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Studies on cellulose producing *Gluconacetobacter xylinum* isolated from fermented sugarcane juice

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ABSTRACT

Microbial cellulose synthesised by *Gluconacetobacter xylinum* is a new biopolymer with a high tensile strength and thermal stability. It is expected to be a new industrial material for the production of cellulose based products. Considering this, a study was undertaken to isolate elite cellulose producing *G. xylinum* strain from various sugary rich sources at TNAU, Coimbatore during 2010-2012. Cellulose producing strain was isolated, characterised and selected based on the pellicle type, colony morphology and amount of cellulose production. The selected strain *G. xylinum* 'sju-1' isolated from high sucrose containing sugarcane variety 'Co1148' had a stable and consistent production of cellulose pellicle. *G. xylinum* 'sju-1' produced 14.10 g / L of cellulose with thickness of 13 mm. On the other hand, *G. xylinum* NCIM 2526 produced only 11.40 g/L of cellulose. The weight of the cellulose produced by 'sju-1' isolate was found to be significant at $p<0.05$ when compared to the cellulose produced by *G. xylinum* NCIM 2526. The higher water holding capacity (85.50 %) and crude fibre content (11.25 g) of cellulose produced by *G. xylinum* 'sju-1' clearly indicated that the product was highly juicy and fibrous in nature. The results of the present investigation proved that the sugarcane juice naturally harbours *G. xylinum*. Therefore, the isolated strain 'sju-1' can be exploited for high quality cellulose manufacturing at the industrial level by mass production using sugarcane industry wastes also.

Keywords: Bacterial cellulose, *Gluconacetobacter xylinum,* Sugarcane juice, Fermentation.

Gluconacetobacter xylinum (formerly *Acetobacter xylinum*) is one of the nature's most prolific cellulose producing bacteria. It is found in soil, rotten fallen fruits, sometimes in symbiosis with plants such as sugarcane or coffee plants. Bacterial cellulose fibrils are highly amorphous and free from lignin and hemicelluloses. Many potential high value markets exist for thin film bacterial cellulose, including acoustic diaphragms, artificial skin, speciality papers, food thickeners, organic dietary fibre food *nata*, artificial blood vessels, liquid loaded medical pads, super-sorbers and speciality membranes (Thompson and Hamilton 2001).

Cellulose producing *Acetobacter* has been isolated from rotting mango fruits, guava, custard apple, chikku, citrus, oranges, dates, mulberry, apple, radish, tomato beer, *Kombucha* tea, malt vinegar and from fermenting vinegar. Kim *et al*. (2007) isolated *Gluconacetobacter* sp. RKY5 from persimmon vinegar and optimized the conditions for maximum cellulose production. Park *et al*. (2003) has isolated sulfaguanidine resistant mutants of *Acetobacter xylinum* subsp. *sucrofermentans* from rotten apples. *G. xylinum* previously characterized for cellulose production has been isolated from fruit sources and isolates from sugarcane juice have not been extensively characterized. Previously studied strains from fruit have failed in long-term subculturing under static conditions (Lisdiyanti *et al*. 2001). The scale of bacterial cellulose production, processing and use is relatively small because of problems associated with the selection of sufficiently efficient producers. Hence, a study was undertaken to isolate cellulose producing *G. xylinum* from high sucrose containing sugarcane variety 'Co1148 ' possessing an elite Brix value categorisation of 21.59 collected from Sugarcane Breeding Institute (SBI), Coimbatore, Tamil Nadu.

MATERIALS AND METHODS

Isolation of cellulose producing bacteria

The present investigation on the production of cellulose by *G. xylinum* 'sju-1' was conducted at the Department of Agricultural Microbiology, Tamil Nadu Agricultural University (TNAU), Coimbatore during 2011 - 2012. A total of 28 samples of rotting fruits and juices were collected from different places of Coimbatore, Tamilnadu. Preliminary screening was performed by the methods suggested by Sowden and Colvin (1978).

Screening the ability of bacterial isolates for cellulose production

Ability of bacterial isolates in cellulose production was examined by inoculating Hestrin-Schram medium, 2% glucose,

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0.5% yeast extract, 0.5% peptone, Na2HPO4 and 0.115% citric acid (Hwan *et al*. 2004) with 100 ìl of fresh culture of each bacterial isolate and incubated at 30^{0}C for one week. Cellulose production was investigated by the appearance of white pellicle on the surface of culture medium. According to the criteria of Gallardo-De-Jesus and Elvira (1971), isolates with more than 5 mm thickness were selected for further study. The best isolate was morphologically and biochemically characterized. The screened isolate was grown in HS medium and used as inoculum for bacterial cellulose production. The reference culture, *G. xylinum* NCIM 2526 was obtained from National Collection of Industrial Microorganisms (NCIM), Pune, India to compare the efficiency of screened isolate.

Starter culture and cellulose recovery

The screened isolate was inoculated in 1.0 L of sterilised HS medium and incubated at 30^{0}C for 14 days. Similarly *G. xylinum* NCIM 2526 was also inoculated separately in the above said medium to compare the cellulose producing pattern of the two strains 'sju-1' and 'NCIM 2526', respectively. After the incubation period, bacterial cellulose was formed as a white pellicle floating at the top of the medium. Cellulose was then extracted from the production medium by filtering through filter paper No.1, and washed with tap water, heated in water bath with 0.5 per cent NaOH at 80^{0}C for 15 min to remove microbial cells and other medium constituents. Then the pellicle was washed scrupulously with tap water to remove the acid flavour. This washed pellicle obtained was pure bacterial cellulose and the dry weight (g/ L) was recorded. The thickness (mm) was measured using a digital caliper. Water Holding Capacity (WHC) of the bacterial cellulose was estimated as per the procedures given by Bielecki *et al*. (2005). The crude fibre content was analysed using fibra plus auto analyser by the procedures of Soest and McQueen (1973).

RESULTS AND DISCUSSION

Morphological and biochemical characteristics

Amongst the various strains, sugarcane juice isolate namely 'sju-1'produced maximum cellulose thickness of about 13.00 mm. Hence based on the grading of cellulose pellicle, 'sju-1' was selected as the best cellulose producing isolate. Table 1 shows the biochemical characteristics of the strain 'sju-1'. The colonies of the bacteria were milky white, opaque with perfect margin. The selected strain 'sju-1' was found to be Gram negative and negative to endospore formation. The isolate was able to oxidize ethanol to acetic acid and over oxidation of acetic acid to CO_2 and H_2O. Formation of clearing zone in $CaCO_3$ agar medium clearly proves that the strain belonged to Acetic Acid Bacteria (AAB). The most important biochemical characteristics such as production of dihydroxyacetone from glycerol, oxidation of ethanol to acetic acid and production of water insoluble surface pellicle was confirmed to be positive for *G. xylinum* (Fig.1). Hutchens *et al*. (2007) have reported

Table 1 Morphological and Biochemical characteristics of the cellulose producing strain 'sju-1'

Morphological characters	
Colonies	Circular, convex, smooth brownish white opaque colonies with entire margin
Cell Shape	Short rods
Arrangement	Singly and in pairs
Gram reaction	Negative
Spore	Negative
Biochemical characteristics	
Reaction to litmus milk	Negative
Indole production	Negative
Nitrate reduction	Negative
Gelatin hydrolysis	Negative
Catalase reaction	Negative
Dehydroxyacetone from glycerol	Positive
Oxidation of ethanol to acetic acid	Positive
Production of water insoluble surface pellicle	Positive
Identification of isolate	***Gluconacetobacter xylinum***

that 10 % sucrose concentration could produce good and high quantity of bacterial cellulose. It is interpreted that in the present study the source of inoculum that is the fermented sugarcane juice with TSS of 21.59° Brix has substantially produced a best cellulose secreting bacteria. Premjet *et al*. (2007) have also indicated that *G. xylinum* develops well in sugarcane molasses and improves the bacterial cellulose production. In the present investigation, the strain isolated from fermented sugarcane juice yielded cellulose in quantities of commercial interest. A thick layer of cellulosic mat was formed on the surface of the HS medium by the two strains namely

Fig. 1 Cellulose pellicle formed by G. xylinum 'sju-1'

Table 2 Physico-chemical properties of bacterial cellulose

STRAINS	Characteristics of bacterial cellulose					
	Wet weight of cellulose g/L	Dry weight of cellulose g/L	Moisture (%)	Thickness (mm)	Water Holding Capacity (%)	Crude fibre (g)
G. xylinum 'SJU-1'	180.00 ±0.82	14.10 ±0.26	92.27 ±0.05	13.00 ±0.14	85.50 ±0.25	11.25 ±0.22
G. xylinum 'NCIM 2526'	160.50 ±0.44	11.40 ±0.15	92.89 ±0.29	8.00 ±0.01	84.44 ±0.12	11.00 ±0.04
CD at 5%	1.03	0.18	0.11	0.46	0.08	0.09
SEm	0.37	0.06	0.04	0.16	0.03	0.03

'sju-1' and NCIM 2526. *G. xylinum* 'sju-1' isolate produced 14.10 g/ L of cellulose with thickness of 13 mm and NCIM 2526 produced only 11.40 g/ L of cellulose with 8 mm thickness (table 2). Cellulose produced by 'sju-1' isolate was found to be highly significant at $p<0.05$ when compared to the cellulose produced by *A. xylinum* NCIM 2526. Earlier reports of cellulose production using NCIM 2526 on mature coconut water was found to produce 8.0 mm thickness of cellulose (Jagannath *et al*. 2008). The higher water holding capacity and crude fibre content of 85.50 % and 11.25 g respectively clearly indicated that the product is highly juicy and fibrous in nature.

Isolation of an efficient and stable cellulose producing strain still remains to be a challenging task, since *G. xylinum* cells may revert to cel^- mutants under agitated conditions. Hence, screening and selection of best isolate with consistent cellulose production under multiple sub culturing is the need of the hour. The native isolate namely *G. xylinum* 'sju-1' from fermented sugarcane juice with highest recorded yield opens up a great opportunity in the area of cellulose research.

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