Bulletin of Environment, Pharmacology and Life Sciences
Bull. Env. Pharmacol. Life Sci., Special Issue [1]2022 : 592-595

Online ISSN 2277-1808
Journal's URL:http://www.bepls.com
CODEN: BEPLAD

ORIGINAL ARTICLE **OPEN ACCESS**

Influence of Salinity on Bacterial Exoployssaccharide Production

Belorkar SA*and Sahu KK
Department of Microbiology and Bioinformatics,
Atal Bihari Vajpayee University, Bilaspur-495001, (CG) India
E-mail-seema.belorkar@gmail.com

ABSTRACT
Water is presently suffering contamination due to heavy metals, aromatic molecules and dyes which are increasing the its toxicity. Removal of toxic pollutants is the utmost important remediation necessity in water treatment protocols. Natural polymers are gaining popularity as successful adsorbents for the pollutants. Polysaccharides production is important as they can be modified for their use in water treatment and for formulation of low cost adsorbents. The present work was initiated by isolation of microbes from water. The preliminary screening of 40 isolates revealed four potent EPS producer namely B-3, B-8 B-15 B-21. The secondary screening exhibited the highest EPS production of 0.7 mg/ml by isolate B-8. Such strains can be further studied for optimization to increase the yield and its application in waste water treatment.
Key words- Exopolysaccharides, waste water treatment, Adsorption, toxic pollutants

Received 11.03.2022 Revised 16.03.2022 Accepted 02.04.2022

INTRODUCTION

Water is a basic necessity, but its availability for human use is hardly about 1%. Current global water crises are due to rapid increase in population, climatic variation, environmental pollution, urbanization, industrialization and contamination of existing water reservoirs. With increased amounts of wastewater, limited space and stricter regulations and quality controls, the demand for new wastewater treatment processes is growing. This has led to the development of new biofilm-based techniques that have high capacity [1]. The development and persistence of biofilms are affected not only by the surrounding environment but also by the variety of species present [2]. Recent observations have shown that factors other than growth rate are important for the relative occurrence and spatial distribution in biofilms [3, 4]. The mechanisms controlling the microbial interactions in multispecies biofilms are not yet fully understood [5, 4]. Microbial ecologists advocate culture-independent, in situ methods to gain further knowledge of individual species in biofilms, their spatial distribution and activity [6].The present work here includes the isolation of EPS producing bacteria from waste water, their preliminary and secondary screening of the isolates. The extraction and estimation of EPS produced [7].

Natural polysaccharides are bestowed with qualities like low price, excellent compatibility, presence of hydrophilic functional groups, hydrophilic nature, easily degradable nature make them of paramount importance(.Hydrogels are mainly synthesized by Polysaccharides. EPS are found to be associated with proteins like lectins which play important role in biofilm production. The complexity of EPS has to be investigated regarding its nature, associated proteins which confer the EPS with water purification ability [8].

MATERIAL AND METHODS

Collection of waste water sample

Waste water is rich in organic matter and excellent source of microorganisms. Sewage water, drainage and industrial effluents were selected as samples.The sample was made to age for 7 days in sterile bottles in which it was collected. The water was left undisturbed for the settlement of sludge. Further they were brought in the laboratory for further investigation. It was inoculated in basal media [1].

Isolation of microorganisms and Preliminary screening

The medium used for isolation was EPS medium KH_2PO_4-0.12gm, $MgSO_4.2H_2O$-0.02gm, K_2HPO_4 -0.15gm, $CaSO_{4.2}H_2O$-0.01gm, $FeCl_3$ -0.2gm. 1ml water sample was added to 9ml of sterilized EPS media to make suspension. The suspension was vigorously shaken and allowedto form uniform suspension. Serial

dilution of the suspension was done in EPS medium. The fifth dilution was transferred to the preliminary screening plate. Pour plate method was used to inoculate the suspension on agar plates. EPS production was detected by Congo red. 2ml of 1% Congo red solution was poured into petriplate for indication of bacterial colony. The colonies which showed pinkish in colored were isolated and maintained pure culture on nutrient agar slants.After preliminary screening 4 isolates were selected on the basis of EPS production [3].

Secondary screening

The selected isolates from preliminary screening were subjected for secondary screening using EPS production medium Peptone Yeast Extract Glucose Broth containing Glucose - 4.0gm, Peptone -1gm, Yeast extracts-3gm in 100 ml distilled water. Sample from pure culture was inoculated into both the conical flasks and incubated. Bacterial EPS was detected by the Congo red plate method.

Precipitation of EPS

The selected bacterial strains were inoculated for production of EPS for 48hr in the production media (Yu et al2004).Centrifugation was used for the removal of cell debris. The sample was centrifuged for 15 min at 12000rpm. Supernatant was removed and added with double volume of ice cold ethanol and incubated for 24 hr at 4°C. It was centrifuged for 30 min at 20,000 rpm [2].

Exopolysaccharide estimation

EPS sample was analyzed by Phenol sulphuric acid method. 0.1ml EPS sample was added with 2.0ml distilled water. 1ml of 6% phenol and 5.0ml of 95% $H_2SO_4$was added inice bath. Sample was incubated for 10mins and absorbance was read at 490nm. Distilled water was used as blank. [2]

Influence of salt concentration on EPS production

NaCl was added in the EPS medium in range of 0.01% to 0.08% and its influence on growth and EPS production was investigated till 48h after every 2h interval.

RESULTS AND DISCUSSION

Isolation of microorganisms

The sources of water samples selected for microbial isolation were sewage, domestic drainage and industrial effluents. The isolation of the bacteria was done in EPS medium. The Cfu/ml of the diluted samples are recorded in Table-I. The results are the mean of the triplicates. The plate inoculated with sterile distilled water was considered to be the control.

Table 1: Isolation of bacteria from the collected water samples

Samples	Cfu/ml
Sewage	65*10^8 ± 0.12[a]
Drainage	122 *10^8 ± 0.25[b]
Effluent	45 *10^8 ± 0.23[c]

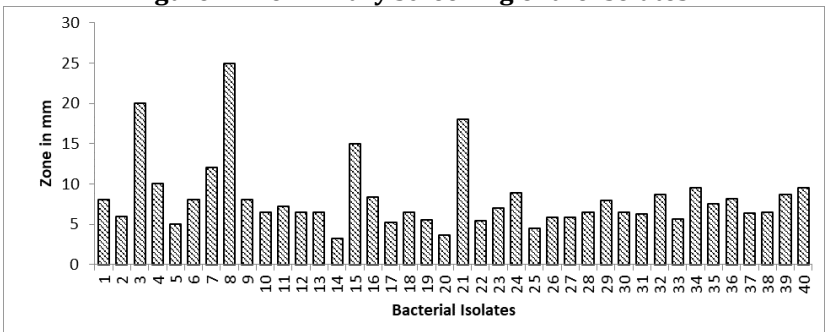
All the values of cfu are statistically significant in comparison to the control (p<0.01)
Different superscripts represent values which are significantly different (Tukeys test).

PRELIMINARY SCREENING

The plate method revealed 4 isolates with red colour zone after congo red treatment. The 40 isolates were screened in which 4 isolates indicated potential EPS production. Figure 1 represents the potential of the isolates for EPS production in form of red color zone in mm.

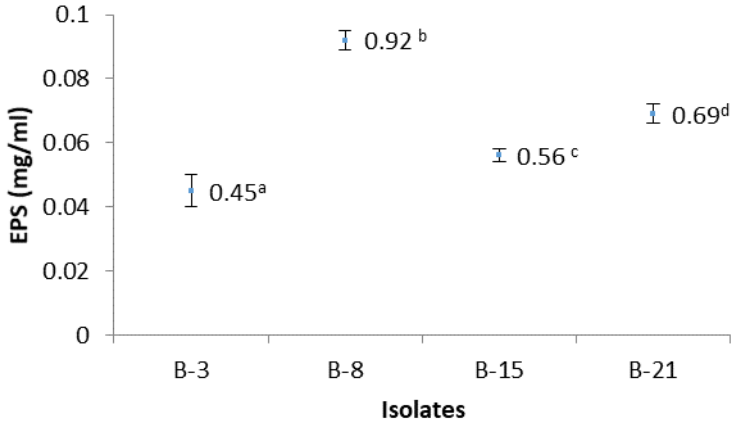
The B-3, B-8, B-15, B-21 isolates were selected for secondary screening as the zone of EPS production was found to be 20, 25, 15 and 18 mm respectively. B-8 isolate exhibited maximum EPS production. Figure-1 represents the preliminary screening of bacterial isolates for EPS production by congo red plate method.

Figure:1 Preliminary screening of the isolates



The secondary screening of the selected isolates is tabulated in Table-3. The highest EPS production 0.092 ± 0.03mg/100 ml was recorded for isolate 8 by spectrophotometric analysis.

Figure 2 Production of EPS by the screened isolates

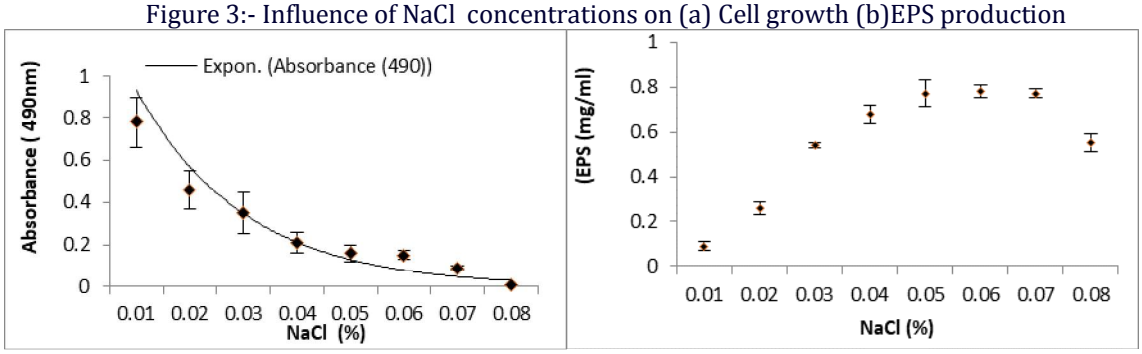


Note- All values in this figure with superscripts are significant ($p < 0.05$); Different superscripts represent values which are significantly different.

Effect of Salinity on EPS Production and Cell Growth

The experiments of influence of NaCl on growth and EPS production revealed a peculiar relationship as shown in Figure 3a and 3b respectively. NaCl influenced the growth negatively with increase in concentration. Contradictorily, The EPS production increased with the concentration of NaCl but at higher concentrations remained stable. This observation was in agreement with the fact that EPS production by bacteria is a response mechanism for extreme environments.

Figure 3:- Influence of NaCl concentrations on (a) Cell growth (b)EPS production



CONCLUSION

The present work has opportunities of future work on nutritional amendments in the culture medium, experimental designing for optimum production and yield enhancement parameters of EPS production

REFERENCED

1. Lazarova V &Manem J (2000) Innovative biofilm treatment technologies for water and wastewater treatment. Biofilms II:Process Analysis and Applications (Bryers JD, ed), pp. 159–206.Wiley-Liss, New York.
2. Simões LC, Simões M, Vieira MJ.(2007). Biofilm interactions between distinct bacterial genera isolated from drinking water. Appl Environ Microbiol. ;73(19):6192-200. doi: 10.1128/AEM.00837-07. Epub 2007 Aug 3. PMID: 17675433; PMCID: PMC2075010.
3. Stewart PS, Camper AK, Handran SD, Huang C, Warnecke M.(1997). Spatial Distribution and Coexistence of Klebsiella pneumoniae and Pseudomonas aeruginosa in Biofilms. Microb Ecol. ;33(1):2-10. doi: 10.1007/s002489900002. PMID: 9039760.
4. Komlos J, Cunningham AB, Camper AK, Sharp RR. (2005). Interaction of *Klebsiella oxytoca* and *Burkholderiacepacia* in dual-species batch cultures and biofilms as a function of growth rate and substrate concentration. Microb Ecol.;49(1):114-25. doi: 10.1007/s00248-003-1066-z. Epub 2005 Jan 28. PMID: 15688257.
5. Cowan SE, Gilbert E, Liepmann D, Keasling JD. (2000). Commensal interactions in a dual-species biofilm exposed to mixed organic compounds. Appl Environ Microbiol.;66(10):4481-5. doi: 10.1128/AEM.66.10.4481-4485.2000. PMID: 11010902; PMCID: PMC92328.
6. Sheng GP, Yu HQ, Li XY. (2010). Extracellular polymeric substances (EPS) of microbial aggregates in biological wastewater treatment systems: a review. Biotechnol Adv. ;28(6):882-94. doi: 10.1016/j.biotechadv.2010.08.001. Epub 2010 Aug 10. PMID: 20705128.

7. Yadav MK, Short MD, Aryal R, Gerber C, van den Akker B, Saint CP. (2017). Occurrence of illicit drugs in water and wastewater and their removal during wastewater treatment. Water Res. 1;124:713-727. doi: 10.1016/j.watres. Epub 2017 Jul 26. PMID: 28843086.
8. Kwiecień I, Kwiecień M. (2018). Application of Polysaccharide-Based Hydrogels as Probiotic Delivery Systems. Gels. 22;4(2):47. doi: 10.3390/gels4020047. PMID: 30674823; PMCID: PMC6209284.

CITATION OF THIS ARTICLE

Balamurugan.K , Mulagani P Evangelin, Prem Kumar P. Synthesis and Antimicrobial Activity Evaluation of 2, 2, 2-trifluoro-1-(6-substituted-4-(thiophen-2-yl) cinnolin-3-yl) ethanone Derivatives. Bull. Env.Pharmacol. Life Sci., Spl Issue [1] 2022 : 592-595