INTRODUCTION

β (1-3) glucan, a secondary metabolite, produced by different groups of bacteria and yeasts. These are natural non-toxic and biodegradable polymers, present mostly at the surface of cells. Agrobacterium sp. ATCC 31749 produces a water-insoluble extracellular unbranched homo β (1-3) glucan at a neutral pH. At a temperature of 54°C or above, this forms a firm gel, and due to this nature, it has an industrial scale production by the fermentation of Agrobacterium sp. Food and chemical industries use this as a gelling agent. A complex media containing yeast extract as the nitrogen source are used as the production medium. A mineral salt medium with suitable pH of the growth of culture has been developed for the production of β (1-3) glucan. For the improved productivity, a two-stage continuous process was performed. The post-stationary phase at a nitrogen-depleted aerobic condition was favorable for the production of β (1-3) glucan. Aniline blue, a specific dye which is absorbed by β (1-3) glucan producing microorganisms, was used for the screening. It produces blue-colored colonies. Rhizobium sps. are also involved in the production of β (1-3) glucan. About 12,000 glucose units are present in it. These are soluble in dilute bases (0.25 M NaOH), dimethyl sulfoxide (DMSO), and aprotic reagents such as N-methylmorpholine-N-oxide. Depending on the method of preparation, β (1-3) glucan shows different structural variations ranging from endless microfibrils to spindle-shaped fibrils of various length when precipitated with NaOH or DMSO solution. The present study was performed for the production of β (1-3) glucan under the presence of different carbon and nitrogen sources in the MSM medium.

MATERIALS AND METHODS

Sample Collection and Isolation of Agrobacterium sp.

The root nodules obtained from the different areas of Coimbatore and Palakkad were washed, and 1% mercuric chloride solution was used for surface sterilization. The nodule extract was serially diluted and streaked on YEMA medium. The plates were incubated at room temperature for 5–7 days.
Screening for the β (1-3) Glucan Production

Yeast extract mannitol medium containing aniline blue reagent was prepared. The isolated organisms were inoculated and incubated for 3–4 days at room temperature. The plates were observed for the formation blue-colored colonies.

Characterization of Positive Agrobacterium sp.

The tests performed for the identification of Agrobacterium sp. are citrate utilization test, Hofer’s alkaline agar test, Congo red test, 3-ketolactose agar test, and growth in glucose peptone agar.

Seed Culture Preparation and Production

Seed culture media containing peptone 0.5 g, yeast extract 0.5 g, and 1% of sucrose were used for the preparation of inoculums and incubated for 24–48 h incubation at room temperature. Minimal salt medium containing KH2PO4, 3.0; Na2HP04, 6.0; MgSO4, 0.5; (NH4)2HP04, 0.1, trace element solution – 10 mL (5 g FeSO4.7H2O, 1 g CoCl2.6H2O, 2 g MnSO4, citrate, and 1 g ZnCl2 per liter of 0.1 mol/l HCl), and 2% sucrose as carbon source was used as optimized production medium to which 50 mL of the seed culture was added. The pH 5.5 was maintained and the flasks were incubated in a shaker at 180 rpm for 76 h at room temperature.

Extraction of β (1-3) Glucan

Following 76 h of incubation, the culture medium was centrifuged at ×12,000 g for 30 min. 0.5N sodium hydroxide at 4°C was added to the extracted pellet and kept undisturbed for 3 h at the same temperature. Successive centrifugation at ×12,000 g for 40 min was performed. The supernatant was retained to which 10% acetic acid was mixed for the precipitation of β (1-3) glucan, and the precipitate was washed twice with water, acetone, and ether and stored for further study.

Confirmation of the Agrobacterium sp.

The organism was subjected to 16S rRNA sequencing at Yaazh Xenomics lab, Coimbatore, for the confirmation of the isolate.

Fourier-transform Infrared (FT-IR) Analysis

FT-IR spectroscopy was performed for the characterization of extracted β (1-3) glucan, and band transmittance was observed.

RESULTS AND DISCUSSION

Collection and Isolation of Agrobacterium sp.

The red-colored mucoid colonies were observed on YEMA medium. Such fifty colonies were selected, pure cultures, and screened.
Characterization of *Agrobacterium* sp.

Five of the isolates showed Prussian blue color for citrate utilization test [Figure 4]. They produced red-colored colonies on YEMA medium with Congo red dye, yellow color on the 3-ketolactose agar test [Figure 5] and Hofer’s alkaline agar test [Figure 6], and also growth on glucose peptone agar.

**Production and Extraction of β (1-3) Glucan**

The isolate which gave a higher yield of β (1-3) glucan was selected for the production [Table 1]. Approximately 0.12 g of β (1-3) glucan was extracted from 100 mL of the production medium [Figure 7]. The extraction of curdlan by centrifugation method was given in a report by Lee et al., 1999.[23]

The isolate was confirmed to be *Agrobacterium fabrum* by 16S rRNA sequencing. The GenBank accession number is MF521602.

**FT-IR Analysis**

The FT-IR spectrum showed bands corresponding to –OH, -CH₂, CH, and CH₂ at spectrum transmittance 3425.58, 2927.94, 1346.31, and 1323.17 cm⁻¹, respectively. The presence of (1→3)-β-glycosidic bonds is indicated by an absorption band at 883.40 cm⁻¹ [Figure 8].[24] The result obtained was compared with the previous FT-IR reports of the compounds produced by the strains of *Rhizobium radiobacter* ATCC 6466[25] and *Agrobacterium* sp. ATCC 31750.[26]

**CONCLUSION**

β (1-3) glucan is a biopolymer produced by different types of organisms. *A. fabrum* was isolated, identified, and screened for the production of β (1-3) glucan from among 50 isolates of *Agrobacterium* sp. obtained through serial dilution of root nodules from

<table>
<thead>
<tr>
<th>Samples</th>
<th>Cell weight g/100 mL</th>
<th>β (1-3) glucan g/100 mL</th>
</tr>
</thead>
<tbody>
<tr>
<td>A4</td>
<td>0.12</td>
<td>0.05</td>
</tr>
<tr>
<td>A16</td>
<td>0.09</td>
<td>0.10</td>
</tr>
<tr>
<td>A20</td>
<td>0.11</td>
<td>0.09</td>
</tr>
<tr>
<td>A43</td>
<td>1.2</td>
<td>0.12</td>
</tr>
<tr>
<td>A48</td>
<td>0.04</td>
<td>0.07</td>
</tr>
</tbody>
</table>
different regions of Coimbatore and Palakkad. The production was carried out in an optimized medium, and about 0.12 g of β (1-3) glucan was produced from 1.2 g/100 mL cell weight. This compound is stored for further study on cancer.

REFERENCES

23. Lee JH, Lee IF, Kim MK, Park YH. Optimal pH control of

