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Characterization and optimization of bacterial cellulose produced by *Acetobacter* spp.

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Abstract

Aim : The study aimed to search for a potential bacterial strain capable of producing maximal bacterial cellulose under optimized conditions for future scale up.

Methodology : Eight cellulose producing bacterial strains were isolated from sugarcane juice using Hestrin and Schramm (HS) medium. These isolates were identified through 16S rDNA based on molecular phylogenetic approach and the cellulose mat was analyzed for their physico-chemical properties. Morphological, chemical and physical properties of cellulose mat was studied by Scanning Electron Microscopy, Fourier Transform Infrared Spectroscopy spectrum, High Performance Liquid Chromatography and Differential Scanning Calorimetry. Optimal nutrient composition for maximum cellulose production by the isolate was carried out by response surface methodology.

Results : 16S rDNA sequence analysis revealed that the isolates belonged to *Acetobacter senegalensis*, *Acetobacter thailandicus*, *Acetobacter lambici*, *Acetobacter lovaniensis* and *Acinetobacter baumannii*. Among them, *A. senegalensis* MA1 produced the maximum cellulose mat of dry weight 3.6 g per 500 ml of HS medium after 2 weeks of incubation. The morphology of bacterial cellulose produced by *A. senegalensis* MA1 had a pellicle shaped distinguished network structure and the rod shaped bacterium was attached to the bottom of cellulose mat. HPLC analysis revealed that the peaks obtained from bacterial cellulose were almost similar to carboxymethyl cellulose (CMC) peak. In cellulose mat, the weight of carbon, oxygen were 60.13 and 25.12, respectively. Analysis of differential scanning calorimetry showed endothermal peak at 139.8°C., indicating the melting point of bacterial cellulose mat. Through response surface methodology analysis, 5 g of glycerol and 1.2 g of yeast extract per 100 ml medium was optimized to get maximum cellulose production (2.04 g) from *A. senegalensis* MA1.

Interpretation : Optimized condition for maximum bacterial cellulose production may be applied at industrial scale for commercial utilization.

Key words: *Acetobacter* spp., Bacterial cellulose, Response Surface Methodology, 16S rDNA

Isolation of cellulose producing bacteria

Molecular characterization and phylogenetic analysis

Morphological, chemical and physical analyses

Optimization of cellulose mat production through RSM

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Introduction

Cellulose is the most abundant natural polymer on earth, mostly produced by vascular plants, animals and microorganisms. Bacteria are the major producer of cellulose, which possess considerably different properties than plant cellulose. Plant and bacterial cellulose are chemically same but differ in their physico-chemical properties possessing several advantages over plant cellulose. Bacterial cellulose is considered pure due to lack of hemicellulose or lignin (Phisalaphong and Jatupaiboon, 2008). In addition, bacterial cellulose possess higher water holding capacity, mechanical strength and highly pure fiber network (Lin et al., 2016). Notably, bacterial strains belonging to genera *Gluconacetobacter*, *Acetobacter*, *Agrobacterium*, *Aerobacter*, *Achromobacter*, *Alcaligenes*, *Azotobacter*, *Rhizobium*, *Sarcina*, *Salmonella*, *Escherichia*, *Komagataeibacter*, etc., have been reported to produce cellulose (Cheng et al., 2009; Lin et al., 2016). Among these, *Acetobacter xylinum* is highly investigated for biochemical synthesis of bacterial cellulose and to the best of our knowledge, hardly any other species of this genus has been reported for cellulose production till date (Matsuoka et al., 1996; Lestari et al., 2014).

Because of the unique properties, resulting from the ultrafine reticulated structure, bacterial cellulose has multitude application in paper, textile and food industries and as a biomaterial in cosmetics and medicine, artificial skin, artificial blood vessels, high fidelity speakers and multifunctional sheets (Rainer and Luiz, 1998; Rezaee et al., 2005). Bacterial cellulose is also used extensively in wound healing and tissue engineering. Despite the purity and high quality of bacterial cellulose, its production, processing and uses are relatively low due to the fact that lack of efficient cellulose producer, low productivity, difficulty in scale up production and high cost of production, limits its commercial applications (Farag et al., 2016). Hence, search for newer bacterial cellulose producing strain is indispensable for cellulose production.

It appears that cellulose production by bacteria is strictly linked with cellular metabolism and culture conditions, particularly carbon and nitrogen sources, temperature, pH and aeration (Chawla et al., 2009). To increase the production of bacterial cellulose, researchers have tried various combination of physico-chemical and biochemical parameters in the culture medium (Chawla et al., 2009). In view of the above, the present study was carried out to isolate efficient bacterial cellulose producing bacterial strain and maximize its production capacity by optimizing nutrient composition of growth medium.

Materials and Methods

Isolation of cellulose producing bacteria : Cellulose producing bacteria was isolated by using Hestrin–Schramm medium (Hestrin and Schramm, 1954). After 2 weeks of incubation, the bacterial culture was isolated from the mat formed on the surface of broth and purified by four streak method and preserved in 50%

glycerol at -20°C for further use. The morphological characters of cellulose mat producing bacteria were carried out by following the method of Gerhardt et al. (1981).

Molecular characterization and evaluation of phylogenetic relationship: Total genomic DNA from cellulose producing bacterial isolates was extracted using HiPurA™ bacterial genomic DNA purification kit (Himedia, India). The isolated DNA were characterized and identified by 16S rRNA gene sequencing using universal primers, 27F (5' AGAGTTTGATCMTGGCTCAG 3') and 1492 R (5' TACGGYTACCTTGTACGACTT 3') as described by Anandham et al. (2008).

Purification of bacterial cellulose mat : Hyper cellulose producing bacterial isolate MA1 was inoculated in HS medium incubated for 2 weeks under static condition at 28±2°C. The mat was removed carefully, boiled in 2% NaOH solution for 60 min and thoroughly washed 2-3 times with distilled water. Further, cellulose mat was dried in hot air oven at 65°C for 24 hr and dry weight was measured (Hungund and Gupta, 2010). Purified mat was subjected to further characterisation.

Scanning Electron Microscope and Fourier Transform Infrared Spectroscopy analysis of bacterial cellulose : In order to observe morphology of bacterial cellulose, the mat treated with NaOH was dried completely, to remove the moisture coated with gold using an ion sputter coater and fiber of cellulose mat was observed under SEM operated at 20 kV (Bruker, USA). FT-IR spectrum revealed that the composition of cellulose mat was studied in comparison with carboxy methyl cellulose (CMC). Dried bacterial cellulose was finely powdered and a 5 mg of sample was taken for FT-IR analysis (Jasco, Japan). The analysis was carried out at 20°C in anhydrous condition with air as background. Thirty two scans at 2 cm⁻¹ resolution were collected for the sample in the scanning range of 4000-400 cm⁻¹ wavelength (Usha Rani and Anu Appaiah, 2013).

Dried bacterial cellulose was hydrolysed with 2 ml of 90% formic acid at 105°C in a sealed tube for 6 hr. Excess acid was removed by flash evaporation on a water bath at 40°C (Farag et al., 2016). Dried powder was collected and diluted with distilled water, the sample was then injected into HPLC. Acetonitrile and water was used as mobile phase (80:20) at a flow rate of 2 ml min⁻¹. Energy dispersive analysis of X-ray was used to determine the chemical structure of cellulose mat. The experiment was performed in parallel with SEM analysis and detected by Energy Dispersive Analysis of X-ray mode in SEM operated at 20 kV (Bruker, USA). The melting behavior of bacterial cellulose was investigated using Differential Scanning Calorimeter in Helium atmosphere (NETZSCH, German).

Thermal degradation behavior of bacterial cellulose was characterised using Thermal Gravimetry Analysis (NETZSCH, German). A sample size of approximately 6 mg was used and heated from room temperature to 550°C at the rate of 10°C min⁻¹. Nitrogen atmosphere was used for characterisation to provide an

inert atmosphere (Lee *et al.*, 2009). Thermal behavior of bacterial cellulose was assessed based on the developed peak. The effect of different pH (4, 4.5, 5, 5.5, 6, 6.5, 7, 7.5 and 8) on production of bacterial cellulose by *A. senegalensis* MA1 was studied in the HS medium. To study the effect of different carbon sources on bacterial cellulose production, sucrose, glucose, fructose, maltose, inositol, glycerol and mannitol were used at 2% concentration instead of glucose. To investigate the effect of nitrogen source on bacterial cellulose production yeast extract, peptone, ammonium sulphate, sodium nitrate and yeast extract plus peptone (1:1) were added at 1% in HS medium (Farag *et al.*, 2016).

To determine the optimum level of carbon source (glycerol) and nitrogen source (yeast extract) for maximum cellulose production, Response Surface Methodology was followed. RSM was employed in a central composite design (CCD). The range and level of selective medium components (glycerol and yeast extract) investigated in this study are as follows

	Names	Units	Low	High	-alpha	+alpha
A Numerical	Glycerol	g	0.1	10	-3.27817	12.2782
B Numerical	Yeast extract	g	0.05	2.5	-0.457412	3.00741

Trial for 13 sets of media with different concentration of components were inoculated with 5% of *A. senegalensis* MA1 and incubated at $28 \pm 2^\circ\text{C}$ for 14 days under static condition. The following second order polynomial equation was adopted to study the effect of variable to the response.

$$Y = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_{11} X_{12} + \beta_{22} X_{22} + \beta_{12} X_1 X_2$$

where, Y is the response (cellulose yield, g l⁻¹), β_0 is the constant term, $\beta_1, \beta_2, \beta_3$ are the coefficient of linear terms and $\beta_{11}, \beta_{22}, \beta_{33}$ are the coefficient of quadratic terms and β_{12}, β_{23} are

coefficient of cross product terms. X_1 and X_2 represent the factors for glycerol and yeast extract. Analysis of variance (ANOVA) was adopted to determine statistical adequacy of model test. Overall model significance was validated using Fischer's F- test and its associated probability. The quality of polynomial model equation was judged statistically through coefficient of determination (R^2) and adjusted R^2 . Three dimensional response surface plots were drawn to illustrate the relationship between the response and experimental level of each independent variable.

Results and Discussion

In the present study, eight bacterial strains producing bacterial cellulose designated, S5D, S5D₁, S6M, S6M₁, S6M₄, S6M_A, S7B and MA1 were isolated from sugarcane juice in HS broth maintained at pH 6.0 after 14 days of incubation. Isolation of similar cellulose producing bacterial strains from different sources such as citrus fruit juice (Kim *et al.*, 2017), rotten apple (Hungund and Gupta, 2010), temple wash water (Raghunathan, 2013), rotten fruits (Son *et al.*, 2002) and coffee cherry husk (Usha Rani and AnuAppaiah, 2013) have been reported. The phenotypic properties of cellulose producing strains isolated in this study is summarized in Table 1.

The result of 16S rDNA sequence analyses report revealed that selected cellulose producing bacterial strains belonged to two different genera. Among the eight isolates, seven strains MA1, S6M₄, S6M, S6M_A, S6M₁, S5D and S7B exhibited 100% 16S rDNA sequences similar to *Acetobacter senegalensis*, *A. thailandicus*, *A. lambici* and *A. lovaniensis*, respectively (Table 2) whereas S5D1 exhibited 99.52% 16S rDNA sequence similar to *Acinetobacter baumannii*. After 2 week of incubation, all the cellulose producing bacterial isolates formed bacterial cellulose mat on the top of HS broth. Among them, *A. senegalensis* MA1 produced maximum cellulose mat and its dry weight was 0.72g 100 ml⁻¹ of HS broth (Table 2; Fig. 1). Similar observations were

Table 1: Phenotypic properties of cellulose producing bacterial strains isolated in this study

Isolates	Fermentation of carbohydrate						IMVIC test			Hydrolysis of carbohydrate			
	Glucose		Sucrose		Lactose		Indole production	MR	VP	Citrate	Starch	Gelatin	CMC
	Acid	Gas	Acid	Gas	Acid	Gas							
S5D	+	—	+	—	—	—	+	—	—	—	—	—	—
S5D ₁	+	—	+	—	—	—	+	—	—	—	—	—	—
S6M	+	—	+	—	—	—	+	—	—	—	—	—	—
S6M ₁	+	—	+	—	—	—	+	—	—	—	—	—	—
S6M ₄	+	—	+	—	—	—	+	—	—	—	—	—	—
S6M _A	+	—	+	—	—	—	+	—	—	—	—	—	—
S7B	+	+	+	—	—	—	+	—	—	—	—	—	—
MA1	+	+	+	—	—	—	+	—	—	—	—	—	—

+, Positive; -, Negative; VP - Voges Proskauer; MR - Methyl red; CMC - Carboxyl methyl cellulose

Fig. 1: Morphological image of bacterial cellulose mat produced by *Acetobacter senegalensis* MA1 treated with NaOH at different magnifications under Scanning Electron Microscope. Wet (A) and Dry (B) cellulose mat produced by *Acetobacter senegalensis* MA1; Cellulose mat magnified up to 298 x (C); 2.32 kx (D); 6.33 kx (E) and 69.2 kx (F).

Table 2 : Taxonomic identification and cellulose production of bacterial strains isolated in this study

Isolates	Closest neighbour	Sequence length (bp)	% Similarity	NCBI accession No.	Dry weight of cellulose mat (g 100 ml ⁻¹)
S5D	<i>Acetobacter lambici</i> LMG 27439 ^T	1422	100	MG279708	0.144 ± 0.01
S5D ₁	<i>Acinetobacter baumannii</i> ATCC 19606 ^T	1495	99.52	MG279709	0.162 ± 0.02
S6M	<i>Acetobacter senegalensis</i> LMG 23690 ^T	1433	100	MG279702	0.300 ± 0.01
S6M ₁	<i>Acetobacter thailandicus</i> Ad25 ^T	1402	100	MG279703	0.122 ± 0.004
S6M ₂	<i>Acetobacter senegalensis</i> LMG 23690 ^T	1430	100	MG279704	0.360 ± 0.03
S6MA	<i>Acetobacter estunensis</i> LMG 1626 ^T	1439	100	MG279705	0.136 ± 0.01
S7B	<i>Acetobacter lovaniensis</i> LMG 1617 ^T	1438	100	MG279706	0.152 ± 0.06
Ma1	<i>Acetobacter senegalensis</i> LMG 23690 ^T	1370	100	MG279707	0.720 ± 0.01

Table 3 : Analysis of variance (ANOVA) for quadratic model used in response surface methodology

Source	Sum of squares	Df	Mean square	F-value	p-value Prob> F
Model	9.30	5	1.86	48.49	< 0.0001
A-glycerol	2.90	1	2.90	75.63	< 0.0001
B-yeast extract	3.77	1	3.77	98.24	< 0.0001
AB	1.640E-003	1	1.640E-003	0.043	0.8421
A2	4.25	1	4.25	110.79	< 0.0001
B2	6.09	1	6.09	158.83	< 0.0001
Residual	0.27	7	0.038		
Lack of Fit	0.11	3	0.038	0.97	0.4877
Pure Error	0.16	4	0.039		
Cor Total	9.57	12

Fig. 2: FT-IR spectrum of bacterial cellulose (produced by *Acetobacter senegalensis* MA1) in comparison with standard CMC. Red and Green lines indicate CMC and bacterial cellulose.

Fig. 3: HPLC analysis of cellulose mat produced by *Acetobacter senegalensis* MA1 (A) and Carboxy methyl cellulose (B) Elemental composition analysis of fresh bacterial cellulose mat (C) and 2% NaOH treated mat (D) in Energy Dispersive Analysis X-ray (EDAX); Thermal decomposition behaviour of bacterial cellulose mat in Thermogravimetric Analysis (E); Analysis of crystallization and melting behaviour of cellulose mat in Differential Scanning Calorimetry (F).

made by Son *et al.* (2001) and Farag *et al.* (2016) for *Acetobacter* sp. A9. Kiziltas *et al.* (2015) studied the production of bacterial cellulose by *G. xylinum*; their results showed that the maximum production (0.15 g l^{-1}) occurred at pH 8 and 28°C using a medium formulated with a residual material originating from pulp mills and lignocellulosic biorefineries. The quantity of bacterial cellulose produced by *A. senegalensis* MA1 in the present study was higher compared to other studies (Son *et al.*, 2001; Kiziltas *et al.*, 2015; Farag *et al.*, 2016).

Bacterial cellulose produced by *A. senegalensis* MA1 had a pellicle shaped distinguished network structure. The rod shaped bacterium was clearly observed at 9.11 KX magnification which confirmed that bacterium was attached at the bottom of cellulose mat (Fig. 1). Earlier, Farag *et al.* (2016) used SEM to monitor morphology and bacterial cellulose pellicles structure of *Achromobacter* sp. M15. The bacterial cellulose produced by *Gluconacetobacter oxydans* showed distinguished network structure and composed of all the components as that of pure

Fig. 4: Surface and contour plots showing interaction effect between glycerol and yeast.

cellulose without any other impurities in SEM analysis (Yamanaka *et al.*, 2000). Clear network of cellulose fibres produced in bacterial cellulose indicated micro-structure similarity with cellulose materials. FT-IR spectrum was used for the confirmation of bacterial cellulose mat produced by *A. senegalensis* MA1 and compared with carboxy methyl cellulose (Fig. 2). The absorption peak at 3334 cm^{-1} in bacterial cellulose and 3292 cm^{-1} in carboxy methyl cellulose spectrum showed broad vibration of OH groups in cellulose molecules, another absorption peak at 2918 cm^{-1} in bacterial cellulose, 2919 cm^{-1} in carboxy methyl cellulose indicated the stretching vibration of C-H bonding. The absorption peak at 1588 cm^{-1} for both bacterial cellulose and carboxy methyl cellulose, revealed the presence of carbonyl group (C=O). The absorption peak at 1413 cm^{-1} in bacterial cellulose and 1438 cm^{-1} in carboxy methyl cellulose showed CH_2 bonding, whereas absorption peak at 1322 cm^{-1} in bacterial cellulose confirmed CH deformation.

The peak obtained from bacterial cellulose was almost similar to carboxy methyl cellulose peak. Hence, the mat produced by *A. senegalensis* MA1 was confirmed as cellulosic mat. Similarly, Farag *et al.* (2016) described that the FT-IR spectra of bacterial cellulose, where the absorption peak at $3427\text{--}3438\text{ cm}^{-1}$ spectrum as broad band indicated free O-H stretching vibration of OH groups in cellulose molecules. Another absorption peak at 2856 cm^{-1} and 2922 cm^{-1} and $1436\text{--}1447\text{ cm}^{-1}$ showed stretching vibration of C-H and CH_2 , respectively. Bacterial cellulose mat produced by *A. senegalensis* MA1 was subjected to

HPLC analysis and compared with carboxy methyl cellulose (Fig. 3). The peaks obtained from the compounds were more or less similar in height. The first peak was noted at 1.8 min carboxy methyl cellulose and 1.76 min in bacterial cellulose mat. Similarly, the second was obtained at 2.53 min carboxy methyl cellulose and 2.5 min in bacterial cellulose mat. The third peak was observed at 3.03 min carboxy methyl cellulose and 3.45 min in bacterial cellulose mat respectively. Previously, the monosaccharide of bacterial cellulose hydrolysate of *Acromobacter* sp. M15 was measured by HPLC by Farag *et al.* (2016) where glucose was the major sugar (98.75%). In the present study, the bacterial cellulose mat produced by MA1 was similar to carboxy methyl cellulose.

The energy dispersive analysis of X-ray was performed to determine the elements present in bacterial cellulose mat produced by the isolate MA1. In treated bacterial cellulose mat, the bacterial cells might have been removed completely and exhibited a proportion of carbon (60.02%) and oxygen (36.83%) (Fig. 3). In addition, the presence of sodium, calcium, magnesium, silicate and phosphorus in bacterial cellulose mat was observed, while in untreated cellulose mat the weight of the elements such as carbon, oxygen, sodium, phosphorus, potassium and calcium were 60.13, 25.12, 5.6 and 1-2%, respectively. Sofla *et al.* (2016) reported that the EDX of cellulose nano crystal showed peaks for carbon, oxygen and other elements corresponding to their binding energies. cellulose nano crystal (CNC) contained 0.2% sulphur impurity with carbon (65.3%) and oxygen (31.4%) as main component.

The crystallisation and melting behaviour of bacterial cellulose mat was carried out by Differential Scanning Calorimetry. The endothermal peak at 139.8°C indicated the melting point of bacterial cellulose mat. Whereas a broad exothermic peak around 330°C could be attributed to partial pyrolysis due to fragmentation of carbonyl and carboxylic bonds from anhydrous glucose units giving carbon or monoxide carbon. An exothermic peak observed around 200°C was assigned to cellulose acetate crystallisation. Zhang *et al.* (2011) studied that the crystallisation and melting behavior of bacterial cellulose *A. xylinum* through Differential Scanning Calorimetry was in accordance with the present study. Thermal decomposition behavior of bacterial cellulose mat was examined by thermogravimetric analysis (Fig. 3). The change in initial mass at lower temperature ranging from 200°C to 360°C was attributed to the removal of small molecular fragments such as hydroxyl and methyl hydroxyl group. The second mass change ranging between 360°C to 600°C showed degradation of polymeric chains and six member cyclic structure, pyran. The vertical line indicated changes in sample from the original state further gradually decreased till 500°C. Thermal gravimeter spectrum of bacterial cellulose showed a weak loss of weight due to evaporation of water (85°C) and also a quick drop in weight at 300°C and complete degradation of cellulose took place between 275 and 400°C (Deepa *et al.*, 2011). Thermal gravimeter curve showed the maximum rate of degradation at 370°C for bacterial cellulose/cane sugar. However, bacterial cellulose/honey possessed higher degradation at 450°C mainly due to higher crystalline rate. These results clearly evidenced higher thermal behaviour with developed bio-nanocomposites than pure bacterial cellulose mat (Zhang *et al.*, 2011). The pH of the culture medium is an important factor that affects cell growth and production of bacterial cellulose mat. The maximum bacterial cellulose production (2.1 g f.wt. with 0.07g d.wt. per 100ml medium) was obtained at pH 5. However, the maximum cellulose yield was also reported at pH 7.0 (Raghunathan, 2013). The optimum pH of the culture medium for bacterial cellulose production ranged from 4.0 to 6.0 and cellulose yield decreased below pH 4. Similar to our study, Son *et al.* (2001) reported high cellulose production at broader pH range 4.5 - 7.5, with maximum production at pH 6.5. Loose mat was formed with carbon, sucrose, except lactose. In all the carbon sources tested, except lactose, the cellulose mat was formed. Among these carbon sources glycerol produced maximum bacterial cellulose (1.5 g wet weight and 0.11g dry weight per 100 ml medium). Similarly, mannitol was found to be the best carbon source as it produced the maximum cellulose (1.38 g l⁻¹) in *Acetobacter* sp. (Raghunathan, 2013). Fructose was found to be the best carbon source at 2% concentration for the production of bacterial cellulose by *A. xylinum* (Son *et al.*, 2002).

Various nitrogen sources were used in combination to study the influence on the production of cellulose mat. Among these, yeast extract alone as well as yeast extract in combination with peptone (1:1) produced higher bacterial cellulose yield of 0.14 g and 0.08 g of d.wt. per 100 ml medium.

Cellulose-producing bacteria also need a particular complex nitrogen source as it is the main component of protein necessary for cell metabolism, and comprises 8-14% of dry cell mass of bacteria. The effect of various nitrogen sources on the production of bacterial cellulose has already been reported (Matsuoka *et al.*, 1996). Peptone supported the maximum cellulose yield (0.86 g l⁻¹), when compared to the rest of the nitrogen sources evaluated (Raghunathan, 2013). To optimise carbon (glycerol) and nitrogen (yeast extract) source for maximum bacterial cellulose production, a central composite design consisting a set of 13 experiments was conducted.

The model was highly significant as evident from F-value 48.49 and very low P value < 0.0001 (Table 3). The insignificant lack of fit value (0.11) of the model suggested that the obtained experimental data were good. The predicted R² of 0.8904 was in reasonable agreement with the adjusted R² value of 0.9519. Moreover, R² value of 0.9719 indicated that the model could explain 97.4% of total variations. Response Surface Methodology graph exhibited the maximum dry weight of cellulose mat (2.04 g), which was obtained by adding 5.05 g glycerol and 1.275 g yeast extract per 100 ml of Hestrin and Schramm medium as carbon and nitrogen sources, respectively (Fig. 4). Glycerol is one of the low-cost substrate for fermentation and is effectively utilized by *A. senegalensis* MA1, in addition substrate to product conversion ratio was found higher. Recently, Agustin *et al.* (2018) reported the maximum bacterial cellulose production using 4.95 % glycerol as a cheap carbon source. Similarly yeast extract was found effective nitrogen source in bacterial cellulose production. A significant improvement in cellulose synthesis by *A. senegalensis* MA1 may have future implications in large scale production of bacterial cellulose. In addition, research should be focused on the use of agricultural and industrial waste products as a low cost substrate for the production of bacterial cellulose to maximise the profit.

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