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FTIR Spectral and Microarchitectural Analysis of Cellulose Produced by *Lactococcus lactis* Under Agitated Condition

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The demand for cellulose is accelerating in the paper making industry. Alternate sources of cellulose has to be traced in order to reduce the demand for plant cellulose. Hence, in this study bacterial cellulose has been chosen as an option. In this study, the potential of soil bacteria *Lactococcus lactis* to produce cellulose has been assessed. The results obtained indicate that the inocula size of the bacteria had a vital role in altering the quantity of cellulose produced. Among the inocula size, 100 µl of broth culture exhibited highest production of cellulose. The cellulose produced was characterised spectrally and its microarchitectural study reveal its crystalline nature. FTIR spectra of the bacterial cellulose produced depict the signature peak of bacterial cellulose. Based on these observations, it could be concluded that *Lactococcus lactis* produce cellulose. Further studies has to be carried out to optimize the bacterial cellulose production.

Keywords: *Lactococcus lactis* , cellulose, glucose, FTIR, SEM.

Cellulose, the major polymer produced in the biosphere serves as the raw material in the high fidelity acoustic speakers, high quality paper and dessert foods, wound dressings, artificial skin, dental implants, membrane dialysis, drug carrier for controlled release, wet-end additive for paper making process[1-5]. Cellulose is synthesized by plants, animals (Tunicates), bacteria, algae and plankton[3,6].

Bacterial cellulose is preferred over plant cellulose due to his high purity, high degree of polymerization, crystallinity index, high tensile strength and water holding capacity[3]. One of the major bottleneck in BC application is industry is its low productivity. Microorganisms, production methods, carbon and nitrogen sources, temperature, pH and reactor type influence the bacterial cellulose production[7,4,8]. Production of cellulose by bacteria like *Gluconacetobacter xylinum*[9,10], *Sarcina, Agrobacterium, Rhizobium, Acetobacter*[11]*Sucrofermentans* BPR2001[12], *Enterobacter sp*.RVII, *Pseudumonas sp.*,RVI4, *Gloconacetobacter* sp.,[13] have been documented.

Many researchers have evaluated the use of low cost natural carbon sources like coconut water, fruit juices, corn steep liquor, date syrup, dates molasses, sugarcane juice[14,15,16,12,17,18,19,2,20]a nd synthetic carbon sources like glucose, ethanol etc.,[21,15,22,12,23,24,13,25,26]. Bacterial cellulose has been produced under static condition [27,22,28,23] and agitated condition[21] .Some researchers have suggested that static condition is suitable for bacterial cellulose production [14,29,16,12,30].The accelerating demand for cellulose based products may shrink the forest cover. Alternative sources of cellulose could be a sustainable option to minimize the pressure on plant cellulose. Hence, the present study was designed to tap the potential of soil bacteria to produce cellulose and also to characterize cellulose through FTIR and SEM images.

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MATERIALS AND METHODS

Collection of soil and isolation of bacteria

Soil was collected from garden in a bottle aseptically. 1 g of soil was dissolved in 100 ml sterile distilled water and serially diluted. 1µl of dilutions of 10^{-3}, 10^{-5} and 10^{-7} were inoculated on nutrient agar plates and incubated at 37 ºC for 48 hours. The bacterial colonies were isolated and identified according to the methods mentioned in Bergeys Manual of Determinative Bacteriology [31]. Among the bacterial isolates, dominant bacteria *Lactococcus lactis* was evaluated for its potential to produce cellulose.

Inoculation of *Lactococcus lactis* in HS medium [32]

HS medium (2 % w/v D-glucose, 0.5 % w/v peptone, 0.5 % w/v yeast extract, 0.27 % w/v di- sodium hydrogen Phosphate (Na_2HPO_4) and 0.115 % w/v citric acid) was taken in a 250 ml conical flask and 100 µl, 200 µl and 300 µl of *Lactococcus lactis* broth culture of 24 hours was inoculated. The experiment was conducted in triplicates. The culture was incubated at 37 ºC in agitated condition in a orbital shaker at 100 rpm for a period of 15 days. Wet BC pellicles produced were pre- heated and weighed. The wet BC pellicles produced was filtered from the media and washed with running water and immersed in 2 % w/v sodium hydroxide and boiled for 30 minutes and dried it in the oven at 70 ºC for 6 hours [15].

Table 1. Identification of bacteria by biochemical analysis

S. No	Test	Result
1.	Gram staining	+ve
2.	Oxidase	-ve
3.	Catalese	-ve
4.	Indole	-ve
5.	MR	+ve
6.	VP	+ve
7.	Citrate	-ve
8.	Urease	-ve
9.	TSI slant/butt	AK/A
10.	Glucose	+ve
11.	Sucrose	+ve
12.	Lactose	+ve
13.	Fructose	+ve

Evaluation of bacterial cellulose properties:

Weight (g) of cellulose was measured in the analytical balance.The moisture content (% w/w) of bacterial cellulose was determined based on the weight loss of bacterial cellulose when dried at 75 °C[14].Bacterial cellulose production was determined by the method of Hongmel Lu *et al*., [33].

Observation of bacterial cellulose film under Scanning Electron Microscope (SEM)

BC dry films produced were observed under SEM to study morphology and microstructure of cellulose fibres. Prior to examining, the sample were gently fixed on an Aluminium stab with two side adhesive tape and coated with 15 - 20mm thick layer of gold. The samples were then examined under scanning Electron Microscope (Spectrum 2).

FTIR Spectroscopy

FTIR spectra of bacterial cellulose samples were recorded with a BIO-RAD spectrometer (model FTS 40A) using the KBr (Potassium bromide) disc technique (1 mg of BC powder / 300 mg KBr) in the range of 4000 - 400 cm^{-1} . The FT-IR spectra were recorded at a resolution of 2 cm^{-1} and at an accumulation of 32 scans.

RESULTS

The biochemical test reveals that the isolated bacteria is ***Lactococcus lactis*** **(**Table 1**)**. Highest BC moisture content was recorded in bacterial cellulose produced by 100 µl of

Table 2. Moisture content and the quantity of cellulose produced by *Lactococcus lactis*

Inocula size(µl of *Lactococcus lactis* broth culture)	Moisture content(%)	Bacterial cellulose produced (g/ L)
100	4.60	41.4
200	0.20	1.70
30	0.49	4.25

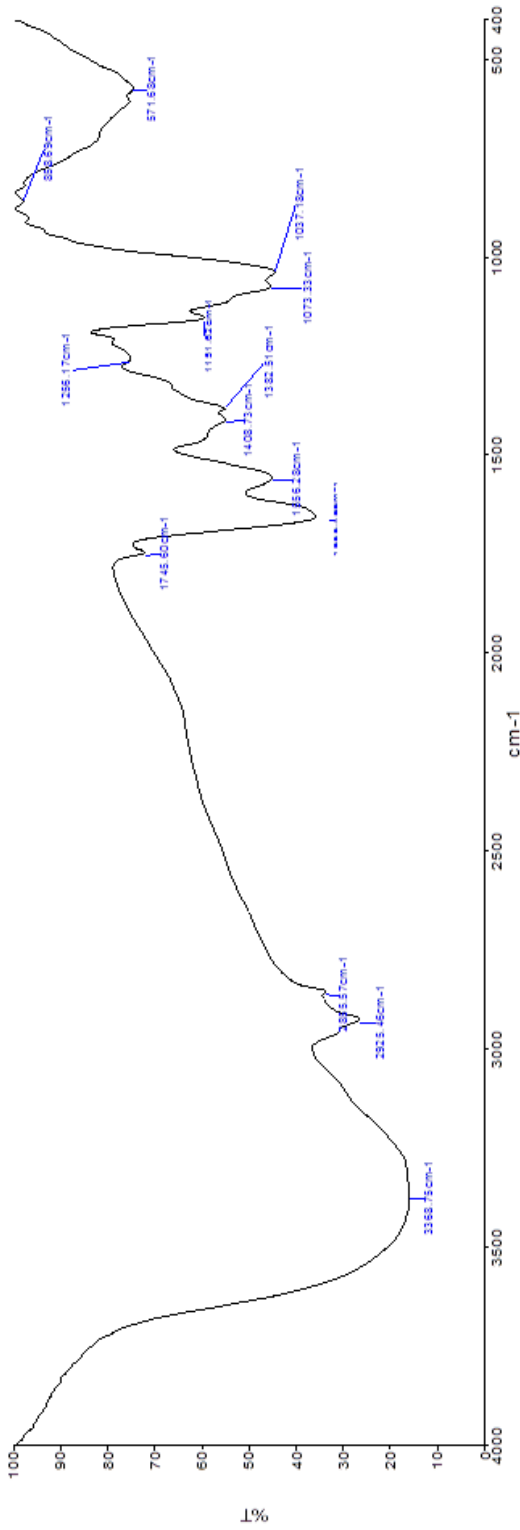

Fig. 1. FTIR Spectra of BC produced by *Lactococcus lactis* (100 µL)

Lactococcus lactis broth culture (4.6 %). Highest quantity of BC was produced at 100 µl of *Lactococcus lactis* broth culture (41.4 g/ L)(table 2).

The signature peak of bacterial cellulose produced by *Lactococcus lactis* reveal the presence of –OH (3368 cm^{-1}), ½(CH_2) (2925 cm^{-1}), ½(CH_2) (2865 cm^{-1}), C = O (1745 cm^{-1}), COOH (1655 cm^{-1}), Amide II (1556 cm^{-1}), CH (1408 cm^{-1}), CH (1382 cm^{-1}), (1256 cm^{-1}), C- O – C (1151 cm^{-1}), C- O – C (1073 cm^{-1}), (1037 cm^{-1}) and CH (858 cm^{-1}) at inoculum density of 100 µL. (Fig. 1) (Table 3). The SEM Image reveals the BC structure. It is crystalline in nature (fig 2).

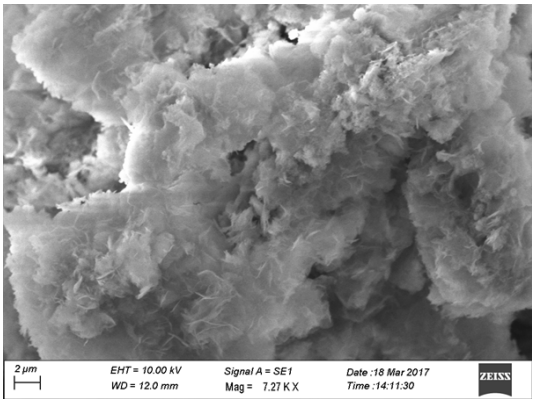

Fig. 2. SEM image of bacterial cellulose produced by *Lactococcus lactis* (100 µl / ml)

DISCUSSION

The present finding agrees with that of Faridah *et al.*,[27] who have reported that cellulose produced by *A. xylinum* under different sugar concentrations (7.5 % and 10 %) does not alter the functional groups but causes changes in the intensity of absorption peak in the FTIR spectra of cellulose and have concluded that the concentration of sugar does not alter the microstucture of the cellulose. Similarly, Hestrin and Schramm medium containing different carbon sources influence the yield of cellulose by *Gluconactobacter xylinus*strain ATCC 53524 but doesnot affect the molecular and microscopic features of cellulose[21]. Hungund *et al.*,[15]have also observed that highest cellulose yield in combination with fructose and sucrose (1:1) in Hestrin and Schramm medium by *Gluconactobacter persimmonis.* Yodsuwan *et al*.,[22] also have reported that mannitol and fructose enhanced the cellulose yield of *Acetobacter xylinum* strain TISTR 975.*Gluconoacetobacter hansenii* yielded cellulose in the range of 0.81g/ L to 0.84 g/ L in standard HS medium after a period of 14 days[39].

The present result partially agrees with that of Ragaswamy *et al*.,[13] who have stated that *Gluconacetobacter* sp., RV28 produced 4.7 g/ L of cellulose at optimum growth conditions of temperature (30 ºC), pH (6.0), sucrose (2%),

Table 3. Band Assignment of FTIR spectra of Cellulose produced by *Lactococcus lactis* (100 µl / ml)

Wave Number (cm^{-1})	Intensity	Functional Group	References
3368	s	OH	23, 24, 34,35,36,37, 38,39,40, 41, 42,43, 44,45
2925	s	$q_s(CH)_2$	2, 24,25, 35,36, 38, 39, 40,41, 43, 44,46,47,48,49,50
2865	s	$q_s(CH)_2$	35, 39, 41, 46, 47
1745	m	C=O	23, 47, 51
1655	m	COOH	2,24, 25,37, 40,
1556	S	Amide II absorption	23, 47
1408	s	CH	25, 35, 38,39, 45
1382	s	Planar CH	24, 36,39, 45,47
1256	m	O-C	51
1151	m	C-O-C	25, 38,39, 42, 44,47,54,55,56
1073	s	C-O-C	2, 24, 41,56
1037	s	C-O-C	25, 35, 51
858	w	CH out of plane bending vibrations	43,56

s – strong, m – medium, w - weak

peptone (0.5 %) and inoculum density (5 %) under static condition. Auta *et al.*, [24] have reported that *Gluconacetobacter xylinus* produced an average dry yield of 1.4 ± 0.09 g/ L cellulose after 9 days by using glucose as a carbon source under static condition at 30 ºC. This finding is in consistent with the present observation.

The present observation gains support from the findings of Gayathri and Gopalaswamy[2] who have reported that *Acetobacter xylinum* produced 11g/L bacterial cellulose in HS medium after 14 daysof fermentation period. Castro *et al*.,[23] have demonstrated that *Gluconacetobacter medellensis* produced optimum cellulose in HS medium modified with glucose (4.2 g/ L) followed by sucrose and fructose under static condition at 28 ºC. These findings are in conformity with the observations of Barbara Surma- Slusarska *et al*.,[28]who have reported that *Acetobacter xylinum* yielded highest bacterial cellulose using glucose and mannitol when compared to other carbon sources (arabinose, mannose, galactose and xylose) at 30 ºC after 7 days under static condition. Alaa Raheem Kazim[19] have reported that dates molasses enhances the production of cellulose by *Pseudomonas sp.,* when compared to other carbon sources (glucose, fructose, maltose, ethanol) and have attributed it to the nutrient content of dates. These observations are in harmony with the findings of Masaoka *et al.*,[26] who have reported that bacterial cellulose production by *Acetobacter xylinum* was enhanced when glucose was used as a carbon source at 30 ºC statically.

The crystalline nature of bacterial cellulose observed in te SEM image is similar to our previous findings[35]. We have observed the crystalline nature of cellulose produced by *Actinomycetes sp*. and *Pseudomonas sp*. The crystalline nature of cellulose produced by bacteria using glucose as a carbon source have been reported (*Gluconacetobacter* sp.[13], *Acetobacter xylinum sub sp. Sucrofermentans* BPR2001[12], *Acetobacter xylinum*[30] ,*Acetobacter xylinum*[2], *Achromobacter sp.,*[41], *Acetobacter aceti*[57], *Acetobacter xylinum*[29], *Gluconacetobacter*[23],*Gluconacetobacter xylinus* strain ATCC53524 [21]. The results obtained indicate that *Lactoccocus lactis* could be used to produce cellulose but further investigations have to be carried out to optimise the bacterial cellulose production.

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