EUROPEAN JOURNAL OF BIOMEDICAL AND PHARMACEUTICAL SCIENCES
http://www.ejbps.com

ISSN 2349-8870
Volume: 4
Issue: 12
612-614
Year: 2017

EPS PRODUCTION AND BIOFLOCCULANT ACTIVITY OF BACTERIAL ISOLATES

Nisha P.*[1], Anu Elias[2] and Anu M. Baby[2]

[1]Dept of Biosciences, MES College, Marampally, Ernakulam, Kerala.
[2]P.G. Department of Biochemistry, S. S. V. College Valayanchirangara, Ernakulam, Kerala.

***Corresponding Author: Nisha P.**
Dept of Biosciences, MES College, Marampally, Ernakulam, Kerala.

Article Received on 14/10/2017 **Article Revised on 05/11/2017** **Article Accepted on 25/11/2017**

ABSTRACT
River pollution is the major issues, this study is focused to reduce the same by using bacterial sources. Bacterial species were isolated from water samples and identified as *Bacillus* sp-1, *Bacillus* sp-2, *Micrococcus* sp-1, *Micrococcus* sp-*2 and E coli.* These organisms are capable to produce Exopolysacharides. The higher EPS producing organism was *Bacillus* sp 2 compared to other organisms. In industries the bacterial EPS are used as a bioflocculants. In this study it is revealed that the EPS from the study organisms were showing bioflocculant activities both in deionized water and in Periyar river water. The bioflocculants were also exhibited an antibacterial activity against 3 organisms. Periyar River is being polluted dangerously with industrial wastes, which affects the life of an organisms. The application of the present study is, the EPS produced by these organisms may used as a bioflocculants and can be used to reduce the pollution in Rivers or other water bodies.

KEYWORDS: *Bacillus* sp-1, *Bacillus* sp-2, *Micrococcus* sp-1, *Micrococcus* sp-*2 and E coli.*

INTRODUTION
Bioflocculants are basically polymers produced by microbes during their growth, with their flocculating activity being dependent on the characteristics of the flocculants. Bioflocculants has an advantages such as biodegradable, safety, strong effect and harmlessness to humans and the environment and are potentially be applied in drinking and wastewater treatment, downstream processing and fermentation processes, etc (Salehizadeh and Shojaosadati, 2001). In recent years, many bioflocculant-producing microorganisms including bacteria, fungi and actinomycetes have been reported to produce extracellular polymeric substances, such as polysaccharides, functional proteins and glycoprotein, which function as bioflocculant.

MATERIALS AND METHODS
Sample Collection and Isolation of Microorganisms
Water samples were collected from different places nearby college and aseptically transfer to the laboratory and isolation of organisms was done by TPC.

Identification
The selected organisms were identified based on morphological, biochemical and physiological characters according to Bergey's manual of determinative bacteriology.

Production of EPS
EPS was extracted according to the method followed by Ohno *et al* (2000). Pre-inoculum was inoculated into YMG broth and incubated at room temperature for 5 days at 120 rpm. The culture broth was centrifuged at 10,000 rpm for 20 min, after removing the pellet, transfer the supernatant. The supernatant was mixed with 3 volumes of ethanol and left overnight at 4°C. The weight of the precipitated EPS was measured after drying 80°C for 24 hour.

Extraction of the Bioflocculant
The pre - inoculum was added into YMG broth and after the incubation at room temperature for 5 days at 120 rpm, media was centrifuged at 10,000 rpm for 20 min, remove the pellet. The supernatant was used as the source of bioflocculant for the further studies.

Measurement of Flocculating Activity of EPS
Using a suspension of kaolin clay as test material. Flocculating activity was determined according to Kurane *et al*., 1994. as modified by Gao *et al*., 2009. A suspension of kaolin clay (4g/l) in deionized water at pH 7 was used as a stock solution for the subsequent assays. The following solutions were mixed in a test tube kaolin clay suspension (9 ML), 1% biofloculant (0.1ML) & 1% $CaCl_2$ (0.9 mL). A control in which the bioflocculant was replaced with deionized water was also included & measured under similar conditions. The final volume of all mixtures was made up to 10mL with deionized water.

The solutions were mixed gently & allowed to settle for 5 min at room temperature. The optical density (OD) of the clarifying upper phase solution was measured at 550 nm.

Measurement of Flocculating Activity of EPS using Periyar River water

Various quantities of bioflocculants like 0.5 ml, 1 ml, 2 ml, 4 ml & 8 ml from all the organisms were subjected to flocculating activity using Periyar River water.

Antimicrobial activity of bioflocculant - Disc Diffusion method

The bioflocculant from all the study organisms were subjected to antimicrobial activity against 3 test organisms such as *Pseudomonas* sp, *Staphylococcus aureus* and *Klebsiella* sp.

RESULTS

5 organisms AA1,AA2,-AA3,AA4,AA5, were selected for the study and were identified as *Bacillus* sp 1, *Bacillus* sp2, *E coli*, *Micrococcus* sp1 and *Micrococcus* sp 2 respectively.

Production of EPS

The results of EPS production from the organisms showed that, higher EPS was produced by *Bacillus* sp-2 12.436gm/100ml. Least EPS was produced by *Micrococcus* sp- 1, 2.8955gm/100ml (Table 1).

Measurement of Flocculating Activity of EPS using Periyar River water

The results of bioflocculant activity with Periyar River water (Table- 3) revealed that, the bioflocculants produced by the study organisms were actively capable to produce floccules. The higher optical density was measured for *Micrococcus* sp-2 was 0.69(8ml). A similar by Shimforuya *et al.,* [1995] was observed by *Streptomycetes griseus* produced a bioflocculant with flocculating activity increasing with the increase of cultivation time. Deng *et al.* [2005] reported bioflocculant produced by *Aspergillus parasiticus* to attain the highest flocculating activity in 96 h. Bioflocculant produced by *Serratia ficaria* reached its maximum flocculating activity on the third day of cultivation Gong W *et al.*,[2008] whilst in case of *Citrobacter* sp. TKF04 bioflocculant exhibited maximum flocculating activity within one day Fujita M *et al*., [2000]. Zhang *et al* (2002) studied exopolysaccharide and its bioflocculant activity using myxobbacteria *Sorangium cellulosum.*

Antimicrobial activity of Crude Pigment- Disc Diffusion method

The antimicrobial activity of bioflocculant, produced by these 5 organisms were shown the bactericidal activity (Table- 4) against the test organisms in all the concentrations of samples. Maximum zone of inhibition was produced by the bioflocculant of *Bacillus sp*-2 against *Staphaylococcus sp*, 1.4 mm at 100 µl. Bioflocculant produced by *Bacillus* sp-1 produced least zone of inhibition against *Staphaylococcus sp*, 0.2 mm at 25 µl.

Table 1: Production of EPS.

Sl.No	Organisms	EPS production in gm /100ml
1	*Microoccus* sp-1	2.8955
2	*Microoccus* sp-2	7.9525
3	*E coli*	4.574
4	*Bacillus* sp -1	7.476
5	*Bacillus* sp -2	12.436

Table 2: Flocculating Activity of EPS of Organisms.

Sl.No	Organisms	Flocculating Activity	
		Initial	Final
1	*Microoccus* sp-1	0.03	0.05
2	*Microoccus* sp-2	0.14	0.15
3	*E coli*	0.113	0.114
4	*Bacillus* sp -1	0.30	0.31
5	*Bacillus* sp -2	0.37	0.38

Table 3: Flocculation Activity of Organisms in Periyar Water.

S.No	Organism	Volume of Flocculant									
		0.5 ml		1 ml		2 ml		4 ml		8 ml	
		Optical Density at 550nm									
		Initial	Final	Initial	Final	Initial	Final	Initial	Final	Initial	Final
1	*Micrococcus* sp-1	0.30	0.36	0.07	0.10	0.21	0.24	0.02	0.09	0.07	0.10
2	*Micrococcus* sp-2	0.01	0.04	0.30	0.35	0.40	0.47	0.56	0.59	0.65	0.69
3	*E.coli*	0.01	0.04	0.07	0.10	0.34	0.43	0.39	0.45	0.48	0.51
4	*Bacillus* -2	0.06	0.10	0.15	0.20	0.20	0.25	0.30	0.35	0.40	0.44
5	*Bacillus* -2	0.02	0.08	0.18	0.20	0.18	0.20	0.22	0.28	0.30	0.38

Table 4: Antibacterial Activity of Bioflocculants.

S. No	Bio flocculant Source	Test organism	Zone of inhibition in mm		
			25 µl	50 µl	100 µl
1	*Micrococcus sp-1*	*Pseudomonas* sp	0.4	0.9	1.2
2		*Staphaylococus aureus*	0.5	0.8	1.1
3		*Klebsiella* sp	0.9	1.1	1.3
4	*Micrococcus sp-2*	*Pseudomonas* sp	0.5	0.6	1.1
5		*Staphaylococus aureus*	0.8	1	1.2
6		*Klebsiella* sp	0.5	0.8	1
8	*E.coli*	*Pseudomonas* sp	0.6	0.9	1.1
9		*Staphaylococus aureus*	0.7	0.9	1.2
10		*Klebsiella* sp	0.5	0.7	1.1
11	*Bacillus* sp -1	*Pseudomonas* sp	0.4	0.9	1.1
12		*Staphaylococus aureus*	0.2	0.4	0.8
13		*Klebsiella* sp	0.4	0.7	1.3
14	*Bacillus* sp -2	*Pseudomonas* sp	0.5	0.8	1.4
15		*Staphaylococus aureus*	0.6	1	1.2
16		*Klebsiella* sp	0.3	0.5	0.9

CONCLUSION

Bacterial species isolated from water samples were identified as *Bacillus* sp-1, *Bacillus* sp-2, *Micrococcus* sp-1, *Micrococcus* sp-*2 and E coli.* These organisms are Exopolysacharides producers and from the results, the higher EPS production was performed by *Bacillus* sp 2, 12.436gm/100ml. These EPS has the ability to form floccules, these Bioflocculants from all these organisms has the flocculation activity in polluted water source such as Periyar water. The higher flocculation activity was measured for *Micrococcus* sp-2 was 0.69(8ml). These bioflocculants also has an antibacterial activity against *Pseudomonas* sp, *Staphylococcus aureus* and *Klebsiella* sp. By using these bioflocculants from study organisms, we can diminish the pollution level in polluted water sources.

REFERENCES

1. Salehizadeh, H. and Shojaosadati, S. A. Extracellular biopolymeric flocculants: recent trends and biotechnology importance. Biotechnology Advanced, 2001; 19(5): 371-385.
2. Ohno, N., N. Miura, M. Nakajima and T. Yadomae,. Antitumor 1-3- -glucan from cultured fruit body of *Sparasis crispa*. Boil. Pharm. Bul., 2000; 23: 866- 872.
3. Kurane R, Matsuyama H. Production of a bioflocculant by mixed culture. Biosci Biotechnol Biochem. 1994; 58: 1589–94. View Article
4. Gao Q, Zhu XH, Mu J, Zhang Y, Dong XW. Using *Ruditapes philippinarum* conglutination mud to produce bioflocculant and its applications in wastewater treatment. Bioresour Technol. 2009; 100: 4996–5001. View Article
5. Shimofuruya H, Koide A, Shirota K, Tsuji T, Nakamura M, Suzuki J. The production of flocculating substance(s) by *Streptomyces griseus*. Biosci. Biotechnol. Biochem. 1995; 60: 498–500.
6. Cosa S, Mabinya LV, Olaniran AO, Okoh OO, Okoh AI. Bioflocculant Production by *Virgibacillus* sp. Rob Isolated from the Bottom Sediment of Algoa Bay in the Eastern Cape, South Africa. Molecules. 2011; 16: 2431–2442. [PubMed]
7. Deng S, Yu G, Ting YP. Production of a bioflocculant by *Aspergillus parasiticus* and its application in dye removal. Colloids Surf. B Biointerfaces. 2005; 44: 179–186.
8. Gong W, Wang S, Sun F, Liu XW, Yue QY, Gao BY. Bioflocculant production by culture of *Serratia ficaria* and its application in wastewater treatment. Bioresour. Technol. 2008; 99: 4668–4674. [PubMed]
9. Fujita M, Ike M, Tachibana S, Kitada G, Kim SM, Inoue Z. Characterization of a bioflocculant produced by citrobacter-tkf 04 from acetic and propionic acids. J. Biosci. Bioeng. 2000; 89: 40–46. [PubMed]
10. Zhang J, Wang R, Jiang P, Liu Z Production of an exopolysaccharide bioflocculant by Sorangium cellulosum. Lett Appl Microbiol. 2002; 34(3): 178-81.