

# Preparation of kombucha tea review on: Different kinds of microorganism's used for development of the kombucha.

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**ABSTRACT** –Kombucha is a refreshing probiotic beverage like an apple cider, slightly acidic in nature due to combination of the bacteria and yeast during fermentation. It is provided the beneficial effects to human being: lower-down the cholesterol level in blood, effects on hypertension, boost the immune system and prevent various types of cancers. Main objective of this review paper is to check the functional properties and potentials of different kinds of the microorganisms used for the different types of kombucha.

**Key points** – Kombucha production, Lactic acid bacteria, Acetic acid bacteria, Culture activation.

## I. INTRODUCTION

Kombucha is a potential source of probiotic beverage. It's also known as functional food (Jayawardena 2015; Hasler 2009). Its slightly acidic and refreshing beverage made up from the Symbiotic Culture of the Bacteria and Yeast (Jayabalan, 2018). It produce a flavour like an apple cider after the tea fungus added in the tea broth specially made in home (Jayabalan, 2014). GT's Living Foods was the first started selling bottled kombucha beverage in 1995 to Los Angeles Health Food Store, Erewhon. It is known to be the first commercial retailing of kombucha in the modern world (Dutta and Paul, 2019). It produces a specific flavour depend upon the how long its left to ferment process, type of tea used and addition of the of the other ingredients like herbs, fruits etc. Starter culture added in the sweetened tea at the time of fermentation process start. Bacteria and yeasts present in culture it helps in breakdown of tea sugars and converted into alcohol, carbon dioxide and acids (Maulina, 2017). SCOBY form a fibrous like structure during the fermentation process start after the inoculation of starter culture with the help of suitable environment and concentration of the substrates. Substrates are used for the preparation of kombucha are carbon source i.e. sugar and acetic acid bacteria produce a fibrous

network used as a secondary metabolites of fermentation process (Jayabalan 2014).

Kombucha is composed of two parts one is floating cellulose pellicle layer and the sour liquid broth (Chen and Liu, 2000). Microbial composition depends upon the starter culture origin. Cellulose structure has a speciality i.e. water absorption capacity its about 99% moisture content. In aerobic condition fermentation process complete in 7-14 days. At the time of fermentation process *A. xylinum* produces a slim cellulose film this film attached with group of bacteria and yeast. It is a fungus - like mixture of cellulose and microorganisms (Sreeramulu, 2000). Reason behind this due its crystalline nature and fibrous nature, it having length about 25-100 nm and thickness about 100 time thinner than fibrous plant cellulose (Lee 2014).

Kombucha generally provides about 30 calories, five grams of carbohydrates, five milligrams of sodium, and negligible fat. Most kombucha tea also contains acetic acid, lactic acid, and good bacteria. Kombucha beverage contains variety of compounds which is produced by the starter culture SCOBY. Compounds contains organic acids, mainly acetic, gluconic, and glucuronic acid (GlcUA), although citric, L-lactic, malic, tartaric, malonic, oxalic, succinic, pyruvic, and usnic acids may also be found (Malbasa 2002). Kombucha provides a beneficial effect due to the presence of tea polyphenols, gluconic acid, glucuronic acid, lactic acid, vitamins, amino acids, antibiotics and lot of micronutrients produced during fermentation (Vijayaraghavan, 2000). These components are acetic acid (mildly antibacterial), butyric acid, B-vitamins (Aleksandra, 2007), alcohol, gluconic acid, lactic acid, malic acid, oxalic acid and usnic acid.

Commonly local SCOBY contains at least five yeast strains: *Saccharomyces ludwigii*, *Saccharomyces cerevisiae*, *Saccharomyces bisporus*, *Torulopsis sp.* and *Zygosaccharomyces*

sp., This study conducted by Markov and Cvetkovic in 2001. Some Acetic acid bacteria are, *Acetobacter xylinum*, *Acetobacter xylinoides* or *Bacterium gluconicum* and Yeasts are *Saccharomyces cerevisiae*,

*Zygosaccharomyces bailii*, *Schizosaccharomyces pombe*, *Saccharomyces ludwigii*, *Zygosaccharomyces rouxii*, *Torulaspora delbrueckii*, *Brettanomyces bruxellensis*, *Brettanomyces lambicus*, *Brettanomyces custersii*, *Candida sp.* or *Pichia membranaefaciens* (Chen and Liu 2000; Teoh 2004). Osmophilic yeast and bacterias having scientific name is *Medusomyces gisevii*.

Regular consumption of the Kombucha, prevents degradation of polyunsaturated fatty acid in the human body, due to its glucuronic acid content that takes part in glucuronidation, increasing polyphenol bioavailability, that's helps to neutralize free radical to promote lipid peroxidation (Jayabalan, 2008). It increases the level of intestinal microbiota, reduce level of nutrient absorption and/or reduce the risk of chronic non-communicable diseases (Crowe and Francis, 2013). Green and black tea extracts can be reduced blood sugar level and prevent absorption of sugar by another manner (Zeyuan and Bingying 1998). Decrease the level of cholesterol and blood pressure, improve immune system and gastrointestinal function (Leal, Suarez, and Jayabalan, 2018). Kombucha provides the therapeutic benefit after consumption result gives weight loss, cure the cancer and AIDS. Free radical scavenging and activities of antioxidant it has been recently reported by Jayabalan, 2008 and malabasa 2011.

## II. DISCUSSION

### ➤ Production of kombucha -

The amounts of tea, sugar, and tea fungus differ in different places according to their requirement. The standard procedure for the kombucha are followed: 1000 ml Tap water for boiling purpose and add 50 g sugar at the time of boiling water with continuous stirring (Greenwalt and a Ledford, 2000; Nummer, 2013; Jayawardena, 2015; Torie 2018). Add 5g of tea leaves or powder in boiled water, green tea was used by Farnwort, 2000 this scientist as well as used black tea and alternative option are tea bags (Morshedi, 2010). The After extraction of flavour and colour powder or leaves removed with the help of filtration medium (Jayabalan, 2014). Tea broth cooled at room temperature approximate 20 °C then add Erlenmeyer flask was inoculated with 5% culture, its obtained from the culture collection of CBB, Fuzhou University, which is composed of

*Saccharomyces cerevisiae* Meyen ex Hansen (108 CFU/mL), *Gluconacetobacter sp.* (108 CFU/mL), and *Lactobacillus plantarum* (108 CFU/mL), with the ratio of 1:1:1 and reported to be optimal strains for kombucha fermentation this study conducted by (Fu, 2014). Tea fungus that contains medicinal and nutritional value (Loncar and others 2000). Starter culture added in tea broth it helps to reduce the pH of solution and inhibit the undesirable growth of microorganism. Sterile instruments used at the time of kombucha production for avoid the cross-contamination of the foreign matter (Ledford, 2000). Jar that contains tea broth it put for the fermentation process in suitable environment and avoid the drosophila fruit flies by covering of paper towel to jar. Temperature for incubation to kombucha at 20 -22°C its suitable for the fungus activity to form a fibrous structure in broth in fermentation period. In next few days mother culture forms a newly culture i.e. daughter culture it will be star to float on surface and clear thin gel like membrane across the present area. After 10 to 14 days, a new tea fungus will have developed on the surface of the tea as a disc of 2-cm thickness covering the whole diameter of the beaker. The mother culture remains its original volume and sink at the bottom site of jar. kombucha beverage is rich in organic acids: acetic acid, gluconic acid and vitamins as well as polyphenols (Jaybalan, 2014).

This study was conducted by Francesca and Galiano in 2018 about the kombucha prepared from consortium of symbiotic microorganisms using two traditional substrates, green and black teas, and a never tested substrate, the tea obtained from rooibos (*Aspalathus linearis*) leaves. (Kanuric, 2012) in this study analysed two type of milk used for production of the kombucha that contain low fat (0.9%) and reduced fat (2.2%) with normal kombucha culture it's a newly produced product. In 2018 scientist Chunhai Tu and Sijie Tang conducted study on liquid soya whey extracted from soya protein and tofu its fermented product from manufactured industry investigate the changes of metabolic process, also the improvement of antimicrobial activity and antioxidant capacity at the time of fermentation. In 2019 scientist Zubaidah, Ifadah and Afgani was studied on changes in different flavours extracted from the various cultivars of snake fruit at the time of fermentation of kombucha. That's fruits contain salak Suwaru (Malang, Jawa Timur), salak Pondoh (Sleman, Yogyakarta), salak Madura (Bangkalan, Madura) and salak Bali (Karangasem, Bali) its Indonesian fruits. Herbal used for extraction flavour of thyme, lemon verbena, rosemary, fennel,

and pepper mint added in tea broth this study conducted by Battikh in 2012.

#### ➤ Used Microorganisms -

##### 1. Lactic acid bacteria–

Kombucha culture contains a large proportion of lactic acid bacteria, which primarily makes it a suitable inoculum for milk fermentation (Malbasa 2009). Lactic acid bacteria are used in production of the fermented products at industrial level as well as improve flavour in products like yoghurt, cheese, kefir and cabbage pickle it concluded by Dufresne and Farnworth in 2000. These provides health benefits include stimulation of the human immune system and antimicrobial activity. Adding LAB to any food products it can improve biological activities such as food preservation, wheat bread and cocoa fermentation, and D-Saccharic acid 1,4 Lactone (DSL) production in kombucha this study conducted by Nguyen in 2015. Now a day production lactic acid bacterium increased by modern and traditional way. Presently lactic acid bacteria reported production from kombucha culture up to 30% its stated by Marsh 2014; Yang 2010. Newly research conducted Farnworth and Mainville in 2003 its stated that lactic acid bacteria interaction with kefir its probiotic beverage made up from kefir grains with yeast and bacteria fermentation starter culture and acetic acid bacteria from kombucha with respect to growth rate, biomass and secondary metabolites.

The positive stimulation in growth rate, bio-mass and secondary metabolites provided by the co-cultures of *Lactobacillus* spp. in kombucha, the human health benefits. Therefore, we focused on the significance of *Lactobacillus* spp. in mixed culture kombucha in enhancing its three important biological functions: glucuronic acid (GlcUA) production, antibacterial activity and antioxidant ability. This study conducted by yang in 2010, they isolate *Lactobacillus* strains from kefir, kombucha and pickle cabbage. Culture of this strains obtained from the sweetened black tea it used as kombucha substrate. They produce higher quantity of the healthy kombucha tea from the *Lactobacillus* species. And combination of the *Lactobacillus* species with acetic acid bacteria in kombucha it improves the D-Saccharic acid 1,4 Lactone concentration which determine the level of glucuronic acid in a glucuronate pathway. Strains of genus *Acetobacter*, *Lactobacillus* according to the sequence-based analyses stated that by Tang 2018. Soymilk is suitable substrate for the growth of lactic acid bacteria and kefir culture sated by Garcia in 2014.

One role of acetic acid bacteria in *Lactobacillus* its increase cellulose production by glucon- *acetobacter* in co-culture (Seto, 2006). Recently, lactic acid bacteria shown to support to growth of *Gluconacetobacter* in kombucha (Yang 2010). In milk substitute kombucha exhibit protein gelatinization at higher pH than commercial yogurt, better texture provide to the probiotic beverage in milk contains (Hrnjez, 2014).

##### Acetic acid bacteria–

Acetic acid is the organic compound responsible for the acetic flavour and taste produces like vinegar. Acetic name comes from Latin word called as *acetum*, which means vinegar (Bramforth, 2014). Different studies conducted by Balentine, in 1997 they conclude that, microbial commodity that contains acetic acid bacteria present in tea fungus is *Acetobacter xylinum* and other species are *Acetobacter xylinoides*, *Bacterium gluconicum* (Reiss, 1994), *Acetobacter pasteurianus*, and *Acetobacter aceti* (Liu, 1996). Recently identified new bacterial strain are *Gluconacetobacter* sp. A4 its investigated by (Wang, 2010), *Gluconobacter oxydans* (Greenwalt, 2000; Kurtzman, 2001), *Acetobacter nitrogenifigens* sp. nov., and *Gluconacetobacter kombuchae* sp. nov. (Dutta and Gachhui, 2006, 2007), and *Komagataeibacter* (Chakravorty, 2016). After 7 days of fermentation, *Gluconacetobacter* species produced more than 90% population of bacteria and *Acetobacter* increases population of bacteria about 3-5% after completion of 21 days of fermentation (Danilo Ercolini, 2017). Initial load of acidic acid bacteria on media are 10<sup>5</sup> -10<sup>6</sup> CFU/ml. After 7 days completion of fermentation process AAB increased about 1.5 log. Acidity decreased from 3.5 to 2.5 finished fermentation process (Jayabalan, in 2007; 2014).

Main microbes present in tea fungus are acetic acid bacteria: *Acetobacter xylinum*, *Acetobacter aceti*, *Acetobacter pasteurianus*, *Gluconobacter oxydans* (Greenwalt, 2000) and yeasts: *Saccharomyces* sp., *Zygosaccharomyces* sp., *Torulopsis* sp., *Pichia* sp., *Brettanomyces* sp. this microbe responsible for the production of kombucha. **Table 1.** Most important acetic acid bacteria species found in kombucha were identified as *Acetobacter xylinoides*, *A. pasteurianus*, *A. xylinum*, *A. aceti*, and *Bacterium gluconicum* (Malbasa, 2008 and 2011; Greenwalt, 2000; Chu and Chen, 2006). Moreover, the genus *Gluconacetobacter* has recently been subdivided into three genera: *Nguyenibacter*, *Komagataeibacter* and *Gluconacetobacter* (Vu 2013; Yamada, 2012). Acidic acid bacteria and osmophilic yeasts

are the dominant microorganisms during Kombucha 64 fermentation. They produce a cellulosic pellicle tea fungus floating on the fermented liquid, where 65 they remain embedded and that can be transferred to propagate the inoculum (Francesca De Filippis 2017). *Acetobacter* (formerly *Bacterium*) *xylinum* is the most commonly mentioned bacterium from the Kombucha consortium.

Enumeration of acetic acid bacteria and yeast by using two media that's are PDA (potato dextrose agar) and GYCA media (Chen and Liu, 2000). When glucose and fructose acts as a metabolite they produce ethanol and acetic acid by using microorganism (Spedding, 2015). In particular, authors reported the dominant presence of *Komagataeibacter* spp. and *Acetobacter* spp. and less than 1% of *Lactobacillus* spp., among bacteria; *Zygosaccharomyces* spp. and *Brettanomyces* spp. are the most abundant yeasts (Marsh and Sullivan 2014). Regarding therapeutic effects, functionality and safety aspects of kombucha consumption reported that *Acetobacter xylinoides*, *A. pausterianus*, *A. xylinum*, *A. aceti* and *Bacterium gluconicum* are the most common species of bacteria found in kombucha cultures. Regarding therapeutic effects, functionality and safety aspects of kombucha consumption reported that *Acetobacter xylinoides*, *A. pausterianus*, *A. xylinum*, *A. aceti* and *Bacterium gluconicum* are

the most common species of bacteria found in kombucha cultures (Viduranga, 2015).

Modification in temperature at the time of incubation process acetic acid bacteria will increase the concentration of organic acid, which is helpful to health promoting effect at the time of daily consumption of kombucha beverage (Jayabalan 2007 and 2014). Genus of *ssAcetobacter* was consistently found in Irish- sourced kombucha only where proportions were highest in the pellicle (1.9%) but represented <1% of the bacterial population in the fermentation. Reclassification of *A. xylinum* to *G. xylinus* according to the high level of sequence homology (Yamada 1997), a species considered to be the dominating bacterium and principal contributor of bacterial cellulose in kombucha (Mikkelsen 2009, Strap 2011).

**Other species –**

Recently Dutta and Gachhui in 2007 identified new species other than lactic acid bacteria and acetic acid bacteria. *Komagataeibacter* and *Gluconacetobacter* species identified in kombucha including *Komagataeibacter kombuchae*, *Gluconacetobacter SpA4* (Yang 2010), *Glucoacetobacter sacchari* (Trovatti 2011), *Acetobacter aceti* (El-salam 2012), *Acetobacter nitro-genificans* (Dutta and Gachhui, 2007), *Acetobacter pasteurianus* (Chen and Liu, 2000; Liu 1996) and *Acetobacter liquefaciens* (Zhang 2011).

<i>Acetobacter xylinum</i>	Kwanashie1990; Mollenda1928; Roussin 1996; Sievers 1995
<i>Acetobacter aceti</i>	C. Liao. 1996
<i>Acetobacter pasteurianus</i>	C. Liao. 1996
<i>Gluconobacter</i>	C. Liao. 1996
<b>Yeasts</b>	
<i>Brettanomyces</i>	Mayser,1995
<i>Brettanomyces bruxellensis</i>	C. Liao. 1996
<i>Brettanomyces intermedius</i>	Herrera, 1989
<i>Candida</i>	Jankovic, 1994
<i>Candida famata</i>	Herrera, 1989
<i>Mycoderma</i>	Jankovic, 1994
<i>Mycotorula</i>	Jankovic, 1994
<i>Pichia</i>	Hesseltine, 1965; Jankovic, 1994
<i>Pichia membranaefaciens</i>	Herrera, 1989
<i>Saccharomyces</i>	Kwanashie1990; Mayser,1995
<i>Saccharomyces cerevisiae subsp. aceti</i>	Herrera, 1989
<i>Saccharomyces cerevisiae subsp. cerevisiae</i>	Herrera, 1989; C. Liao. 1996
<i>Torula</i>	Jankovic, 1994
<i>Torulasporea delbrueckii</i>	Herrera, 1989
<i>Torulopsis</i>	Jankovic, 1994
<i>Zygosaccharomyces</i>	Hesseltine 1965; Mayser,1995; Roussin 1996; Sievers 1995
<i>Zygosaccharomyces bailii</i>	Herrera, 1989; C. Liao. 1996
<i>Zygosaccharomyces rouzii</i>	Herrera, 1989

**Table 1** List of Microbes

### Activity of fungus culture–

Acetic acid bacteria produce “tea fungus” or cellulose floating network on the surface of tea broth, cell mass attached with bacteria and yeast (Sreeramulu 2000). Osmophilic yeast and bacteria responsible for the development of semi-solid structure known as tea fungus. It having scientific name: *Medusomyces gisevi*. There are many types of bacteria used for the kombucha fungus that’s are *Aerobacter*, *Agrobacterium*, *Azotobacter*, *Rhizobium*, *Salmonella* and *Gluconacetobacter* (Villarreal-Soto, 2018; Mohite and Patil, 2014). For development of the kombucha two stage of fermentation occurred in first stage increase the population of cellulose producing bacteria by consumption of dissolved oxygen in tea broth. Second stage of fermentation is sucrose is

converted into ethanol and acetic acid with the help of beneficial microbes and produce carbon dioxide. Symbiotic culture of bacteria and yeast type of activity in tea broth shown in Figure 1 (May, 2019). During this time, the microorganism synthesizes certain amount of fibrous network in the liquid medium. As fermentation time increases, then the membrane thickness is increased by the generation of new layers on the surface, forming a suspended structure in the culture medium. The development of the biofilm along with hydrogen and C-H bonding will continue through all fermentation, its synthesis will reach its limit when it grows downward entrapping all bacteria, which then will become inactive due to insufficient oxygen supply (Esa, Tasirin and Rahman, 2014).

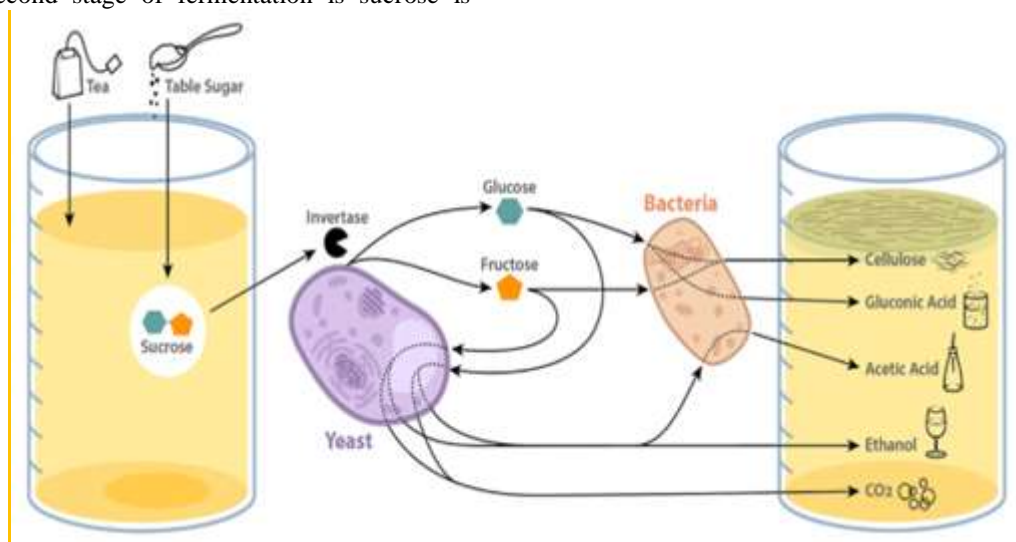


Figure 1 Activity of yeast and bacteria in kombucha

Tea fungus contains no spores present, it reproduces on basis of sprouting phenomenon. Ability of tea fungus it produces fibrous structure with the help of mother culture that’s added at the time of fermentation. Mother culture float at top side when they added then its slowly immersed in tea broth. Immersion time it produces small threat like structures in broth medium (Dutta and Paul, 2019). Cellulose produced during the fermentation of *A. xylinum* as a thin film in 7- 12 of fermentation days. Symbiotic culture is a powerful inhibition for potential contaminating bacteria (Jayabalan 2010). Cellulose created on the stable medium of broth. The fermented tea is produced by the action of a floating microbial colony consisting of aerobic bacteria and yeasts. The colony’s appearance often resembles a surface mushroom but is actually a floating cellulose colony produced during microbial growth. Production of the floating colony facilitates aeration for the aerobic

microorganisms (Ledford, 2000; Asai, 1968). With every batch of Kombucha a new film is formed, and this new film is the colony that can be used to make subsequent batches of Kombucha. The initial weight of SCOBY before to inclusion in the green tea substrate was 2.3 g, at 1-day fermentation of cellulose weight to 2.5g, day 2.7g, 5th day 5.8g, 7th day 8.7g, day 9th 10.3g and 11th day 11.9g (Health, 2017). As per fermentation time increases it increases weight of floating cellulose. Total sugar present in the kombucha its completely breakdown in to monosaccharides its easy to digest for microorganism (Maulina Nurikasari 2017).

### III. CONCLUSION

Kombucha drink consumed whole world as a refreshing beverage and helps to improve medicinal value in human being. Different kinds of kombucha prepared from the different sources i.e.

fruits, vegetables extract, sugar industry by-product i.e. molasses etc. It is made up from the different kind of sugary sources by traditional as well as modern way. It is a good source fibre obtained from the activity of themicrobes during fermentation process. Flavours development occurred due to the activity of microbes at the time of fermentation process. Microbes play important role in fermentation, without microbes don't occurred fermentation process. This microorganism helps to improve the production for required species. Microbes play important role due to antimicrobial property helps to health benefits for human.

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