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Regular Article

Systematic comparison of the functional physico-chemical characteristics and biocidal activity of microbial derived biosurfactants on blood-derived and breast cancer cells

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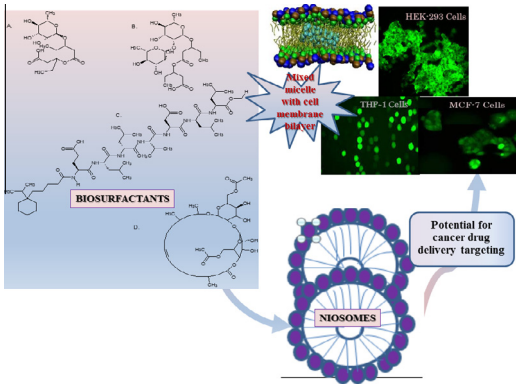
GRAPHICAL ABSTRACT



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ABSTRACT

Hypothesis: The cytotoxicity of biosurfactants on cell membranes may be influenced by composition of their hydrophilic head and hydrophobic tails. It is hypothesised that they form mixed micelles which exert a detergent-like effect that disrupts the plasma membrane. The functional physico-chemical and biocidal characteristics of four biosurfactants were concurrently investigated to determine which of their structural characteristics may be tuned for greater efficacy.

Experiments: Rhamnolipid-95, rhamnolipid-90, surfactin and sophorolipid were characterised using FTIR, LC-MS, HPLC, surface tension and critical micelle concentration. Their biocidal activity against HEK 293, MCF-7 and THP-1 cell lines were investigated by MTT assay, using doxorubicin as cytotoxic control. Growth curves were established for all cell lines using trypan blue (TB) and MTT assays, corresponding doubling time (DT) and growth rate were obtained and compared.

Findings: HEK 293 cell-line had the highest growth rate amongst the three cell lines. For TB assay, growth of HEK 293 > THP-1 and for MTT, HEK 293 > MCF-7 while the DT was in the order of THP-1 > MCF-7 > HEK 293. Sophorolipid showed anti-proliferative activity comparable to doxorubicin on THP-1 > MCF-7 > HEK 293. THP-1 showed high sensitivity to sophorolipid with IC_{50} of 10.50, 25.58 and 6.78 (μg/ml) after 24, 48 and 72 h respectively. However, sophorolipid was cytotoxic from 24 to 72 h on HEK 293 cell lines with IC_{50} of 21.53, 40.57 and 27.53 μg/ml respectively. Although, doxorubicin showed higher anti-proliferative activity than all biosurfactants, it had poorer selectivity index for the same time durations

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compared to the biosurfactants. This indicates that biosurfactants were more effective for slowing the growth of the tested cancer cell lines and hence may be potential candidates for use in human cancer therapy. Physico-chemical characteristics of the biosurfactants suggest that their mechanism of action may be due to activity on the cell membrane.

1. Introduction

Surfactants are amphiphilic compounds that consist of a hydrophilic head and hydrophobic tail and have surface activity [17]. The hydrophilic part can be a charged polar group (e.g. sulphate), a zwitterionic group (e.g. glycine) or uncharged polar group (e.g. poloxamers), whereas the hydrophobic part can be a non-polar group, comprising a single carbon chain or up to four alkyl chains [39]. Based on the nature of their polar head group, surfactants can be classified as anionic, non-ionic, zwitterionic and cationic [16,28,33]. They are synthesised from petrochemical sources such as sodium lauryl sulphate, polyoxyethylene glycol octylphenol ethers, phospholipids and alkyltrimethylammonium salts [34] and used in the formulation of detergents, personal care products and cleaning agents [34], and this is possible through their surface active and interfacial properties.

Surfactants are also secreted by mammals (pulmonary surfactants), plants (lecithin), and microorganisms and these are referred to as biosurfactants to differentiate them from the chemically synthesised ones. Microbial biosurfactants (BSs) exist as low and high molecular weight compounds such as rhamnolipids, sophorolipids, surfactin, trehalose lipids and emulsan. They are further subdivided into glycolipids (rhamnolipids, sophorolipids), lipopeptides, (surfactin) and polymeric BSs (emulsan). Due to their natural origins, BSs are recognised as non-toxic or of low toxicity, biodegradable and therefore potential alternatives to synthetic surfactants [15]. In addition, BSs are multifunctional compounds that also have biotechnological and biomedical applications. The BSs investigated in this study (rhamnolipids, surfactin and sophorolipids) were selected on the basis of their therapeutic and biophysical properties as well as their ready availability. Rhamnolipids and sophorolipids are members of the same glycolipid sub-class while surfactin is a macrolide lipoheptapeptide [20] and are amongst the most widely characterised biosurfactants and also used in several applications including bioremediation [9].

Rhamnolipids are synthesised from *Pseudomonas aeruginosa* [4] and exist as a family of congeners, some of which have isomers. The most prominent congeners are mono-rhamnolipids and di-rhamnolipids with molecular formulas Rha-C_{10}-C_{10} and Rha-Rha-C_{10}-C_{10} respectively (Fig. 1). The structural units were further elucidated as being composed of two β-hydroxydecanoic acids linked through an ester bond to two rhamnose moieties via a 1,3 glycosidic linkage (Fig. 1). The ratio of mono to di-rhamnolipids (Fig. 1A and B) produced by bacteria depends on the carbon sources utilised during biosynthesis [22]. The di-rhamnolipid congener, Rha-Rha-C_{10}-C_{10} is considered to be the most common of its class [1] and a higher percentage of di-rhamnolipids is produced when hydrophilic substrates such as hydrocarbons are used during synthesis.

Surfactin, is predominantly secreted by *Bacillus subtilis* and its chemical structure consists of a cyclic lactone ring surrounded by seven amino acid residues interlinked with a β-hydroxyl fatty acid whose chain length varies from 12 to 16 carbon atoms (Fig. 1C). Surfactin adopts a β turn, and forms a β helical sheet and the amino acid sequence is expressed as LLDLLDL and surrounded by a heptapeptide ELLVDLL [20]. Additionally, the hydrophobic amino acid residues are situated at positions 2, 3, 4, 6 and 7 while negatively charged amino acid residues are situated at positions 1 and 5 [18,37].

Gorin et al. [13] identified the yeast fungi, *Candida apicola* as a producer of sophorolipids. Since then, sophorolipids have been found to be secreted extracellularly from several other non-pathogenic yeasts, however, *Candida bombicola* has been the subject of many investigations. Sophorolipids exist as acidic or lactonic forms, with the latter resulting from internal esterification of the carboxylic acid group to a lactone ring. Additionally, structural variations could exist as a result of differences in hydroxylation of the terminal carbon atom via acetylation of the hydroxyl sophorose sugar at C6′, C6″ or C4″ which may be diacetylated, monoacetylated or deacetylated. Structural variation could also be due to the possession of one or more saturation bonds. Lactonic sophorolipids (Fig. 1D) are non-ionic BSs, however, the acidic forms can be converted into anionic, cationic, or zwitterionic forms by coupling the carboxylic end with di-carbodiimide. Sophorolipid isoforms exhibit different biological and chemical behaviours. For example, lactonic sophorolipids have better surface tension with cytotoxic, biocidal, spermicidal and hydrophobic properties while acidic sophorolipids have better foaming, detergent, solubility, cosmetic and bio-remediation properties.

Due to their structural novelty and diverse biophysical properties, lipopeptides, glycolipids and other BSs have recently emerged as possible broad-spectrum agents for cancer chemotherapy [14]. The cytotoxic activity of the selected BSs on cancer cells has been reported by a number of studies [5–8,12,31,32,38,43]. However, the pharmacological effects of BSs on blood derived monocytic cancer cells has not been reported. In this study, the biological (e.g. anti-cancer) activities of these BSs is of particular interest. Furthermore, this research sheds new light on the action of lactonic sophorolipids on breast cancer cells. The aim of this work therefore was to functionally characterise the physico-chemical properties and systematically compare the surface active properties and cytotoxicity of the four selected biosurfactants against blood derived and breast cancer cell lines using MTT assay.

2. Materials and methods

2.1. Chemicals and reagents

R-95™ rhamnolipid (BS1a), R-90™ rhamnolipid (BS1b), surfactin (BS2) and 1′,4″-sophorolactone 6′,6″-diacetate (BS3) from yeast, doxorubicin hydrochloride, penicillin/streptomycin and MTT (3-[4,5-dimethyl-2-thiazolyl]-2,5-diphenyl-2H-tetrazolium bromide) were purchased from Sigma Aldrich (Gillingham, UK). Dulbecco's modified eagles medium (DMEM), supplemented with 10% heat inactivated foetal bovine serum [Origin: EU approved (South American)] in PET bottle were obtained from Gibco, UK. All other reagents were of analytical grade and used as received.

2.2. Physico-chemical characterisation

2.2.1. Attenuated total reflectance Fourier transform infrared spectroscopy (ATR-FTIR)

Before ATR-FTIR analysis the diamond crystal surface was cleaned and background spectra were collected. Samples were