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Biopolymers from Lactic Acid Bacteria

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Abstract: Lactic Acid Bacteria (LAB) are widely used in the fermented food industry worldwide. Certain LAB is able to produce Exopolysaccharides (EPS). Physiochemical properties of EPS make its application in commercialization. For this purpose, in this study, LAB were isolated from 10 different fermented milk products such as home made curd, dairy curd, yoghurt, raw milk, etc. 18 isolates (12 Lactococcus and 6 Lactobacillus) were isolated from samples by streaking the samples directly on deMan, Rogosa and Sharpe (MRSA) medium. Fermentation of EPS was carried out using modified Exopolysaccharide Selection Medium (mESM). Further extraction of EPS was carried out by centrifugation, evaporation, followed by ethanol extraction method. The presence and concentration of EPS was calculated by Phenol sulphuric acid method. Characterization of EPS was done by using FTIR technique.
Keywords: LAB, EPS, MRSA medium, mESM.

I. INTRODUCTION

Majority of the polysaccharides used in foods are of plant, animal and algae origin [1]. But most of them are enzymetically or chemically modified to improve their rheological properties and therefore their use is strongly restricted for food applications. An alternative source of biopolymers is microbial EPS [2]. Since ancient times, Lactic Acid Bacteria (LAB) have been empirically exploited as starter cultures , to improve the preservation, nutritional value and sensorial characteristic of a variety of fermented foods and products derived from animal and vegetable origins. In addition to their main feature which is lactic acid production, certain LAB can form other compounds also such as vitamins, bioactive peptides, antibacterial compounds, aroma compounds, low calorie sugars, EPS – exopolysaccharides [3]. Homo and Hetero are the types of exopolysaccharides. EPSs of microbial origin are ubiquitous in nature. EPS have been proved to show important health benefits like antioxidant, cholesterol lowering, antitumor, antiviral, and immunomodulatory activities. Also they reduce formation of pathogenic biofilms, which help in modulation of adhesion to epithelial cells and increase levels of *bifidobacteria* showing a prebiotic potential. Microbial polymers are used in food, textile, detergents, beverages, pharmaceutical, biotechnology, agricultural, paper, paint, and petroleum industries, drug delivery and cancer therapy and in formulation of culture media[4]. EPS produced by LAB, are widely used to improve the body and texture of Yoghurt and other fermented milk products like dahi. Microbial polysaccharides have rheological properties that match the industrial demands and can be produced in large amounts and high purity.

II. MATERIALS AND METHODS

A. Collection of Samples

The lactic acid bacterial isolates were obtained from fermented dairy products, such as homemade curd, dairy curd, raw milk and yoghurt. In all total 10 different samples were collected.

B. Isolation and Identification of Lactic Acid Bacteria

From each sample, a loopful of culture was directly streaked on sterile MRS agar medium, and plates were incubated at 37º C for 24 to 48hr anaerobically. 15 isolates were characterized by microscopic, morphological, examination by Gram's staining, metachromatic granule staining and catalase activity. All the strains were maintained by monthly sub-culturing from 48 hrs MRS agar cultures.

C. Production of EPS by the LAB strains Using mESM medium

The identified isolates were cultivated in modified Exopolysaccharides Selection Medium (mESM).

1) A loopful of each of the working cultures was transferred into 100ml conical flasks containing 10ml of mESM broth and the broths were incubated anaerobically for 24hrs at 30ºC.

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2) 10ml inoculum was transferred into 200-ml conical flasks containing 90ml of mESM broth and incubated at 30ºC for 36hrs anaerobically.
3) After incubation samples were taken and analysed for EPS production. (Ishola, R. O., & Adebayo-Tayo, B. C. 2012). Screening of lactic acid bacteria isolated from fermented food for bio-molecules production. *AU Journal of Technology*, *15*(4)).

D. Isolation, Purification and Quantification of EPS Produced by the LAB isolates

The EPS were isolated according to the method of Garcia-Garibay and Marshall (1997).

1) The lactic acid culture was treated with 17% (w/v) of 80% trichloroacetic acid solution and centrifuged at 16,000 rpm at 4ºC for 30 min.
2) The clarified supernatant was concentrated 5 times by evaporation using a rotavap evaporator.
3) The EPS were precipitated by adding 3 volumes of cold absolute ethanol, and stored overnight at 4ºC.
4) Finally, the recovered precipitates were re-dissolved with distilled water and dialysed against the same solution for 24hrs at 4ºC.

The total amount of carbohydrates in the polysaccharides was determined by the phenol-sulfuric acid method described by (DuBois *et al.*, 1956).

III. RESULTS AND DISCUSSION

Fig.1 Screening of Lactic Acid Bacteria on MRS agar plates from different fermented milk products.

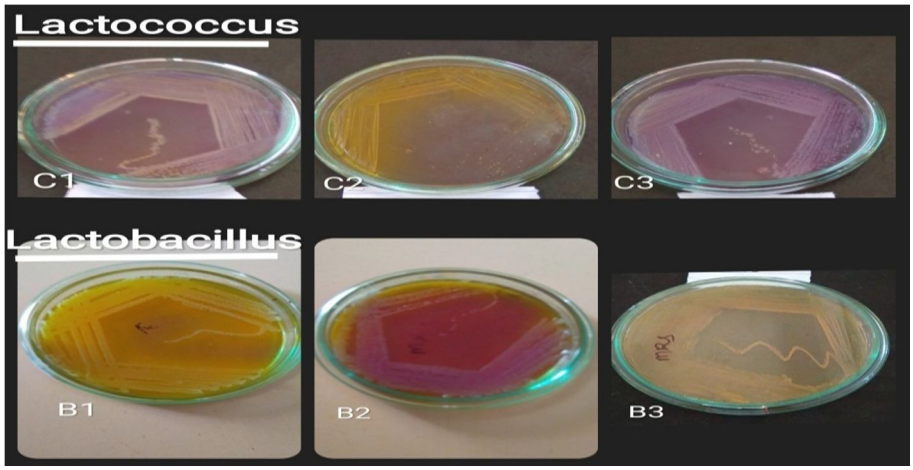

Fig.2 Lactic Acid Bacteria isolates on MRS agar plates.

From different fermented milk products, lactic Acid Bacteria were isolated. From homemade curd, 4 *Lactococcus* and 3 Lactobacillus, from dairy curd, 3 *Lactococcus* and 3 *Lactobacillus*, from raw milk, 3 *Lactococcus*, from Yoghurt, 2 *Lactococcus* were isolated. Thus total 18 Lactic Acid Bacteria were isolated from homemade curd, dairy curd, raw milk and yoghurt. Among them, 6 were *Lactococcus and* 12 were *Lactobacillus.* The isolates were initially differentiated on the basis of their cultural and morphological studies after that by microscopic examination – Gram's staining, metachromatic granules staining and catalyse activity, they were confirmed. The isolates were gram positive, long and thick rods/cocci shaped and catalase positive.

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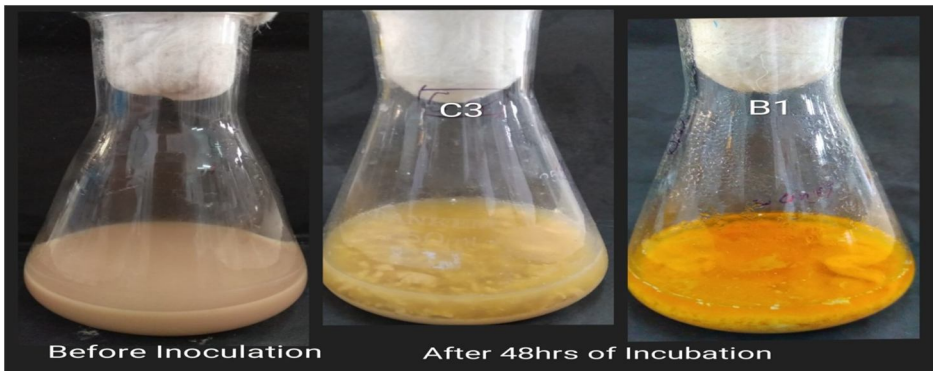

Fig.3 Fermentation of EPS by using mESM.

By using modified Exopolysaccharide Selection Medium, fermentation for EPS production was carried out and all isolates gave the positive results.

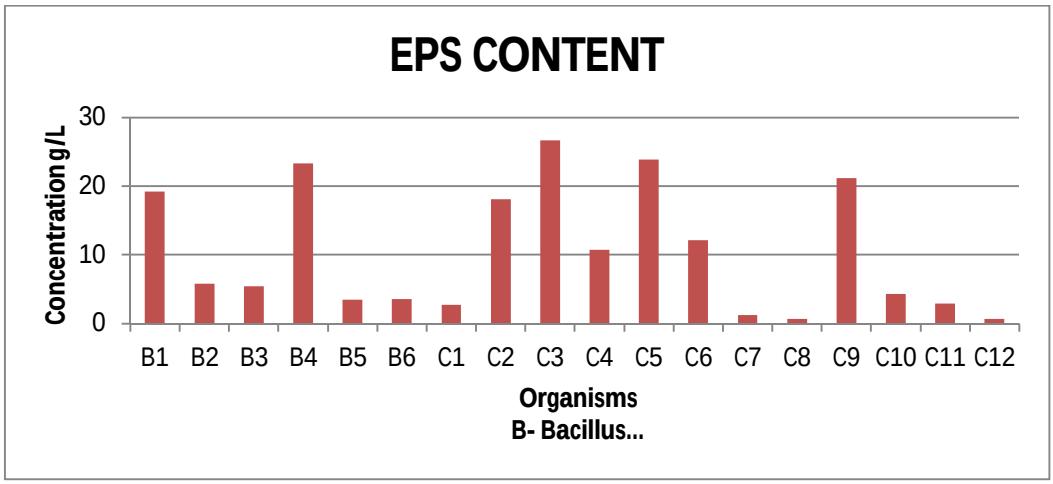

Fig.4 Total EPS content present in the individual isolates.

Phenol- sulphuric acid method was performed to determine the total concentration of exoopolysaccharides present in the individual organisms, after the evaporation and ethanol-extraction methods. All isolates gave the EPS production in the range of 0.65 to 26.7 g/L. C3, C5, B1, B4 gave higher production of exopolysaccharides. Among 18 LAB isolates screened during this study, all were found to be potential EPS producers. This result is similar to the work of Ishola, R. O., & Adebayo-Tayo, B. C.2012 [5] in which thirty five LAB were screened for EPS production and all were found to be potential EPS producers. This result is in contrast to the work of Van Geel-Schutten *et al.*, 1998 [6] in which 60 lactobacillus strains were active producers of EPS among 82 isolates screened. This work is also in contrast with the work of Adebayo-Tayo and Onilude, 2008[7] in which out of 119 isolates screened, 103 isolates had best EPS producers.

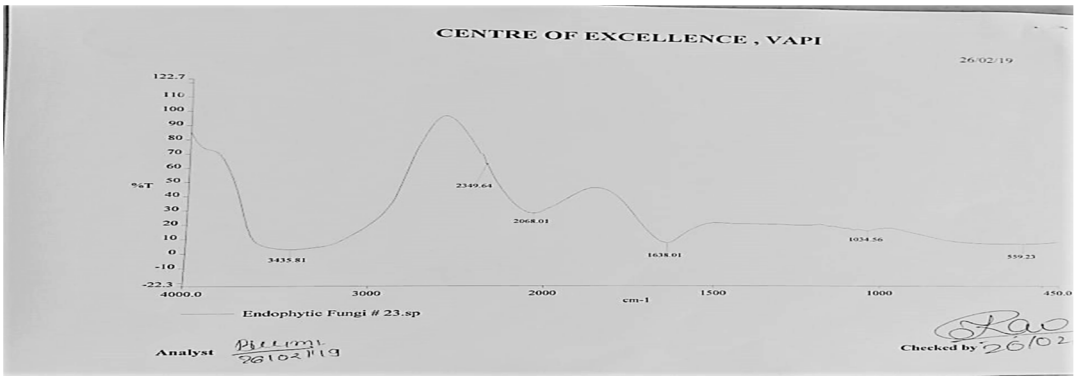

Fig.5 FTIR analysis for characterization of EPS.

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Among 18 isolates, C3 gave the highest EPS production, thus EPS produced by this organism was characterized by FTIR methods. FTIR analysis of this EPS of C3, revealed the distribution of functional groups within the EPS such as carboxylic, hydroxyl, methylene, amino and alkin groups. The presence of various functional groups may be attributed to the existence of potential EPS.

IV. CONCLUSION

A. Production, extraction, and purification of EPS from LAB which were isolated from milk products, was successfully done by evaporation, ethanol extraction and dialysis method and quantification of EPS was done by Phenol-Sulfuric acid method.

B. Characterization of EPS of highest EPS producing organism C3 was done by the method FTIR.

V. ACKNOWLEDGMENT

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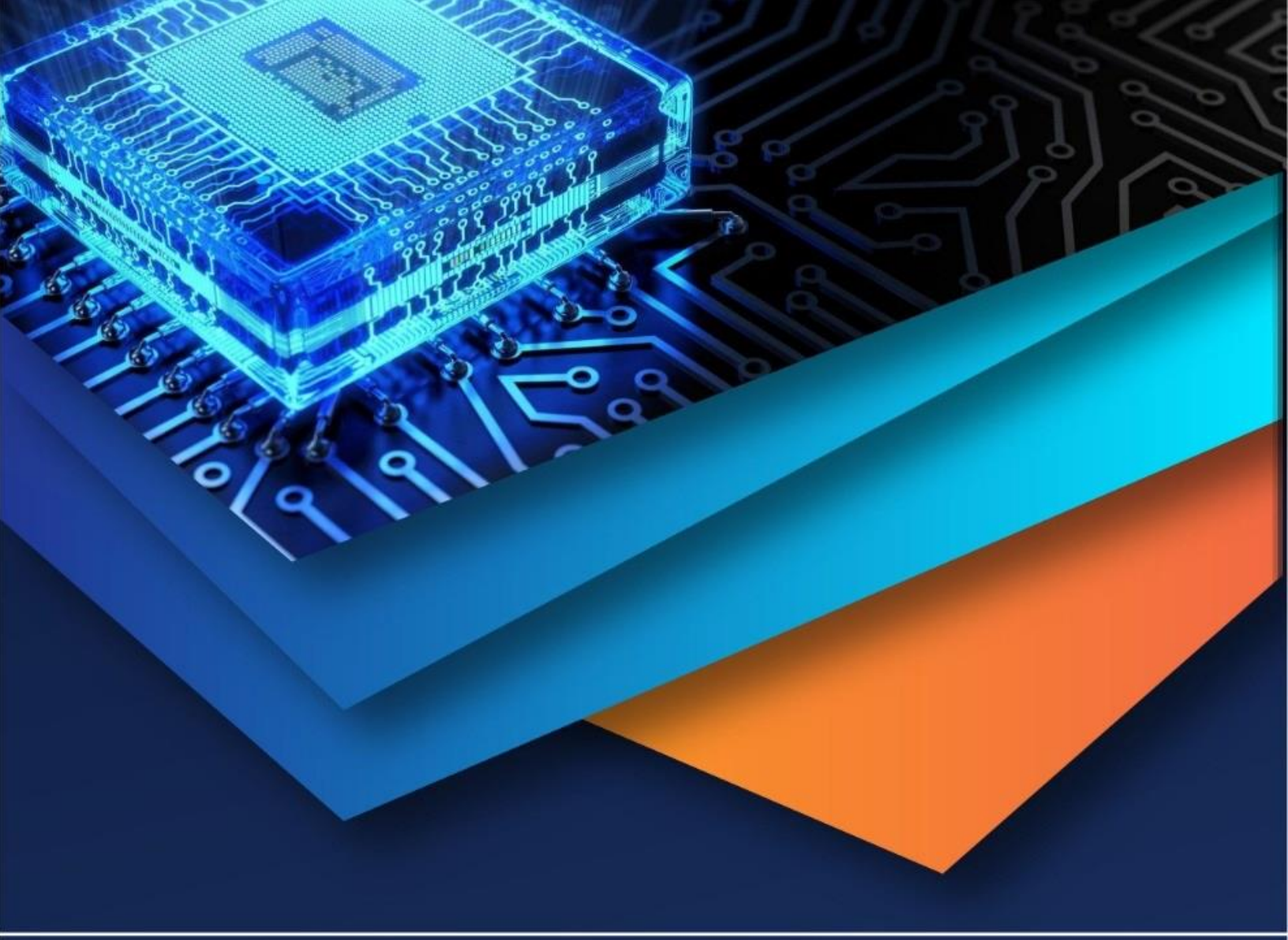

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