# Isolation of *Acetobacter xylinum* from Kombucha and Application of Cellulose Material Produced by Bacteria from Some Culture Media for Drug Carrier

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Abstract: Bacterial cellulose (BC) can be produced by bacteria in culture media. BC contain some superior properties such as a nanosized ultrafine fiber network, high water-holding capacity and ease of fabrication into a desired shape, which are capable of drug absorbing to form a prolonged release therapy to improve drug bioavailability. BCs were prepared and evaluated for drug carrier using curcumin and famotidine as model drugs. Acetobacter xylinum was successfully isolated from Kombucha. BC was produced by Acetobacter xylinum in the standard medium (SM), coconut medium (CM) and rice medium (RM). The BC-CM, and BC-RM have characteristics the same as the BC-SM, and BC can be fabricated with the desired thickness and diameter in culture media. BCs were absorbed the famotidine in optimal condition with no difference in famotidine loading (20mg) and entrapment efficiency (89-90%). The amount of loaded curcumin and entrapment efficacy of BC-SM and BC-CM were higher than them of BC-RM. Investigation of the BC structure by SEM showed that the cellulose fibers of BC-SM and BC-CM have a stable structure, without modifications in structure when loading under optimal condition. The results indicate the potential for using BC-SM and BC-CM to produce the drug carrier.

Keywords: Bacterial cellulose (BC); curcumin; drug carrier; famotidine; kombucha

#### 1. Introduction

Green tea, derived from the leaves of *Camellia sinensis*, is a widely consumed beverage whose antioxidant activity and other beneficial health effects have gained considerable notoriety in the recent years. The Kombucha contains bacteria producing bacterial cellulose (BC). The metabolites of bacteria during the fermentation contain BC, which has been used as a drink called Kombucha tea. BC has the structure of super-thin nano-fibers with great tensile and mechanical strength. Some studies proved that BC has the potential of being a delivery system by its properties. Amin et al. [1] have reported the use of BC on coconut jelly (made from coconut juice after the fermentation of bacteria strain Acetobacter xylinum) in the coating for Paracetamol by spraying technique. Their results have indicated that BC membranes were able to increase releasing time of the drug and improve the efficiency of drug use. Huang et al. [2] have tested BC membrane from the fermentation of bacteria Gluconacetobacter xylinum in the standard medium (Hestrin-Schramm) for transporting and releasing berberine in vitro. The experiment has controlled the drug releasing of BC in artificial models, including stomach and intestine. The gained information has shown that berberine released with a low rate in acidic condition, normal in alkaline condition and high releasing rate in neutral pH condition. Famotidine is one of the gastrointestinal drugs, which is used by drink or injection, soluble in acids and weakly dissolved in water [3]. It has the effect of reducing gastric secretion by inhibiting histamine at the H<sub>2</sub> receptor in the gastric mucosa wall, decreasing the secretion of HCl in gastric juice, the healing of gastric ulcers. However, the low bioavailability of famotidine (about 40-45%) has prevented its therapeutic applications [3]. Drinking is one of the most favorite and traditional ways to distribute drugs, reducing costs and pressure on patients [4]. Therefore, a system has been designed to help the drug to absorb and release slowly increases the bioavailability of the drug. Satishbabu et al. [5] have assessed slowly releasing drug rate of famotidine on the cod liver oil combined with calcium alginate granules. Anraku et al. [6] have studied the slow release of famotidine from tablets: chitosan/sulfobutyl ether  $\beta$  - cyclodextrin composites. Zhu et al. [3] have studied the multiple-unit floating-bioadhesive cooperative minitablets for improving the oral bioavailability of famotidine in rats. Their studies the multiple-unit floating-bioadhesive proved that cooperative minitablets may be a promising gastro-retentive delivery system for drugs that play a therapeutic role in the stomach. Maday et al. [7] have evaluated the acid function of carboxymethyl - beta - cyclodextrin in improving the chemical stability, oral - route bioavailability and bitter taste of famotidine. Fahmy et al. [8] have tested the rate of release of Famotidine through the construction of liquisolid tablets in both in vitro and in vivo. Gao et al. [9] have studied the pharmacokinetics and bioavailability of famotidine on 10 Chinese volunteers. Although, curcumin exhibited good therapeutic efficacy toward a variety of diseases including cancer, its use is fettered due to its poor aqueous solubility, low bioavailability and rapid degradation. Our research aims to isolate Acetobacter xylinum from Kombucha and apply the cellulose material produced by bacteria in some culture media for carrier of cucurmin or famotidine.

### 2. Material and Methods

#### 2.1. Materials and equipment

Bacterial strain: *Acetobacter xylinum* producing cellulose (BC) used in this study was isolated from Kombucha at the clean laboratory of microorganism, Institute of Scientific Research and Applications (ISA) – Hanoi Pedagogical University 2 (HPU2), Vietnam.

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Materials and chemicals: Kombucha was used as the natural sources for the isolation of Bacteria producing cellulose (BC). The coconut water and rice water were used as nutrient sources for the production of BC by *Acetobacter xylinum*. Curcumin 95% (Apollo, India), famotidine 99.5% (Sigma, USA), yeast extracts (Sigma-Aldrich, USA), peptone (ECHA, European Union), and other analytical grade chemicals were used.

Equipment: Field emission scanning electron microscopes (FE-SEM, Hitachi, Japan), fourier transform infrared spectrophotometer (FTIR, Shimadzu, Japan), spectrograph UV-Vis 2450 (Shimadzu, Japan); analytic scale (Sartorius, Switzerland); magnetic stirrer (IKA, Germany); low speed rotator (Orbital Shakergallenkump, Anh); shaker (Lab companion, SKF-2075, Korea); oven, incubator (Binder, Germany); antiseptic cabbin (Haraeus); antiseptic autoclave (HV-110/HIRAIAMA, Japan).

#### 2.2. Isolation of Acetobacter xylinum from Kombucha

Kombucha were prepared using low-cost green tea purchased from Thai Nguyen Province in Vietnam. Briefly, 20g the green tea leaves was added to 1000mL boiled water and allowed to infuse for 10-15 minutes. The infusion was filtered to remove the tea leaves. 100g sugar was dissolved in hot aqueous green tea extract, and preparation was left to cool to room temperature. The aqueous green tea extract was then poured into sterile glass Erlenmeyer flasks. The flasks were then covered with sterile muslin cloth and incubated at 30°C in the dark for 19 days (Figure 1).



Figure 1: Preparation of Acetobacter bacteria from Kombucha

All the flasks were observed for formation of thin cellulosic film (BC) at air liquid interface. Those flasks with BC growth were selected and purified the culture by repeated streaking on HS agar plates to obtain isolated colonies. Each distinct isolate was inoculated on screening media, that is, the enrichment media used was GY (glucose - yeast extract). Inoculated broth was incubated in GY at 30°C for 2 days. Isolation was carried out on two different selective media for isolation of Acetobacter bacteria, GEM (glucose-ethanol medium) and GYC (glucose - yeast extract - calcium carbonate medium). The morphology and Gram nature of Acetobacter bacteria isolated on the selective media was determined. Its biochemical characterization involved catalase, oxidase, over oxidation of ethanol by use of Carr medium, oxidation of acetate and oxidation of lactate [10], [11].

After receiving the *Acetobacter xylinum* from the Kombucha, *Acetobacter xylinum* were cultured in some nutrient media (SM, CM, RM) to produce BC-SM, BC-CM, and BC-RM.

### 2.3. Fabrication and characterization of BC

Acetobacter xylinum were fermented in three culture media: SM [12], [14] including glucose (20g), pentone (5g), disodium phosphate (2.7g), yeast extracts (5g), citric acid (1.15g), double-distilled water (1000mL); CM [13], [14] including glucose (20g), peptone (10g), disodium phosphate (0.5g), amonia sulfate (0.5g), ripe coconut water (1000mL); RM [14] including glucose (20g), peptone (10g), disodium phosphate (0.5g), ammonia sulfate (0.5g), rice water (1000mL).

Treatment of the BCs before drug absorption: The BCs obtained from culture media were treated with 0.3 M NaOH solution in an autoclave at 113°C for 15 minutes to remove bacterial cells, debris and other culture medium impurities. The BCs were thoroughly rinsed with distilled water until reaching neutral pH and stored at 4°C for further use [13], [15], [16].

Evaluation of the purity of the BC: The present of D-glucose in the BC was determined by Fehling reagent. If there is a Dglucose present in the BC, the Fehling reagent will give a reddish precipitate [17], [18]. The present of protein in BC was determined by the precipitation reaction of the protein with trichlor-acetic acid [17], [18].

Determination of the amount of the BC formed: The wet weight of the purified BC was measured and then dried at 60 °C until a constant weight was obtained [13], [15], [16].

Determination of the structure of the BC: The samples were heated in 40°C in 20 minutes, cover then a thin platinum layer and put into the sample chamber. The field emission scanning electron microscopes (FE-SEM, Hitachi S-4800 with magnification M=20-800,000, resolution  $\delta = 1.0$ nm, piezoelectric accelerator U = 10kV) was used for examination of the samples.

Determination of the interaction of the BC to drug: The samples are directly measured by reflectometry in 20°C, moisture 40-43%. The fourier transform infrared spectrophotometer (FT-IR) was used for examination of the samples.

## 2.4. Evaluation of drug loading and entrapment efficiency

The BCs with a diameter of 1.5cm and a thickness of 1cm created from culture media (SM, CM, RM) are absorbed curcumin or famotidine in the optimized conditions [14], [20]. After a period of maximum loading, the spectrophotometry UV-2450 was performed to determine an excessive amount of drug in the solution [2], [19] at the time of sampling, to determine the drug concentration, determine the drug dose in the solution. The amount of loaded drugs into BC are calculated according to formula 1:  $m_{ab} = m_1 - m_2$  (mg) (1). Where:  $m_{ab}$  is the amount of drugs that are loaded into the BC;  $m_1$  is the initial dose in solution;  $m_2$  is the excessive amount of drug existing in the solution after a certain period of time BC absorbs the drug. The drug

Volume 8 Issue 1, January 2019 <u>www.ijsr.net</u> Licensed Under Creative Commons Attribution CC BY entrapment efficiency (EE) of BCs is calculated according to formula 2 [4]. EE (%) =  $(m_{ab}/m_1)x100\%$  (2).

#### 2.5. Statistical analysis

All results are analysed, processed by Excel 2010 and is performed by the mean  $\pm$  standard deviation. Check the hypothesis of the mean value of the two samples the two-way ANOVA test, and it was concluded that there were significant differences between the means at the significance level of p < 0.05.

## 3. Result and discussion

#### 3.1 Isolation and screening of Acetobacter xylinum

Based on the morphological and biochemical characters, the isolate was identified as *Acetobacter xylinum* as per Bergey's manual. The major differentiating biochemical characters were; gram negative short rod, catalase positive, oxidase negative, growth on acetic acid and ethanol positive and production of cellulose positive [11], [17].

In our study, seventeen bacterial isolates were obtained from Kombucha which are found to produce cellulose material. The enriched isolates after incubation of 2-4 days showed turbidity indicating presence of organisms in the sample. Isolated colonies with zone of clearance around them after incubation at  $30^{\circ}$ C on GYC and GEM selective media confirmed the growth of Acetobacter bacteria (Figure 2).



Figure 2: Growth of isolated colonies on GYC medium

The isolate was gram negative short rod, catalase positive and oxidase negative. Complete oxidation of acetate and lactate was observed with no brown pigmentation on GYC medium. An initial colour change from green to yellow and reversion to green colour on further incubation was observed on the Carr medium due to the growth of the isolate (Figure 3).



Figure 3: Change in colour of Carr medium due to growth of Acetobacter bacteria

The isolate classified under Acetobacter genera are catalase positive, oxidase negative, oxidize lactate and acetate to  $\rm CO_2$ 

and  $H_2O$ . Acetobacter strains are able to overoxidise ethanol to acetic acid and finally to  $CO_2$  and  $H_2O$ . As tricarboxylic acid cycle is functional in Acetobacter strain, genera is able to overoxidise organic acids. Ability to oxidise acetic acid to  $CO_2$  is a major distinguishing feature between genera Acetobacter and Gluconobacte [11]. In our study similar results were observed on Figure 4, therefore isolated strain in present study was confirmed *Acetobacter xylinum*.



Figure 4: Growth of isolated colonies with  $CaCO_3$  (a) and without  $CaCO_3$  (b)

After the isolation, it was observed that 17 strains of *Acetobacter xylinum* were able to produce thin cellulosic film (BC) at air liquid interface in glass Erlenmeyer flasks. Further static culturing in flasks shows that most of them could only produce small amount of BC. Among them, 3 strains of *Acetobacter xylinum* were tested to be able to produce more than 2.5 g/L. The strain with a yield of 6.5 g/L was singled out to be the target strain of *Acetobacter xylinum* for fabrication of BC.

## **3.2** Fabrication and characterization of BC

The BCs (BC-SM, BC-CM and BC-RM) with a diameter of 1.5cm and a thickness of 1cm were produced by *Acetobacter xylinum* in the culture media (SM, CM, RM) from 10 to 14 days.

According to previous studies, it is possible to create the BCs with different shapes and thickness depending on the intended use [2], [14]. In this study, the BCs with a thickness of 1cm (depending on the time of culture) and a diameter of 1.5cm (depending on the size of the culture well) were created for the application via oral route.

The thickness of the BC in different positions is measured by a ruler. The results showed that the thickness, and the diameter of the BCs created from the culture media were relatively uniform.

Fehling reagent is used to detect the presence of D-glucose in the BCs. The research results showed that there was no reddish brown precipitate. Therefore, the BCs didnot contain D-glucose.

The protein in the BCs was determined by the reaction of precipitation of protein with trichlor-acetic acid. The test results did not detect the presence of protein in the BCs.

To determine the amount of the BC formed, the purified BCs were dried at  $60^{\circ}$ C until reaching a constant mass. The results showed that the dried mass of the BC created in SM was highest.

Volume 8 Issue 1, January 2019 <u>www.ijsr.net</u> Licensed Under Creative Commons Attribution CC BY A field emission scanning electron microscope (FE-SEM, Hitachi, Japan) was used to visualize the surface morphology of the samples.

SEM images of the BCs (BC-SM, BC-CM, and BC-RM) before and after loading drugs were shown in Figure 5. As the results, BCs have the homogeneous fiber structure network.



**Figure 5:** The FE-SEM images of BC-SM, BC-CM and BC-RM (A, C, E) and famotidine loaded BC-SM, famotidine loaded BC-CM and famotidine loaded BC-RM (B, D, F)

## 3.3 Evaluation of drug loading and entrapment efficiency

Perform the experiment of the curcumin or famotidine absorption into BCs which is produced from different culture media based on optimal conditions [14], [20]. At the end of the experiment, the sample was removed from the absorbent solution to measure OD, based on the drug's calibration curve to calculate the amount of loaded curcumin or famotidine and the curcumin or famotidine entrapment efficiency of the BCs. The results in Table 1 showed that there were no differences in the amount of loaded famotidine and famotidine entrapment efficacy of BCs which were produced from different culture media.

| Table 1: | Evaluation | of famotidine | loading | and |
|----------|------------|---------------|---------|-----|
|          | entrapmer  | nt efficiency |         |     |

| BC types         | BC-SM         | BC-CM           | BC-RM         |  |  |  |
|------------------|---------------|-----------------|---------------|--|--|--|
| Loaded drug (mg) | $20.03\pm0.5$ | $20.07\pm0.8$   | $20.05\pm0.7$ |  |  |  |
| Efficiency (%)   | $89.10\pm0.9$ | $90.00 \pm 0.7$ | $89.25\pm0.9$ |  |  |  |

The results in Table 2 showed that there were differences in the amount of loaded curcumin and curcumin entrapment efficacy of BCs which were produced from SM and CM media and RM medium. The amount of loaded curcumin and curcumin entrapment efficacy of BC-SM and BC-CM were higher than them of BC-RM.

Table 2: Evaluation of curcumin loading and entrapment

| efficiency       |               |                 |                |  |  |  |  |
|------------------|---------------|-----------------|----------------|--|--|--|--|
| BC types         | BC-SM         | BC-CM           | BC-RM          |  |  |  |  |
| Loaded drug (mg) | $14.31\pm0.1$ | $13.93\pm0.8$   | $8.47\pm0.5$   |  |  |  |  |
| Efficiency (%)   | $15.90\pm0.8$ | $15.48 \pm 0.4$ | $9.41 \pm 0.7$ |  |  |  |  |

## **3.4** Determination of the interaction of BC to famotidine by FT-IR

The FT-IR spectra of famotidine, BC-SM, BC-CM, BC-RM, famotidine loaded BC-SM, famotidine loaded BC-CM, and famotidine loaded BC-RM are shown in Figure 6, 7, 8, 9, 10, 11 and 12.



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Figure 11: FT-IR spectra for famotidine loaded BC-CM



Figure 12: FT-IR spectra for famotidine loaded BC-RM

The FT-IR spectra of BCs (BC-SM, BC-CM, BC-RM) in Figure 7, 8, 9 showed peaks at 1039, 1159, 2895, and 3315 cm<sup>-1</sup>, representing C-O stretching vibration, C–O–C stretching of the ether linkage (1,4- $\beta$ -D-glucoside), C–H stretching and O–H stretching of intermolecular hydrogen bonds, respectively [2], [20]. FT-IR spectra of the famotidine in Figure 3 showed that the famotidine exhibited various peaks due to the presence of specific functional groups. Peaks of the major functional groups of the famotidine were obtained at 1284.50, 1535.23, 3101.32 and 3394.48.

The results of the FT-IR spectra in Figures from 6 to 12 showed that the same peaks of famotidine functional groups were present in the FT-IR spectra of the famotidine loaded BCs and other peaks of BCs were present. It was observed that there were no changes in these main peaks in FT-IR spectra of a mixture of famotidine and BCs (Figures 10-12). In the famotidine loaded BCs, no additional peaks attributable to the formation of a complex appeared, but variations in the relative intensities of the characteristic peaks for BCs and the famotidine could be observed. The FT-IR study revealed no physical or chemical interactions of famotidine with BCs as evident from Figures 10-12. Consequently, between the BCs and the famotidine without the formation of covalent bonds, the famotidine loaded BCs; therefore, only involved noncovalent bonding forces. The results in this study are also consistent with other studies [2] [20].

#### 3.5 Discussion

These results are consistent with other studies about the structure of BC, including nano-sized cellulose fibers that

make up the three-dimensional structure network [2] [20] [21]. According to Huang et al [2] have compared SEM images of BC-SM generated from Gluconacetobacter xylinum after 24 hours treatment of some conditions (doubledistilled water, artificial medium of stomach and intestine, NaOH medium) showed that: porosity of the BC cultured in SM in acidic and alkaline media increasing when compared to neutral medium (double-distilled water). It affirmed that have the contraction of cellulose fibers in these two conditions, and neutral medium does not affect to the cellulose fibers. The results from this reseach showed that BC is drug loaded and non-loaded with no apparent difference in results consistent with other studies [2] [20]. For the BC-SM or BC-CM, the cellulose fibers have the stable structure, with no significant changes in structure when famotidine loaded under optimal (experimental) conditions. For the BC-RM, the spatial structure of the cellulose fibers is noticeably altered after famotidine loading, the size of the holes in the famotidine loaded BC-RM changes, the cellulose fibers of BC-RM are loosely linked; the structure of BC-RM is unstable.

Compared to the BC produced from the standard culture of pure bacteria strain (*Gluconacetobacter xylinum*) [2], the BC structure in this study was not significantly different. It is concluded that the BCs of the study have obtained by Acetobacter bacteria from Kombucha in three types of culture media were effective in generating the famotidine delivery system.

## 4. Conclusion

Acetobacter xylinum was successfully isolated from Kombucha. BC-CM and BC-RM have characteristics the same as the BC-SM, and BCs can be fabricated with the desired thickness and diameter in all three types of culture media. BCs were absorbed the famotidine in optimal condition with no difference in famotidine loading (20mg) and entrapment efficiency (89-90%). The amount of loaded curcumin and entrapment efficacy of BC-SM and BC-CM were higher than them of BC-RM. Investigation of BC structure by SEM showed that the cellulose fibers of BC-SM and BC-CM have a stable structure, without modifications in structure when curcumin or famotidine loading under optimal conditions. The results indicate that the potential for using BC-SM and BC-CM to fabricate the drug carier.

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