Bacterial Cellulose of Kombucha Mushroom Tea

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Abstract: Kombucha is composed of yeast and acetic acid bacteria especially, *Acetobacter Xylinum* which forms a cellulose pellicle on tea broth. The yield and properties of cellulose produced were investigated in this study; the tea broth was fermented naturally for 14 days in the presence of different amounts of black tea and sucrose as nitrogen and carbon sources. 8.7g/L black tea produced highest weight of bacterial cellulose (55.46g/L) and 100g/L sucrose also exhibited high amount of pellicle (63.58g/L). The bacterial cellulose production increased with the increase of surface area and depth of the broth. Temperature was essential factor on growth, where the pellicle was formed at range (20°C - 50°C) and higher temperature over 50°C depressed the bacterial cellulose formation. Bacterial pellicle was separated from kombucha tea preparation and purified; the pellicle was reticulated structure consisting of fine cellulose threads, where it could be detected by carrying out transmission, scanning electron microscope and FT.IR spectroscopy.

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1. Introduction

Kombucha is healthy beverage produced by the fermentation of sweetened black tea by a symbiotic relationship between acetic acid bacteria and yeasts (Durfresne and farnworth, 2000; teoh *et al*., 2004 ; Malbasa *et al*., 2011). The microbial composition has well studied, the main acetic acid bacteria include *A. acetic, A. xylinum* and others (liu *et al*., 1996; Balentinc, 1997; Teoh *et al.,* 2004), yeasts break sucrose into glucose and fructose (Jonas and farah, 1998), glucose subunit that form the cellulose microfibril are excreted through pores in the cell wall of the acetic acid bacteria as *A. xylinum* (son *et al*., 2003; keshk and sameshima, 2005; Mikkelsen *et al*., 2009; Jung *et al*., 2010).

Cellulose is the most abundant bio-polymer and has been widely accepted as the excipient in pharmaceutical and industries. It has found its utility in tablet formation (Yu and Atalla , 1996).

The molecular formula of bacterial cellulose $(C_6H_{10}O_5)^n$ is the same as that of plant cellulose, but their physical and chemical are different (Yoshinaga *et al*., 1997; Rezaee *et al*., 2008) where the bacterial cellulose has not hemicelloulse and lignin .

The main objective of the present study was to investigate the microbial cellulose and affected it by some factor as nitrogen and carbon sources, surface area, depth and temperature during fermentation of a natural broth.

The bacterial cellulose was determined by scanning electron microscope and FT.IR spectroscopy. Also the symbiosis relationship between Acetic acid bacteria and yeasts of kombusha tea were examined by transmission and scanning electron microscopy.

2. Material and Methods

2.1. Microorganism

Kombucha Mushroom tea was obtained from (soheir. S. Abd El-Salam). The tea sample was activated every 2 weeks by the procedure described by (Chen and liu, 2000).

2.2. Culture media and cultivation

Substrate for kombucha fermentation was prepared by adding 70g/L of commercial sucrose to tap water and after boiling 5g/L of dry black tea was added. The tea leaves were steeped for 15minutes and removed by filtration, after cooling to about 30°C and it incubated under aerobic conditions at 28°C.

2.3. Effect of different concentrations of black tea on the bacterial cellulose production

Sweetened tea broth was prepared by different concentrations of black tea 4.4, 8.7, 17, 35, 70 g/L. these concentrations of tea were chosen on the wet weight of cellulose produced in g/L.

2.4. Effect of various concentrations of sucrose on the bacterial cellulose formation

Different amounts of sucrose as carbon source were tested on wet bacterial cellulose pellicle formation in g/L, these amounts were 70, 100, 130, 160 and 190g/L.

The bacterial cellulose produced in different concentrations of tea or sucrose was weighed according to the following equation:

- weight of bacterial cellulose (g/L) = total weight of beaker containing cellulose + tea broth – weight of beaker only + tea broth
- the yield of the cellulose:-

yield % = wet weight of bacterial cellulose (g/L) ÷ tea or sucrose concentration (g/L)

2.5. Effect of surface area and depth of culture medium on bacterial cellulose production

Tea broth with 100g/L sucrose and 8.7g/L black tea was prepared in different container's size. The bacterial cellulose produced was weighed after 14 days of the fermentation. Then, the effect of surface area and depth of the culture medium on cellulose production was examined.

2.6. Effect of different temperature on bacterial cellulose production

Tea broth was prepared by adding 100g/L sucrose and 8.7g/L black tea and incubated at different temperature (20^{o}C , 30^{o}C, 40^{o}C, 50^{o}C, 60^{o}C, 70^{o}C and 80^{o}C) and tested their effect on bacterial cellulose formation.

2.7. Purification of bacterial cellulose

After cultivation, the bacterial cellulose was formed at the interface of air; thickened pellicle sinks to the bottom of fermentation vessel. Bacterial pellicle isolated from culture media was washed with distilled water and cut into small pieces, then transferred to flask containing 4% NaoH (W/V) and boiled at 100 $\pm$ 5^{o}C for 20 min in order to remove bacterial cells. The alkali treated pellicle was washed with distilled water (Surma – Slusarska *et al.,* 2008) and the bacterial cellulose was bleached with 10% of hydrogen peroxide in order to achieve maximum brightness (Gupta and Johnson, 1991). Bleached product was washed with distilled water and filtered then drying in an oven at 40^{o}C overnight. The dried product was size reduced in mixer and stored in closed container (Netravali, 2010).

2.8. Electron microscope

2.8.1. Transmission

The kombucha is known a powerful symbiotic relationship between bacteria and yeast has been investigated by transmission electron microscopy (Jeol Jem - 1010) put a drop of the sample on grid coated carbon then, stained by urinayl acetene and washed by distilled water. The magnification power was 20.000x for yeasts and 25.000x for bacteria.

2.8.2. Scanning

Shape and morphology of bacterial cellulose was studied by using scanning electron microscopy (Jeol JSM – T100). Samples was put on copper support, cover with a 1nm thick layer of gold for 60 second**.**

2.9. FT-IR spectroscopy

The thin samples of bacterial cellulose were prepared according to (kai and keshk, 1999). FT-IR Spectra were recorded on varian 600 infrared spectrometer (National Research center) IR. Spectrometer lab).

3. Results

Black tea used to have the greatest bacterial cellulose. At concentration of 4.4g/L, the inhibitory affect was observe of but 8.7g/L black tea was exhibited a maximum value of bacterial pellicle compared with another set of three tea concentrations, where the weight of bacterial pellicle decrease with the increase of tea concentrations as in table (1).

Table 1. Effect of different tea concentrations on the yield of bacterial cellulose

Tea concentration (g/L)	Wet weight of bacterial cellulose (g/L)	Yield %
4.4	23.40	531.82
8.7	55.46	637.47
17	28.00	164.71
35	25.85	73.86
70	24.60	35.14

Also, the bacterial cellulose depends on the supply of a carbon source, it cannot produce the cellulose. In table (2), the experimental results conducted that the concentration of sucrose at 100g/L produced the highest yield of pellicle and increasing sucrose concentration from (130g/L - 190g/L) produced a gradual decrease in the yield.

Table 2. Effect of different sucrose concentrations on the yield of bacterial cellulose

Sucrose concentration (g/L)	Wet weight of bacterial cellulose (g/L)	Yield %
70	31.50	45.00
100	63.58	63.58
130	29.45	22.65
160	27.00	16.88
190	24.95	13.13

Results in table (3) shows the amount of bacterial cellulose produced in cultures with different volumes and surface areas, the bacterial cellulose production increased with an increase of surface area. As the metabolic processes of kombucha tea depend on fresh air, it is very important to supply sufficient oxygen. The round container with the greatest surface area (176.6cm^2), depth (36.8cm) produced highest bacterial cellulose (85g/L) while, other container has surface area (28.26cm^2), depth (40.2cm) produce bacterial cellulose (20.5g/L). Thus, surface area played an important role in the formation of bacterial cellulose than volume and depth.

Table 3. Effect of culture surface area & depth of containers on the bacterial cellulose

No. of container	**Diameter (cm)**	**Depth (cm)**	**Surface area (cm^2)**	**Surface area depth(cm)**	**Wet weight of pellicle (g/L)**
1	5	5.1	19.60	3.84	7.9
2	6	40.2	28.26	0.70	20.50
3	10	8.4	78.50	7.85	39.80
4	15	6.8	176.63	11.78	85.30

Bacterial cellulose production was strongly affected by the incubation temperature. A gradual drop occurred in fermentation tea broth with high temperature (60^{o}C - 80^{o}C) where the bacterial pellicle was not formed and tea broth was dark brown in colour, odourless and clear table (4).

Table 4. effect of different temperature on the bacterial cellulose of kombucha tea

Temperature (^{o}C)	**Colour**	**Clearity**	**Pellicle formation**	**Odour**
20	Normal	Turbid	Formed	+ve
30	Normal	Turbid	Formed	+ve
40	Normal	Turbid	Formed	+ve
50	Normal	Turbid	Formed	+ve
60	Normal	Clear	Nil	-ve
70	Dark	Clear	Nil	-ve
80	Dark	Clear	Nil	-ve

Kombucha mushroom tea composed of acetic acid bacteria and yeasts (Fig. 1), the symbiotic relationship between yeasts and Acetic acid bacteria was illustrated by transmission electron microscopy at magnification 20.000x, 10.000x (Fig . 2).

While scanning electron microscopy shows that acetic acid bacteria able to form cellulose pellicle which consisting of fine thread like fibrils and densely packed reticulated structure (Fig. 3).

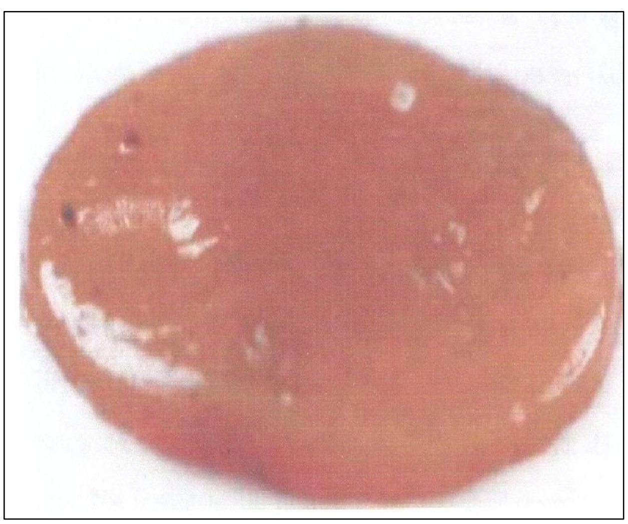


Figure 1. bacterial cellulose pellicle grown in natural tea broth

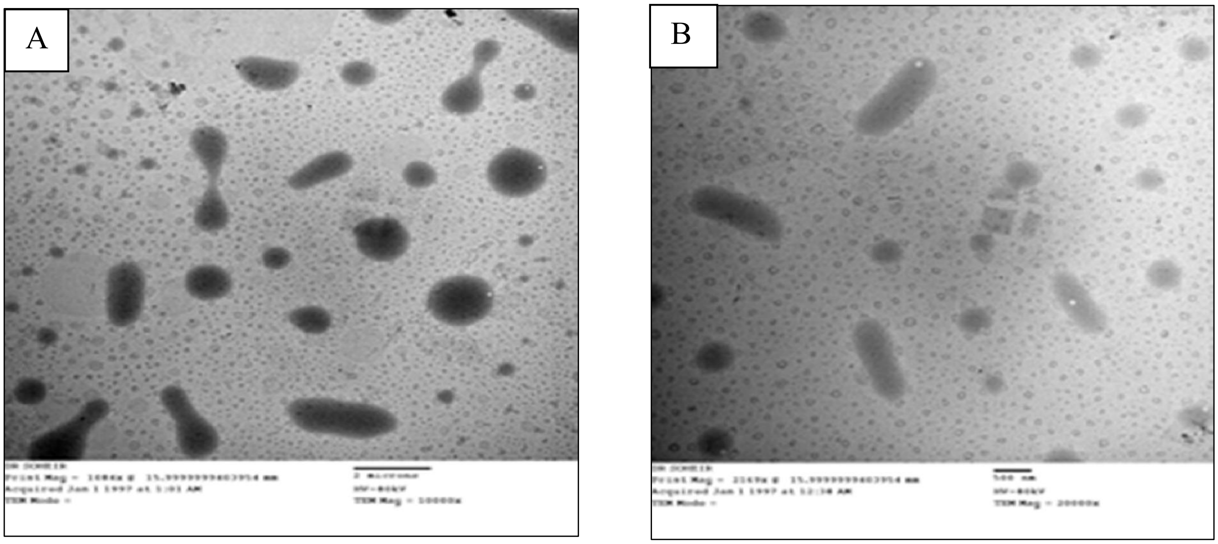


Figure 2. transmission electron microscopy illustrating symbiotic relationship between acetic acid bacteria and yeasts at magnification (A) 10,000x and (B) 20,000x

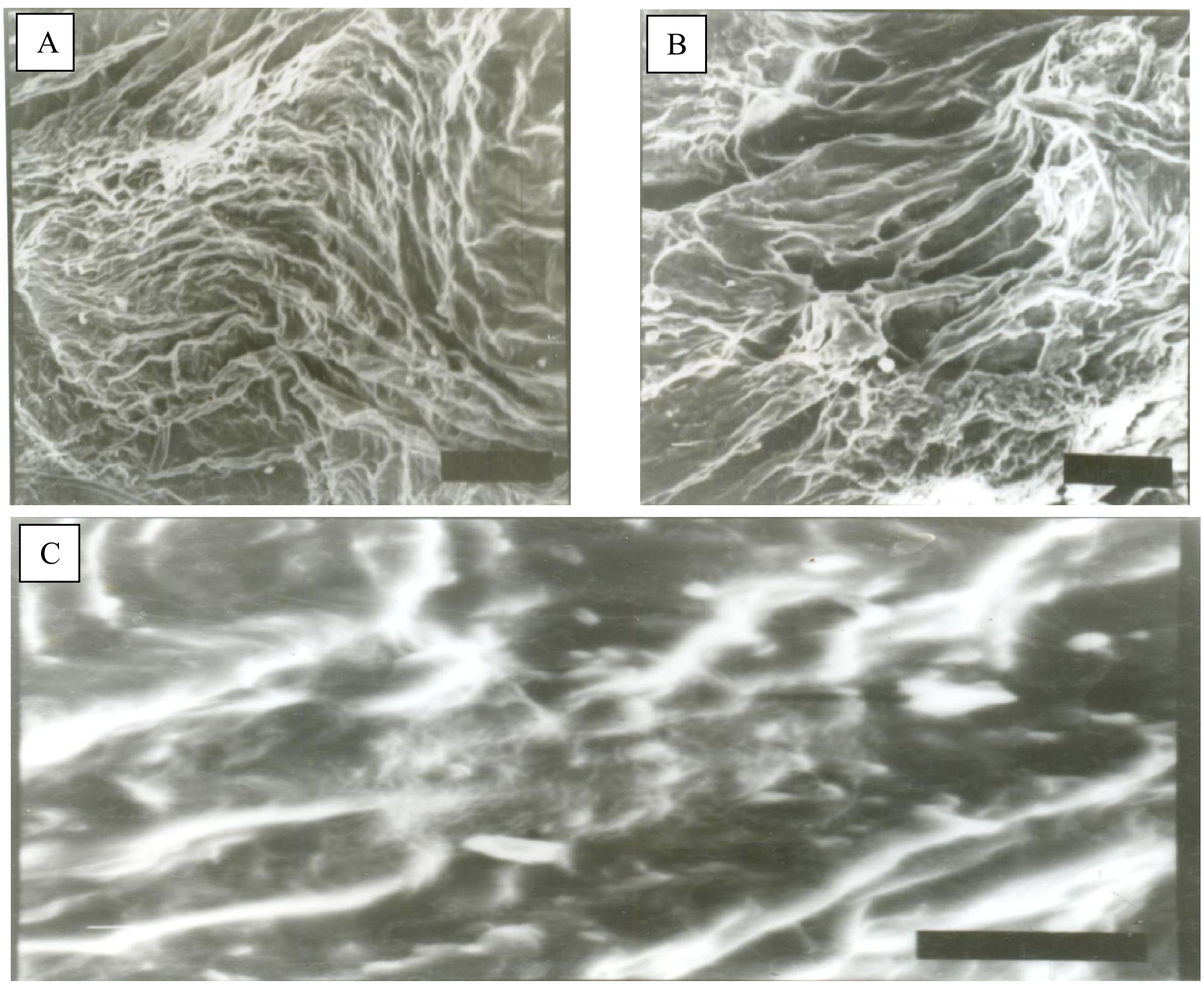
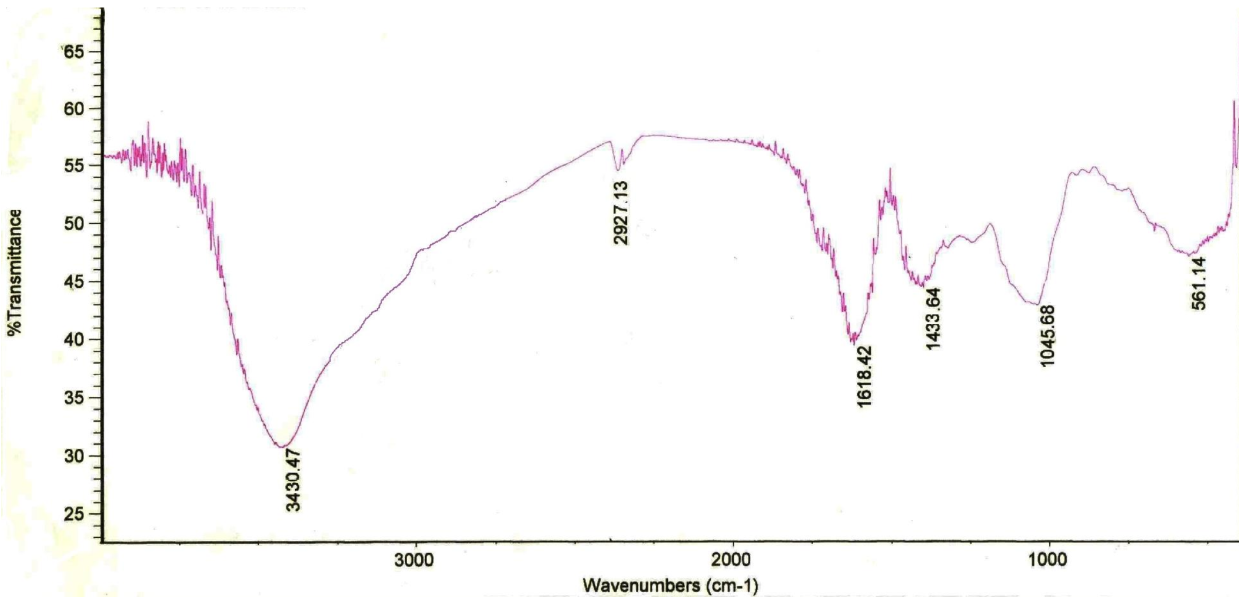


Figure 3. Scanning electron micrographs of the surface structure of the bacterial cellulose at magnification (A) 200x, (B) 400x and (C) 1000x

As shown in (Fig. 4), the conformational characteristics of the bacterial cellulose were determined by FT- IR spectroscopy. The FT-IR spectra showed characteristic cellulose peaks of bacterial cellulose as 3430cm^{-1} for hydroxyl groups stretching vibration, at 2927 cm^{-1} for C-H stretching vibration and also at 1433 for C-H bending vibration, at 1045cm which corresponds to C-O-C and C-O-H of the sugar ring.



Figutr 4. FT-IR spectroscopy of bacterial cellulose

4. Discussion

Kombucha is a popular beverage among traditional fermented foods across the world. It is symbiotic relationship between acetic acid bacteria and yeasts in a sugar tea , that is confirmed by transmission and scanning electron microscopy in this work (Blanc, 1996; chen and liu, 1997 Teoh *et al*., 2004).

Cellulose produced during the fermentation by Acetic acid bacteria especially, *A-Xylinum* appears as thick film on the top of tea broth which enhances the association formed between bacteria and yeasts (Balentine *et al*., 1997, Green walt *et al*., 1998).

By scanning electron microscope technique at different magnification in this research, bacterial cellulose layer is constiluted by compact cellulose network structure which attributed to (Klemm , *et al*., 2001 and Iguchi *et al.,* 2000).

Tea used in different concentrations, the microbial cellulose is obtained at 8.7g/L tea, as (Desilva and saravanapavan 1966) discussed the tea prepared with 10g/L (1%) tea w/v.

In the present study, results conducted that the concentration of sucrose present in the tea broth effects on the synthesis of bacterial cellulose, these results similar to the report by (Masaoka *et al*., 1993). With increase sucrose concentration produced a gradual decrease in the yield, this is finding agree with an earlier report published by (Embuscado *et al*., 1994).

According to (Iguchi *et al*., 2000), in a static culture , the bacterial cellulose pellicle is formed interface of the surface. The present study was conducted to the influence of the surface area and depth of the culture medium on pellicle formation; this is agreed with (Frank, 1995 and Okiyama *et al*., 1992), who found that a deep container generated a small amount of bacterial cellulose.

Temperature effects on the cellulose pellicle production , where the high temperature prevents the growth of bacterial cellulose and pellicle formation and this is similar to (Jayabalan, 2008).

The position and intensity of absorption bands of a substance are specific. The infrared spectrum by FT-IR (fourier transformed infrared spectroscopy) is highly characteristic for a substance (Gunzler and Gremlich, 2002) and can be used to identify the microbial cellulose.

In this study, the chemical nature of the microbial cellulose polymer was confirmed by the infrared spectrum, also, microbial cellulose is free lignin or hemicellulose, which is often present in plant cellulose (Bertocchi *et al*., 1997) and the sample of microbial cellulose as in the polymorphic forms of native cellulose similar to those obtained by plants as indicated spectra, that is according to (Nelson and O' Connor, 1964a).

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