VOLUME - 14, ISSUE - 05, MAY - 2025 • PRINT ISSN No. 2277 - 8160 • DOI : 10.36106/gjra

Original Research Paper | Microbiology

EXTRACTION OF EXOPOLYSACCHARIDE PRODUCING BACTERIA FROM SOIL FOR THE DEVELOPMENT OF ANTIMICROBIAL PACKAGING MATERIAL FOR FOOD PRESERVATION

K. Madhanasundareswari* Associate Professor, Sri Ramakrishna College of Arts & Science for Women. *Corresponding Author

K. R. Uthra Das M.Sc. Microbiology, Sri Ramakrishna College of Arts and Science for women.

ABSTRACT Exopolysaccharide are high molecular weight polysaccharides that are secreted by microorganisms from the surrounding environment. They are produced by variety of bacteria, fungi and algae often as a part of biofilm formation or to provide protection from environmental stresses. Polysaccharides are important potent molecules with their structural and compositional complexity which led to wide range of applications in various industries. The exopolysaccharides of microbial origin are released in response to extreme environmental conditions for the purpose of survival. The study focusses on the isolation of exopolysaccharide producing bacteria from soil sample. Screening for the EPS production by the isolates is determined by the dry weight determination of precipitates of EPS. After that the extracted EPS solution was used for several applications. In this study EPS solution was used for food preservation. EPS solution for coating purpose to enhance the shelf-life of the fruits and vegetables. The EPS producing isolates were further grown in the presence of carbon sources like glucose, lactose, maltose and sucrose to determine the best utilizable carbon for their growth. The most utilizable carbon source for maximum growth of EPS producing isolates was determined to be sucrose with 2% concentration. Bacterial isolates were screened for their ability of antibiotic resistance.

KEYWORDS : Exopolysaccharides, Screening, Carbon source, Antibiotic resistance.

INTRODUCTION

Exopolysaccharides are high-molecular weight polysaccharides that are secreted by microorganisms from the surrounding environment. Microbial cells generally contain three types of polysaccharides with different functions – a) intracellular polysaccharide which provides a mechanism for carbon and energy storage, b) structural polysaccharides such as lipopolysaccharide and teichoic acids that are presented as integral components of cells walls, c) extracellular polysaccharide or exopolysaccharides (EPS) which are water soluble gums with unique and novel properties (Ayer *et al*., 2005).

The unique structural and physical properties of exopolysaccharides make them versatile agents, exhibiting a range of valuable functions including emulsification, gelation, thickening, stabilization, suspension, film coating, coagulation and viscosification, highlighting their broad applications in both industrial and research settings. In (2009), Schmid *et al*. found evidences for plant origin of *Cronobacter* and investigated biochemical traits associated with plant microorganisms in *Cronobacter* species and found that all strains were able to produce indole acetic acids, produce siderophores and solubilize mineral phosphate. The strains were also able to colonize maize and tomato roots. Polysaccharides are complex, potent molecules with diverse structural and compositional properties, rendering them versatile tools in various industries. Microbial exopolysaccharides, in particular are produced as a survival strategy in response to harsh environmental conditions, showcasing their adaptive significance.

Exopolysaccharides are complex high-molecular weight polysaccharides produced by wide range of microorganisms including bacteria, fungi and algae. These extracellular polymeric substances are secreted into the surrounding environment, where they play a crucial role in the survival and adaption of microorganisms to various ecological niches. With their unique structural and compositional properties, EPS have several significant attentions in recent years due to their potential applications in various industries. EPS can be used as moisturizers, anti-aging agents, and skin protectants. Exopolysaccharides are used in various industries as in food, pharmaceuticals, textiles, cosmetics, metal mining, oil recovery and other industries (Kaur *et al*.2013; Sivakumar *et al*., 2012). In medical fields exopolysaccharides are used as anti-tumor, anti-viral, immune-modulator agents for drug delivery devices, in vaccine preparations, wound healing (Morris and Harding 2009; Emmanuel *et al*., 2012). Exopolysaccharide is used for the coating purpose in food items to enhance their shelf-life. EPS coating can regulate moisture loss, reducing transpiration and maintaining fruits and vegetables freshness and can inhibit the growth of microorganisms, reducing spoilage and extending shelf life. EPS coatings can help maintain the texture and crispness of fruits and vegetables and also can reduce respiration rates, slowing down the ripening process. The factors affecting EPS coating that efficacy the concentration of EPS in the coating solution can affect its efficacy.

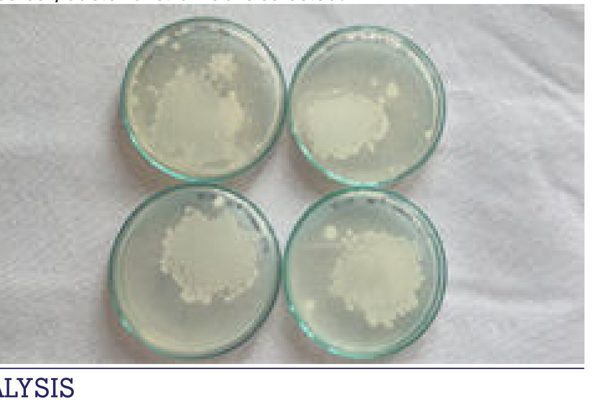
MATERIALS AND METHODS

Collection Of Sample

Soil sample was collected from rhizosphere of maize plants for the isolation of bacterial isolates. Total 50gms of soil from Palakkad Kerala.

Isolation Of Bacteria From Sample

Various types of bacterial strain were isolated from the soil sample by serial dilution upto 10^{-8} was made. 0.1 ml of each dilution was suspended on the nutrient agar plate by spread plate technique. Based on the colony morphology, size, and colour, bacterial strains are selected.



Primary Screening Of Bacteria For EPS Production

The isolates were tested for the EPS production. These isolated colonies were streaked on nutrient agar plate supplemented with 2% sucrose. Isolate shows positive for the EPS production by forming mucoid colonies on the nutrient agar plate.

Secondary Screening Of Bacteria For EPS Production

EPS positive isolates from the primary screening was taken and used for secondary screening. Nutrient broth with 2% sucrose was prepared and inoculated. Then it was incubated in the rotary shaker for 3days in each day OD was taken at 490nm and 610nm after 24, 48 and 72hours of incubation for growth status and EPS production. The positive isolate that showed the highest OD of 1.60 indicates the EPS production.

Extraction Of Exopolysaccharide

Exopolysaccharide was extracted from the isolate. The fermentation broth was centrifuged at 4000rpm for 30mins. Pellet was discarded and the supernatant was taken and it was diluted with cold ethanol thrice its level.

Extracted EPS

Optimization Of Carbon Source For EPS Production

Different carbon sources in different concentration were used for the optimization studies for EPS production on selected isolates. Among the carbon sources lactose and maltose shows higher rate of growth than fructose and glucose.

Table 1: Showing Result For Carbon Source Production

Isolate – S1	Biochemical Tests
Indole	Negative
MR	Negative
VP	Positive
Citrate	Positive
Catalase	Positive
Oxidase	Negative
Urease	Positive
Nitrate	Positive
Maltose	Negative
Glucose	A/G
Lactose	Positive
Sucrose	Negative

RESULT

IDENTIFICATION OF POTENTIAL ISOLATES

MORPHOLOGICAL AND BIOCHEMICAL IDENTIFICATION

Morphological Identification: Cultural and morphological identification of 5 bacterial isolates selected for the study were observed as Gram Positive *Bacilli*.

Biochemical identification: The EPS producing bacterial isolates were further identified by the biochemical test.

Table 2: Showing Result For Biochemical Tests

Carbon source	Sample
Glucose 2%	A/G production
Glucose 4%	No gas production
Lactose 2%	A/G production
Lactose 4%	A/G production
Maltose 2%	A/G production
Maltose 4%	A/G production

Antimocrobial Activity Of EPS By Agar Well Diffusion Method

Muller Hinton agar was prepared in the plate and test organism (*E.coli* , *Staphylococcus aureus*) are swabbed uniformly. Wells were made by sterile cork borer and different quantity of the extract was added in each well. The zone of inhibition was measured.

It is to assess their effectiveness in inhibiting microbial growth. EPS extract of 50μl & 75μl exhibit large zone of inhibition against *E.coli* & *Staphylococcus*.

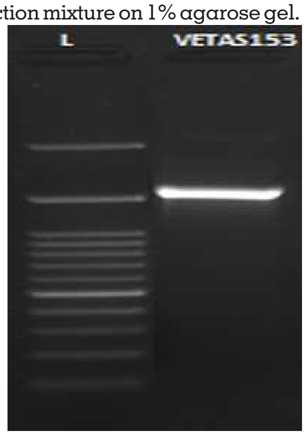
Molecular Identification

Isolation of DNA

The genomic DNA was isolated for potential isolate by using C-TAB method. The isolated DNA was identified by using 0.8% agarose gel followed by observation on ultraviolet trans illuminator revealed sharp high molecular weight bands of DNA that indicates the DNA was of good quality and suitable for PCR analysis.

16S rRNA Sequencing

The 16S rRNA of the bacterial isolate was amplified by performing PCR by using total DNA isolated from organism using universal 16S primer. The confirmation of amplification product was done by agarose gel electrophoresis by running 5µl of PCR reaction mixture on 1% agarose gel.



The amplified product was sequenced in Veta Genomics Research institute Trissur, Kerala. The obtained sequences were compared using BLAST analysis. 16S rRNA gene of the bacterial isolate showed 100% identical to *Bacillus thuringiensis* submission ID of NBRC101235 and finally Phylogenetic tree was constructed.

Phylogenetic Tree Construction

Application Of EPS On Fruits And Vegetables To Increase The Shelf-life

When compared to control samples such as fruits and vegetable the EPS treated samples withstand more days as in the form of fresh nature. This may be due to formation of thin film on the vegetables by the applied EPS. This acts as a barrier for the bacteria, so contamination of vegetables prevented. But in the case of normal control vegetables the bacteria and other contaminants easily evade and destroy it.

Control

After 10 days– No Spoilage

After 15 days – No Spoilage Control

DISCUSSION

Exopolysaccharides that has many advantages and applications. Exopolysaccharides are the high-molecular weight polymers composed of sugar residues produced by microorganisms such as bacteria and yeast. These polysaccharides are secreted into the surrounding environment where they play various roles in the microbial life cycle. Isolate have screened and identified it is capable of producing EPSs by call and colony phenotypes in solid medium. This visual cell classification tool has been widely used for many years to identify EPS producing bacteria from naturally occurring microbiota as a pre selection process.

It is well known that *Bacillus* are useful microorganisms in dairy technology, along with documented history of use in foods. To date a number of *Bacillus* strains have been screened for their ability to produce exopolysaccharides as well as their health beneficial effects. Moreover, production of exopolysaccharides is dependent on specific growth phase of bacteria and media components. Hence favourable media composition especially carbon sources (glucose & fructose) and bacterial growth should be optimized in order to get better exopolysaccharide yield from lactic acid bacteria.

In the present study, bacterial strain were isolated from the soil sample and screened for exopolysaccharide production. The strain were identified as higher level EPS producers. It is identified as *Bacillus thuringiensis* by 16S rRNA sequencing. The organism was able to produce heteropolysaccharide capsule composed of glucuronic acid, D-glucose, D-galactose, D- fructose and D- mannose.

Different simple sugars were used as carbon sources instead of compound material. The effects of these simple sugars on EPS production was examined. Due to its many advantageous properties like availability, ease of storage, high sucrose and other nutrient contents, and the low cost, molasses has been used as a substrate for fermentation production of commercial polysaccharides like curdlan, xanthan, dextran, scleroglucan and gellan (Mao *et al*., 2011).

The sucrose carbon source at 2% concentration was found to be the most utilizable carbon source by the EPS producing isolate. Among the carbon sources lactose and maltose shows higher rate of growth than fructose and glucose. Antimicrobial activity of EPS by agar well diffusion method.The extracted Exopolysaccharide has different applications and in this study EPS was used for the coating purpose. The Exopolysaccharide that is coated on the fruits and vegetables to enhance their shelf life. It was kept in the room temperature for about 20 days the control sample that was spoiled in 5-6 days and the coated sample that withstand for 20 days without any change in its texture, colour and smell.

REFERENCES:

[1] Kordi, S., Saidi, M., & Ghanbari, F. (2013). Induction of drought tolerance in sweet basil (Ocimum basilicum L.) by salicylic acid. *International Journal of Agricultural and Food Research,* 2(2), 18-26.
[2] T. Sivakumar, S. Sivasankara Narayani, T. Shankar and P. Vijayabaskar (2012). Optimization Of Cultural Conditions For Exopolysaccharides Production By *Frateuria aurentia.International Journal of Applied Biology and Pharmaceutical Technology,*3(3),133-143.
[3] Schmid, J., Sieber, V., & Rehm, B. (2015). Bacterial exopolysaccharides: Biosynthesis pathways and engineering strategies. *Frontiers in Microbiology*, 6, 49.
[4] Y Chen, W Mao, H Tao, W Zhu, X Qi, Y Chen, H Li (2011). "Structural characterization and antioxidant properties of an exopolysaccharide produced by the mangrove endophytic fungus Aspergillus sp. Y16" Bioresource,102(17), 8179-8184.
[5] Varinder Kaura, M.B. Beraa*, P.S. Panesara, H.K. Chopra (2013). Production and Characterization of Exopolysaccharide Produced by Alcaligenes Faecalis B14 Isolated from Indigenous Soil. *International Journal of Biotechnology and Bioengineering Research,*4(4), 365-374.
[6] Wenjun Mao, Chenglong Chen, Xianglan Kong, Qianqun Gu, Na Li, Xue Liu, Baofeng Wang, Shuyao Wang, Bo Xiao(2014). Structural elucidation of the exopolysaccharide produced by the mangrove fungus *Penicillium solitum,* Carbohydrate Polymers.111,485-491.