Chapter

Anti-cancer biosurfactants

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Abstract Microbial biosurfactants are amphiphilic compounds and surface-active biomolecules produced by several microorganisms. Recently, biosurfactants have emerged as promising agents for cancer therapy since a high diversity of these molecules has shown the ability to induce cytotoxicity against many cancer cell lines, thus regulating cancer progression processes. In this sense, microbial biosurfactants are a potential alternative to current cancer therapeutics and as drug delivery systems of anti-cancer drugs. This chapter covers the current knowledge of microbial surfactants with anti-cancer potential, providing information on their production, structure, and research on different cancer cell lines, such as breast and lungs

cancer, leukaemia, melanoma and colon cancer. The potential application of biosurfactants in drug delivery is also reviewed.

1 Introduction

Cancer is the second-highest cause of death worldwide. In 2018, the World Health Organization (WHO) presented data estimating 9.6 million deaths and new cases rising to 18.1 million [1]. Chemotherapy remains one of the major options in cancer treatment, despite having known limitations and undesirable side effects [2]. Chemotherapeutic drugs target non-specific highly proliferative cells, presenting toxicity to normal tissues, and interfering with the life quality of cancer patients [3]. Thus, many efforts have been done to find new drugs for cancer treatment focusing on novel agents that selectively target cancer cells [4]. Recently, due to their ability to control some functions on mammalian cells, biosurfactants have demonstrated their potential to be applied as anti-canceragents [5].

Biosurfactants are amphiphilic compounds with surface and emulsifying activity produced by microorganisms. With a wide range of chemical structures, these compounds show diverse properties and physiological functions [6]. Some of the natural roles of these compounds include an increasing of the surface area and bioavailability of hydrophobic water-insoluble subtracts, heavy metal binding, bacterial pathogenesis, antimicrobial activity, capacity of regulating the attachment and removal of microorganisms from surfaces, quorum

sensing and biofilm formation [7]. Furthermore, biosurfactants usually have lower toxicity and higher biodegradability than synthetic surfactants [6, 8]. Some biosurfactants additionally have potential as biologically active compounds, being suitable therapeutic alternatives to synthetic drugs [9, 10]. Biosurfactants have been explored in gene delivery [11], drug delivery [12], as adjuvants in immunology [13], as antimicrobial [14], antifungal [15] and antiviral [16] agents, and as anti-cancer therapeutics [4].

The anti-cancer activity of biosurfactants is related to their ability to inhibit cancer cells growth [17], apoptosis (cell death) induction [18], activity on differentiation [19], necrosis [20] and cell cycle arrest [18]. These biosurfactants comprise mannosylerythritol lipids (MELs) [21, 22], succinoyl trehalose lipids (STLs) [23], sophorolipids [24], rhamnolipids (RLs), surfactin [25], serrawettins [26, 27] and monoolein [28]. In addition to the high potential of biosurfactants as anti-cancer therapeutics, these molecules can also be incorporated or used as vehicles or drug delivery systems (DDS) of anti-cancer drugs [25].

This book chapter discusses the current research and knowledge of microbial biosurfactants as anti-cancer drugs, with emphasis on their structure, production, DDS and potential anti-cancer activity in the treatment of breast and lung cancer, leukaemia, melanoma and colon cancer.

2 Biosurfactants classification and structure

Based on their molecular weight, biosurfactants can be classified into two categories: low molecular weight biosurfactants and high molecular weight biosurfactants. The first class encompasses glycolipids, lipopeptides, lipoproteins, fatty acids and phospholipids, whereas the second one comprises polymeric biosurfactants [29]. In this chapter, only biosurfactants with anti-cancer properties are reviewed.

2.1 Mannosylerythritol lipids (MELs)

Mannosylerythritol lipids (MELs) were firstly characterized in 1970 by Bhattacharjee et al. (1970) [30]. MELs are glycolipids with a mixture of partially acylated derivative of 4-*O*-β-D-mannopyranosyl-D-erythritol, containing fatty acids as the hydrophobic groups [30]. The structural variants of MELs arise due to: i) the number and the position of acetyls group on mannose and erythritol; ii) the number of acyl groups in mannose and erythritol; and iii) the fatty acid chain length and saturation [31, 32]. Based on the degree of acetylation at C-4' and C-6' position in mannopyranosyl, MELs are classified as MEL-A, MEL-B, MEL-C and MEL-D (Fig. 1). MEL-A represents the diacetylated compound, whereas MEL-B and MEL-C are monoacetylated at C-6' and C-4', respectively. The completely deacetylated structure is known as MEL-D [31, 32]. Novel types of MELs have been reported and named as mono-acylated and tri-acetylated MEL, in which C-2',

C-4', and C-6' of mannopyranosyl are linked with OAc or OH [31]. Fukuoka et al.c(2008) [33] discovered a diastereomer type of MEL-B with a sugar moiety, identified as 1-*O*-β-D-mannopyranosyl-erythritol, and stereochemically different from the 4-*O*-β-D-mannopyranosyl-erythritol of conventional MELs. Morita et al. (2009) [34] reported a new MEL containing mannitol as the hydrophilic part instead of erythritol, and a mannosyl-mannitol lipid. The hydrophobic part of MELs contains C2:0, C12:0, C14:0, C14:1, C16:0, C16:1, C18:0 and C18:1 fatty acids. The fatty acid profiles of MELs are of a wide diversity with the variation of species (same genus), with one MEL produced as a major product [31]. For instance, MEL-A was produced as a major MEL, with C6:0, C12:0, C14:0, and C14:1 from *Candida pseudozyma* sp. SY16 [35], while MEL-C was the major MEL produced by *Pseudozyma hubeiensis* KM-59 with C6:0 (21.3%), C10:0 (9.5%), C12:0 (16.3%), and C16:2 (30.3%) [36].

FIGURE 1

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2.2 Succinoyl trehalose lipids (STLs)

Succinoyl trehalose lipids (STLs) are glycolipids produced from *n*-alkanes. STLs represent the most promising type of trehalose lipids

produced by *Rhodococcus* sp. strain SD-74 [37]. The chemical structure of STLs has one or two succinic acids and two or three fatty acids attached to a trehalose moiety, being also reported as a “di-succinylated” trehalose lipid and a “mono-succinylated” trehalose lipid [37, 38]. The major component of STL-1 is characterized as 3,4-di-*O*-palmitoyl- 2,2′-di-*O*-succinoyl-*α,α*-trehalose, and that of STL-2 is identified as 2,3,4-di-*O*-alkanoyl-O-succinoyl-*α,α*-trehalose, whereas STL-3 is shown to be 2,3,4,2′-mono-*O*-succinoyl-tri-*O*-alkanoyl-trehalose (Fig. 2) [39]. The exact location of the acyl chains in STL-2 and STL-3 is not confirmed. However, based on the structure of STL-1, it is believed that the succinic acid is present at the O2 position [39]. Uchida et al. [40] reported the production of two main types of STL homologues by *Rhodococcus* sp. strain SD-74, STL-1 and STL-2. These compounds present hydrophobic acyl groups of both STLs and have the same carbon chain length as the *n*-alkane used as the substrate. Tokumoto et al. (2009) [38] reported the structural characterization of STL-1 (Fig. 2), with trehalose lipid having two succinoyl and hexadecanoyl residues as previously reported by Uchida et al. (1989) [40]. The fatty acid profile of the purified STL-1 was evaluated by Gas Chromatography–mass spectrometry (GC-MS). C16 (63.5%) was the major fatty acid found, while C14 (26.6%) and C12 (9.9%) were found in less amounts.

FIGURE 2

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2.3 Sophorolipids

Sophorolipids are biosurfactants composed of a residue of sophorose (acetylated 2-*O*-β-D-glucopyranosyl-D-glucopyranose), a disaccharide consisting of two glucose residues linked by the β-1,2' bond, and by a long-chain hydroxy fatty acid (Fig. 3 (A)) [41]. These glycolipids differ in the number and position of acetate groups as *O*-substituents in the carbohydrate residue and in the structures of fatty acid residues [42]. Sophorose can be acetylated in the 6- and/or 6'-positions, and one terminal or subterminal hydroxylated fatty acid is β-glycosidically linked to the sophorose molecule [41]. The hydroxy fatty acid residue is generally C16 or C18 and may have one or more unsaturated bonds (Fig. 3 (A)) [41]. Moreover, the carboxylic group of fatty acid is either free (acidic or open form) or internally esterified at the C-4'-position (lactonic form) (Fig. 3 (B)) [42]. Sophorolipids can exist as lactones, and as monomeric or dimeric forms containing C22 fatty acid residues [43]. Sophorolipids from *Starmerella bombicola* and *Candida batistae* differ in the position of the hydroxylic group in the fatty acid residue: the fatty acid residues in sophorolipids from *S. bombicola* are hydroxylated mainly in ω-1 position, while from *C. batistae* are hydroxylated mainly in ω-position [44]. *Candida apicola* is able to produce di-*O*-acetyl, mono-*O*-acetyl, and non-acetyl sophorolipids in the free-acid and lactone forms [41]. The sophorolipid produced by *Rhodotorula bogoriensis* contains C22 fatty acid residue as an aglycone (Fig. 3(B)) [43].

FIGURE 3
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2.4 Rhamnolipids (RLs)

Rhamnolipids (RLs) are glycolipid biosurfactants produced by several bacteria [45]. The discovery of RLs (Fig. 4) goes back to 1946 when Bergström et al. (1946) [46, 47] reported an oily glycolipid produced by *Pseudomonas pyocyanea* (now *P. aeruginosa*). Later, in 1965, Edwards and Hayashi (1965) [48] found that the linkage between the two rhamnose moieties is an α-1,2-glycosidic linkage, as determined by periodate oxidation and methylation. Based on that, the authors chemically described these RLs as 2-O-α-1,2-L-rhamnopyranosyl-α-L-rhamnopyranosyl-β-hydroxydecanoyl-β-hydroxydecanoate. This was the first discovered glycolipid containing a link between a sugar and a hydroxylated fatty acid residue. Overall, eight RLs congeners were identified until the mid-1980s [45].

In the last decade of the twentieth century, a significant number of new RLs were identified, and new congeners are still regularly reported. Thus, in view of the structures, RLs can be described as glycosides composed of rhamnose moieties (glycon part) and lipid moieties (aglycon part) linked to each other via an O-glycosidic linkage. The glycon part is composed of one (mono-RLs) or two (di-RLs) rhamnose moieties linked to each other through an α-1,2-glycosidic linkage. The aglycon part, however, is mainly one or two (in few cases

three) β- hydroxy fatty acid chains (saturated, mono-, or polyunsaturated and of chain length varying from C8 to C16) linked to each other through an ester bond formed between the β-hydroxyl group of the distal (relative to the glycosidic bond) chain with the carboxyl group of the proximal chain (Fig. 4). In most cases, the carboxyl group of the distal β-hydroxy fatty acid chain remains free; few congeners, however, have this group esterified with a short alkyl group [45]. Similarly, the 2-hydroxyl group of the distal (relative to the glycosidic bond) rhamnose group remains mostly free, although in some rare homologues it can be acylated with long-chain alkenoic acid [45].

Variations in the chemical structures of bacterially produced RLs give rise to a large pool of RL homologues that approaches 60 structures. The differences among these homologues come from modifications in the glycon and/or the aglycon parts, taking in consideration that variation in the aglycon part contributes largely to the biodiversity of RLs [45].

FIGURE 4

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2.5 Myrmekiosides

Myrmekiosides are glycolipids with a unique mono-O-alkyldiglycosylglycerol structure isolated from the marine sponges *Myrmekio-*

derma sp. and *Trikentrion loeve*. These compounds consist of a glycerol backbone bearing an alkyl chain and a xylose at the terminal hydroxyl positions, and a mono- or diglucosyl unit or a monoglucosamine residue attached to C-2’ of the glycerol (Fig. 5). Among these compounds, myrmekiosides A–C, contain the same sugar moieties but have different O-alkyl chains. Myrmekioside E-2 is a peracetylated derivative of myrmekioside E, which contains xylose and N-acetylglucosamine and a long alkyl chain with a terminal alcohol group [49].

FIGURE 5

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2.6 Cyclic lipopeptides (CLPs)

Cyclic lipopeptides (CLPs) are versatile molecules produced by a variety of bacterial genera [50]. Structurally, CLPs consist of an oligopeptide with a peptidically linked N-terminal fatty acid. The linear or branched lipid tail can vary in length (typically C6−C18) and in the degree of oxidation [51]. The C-terminus of the oligopeptide (up to 25 amino acids) forms a lactone or lactam with a hydroxyl, phenol, or amino functional group that is either present in the side chains of the peptide or is part of the lipid moiety, thus giving rise to macrocycles of varying sizes (typically 4−16 amino acids) [51]. Given that CLPs are biosynthesized by nonribosomal peptide synthetases, both

nonproteinogenic (e.g., D-configured or β-amino acids) and modified amino acids (e.g., 4-chlorothreonine) can be present in the peptide [51].

2.6.1 Amphisin, tolaasin and syringomycin CLPs

The chemical structure analysis of all CLPs from *Pseudomonas* sp. could be clustered into two major groups, each one consisting of subgroups (i.e. amphisin, tolaasin, and syringomycin groups) [50]. The two major groups vary primarily in the number of amino acids in the cyclic peptide moiety, while each of the subgroups could be differentiated by substitutions of specific amino acids in the peptide moiety [52]. Amphisin CLPs, including amphisin and tensin, consist of 11 amino acids in the peptide part coupled to 3-HAD. For both tensin and amphisin, the structures are mainly helical, with the cyclic peptide wrapping around a water molecule. On the other hand, CLPs in the tolaasin group are much more diverse due to multiple variations in both the composition and length of the peptide chain (19 to 25 amino acids) and lipid tail (3-HDA or 3-hydroxyoctanoic acid [3-HOA]). The peptide part of tolaasin contains several unusual amino acids, including 2,3-dihydro-2-aminobutyric acid (Dhb) and homoserine (Hse), the first always being in front of the allo-Thr residue. The cyclic part of the peptide moiety contains from five to eight amino acids and the lactone ring is formed between the C-terminal amino acid and the allo-Thr residue. Finally, CLPs in the syringomycin group harbor unusual amino acids, including Dhb,2,4-diamino butyric acid (Dab),

and the C-terminal 4-chlorothreonine (Thr[4-Cl]). Furthermore, the lactone ring is formed between the N-terminal Ser and the C-terminal Thr(4-Cl). The fatty acid tail of CLPs in the syringomycin group may consist of a 3-hydroxy or 3,4-dihydroxy fatty acid composed of 10 to 14 carbon atoms [50].

The structure of other CLPs from *Pseudomonas* sp. has been elucidated in the past years. For example, Pseudofactin II, is a novel compound identified as CLP with a palmitic acid connected to the terminal amino group of eighth amino acid in peptide moiety. The C-terminal carboxylic group of the last amino acid forms a lactone with the hydroxyl of Thr3 [53].

2.6.2 Iturin and fengycin CLPs

CLPs produced by other bacteria have been also reported. Iturin (Fig. 6 (a)) from *Bacillus subtillis* is a cyclic peptide containing 7 amino acids (heptapeptides) linked to a fatty acid (β-amino) chain that can vary from C14 to C17 carbon molecules [54]. Mixirins A–C (three cyclic acylpeptides) belonging to the iturin class have been also isolated from marine bacterium *Bacillus* sp. [55].

Fengycin (Fig. 6 (b)), also produced by *B. subtillis*, contains a lactone ring in the β-hydroxy fatty acid chain that may be saturated or unsaturated [54]. The structure of fengycin contains a peptide chain of ten amino acids linked to a fatty acid chain. The length of the fatty acid

chain can vary from C14 to C17 carbon atoms for fengycins, thus giving different homologous compounds and isomers (Fig. 6 (b)). Members of fengycin family exhibit heterogeneity at the 6th position in the peptide moiety as well as in chain length of the β-hydroxy fatty acid, being classified as fengycin A (contains Ala at position 6) and fengycin B (contains Val at position 6) [54].

FIGURE 6

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2.6.3 Surfactin CLP

The surfactin biosurfactant, produced by different strains of *B. subtilis*, is the most studied CLP. Surfactin is constituted by a heptapeptide with the chiral sequence interlinked with a β-hydroxy fatty acid chain of C12-C16 carbon, which forms a closed cyclic lactone ring structure (Fig. 7) [25, 56]. Hydrophobic amino acids of surfactin molecule are located in positions 2', 3', 4', 6' and 7', while hydrophilic glutamyl and aspartyl residues are located in position 1' and 5', giving the molecule two negative charges [25]. Two conformations of surfactin, S1 and S2, have been found by Bonmatin et al. (1994) [57]. Both display a saddle-shaped conformation, where the two charged side chains are gathered on the same side. They form a "claw" and provide a polar head opposite to a hydrophobic domain [56]. Baumgart et al. [58] reported that three surfactin compounds were produced

by *B. subtilis* ATCC 21332 and OKB 105. Two were different from the basic structure mentioned above in the amino acid Leu, which was replaced by Val and Ile, respectively. Liu et al. (2007) [59] identified surfactins produced by *B. subtilis* when incubated with the same culture medium and found three kinds of surfactins with different peptide moieties: i) a surfactin with amino acid sequence of N-Asp-Leu-Leu-Val-Glu-Leu-Leu-C; ii) a surfactin with amino acid sequence of N-Glu-Leu-Leu-Val-Asp-Leu-Leu-C; and iii) a surfactin with the peptide chain methyl esterified. Meanwhile, the fatty acid moiety of these surfactins was identified to be diverse, including iso C12, iso C13, anteiso C13, iso C14, n C14, iso C15, anteiso C15, n C15, anteiso C16, and anteiso C17 beta-hydroxy fatty acids [59].

FIGURE 7

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2.7 Rakicidns and apratoxins

Rakicidins are the fifteen-membered depsipeptides consisting of three amino acids and a 3-hydroxyfatty acid. Up to date, four congeners (Fig. 8), rakicidins A and B from *Micromonospora* sp. and rakicidins C and D from *Streptomyces* sp., have been reported [60]. All these compounds share the common amino acid components, 4-amino-2,4-

pentadienoate, glycine and hydroxyaspargine (or glutamine in rakicidin C), and a 3-hydroxyfatty acid unit that varies in the chain length and methylation pattern [61].

FIGURE 8

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Marine cyanobacteria produce a number of secondary metabolites that possess interesting molecular architectures and biological properties, namely apratoxins. Apratoxins are cyclodepsipeptides that feature a proline residue, N-methylated amino acids, a modified cysteine residue, and a dehydroxylated fatty acid moiety (Fig. 9) [63]. A total of seven apratoxins has been characterized (A–G), showing a number of interesting modifications, including absence of an N- or C-methyl group at various locations (B, C, E and G), an additional polyketide synthases module early in the biosynthetic sequence (D), or replacement of a terminating proline residue with an N-methyl alanine (F and G) [64].

FIGURE 9

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2.8 Serrawettins

Serrawettins are produced by different *Serratia* genus and were first isolated in 1985. Serrawettin W1 is produced by strains of *S. marcescens,* while serrawettin W2 is produced by *S. marcescens* and *S. surfactantfaciens* strains [27].

The general structure of serrawettin W1 (also designated as serratamolide A), Fig. 10, includes a symmetric dilactone structure composed of two L-serine amino acids linked to two β-hydroxy fatty acids (comprising 3- hydroxydecanoic acids) [65]. However, different homologues have been described, namely serratamolides B to G, which is related to the variation in the length of the fatty acid chain (C8 to C14) and presence or absence of double bonds in the structure of serrawettinW1 (serratamolide A) [27].

Differently, serrawettin W2 includes five amino acids (D-leucine/ isoleucine-L-serine-L-threonine-D-phenylalanine-L-isoleucine/ leucine) bonded to a β-hydroxy fatty acid moiety, and variations in the first, second or fifth amino acid positions or the length of the fatty acid chain (C8 or C10) result in serrawettin W2 analogues [27].

FIGURE 10

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2.9 Monoolein

Monoolein (1-Monoolein (1-(cis-9-Octadecenoyl)-rac-glycerol)) is composed of a hydrocarbon chain (oleic acid), which is attached to a glycerol backbone by an ester bond (Fig. 11). The remaining two hydroxyl groups from the glycerol moiety confer polar characteristics to this portion of the molecule [67, 68]. The glycerol moiety may form hydrogen bonds with water in an aqueous environment and is commonly referred to as the "head" group [68]. The hydrocarbon chain (usually referred as the 'tail'), featuring a cis double bond at the 9, 10 positions, is strongly hydrophobic [67, 68], thus rendering monoolein as an amphiphilic molecule.

FIGURE 11

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2.10 Fellutamides

Fellutamides are a small family of marine-derived lipopeptide natural products, characterized by a C-terminal aldehyde and a (3R)-β-hydroxy alkanoate tail (Fig. 12). Fellutamides A, C and D contain the non-ribosomal amino acid β-L-threo-hydroxy-glutamine, while fellutamides A and B both possess a β-hydroxylated fatty chain amide derived from (3R)-hydroxy lauric acid [69]. However, two different

compounds were named fellutamide C in the literature around the same time-frame. To resolve the ambiguity between them, Singh's fellutamide C structure has been renamed, as fellutamide E [70].

FIGURE 12
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3 Biosurfactants production

The production of biosurfactants should be performed using safe and nonpathogenic microorganisms to prevent problems with pathogenicity [71]. Furthermore, most biosurfactants are considered secondary metabolites, playing crucial roles for the survival of the producing-microorganisms, either through facilitating nutrient transport, by promoting microbe-host interactions or as biocide agents [72]. Accordingly, many biosurfactant applications substantially depend on whether they can be economically produced. Thus, efforts in the process optimization have been extensively carried out. Biosurfactants production from low-cost raw materials and cheap substrates can decrease the production cost [73]. Accordingly, substrates such as olive oil mill effluents, corn steep liquor, vegetable cooking oil waste, animal fat, soap stock, dairy industry waste, among other, have been deeply investigated [74]. The selection of the waste biomass should

ensure the proper balance of nutrients to allow microbial growth and consequent biosurfactant production [74]. However, it is necessary to have in mind that the use of low-priced substrates for biosurfactants production does not bring only advantages, but also some disadvantages, as summarized in Fig. 13. Banat et al. (2014) [75] discussed this topic (renewable sources) and the cost-effectiveness of the related processes in their review.

In summary, the development of economically competitive biosurfactant production processes is urgent, and must include the optimization of culture conditions and development of cost-effective recovery processes to improve the yield and quality of biosurfactants.

FIGURE 13

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3.1 Factors involved in biosurfactants production

The production of biosurfactants is usually affected by the type of carbon and nitrogen sources and by different elements such as phosphorous, magnesium, ferric, and manganese ions present in the broth medium [29]. Moreover, the growth conditions of the culture can be affected by the biosurfactant synthesis procedure, i.e., agitation speed, temperature, pH, aeration and dilution rate [29]. During their produc-

tion several screening methods have been applied to identify and select efficient factors/conditions to achieve a high yield and quality of biosurfactants [29]. These factors are discussed below.

3.1.1 Carbon source

The growth of microorganisms and the production of biosurfactants are closely related with the carbon source. Carbon sources used in biosurfactant production can be divided into three categories, including carbohydrates, hydrocarbons, and vegetable oils [76]. Among them, water-soluble carbon sources such as glycerol, glucose, and mannitol, are usually used for the production of biosurfactants [29]. Reports from the literature showed that the use of a mixture of carbon sources in the production of biosurfactants can be more efficient than the use of one source. For example, Cooper et al. (1984) [77] demonstrated that the use of a carbon source composed only of glucose or vegetable oil do not change the biosurfactant yield (1 g/L) by *T. bombicola*. However, when both sources were provided together, the yield increased up to 70 g/L [77]. A similar behaviour was identified in the production of sophorolipids by *C. bombicola*, with the best production yield (120 g/L) obtained using a mixture of carbon sources (sugar and oil) [78]. Furthermore, when an industrial waste (soap stock) was used for the production of sophorolipids by *C. antarctica* and *C. apicola*, yields of 13.4 and 7.3 g/L were obtained, respectively [79]. Additionally, the *Pseudozyma* (*C. antarctica*) converted C12 to C18 *n*-alkanes

into MELs with a yield of 140 g/L using a soybean oil as a carbon source [80].

3.1.2 Nitrogen source

Nitrogen is the second most important factor in the production of biosurfactants by microorganisms since it is essential for growth and for the regulation of proteins synthesis [29]. Different organic and inorganic nitrogen sources, such as peptone, urea, ammonium sulfate, ammonium nitrate, sodium nitrate, meat extract, and malt extract, have been used in the production of biosurfactants [29]. Usually, a high carbon/nitrogen (C/N) ratio decreases the cell growth and increases the cell metabolism [74]. On the other hand, a low C/N ratio leads to the synthesis of cellular material and limits the buildup of products [74]. For example, the production of a biosurfactant by *Arthrobacter paraffineus* was increased when nitrogen sources such as ammonium salts and urea were used, while by *P. aeruginosa* and *Rhodococcus* sp. the highest yield was obtained when nitrates were used [29]. In a similar work, Mulligan and Gibbs (1989) [81] reported the biosurfactant production by *P. aeruginosa* using nitrates, ammonium and amino acids as nitrogen sources. In general, when compared to ammonium, the assimilation of nitrate is usually lower, simulating the nitrogen limitation, which is favorable to the production of a biosurfactant [74]. As an example, the production of RLs biosurfactants by *Pseudomonas* sp. strain DSM-2874 with the commencement of the

stationary phase of growth has been studied upon exhaustion of nitrogen, i.e., the addition of nitrogen source resulted in the inhibition of rhamnolipid synthesis [82]. Amani et al. (2013) [83] showed a maximum rhamnolipid production after nitrogen limitation (120 mg/L). According to Hommel et al. (1987) [84], the amount of nitrogen appears to be a mandatory factor for a high biosurfactant yield.

3.1.3 Effect of ions

The addition of multivalent cations such as magnesium, ferric, manganese, among others, into the culture media has been found to affect the production of biosurfactants [29]. More specifically, the limitation of multivalent cations is reported to enhance the production of biosurfactants [29]. For instance, better yields of RLs produced by *B. subtilis* were achieved by limiting the concentrations of salts of sodium, magnesium, potassium, calcium, and trace elements [29]. Furthermore, the addition of other chemicals like EDTA, ethambutol, chloramphenicol and penicillin influence the production of biosurfactants [85]. The regulation of biosurfactants production by these compounds is either through their effect on the solubilization of nonpolar hydrocarbon substrates or by the increased production of water-soluble (polar) substrates.

3.1.4 Physical factors

Growth culture conditions, namely temperature, pH, agitation speed and oxygen also influence the biosurfactants production [29]. For example, at a pH range between 6.0-6.5, the production of rhamnolipid by *Pseudomonas* sp. was maximum, decreasing significantly at pH values above 7.0. On the other hand, the production of surfactin by *B. subtilis* was favored at neutral pH [86], whereas the production of sophorolipids by *C. batistae* was maximized at pH 6.0 [44].

Overall, the most favorable temperature for the production of biosurfactants by different fungi is around 30°C, as observed for different species of *Candida* [74]. The same temperature conditions were verified in the production of surfactin by *B. subtilis* [87]. On the other hand, the highest rhamnolipid concentration (1892 mg/mL) was reached when the fermentation of *P. aeruginosa* was carried out at 42°C [88].

Maximum production of Rhamnolipid by *P. aeruginosa* was found after five days of incubation [76], whereas the incubation periods for *C. bombicola* in the production of sophorolipids were from seven to eleven days [82, 89]. Finally, the agitation is also an important factor since it is responsible for an efficient oxygen transfer from the gas phase to the aqueous phase. Oliveira et al. (2009) [90] found that the increase in agitation favored the production of RLs by *P. alcaligenes*. Wei et al. [91] evaluated the agitation speeds (between 50 and 250 rpm) in the production of rhamnolipid by *P. aeruginosa* and observed a better result at 200 rpm. However, other studies have concluded that

high agitation speed (>500 rpm) had a negative effect on surfactin production by *B. subtilis* (2018) [92].

4 Anti-cancer activity of biosurfactants

Biosurfactants have been investigated as anti-cancer agents due to their promising intercellular recognition steps, which comprise signal transduction to selectively inhibit the proliferation of cancer cells [93]. The first approach in the treatment of cancer involves the induction of the terminal differentiation and apoptosis (main way of programmed cell death) pathways of cancer cells [93, 94]. Diverse mechanisms have been proposed to explain the anti-cancer activity of biosurfactants, such as: i) delay of cell cycle progression; ii) inhibition of signaling pathways; iii) reduction of angiogenesis; vi) activation of natural killer T (NKT) cells; and v) induction of apoptosis through death receptors in cancer cells (Fig. 14) [95]. Moreover, biosurfactants are able to disrupt cell membranes by lysis and increasing the membrane permeability [5].

FIGURE 14

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Hannun and Bell (1989) [96] reported a review article regarding the discovery of glycosphingolipids and lysosphingolipids as active compounds in the modulation of cell proliferation in oncogenesis and cell differentiation [96]. Since then, a diversity of compounds, such as polar compounds, glucocorticoids, short-chain fatty acids and retinoids, were found to induce features of differentiation in cancer cell lines and can trigger apoptotic events [97, 98]. The current state of biosurfactant as potential therapeutic applications of cancer (breast and lungs cancer, leukaemia, melanoma and colon cancer) are presented in Table 1 and discussed in this section.

TABLE 1

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4.1 Breast cancer

Breast cancer is one of the highly common cancers in women, affecting 1 in 8 women, with more than one million new cases per year [128, 129]. Early detection of breast cancer can be an effective strategy to reduce the number of breast cancer; however, in an advanced stage, conventional cancer treatments are required.

Despite improvements in early detection and treatment, around 50% of patients will either not succeed in chemotherapy or will develop resistance to chemotherapeutic drugs [130]. Biosurfactants have been

reported as a potential therapeutic alternative to combat breast cancer. Cao et al. (2009, 2010) [99, 131] demonstrated that surfactin induced apoptosis in human breast cancer MCF-7 cells. This biosurfactant produced reactive oxygen species indicating the involvement of reactive oxygen species generation in surfactin-induced cell death [131]. Duarte et al. (2014) [95] also studied the antitumor capacity of surfactin from *B. subtilis* 573 against two breast cancer cell lines, T47D and MDA-MB-231. The results show that the biosurfactant leads to a decrease in cells viability and proliferation by induced cell cycle arrest at G1 phase after 48h of exposure, without negatively affecting normal fibroblasts [95]. In an additional work, a surfactin from *Micromonospora marina* was tested on MCF-7 breast cancer cell lines [101]. This compound presented cytotoxicity against cancer cells by inducing apoptosis and cleaving mitochondrial membrane, while did not affecting normal cells [101].

Other types of biosurfactants studied against breast cancer cell lines are iturins (lipopeptides), produced by *Bacillus* strains. Iturins were found to significantly lead the apoptosis induction in breast cancer cells, MDa-MB-231 and MCF-7 [102–104]. Iturin A was evaluated in human breast cancer *in vitro* and *in vivo*. The results showed that the proliferation of MDA-MB-231 and MCF-7 cancer cells was significantly inhibited by inducing apoptosis [102]. Moreover, iturin A inhibited tumour growth with reduced expressions of Ki-67, CD-31, P-Akt, P-GSK3β, P-FoxO3a and P-MAPK proteins [102].

Sophorolipids produced by *Starmerella bombicola*, *namely* C18:0, C18:1, C18:2 and C18:3, were evaluated in what concerns their cytotoxic towards MDA-MB-231 breast cancer cells [24]. A high cytotoxic effect was observed in cancer cells at the sophorolipids concentration close to the Critical Micelle Concentration (CMC). The higher cytotoxicity was obtained with C18:0 and C18:1, being the compound C18:1 able to increase intracellular reactive oxygen species (ROS), involved in cancer cell death, and inhibit the cells migration without cellular damage. This can be a potential strategy in the management of tumour growth in early stages [24].

RLs, the most popular glycolipid biosurfactants, were studied by Rahimi et al. (2019) [105]. In this work, the cytotoxic effect of mono and di-RLs produced by *P. aeruginosa* on MCF-7 breast cancer cells was explored. Both compounds studied were found to induce the expression of the p53 gene [105]. A new glycolipid biosurfactant produced by *Planococcus maritimus* was evaluated as anti-cancer agent in MCF-7 cell line with a high cytotoxicity effect [132, 133]. The anticancer capacity was associated to hydrophobic and Van der Waal interactions; however, the mechanism involved was not presented [132, 133].

Rakicidins from *Micromonospora* are also known as breast anti-cancer agents. Even though, rakicidin derivatives C and D containing short lipid chains have no cytotoxicity, derivative E was found to inhibit the invasiveness of aggressive breast cancer cells [134].

4.2 Lung cancer

Lung cancer is the second most common cancer after prostate cancer in men and breast cancer in women. Recently, lung cancer surpassed heart disease as the leading cause of smoking-related mortality. Unfortunately, most lung cancer patients are diagnosed in an advanced stage. Thus, researchers are continuously looking for improved diagnosis and better alternatives of lung cancer treatment [135], which include the use of biosurfactants as suitable alternative therapeutic agents. Lipopeptides, such as surfactin, have been explored for their potential in lung cancer treatment. A surfactin produced by *B. atrophaeus* was studied by Routhu et al. (2019) [107]. This biosurfactant presented cytotoxicity against A549 lung carcinoma cell line [107]. The anti-cancer activity occurred due to cancer cell inhibition, cycle progression in G_0/G_1 and induced apoptosis via ROS accumulation [107].

Wrasidlo et al. (2008) [108] studied lipopeptide somocystinamide A obtained from the cyanobacteria *Lyngbya majuscula*. This biosurfactant exhibited a significant cytotoxicity against the A549 lung cancer cell line. The anti-proliferative activity was largely attributable to the induction of programmed cell death, with accumulation and aggregation of ceramide in treated cells and subsequent colocalization with caspase 8 [108].

A biosurfactant of the glycolipoprotein class produced by *Acinetobacter M6* strain demonstrated anti-cancer activity against A549 cancer

cells. The overall results showed that the cell viability decreased with increasing biosurfactant concentrations and incubation time [109].

Fellutamides biosurfactants isolated from the sponge-derived fungus *Aspergillus versicolor* displayed cytotoxic effects against A549 lung cancer cell lines [110, 111]. Fengycin produced by *B. subtilis* can block cell lung cancer cell 95D and inhibit the growth of xenografted 95D cells in nude mice [112]. This biosurfactant can inhibit the proliferation of cancer cells due to cell cycle arrest at the G0/G1 phase and promoting apoptosis via mitochondrial pathway, with increasing of caspase activity [112].

Rakicidin B (FW523-3) from *Micromonospora chalcea* has also been reported to be active against lung cancer cell lines, A549 and 95D [113]. This rakicidin derivative induced apoptosis by activation of caspase 3, 7 and 9, while blocking MAPK and JNK/p38 signalling pathways. Rakicidin B inhibited tumour cell growth and induced tumour cell apoptosis via the mitochondrial and MAPK pathways [113].

Another type of biosurfactants for lung cancer are apratoxins. For example, two apratoxin analogues, namely apratoxin A sulfoxide and apratoxin H, isolated from the cyanobacterium *Moorea producens*, were studies against human NCI-H460 lung cancer cells [114].

Myrmekioside, an o-alkyl-diglycosylglycerol, is a glycolipid biosurfactant produced by *Myrmekioderma dendyi*. Myrmekioside derivatives E-1, E-2 and E-3 displayed different anti-cancer activity against two human lung cancer cells, NSCLC-N6 and A549 [136]. The authors concluded that the difference between the cytotoxicity of myrmekiosides E-1, E-2 and E-3 was due to their different polarities.

Peracetylated myrmekioside E-2 has a liposoluble character, being this molecule more able to penetrate the lipid bilayer and cross more readily the cell membrane [136]. Liu et al. (2018) [116] isolated two new cyclopepetides, dolyemycins A and B from *Streptomyces griseus*. These molecules presented anti-proliferative activity against lung cancer A549 cells. The mechanism of action of these molecules on cancer cells was yet not presented [116].

4.3 Leukaemia

Leukaemia is a group of highly heterogeneous cancer types of the blood, characterized by the excessive cell proliferation of lymphoid or myeloid origin in the peripheral blood and bone marrow [137]. Leukaemia is sub-divided in four types: i) acute myeloid leukaemia; ii) acute lymphoblastic leukaemia; iii) chronic myeloid leukaemia, and iv) chronic lymphocytic. All of them are responsible for 8 % of all cancers and is the most common cancer in children [138]. Besides several options for leukaemia treatment such as chemotherapy, bone marrow transplantation, and radiation therapy, new approaches using biosurfactants have also been studied.

STLs produced by *Rhodococcus erythropolis* and MELs produced by *Candida antarctica* T-34, both glycolipid biosurfactants, were evaluated in human HL-60 promyelocytic leukaemia cells [139]. In this work, MELs and STLs have shown to markedly induce HL-60 cell differentiation towards granulocytes instead of cell proliferation, suggesting that the differentiation-inducing activity of MELs and STLs

are not due to a surfactant-like effect but due to a specific action on the plasma membrane [139]. In another work from the same research group, STL-1 from *R. erythropolis* SD-74 significantly inhibited human monocytoid leukaemia cell line U937 growth, and also induced its morphological changes by a monocyte-macrophage lineage [140]. The authors concluded that STL-1 presents low cytotoxicity for normal human cells [140]. STL-3 with saturated even-number or odd-number carbon chains, and unsaturated or halogenated fatty acids, induced the differentiation of human HL-60 promyelocytic leukaemia cell line [23]. The results proved that STL-3 and its analogues on HL-60 cells depended on the structure of the hydrophobic moiety of STL-3 [23].

The cyclic lipopeptide biosurfactant from *Bacillus natto* T-2 inhibited the growth of human leukaemia K562 cells by inducing apoptosis [118]. Surfactin cyclic lipopeptide induced apoptosis in human leukaemia K562 cells through Ca^{2+} regulating extracellular-related protein kinase ERK activation [141].

The anti-cancer potential of RLs was studied in blood human chronic myeloid leukaemia K562 cells [119]. The study showed an antiproliferation activity of cancer cells, without affecting healthy blood cells. This phenomenon was related to the stiffness of the cells, since K562 cells are characterized by a greater corticol membrane tension than healthy blood cells [23].

The lipopeptide iturin obtained from *B. subtilis* was evaluated for the treatment of chronic myelogenous leukaemia, using K562 cells [120]. The biosurfactant showed an anti-proliferative activity against the

cancer cells and acted via 3 pathways. Iturin inducted paraptosis in the presence of a caspase inhibitor, inhibited of autophagy progress, and induced apoptosis by causing ROS burst [120].

The use of serratamolide (serrawettin W1) as a chemotherapeutic agent against various cancer types was patented in 2005 by Tomas et al. (2005) [26]. The studies were based on acute human T cell leukaemia cells (jurkat clone E6-1) and peripheral blood acute human lymphoblastic leukaemia (Molt-4) [26]. This biosurfactant was found to induce apoptosis, reducing cancer cell viability, with no negative effects on healthy cell lines [26].

Chiewpattanakul et al. (2010) [28] discovered a new biosurfactant, monoolein, produced by the fungus *Exophiala dermatitidis*. In U937 leukaemia cell lines, this molecule presents an anti-proliferative activity, in addition to not showing toxic effects on healthy cells [28]. Monoolein acts by morphologically modifying the cell and its DNA, including cell shrinkage, membrane blebbing, and DNA fragmentation [28].

4.4 Melanoma

Melanoma is the most destructive form of skin cancer and advances in therapies are slow. This concern raises the need to discover new therapeutic agents. Some studies from the literature have been shown that biosurfactants induce apoptosis and growth arrest in melanoma tumour cells. The work of Zhao et al. (1999) [121] corresponds to the first evidence that MELs markedly inhibit the growth and apoptosis

of mouse melanoma B16 cells in a dose-dependent manner. In the following work of Zhao et al. (2000) [142], MELs were used as a potent inhibitor for the proliferation growth of mouse melanoma B16 due to the condensation of chromatin, DNA fragmentation, and sub-G1 arrest, thus inducing B16 cell apoptosis [142]. MELs from *C. Antarctica* induced cell differentiation by promoting apoptosis via condensation of chromatin, DNA fragmentation and sub-G1 arrest [22]. These findings indicate that MELs biosurfactants induce the expression of differentiation markers of melanoma cells, and the enhanced production of melanin, which is an indication that MELs triggered both apoptotic and cell differentiation programs [22, 103, 127].

Other biosurfactants with biological activity for melanoma cancer cells include pseudofactin I+I (PFII), a cyclic lipopeptide biosurfactant from *Pseudomonas fluorescens* BD5 [122]. This type of surfactant was used to investigate the effect of PFII on A375 melanoma cells [122]. Melanoma A375 cells exposed to PFII had an apoptotic death through DNA fragmentation. The authors concluded that melanoma A375 cell death was the consequence of plasma membrane permeabilization by the surfactant micelles [122]. Abdelli et al. (2019) [143] studied a surfactin produced by *Bacillus safensis* and its anti-cancer activity against B16F10 mouse melanoma cells (and T47D breast cancer cells). The results showed potential cytotoxic activity against both cell lines [143].

4.5 Colon cancer

Colon cancer normally starts from benign lesions, and due to the accumulation of DNA damage the lesion became malign [144]. Besides many efforts done to develop a more effective therapeutic, colon cancer still remains a major life-threatening malignancy [145]. Thus, novel effective therapeutic strategies for combating this cancer type are mandatory, in which biosurfactants may play a role. Three new cyclic acylpeptides (itaurin based-biosurfactants) named as mixirins A ($C_{48}H_{75}N_{12}O_{14}$ (18 unsaturations)), B ($C_{45}H_{69}N_{12}O_{14}$) and C ($C_{47}H_{73}N_{12}O_{14}$) produced by a *Bacillus* sp. were evaluated for anti-tumor activity in colon tumour cells (HCT-116) [104]. These mixirins are cyclic octapeptides, and comprise a mixture of L- and D-amino acid with an unusual amino alkanoic acid. Mixirins A, B and C demonstrated to be cytotoxic and inhibited the growth of human colon tumour cells (HCT-116), being the variant A the most effective [104]. Surfactin and fengycin isoforms lipopeptides produced by marine bacterium *B. circulans* DMS-2 presented a considerable and selective anti-proliferative activity against the human colon cancer cell lines HCT-15 and HT-29 [17]. The effect of surfactin from *B. subtilis* was evaluated on the anti-tumour activity of a human colon carcinoma cell line, LoVo cells [126]. Surfactin strongly inhibited the proliferation of LoVo cells through apoptosis induction, cell cycle arrest and survival signalling suppression on LoVo cells. In this work, the anti-pro-

liferative activity of this surfactin was mediated by inhibiting extracellular-related protein kinase and phosphoinositide 3-kinase/Akt activation, as assessed by phosphorylation levels [126].

A novel isoform of a marine lipopeptide biosurfactant was evaluated as an anti-cancer agent in human colon adenocarcinoma cell line HT-29 [123]. The results presented for the first time an anti-proliferative activity of biosurfactants in nanomolar concentrations by programmed cell death, and at the same time, with no antioxidant activity [123].

Serrawettin W2 produced by *S. surfactantfaciens* showed antitumour activity against CaCo-2 cell lines (human colon adenocarcinoma cells) [124]. This biosurfactant can suppress the growth of cancer cell lines, without negatively the viability of healthy cell lines [124]. Burgos-Díaz et al. (2013) [125] studied a new biosurfactant produced by *Sphingobacterium detergens* against CaCo-2 human colon cancer cells. The results showed an anti-proliferative effect and apoptosis activity on cancer cells. However, more studies are required to fully understand the apoptosis activity of this biosurfactant [125]. Finally, surfactin from *B. subtilis* exhibited an anti-proliferative effect in LoVo cells (human colon carcinoma cell line) by apoptosis induction, cell cycle arrest and survival signalling suppression [105].

5 Biosurfactants as Drug Delivery System (DDS)

A DDS is designed to induce the introduction of a therapeutic substance into the body, while improving its safety and efficacy [146, 147]. The two main characteristics of controlled DDSs are: i) an optimal drug loading capacity, which improves drug bioavailability and reach the target; ii) a controlled drug release [146, 147]. Since chemotherapy is limited by the anti-cancer drugs' poor penetration into tumour tissues, along with their severe side effects on healthy cells, novel biosurfactants-based DDS constituted by liposomes, niosomes, transferosomes and nanoparticles have been developed [12].

5.1 Liposomes

Liposomes are bilayered lipid vesicles useful for hydrophobic and hydrophilic drugs encapsulation, sustained drug release, degradation protection and therapeutic efficacy increase, and have low adverse effects. Among these, MEL-A was applied in order to improve gene transfection efficiency of a cationic liposome. MEL-liposome (MEL-L) composed of 3β-[N-(N′,N′-dimethylaminoethane)- carbamoyl] cholesterol (DC-Chol), dioleoyl phosphatidylethanolamine (DOPE) and MEL-A exhibited efficiency in DNA transfection into cells through increasing the lipoplexes association with the cells in serum [11, 148–150]. Apart from being described as an efficient vector for DNA transfection into cells, clinical trials with advanced melanoma patients by injection of DNA-liposome complexes into tumor nodules

occurred without complications in metastatic melanoma patients treated with catheter injection of DNA-liposomes into tumour masses were well tolerated, displaying the safety of therapeutic direct gene transfer in humans [151–155].

Maitani et al. (2006) [156] produced 300-nm-sized aggregated liposome-plasmid DNA (pDNA) complexes (lipoplexes) through the addition of biosurfactants, such as MEL-A and β-sitosterol β-D-glucoside (Sit-G), to cationic liposomes, which can be applied in intratumoral and intravenous injections. Sit-G-liposome exhibited potential as a vector in gene-based therapy, since it demonstrated low cytotoxicity and displayed high luciferase gene transfection efficiency in the presence of serum of human hepatoblastoma cell line (HepG2) cells [156, 157].

Overall, liposomes drug delivery has gathered attention in cancer therapy. In fact, liposomes became the first nanoparticles to reach clinical trials, mainly in breast cancer therapy [158]. Furthermore, triggered release liposomes have been introduced in order to guarantee successful treatments through an efficient and immediate drug release in tumor tissues due to: i) inner stimulants (pH and enzyme); and ii) outer stimulants (local heating, ultrasound, magnetic field, light) [158].

5.2 Niosomes

Niosomes are non-ionic biosurfactant bilayer vesicles formed by biosurfactants hydration, with or without mixtures of cholesterol or other

lipids, whose stability, low-cost, biodegradability, biocompatibility, non-immunogenicity, and structural characterization flexibility reinforce their potential as drug delivery vehicles [159]. Their amphiphilic nature is essential for encapsulating lipophilic or hydrophilic drugs, where the hydrophilic core is the ideal medium for incorporating hydrophilic drugs and hydrophobic drugs are predominantly confined to the lipid layer (Fig. 15) [159]. The properties of niosomes are adjustable by changing the vesicles' composition, surface charge, size, lamellarity, tapped volume and concentration. However, niosomes' stability depends on the biosurfactant type, encapsulated drug nature, storage temperature, detergents, membrane-spanning lipids use, interfacial polymerisation of surfactant monomers in situ, and charged molecule inclusion [159–161].

Wu et al. (2017) [25] revealed that surfactin could be included into nano-formulations, such as niosomes, since it effortlessly positions itself within the hydrophobic/hydrophilic core-shell structure of such nano-formulations due to its amphiphilic structure and surface-active properties [25]. Recently, Haque et al. (2017) [162] showed sophorolipids-based niosomes for amphotericin B (AmB) delivery against *Candida albicans* in a cost-effective way [162].

FIGURE 15

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5.3 Nanoparticles

Microbial biosurfactants are growing as exciting options for quick nanoparticles synthesis [163–166]. Kasture et al. (2008) [163] described silver nanoparticles synthesis using sophorolipids biosurfactants, as reducing and capping agents [163]. Reddy et al. (2009) [165, 167] showed that surfactin can be used as a stabilizing agent for silver and gold NPs synthesis [165, 167]. Palanisamy and Raichur (2009) [168] reported an eco-friendly alternative method, using RLs for microemulsion synthesis of spherical nickel oxide NPs [168]. Maity et al. (2011) [169] displayed a reverse microemulsion technique using surfactin as a green process for nanocrystalline brushite particles (nanospheres and nanorods) synthesis [169].

Surfactin combined with other chemotherapeutic drug can be loaded into nano-formulations, and used as an adjuvant in anti-cancer treatment [25]. Taking this into account, Huang et al. (2018) [170] took advantage of the anti-cancer drug doxorubicin (DOX) to develop DOX-loaded surfactin nanoparticles (DOX@SUR), which showed higher cytotoxicity against DOX-resistant human breast cancer MCF-7/ADR cells than free DOX, by exhibiting an increased cellular uptake and decreased cellular efflux due to inhibition of the P-glycoprotein expression [170]. Furthermore, DOX@SUR presented higher in vivo tumour inhibition and lower side effects in MCF-7/ADR-bearing nude mice [170]. Consequently, DOX@SUR displayed potential as an anti-cancer drug carrier to reverse multidrug resistance in cancer chemotherapy [170]. Therefore, surfactin nano-

formulations have significant potential in anti-cancer nanomedicine treatment. However, their full potential still remains unexplored [25].

6 Conclusions and future challenges

The ability of biosurfactants to act on cancer cells, without negatively affecting healthy cells, makes of their use as anti-cancer agents an excellent alternative to current treatments, especially when compared to chemotherapy. However, their application in this field is still a challenge. Firstly, the immensity of microorganisms and their metabolites leads to the continuous discovery of new biosurfactants, with the existing ones being unexplored in what concerns their potential of application. Secondly, only few studies were devoted to the understanding of the mechanisms of action of biosurfactants. Furthermore, some studies used semi-purified fractions with biosurfactants, affecting the interpretation of results and the understanding of the underlying mechanisms. Lastly, many of the existing studies are in the stage of *in vitro* testing with cell lines. In order to reach the *in vivo* stage, a significant amount of work is still required up to their final approval by the respective health regulatory agencies.

DDS using biosurfactants are an additional area with relevant therapeutic potential. Additional research on the interactions between DDS constituents and DDS interaction with cells is still required. Overall, this field is still in its infancy with a small number of works reported

up to date. However, given the promising results reported, this field of research will certainly increase in the following years.

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FIGURES AND TABLES

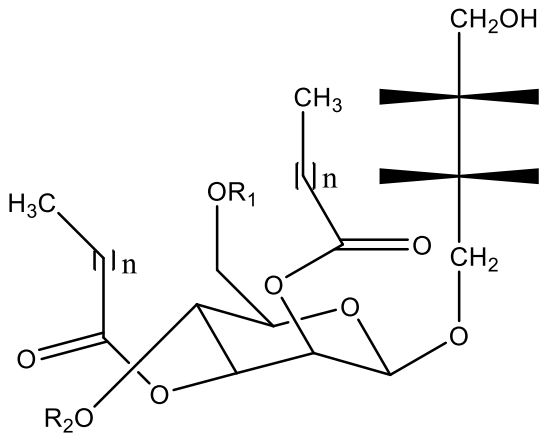


Fig. 1. Structure of mannosylerythritol lipids (MELs): MEL A: R_1 = R_2 = Ac; MEL-B R_1=Ac, R_2 = H; MEL-C: R_1 = H, R_2 = Ac; MEL-D: R_1 = H, R_2 = H. *n* =6-10. Adapted from Arutchelvi et al. (2008) [32].

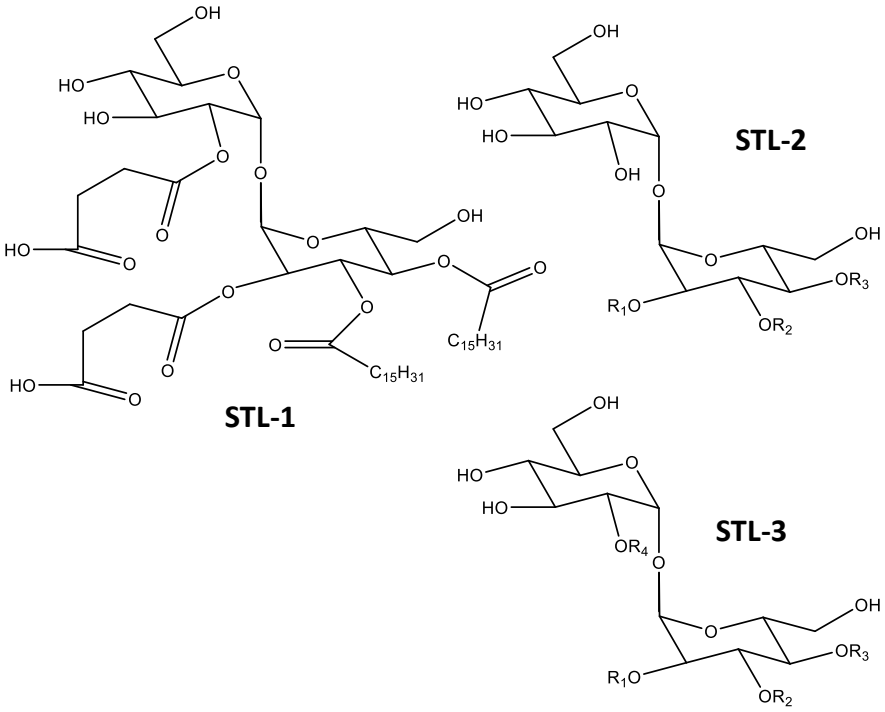


Fig. 2. Structures of succinoyl trehalose lipids (STLs): STL-2: R1-R3 = 1×succionyl + 2×alkanoyl; STL-3: R1-R4 = 1×succionyl + 3×alkanoyl. Adapted from Jana et al. (2017) [39].

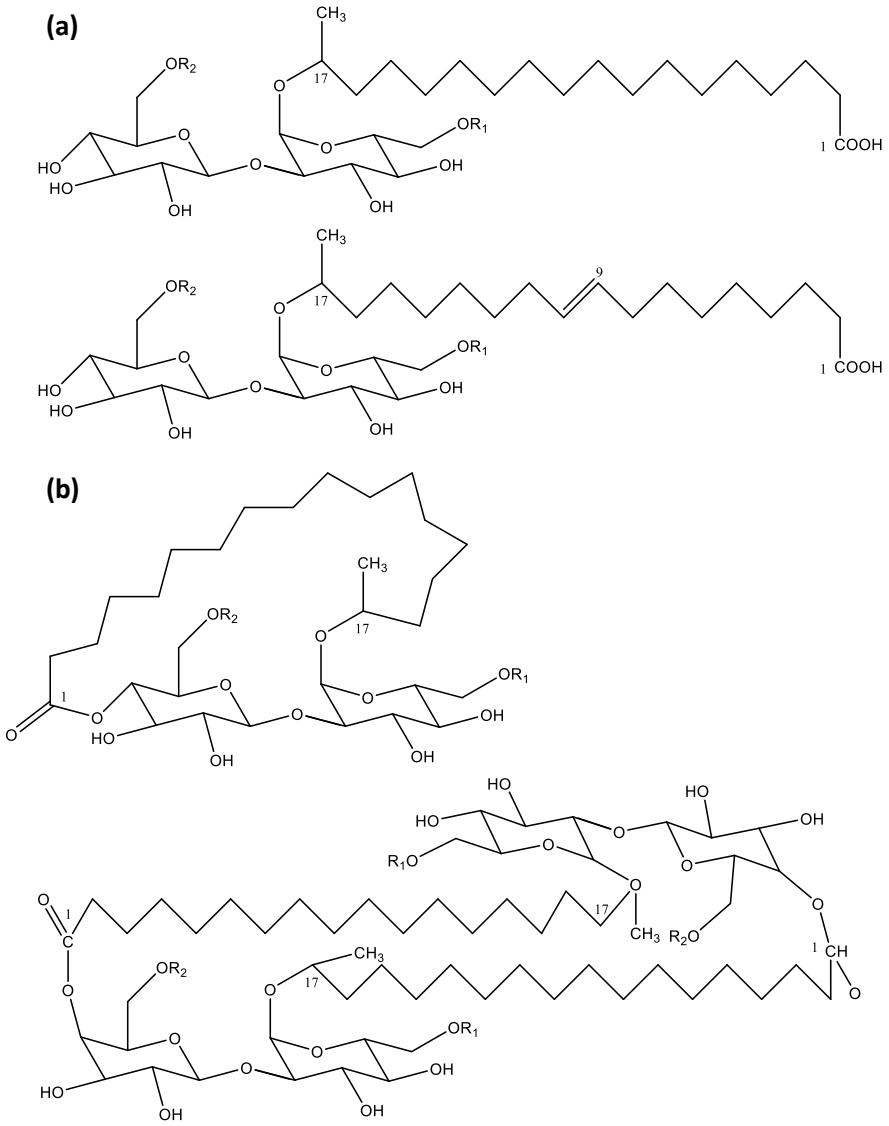


Fig. 3. Structures of sophorolipids in acid form (**a**) and in lactone form (**b**). Adapted from Kulakovskaya et al. (2014) [42].

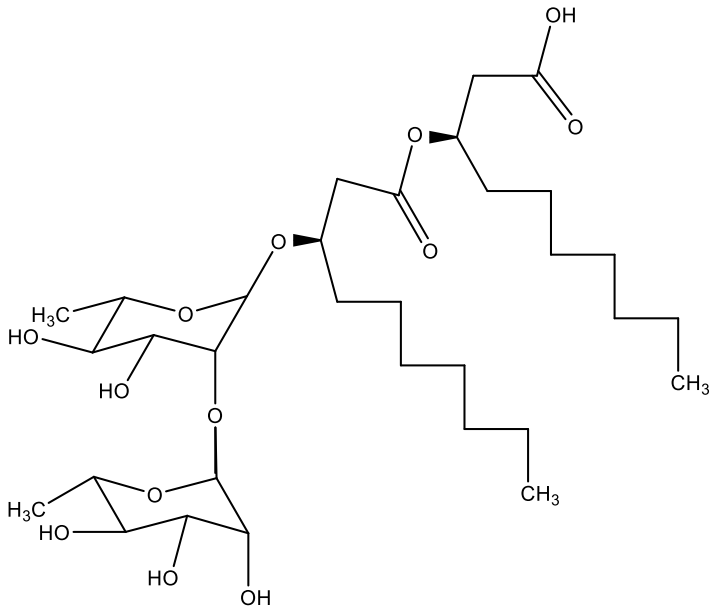


Fig. 4. Chemical structure of the first identified rhamnolipid; simply named as α-L-rhamnopyranosyl-α-L-rhamnopyranosyl-β-hydroxydeca-noyl- β-hydroxydecanoate and symbolized as Rha-Rha-C10-C10. Adapted from Abdel-Mawgoud et al. (2010) [45].

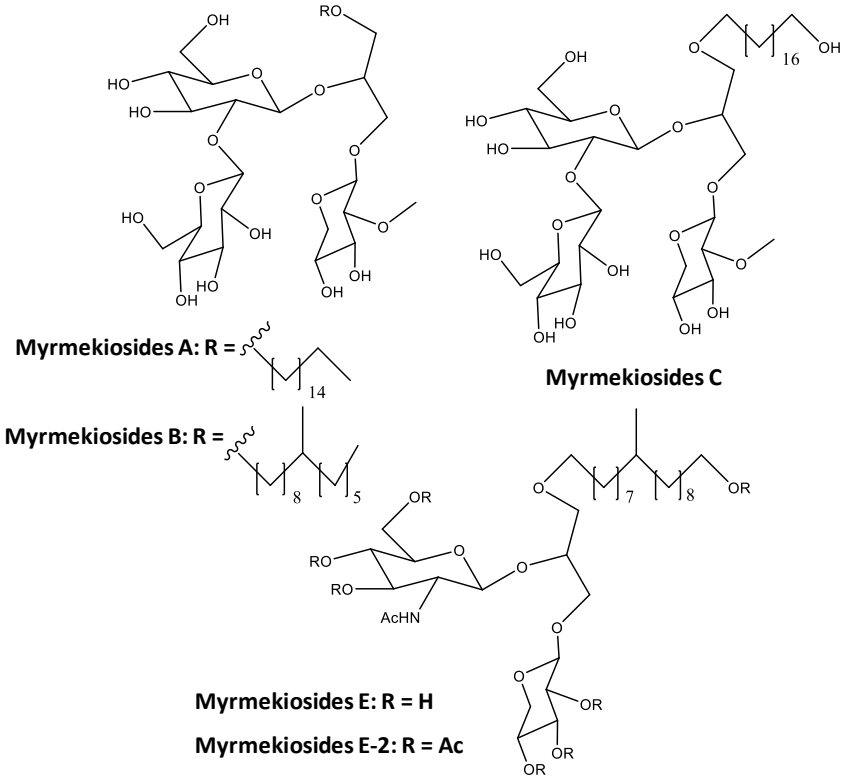


Fig. 5. Structure of myrmekiosides. Adapted from Zhang et al. (2015) [49].

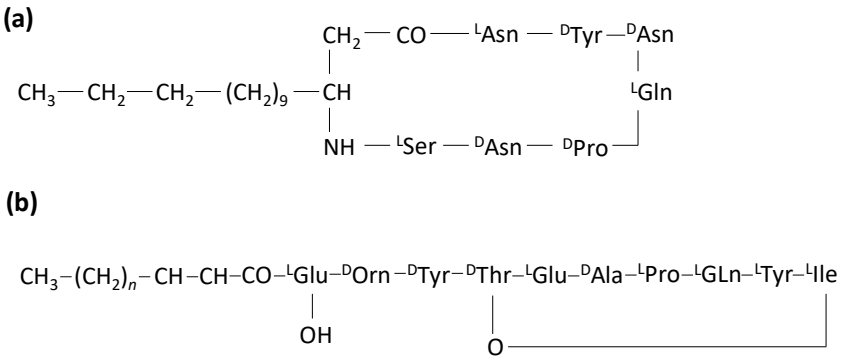


Fig. 6. Primary structure of iturin (**a**); Primary structure of fengycin, *n* = 14-17 (**b**). Adapted from Meena and Kanwar (2015) [54].

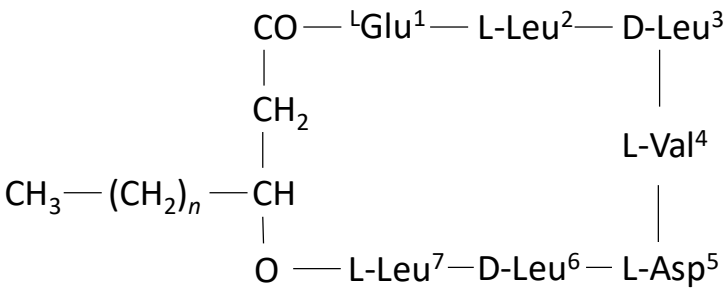


Fig. 7. Primary structure of surfactin, *n* = 9–11. Adapted from Wu et al. (2017) [25].

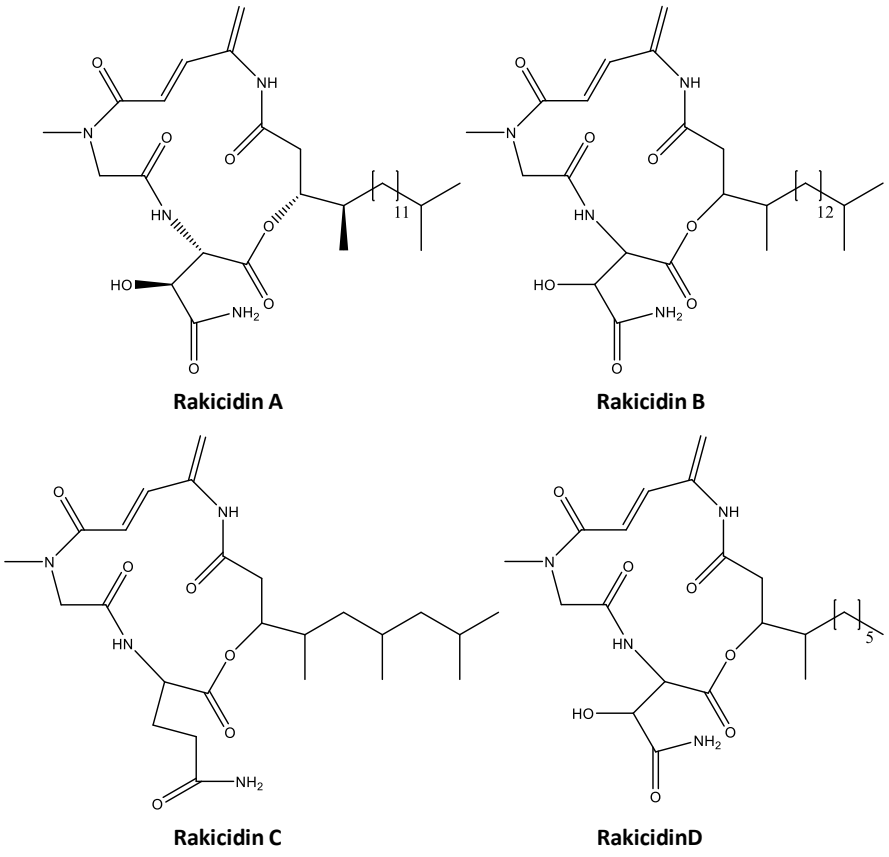


Fig. 8. Structure of rakicidins A, B, C, and D. Adapted from Sang et al. (2016) [62].

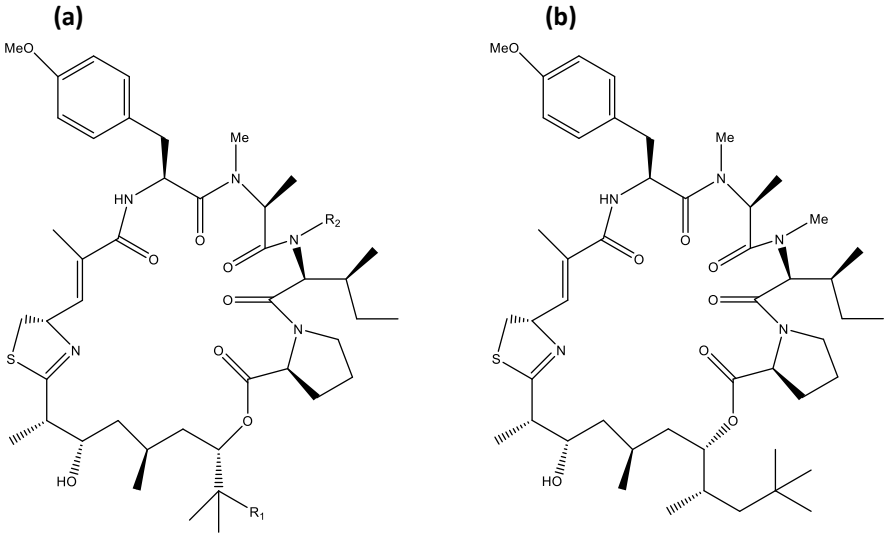


Fig. 9. Chemical structures of apratoxins: (**a**) apratoxin A: R_1 = Me, R_2 = Me; apratoxin B: R_1 = Me, R_2 = H; apratoxin C: R_1 = H, R_2 = Me; (**b**) apratoxin D. Adapted from Masuda et al. (2014) [63].

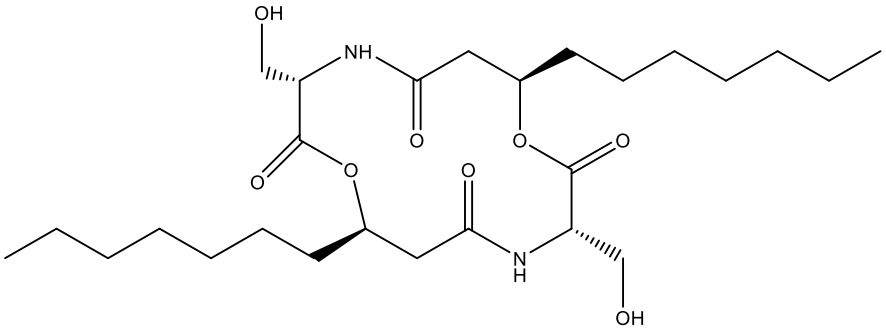


Fig. 10. Structure of serrawettin W1 (serratamolide A). Adapted from Shanks et al. (2012) [66].

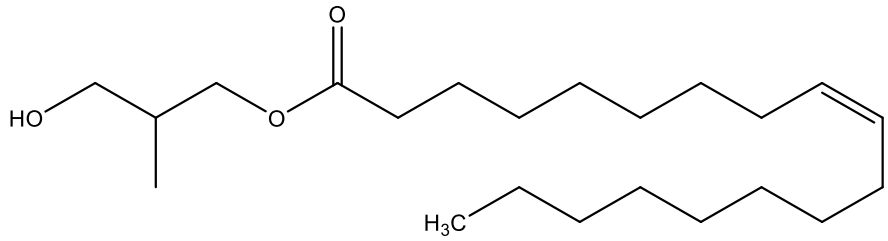
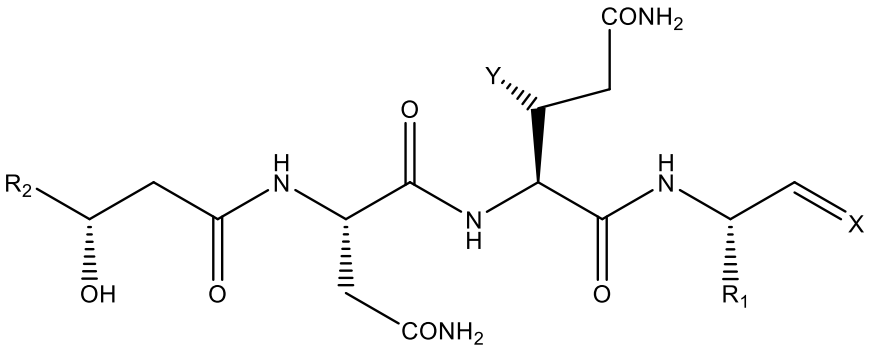


Fig. 11. Structure of monnolein. Adapted from Kulkarni et al. (2011) [67].



Fellutamide	R_1	R_2	X	Y
A	iBu	C_9H_{19}	O	OH
B	iBu	C_9H_{19}	O	H
C	iBu	C_9H_{19}	H, OH	H
D	iBu	$C_{11}H_{23}$	O	OH
E	iPr	$C_{11}H_{23}$	O	OH
F	iAmyl	C_9H_{19}	OH, OH	H

Fig. 12. Structure of fellutamides. Adapted from Pirrung et al. (2016) [70].

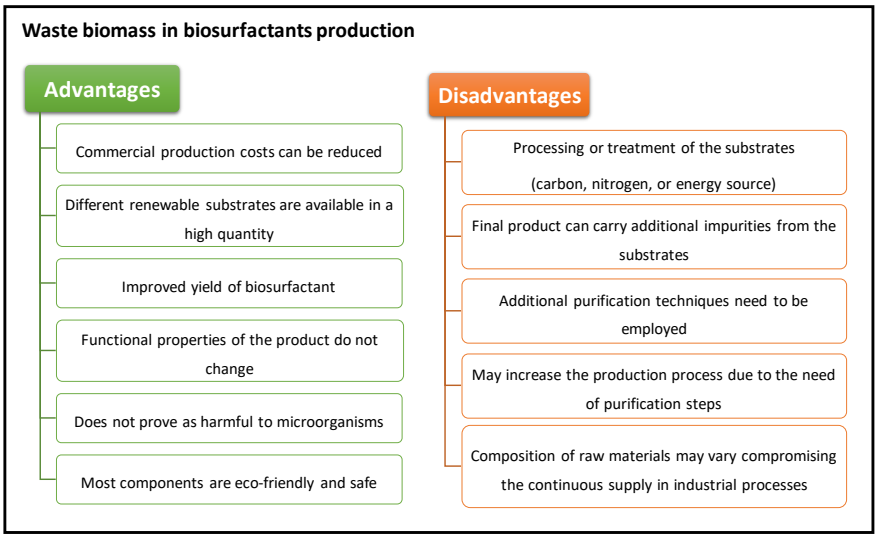


Fig. 13. Advantages and disadvantages associated with the use of waste biomass in biosurfactants production. Adapted from Banat et al. (2014) [75].

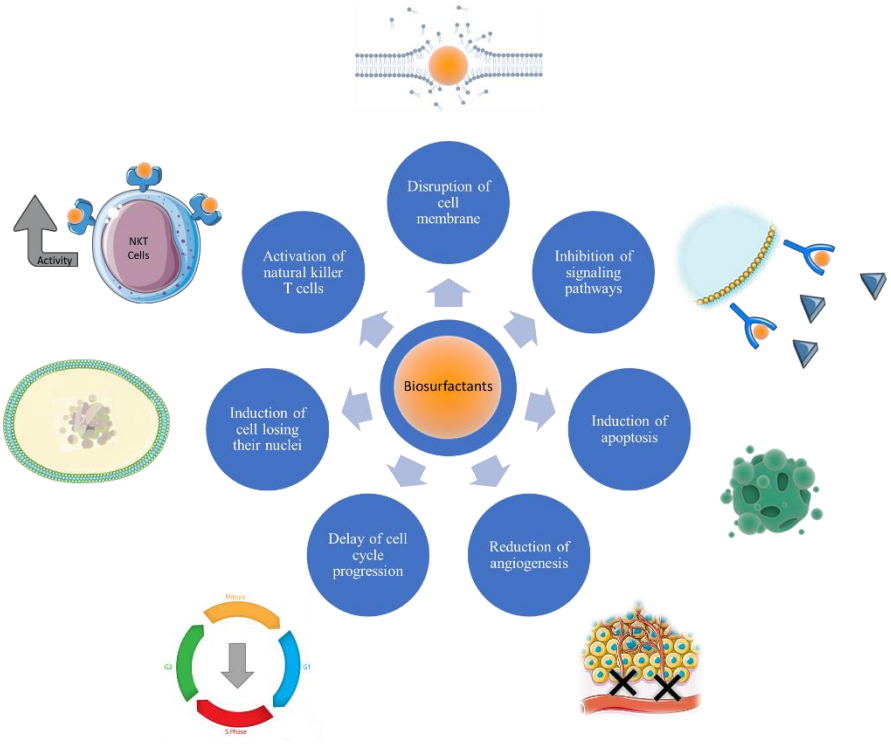


Fig. 14. Mechanisms to exemplify the anti-cancer activity of microbial biosurfactants.

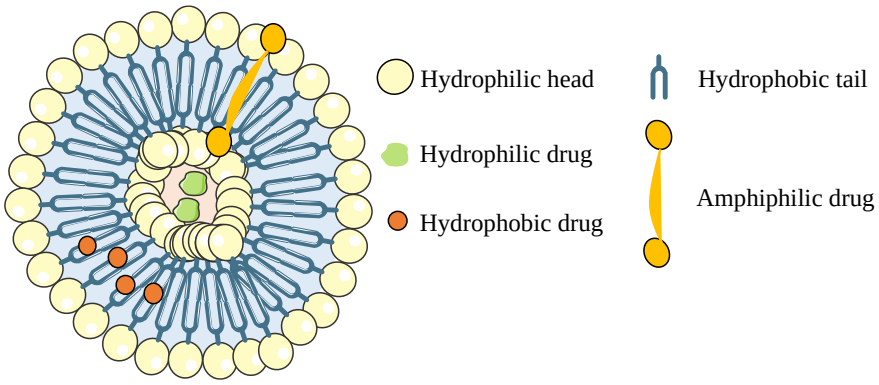


Fig. 15. Graphic illustration of a niosome.

Table 1 Antitumor activity of biosurfactants against cancer cells.

Cancer type	Biosurfactant	Activity	References
Breast cancer	Surfactin	Induces apoptosis, inhibits proliferation, reduces cell viability and induces cell cycle arrest at G1 phase	[99–101]
	BioEG from *Lactobacillus paracasei*	Induces cell cycle arrest at G1 phase	[95]
	Iturin from *Bacillus* sp.	Leads the apoptosis induction and inhibits tumour growth	[102–104]
	Sophorolipids from *Starmerella bombicola*	Interferes with cell migration and intracellular ROS increase	[24]
	RLs from *Pseudomonas aeruginosa*	Induces p53 gene	[105]
	Rakicidins from the *Micromonospora*	Interferes with the invasiveness	[106]
Lungs cancer	Surfactin	Induces cell cycle arrest at G0/G1 phase and induces apoptosis	[107]

	Somocystinamide A from *Lyngbya majuscula*	Induces apoptosis	[108]
	Glycolipoprotein from *Acinetobacter M6*	Decreases cell viability and induces cell cycle arrest at G1 phase	[109]
	Fellutamides from *Aspergillus versicolor*	Cytotoxic effects	[110, 111]
	Fengycin from *Bacillus subtilis*	Induces cell cycle arrest at the G0/G1 phase and promotes apoptosis	[112]
	Rakicidin B from *Micromonospora chalcea*	Induces apoptosis	[113]
	Apratoxins from *Moorea*	Cytotoxic effects	[114]
	Myrmekioside from *Myrmekioderma dendyi*	Cytotoxic effects	[115]
	Dolyemycins A and B	Anti-proliferative effects	[116]
Leukaemia	STL from *Rhodococcus erythropolis*	Induces cell differentiation, inhibit growth and induce morphological changes	[117]
	MEL from *Candida Antarctica*	Induces cell differentiation	[117]

	Cyclic lipopeptide from *Bacillus natto*	Inhibits cell growth by inducing apoptosis	[118]
	RLs	Antiproliferation	[119]
	Iturin from *Bacillus subtilis*	Paraptosis inducing, apoptosis, and inhibition of autophagy	[120]
	Serratamolide from *Serratia marcenses*	Induce apoptosis	[26]
	Monoolein from *Exophiala dermatitidis* SK80	Morphological cell changes such as cell shrinkage, membrane blebbing, and DNA fragmentation	[28]
Melanoma	MEL	Inhibits cell growth and induce apoptosis	[121]
	PFII from *Pseudomonas fluorescens*	Induce apoptosis	[122]
	MEL from *Candida Antarctica*	Induces cell differentiation by promoting apoptosis by the condensation of chromatin, DNA fragmentation and sub-G1 arrest	[23]

	Mixirins from *Bacillus* sp.	Cytotoxic effects and inhibits cancer growth	[104]
Colon cancer	Surfactin and fengycin from *B. circulans*	Selective anti-proliferative activity	[17]
	Marine lipopeptide	Anti-proliferative activity	[123]
	Rakicidins	Cytotoxic effects	
	Serrawettin W2 from *Serratia surfactantfaciens*	Selective suppresses the growth of cancer cell lines	[124]
	New molecule from *Sphingobacterium detergens*	Anti-proliferative effects and apoptosis activity	[125]
	Surfactin from *Bacillus subtilis*	Apoptosis induction, cell cycle arrest and survival signalling suppression	[126]

Viscosin from *Pseudomonas libanensis*	Inhibits migration of metastatic cells	[127]