Research Article

Suitability of Growth Media and Carbon Source for EPS Production by *Lysinibacillus macroides*

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ABSTRACT

Exopolysaccharides (EPS) are carbohydrate polymers excreted by bacteria and fungi in the external environment of their cell walls. This study aims to obtain EPS-producing bacteria from the rhizosphere soil of the legume plant. Isolation and screening of potential organisms were performed using the serial dilution method and YEM agar. Based on the mucoid characteristics, 07 isolates were selected for further EPS production from 12 obtained isolates. These 07 isolates were again inoculated in YEM broth and incubated. After incubation, extraction and purification of EPS were carried out by centrifuging the medium at 10000 rpm. Then the supernatant was recovered and chilled ethanol (3 times the volume) was added. Isolate No. KR7 was found more potential with 4.4 g L^{-1} EPS production in YEM broth. 16S rRNA sequencing identified it as *Lysinibacillus macroides*. Different growth media were used to obtain the highest EPS and found Media-6 suitable for that with 16.5 g L^{-1} EPS production. Bagasse can be a cheaper carbon source with 5.5 g L^{-1} EPS production.

Keywords: Bagasse, Exopolysaccharide, Growth media, Modified YE medium.

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INTRODUCTION

A number of bacteria are synthesizing biodegradable Exopolysaccharides (EPS) biologically having a high molecular weight.[1] Bacteria utilize any carbon source for the production of these secondary metabolites. Polysaccharides occur as a storage polysaccharide, an important constituent, in plants as well as in microbial cell walls They may be either endopolysaccharides or exopolysaccharides, depending on their position. They are classified into two categories: (1) Homopolysaccharide – having only one type of sugar moiety e.g., dextran, cellulose, pullulan, etc., (2) Heteropolysaccharide – containing two or more sugar moiety e.g., xanthan and gellan.[2] Bacterial EPS are classified based on their degree of attachment with the cell surface as capsular or slime EPS.[3] Some species of Gram-positive bacteria like *Bacillus*, *Streptococcus*, *Leuconostoc*, *Lactobacillus*, *Diplococci,* etc. are reported as a producer of bacterial EPS. On the other hand, some species of Gram-negative bacteria are also reported as EPS producers such as *Enterobacter*, *Klebsiella*, *Pseudomonas*, *Azotobacter*, *Rhizobium*, *Xanthomonas,* etc.[2] Many factors are influencing EPS production like incubation time, pH, incubation temperature, and media composition, especially carbon and nitrogen sources.[4]

In soil, an important and highly rich source of organic material is available naturally, known as the rhizosphere. This source provides organic matter to the plant roots either directly (by releasing and decomposition of soil root material) or indirectly (by root exudation) and in this way, it stimulates microbial activity as well as biomass in the soil.[5]

The Rhizosphere is a very rich source of organic materials and contributes to improving organic matter in the soil directly or indirectly by stimulating microbial activities and biomass.[5] At different niches, it contains various types of bacteria capable of producing EPS. So, depending on the specific natural habitats, the

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physiological activity of the EPS varies. It provides protection to the cells against different adverse environmental effects like predation, desiccation, and antibiotic susceptibility. Bacterial EPS can be used as a gelling agent and emulsifier mainly in processes like environment bioremediation, biofilm formation, surface attachment, plant-microbe symbiosis, microbial aggregation, etc.[6,3]

Presently, the production cost of EPS is the major concern at the time of large-scale production. Among total production costs, more than 50% of the cost accounts for the carbon source.[7] So, to reduce the EPS production cost, many researchers are trying to use various agricultural wastes as cheap carbon sources like fruit waste,[8,9] pineapple waste,[10] citrus fruit waste,[11,12] milk whey,[13] olive mill wastewater,[14] beet molasses,[15] sugarcane vinasse,[16] cane molasses,[17,4] rice bran,[17,7,4] wheat bran sawdust, sweet whey, potato waste, corn steep liquor, glucose syrup,[17] corn straw, coconut shell, corn cob, passion fruit peel,[18] etc.

The aim of the present study is to isolate and screen bacteria from rhizosphere soil capable to produce EPS and find out suitable growth media for the maximum EPS yield. This study also includes EPS extraction, culture identification, and the use of different agricultural residues as cheap carbon sources.

MATERIALS AND METHODS

Sample collection

In sterile containers, about 10 g of rhizosphere soil sample was collected from a Legume Plant (*Cajanus cajan*), of the garden of Excel Industries Limited, MSW Treatment Plant, Pirana, Ahmedabad. All samples were stored at 4°C until further use.

Isolation, screening and characterization of EPS-producing micro-organisms

Serial dilution of the Rhizospheric soil sample was made in the laboratory under aseptic conditions. First of all, 1 g of soil was aseptically added to a 9 mL sterile distilled water tube and vortexed. The tube was kept in a stationary condition to obtain soil suspension. Now 1 mL suspension was aseptically pipetted and transferred to the next tube of 9 mL sterile distilled water and mixed well. This gives 10^{-1} dilution. Similarly, further dilutions of 10^{-2}, 10^{-3}, 10^{-4}, and 10^{-5} were prepared. A spread plate method on a sterile Yeast Extract Mannitol (YEM) agar plate was performed from the dilutions 10^{-4} and 10^{-5} to obtain isolated and differentiated colonies. The plates were incubated in the incubator for 48-72 hr at 32 ± 2°C.

Composition of YEM Agar (g L^{-1})

Mannitol, 10.0; Yeast Extract, 1.0; Dipotassium Hydrogen Phosphate, 0.5; Sodium Chloride, 0.1; Magnesium Sulphate, 0.2; Congo Red, 0.025; Agar, 20.0; Final pH, 7.4 ± 0.2]. After incubation, all mucoid and moist colonies were selected and purified by further plating on sterile YEM agar plates by the streak plate method. All the purified bacterial colonies were further screened out for the production of EPS.

The primary criteria for bacterial colony selection were its consistency and appearance. All mucoid colonies on the YEM agar medium showing glistening and slimy appearance were primarily isolated as EPS producers.[19] The isolates were studied according to Gram staining and colonial characteristics.[20]

Production, extraction and estimation of EPS

Screened bacteria were taken for EPS production. Cultures were streaked on slants of YEM agar for maintenance and stored at 4°C. 50 mL of YEM broth was taken for EPS production in 250 mL flasks. Media were sterilized at 121°C for 20 min. Sterilized media were inoculated with 1 mL of inoculum and incubated for 72 hr at room temperature (32 ± 2°C) on a rotary shaker (120 rpm).[21]

After incubation, 5% w/v Trichloroacetic Acid (TCA) was added to the flask and incubated at room temperature for 30 min with agitation.[22-25] Proteins precipitated in the medium were separated from cells by centrifugation at 10,000 rpm for 20 min. After centrifugation, the supernatant was recovered; three volumes of ice-cold ethanol were added and kept overnight at 4°C. Precipitated EPS was centrifuged at 10,000 rpm again for 20 min. to separate it. Collected EPS was 3 times washed with cold ethanol and centrifuged in the same way mentioned above. After 3 washes, EPS was dried at 65°C and weighed to know the quantity of the EPS produced by each isolate.[2]

16S rRNA sequencing for identification of the isolate

The isolate showing the highest amount of EPS production was selected for identification. Culture isolate was prepared on slants and sent to the National Chemical Laboratory (NCL), Pune for identification.

Production of EPS in different growth media

Different researchers used different growth media to study the production of EPS depending on the culture they used. The suitability of the growth medium is totally depending on the strain producing the EPS. In the present research work, eleven growth media were

studied as a growth media to find out their suitability for EPS production. These media compositions (g L^{-1}) are as below:

Media-1 (De Man Rogasa and Sharpe Medium): glucose, 20.0; beef extract, 10.0; peptone, 10.0; sodium acetate, 5.0; yeast extract, 5.0; dipotassium hydrogen phosphate, 2.0; ammonium citrate, 2.0; Tween-80, 1.0; magnesium sulphate, 0.1; manganese sulphate, 0.05; pH, 6.5 ± 0.2;[26-28,2]

Media-2 (Commercial Medium): glycerol, 120.0; glutamic acid, 20.0; citric acid, 12.0; ammonium chloride, 7.0; magnesium sulphate, 0.5; ferric chloride, 0.04; dipotassium hydrogen phosphate, 0.5; calcium chloride, 0.15; pH, 6.5;[29]

Media-3 (Mineral Medium): glucose, 25.0; magnesium sulphate, 0.2; potassium dihydrogen phosphate, 1.0; dipotassium hydrogen phosphate, 2.0; ammonium chloride, 1.0; yeast extract, 0.01; pH, 7.0;[30]

Media-4 (Yeast Extract Medium): mannitol, 10.0; yeast extract, 1.0; sodium chloride, 0.1; dipotassium hydrogen phosphate, 0.5; magnesium sulphate, 0.2; Congo red, 0.025; pH, 7.4 ± 0.2;[2]

Media-5 (Basal Medium): glucose, 10.0; yeast extract, 0.5; peptone, 2.0; dipotassium hydrogen phosphate, 2.0; magnesium sulphate, 0.5; ammonium sulphate, 0.5; sodium chloride, 0.1;[31]

Media-6 (Modified YE Medium): yeast extract, 1.0; potassium dihydrogen phosphate, 1.0; magnesium sulphate, 0.5; sucrose, 30.0; pH, 7.0;[2]

Media-7 (EPS Production Medium): glucose, 5.0; yeast extract, 3.0; peptone, 5.0; potassium dihydrogen phosphate, 3.0; magnesium sulphate, 0.2;[32]

Media-8 (Liquid Medium): sucrose, 20.0; yeast extract, 2.0; peptone, 2.0; pH, 7.0;[33]

Media-9 (Nutrient Broth): yeast extract, 1.5; beef extract, 1.5; peptone, 5.0; sodium chloride, 5.0; pH, 7.4 ± 0.2;[6]

Media-10 (EPS Medium): casein hydrolysate, 15.0; sodium acetate, 12.0; yeast extract, 5.0; dipotassium hydrogen phosphate, 10.0; L-cystine, 2.5; sodium chloride, 0.5, sucrose, 50.0; pH, 7.1 ± 0.2;[2]

Media-11 (Modified EPS Medium): sucrose, 50.0; sodium acetate, 12.0; yeast extract, 5.0; dipotassium hydrogen phosphate, 10.0; sodium chloride, 0.5; pH, 7.1 ± 0.2.[2]

50 mL of each of the 11 media was taken for EPS production in 250 mL flasks. All the media were sterilized by autoclaving at 121°C for 20 min, inoculated with 1 mL of inoculum and incubated for 72 hr at room temperature (32 ± 2°C) on a rotary shaker (120 rpm). After incubation, EPS was extracted from each media as above and estimated EPS production in the respective media.

Use of agriculture residue as a carbon source

Different cheap agriculture residues like bagasse, coconut waste, groundnut shell, rice bran, and wheat husk were studied as a replacement for costly carbon sources for EPS production. One of the above 11 media, the flask that showed the highest production of EPS was selected for this study. 3% of each agriculture residue was added to the media instead of a carbon source and EPS production was estimated as mentioned above.

RESULTS

Isolation, screening and characterization of EPS-producing micro-organisms

Different 12 bacterial colonies were isolated from the soil sample collected from the rhizosphere on the basis of unique colonial characteristics on the YEM agar medium. Out of 12 different isolates, 07 isolates (58%) were selected on the basis of mucoid colony characteristics. Morphological and colonial characterization helps in the partial identification of microorganisms. The colonial and morphological characteristics of all 12 isolates are shown in Tables 1 and 2, respectively. The results of Table 1 reflect that 42% of isolates were producing medium-sized colonies, 33% of isolates were producing large-sized colonies, whereas only 25% of colonies were small-sized. It was also observed that 67% of colonies were round in shape. The results shown in Table 2 indicate that 58% of isolates were producing mucoid colonies with smooth surfaces which were taken further for EPS production. Table 2 showed that all the isolates were Gram-negative in nature. Among them, 50% were long rods and 50% were short rods.

Production, extraction and estimation of EPS

All the selected isolates were grown in the YEM broth and after suitable incubation time, EPS was extracted and estimated. For the quantification of ESP, it was dried and weighed. The results showed that Isolate No. KR7 has the maximum capacity to produce EPS (4.4 g L^{-1}) followed by Isolate No. KR4 (3.6 g L^{-1}) (Figure 1). EPS looked like a thin transparent layer on the surface of extraction media, as shown in Figure 2.

Identification of Isolate No. KR7 by 16S rRNA sequencing

For the molecular identification by 16S rRNA sequencing, a culture sample of Isolate No. KR7 was sent to NCL, Pune. The 16S rRNA sequence of KR7

Table 1: Colonial characteristics of all isolates.

Isolate No.	Size	Shape	Margin	Elevation	Surface	Consistency	Opacity
KR1	M	R	Entire	Flat	Rough	Dry	Opaque
KR2	S	P	Undulate	Flat	Rough	Powdery	Opaque
KR3	**L**	**R**	**Entire**	**Convex**	**Smooth**	**Mucoid**	**Transparent**
KR4	**S**	**R**	**Entire**	**Convex**	**Smooth**	**Mucoid**	**Translucent**
KR5	**M**	**R**	**Entire**	**Convex**	**Smooth**	**Mucoid**	**Translucent**
KR6	L	IR	Undulate	Raised	Rough	Dry	Opaque
KR7	**L**	**R**	**Entire**	**Convex**	**Smooth**	**Mucoid**	Translucent
KR8	M	R	Entire	Convex	Smooth	Mucoid	Transparent
KR9	S	R	Entire	Convex	Smooth	Mucoid	Translucent
KR10	M	R	**Entire**	**Convex**	**Smooth**	**Mucoid**	**Translucent**
KR11	L	IR	Undulate	Flat	Rough	Dry	Opaque
KR12	M	IR	Undulate	Raised	Rough	Dry	Opaque

M=Medium, S=Small, L=Large, R=Round, P=Punctiform, IR=Irregular.

Table 2: Morphological characteristics of all isolates.

Isolate No.	Gram Reaction	Size and Shape	Arrangement
KR1	Gram Negative	Long Rods	Cluster
KR2	Gram Negative	Short Rods	Scattered
KR3	**Gram Negative**	**Short Rods**	**Cluster**
KR4	**Gram Negative**	**Long Rods**	**Scattered**
KR5	**Gram Negative**	**Long Rods**	**Cluster**
KR6	Gram Negative	Long Rods	Scattered
KR7	**Gram Negative**	**Short Rods**	**Scattered**
KR8	**Gram Negative**	**Long Rods**	**Cluster**
KR9	**Gram Negative**	**Long Rods**	**Cluster**
KR10	**Gram Negative**	**Short Rods**	**Scattered**
KR11	Gram Negative	Short Rods	Scattered
KR12	Gram Negative	Short Rods	Cluster

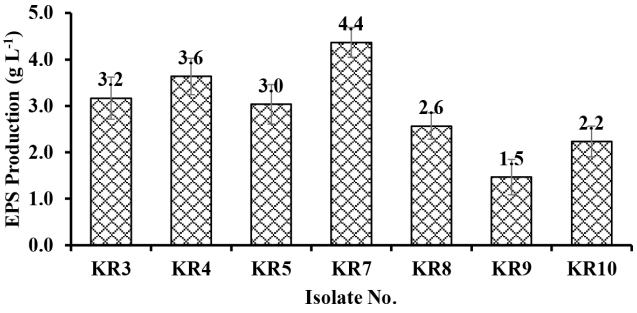

Figure 1: EPS produced by different isolates.

shows 99.70% similarity with *Lysinibacillus macroides* strain LMG 18474 16S ribosomal RNA gene, a partial sequence having accession no. NR_114920.1. The total and maximum score obtained for alignment was 2407 with 99% query coverage and 0.0 E-value. Thus, isolate no. KR7 was identified as *Lysinibacillus macroides*. The

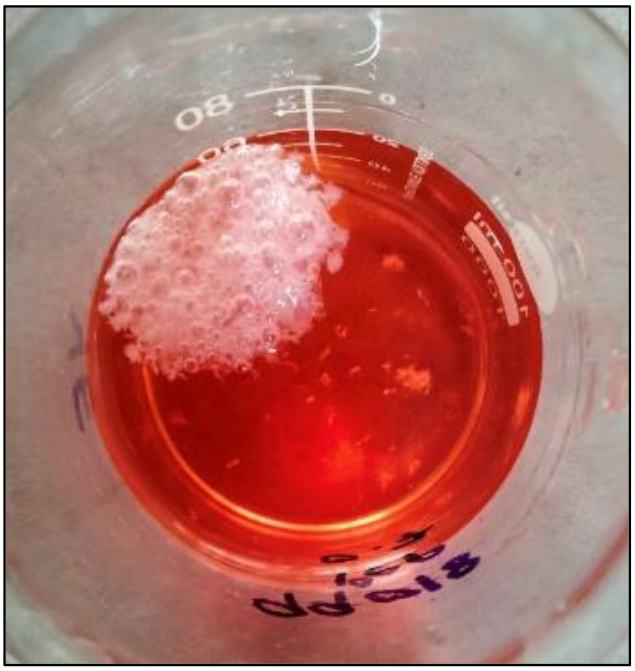

Figure 2: EPS produced by Isolate No. KR7.

phylogenetic tree of the strain *Lysinibacillus macroides* with the closest similarity using the neighbour-joining method is shown in Figure 3.

EPS production in different growth media

11 different growth media were studied for their suitability for EPS production. EPS production was observed in all the media in various quantities. As per the results obtained, we found Media-6 (modified YE medium) the most suitable with 16.5 g L^{-1} of EPS production followed by Media-10 (8.4 g L^{-1}) and Media-11 (7.9 g L^{-1}). Results showed that the media containing sucrose (30 g L^{-1}) and yeast extract (1 g L^{-1}) as a carbon

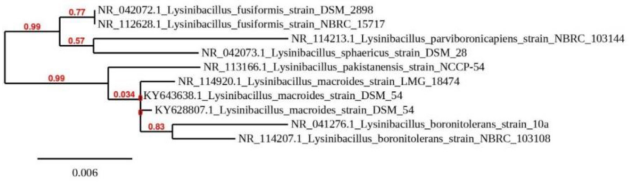

Figure 3: Phylogenetic tree of ***Lysinibacillus macrolides***.

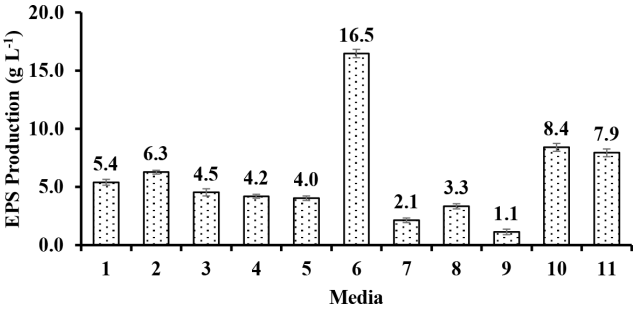

Figure 4: EPS production in different growth media.

and nitrogen source, respectively, and a carbon-to-nitrogen ratio of 30:1, produces the highest number of EPS. Figure 4 represents EPS production in all different growth media.

Use of agriculture residue as a carbon source

Modified YE medium was obtained as a suitable media for the highest EPS production and so it was taken for further study. The carbon source of this media (sucrose) was replaced by different cheap agriculture residues (3% each) like bagasse, coconut waste, groundnut shell, rice bran, and wheat husk. A similar experiment was carried out for EPS production. The results indicated that the media containing bagasse as a carbon source was able to produce maximum EPS with 5.5 g L^{-1} of production followed by wheat husk (3.3 g L^{-1}). Figure 5 represents the EPS production in different media containing different agriculture residues as a carbon source.

DISCUSSION

Bacteria are able to produce EPS in different nutritional environments. Many researchers investigated various growth media suitable for EPS production. In the present study, we have used 11 different media to check their suitability for maximum EPS production and found Media-6 (modified YE medium) the most suitable for EPS production (16.5 g L^{-1}), a similar result reported by Vaishnav *et al.*[8] for the EPS production from *Bacillus* species SRA4 (14.2 ± 0.6 g L^{-1}) in the same media. They investigated EPS production in the same medium for other bacterial species of *B. subtilis*, *B. soneresis* and *B. tequilensis* with the results of 6.95 ± 0.5, 4.9 ± 0.8 and

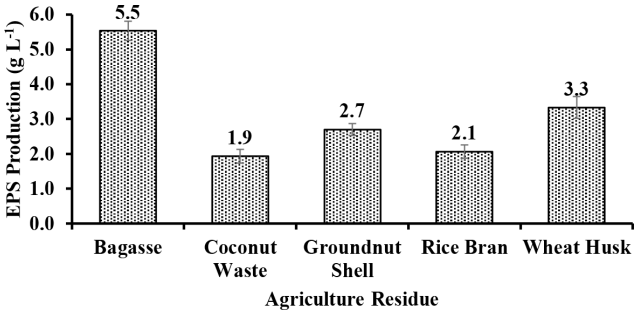

Figure 5: EPS production by using different agriculture residues as carbon source.

6.7 ± 0.5 g L^{-1}, respectively. In contrast, Vaishnav *et al.*[9] also observed maximum EPS production of 12.85 g L^{-1} from *Xanthomonas campestris* in the medium containing 2.0% casein hydrolysate, 0.5% yeast extract and 0.75% sodium acetate.

The EPS production of 504.6 mg L^{-1} from *Lactobacillus delbrueckii* ssp. *bulgaricus* RR, and 454.67 g L^{-1} from bacterial strain RJ-5 were reported in the Media-1 (MRS medium).[26,28] The EPS production from 5.0 to 36.4 g L^{-1} was also reported for 25 different isolates in the Media-3 (mineral medium)[30] whereas Prasertsan *et al.*[31] reported EPS production of 2.23 g L^{-1} from *Enterobacter cloacae* WD7 in the Media-5 (basal medium). Indira *et al.*[32] isolated EPS-producing bacteria in Media-7 (EPS production medium) from the dairy effluents. Different amounts of EPS production from *Bacillus* species were also investigated in the past by various researchers.[33,6] Vaishnav[2] reported different quantities of EPS produced by Bacillus species and *Xanthomonas campestris* using different types of media such as Media-1 (MRS medium), Media-4 (yeast extract medium), Media-6 (modified yeast extract medium), Media-10 (EPS medium) and Media-11 (modified EPS medium).

Various low-cost agriculture residues were studied as a carbon source for EPS production. Agriculture residues can be transformed into biopolymers through various conversion processes, including chemical, enzymatic and microbial routes. These processes involve the extraction of cellulose, hemicellulose and lignin components from the agriculture residues, followed by their conversion into biopolymer precursors. These precursors can be further processed to obtain biopolymers with desirable properties for various applications.

Bagasse, a residue obtained from sugarcane processing, has been extensively studied for biopolymer production. We obtained 5.5 g L^{-1} of EPS production using bagasse as a carbon source. In 2016, Getachew and Woldesenbet[34] reported similar production of PHB (5.0 ± 0.08 g L^{-1}) from *Bacillus* species. Razack *et al.*[4] reported 4.86 g L^{-1}

EPS production from *B. subtilis* by using 2% bagasse as a substrate. The higher pullulan production was also reported by the fungi *Aureobasidium pullulans* (up to 25 g L^{-1}) by using bagasse as a carbon source.[35] Bagasse-based biopolymers have demonstrated good mechanical strength and thermal stability, making them suitable for applications such as packaging materials and disposable utensils.

Coconut shell, a by-product of the coconut industry, has shown potential for biopolymer production. Ogidi *et al.*[36] documented 2.1 g L^{-1} of EPS production from *Pleurotus pulmonarius* by supplementing 4 g L^{-1} coconut husk in the growth media which is almost similar to our results. On the other hand, the higher xanthan production of 5.5 ± 3.8 g L^{-1} was reported for the coconut waste as a substrate.[18] Coconut shell-based biopolymers exhibit excellent water resistance and barrier properties, making them suitable for applications in food packaging and agricultural films.

Groundnut shell, a by-product of the peanut industry, can be utilized for biopolymer production for along. Groundnut shell enhances the EPS production from 3.5 to 5.6 with increasing concentration from 4 to 20 g L^{-1}, which is a slightly higher yield than ours.[36] Groundnut shell-based biopolymers offer advantages such as biodegradability and low cost, making them attractive for applications in sectors like agriculture and packaging.

Rice bran is a by-product of rice milling which has been explored for biopolymer production. Many researchers reported rice bran as a substrate for EPS production. 2.14 g L^{-1} of EPS production from *B. subtilis* was reported, which is similar to our results.[4] Singh and Kaur[37] reported a 5.48% EPS yield by *A. pullulans* at the substitution of 3.88% rice bran concentration in the growth media, whereas Sirajunnisa *et al.*[38] recorded maximum EPS production from *P. fluorescens* CrN6 by supplementing 5.02% rice bran. Rice bran-based biopolymers possess good film-forming properties and have shown potential for applications in coating materials and drug delivery systems.

The wheat husk is an agricultural residue obtained during grain processing. It can serve as a feedstock for biopolymer production. By using wheat husk as a substrate, a similar result of 3.2 g L^{-1} production of EPS from the fungi *A. pullulans* ATCC 42023 has been reported.[17] Some mushroom species such as *Ganoderma lucidum* was also reported as EPS producer in a small amount (1.05 g L^{-1}) by Liu and Zhang.[39] Wheat husk-based biopolymers exhibit good mechanical properties and have potential applications in areas such as construction materials and packaging.

CONCLUSION

A total of 12 bacterial colonies with different characteristics were isolated from the rhizosphere soil sample. From which, 07 isolates were found as EPS producers. Among them, Isolate No. KR7 was identified as *Lysinibacillus macroides* and observed capsulated in capsule staining, with the highest amount of EPS production (4.4 g L^{-1}). The results of this study revealed that Media-6 (modified YE medium) containing sucrose (30.0 g L^{-1}), yeast extract (1.0 g L^{-1}), potassium dihydrogen phosphate (1.0 g L^{-1}), and magnesium sulfate (0.5 g L^{-1}) produced the highest number of EPS (16.5 g L^{-1}). The present study also shows that bagasse can be used as a cheaper source of carbon for EPS production. Further study can be done for the optimization of the media and culture conditions to obtain maximum EPS.

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STATEMENTS AND DECLARATIONS

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ABBREVIATIONS

EPS: Exopolysaccharides; **YEM:** Yeast Extract Mannitol; **TCA:** Tri-Chloro Acetic acid.

SUMMARY

The present study summarises that EPS can be produced in different types of growth media, and among them, the modified YE medium is found the most suitable for maximum EPS production. Various agriculture residues such as bagasse, coconut waste, groundnut shell, rice bran and wheat bran can be used as a low-cost replacement for complex carbon sources. The results of this study indicated that bagasse might be a promising

substrate in the future. A significant number of EPS can be produced by using bagasse as a cheaper substrate than complex and synthetic carbon sources. Further studies can be done with bagasse concentration and its optimization to make it an economical source for EPS production.

REFERENCES

1. Kaur V, Bera MB, Panesar PS, Chopra HK. Production and characterization of exopolysaccharide produced by *Alcaligenes faecalis* B14 isolated from indigenous soil. Int J Biotechnol Bioeng Res. 2013;4(4):365-74.
2. Vaishnav AM. Bacterial exopolysaccharides production from fruits and potato waste. Available from: http://hdl.handle.net/10603/212032 [Ph.D. thesis]. Gujarat, India: Gujarat University; 2017.
3. Sonawdekar S, Gupte A. Production and characterization of exopolysaccharide produced by oil emulsifying bacteria. Int J Curr Microbiol Appl Sci. 2016;5(2):254-62. doi: 10.20546/ijcmas.2016.502.028.
4. Razack SA, Velayutham V, Thangavelu V. Medium optimization for the production of exopolysaccharide by Bacillus subtilis using synthetic sources and agro wastes. Turk J Biol. 2013;37(3):280-8. doi: 10.3906/biy-1206-50.
5. Amellal N, Burtin G, Bartoli F, Heulin T. Colonization of wheat roots by an exopolysaccharide-producing Pantoea agglomerans strain and its effect on rhizosphere soil aggregation. Appl Environ Microbiol. 1998;64(10):3740-7. doi: 10.1128/AEM.64.10.3740-3747.1998, PMID 9758793.
6. Vidhyalashmi R, Nachiyar CV. Microbial production of exopolysaccharides. J Pharm Res. 2011;4(7):2390-1.
7. Saranya Devi ES, Vijayendra SVN, Shamala TR. Exploration of rice bran, an agro-industry residue, for the production of intra- and extra-cellular polymers by Sinorhizobium meliloti MTCC 100. Biocatal Agric Biotechnol. 2012;1(1):80-4. doi: 10.1016/j.bcab.2011.08.014.
8. Vaishnav A, Upadhayay K, Tipre D, Dave S. Utilization of mixed fruit waste for exopolysaccharide production by Bacillus species SRA4: medium formulation and its optimization. 3 Biotech. 2020;10(12):550. doi: 10.1007/s13205-020-02545-2, PMID 33269184.
9. Vaishnav A, Upadhyay K, Koradiya M, Tipre D, Dave S. Valorisation of fruit waste for enhanced exopolysaccharide production by Xanthomonas campestris using statistical optimisation of medium and process. Food Biosci. 2022;46:101608. doi: 10.1016/j.fbio.2022.101608.
10. Pyar HA, Liong MT, Peh KK. Potentials of pineapple waste as growth medium for Lactobacillus species. Int J Pharm Pharm Sci. 2014;6(1):142-5.
11. Green M, Shelef G, Bilanovic D. The effect of various citrus waste fractions on xanthan fermentation. Chem Eng J Biochem Eng J. 1994;56(1):B37-41. doi: 10.1016/0923-0467(94)87029-2.
12. Bilanovic D, Shelef G, Green M. Xanthan fermentation of citrus waste. Bioresour Technol. 1994;48(2):169-72. doi: 10.1016/0960-8524(94)90205-4.
13. Yang ST, Silva EM. Novel products and new technologies for use of a familiar carbohydrate, milk lactose. J Dairy Sci. 1995;78(11):2541-62. doi: 10.3168/jds.S0022-0302(95)76884-9, PMID 8747342.
14. Lopez MJ, Ramos-Cormenzana A. Xanthan production from olive-mill wastewaters. Int Biodeterior Biodegrad. 1996;38(3-4):263-70. doi: 10.1016/S0964-8305(96)00059-5.
15. Roukas T. Pretreatment of beet molasses to increase pullulan production. Process Biochem. 1998;33(8):805-10. doi: 10.1016/S0032-9592(98)00048-X.
16. Ventorino V, Nicolaus B, Di Donato P, Pagliano G, Poli A, Robertiello A, et al. Bioprospecting of exopolysaccharide-producing bacteria from different natural ecosystems for biopolymer synthesis from vinasse. Chem Biol Technol Agric. 2019;6(1):1-9. doi: 10.1186/s40538-019-0154-3.
17. Hafez AA, Abdelhady HM, Sharaf MS, El-Tayeb TS, El-Kheima S. Bioconversion of various industrial by-products and agricultural wastes into pullulan. J Appl Sci Res. 2007;3(11):1416-25.
18. Dos Santos FP, Jra AM, Nunesa TP, de Farias Silvab CE, de Souza Abud AK. Bioconversion of agro-industrial wastes into xanthan gum. Chem Eng. 2016;49:145-50. doi: 10.3303/CET1649025.
19. Fusconi R, Godinho MJ. Screening for exopolysaccharide-producing bacteria from sub-tropical polluted groundwater. Braz J Biol. 2002;62(2):363-9. doi: 10.1590/s1519-69842002000200020, PMID 12489408.
20. Angel SJ. Optimization and characterization of biodegradable adhesives (polyglycolic acid and exopolysaccharides) produced by bacterial isolates. Available from: http://hdl.handle.net/10603/156206 [Ph.D. thesis]. Tamil Nadu, India: Periyar University; 2015.
21. Pawar ST, Bhosale AA, Gawade TB, Nale TR. Isolation, screening and optimization of exopolysaccharide producing bacterium from saline soil. J Microbiol Biotechnol Res. 2013;3(3):24-31.
22. Zhang L, Liu C, Li D, Zhao Y, Zhang X, Zeng X, et al. Antioxidant activity of an exopolysaccharide isolated from Lactobacillus plantarum C88. Int J Biol Macromol. 2013;54:270-5. doi: 10.1016/j.ijbiomac.2012.12.037, PMID 23274679.
23. Shao LI, Wu Z, Zhang H, Chen W, Ai L, Guo B. Partial characterization and immunostimulatory activity of exopolysaccharides from Lactobacillus rhamnosus KF5. Carbohydr Polym. 2014;107:51-6. doi: 10.1016/j.carbpol.2014.02.037, PMID 24702917.
24. Fontana C, Li S, Yang Z, Widmalm G. Structural studies of the exopolysaccharide from Lactobacillus plantarum C88 using NMR spectroscopy and the program CASPER. Carbohydr Res. 2015;402:87-94. doi: 10.1016/j.carres.2014.09.003, PMID 25497338.
25. Dave SR, Upadhyay KH, Vaishnav AM, Tipre DR. Exopolysaccharides from marine bacteria: production, recovery and applications. Environmental Sustainability. 2020;3(2):139-54. doi: 10.1007/s42398-020-00101-5.
26. Kimmel SA, Roberts RF. Development of a growth medium suitable for exopolysaccharide production by Lactobacillus delbrueckii ssp. bulgaricus RR. Int J Food Microbiol. 1998;40(1-2):87-92. doi: 10.1016/S0168-1605(98)00023-3, PMID 9600614.
27. Alhudhud M, Humphreys P, Laws A. Development of a growth medium suitable for exopolysaccharide production and structural characterisation by Bifidobacterium animalis ssp. lactis AD011. J Microbiol Methods. 2014;100:93-8. doi: 10.1016/j.mimet.2014.02.021, PMID 24632517.
28. Chen Z, Shi J, Yang X, Liu Y, Nan B, Wang Z. Isolation of exopolysaccharide□producing bacteria and yeasts from Tibetan kefir and characterisation of the exopolysaccharides. Int J Dairy Technol. 2016;69(3):410-7. doi: 10.1111/1471-0307.12276.
29. Shih IL, Van YT, Yeh LC, Lin HG, Chang YN. Production of a biopolymer flocculant from Bacillus licheniformis and its flocculation properties. Bioresour Technol. 2001;78(3):267-72. doi: 10.1016/S0960-8524(01)00027-X, PMID 11341686.
30. Bala Subramanian S, Yan S, Tyagi RD, Surampalli RY. Extracellular Polymeric Substances (EPS) producing bacterial strains of municipal wastewater sludge: isolation, molecular identification, EPS characterization and performance for sludge settling and dewatering. Water Res. 2010;44(7):2253-66. doi: 10.1016/j.watres.2009.12.046, PMID 20122709.
31. Prasertsan P, Wichienchot S, Doelle H, Kennedy JF. Optimization for biopolymer production by Enterobacter cloacae WD7. Carbohydr Polym. 2008;71(3):468-75. doi: 10.1016/j.carbpol.2007.06.017.
32. Indira M, Venkateswarulu TC, Chakravarthy K, Reddy AR, Babu DJ, Kodali VP. Morphological and biochemical characterization of exopolysaccharide producing bacteria isolated from dairy effluent. J Pharm Sci Res. 2016;8(2):88.
33. Mohamed S, Shimaa R, El Awady M, El Sayed O. Screening of bacterial antioxidant exopolysaccharides isolated from Egyptian habitats. J Chem Pharm Res. 2015;7:980-6.
34. Getachew A, Woldesenbet F. Production of biodegradable plastic by polyhydroxybutyrate (PHB) accumulating bacteria using low-cost agricultural waste material. BMC Res Notes. 2016;9(1):509. doi: 10.1186/s13104-016-2321-y, PMID 27955705.
35. Terán Hilares RT, Resende J, Orsi CA, Ahmed MA, Lacerda TM, Da Silva SS, et al. Exopolysaccharide (pullulan) production from sugarcane

bagasse hydrolysate aiming to favor the development of biorefineries. Int J Biol Macromol. 2019;127:169-77. doi: 10.1016/j.ijbiomac.2019.01.038, PMID 30639656.

36. Ogidi CO, Ubaru AM, Ladi-Lawal T, Thonda OA, Aladejana OM, Malomo O. Bioactivity assessment of exopolysaccharides produced by Pleurotus pulmonarius in submerged culture with different agro-waste residues. Heliyon. 2020;6(12):e05685. doi: 10.1016/j.heliyon.2020.e05685, PMID 33336098.
37. Singh RS, Kaur N. Understanding response surface optimization of medium composition for pullulan production from de-oiled rice bran by Aureobasidium pullulans. Food Sci Biotechnol. 2019;28(5):1507-20. doi: 10.1007/s10068-019-00585-w, PMID 31695950.
38. Sirajunnisa AR, Vijayagopal V, Sivaprakash B, Viruthagiri T, Surendhiran D. Optimization, kinetics and antioxidant activity of exopolysaccharide produced from rhizosphere isolate, Pseudomonas fluorescens CrN6. Carbohydr Polym. 2016;135:35-43. doi: 10.1016/j.carbpol.2015.08.080, PMID 26453848.
39. Liu SR, Zhang WR. Optimization of submerged culture conditions involving a developed fine powder solid seed for exopolysaccharide production by the medicinal mushroom Ganoderma lucidum. Food Sci Biotechnol. 2019;28(4):1135-45. doi: 10.1007/s10068-018-0536-5, PMID 31275713.

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