ABSTRACT

An exopolysaccharide (EPS) was isolated and purified from *Bacillus coagulans* RK-02 grown in glucose mineral salts medium by acid and alcohol precipitation followed by gel filtration chromatography. The growth kinetics of the organism and the time course of accumulation of EPS were studied. The experimental data of growth and product formation were fitted into the models of Luedeking-Piret and Kono-Asai to confirm that the production of the EPS was partially growth associated. The molecular weight of the purified EPS was determined by size exclusion chromatography using standard dextrans of known molecular weights and its homogeneity was checked by agarose gel electrophoresis. The probioactive properties of the EPS were studied to assess its therapeutic and commercial application potentials. The antioxidant and immunostimulatory activities of the EPS were evaluated in both *in vitro* and *in vivo* experiments. The antihyperglycemic activity was tested in both the normal and diabetic rats. The emulsifying activity of the EPS was measured by using different hydrophobic substrates. The monomeric composition of the EPS was determined by HPLC. The glycosidic linkages between the monomers in the molecular structure were determined by Gas Chromatography-Mass Spectroscopy (GC-MS) and Nuclear Magnetic Resonance (NMR) spectroscopy.

From the growth and time course profiles, it was observed that *B. coagulans* RK-02 produced a maximum EPS concentration of 110 mg/l during the exponential and stationary growth phases. The average molecular weight of the EPS was found to be 3×10^4 dalton by gel filtration chromatography, using FITC labeled standard dextrans for calibration. The agarose gel electrophoresis results confirmed that the *B. coagulans* produced a homogenous polysaccharide. The EPS showed very significant antihyperglycemic activities by reducing the blood glucose level by 68 % at the dose of 200 mg/Kg body weight of the model animal. The EPS exhibited very good antioxidant activities in both *in vitro* and *in vivo* studies. The purified EPS also showed excellent emulsifying activities in xylene and kerosene as compared to commercial plant polysaccharides. FTIR spectroscopic results indicated the presence of hydroxyl groups, anomeric regions, α- glycosidic linkages and mannose residue in the EPS. HPLC and GC analyses revealed that the EPS was a heteropolymer of glucose, mannose, galactose, fucose and glucosamine as monomers. The GC-MS and NMR analyses confirmed the presence of 1→3 and 1→6 glycosidic linkages in the backbone. The structure-function relationships for each of the probioactive properties have been adequately explained. Due to its novel monosaccharide composition, glycosidic linkages and highly significant therapeutic and emulsification activities, the EPS can find potential commercial applications in dairy, food, environmental, oil recovery, nutraceutical and pharmaceutical industries.

Key words: *Bacillus coagulans*; Exopolysaccharide; antioxidant activity; antihyperglycemic activity; emulsifying activity; HPLC analysis; FTIR; NMR