Eco. Env. & Cons. 27 (February Suppl. Issue) : 2021; pp. (S226-S228)
Copyright@ EM International
ISSN 0971–765X

Investigating the promising potential of bacterial EPS for mitigating environmental issues

Ravneet Chug, Manishita Das Mukherji, Era Upadhyay and Vinod Singh Gour

Amity Institute of Biotechnology, Amity University Rajasthan, Jaipur, India

(Received 2 May, 2020; Accepted 10 September, 2020)

ABSTRACT

Extracellular polymeric substances extracted from microorganisms are a complex mixture of diverse polymers of polysaccharides, proteins, lipids, nucleic acids, uronic acid, humic acid. These heterogeneous biomolecules have a vast potential in bioremediation/metal removal and their proficiency can also be emphasized by the fact that after extraction these are not vulnerable to deviation in the climatic conditions. Considering the above facts, in the current study experiments have been conducted to study bioremediation potential of EPS extracted from bacteria in a view to suggest better alternatives to conventional physicochemical methods of bioremediation.

Key words : *Extracellular polymeric subsances, Bioremediation, Environmental restoration*

Introduction

Environmental pollution due to an increase in the number of industries is an issue of intense concern in today's era pertaining to human health. Increase in the number of pollutants drastically alters the nature of environment. Metal ions are the usual constituent of the earth's crust and are also obtained from anthropogenic sources. These may act as necessary micronutrients for living organisms at their threshold concentration but can be poisonous above a certain limit. These are nondegradable and persistant (Ayangbenro *et al*., 2018; Camacho-Chab *et al*., 2018).

Extracellular polymeric substances (EPS), which are the complex biomolecules are composed of a mixture of proteins, polysaccharides, lipids and their derivatives. These are constantly produced by the microorganisms during their growth and metabolism. The composition of the EPS obtained from various microbial aggregates has been reported to be diversified. They play an essential role in the aggregation of microbial cell, in formation of biofilms, in immunomodulation, in food industries as gelling agents, in the sequestration of heavy metal comtaminants, etc (Marvasi *et al*., 2010; More *et al*., 2014). The ionizable functional groups of EPS, for example, carboxyl, amine and hydroxyl assist them in the metal ion sequestration (Joshi and Juwarkar, 2009; Ha *et al.,* 2010; Chien *et al*., 2013).

Various microorganisms have been previously identified and reported in terms of their EPS production. Past investigations have revealed that the potential of microorganisms to remove metals may be correlated to their EPS production (Kýlýc *et al*., 2008; Chug *et al.,* 2016). The present study examined the impact of environmental factors like pH temperature, metal ion concentration on EPS production by *Pseudomonas aeruginosa* which was found to increase when the culture was being exposed toincreasing metal ion concentration Cr (VI). Enhancement in the EPS production was considered to be an adjustment of microorganisms towards increasing stress.

Materials and Methods

Bacterial cultures

The heavy metal resistant bacteria use in this study was *P. aeruginosa.* It was isolated from tannery effluents contaminated with heavy metals. It was identified according to 16S rRNA analysis.

EPS production

For EPS production, microbial culture was inoculated on nutrient agar media in petri dishes. The composition of the nutrient agar was 3 g of peptone, 5 g of yeast extract, and 15 g of agar in 1 L of double distilled water. To decide the ideal pH for the highest EPS production, the pH of the media supplemented with 100 mg/L Cr(VI) was adjusted to 6, 7, 8 and 9 using 0.1M HCL and 0.1 MNaOH. Chromium stock solution was prepared by diluting $K_2Cr_2O_7$ to a concentrationof 10 g/L of chromium. The influence of different temperatures and various Cr(VI) concentrations on EPS production by the bacterial culture was also determined.EPS was extracted after 48 hours according to the method described by Lin *et al*., 2011. Extracted EPS was then lyophilized and weighed.

Results and Discussion

Recognition of bacteria

The bacterial isolate was first studied under the microscope using standard method of GramStaining. It was found that the culture was Gram-negative and rod-shaped. It was then further identified by 16S rRNA sequencing. Phylogenetic analysis was also done by BLAST search and it was then found that the bacterium had a similarity >99% to *Pseudomonas* sp.

EPS production at various pH levels, temperature and different Cr (VI) concentration

Four different pH values 6, 7, 8 and 9 were initially chosen to determine the ideal pH for maximum EPS production bythese bacteria. Maximum EPS production was found at neutral pH (Table 1), also reported by Liu *et al.,* 2010. EPS was weighed at each stage. At pH 7 *P. aeruginosa,* showed variation in EPS production with variation in temperature (32 °C, 37 °C, 40 °C) (Table 2). At pH 7 and temperature 37°C Cr (VI) concentration was varied (10 mg/L, 20 mg/L, 30 mg/L, 40 mg/L, 50 mg/L) (Table 3). An increasing trend in EPS production was initially observed till 40 mg/L concentration which then decreased. So, Maximum Tolerable Concentration (MTC) of Cr (VI) was found to be 40 mg/L. Beyond this EPS production was decreasing. It can be concluded that Cr (VI) removal is interrelated to the quantity of EPS produced by the microorganism as also reported by Kýlýc *et al*., 2008 and Chug *et al.,* 2016. EPS production was highly affected by the temperature variation, Cr (VI) concentration and also the pH. The isolate has got the potential to sequester heavy metal from waste water owing to its ability to biofilm formation and EPS production.

Table 1. Effect of pH on EPS yield of *P. aeruginosa* in 50 mL culture after 48 hrs at 37 °C.

pH	EPS yield in mg
6.0	11.80
7.0	15.60
8.0	12.50
9.0	11.90

Table 2. Effect of temperature on EPS yield of *P. aeruginosa* in 50 mL culture after 48 hrs at 37 °C.

Temperature	EPS yield in mg
32°C	10.83
37°C	17.05
40°C	13.50

Table 3. Effect of varying concentration of Cr (VI) on EPS yield of *P. aeruginosa* in 50 mL culture after 48 hrs at 37 °C.

Cr(VI) (mg/L)	EPS yield in mg
10	18.80
20	21.38
30	13.50

References

Ayangbenro, A. and Babalola, O. 2018. Metal (loid) Bioremediation: Strategies Employed by Microbial Polymers. *Sustainability.* 10 (9) : 3028.

Camacho-Chab, J., Castañeda-Chávez, M., Chan-Bacab, M., Aguila-Ramírez, R., Galaviz-Villa, I., Bartolo-Pérez, P. and Ortega-Morales, B. 2018. Biosorption of cadmium by non-toxic extracellular polymeric substances (EPS) synthesized by bacteria from marine intertidal biofilms. *International Journal of Environmental Research and Public Health.* 15 (2) : 314.

Chien, C.C., Lin, B.C. and Wu, C.H. 2013. Biofilm forma-

tion and heavy metal resistance by an environmental *Pseudomonas* sp. *Biochemical Engineering Journal*. 78 : 132-137.

Chug, R., Gour, V. S., Mathur, S. and Kothari, S. L. 2016. Optimization of extracellular polymeric substances production using *Azotobacter beijreinckii* and *Bacillus subtilis* and its application in chromium (VI) removal. *Bioresource Technology.* 214 : 604-608.

Ha, J., Gélabert, A., Spormann, A.M. and Brown, G.E. 2010. Role of extracellular polymeric substances in metal ion complexation on *Shewanellaoneidensis*: batch uptake, thermodynamic modeling, ATR-FTIR, and EXAFS study. *Geochimica et Cosmochimica Acta.* 74 (1): 1-15.

Joshi, P.M. and Juwarkar, A.A. 2009. *In vivo* studies to elucidate the role of extracellular polymeric substances from *Azotobacter* in immobilization of heavy metals. *Environmental Science & Technology*. 43 (15) : 5884-5889.

Kýlýç, N. K. and Dönmez, G. 2008. Environmental conditions affecting exopolysaccharide production by *Pseudomonas aeruginosa, Micrococcus* sp., and *Ochrobactrum* sp. *Journal of Hazardous Materials.* 154 (1) : 1019-1024.

Lin, M.H., Yang, Y.L., Chen, Y.P., Hua, K.F., Lu, C.P., Sheu, F., Lin, G.H., Tsay, S.S., Liang, S.M. and Wu, S.H. 2011. A novel exopolysaccharide from the biofilm of *Thermus aquaticus* YT-1 induces the immune response through Toll-like receptor 2. *Journal of Biological Chemistry.* 286 (20) : 17736-17745.

Liu, W.,Wang, K., Li, B., Yuan, H. and Yang, J. 2010. Production and characterization of an intracellular bioflocculant by *Chryseobacteri umdaeguense* W6 cultured in low nutrition medium. *Bioresource Technology*. 101 (3) : 1044-1048.

Marvasi, M., Visscher, P.T. and Martinez, L.C. 2010. Exopolymeric substances (EPS) from *Bacillus subtilis*: polymers and genes encoding their synthesis. *FEMS Microbiology Letters*. 313 (1) : 1-9.

More,T.T., Yadav, J.S.S., Yan, S., Tyagi, R.D. and Surampalli, R.Y. 2014. Extracellular polymeric substances of bacteria and their potential environmental applications. *Journal of Environmental Management.* 144 : 1-25.