

Abstract

Sophorolipids (SLs) belong to the category of glycolipid biosurfactants. They consist of a dimeric sugar- sophorose as a carbohydrate head and a long hydroxy fatty acid chain as tail. This amphiphilic nature allows them to form unique structures such as micelles and bilayers in heterogenous systems. SLs find applications in wide range of fields including Petroleum, food, pharmaceuticals, cosmetics, laundry and bioremediation. SLs are synthesized by employing non pathogenic yeast strains and they confer several physiological advantages to the producer. Biosurfactants are coming up as emerging class of biomedical compounds. They are a suitable alternative to synthetic medicines and antimicrobial agents, and could be used as safe and effective therapeutic agents or probiotics, especially at a time when drug resistance among causal organisms for many life-threatening diseases is on the rise. SLs offer the advantages of biodegradability, low ecotoxicity and the production based on renewable-resource substrates. The US FDA has also approved biosurfactants/sugar esters for the use in food and pharmaceuticals. Keeping this in mind; several basic and applied aspects of sophorolipids have been explored and the work has been reported in the thesis.

The thesis has been divided into 5 chapters

Chapter I. Introduction

The *first chapter* is an introduction to the thesis. It includes brief account on surfactants, current market share of Biosurfactants, types, their advantages over chemical ones and producing microorganisms. SL stands as a promising biosurfactant. The features which make it so are described along with its physiological role and the biosynthetic pathway. Owing to the unique nature; sophorolipids find applications in wide range of areas. The chapter gives brief literature review of the applications. With this background, the scope and objectives of the thesis have been defined.

Chapter II. Production of sophorolipids using non edible oils and their use as an alternative/ additive to laundry detergents

The *second chapter* is about the attempt to reduce the SL production cost by using non-edible oils namely Jatropha and Pongamia. Through optimization of

parameters and resting cell method, the yields 15.25g/l and 19.3g/l could be achieved for Jatropha oil derived SL (SLJO) and Pongamia oil derived sophorolipid (SLPO) respectively. Both SLs showed good surfactant property with the CMC values 9.5mg/l for SLJO and 62.5mg/l for SLPO. Keeping the prospective use of these SLs in mind, the physicochemical characterization, emulsion stability, antibacterial and stain removal studies in comparison with commercial detergent were done. Based on the results, it can be said that SLs have utility as fabric cleaner with advantageous properties such as skin friendly nature, antibacterial action and biodegradability. SLs enhance the detergent performance, so less quantity of detergent can be sufficient for desired cleaning effect. Thus the harm caused to environment through detergent usage can be reduced by replacing synthetic surfactants with green surfactant molecules.

Chapter III(a). Sophorolipid biosurfactants act in synergy with antibiotics to enhance their efficiency

In the *third chapter (part a)* the effect of co-administration of SL and antibiotics has been evaluated. SLs are amphiphilic in nature and therefore can span through the bacterial cell membrane. Thus the co-administration is expected to facilitate the entry of antibiotic molecules. During the experiments, effect of SL-antibiotic co-administration was evaluated against 3 different index bacteria namely, *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*. Also the antibiotics differed in their mode as well as site of action. In accordance with the anticipation, SL-antibiotic combination achieved the bacterial inhibition within shorter period as compared to the antibiotic alone. The Scanning electron micrographs of the bacterial cells treated with combinations confirmed the damage to cell membrane with all 3 test bacteria at sublethal concentrations of both inhibitory agents.

Chapter III(b). Exploration of antiviral activity of SLs

The *third chapter (part b)* describes antiviral action of Oleic, Linoleic acid derived SL against viruses differing in their genetic makeup such as plus stranded RNA, segmented single negative stranded RNA and double stranded DNA viruses. The antiviral activity was tested in 3 different modes- direct treatment of virus, rapid

culture assay for Influenza virus and treatment of host cell line with SL prior to viral challenge in order to check if SL gives any antiviral immunity. Coxsackieviruses CV (B1-CVB6, CA7, CA9), murid herpesvirus, strain MHV-68, Influenza virus A/Mississippi/1/85 (H3N2) have been used for the studies.

It was observed that direct treatment of virus indicated $1 \log_{10}$ - $4.5 \log_{10}$ reduction in the virus titers. Pretreatment of cell cultures GMK and Hep-2 prior to Coxsackie virus infection showed a reduction in virus titer $1 \log_{10}$ - $2 \log_{10}$. Similar results were obtained on the VERO, BHK and 3T3 cells with gamma-herpesvirus MHV-68. Visible reduction of Influenza A virus replication on MDCK cells was obtained at the concentration $100 \mu\text{g/ml}$ of SLLA (SL derived from Linoleic acid). Thus it can be concluded that direct treatment of viruses was more effective than indirect treatment and the inhibitory action can be attributed to the amphiphilic structure of SL which might be killing the viruses by disturbing the membrane lipid order.

Chapter IV. Differentiation inducing ability of SLs against glioma cells

The *forth chapter* deals with the differentiation inducing ability of SLs. The effects have been investigated on LN229 - a glioma cell line which has been reported for the first time. In response to different SL forms, marked difference in cell density was observed as compared to the control cells along with various morphological changes such as formation of long thread like extensions arising from ends of the cells, cell alignment, cell elongation and bundle formation in dose dependent manner. In this chapter the morphological evidence of the potential of SLs as differentiation inducers has been presented. The finding suggests the utility of SLs as a pharmaceutical agent for the treatment of glioblastomas. With the use of SL, the cancerous cells undergoing uncontrolled proliferation can be forced to differentiate thus tumor progression may be arrested.

Chapter V(a). Glycolipid production by a novel yeast- *Pichia caribbica* (HQ222812) with xylose as a head group and its advantageous properties

The *fifth chapter (part a)* deals with the use of a new xylose fermenting yeast- *Pichia caribbica* for biosurfactant production so as to achieve the less explored head group diversity in SL structure. The media and fermentation parameters

have been optimized to achieve maximum yield of 7.5g/l. The physicochemical properties of the xylolipid biosurfactant have been assessed. It reduced the surface tension of distilled water from 70mN/m to 35.9mN/m with the remarkably low CMC value 1.0 mg/l as compared to typical SLs (reported CMC range-40-100mg/l). Structural characterization was done using FTIR and HR-MS to identify the structure putatively. 17-L-[(β -D-xylopyranosyl)-oxy]- Δ 9-heptadecanoic acid correlated to m/z 415 majorly constituted the product. Control experiment was performed in which glucose was provided as the hydrophilic carbon. This product was also subjected to HR-MS analysis to determine its chemical nature and found to be different from xylolipid. Presence of xylose as head group was anticipated to give altered physicochemical and biological activities. In accordance to the same, low CMC value and better inhibitory action was demonstrated against *Staphylococcus aureus*, a gram positive bacterium.

Chapter V(b). Crystalline xylitol production by a novel yeast- *Pichia caribbica* (HQ222812) and its application for quorum sensing inhibition in gram negative marker strain *Chromobacterium violaceum* CV026

The *fifth chapter (part b)* is about quantitative production of xylitol from D-xylose with the yield of 0.852 gm/gm and volumetric productivity of 1.83 gm/l/h. A safe procedure for product extraction has been described. The ability of xylitol to act as a quorum sensing antagonist in gram negative marker strain *Chromobacterium violaceum* CV026 has been demonstrated for the first time.

Conclusions

The last chapter summarizes the work presented in the thesis and emphasizes on possible further research in this area.