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Physico-chemical characterization of β-Glucan produced from *Bacillus cereus* LVK13 (KC 898956)

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Abstract

The present study aimed to produce and characterize β-glucan from an indigenous soil isolate *Bacillus cereus* LVK13 (KC 898956). Thin-layer chromatography resulted an R_f value of 0.58 that corresponded to glucose. Vibrational frequencies of Fourier Transform Infrared Spectroscopy at 2885 cm^{-1}, 1188 cm^{-1} and 887 cm^{-1} signified the existence of -CH_2-, C=O bonds and β-configuration. Further, β configuration of monomeric glucose and β-(1,3)-D-glucan backbone were retrieved from the spectrum of ^{1}H and ^{13}C NMR. X-Ray diffraction pattern reflected low crystalline nature of the product. The results of physico-chemical analysis substantiate that β-glucan produced from the indigenous strain *Bacillus cereus* LVK13 can be used widely in medical, health, food and environmental sectors.

Key words

Bacillus cereus LVK13, β-1,3-glucan, FTIR, NMR, XRD

Introduction

Exopolysaccharides are high molecular weight polymers synthesized by a series of microorganisms and have eco-friendly comprising properties like renewability, non toxicity and biodegradability (Sunil *et al.,* 2013; Freitas *et al.,* 2011). Various sources of β-glucan include cereals, mushroom, yeast and bacteria (Gummadi and Kumar 2005). Use of bacteria has added advantage of being economically viable owing to its easier production strategies. Previous works have reported bacterial β-glucan production by *Agrobacterium sp* (Lee *et al.,* 1999 a), *Paenibacillus* sp (Jung *et al.,* 2007), *Alcaligenes* sp (Jianrong *et al.,* 2008) and *Bacillus* sp (Gummadi and Kumar, 2005), etc.

β-glucan is one of the exopolysaccharides notable for its broad spectrum of applications in drug delivery (Kanke *et al.,* 1995), heavy metal adsorption, admixture to concrete, food additive and pharmaceutical industries (Lee *et al.,* 1999 b) etc. Riddance action of pathogens by unique innate immunomodulatory property of β-glucan facilitates its use in cancer treatment (Steele *et al.,* 2005). It occupies a pivotal position in food industries since they have a notable physical characteristics with gel forming property (Harada *et al.,* 1968). Possession of these many credentials both in application and properties, makes production of β-glucan from a bacterial source an essential one. Among the bacterial sources, the genetic and structural complexity of *Bacillus* species triggers its selection for β-glucan production (Sirajunnisa *et al.,* 2013).

Since characterisation of a product is as important as that of production, the present study aimed at production and further characterization of β-glucan from *Bacillus cereus* LVK13 (KC 898956).

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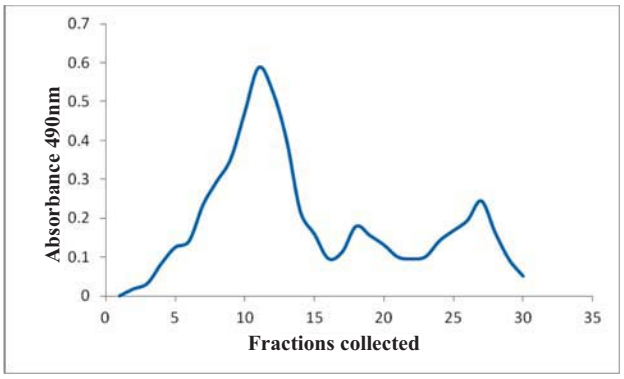

Fig. 1: Phenol sulfuric acid assay of β-glucan

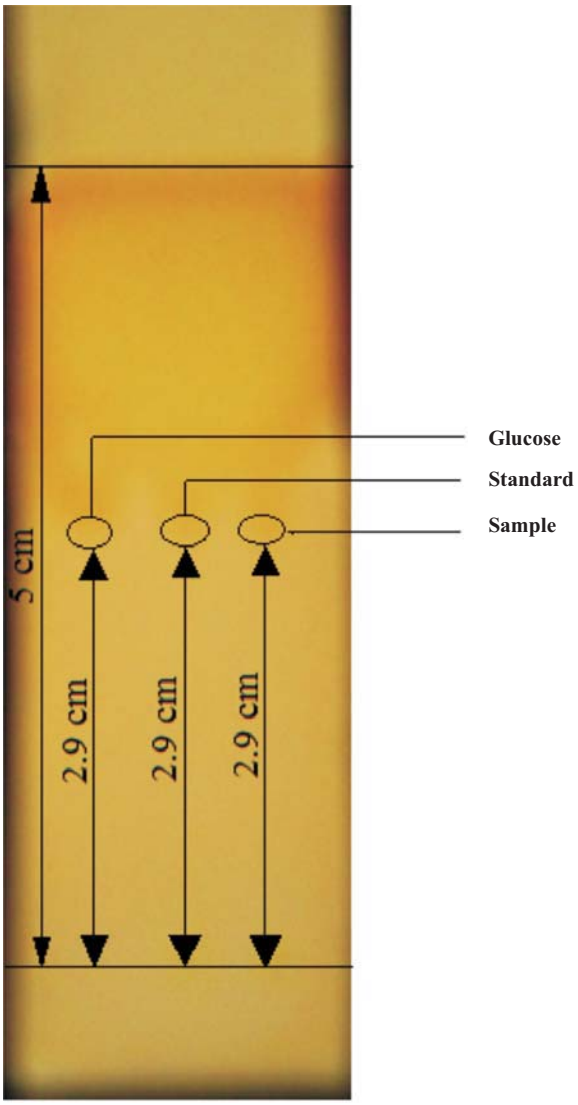

Fig. 2 : Thin layer chromatography of β-glucan from *Bacillus cereus* LVK13

Materials and Methods

Microorganism : The entire study was carried out with *Bacillus cereus* LVK13 (KC 898956), an indigenous soil isolate from the agricultural fields in Sathyamangalam (Taluk), Erode of Tamil Nadu (State), India and was maintained at 4°C as slant cultures using nutrient agar for further studies.

Fermentative production of β-Glucan : Ten ml of log phase cells of *Bacillus cereus* LVK13 developed using nutrient broth was used as an inoculum to 1 litre of mineral salt medium and shake flask experiments were carried out at 180 rpm for 4 days at 30°C (Jung *et al.,* 2007).

Separation and purification of β-Glucan : Supernatant from fermentation media was separated by centrifugation for 15 min at 5000 rpm. β-glucan was precipitated from the resultant supernatant by incubating at 4°C for 12 hrs with 3 volumes of ice cold ethanol. After overnight incubation, pellets were recovered, washed with distilled water and the crude glucan was concentrated by lyophilization. A known quantity of 0.1 M NaCl was used to dissolve the lyophilized crude β-glucan and purified by size-exclusion chromatography using Sephadex G-100 in a glass column. Thirty fractions of 10 ml each were collected at 0.1 ml min^{-1} using 0.1 M NaCl as elution buffer (Jung *et al.,* 2007). Carbohydrate content of eluted fractions were determined by phenol-sulfuric acid method (Dubois *et al.,* 1956).

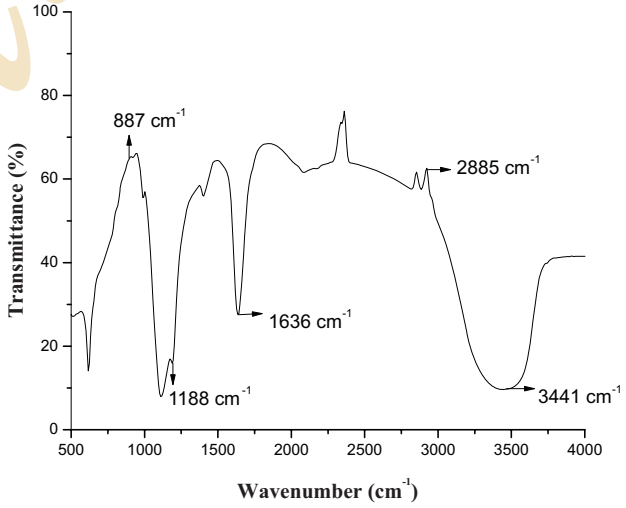

Fig. 3 : FTIR spectrum of β-glucan from *Bacillus cereus* LVK13

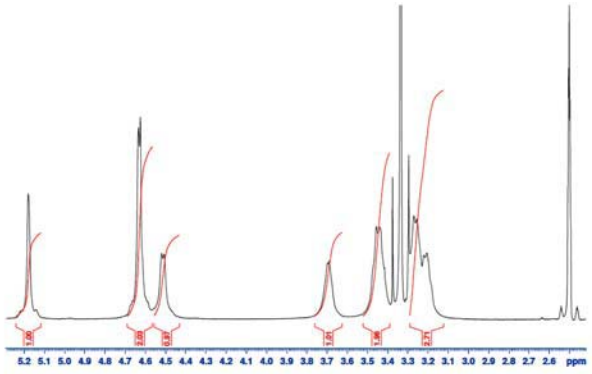

Fig. 4 : ^{1}H NMR spectrum of β-glucan from *Bacillus cereus* LVK13

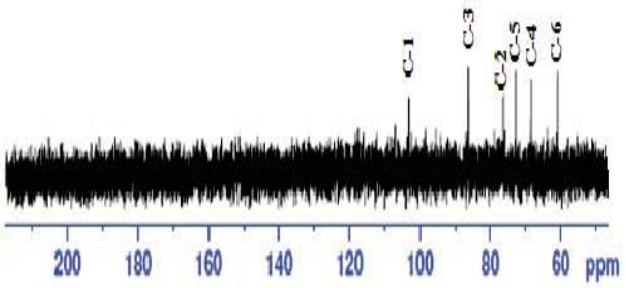

Fig. 5 : ^{13}C NMR spectrum of β-glucan from *Bacillus cereus* LVK13

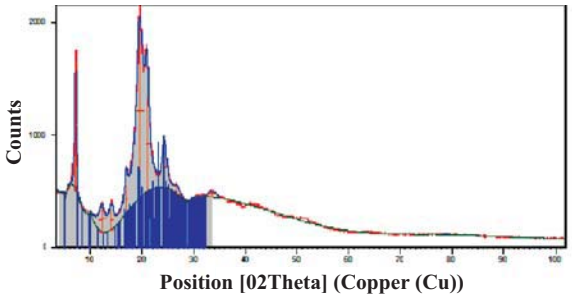

Fig. 6 : X-ray diffraction pattern of β-glucan from *Bacillus cereus* LVK13

Physico-chemical characterization of β-Glucan : Characterization of β-glucan was carried out by Thin layer chromatography, Fourier transform infrared and Nuclear magnetic resonance spectroscopy. TLC examination of purified β-glucan was performed by loading the neutralized hydrolysate as reported by Jung *et al., (*2007) onto the TLC plate along with glucose (1mg ml^{-1}) and β-1,3-glucan from *Sigma-Aldrich (*Product No. *89862)* as reference. Butanol and ethyl acetate (1:1) was used as solvent system and the development of plate was visualized in a iodine chamber.

Functional groups of β-glucan was determined by FTIR. A mixture of about 0.3 g dry potassium bromide and 0.005 g β-glucan was ground to a fine powder. The pellets of fine powder were made using dye by applying 10-16 kpsi pressure by the plunger of the pellet making machine for 1-2 min and the transmittance was measured using FT-IR analyzer (ABB MB 3000) from 500 to 4000 cm^{-1}. Chemical structure of β-glucan was determined based on its nuclear magnetic resonance using Bruker-300 Ultrashield. NMR tube containing 20 mg of sample diluted in 1ml deuterated DMSO at 80°C and the chemical shift resonances were evaluated according to the established method (Mursito *et al.,* 2010; Kalyanasundaram *et al.,* 2012).

β-glucan was physically characterized by X-ray diffraction measuring 2θ (5-50°) diffraction of the disc filled with purified β-glucan by X-ray diffractometer (X'Pert PRO).

Results and Discussion

The fractions from gel filtration column and their corresponding absorbance according to the concentration of product by phenol-sulfuric acid assay are represented in Fig. 1. The fractions containing higher carbohydrate concentration such as 9, 10 and 11 were pooled and lyophilized to obtain pure β-glucan.

The R_f value of hydrolysate was found to be 0.58 as that of β-glucan standard and glucose (Fig. 2). From TLC, it was found that β-glucan is a polymer of glucose (Jung *et al.,* 2007). In FT-IR spectrum (Fig. 3), vibrational frequencies were observed at 3441 cm^{-1}, 2885 cm^{-1}, 1636 cm^{-1} and 1188 cm^{-1}, indicating O-H stretch, -CH_2- bond, C-H group and C=O bond, respectively. The characteristic signal pattern and bands at anomeric region anticipated for a carbohydrate moiety was identical to β-1,3 glucan obtained from Sigma Aldrich (Spectra not shown). A characteristic peak observed near 890 cm revealed β-configuration of D-glucopyranosyl residues. The presence of peak near 890 cm^{-1} and no peak at 840 cm^{-1} (Hans *et al*., 2008) confirms that the glucan produced by *Bacillus cereus* LVK13 has only β-configuration.

NMR spectra of β-glucan produced by *Bacillus cereus* LVK13 in this study was found to be almost corresponding to β-1,3 glucan obtained from Sigma Aldrich

(Spectra not revealed). ^{1}H and ^{13}C NMR is used to examine organic molecules containing hydrogen and carbon atoms respectively in which absorption occured in the range of 0 to 230 δ. ^{1}H NMR obtained for β-glucan (Fig. 4) showed anomeric protons (4.62-5.17 ppm) and sugar protons (3.21-3.70 ppm) and a higher field signal at 4.519 confirmed the characteristic β configuration of glucan. ^{13}C NMR spectra (Fig. 5) analysis indicated peaks at 103.06 ppm (C1), 76.33 ppm (C2), 86.24 ppm (C3), 68.43 ppm (C4), 72.84 ppm (C5) and 60.88 ppm (C6) were in accordance to the signals of β(1,3)D-glucan backbone chain. These results were in accordance with Mursito *et al.* (2010) where hetero multiple bond connectivity results of β-glucan exhibited carbon signal (C3) at 86.87 ppm and proton signal at 4.41 ppm substantiating the existence of β-(1,3)-D-glucan backbone chain.

Crystalline nature of β-glucan was assessed by X-ray diffractometer. X-ray diffraction pattern of β-glucan (Fig. 6) showed 2θ values at 7.136°, 12.25°, 19.578°, 20.953° and 33.5° with d-spacing values at 12.38 nm, 7.22 nm, 4.53 nm, 4.24 nm and 2.67 nm respectively, inferring the low crystalline nature of β-glucan. As compared to the earlier reports (Marchessault *et al*., 1979; Yang *et al.*, 2006; Shih *et al.,* 2009), β-glucan form *Bacillus cereus* LVK13 displayed the partially crystalline nature with x shaped pattern characteristic of helical conformations.

Thin layer chromatography confirmed that β-glucan consisted only of glucose residues. FT-IR portrayed β-linkage and peaks obtained in ^{1}H and ^{13}C NMR indicated the existence of anomeric protons, β configuration and (1, 3)-β-D-glucan backbone respectively. A detailed investigation on scale-up and applications of β-1,3 glucan produced by *Bacillus cereus* LVK13 (KC 898956) in various sectors can be the perspective of future work.

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