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Evaluation of Acetobacter Strain for the Production of Microbial Cellulose

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Polysaccharides are a structurally diverse group of biological macromolecules of widespread occurrence in nature. The production of biopolymers through biotechnological means attracted the keen attention of researchers due to wide range of their applications. Microbial cellulose is homopolysaccharide having wide industrial applications and it could be Biotechnology's next high-value product. Microfibrilar structure of microbial cellulose is responsible for most of its properties such as high tensile strength, higher degree of polymerization and high crystallinity index. Fibrils of bacterial cellulose are about several times thinner than that of plant cellulose making it a highly porous material, which allows transfer of antibiotics or other medicines into the wound while at the same time serving as an efficient physical barrier against any external infection. It is therefore used extensively in wound healing. It is produced by a variety of organisms ranging from unicellular and multicellular. However, there are several genera of bacteria including Agrobacterium, Rhizobium, Psuedomonas, Sarcina and Acetobacter known to synthesize cellulose. This paper is focused on evaluation of Acetobacter aceti the production of microbial cellulose.

Key Words: Microbial cellulose, Acetobacter aceti, FT-IR spectroscopy.

INTRODUCTION

Cellulose is most abundant biopolymer on earth, constitutes a ubiquitous and renewable natural resources. It is extra-cellular polysaccharide produced by variety of organisms produced by variety of organisms ranging from unicellular and multicellular plant to bacteria. Many Bacteria like *Agrobacterium, Rhizobium,*

Pseudomonas, Sarcina and *Acetobacter*[1] are known to synthesize cellulose, but *Acetobacter* species produce sufficient amount of cellulose to warrant commercial interest.

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Microbial cellulose have wide application in various fields such as food, healthcare, cosmetics and beauty, clothing and shoes, outdoor sports, baby care products, audio products like speaker diaphragms etc[2]. It is also being used in paper industry for making electronic paper display packaging industry for building materials; pharmaceuticals, cosmetics, gelling agents and medicines for wound dressing. It is used in preparation of nanocomposites for biomedical purpose[3-5]. The pellet of microbial cellulose can also used in enzyme immobilization[6]. The most important application of microbial cellulose is in clarification of fruit juices and fermentation of *Monascus purpureus* on agricultural-by products to make colourful and functional microbial cellulose[7]. Keeping the above in view, the present work was carried out to optimize the sources and conditions of process parameters for maximum cellulose production by using *Acetobacter aceti* MTCC 2623.

EXPERIMENTAL

Procurement of Micro-organism

Acetobacter aceti MTCC 2623 was procured from Institute of Microbial Technology Chandigarh, India. The starter culture was prepared in media containing yeast extract (5 g/L), peptone (5 g/L), mannitol (25 g/L).

Preparation of Fermentation Media and Production of Microbial Cellulose

The composition of medium was (g/L): glucose (20), yeast extract (5), peptone (5), citric acid (2.7), disodium hydrogen phosphate (1.17). The pH of medium was initially adjusted to 5.5, and fermentation medium was sterilized. *Acetobacter aceti* MTCC 2623 cells taken from the seed culture and was added into a flask (2% inoculum) and were incubated at 28°C for 7 days.

Optimization of Media components and Process parameters

Different carbon and nitrogen substrates (Fig. 1 & 2) were screened for cellulose production. The process parameters such as pH, temperature and incubation time were standardized by varying the respective parameters. The cellulose pellicle produced during the course of the fermentation was purified and measured at the end of each run[1]. The surface properties of the bacterial cellulose were examined by a Perkin-Elmer S2000 Fourier transform infrared spectrometer[5].

RESULTS AND DISCUSSION

Screening of Carbon Sources for Cellulose Production

The results of various carbon sources for maximum cellulose production are shown in Fig. 1. It was observed that the maximum cellulose production was obtained in mannitol (1.8 g/L), followed by glucose (1.5 g/L). Similar trend of cellulose production has also been reported by Jonas and Farah[8]. Mannitol gave best yield but due to the cost factor glucose was selected as carbon source for further experimentation.

Screening of Nitrogen Sources for Cellulose Production

Effects of nitrogen sources on cellulose production were observed. It can be observed from Fig. 2 that peptone, sodium nitrate and methionine were found to be most effective among these nitrogen sources. Matsuoka *et al.*[9] reported that vitamins and amino acids played important role for cell growth and cellulose production. Considering the cost factor sodium nitrate was screened as nitrogen source for furthur experimental studies.

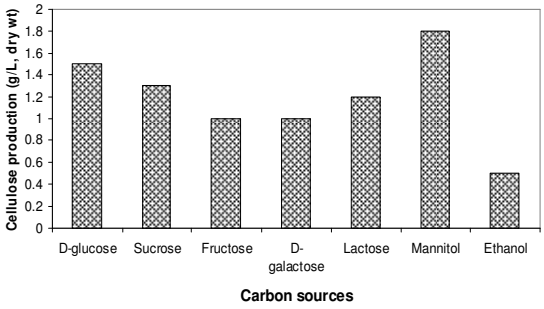

Fig.1 Cellulose production from different carbon sources by *Acetobacter aceti* MTCC 2623

Optimization of Process Parameters for Cellulose Production

The effect of pH indicated maximum cellulose production at 6.5-7.0 at 28°C. The effect of incubation period indicated that with increase in incubation time cellulose production also increased and maximum production of cellulose (1.6 g/L) was observed at 168 hrs (Fig. 3) This range of incubation time has been reported by previous researchers[7,10].

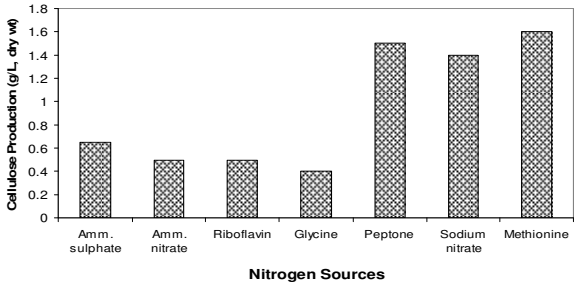

Fig. 2 Cellulose production from different nitrogen sources by *Acetobacter aceti* MTCC 2623

Structure Elucidation of Microbial Cellulose

FTIR spectra of microbial cellulose showed the several strong bands due to OH stretching in the region of 3853–3256 cm^{-1}. Several bands typical for cellulose were shown in the region of 1500 – 1235 cm^{-1}. Further, the IR spectrum obtained showed the same bands as were obtained in authentic cellulose sample.

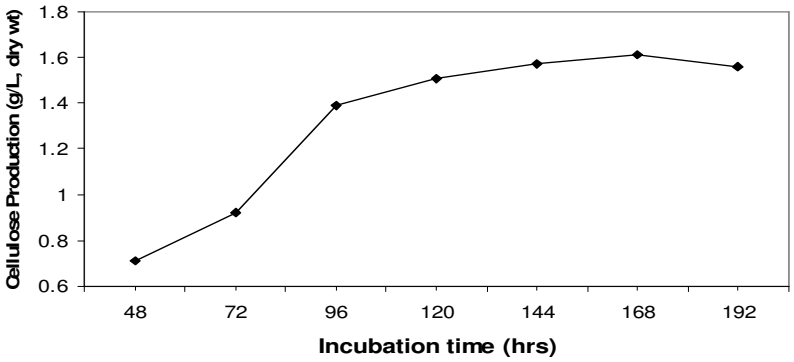

Fig. 3 Cellulose production by *Acetobacter aceti* MTCC 2623 as a function of incubation time

CONCLUSIONS

Among the media components tested, glucose and sodium nitrate was the best carbon and nitrogen source, respectively for production of cellulose with *Acetobacter aceti* MTCC 2623. Maximum cellulose production (1.6 g/L) was obtained at 2%, (w/v) glucose, 1% (w/v) sodium nitrate, pH 6.5-7, temperature 28°C and incubation time 168 hrs with cellulose production. The IR spectrum showed typical bands due to presence of OH group, ether linkage and pyranose ring confirm that the produced material to be microbial cellulose.

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