

## Summary of the Project

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- **UGC approval Letter No. and Date:** No.F.47-588/13 (WRO) dated 28 January 2015
- **Title of the Research Project:** Isolation and characterization of Exopolysaccharide produced by Lactobacilli
- **Date of starting the project:** 29<sup>th</sup> January 2015.**Date of completion of project:** 29<sup>th</sup> January 2017.

## SUMMARY

Lactobacillus genus is a gram positive, facultative anaerobic or micro. aerophilic bacteria. Lactic acid bacteria (LAB) are considered industrially important organisms because of their fermentative ability as well as health and nutritional benefits. Moreover, they are generally regarded as safe (GRAS) for incorporation into food products. LAB consist of a number of diverse genera which include both homofermentative and heterofermentative LAB based on the end product of their fermentation. The ability of lactic acid bacteria to inhibit the growth of various bacteria is well known and attributed to the production of organic acids such as lactic acid and acetic acid, hydrogen peroxide, bacteriocins.

Exopolysaccharides are long chain polysaccharides consists of branched, repeating units of sugars or their derivatives. They are water soluble gums with novel and unique physical properties. Since Lactobacilli are food-grade bacteria known to be of the GRAS (generally recognized as safe) status and well-known probiotic strains, their EPS could be easily utilized in foods in a juridical point of view. The EPS produced by lactic acid bacteria not only be applied as natural additives but also be produced in situ. All these polysaccharides can be homopolymers or heteropolymers of neutral sugars (pentoses and hexoses) or anionic sugars (hexoses), substituted or non-substituted with non-sugar compounds attaining linear or ramified final conformations (Welman & Maddox, 2003; Garai-Ibabe et al., 2010). Probiotics have been

predominantly selected from the genera *Lactobacillus* and *Bifidobacterium*, which belong to lactic acid bacteria group. Some of the common probiotic microbes of this group are *Bifidobacterium bifidum*, *Bifidobacterium breve*, *Bifidobacterium infantis*, *Bifidobacterium longum*, *Lactobacillus acidophilus*, *Lactobacillus casei*, *Lactobacillus plantarum*, *Lactobacillus rhamnosus* (Timmerman et al., 2004). LAB have been extensively studied and established as valuable native inhabitants of the gastrointestinal tract (GIT).

Hence objectives of the Study is i) to isolate lactic acid bacteria having probiotic features as well as exopolysaccharide (EPS) production ii) Selection of Maximum EPS producing strain and iii) characterization of EPS produced by the strain.

In the present study, Lactobacilli were Isolated from commercially available probiotics. A total of 10 isolates were obtained from different probiotics like bifilac, darolac and vibact capsule. Probiotics are serially diluted in MRS medium and the respective dilutions were plated on MRS agar by spread plate technique to isolate Lactobacilli. The inoculated plates were incubated at 30°C for 18-24 hours, after incubation well isolated colonies were observed.

The Isolates were Identified on the basis of conventional method employing cultural, morphological, physiological and biochemical characteristics as described by Bergeys manual of systematic Bacteriology. The colonies were observed as large, irregular, flat, moist colonies on MRS agar plates. Morphological study of isolates revealed the Gram positive bacilli, arranged singly and in very short chains, non-spore forming, non-motile bacteria. The gram's staining and catalase test could support the characterization of lactobacillus spp. After taking these criteria into account gram positive rod shaped, non-spore forming and catalase negative bacteria which indicated the typical basic characteristics of lactobacilli were selected for further studies.

Biochemical classification is carried out to study type of metabolism. The bacilli showed Methyl red, Voges-Proskauer as positive test while Indole and Citrate as negative. The different sugars were used to study carbohydrate fermentation and to identify the isolates. The isolates showed acid production with glucose, lactose, xylose, sucrose, arabinose, maltose, ribose and mannitol.

Preservation and stock cultures: The identified 10 cultures of lactobacilli were transferred on MRS agar slopes and stored at refrigerated temperature.



## Screening of Exopolysaccharide producing Lactic Acid Bacilli.

Preparation of inoculum for EPS production: an inoculum of each *Lactobacillus* was prepared in 50 ml MRS medium in 250 ml Erlenmeyer flasks by inoculating a loop full of culture from agar stabs and incubating at 37 °C for 18 h. The biomass thus obtained is then transferred in individual flask of MRS broth. Bacterial strains are cultivated and individually incubated for 24 h at 30°C, into 250 ml flask containing 50 ml MRS liquid medium. After 24 hrs incubation the contents were centrifuged at 12000 rpm for 10 min followed by precipitation with alcohol .It is then again centrifuged at 8000 rpm for 10 min.The pellet is then washed with equal volume of alcohol and then with distilled water. Characterisation of exopolysaccharide produced by *Lactobacillus* was carried out by Fourier Transform-Infrared (FT-IR) Spectroscopy.

FT-IR spectra of EPS were recorded from 450 to 4600/cm with a resolution of 4.0 cm<sup>-1</sup> and 25 scans by subtracting both the background and atmospheric water at resolution. The sample pellets were prepared by mixing the fine freeze dried EPS (1 mg) with 100 mg KBr. Spectra were recorded using Shimadzu FT-IR spectrophotometer. FTIR revealed characteristic functional groups such as a broad-stretching hydroxyl group at 3,613 cm<sup>-1</sup> and an asymmetrical stretching peak was noticed at 1505 cm<sup>-1</sup>, which corresponds to amide, I>C=O stretch was observed at 1703,N-H stretch at 3286.

Efficient strain of *Lactobacilli* then studied for EPS production at different parameters(effect of carbon sources,PH and temperature) and it is found that among various carbon source, glucose was found to be the best for EPS production with optimum PH 6 and temperature 40<sup>0</sup>C.

Purified EPS can be used in various industries such as food additive to give improved texture and better rheology. *Lactobacilli* gain lot of importance as they are alternatives to the polysaccharides of plant and animal origin.The exopolysaccharides produced by probiotic microbes would develop an increased improvement in the properties of the final product as there will be effect from the probiotic organism as well as the EPS secreted by the same.



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