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Characterisation of Cellulose produced by *Pseudomonas sp*. and *Actinomycetes sp*.

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ABSTRACT

The demand for cellulose in paper making process is accelerating at an alarming rate. Alternate sources of cellulose has to be traced in order to meet this demand. In this study, Pseudomonas sp. and Actinomycetes sp., isolated from the forest soil was tested for their potential to produce cellulose. Characterisation of cellulose produced by Pseudomonas sp. and Actinomycetes sp., by FTIR and SEM indicate that Pseudomonas sp. and Actinomycetes sp., produce cellulose. Furthermore, culture conditions has to be optimized for enhancing the production of bacterial cellulose.

Key words; Cellulose, SEM, FTIR, *Pseudomonas sp*. *Actinomycetes sp*.,

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INTRODUCTION

The ever increasing industrial demand for cellulose from the higher vascular plants for paper making process could disturb the delicate ecological balance of the earth. Thus, alternative sources of cellulose have to be developed. Bacterial cellulose (BC), an exopolysaccharide produced by some bacteria are highly pure with unique, structural and mechanical properties of higher degree of polymerization and crystallinity index [1,2]. Several bacteria are known to produce cellulose, such as those produced by *Gluconacetobacter genus*[3,4], *Acetobacter aceti* [5], *Acetobacter xylinum* [6,7,8]. The present study was an attempt to characterise the cellulose produced by *Pseudomonas sp*., and *Actinomycetes* sp., isolated from forest soil through FTIR and SEM studies .

MATERIALS AND METHODS

BC Production and Culture condition

Pre inoculum preparation

Pseudomonas sp. and *Actinomycetes sp*., isolated from the forest soil were mass cultured in nutrient broth separately. From the mass culture, 1ml of nutrient broth culture of *Pseudomonas* sp. (76 $X10^9$ cfu/ ml) and *Actinomycetes sp*., (53 $X10^9$ cfu/ ml) were separately inoculated into the Hestrin – Schramm medium (HS) (2 % glucose, 0.5 % yeast extract, 0.5 % polypeptone, 0.675 %, NaH_2PO_4 .$12H_2O$, 0.115 % citric acid monohydrate with pH 6.0) [9] for a period of 21 days. After 21days, gelly like cellulose was produced by *Pseudomonas sp*.,. The developed gelly-like cellulose was first purified by washing with deionized (DI) water and then was treated with 1% (w/v) NaOH (sodium hydroxide) at 35 ^{0}C for 24 hours to remove bacterial cells and rinsed with DI water until the pH was 7. The purified sheets were then air dried at room temperature and stored in plastic film. *Actinomycetes sp*., produced cellulose was in the form of crystals. It was purified by washing with deionized water and treated with 1 % (w/v) NaOH at 35 ^{0}C for 24 hours to remove bacterial cells and mixed with DI water until

the pH was 7. These purified crystals were then dried at room temperature and stored in eppendorf tubes.

SEM Analysis
Scanning electron microscopy (SEM- VEGA3 TESCAN) was conducted to observe sample morphology. Samples were sputter coated with gold. The image were taken at 10 Kv accelerated voltage with magnification 1kx to 6 kx.

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.

RESULT

As evinced is this study, strong absorption peaks (2924.73 cm^{-1} and 2855 cm^{-1}) were registered in the FTIR spectra of cellulose obtained from *Pseudomonas sp.,* (Table-1, Fig-1). These peaks have been related to C-H bond stretching. Similar peaks (2925 cm^{-1} and 2856 cm^{-1} attributed to C-H bond stretching) was also observed in the FTIR spectra of cellulose produced by *Actinomycetes* sp., [10] (Table 2, Fig 2).The appearance of absorptions band at 3418 cm^{-1} have been attributed to inter-molecular hydrogen bonds for 6-OH..O-3^{l} [10]. Goelzer et al., have recorded absorption peak in 3000 cm^{-1}-3600 cm^{-1} region in the FTIR spectra of cellulose produced by *Acetobacter xylinum* and have assigned it to O-H stretching frequencies of cellulose [11]. In cellulose crystals, the conformation of the cellulose chains, as well as their strong packing, depends on intermolecular and intra-molecular hydrogen bonds [12].

According to the hydrogen – bond patterns proposed by Gardner and Blackwell there are two classes of hydrogen bonds in cellulose I, the intra molecular hydrogen bond for 2-OH… O-6 and 3- OH… O-5 links, and the inter – molecular hydrogen bonds for 6-OH… 0-3^{l}[13]. The absorption peaks at 2925 cm^{-1} and 1453 cm^{-1} have been assigned to C-H stretching and C-H bending vibration, respectively[14]. The absorption peak around 1117 cm^{-1} (table 1), another signature band of cellulose, corresponds to C-C bonds of monomer unit of polysaccharide [15]. The signature band at 2924 cm^{-1} (table 1) have been attributed to C-H bond stretching vibration, which confirms amorphous characteristic of cellulose [15,16]. The FTIR spectra of cellulose produced by *Actinomycetes sp.,* indicates absorption band at 2925 cm^{-1} and 2856 cm^{-1}, which corresponds to anti symmetrical and symmetrical CH_2 stretching vibration of non- cellulose polysaccharides, respectively (table 2) [17] . 3008 cm^{-1} to 3418 cm^{-1} region correspond to the O-H stretching frequencies of cellulose. The absorption peak was observed at 2925 cm^{-1} and 1453 cm^{-1} for C-H stretching vibration and C-H bending vibration , respectively (table 2) [14]. Existence of characteristic bands at 1110 cm^{-1} indicates signature band of cellulose , which have been assigned to C-C bonds of the monomers units of polysaccharides [15]. The intra- molecular hydrogen bonding of O (2) H… 0(6) and 0(3)H… 0(5), in cellulose are shown at 3455 cm^{-1} – 3410 cm^{-1}. In the FTIR spectrum of cellulose produced by *Actionomycetes sp*., the peaks at 3418 cm^{-1} and 2925 cm^{-1} correspond to O-H and CH_2 stretches, respectively [16].

Table -1 Band assignment of FTIR spectra of Cellulose produced by *Pseudomonas sp.,*

Wave numbers (cm^{-1})	Functional groups	Relative intensity	References
3782	OH	W	Sliverstein et al [18]
3409	OH	M	Sliverstein et al [18]
2924	C-H	W	Dayal et al [15] ; Qin et al .,[16]
2855	va (CH_2)	W	Oh et al [19] ; Movasaghi et al.,[20]
2102	C=C	VW	Sliverstein et al., [18]
1580	C-C	M	Sliverstein et al., [18]
1404	C-H	M	Sharma [21]
1320	Benzene ring with mixed C-H in Plane bending	W	Movasaghi et al.,[20]
1117	C-C	W	Dayal et al., [15]
717	C-H	W	Sliverstein et al [18]

W-weak ; M- Medium; VW – Very Weak

SEM analysis
Cellulose produced by *Pseudomonas sp*., (Fig -3) was jelly like and that of *Actinomycetes* sp., was in the form of

crystal (Fig-5).The SEM images of BC membranes synthesised by *Pseudomonas sp*. and *Actinomycetes* sp., indicate that they are intertwined into a cross linked three - dimensional network (Fig 4, 4a, 6 ,6a).

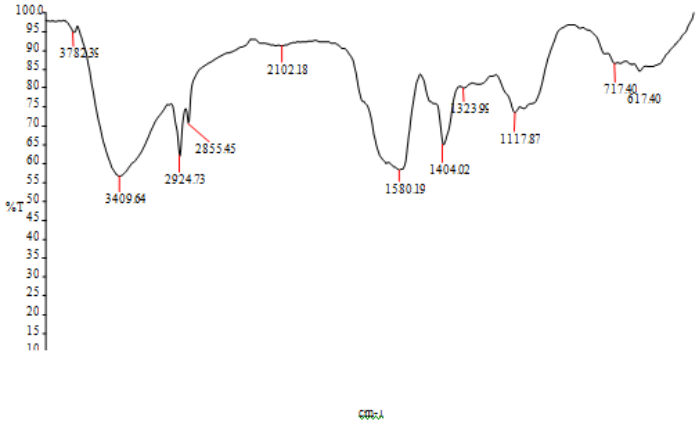

Fig – 1 FTIR spectra of Cellulose produced by *Pseudomonas sp.*,

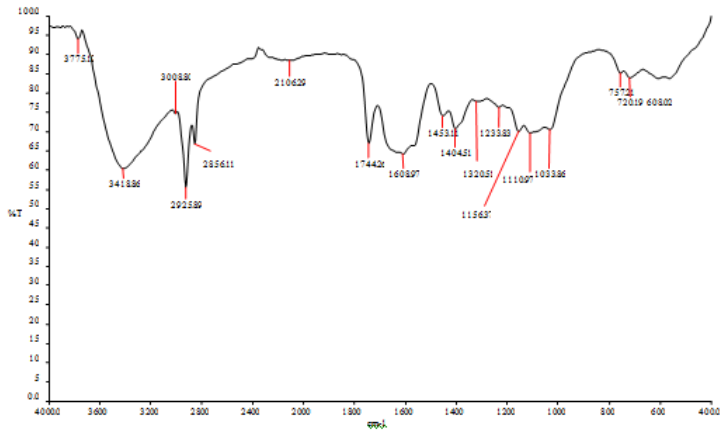

Fig 2 FTIR spectra of Cellulose produced by *Actinomycetes sp.*,

Table-2 Band assignment of FTIR spectra of Cellulose produced by *Actinomycetes sp.*

Wave numbers (cm^{-1})	Functional group	Relative intensity	Reference
3775	O-H	W	Sliverstein et al [18]
3418	ν(N-H)free	M	Oh et al., [19]; Movasaghi et al.,[20] ; He et al.,[10] ; Qin et al .,[16]
3008	OH	VW	Silverstein et al ., [18]
2925	ν(CH_2)	M	Oh et al .,[19] ; Movasaghi etal.,[20]; Omer Shezad et al., [14] ; He et al.,[10] ; Qin et al .,[16]
2856	ν(CH_2)	W	Oh et al .,[19] ; Movasaghi et al.,[20] . He et al.,[10]
2106	C=C	VW	Silverstein et al .,[18]
1744	C=O	W	Silverstein et al ., [18]
1608	C-C	W	Silverstein et al ., [18]
1453	C-H Stretching	W	Omer Shezad et al., [14]
1404	C-H	W	Sharma [21]
1320	Benzene ring with C-H in Plane bending	W	Movasaghi et al.,[20]
1233	C-O-C	W	Silverstein et al ., [18]
1156	va(C-O-C),CH deformation	W	Movasaghi et al.,[20] ;Kacurakora et .al.,[21]
1110	va (C-C) ring (Polysaccharides ,cellulose)	W	Movasaghi et al.,[20] ; Dayal et al.,[15]

1033	O-C-O	W	Silverstein et al ., [18] ; Omer Shezad et al., [14]
757	C-H	W	Silverstein et al ., [18]
720	C-H	W	Silverstein et al ., [18]

W-weak ; M- Medium; VW – Very Weak

Fig -3. **Fig -4.** **Fig -4a.**

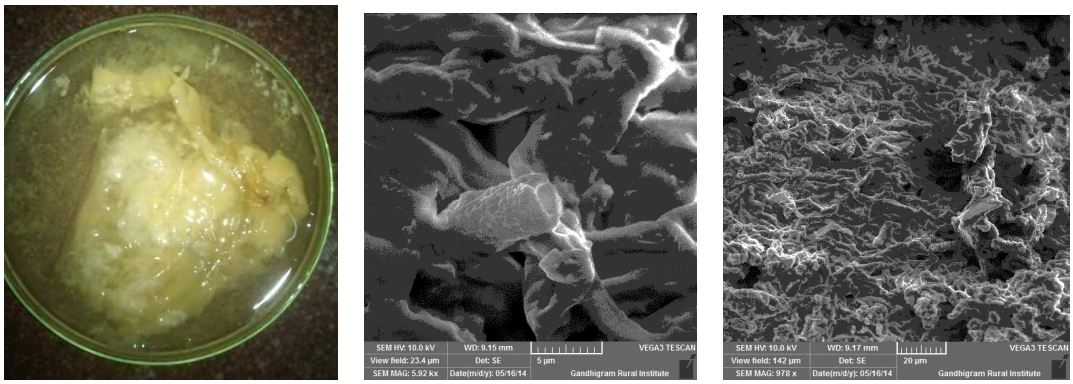

Fig-3: Jelly like Cellulose produced by *Pseudomonas sp*.,
Fig-4 and 4a: SEM image of cellulose produced by*Pseudomonas sp*.,

Fig -5 **Fig-6** **Fig-6a**

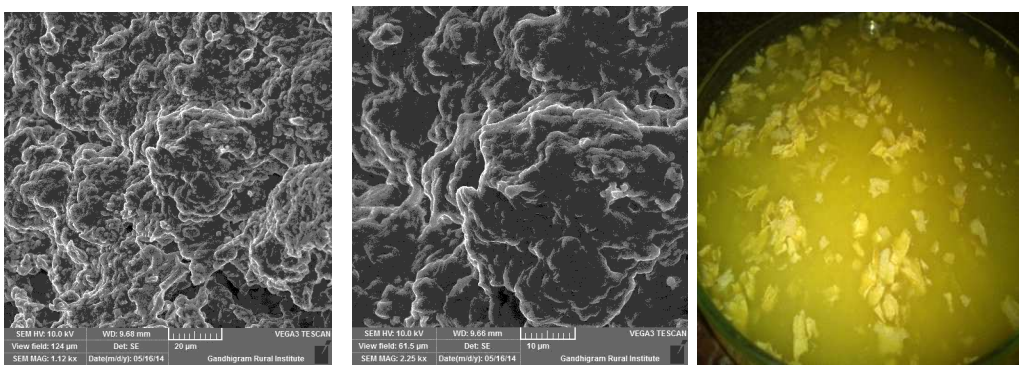

Fig -5: Cellulose crystals formed in HS medium by *Actinomycetes* sp.,
Fig-6 and 6a : SEM image of cellulose produced by *Actinomycetes* sp.,

DISCUSSION

Similar absorption bands were recorded by He et al., in the FTIR spectra of cellulose obtained from bamboo and ramie fibers (2900.5 cm^{-1}) and cotton exhibited doublets at 2894.7 cm^{-1} and 2917.8 cm^{-1}. On the other hand, they noticed triplet at 2902.5 cm^{-1}, 2917.8 cm^{-1} and 2852 cm^{-1} absorption peak in the FTIR spectra of cellulose produced by flax fibers. Furthermore, they have assigned these peaks to C-H stretching vibrations of cellulose, and the antisymmetrical and symmetrical CH_2 stretching vibration of non- cellulose polysaccharides, respectively [10].

Carreira et al., have also produced BC from *Gluconacetobacter sacchari* using HS medium, with glucose as carbon source [4]. As evinced in the study, Ananda Putra et al., have also observed that BC gel produced on both smooth flat PDMS (Polydimethylsiloxane) mold and on ridge PDMS mold showed a metallic glossy surface that comes from light interference [23]. Basavary et al., have demonstrated that the most preferred carbon source of BC biosynthesis are glucose and fructose [24]. Further, they also reported that BC produced by strain GH-2 (*Gluconacetobacter persimmonis*) was in the form of stellate and irregular masses under agitated culture conditions. Gomes et al., reported typical homogeneous tridimensional network of nano and microfibrils of

cellulose produced by *Glucanocetobacter sacchari* using dry olive oil residue. Further, they have reported that the FTIR spectra of cellulosic substrates displayed strong bands at around 3300 cm^{-1}, 2880 cm^{-1} and 1100 cm^{-1} and have associated it to vibration of OH,CH and C-O-C groups of cellulose , respectively [25].

Takayasu Tsuchida and Fumihiro Yoshinaga have demonstrated that *Acetobacter xylinum subsp*. *Sucrofermentans subsp*.nov could be used to produce bacterial cellulose on agitated culture. Further, they have reported that CSL (corn steep liquor) was the most suitable organic nitrogen source for BC production [26]. Gao et al., have also observed absorption bands at 3416 cm^{-1} (-OH stretching) and 2921 cm^{-1} (-CH_2 stretching) in the FTIR spectra of native BC [27].

As observed in this study, Tanskul et al., have also reported for the first time the production of cellulose by *Rodococcus sp*. MI 2 for a period of 14 day inoculation under static, agitated and mixed condition. Further, they have reported that cellulose produced by *Rhodococcus sp.*, was in the form of small granules with few irregular shapes under agitated conditions [28].

CONCLUSION

This study reveals that bacteria could be an alternate option for production of cellulose. Hence, it could reduce the plant cellulose demand in paper making process..

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