PURIFICATION AND CHARACTERISATION OF NON-DIGESTIBLE OLIGOSACCHARIDES DERIVED FROM EXTRACELLULAR POLYSACCHARIDE OF ANTARCTIC FUNGUS *Thelebolus* sp. IITKGP-BT12

ABSTRACT

Depolymerisation of complex polysaccharides is an efficient technique to obtain oligosaccharides that are known to impart health benefits. *Thelebolus* sp. IITKGP-BT12, a recently identified psychrophilic, ascomycetes fungus has been reported to produce a bioactive extracellular polysaccharide (EPS), thelebolan. The current study was aimed at identifying and optimising the most suitable method for hydrolysis of thelebolan into oligosaccharides and focused on further purifying, characterising and evaluating the bioactivity of the same. Among physical, chemical and biological methods, enzymatic hydrolysis was identified as the best method and the optimum hydrolysis conditions obtained using response surface methodology were: reaction time of 24 h, β-(1, 3) endo-glucanase concentration of 0.53 U/mL and substrate concentration of 10 mg/mL. Thelebolan oligosaccharides (TOs) were purified using gel filtration chromatography and their molecular weights were determined using MALDI-TOF. The three major fractions were found to be of hexa-, penta- and trisaccharide with a molecular weight of 990 Da, 828 Da and 504 Da respectively, the yield of total oligosaccharides was approximately 14% (w/w) of the polysaccharide hydrolyzed. The monomeric unit of TOs was confirmed to be glucose using TLC and GC-MS analysis.1D/2D NMR experiments and methylation experiments indicated the presence of β-(1→3) and β-(1→6) linkages in the structure of the purified oligosaccharides. TOs proved to be non-digestible when subjected to simulated digestive conditions. Upon supplementing TOs as sole carbon source for growth of probiotic strains *L. brevis*, *L. acidophilus* and *L. plantarum*, positive prebiotic activity scores were obtained, with *L. brevis* exhibiting the highest score of 0.44 ±0.105 and 0.671± 0.005 after 24 h and 48 h respectively. The ability of TOs to be selectively fermented by probiotic bacteria was confirmed from SCFA analysis through HPLC. Quantitative and qualitative assays affirmed the facilitation of biofilm formation by probiotic bacteria over pathogenic strains in the presence of TOs. *In vitro* studies performed on macrophage cell line, RAW 264.7 indicated that TOs stimulated the production of NO, cytokines IL-6 and TNF-α and enhanced phagocytosis by ~3.7 times. *In vivo* studies on Balb/c mice fed with TO (200 mg/kg body weight) exhibited enhanced SCFA production, with a significant increase in butyrate levels. Immunostimulation of cyclophosphamide treated Balb/c mice subjected to TO treatment was observed in terms of enhanced splenic, thymic indices, increased cytokine production and improved proliferation of splenocytes *ex vivo*. Increase in the expression levels of Dectin-1 in TO treated RAW 264.7 cell line (~2.5 fold) and tissue extracts of mice suggest that immunostimulation occurred via the β-glucan specific Dectin-1 mediated pathway.

Keywords- Characterisation, Enzymatic hydrolysis, Non-digestible oligosaccharides, Response surface methodology, Prebiotic, Immunostimulation