Antiadhesive properties of biosurfactant against Pseudomonas aeruginosa

Anjali Sharma, Sachin Gudhadde, Dr. C.J.Chandekar
Lecturer, Research student, Associate Professor.
Department of Microbiology
Shivaji Science College Nagpur, Maharashtra, India.

Abstract - Biofilms, which are formed by the majority of micro organisms in natural environments, are structures with low sensitivity to drugs. Many laboratories are synthesizing or isolating new compounds preventing the formation of biofilms or causing their elimination. Adhesion is the first stage of biofilm formation and the best moment for the action of antiadhesive and anti-biofilm compounds. Biosurfactants are promising compounds often showing antimicrobial and antiadhesive properties and sometimes penetrating and removing mature biofilms.

Keywords - Biofilm, Pseudomonas aeruginosa, Biosurfactants.

I. INTRODUCTION:

Microbial surfactants-amphiphilic, surface-active, secondary metabolites of bacteria or fungi ranging from low-molecular-mass glycolipids, sophorolipids, rhamnolipid and lipopeptides, to high-molecular mass proteins, lipopolysaccharides and lipoproteins can interact with interfaces and inhibit the adhesion of microorganisms to different surfaces(4,7, 10,12). They are an alternative to synthetic surface-active agents because of their low toxicity and biodegradability(6,15). Another mechanism of biosurfactant action is the permeabilization of bacterial cells(15,19). The rhamnolipid secreted by *Pseudomonas sp.* S-17 permeabilized Gram negative and Gram positive cells, but a strong inhibition of growth was observed only in the case of Gram-positive bacteria(20).

Biofilm disruption was observed after the addition of rhamnolipids from *Pseudomonas aeruginosa* and lipopeptide from *Bacillus spp*. particular group of biosurfactants, lipopeptides, can act as antibiotics and also as antiviral and anti tumor agents. Surfactin from *Bacillus subtilis* can interact with the plasma membranes of bacterial and fungal cells leading to their disruption(3,9,11,16). The effects of biosurfactants on decreased microbial adhesion and detachment from different surfaces can be conveniently utilized in many fields, from medicine to various branches of industry, e.g., antimicrobial or antitumor activities and their surface activity and antiadhesive properties can be suitable for preventing microbial colonization of implants or urethral catheters(5). Microbial surfactants from *Lactobacillus fermentum* and *Lactobacillus acidophilus* adsorbed on glass, reduced the number of adhering uro pathogenic cells of *Enterococcus faecalis* by 77%.

II. MATERIAL AND METHODS:

Antiadhesive property of crude biosurfactant:

To check the antiadhesive property of biosurfactant microtitter plate assay method was performed. In this method, culture of organism was added (50µl) in each well, control well was also maintained. Add crude biosurfactant in each wells at different concentrations 0.08 µg/ml, 0.05 µg/ml, 0.03 µg/ µg/ml, 0.06 µg/ml, 0.12 µg/ml, 0.25 µg/ml, 0.5 µg/ml, 1,2,4 µg/ml respectively(1,5). Microtiter Plate was incubated at 37 ℃ for 24 hours. Crystal violet was added to each well in microtitre plate(17). Finally the concentration of crystal violet which was retained after staining was visualized in each well(21).

III. OBSERVATIONS AND RESULTS:

FIGURE 2: Fourier Transform Infrared Spectroscopy (FTIR) of Biosurfactants.

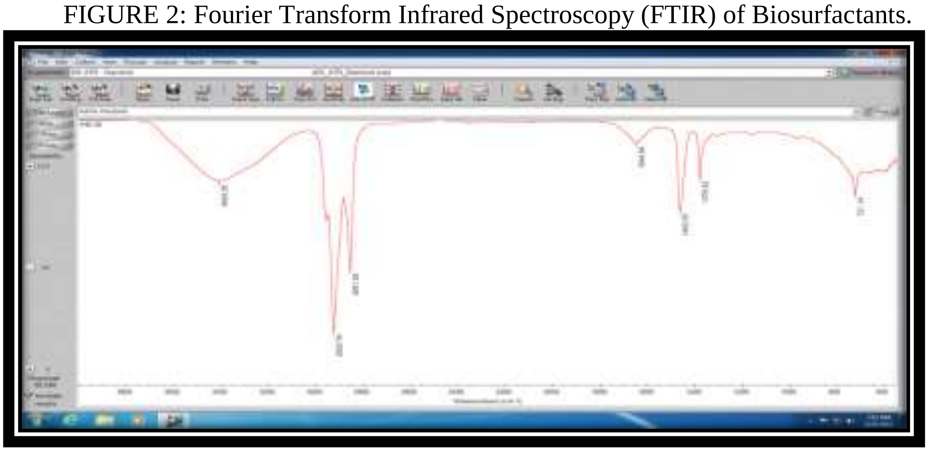

FIGURE 3: Microtitre plate assay for determining MIC

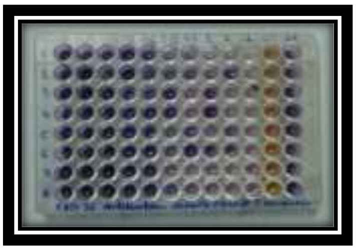
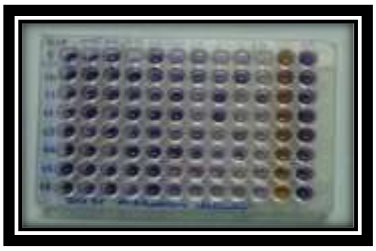


Table 1: MIC values of biosurfactant against bacterial isolates of *Pseudomonas aeruginosa*

Isolates	MIC µg/ml	Isolates	MIC µg/ml	Isolates	MIC µg/ml	Isolates	MIC µg/ml
1	0.25	7	0.25	13	0.5	19	0.12
2	0.5	8	0.25	14	0.03	20	0.03
3	0.5	9	0.25	15	0.25	21	0.25
4	0.5	10	0.10	16	0.5	22	0.25
5	0.25	11	0.5	17	0.06	23	0.12
6	0.25	12	0.5	18	0.12	24	0.06

In the current investigation we studied anti-adhesive activity of biosurfactants against *Pseudomonas aeruginosa*. The biosurfactant at concentration 0.25 µg/ml showed high anti-adhesive activity against *Pseudomonas aeruginosa*.

Various rhamnolipid biosurfactants produced by the strains under investigation were potent hydrocarbon emulsifiers which can make them suitable candidates for promoting environmental remediation. The strain was found to be a close relative of *P. aeruginosa* which was not found to produce copious amounts of pyocyanin and hence devoid of deleterious effects of this pigment[9, 16, 19]. This strain was also found to be a good biosurfactants producer, making it industrially viable strain. Antimicrobial potentials of the rhamnolipid biosurfactants are well documented. The higher antimicrobial potential of this biosurfactant is thought to be because of the equal congener proportions[4].

Biofilms are populations of microorganisms that accumulate at interfaces and are typically surrounded by a matrix of extracellular polymeric substances (EPS). Attachment of microorganisms to surfaces and the development of microbial biofilms at phase boundaries are frequently encountered in natural, technical and medical environments[2,7,18].

In the present study, the anti-adhesive activities of biosurfactants produced by IHD36 strain against total 24 isolates were studied by microtitre plate assay[8,13,14]. The biosurfactant found to be positive for antiadhesive activity. The MIC was found to be 0.03 µg/ml for isolate 14, 20. The MIC was found to be 0.06 µg/ml for isolate 17, 24. The MIC was found to be 0.12 µg/ml for isolate 18 µg/ml, 19 µg/ml, and 23 µg/ml. The MIC were found to be 0.25 µg/ml for isolate 1 µg/ml, 5 µg/ml, 6 µg/ml, 7 µg/ml, 8 µg/ml, 9 µg/ml, 15 µg/ml, 21 µg/ml, 22 µg/ml. The MIC was found to be 0.10 µg/ml for isolate 10. The MIC were found to be 0.5µg/ml for isolate 2 µg/ml, 3 µg/ml, 4 µg/ml, 11 µg/ml, 12 µg/ml, 13 µg/ml, 16 µg/ml.

CONCLUSION

From the present study it was that the biosurfactant by IHD 36 was found to be positive for anti-adhesive activity against the biofilm organisms with MIC in the range of 0.03 µg/ml to 0.5 µg/ml which can be used as a surface coating agent against microbial colonization on different surfaces of implants or urethral catheters.

ACKNOWLEDGMENT

We are thankful to the department of microbiology of Shri Shivaji Science College Nagpur for the support to carry out this research work.

REFERENCES

[1] Anjali Sharma, Dr. A. V. Gomashe, “Biosurfactant production by bacterial species isolated from petroleum oil contaminated soil”. **(2018)** IJCRT | Volume 6, Issue 2 **April 2018** | ISSN: 2320-2882.

[2] Banat IM, Makkar RS and Cameotra SS., “Potential commercial Application of Microbial Surfactants”, Appl Microbiol Biotechnol 2000; 53:495-508.

[3] Barkay, T., Navon-Venezia, S., Ron, E., Rosenberg, E., Appl Environ Microbiol., 1. 6. (1999). *Appl Environ Microbiol.* , 65, 2697.

[4] Boldrin, B., Tiehm, A., Fritzsche, C., & Appl Environ Microbiol., 1. 5. (1993, 1927.). *Appl Environ Microbiol.* , 59.

[5] Donlan R.M. "*Biofilms: Microbial Life on Surfaces I. Emerging.*" J. Infectious Diseases. 2002. 8 (3) 881-890

[6] Evans, G. L.-1. (1968). "Comparative effects of anticoagulants on bacterial growth in experimental blood cultures". . *Amer. J. Med. Technol.* , 103-112.

[7] Hayes P. R., 1. J. (1963). Hayes P. R., 1963, J. Gen. Microbiol., 30: 1. *J. Gen. Microbiol.*, .

[8] Himedia. (1919). A-516,Swastik Disha Business Park,Via Vadhani Ind. Est., LBS Marg, Mumbai-400086, India. Customer care No.: 022-6147 1919 Email: techhelp@himedialabs.com Website: www.himedialabs.com. *HiMedia*

Laboratories Pvt. Ltd. A-516,Swastik Disha Business Park,Via Vadhani Ind. Est., LBS Marg, Mumbai-400086, India. Customer care No.: 022-6147 1919 Email: techhelp@himedialabs.com Website: www.himedialabs.com .

[9] Holden, P., LaMontagne, M., Miller, A. B., Lindow, S., & ., 2. ,. (2002). *Appl. Environ. Microbiol.*

[10] http://biology.clc.uc.edu/fankhauser/Labs/Microbiology/Meat_Milk/Pour_Plate.html, D. B.

[11] Kathi, S., & Khan, A. (2013). *Int J Sci Res Publ.* , 3.

[12] Mueller, J., Chapman, P., Blattmann, B., & Pritchard, P. (1990). *Appl Environ Microbiol* , 56.

[13] Mulligan, C. (2005). CN Mulligan; Environ Pollut.,2005, 133, 183. *Environ Pollut* , 133, 183.

[14] Murty, V. V. (2013). "Production and properties of a lipopeptide biosurfactant by B. subtilis subsp. Inoaquosorum." *J. Microbial. Biotech. Res., ISSN:2231-3168 , 3* (4), 63-73.

[15] M. G. Healy, C.M. Devine, and R.Murphy, "Microbial production of biosurfactants," *Resources, Conservation and Recycling*, vol. 18, no. 1–4, pp. 41–57, 1996

[16] Nitschke, M., Costab, S., & Trends Food Sci Tech., 2. 1. (M Nitschke; SGVAO Costab; Trends Food Sci Tech., 2007, 18, 252.). M Nitschke; SGVAO Costab; Trends Food Sci Tech., 2007, 18, 252. *M Nitschke; SGVAO Costab; Trends Food Sci Tech., 2007, 18, 252.* , M Nitschke; SGVAO Costab; Trends Food Sci Tech., 2007, 18, 252.

[17] Okerentugba PO and Ezerony OU. "Petroleum degrading potentials of single and mixed microbial cultures isolated from rivers and refinery effluentin Nigeria". Afr.J. Biotechnol 2003; 2: 288-292.

[18] Rodrigues, L., Banat, I., Teixeira, J., & Oliveira, R. (2006). *J AntimicrobChemother.* , 57, 609.

[19] Saravanan v, a. v. (2012)." Isolation and screening of biosurfactant producing microorganism from oil contaminated soil". *J. Acad. Indus. Res.* , *1* (5).

[20] Tuleva, B., Christova, N., Jordanov, B., Nikolova-Damyanova, B., Petrov, P., & C., Z. N. (2005). 60, 577.

[21] W. R. Finnerty, "Microbial conversion of hydrocarbons products: commercial perspectives," in *Bioconversion of Waste Materials to Industrial Products"*, A. M. Martin, Ed., Elsevier, Amsterdam, The Netherlands, 1991.