PRODUCTION AND PURIFICATION OF EXOPOLYSACCHARIDES FROM LACTOBACILLUS SPECIES AND ITS MEDICINAL APPLICATIONS

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Introduction:
Lactic acid bacteria are commonly used in probiotics to treat intestinal disorders such as lactose intolerance, acute gastroenteritis, constipation and inflammatory bowel diseases. In addition to production of lactic acid, these bacteria have the potency to produce exopolysaccharides (EPS) that play an important role in cell-cell interaction, colonization and biofilm formation. These polysaccharides help the bacteria sustain both abiotic and biotic stress conditions like temperature, pH, light intensity. The polysaccharides produced have wide applications in food, pharmaceuticals, drug delivery and therapeutics.

Objectives:
1. Production of exopolysaccharide from Lactobacillus acidophilus and Lactobacillus plantarum.
2. Purification and structural characterization of exopolysaccharide
3. Evaluation of medicinal application of exopolysaccharides.

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Methodology:
Production of exopolysaccharides (EPS) from *Lactobacillus acidophilus* and *Lactobacillus plantarum* was done using MRS media with sucrose as a carbon source. Molasses was clarified and further screened as an alternative carbon source for EPS production. Crude EPS was purified using TCA precipitation, dialysis, phenyl sepharose column for protein removal, DEAE cellulose column was used to separate EPS based on their charges. Pure EPS thus obtained was subjected to molecular weight determination by using sephacryl column and taking standard dextrans of molecular weight 10 kDa, 40 kDa and 70 kDa for plotting calibration graph with blue dextran (2000 kDa) used for calculation of the void volume of the column. Medicinal applications were evaluated using RBC membrane stabilization assay and nitric oxide scavenging assay to test anti-inflammatory activities. MTT assay was conducted for studying lymphocyte and macrophage proliferation and ELISA was used to determine *in-vitro* expression levels of IL-2 and IL-10 cytokines.

Results and Conclusion:

1. 3.818 g/L and 2.452 g/L of crude EPS was obtained from MRS media supplemented with sucrose as carbon source whereas molasses as an alternative showed 9.39 g/L and 6.85 g/L of crude EPS production for *L. acidophilus* and *L. plantarum* respectively.

2. Purified EPS from *L. acidophilus* was neutrally charged polysaccharide and that of *L. plantarum* had neutral and anionic polysaccharide.

3. Molecular weight of the exopolysaccharide obtained from *L. acidophilus* was found to be 212.6 kDa and the neutral fraction of exopolysaccharide from *L. plantarum* had a molecular weight of 80.54 kDa whereas that of the anionic fraction of exopolysaccharide from *L. plantarum* had a molecular weight of 30.37 kDa.

4. EPS of both Lactobacillus species demonstrated concentration dependent anti-inflammatory activity by protecting the RBC membrane from lysis evoked by hypotonic solution and effective scavenging of nitric oxide free radicals was observed in *L. acidophilus* and *L. plantarum* with IC_{50} value of 11.12±0.3 μg/ml and 13.21±0.2 μg/ml respectively.
5. EPS from both lactobacillus species significantly increased the capacity of murine macrophages and lymphocytes mitogenicity and also there was significant increase in the levels of IL-2 and IL-10 cytokine secretion. Thus it can be concluded that exopolysaccharides of *L. acidophilus* and *L. plantarum* may be used as a potent anti-inflammatory and immunomodulating agent to build immunity.

Scope for future work:

1. Monomer identification and detection of linkages present in the polysaccharide produced.
2. Optimization of molasses medium for increased production of exopolysaccharide.