CHARACTERISATION OF BIOSURFACTANT PRODUCED BY BACTERIA FROM ENVIRONMENTAL ISOLATES

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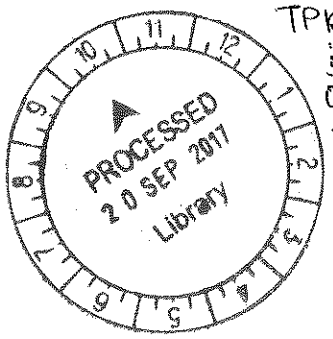
DISSERTATION SUBMITTED IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE OF BACHELOR OF BIOTECHNOLOGY (HONOURS)



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ABSTRACT

Biosurfactants that are widely utilised in various fields such as environmental bioremediation and cosmetic industry have been identified to exhibit antimicrobial and anti-adhesion properties in recent years. Studies show that biosurfactants can inhibit bacterial adhesion through bio-conditioning of a surface by changing the surface's hydrophobicity or by interacting directly with the bacterial cells to modify their surface properties. In this study, 10 environmental bacterial isolates were subjected to preliminary screening assays consisting of blood agar haemolysis test and oil spreading assay to screen for isolates capable of producing biosurfactants. Bacterial isolates 9 and 10 were selected as the biosurfactant producers because they yielded larger diameter in the oil spreading assay compared to other bacterial isolates ($p<0.05$). Both biosurfactants from bacterial isolates 9 and 10 showed emulsification capacity similar to that of the control, Triton X-100 in emulsifying toluene and hexadecane ($p>0.05$). Based on the Bacterial Adhesion to Hydrocarbon (BATH) assay, bacterial cells from the bacterial isolates 9 and 10 shared similar degree of hydrophobicity ($p>0.05$) while the gram staining result showed that both of the bacterial isolates are gram positive bacilli. Biosurfactants from bacterial isolates 9 and 10 inhibited adhesion of *P. aeruginosa* and *S. aureus* bacterial cells at a concentration of 0.1 g/mL ($p>0.05$). Biosurfactant from bacterial isolate 10 showed significant antimicrobial property against both *P. aeruginosa* and *S. aureus* while biosurfactant originated from bacterial isolate 9 only showed significant antimicrobial property against *P. aeruginosa*. Overall result suggests that both biosurfactants from the bacterial isolates 9 and 10 exhibited potential values as biofilm-forming inhibitor and antimicrobial agent to be used in the medical field.

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LIST OF ABBREVIATION

ANOVA	Analysis of variance
BATH	Bacterial Adhesion to Hydrocarbon
CFU/mL	Colony-forming units per milliliter
°C	Celsius
L	Liter
g/L	Gram per liter
NaCl	Sodium chloride
nm	Nanometer
OD	Optical density
PBS	Phosphate-buffered saline
rpm	Rounds per minute
v/v	Volume To Volume

CHAPTER 1

INTRODUCTION

Biosurfactants are amphiphilic biomolecules synthesised by many organisms, especially microorganisms. The amphiphilic nature of the biosurfactant is due to the presence hydrophilic and hydrophobic moieties capable of reducing surface tension and interfacial tensions between individual molecules at the surface and interface, respectively (Karanth, Deo & Veenanadig, 2010). While the hydrophilic moiety could be carbohydrate, amino acid, phosphate group or some other compounds, the hydrophobic moiety is usually a long chain fatty acid (Joshi & Shekhawat, 2014). Microorganisms are known to produce a wide range of biosurfactants on cell surfaces or secreted extracellularly (Karanth et al., 2010) including peptides, fatty acids, glycolipids, phospholipids, lipopeptides, neutral lipids and polymeric biosurfactants (Joshi & Shekhawat, 2014).

The surfactants of biological origin have gained great scientific interest due to their advantageous properties such as structural diversity, reduced toxicity, high substrate selectivity and biodegradability as well as better environmental compatibility (Rufino et al, 2011). These properties make biosurfactants applicable in various fields including environmental bioremediation, food industry, agriculture, cosmetics and medical (Sriram et al., 2011). In recent years, researchers discovered another potential valuable application of biosurfactants, which is antimicrobial property with significant therapeutic and biomedical importance (Das, Mukherjee & Sen, 2008).

According to Sriram et al. (2011), biosurfactants showed efficacious antimicrobial activity against various gram positive and negative bacteria because it is effective in reducing biofilm formation by pathogens. Biofilms are formed by bacterial cells that colonise a surface (Chin et al., 2015). The bacterial cells can adhere to virtually all surfaces, be it biological or artificial, and develop biofilm on it (Madigan, Martinko, Stahl & Clark, 2012). Biofilm formation on medical devices or implants has caused increased cases of

nosocomial infection, thus, to prevent and control microbial biofilm formation is now one of the utmost concerns in the medical sector (Bjarnsholt, 2013; Kuyukina, Ivshina, Korshunova, Stukova & Krivoruchko, 2016).

Research findings suggest that biosurfactants can be used as an anti-adhesive coating agent for medical devices and implants to significantly reduce biofilm formation by pathogens (Fakruddin, 2012). However, not many studies have reported on biosurfactants of environmental isolates origin with such functions. Considering the latent useful medical purposes of biosurfactants, this study aims to characterise biosurfactants with biofilm inhibition and antimicrobial property produced by environmental isolates by assessing the biosurfactants through a series of assay consisting of anti-adhesion assay, emulsification assay, Bacterial Adhesion to Hydrocarbon (BATH) assay and antimicrobial assay.

CHAPTER 2

LITERATURE REVIEW

2.1 BIOFILM

Over the course of billion years, bacterial cells have evolved a phenotype known as biofilm which enables them to colonise surfaces (Chin et al., 2015). Biofilms are assemblages of bacterial cells adhered to a surface and enclosed within a mesh of polysaccharides, fatty acids, proteins or nucleic acids (Madigan et al., 2012). Virtually any surfaces, be it biotic or abiotic, could be colonized by bacteria (Madigan et al., 2012). Table 2.1 shows some of the biofilms that could be formed in different environments (De Beer & Stoodley, 2013).

Chin et al. (2015) stated that changes such as pH, temperature, nutrient levels and ionic strength in the environment will result in the transition of free and planktonic bacterial cells to biofilms producers. The process is known to be triggered by the production of cyclic dimeric guanosine monophosphate (c-di-GMP) which functions as a second messenger where it binds to proteins that regulate the activity of the flagella motor as well as to enzymes that are responsible for the extracellular matrix formation of the biofilm (Madigan et al., 2012). Characklis (1990) identified and condensed the process of biofilm development into three major events: (a) colonisation of cells on a surface, (b) growth of the attached cells into a mature biofilm and (c) detachment of the cells either as a single cell or as large pieces.

According to Madigan et al. (2012), there are at least four reasons proposed for the formation of biofilms. First, biofilms are used as a means of self-defense to increase survival. Second, cells are able to remain in a preferred niche. Third, biofilms allow bacterial cells to live closely associated to each other. Lastly, biofilms seems to be the typical way bacterial cells grow in nature.

Table 2.1 Different types of biofilm that can be formed in different environments (De Beer & Stoodley, 2013).

Environment	Biofilm type	Thickness (m)	Community	References
Natural	Photosynthetic microbial mats, hot springs, and hypersaline lakes	10^{-3} to 1	Mixed algal and bacterial communities	Stal (1994)
	Stromatolites	1	Bacterial	Stal (1994)
	Benthic/river sediments	10^{-6} to 10^{-3}	Mixed bacterial, algal, and protozoan communities	Baty (1996) Costerton (1994)
Medical	Dental plaque	10^{-6} to 10^{-4}	Mixed bacterial community	Kinnement (1996)
	Infectious	10^{-6} to 10^{-3}	Often bacterial or fungal monocultures	Morck (1994)
Industrial	Heat exchangers	10^{-6} to 10^{-3}	Mixed bacterial and fungal communities	Buret (1991) Characklis (1990)
	Drinking water pipes	10^{-6} to 10^{-2}	Mixed bacterial and fungal communities	Camper (1994)
	Wastewater treatment	10^{-4} to 10^{-3}	Mixed bacterial and fungal communities, biofilms, aggregates, and flocs	Van Der Kooji (1994) Lemmers and Griebe (1995)
	Filtration units	10^{-5} to 10^{-4}	Mixed bacterial and fungal biofilms	Flemming (1996)
	Ship hulls	10^{-4} to 10^{-2}	Mixed bacterial and algal and marine macroorganisms	Cooksey (1995)

2.1.1 Medical Concerns on Biofilm Formation

As mentioned, bacteria can develop biofilms on virtually any surfaces. Medical implants are among the surfaces that bacterial cells usually colonized and form biofilms on (Madigan et al., 2012). From short-term devices like urinary catheter to long-term implants such as artificial joints, they are all exposed to bacterial cell adherence (Madigan et al., 2012). Table 2.2 describes some biofilm-forming microbes commonly isolated from selected indwelling medical devices (Donlan, 2001).

Table 2.2 Biofilm-forming microbes commonly isolated from indwelling medical devices (Donlan, 2001).

Indwelling medical devices	**Organisms**
Central venous catheter	Coagulase-negative staphylococci, *Staphylococcus aureus, Enterococcus faecalis, Kiebsiella pneumoniae, Pseudomonas aeruginosa, Candida albicans*
Prosthetic heart valve	Viridans *Streptococcus,* coagulase-negative staphylococci, enterococci, *Staphylococcus aureus*
Urinary catheter	*Staphylococcus epidermis, Escherichia coli, Kiebsiella pneumoniae, Enterococcus faecalis, Proteus mirabilis*
Artificial hip prosthesis	Coagulase-negative staphylococci, β-hemolytic streptococci, enterococci, *Proteus mirabilis, Bacterioides* species, *Staphylococcus aureus,* viridans *Streptococcus, Escherichia coli, Pseudomonas aeruginosa*
Artificial voice prosthesis	*Candida albicans, Streptococcus mitis, Streptococcus salivarius, Rothia dentrocariosa, Candida tropicalis, Streptococcus sobrinus, Staphylococcus epidermidis, Stomatococcus mucilaginous*
Intrauterine device	*Staphylococcus epidermidis, Corynebacterium* species, *Staphylococcus aureus, Micrococcus* species, *Lactobacillus plantarum,* group B streptococci, *Enterococcus* species, *Candida albicans*