test	Fig 2 : lactic acid bacteria on Nutrient Agar

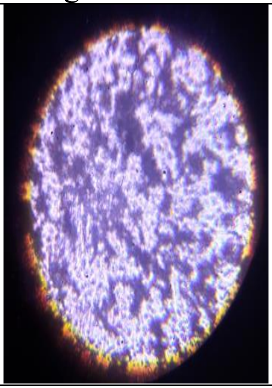
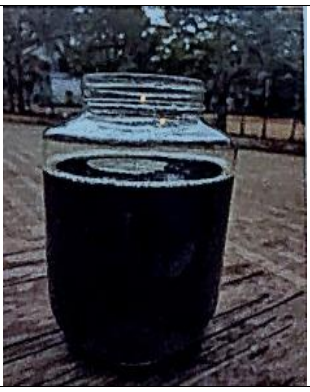

	

Fig 3: Gram Positive Baccilus	Fig 4: Kombucha Fermentation

Results & Discussion:

- The fermentation temperature ranged between 22 °C and 30 °C.
- Gram-negative bacteria indicative of acetic acid bacteria and Gram-positive bacteria were observed.
- Formation of a daughter SCOBY was clearly visible.
- pH values decreased from initial values to between 3 and 5 during 14 days of fermentation.
- Protein and carbohydrate tests were positive, indicating the presence of these biomolecules in kombucha.

Sugar fermentation tests showed acid production, confirming active carbohydrate metabolism by isolated microorganisms. the present study confirms that kombucha fermentation is driven by a dynamic and interactive microbial community dominated by yeasts and acetic acid bacteria. The observed decline in pH and formation of a cellulose pellicle are consistent with progressive organic acid production and bacterial cellulose synthesis.the coexistence of multiple microbial groups enables sequential and parallel metabolic pathways, leading to efficient substrate utilisation and production of diverse metabolites. Qualitative biochemical analyses indicated the presence of proteins and carbohydrates, reflecting both microbial biomass and metabolic by-products. The microbial succession pattern observed during fermentation highlights the importance of oxygen availability, sugar concentration and acid tolerance in shaping community structure. These findings support the concept that controlling fermentation parameters can be used to modulate kombucha composition and functional properties.

Conclusion:

Although kombucha is widely consumed, its microbial dynamics and biochemical mechanisms remain only partially understood. This study demonstrates that kombucha fermentation involves complex interactions between yeasts and acetic acid bacteria that govern acid production, biofilm formation and biochemical transformations. Further research using molecular tools and quantitative metabolomics is required to elucidate precise metabolic pathways and to establish scientifically validated functional claims.

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