Review Article

Methods of purification and characterization of biosurfactants: an overview

Abstract

Biosurfactants are amphiphilic molecules produced by several microorganisms including bacteria, filamentous fungi and yeasts. They are classified according to their chemical composition (glycolipids, lipopeptides, glycoproteins, glycolipopeptides, phospholipids) and their molecular weight (low and high molecular weight). Biosurfactants are currently used in several industrial fields (food industry, pharmacy, medicine, detergents, agriculture, cosmetics, oil recovery and bioremediation) owning to some of their specific properties such as their low toxicity, biocompatibility, biodegradability, environmentally-friendly, stability under extreme conditions (temperature, pH and salinity), structural diversity, production using renewable low-cost substrates, agreement with green chemistry and sustainability. Despite the properties of biosurfactants, there are few studies leading to their structural characterization. The most reported structures are from biosurfactants produced by *Bacillus* spp. (surfactins, fengycins, lichenysines) and *Pseudomonas* spp. (rhamnolipids). The majority of researches performed on biosurfactants emphasized their functional groups characterization. These biosurfactants have yet to be fully characterized at structural level. Hence, in this review, we highlight the different purification and structural characterization techniques which can be combined to provide information on the structure of the biosurfactants independently of its complexity. This will lead to enhance their application in some highly pointed industrial fields such as medicine and pharmacy.

Keywords: Biosurfactants, Microorganisms, Purification, Structural characterization.

Introduction

Biosurfactants are amphiphilic surface-active compounds produced by microorganisms from diverse sources (terrestrial, marine environments, sludge, etc.) including bacteria, molds and yeasts (Nataraj *et al*., 2021a; Mouafo *et al*., 2022). They are either excreted extracellularly or remain bound to the producing cells (Vallejo *et al*., 2021). They are synthetized by microorganisms through different metabolism routes depending of the nature and the composition of the substrates (Figure 1). Once the substrates are intake, some microorganisms produced enzymes that might combine the hydrophilic and hydrophobic components of the substrates leading to the formation of biosurfactants. The substrates can also be metabolized into coenzyme into acetyl coenzyme A, that will be used in *de novo* pathway as the precursor of the synthesis of sugars, lipids and proteins (Tokumoto *et al.*, 2009). Hence, the enzymatic combination of these hydrophilic (sugar, proteins) and hydrophobic (lipids) components synthetized by the microorganisms will lead to the biosurfactants. In some case, the hydrophilic (sugar, proteins) or hydrophobic (lipids) components synthetized by the microorganisms through *de novo* pathway can be combined with carbohydrates, proteins or lipids' components intake from substrates (Sydatk and Wagner, 1987). According to Desai and Banat (1997), microorganisms often used water insoluble substrates for the synthesis of the hydrophobic moiety of the biosurfactants and the water-soluble ones for the synthesis of the hydrophilic moiety. Biosurfactants are classified according to their chemical composition and their molecular weight. With regards to the chemical composition, they can be glycolipids

(rhamnolipids, Mannosylerythritol lipids, trehalolipids, xylolipids), lipopeptides (surfactins, viscosines, fengycins, lichenysines), glycolipopeptides, glycoproteins, phospholipids, neutral lipids, polymeric biosurfactants (emulsan, alasan, biodispersan), and particular biosurfactants (protein–sugar–lipid complex molecules) (Bergevin *et al*., 2017; Nitschke and Silva, 2018; Mouafo *et al*., 2022). Considering the molecular weight, biosurfactants are grouped as low molecular weight (glycolipids, lipopeptides, phospholipids) and high molecular weight (particular biosurfactants, polymeric biosurfactants) compounds (Smyth *et al*., 2014).

Several investigations on the potential replacements of synthetic surfactants by biosurfactants have been conducted this last decade. The main reasons that have impelled these investigations are their low toxicity, biodegradability, environmentally friendly, biocompatibility, structural diversity, stability under extreme conditions of temperature, salinity and pH, production using renewable low-cost substrates, agreement with green chemistry and sustainability (Marchant and Banat, 2012a; Marchant and Banat, 2012b; Mouafo *et al*., 2018a; Nitschke and Silva, 2018). These advantages of biosurfactants associated with their diverse properties (emulsifying, de-emulsifying, foaming, dispersing, wetting, solubilizing, antioxidant, antiadhesive, antimicrobial and antibiofilm agents) confer to these latter's, the ability to be applied in many industrial fields including, cosmetics, detergents, oil recovery and bioremediation, agriculture, pesticides, pharmaceuticals/medicines, and food industry (Nitschke and Silva, 2018; Silva *et al*., 2020; Patel *et al*., 2021; Mouafo and Somashekar, 2020; Mouafo *et al*., 2022). As examples, Wattoo *et al*. (2023) highlighted the application of biosurfactants as emulsifiers and antimicrobial agents in the stabilization and preservation of juices. Reshmy *et al.* (2023) reported the exploitation of the antioxidant properties of biosurfactants in reducing lipids oxidation and thus, improving the preservation of lipids-containing foods. The improvement in the shelf life of raw ground goat meat using biosurfactants was reported by Mouafo *et al.* (2020b). The

authors noticed a significant inhibition of microbial proliferation, lipids oxidation, proteins degradation, and color changes. Fookao *et al.* (2022) demonstrated the ability of biosurfactants to improve the texture and dough stability of milk bread. The beneficial effects of biosurfactants in enhancing the texture profile (Santoso *et al.*, 2023; Sharma and Arya, 2023) and sensory attributes (Rahaman *et al.*, 2023) of starchy foods were recently noticed. Numerous other recent applications of the different properties of biosurfactants in the food industry were summarized in the book of Inamuddin and Adetunji (2023). In view of improving the oral bioavailability of hydrophobic drugs at the target sites, the emulsification properties of biosurfactants were successfully exploited as drug delivery system (Kurozuka *et al.*, 2017; Ohadi *et al.*, 2020; Bjerk *et al.*, 2021). For different administration routes of drugs (intravenous, oral, ocular, nasal and topical), there is nowadays the formulation of microemulsion drug delivery systems with biosurfactants that insures their delivery to targets sites and significantly enhance their bioavailability (Ohadi *et al.*, 2020). Besides, there are also interesting reports highlighting the successful applications of biosurfactants for the bioremediation of heavy metals (Cd, Cu, and Pb) in contaminated soils (Sun *et al.*, 2021). All these industrial exploitations of biosurfactants justify the increasing demand of biosurfactants for which the markets reached 2210.5 million dollars by 2018 (Transparency Market Research, 2013) and the high number of companies (approximately 17) around the world, which currently produced biosurfactants (Randhawa and Rahman, 2014; Sekon and Rahman, 2014).

The activity and structure of biosurfactants which derived from the secondary metabolism of substrates by microorganisms, strictly depend on the producing strain, the medium composition, and the culture conditions (Mulligan *et al.*, 2014; Liu *et al.*, 2015). Although in the literature there are some studies on the full structural characterization of biosurfactants, the majority of these researches concerned lipopeptides' biosurfactants derived from *Bacillus*

spp. (surfactins, fengycins, utirins, lichenysins) and glycolipid biosurfactants derived from *Pseudomonas* spp. (rhamnolipids) (Joshi *et al*., 2016; Sharma *et al*., 2016; Abdalsadiq *et al*., 2018). For the other microorganisms, almost researches performed on biosurfactants emphasized their functional groups characterization (Lara *et al*., 2020; Nataraj *et al*., 2021a, Nataraj *et al*., 2021b; Sakr *et al*., 2021; Vallejo *et al*., 2021; Mouafo *et al*., 2022). These biosurfactants have yet to be fully characterized at structural level. The principal hurdles are the complex nature of some biosurfactants particularly those derived from lactic acid bacteria (Mouafo *et al*., 2022). Hence, several purification and characterization techniques are needed to obtain structural information on the molecule. In this review, we highlight the different purification techniques reported in the literature as well as the different colorimetric, spectrophotometric, chromatographic and spectrometric methods which can be combined to obtain the structure of the biosurfactants independently of its complexity. Relevant data used in this review were from several original articles, review articles, book chapters and books. They are were obtained from PubMed, EMBASE, Google Scholar, Scopus, Google, Web of Science and Google. The search terms were the combination of these four keywords: "biosurfactants", "techniques", "purification" and "structural characterization".

1. Purification techniques of biosurfactants

Several techniques were reported in the literature concerning the purification process of biosurfactants. These techniques included dialysis, membrane filtration, centrifugation, acid precipitation, thin layer chromatography and column chromatography. Currently, no defined commercial method is available for the purification of the biosurfactants. Table 1 summarizes the different methods of purification of biosurfactants according to the producing strain et the nature of the biosurfactant.

1.1. Centrifugation and ultracentrifugation

Biosurfactants extracted for culture media are complex mixture of several compounds. The centrifugation allows the separation of these compounds based on their density even tiny the difference can be. According to the size of biosurfactants, higher gravitational-force instruments or ultra-centrifuges are very often used. That technique has been widely used for purification of biosurfactants. A centrifugation at high speed (13,000×g, 4°C, 20 min) was used by Mouafo *et al.* (2020a) to separate the biosurfactants produced by *L. casei* subsp. casei TM1B from contaminants. A centrifugation at 10,000 rpm, 4 °C, 15 min was applied by Madhu and Paprulla (2013) in the purification process of biosurfactants. It is important to mention that centrifugation and ultracentrifugation are not used as lone purification techniques of biosurfactants. They are always used as a unit operation of a process that included several other unit operations

1.2. Precipitation

Almost biosurfactants are known for their low solubility under acidic conditions (Nelson et al., 2020). That property was exploited by several authors to purify biosurfactants from other contaminants. Madhu and Paprulla (2013) acidified the solution of crude biosurfactants to pH 2 for 18 h, and used acidic water (pH 2.0) to wash the precipitate in order to remove possible contaminants. The precipitation of biosurfactants was also achieved using ice-cold acetone (Bhosale *et al.*, 2019), chilled ethanol (Fookao *et al.*, 2021; Devale *et al.*, 2023) and ammonium sulphate (Vigneshwaran *et al.*, 2021).

1.3. Chromatography

Generally, biosurfactants are produced by microorganisms in association with other molecules. These molecules can be extracted together with biosurfactants following the extraction method chosen. Hence there is a need for purification and chromatography appears a one of the main methods. By the means of the mobile phase the mixture of compounds is flowing through the stationary phase and they are separated. The separation process is based

on the differential partitioning of compounds between the mobile and stationary phases. The most reported chromatographic methods for biosurfactants purification are column chromatography, thin layer chromatography (TLC) and reverse phase chromatography (Rodrigues *et al*., 2006; Sauvageau *et al*., 2012; Mouafo *et al.*, 2020a).

1.3.1. Thin layer chromatography

Based on the properties of the biosurfactants present in the mixture, a solvent is chosen. That solvent into which biosurfactants should be dissolved will move up the plate the spot of the compounds deposited at the bottom of the plate. Depending on the physical properties and the structure of compounds from the mixture, they will move up the plate or stays behind. Then, spots are visualized in UV light or after chemical treatment. The solvent systems generally used for purification of biosurfactants on TLC plates are: petroleum ether/diethyl ether/acetic acid, acetone/acetic acid/water, chloroform/methanol/water, ethyl acetate/methanol/water, chloroform/methanol/water/acetic acid (Sauvageau *et al.*, 2012; Banerjee and Ghosh, 2021; Meena *et al.*, 2021, Dabaghi *et al.*, 2023; Fernandes *et al*., 2023).

1.3.2. Column chromatography

That technique separates a complex mixture of biosurfactants based on their molecular weight, size and their affinity with the stationary phase. The stationary phases used are generally either silica gel (60–120 mesh) or sephadex, while mobile phase consists of a mixture of solvents with different polarities. Rodrigues *et al*. (2006) used an octyl Sepharose 4 FF Prep hydrophobic interaction column and a linear gradient of PBS buffer containing 1.0 M $(NH_4)_2SO_4$ for elution of the complex mixture of biosurfactants from *Streptococcus thermophilus* A. Thavasi *et al.* (2011) and Sharma and Saharan (2016) used silica a gel (60–120 mesh) column with a gradient solvent system (chloroform/methanol) starting from 20:1 to 2:1 (v/v). Reverse phase HPLC using a C-18 column with a gradient solvent system (trifluoroacetic acid/millipore water, 0.05:99.95 and TFA/millipore water/acetonitrile,

0.05:19.95:80) was applied by for purification of biosurfactants from *L. casei* subsp. casei TM1B (Mouafo *et al*., 2020a). A silica gel column (60 Mesh) with chloroform–methanol-water (65:25:4) as mobile phase was successfully used by Banerjee and Ghosh (2021) to purify biosurfactants from *Bacillus oceanisediminis* H2. A Sephadex G-25 column with the sodium phosphate buffer (20 mM; pH 7.5) as elution solvent was used by to purify biosurfactants from *B. velezensis* KLP2016 (Meena *et al*., 2021). Biosurfactants from *B. subtilis* LSFM-05 was purified on a silica gel column (0.03–0.07 mm, 60 A°) using the following solvent system at different polarities: chloroform, methanol, and an aqueous solution of 28% (v/v) ammonium hydroxide (de Faria *et al*., 2021). Vigneshwaran *et al.* (2021) purified biosurfactant from *Brevibacillus* sp. AVN13 using fast protein liquid chromatography.

However, in almost reported studies, the collected active fractions following column chromatography were pooled by the authors (Thavasi *et al.*, 2011; Sharma and Saharan, 2016). This might reduce the probability to discovery new biosurfactants.

1.4. Filtration

This method uses the difference in pressure between two sides of a special membrane to separate biosurfactants from other molecules according to their size. Depending on the membrane pores' size and the pressure, it can be microfiltration, ultrafiltration or nanofiltration (Satpute *et al*., 2010). The crude biosurfactants derived from *L. acidophilus* NCIM 2903 and *L. casei* subsp. casei TM1B was purified from contaminants using filtration through a 0.22 µm membrane pore size (Satpute *et al.*, 2019; Mouafo *et al.*, 2020). It is important to highlight that, filtration is always used as a unit operation included in a purification process that contains other purification techniques like TLC, column chromatography, etc.

1.5. Dialysis

As a good method for sensitive compounds, dialysis enable separation of biosurfactants from accompanying substances simultaneous extracted with biosurfactants such as salts. In the dialysis process, molecules driven by a differential concentration gradient, are transported through a semipermeable membrane based on their size. Hence, contaminants present in the crude biosurfactants will pass through the semipermeable membrane leading to the purification of this latter. In the purification process of biosurfactants, Vecino *et al.* (2017), Ghasemi *et al*. (2018), Satpute *et al.* (2019) and Vigneshwaran *et al.* (2021) used a membrane of molecular weight cutoff 6000–8000 Da to perform dialysis against double demineralized water. The process has led to the obtention of pure biosurfactants.

Globally, the choice of the purification techniques of biosurfactants depends on the producing strain, the culture media and the extraction method. There are some microorganisms that produce only one molecule of biosurfactant as major metabolism compound (*Bacillus* spp., *Pseudomonas* spp.). Depending on the production mode (extracellular or cell-bound), the biosurfactants is extracted from the culture media. The great challenge in the purification process is to separate the biosurfactants from the culture media or cells' components which can be extracted together with the biosurfactants. However, other microorganisms (lactic acid bacteria) produced complex mixture of molecules with different features as biosurfactants. In this case, the choice of the purification technique becomes difficult and requests that several techniques should be used with special care to the screening of desired activity during the process. Hence, at least three different techniques should be used in combination with TLC or column chromatography to strengthen the purity of the biosurfactants (Mouafo *et al*., 2022).

2. Physicochemical characterization of biosurfactants

2.1. Identification of ionic properties of biosurfactants

The different types of biosurfactants vary according to their biochemical composition. Based on their large structural diversity, the biosurfactants might be charged or not. This has led to the classification of biosurfactants depending on their ionic properties. Hence, the different classes are: anionic, cationic, amphoteric and non-ionic. The CTAB agar method was developed and was generally used to detect the production of biosurfactants of glycolipidic nature (Satpute *et al.*, 2008). The method is based on the fact that the biosurfactants can form an insoluble ion pair (precipitation lines) with the cationic cetyl trimethyl ammonium bromide (CTAB) or anionic sodium dodecyl sulfate (SDS). Methylene blue is sometimes added in the culture media to ease the observation of the precipitation line materialized as a blue halo around well containing culture (Satpute *et al.*, 2008). This method was successfully used to confirm the anionic nature of biosurfactants derived from *Lactococcus lactis* (Saravanakumari and Mani 2010) *E. faecium* (Sharma *et al*., 2015), and *Pseudomonas guguanensis* strain Iraqi ZG.K.M (Faisal *et al.*, 2023). However, the method is not suitable for other types of biosurfactants such as lipopeptides or glycoproteins.

2.2. Color of biosurfactants

The color of biosurfactants generally varies according to the producing strain, the culture media composition and the extraction technique. The colors reported in the literature were either whitish, yellowish or brownish (Faisal *et al*., 2023). Fig. 2 presents the color of biosurfactants produced by some lactobacilli strains (Mouafo, 2019).

2.3. Identification of chemical nature

2.3.1. Screening of proteins

The reaction of ninhydrin and amino acids is used to screen the presence of proteins in biosurfactants. After TLC plates development, plates are dried, sprayed with ninhydrin solution and kept at 90°C for 30 min. Appearance of pink or red spots indicates the presence

of amino acids and thus reveals the proteinaceous nature of the biosurfactants (Banerjee and Ghosh, 2021; Faisal *et al*., 2023).

2.3.2. Screening of sugars

At high temperature and in presence of α-naphthol and sulfuric acid, carbohydrates reacted, leading to the formation of a pink complex. That reaction was exploited to screen the presence of carbohydrates in biosurfactants. Plates obtained following TLC development of complex mixture of biosurfactants are sprayed with α-naphthol solution and concentrated sulfuric acid and heated at 100°C for 5 min. Appearance of pink spots indicates the presence of carbohydrates in biosurfactants (Satpute *et al*., 2010). That method was applied directly on a solution of pure biosurfactants by Atta *et al.* (2009). The authors confirmed the presence of carbohydrates in the biosurfactants after the apparition of a violet or purple colour between the two layers formed by the solution of biosurfactants and the reagents. Sen *et al*. (2017) reported that the TLC plates can be sprayed with anthrone reagent for detection of sugars while Satpute *et al.* (2019) noticed that sugars detection can be assessed through TLC plates spraying with diphenylamine. In the studies of Reddy *et al.* (2016), orcinol reagent was sprayed on TLC plates to detect the presence of sugars in biosurfactants.

2.3.3. Screening of lipids

The presence of lipids in biosurfactants is generally assessed on TLC plates. The principle is based on the reaction between iodine vapors and lipids in a close chamber that leads to the development of a yellow color (Sen *et al.*, 2017; Faisal *et al*., 2023). The method is not specific as a positive result might indicate the presence of neutral or polar lipids (Atta *et al*., 2009). According to de Faria *et al.* (2021) and Meena *et al*. (2021), the presence of lipids in biosurfactants can be assessed through the spraying of TLC plates with water followed with

drying. Appearance of white spots on the TLC plates indicates the lipophilic nature of the biosurfactants.

2.3.4. Screening of phosphate groups

Some biosurfactants were reported as containing phosphate groups in their constitution (Rodrigues *et al.*, 2006). A rapid method to assess phospholipids in biosurfactants was developed by Okpokwasili and Ibiene (2006). In that method, a solution of biosurfactants is mixed with nitric acid 6M, the mixture is heated at 70°C for 30 min and a solution of ammonium molybdate 5% (w/v) is added drop by drop. Apparition of a yellow color and formation of a yellow precipitate at the bottom of the tube indicate the presence of phospholipids.

2.4. Identification of the chemical composition

2.4.1. Protein content

In the literature, the protein content of biosurfactants was quantified through different methods. The dye-binding method of Bradford (1976) was used by Ghasemi *et al*. (2019) and Behzadnia *et al*. (2020) to assess the protein content of biosurfactants. In that method, the Coomassie G-250 dye is bound to proteins leading to the formation of a blue complex that absorbs at 595 nm. The dye-binding method of Bradford is relatively rapid and suitable for determination of protein content of biosurfactants with high molecular weight (Smyth *et al.*, 2010). In the study of Morais *et al*. (2017), the method of Lowry *et al*. (1951) was used to quantify protein in biosurfactants. That method combines the Biuret reagent (used to assess protein through the presence of peptide bonds) and the Folin-Ciocalteau phenol reagent (used to assess the residues of tryptophan and tyrosine). The intensity of the blue colored complex developed is measured spectrophotometrically at 660 nm. The Kjeldahl method (AOAC, 1990) was used by Mouafo *et al.* (2018b) to assess the protein content of biosurfactants

derived from three lactobacilli strains. In that method, biosurfactant was digested with a strong acid leading to the release of nitrogen which was titrated. The nitrogen content was then converted to protein content using an appropriate conversion factor.

2.4.2. Lipid content

Lipids are one of the major constituents of biosurfactants. They are mostly responsible for the amphiphilic nature of biosurfactants. The lipid contents of biosurfactants were assessed using the method of Folch *et al*. (1957) by Ferreira *et al.* (2017) and Ghasemi *et al*. (2019) [57]. That method based on the solubility of lipids in solvents of different polarities. When solvent is heated, it evaporated, passed through the sample, extracted lipids and carried these later into the flask where they can be quantified after solvent evaporation. However, as lipids are always complexed in biosurfactants with proteins (lipoproteins), carbohydrates (glycolipids) or the association of proteins and carbohydrates (glycolipoproteins) (Mouafo *et al.*, 2022), the most suitable method to assess their content required to break the bond that hold lipids prior solvent extraction. Generally, hydrolysis with HCl 3N at 100°C for 1 h is used to release these bound lipids (Morais *et al*., 2017).

2.4.3. Sugar content

Sugars are important elements for the composition of some types of biosurfactants such as glycolipids, glycoproteins, and glycolipoproteins. They can be simple sugars, oligosaccharides, or polysaccharides. The most reliable method widely used in the literature to assess the sugar content of biosurfactants is the phenol–sulfuric acid of Dubois *et al*. (1956). In that method, hydrolyzed saccharides of biosurfactants are dehydrated into furfural derivatives while reacting with concentrated sulfuric acid. The furfural derivatives obtained reacted with phenol to form a colored complex that absorb light at 490 nm. The phenol–sulfuric acid was successfully used by Ferreira *et al.* (2017), Mouafo *et al*. (2018a), Mouafo *et*

315 *al.* (2018b), Behzadnia *et al*. (2020), Mouafo *et al*. (2020a) and Devale *et al*. (2023) while
316 identifying the chemical nature of biosurfactants.

317 2.5. Elemental composition of biosurfactants

318 Element analysis provides information that can be used to determine the chemical nature and
319 structure of biosurfactants. The elemental composition of biosurfactants was assessed in the
320 literature with different equipment. Rodrigues *et al.* (2006), used X-ray photoelectron
321 spectroscopy (XPS) to determine the percentage of C, N, O and P in biosurfactants from
322 *Streptococcus thermophilus* A. Based on the higher amount of C and O, the authors concluded
323 on the glycolipid nature of the biosurfactants. The energy dispersive X-ray was successfully
324 used by Habib *et al.* (2023) to assess the elemental composition of biosurfactants from *P.*
325 *benzoelyticum* Pb4, *Bacillus albus* S2i and *Proteus mirabilis* Th1. Ferreira *et al*. (2017), and
326 Vecino *et al.* (2017), used a Carlo Erba EA-1108CHNS-O element analyzer to determine the
327 percentage of C, N, H and S in the biosurfactants from *L. paracasei*. The authors reported
328 respectively the glycoprotein and glycolipopeptide natures of biosurfactants based on their C,
329 N, H and S contents. The glycolipoprotein nature of biosurfactants derived from *L. paracasei*
330 subsp. tolerans N2 was identified by Mouafo *et al.* (2018b) using a Vario EL III elemental
331 analyzer associated with biochemical analyses.

332 **3. Identification of functional groups of biosurfactants**

333 *3.1. FTIR spectroscopy*

334 Fourier transform infrared spectroscopy (FTIR) is a method widely used for the
335 characterization of biosurfactants. It generally leads to determine the chemical nature of
336 biosurfactants based on the functional groups and chemical bonds that are present in the
337 biosurfactants (Jemil *et al*., 2017). In the method, biosurfactants are submitted to radiation
338 ranging from 400 cm^{-1} to 4000 cm^{-1}. When the radiation frequency is close to the resonance

frequency of the biosurfactants that is analyzed, there is absorption of the luminous energy. A decrease of the transmitted energy is then observed. The absorption bands obtained that vary according to chemical bonds and functional groups of constituent elements of the biosurfactants are characteristic of the studied biosurfactants. FTIR was used by several authors to determine the chemical nature of biosurfactants (Morais *et al*., 2017; Vecino *et al*., 2017; Mouafo *et al*., 2018b; Mouafo *et al*., 2020a; Banerjee and Ghosh, 2021; Dabaghi *et al*., 2023; Faisal *et al*., 2023; Habib *et al*., 2023). It is generally performed by 32 scans of the compounds at a spectral resolution of 4 cm^{-1} and a wave number accuracy between 400 and 4,000 cm^{-1}. Background reference used is always potassium bromide pellets.

3.2. Ultraviolet spectroscopy

Several microorganisms produced biosurfactants thus leading to a broad structural diversity. They are mixture of lipids (saturated or unsaturated fatty acids), proteins and sugars that possess several kinds of bonds which can absorb ultraviolet (UV) light in the range of 100 to 800 nm (Wilcox and Wilcox, 1995; Sakr et al., 2021). The ultraviolet spectroscopy method was used by Dehghan-Noudeh *et al.* (2005) and Ismail *et al.* (2013) to identify the lipopeptidic nature of biosurfactants produced by *Bacillus* sp. Authors reported peaks at 215 nm corresponding to α and β-unsaturated ketones, peaks at 260 nm corresponding to aromatic rings thus indicating the presence of aromatic amino acids. Based on these information's, they concluded on the lipopeptidic nature of the biosurfactants. Sakr *et al.* (2021) reported that biosurfactants from *L. plantarum* 60FHE, *L. paracasei* 75FHE, and *L. paracasei* 77FHE absorbed in the far UV region (270-277 nm) and were all glycolipopeptides.

3.3. Fatty acids profile of biosurfactants

The structural diversity of biosurfactants leads to a great variation in their lipid moiety. To obtain detailed structural information on that lipid moiety, the different fatty acid entering in

their constitution should be identified. The most suitable methods for that are GC-FID (gas chromatography coupled with flame ionization detection) and GC-MS (gas chromatography coupled mass spectrometry). In these methods, the lipid moiety of biosurfactants is prior separated from the other moiety through a hydrolytic process that will cleave the link (Smyth *et al*., 2010). The fatty acids obtained are transformed into a volatile derivate (fatty acid methyl esters) which will be easily analyzed in GC-FID using standards or in GC-MS. Fatty acid methyl esters are separated according to their chain length, and their molecular mass registered provide information on their structures. The fatty acids profile of biosurfactants were determined by several authors in the literature using GC-MS (Sharma *et al*., 2015; Morais *et al*., 2017; Vecino *et al*., 2017; Behzadnia *et al*., 2020; De Gregorio *et al*., 2020; Mouafo *et al*., 2020a ; Faisal *et al*., 2023).

3.4. Monosaccharide's profile of biosurfactants

The sugar moiety of biosurfactants is always made of monosaccharides bound through glycosidic links. To identify these monosaccharides, the first step is the separation of the sugar moiety of the biosurfactants from the other moiety which could be lipids, proteins, or both depending on the nature of the biosurfactants. That separation is generally carried out through hydrolysis trifluoroacetic in a sealed tube at 120°C for 4-6 h. Then, the mixture is neutralized with 2 M NH_4OH, reduced with an aqueous solution of sodium borohydride ($NaBH_4$) and then, the sugars are transformed into volatile derivates (N-trimethylsilylimidazole, alditol acetates). The obtained derivates are injected in GC-MS which will provide structural information. The sugar moieties of biosurfactants were successfully identified by Sauvageau *et al*. (2012), Morais *et al*. (2017) and Mouafo *et al*. (2020a) using GC-MS.

3.5. Amino acids profile of biosurfactants

3.5.1. Amino acids profile

Amino acids are constitutive units of peptides that entering into the composition of several class of biosurfactants such as lipopeptides, glycopeptides and glycolipopeptides. The peptide moiety of biosurfactants is linked to other compounds through amide and lactone bonds (for fatty acids chain) or N-glycosidic and O-glycosidic bonds (for sugars) (Joshi *et al.*, 2016). Prior identification of the amino acids of the peptide moiety of the biosurfactants, these bonds should be cloven. That cleavage generally occurs after hydrolysis at high temperature (110°C) with concentrated HCl (6 N) for approximately 24 h in sealed tubes. The amino acid obtained can be analyzed by GC-MS after conversion into volatile derivatives (trimethylsilylation). That method provides information on the amino acid composition of the biosurfactants based on the retention times and masses (m/z). GC-MS was successfully used by You et al. (2015) to identify four amino acids (Val, Leu, Asp and Glu) in the lipopeptide from *Enterobacter* sp. N18 after acid hydrolysis and trimethylsilylation. However, that method does not provide information on the sequence of amino acids.

3.5.2. Amino acid sequence

Amino acids sequence of biosurfactants can be assessed using Edman degradation or Quadruple-time-of-flight tandem mass spectrum (Q-TOF MS/MS). Edman degradation is the earlier method reported in the literature to elucidate the composition and sequence of amino acids of biosurfactants (Smyth *et al.*, 2014). The method was mostly applied for lipopeptides. In that method, the biosurfactant is hydrolyzed and the smaller cleaved peptides are submitted to Edman degradation. Mild alkaline hydrolysis is generally applied to open the ring of lipopeptides. The Edman degradation method required peptides from the biosurfactants to be purified to homogeneity (Smyth *et al*., 2014). The Edman degradation process is conducted in an automated sequencers and the cleaved amino acids are submitted to chromatography. The

retention time of the cleaved amino acid is compared with the one of amino acid standards for identification (Zachara and Gooley, 2000).

Opposite to the Edman degradation technique, Q-TOF MS/MS is applied directly on the entire biosurfactants without a preliminary hydrolysis step (You *et al.*, 2015). In that method, the initial mass (m/z) of the biosurfactants is determined. Then, amino acids from biosurfactants are lost sequentially leading to a decrease of its m/z after MS/MS fragmentation. The m/z lost corresponds to the mass of the amino acid eliminated. The C-term amino acid is often identified using the double hydrogen transfer mechanism of Yang et al. (2006). In that mechanism, the m/z of the C-term amino acid is increased of m/z value of 18 which correspond to the m/z of water. Direct infusion in the mass spectrometer or application of liquid chromatography (LC, HPLC, UPLC) leading to separation of individual peptides before mass spectrometer analysis can be used. The method is less time consuming and required small volume of samples (Smyth *et al.*, 2014). Korenblum *et al*. (2012) used Q-TOF MS/MS to identify Glu-Leu-Leu-Val-Asp-Leu-Leu as the amino acid sequence of biosurfactants produced by *Bacillus* sp. H2O-1. The peptide sequence Gly–Ser–Thr–Leu–Leu–Ser–Leu–Leu was identified from the biosurfactants produced by *P. fluorescens*. BD5 using MALDI TOF/TOF mass spectrometry and MS/MS fragmentation (Janek *et al.*, 2010). The same method was used by Pereira et al. (2013) to identify the sequence of the heptapeptide moiety (L-Glu-L-Leu-D-Leu-L-Val-L-Asp-D-Leu-L-Leu) of the biosurfactants from three strains of *B. subtilis*. Fragments indicating the sequential losses of the amino acid residues Leu/Leu/Asp/Val/Leu/Leu/Glu-OMe was identified in the biosurfactants from *B. subtilis* LSFM-05 by de Faria et al. (2011) while using Fourier transform ion cyclotron resonance mass spectrometry with electrospray ionization (ESI-FTICR-MS).

4. Molecular weight of biosurfactants

The method commonly used to assess the molecular weight of biosurfactants is Sodium Dodecyl Sulphate Polyacrylamide Gel Electrophoresis (SDS-PAGE). The method is recommended for high molecular weight biosurfactants (Smyth *et al*., 2010). In that method, biosurfactants are separated in a gel load with a reducing buffer and SDS. They will be separated according to their molecular mass (Toren *et al*., 2001). Fouad *et al*. (2010) have successfully determined the molecular weight of surlactin (a glycolipid biosurfactants) produced by *L. acidophilus* using SDS-PAGE. The molecular weight of the protein fraction of biosurfactants derived *L. acidophilus* NCIM 2903 was also determined by Satpute *et al*. (2019) using SDS-PAGE. Tricine-SDS-PAGE was performed by Vigneshwaran *et al*. (2021) to identify the molecular mass the biosurfactant *Brevibacillus* sp. AVN13

5. Mass spectrum of biosurfactants

Mass spectrometers provide information on the structure of biosurfactants through an identification of all constitutive elements of the molecule. The method identifies both the moieties of the biosurfactants without a preliminary hydrolysis. The MS spectra provides information on the mass (m/z) of the protonated molecules $[M+H]^+$ of and their sodium $[M+Na]^+$ or potassium $[M+K]^+$adducts. During MS/MS fragmentation, constitutive elements of the biosurfactants are released and identified based on the m/z values. The direct infusion to the mass spectrometer and injection into liquid chromatography (LC) connected to a MS detector system, so as 90% of each fraction is collected and 10% sent to MS detector, are generally used. The only condition is the purity of the biosurfactants. For this purpose, it is always recommended to perform and HLPC-UV or HPLC-DAD to confirm the purify of the compound with the appearance of a well-constructed and differentiated peaks. These peaks correspond to pure fractions. HPLC-ESI-MS, LC-MS/MS, Tandem MS/MS, HRMS and other mass spectrometry techniques were used by several authors to determine the structure of

biosurfactants (de Faria *et al*., 2011; Janek *et al.*, 2013; Pereira *et al*., 2013; You *et al.*, 2015; Oluwaseun *et al*., 2017; Abdalsadiq *et al*., 2018; Adu *et al.*, 2023).

6. Nuclear magnetic resonance of biosurfactants

NMR is an analytic technique that consists to measure the absorption of radio frequencies by atoms of biosurfactants submitted to a magnetic field. That magnetic field allows the resonance of atoms present in the molecule. The different resonance frequencies of the atoms are consigned on a graph which permits to determine the structure of the molecule based on chemical shifts (Abdel-Mawgoud *et al*., 2011). Briefly, NMR required a principal static magnetic field and a magnetic field that oscillates in the field of radio frequencies (10^6-10^7 Hz). Sample is dissolved in solvents (deuterated chloroform, methanol pyridine, acetic acid, dimethyl sulfoxide acetone, benzene) and introduced into an induction coil that generates an oscillating magnetic field. Depending on the nucleus present in the sample and the value of the principal static magnetic field, the sample will absorb and reemit energy in a particular way and at a precise frequency named resonance. Some recent NMR spectrometers are equipped with triple resonance helium-cooled TCI cryoprobe (^{1}H, ^{13}C and ^{15}N or ^{31}P) and provide information on the chemical environment of atoms of H, C, N, and P within the biosurfactants (Hamerly *et al*., 2015).

Proton and carbon NMR are amongst the most used techniques for structure elucidation of biosurfactants. It was used by several authors independently of the biosurfactants producing strain (Monteiro *et al*., 2007; Sauvageau *et al*., 2012; Pereira *et al*., 2013; Sharma *et al.*, 2015 ; Oluwaseun *et al*., 2017; Mouafo *et al*., 2020 ; Dabaghi *et al*., 2023). In the process of identifying the exact position of constitutive atoms (carbons, hydrogen, nitrogen, phosphorus) of the biosurfactants with respect of their chemical environment, two dimensions NMR (COSY, HMBC, ROSY, and HSQC) is often used to strengthen information provided by one dimension ^{1}H and ^{13}C NMR (Sauvageau *et al*., 2012).

7. Successful structure elucidation of biosurfactants.

Successful characterization leading to the proposition of a chemical structure to biosurfactants was achieved by some authors in the literature. Globally, the combination of at least two techniques depending on the biosurfactants and the producing strains, was required.

Nelson *et al.* (2020) used HPLC coupled to high-resolution electrospray ionization mass spectrometry to characterize the biosurfactants from *L. sakei* JN-185, *L. fermentum* JN-119 and *L. plantarum* JN-141. They identified several compounds including glycosyldiglycerides, surfactin C13, iturin A8, octapeptin D, plantaricin A, lichenysin A, sakacin-A, glysperin, plusbacin A3 and laterocin. The biosurfactants from *Pseudomonas putida* BD2 was identified by Janek *et al*. (2013) using TLC and UPLC/ESI-MS/MS. They found that the biosurfactants was a mixture of di-rhamnolipid (Rha-Rha-C10-C10) and phosphatidylethanolamines PE (32:1), PE (33:1). You *et al.* (2015) used the combination of GC-MS, ESI-MS and Q-TOF MS/MS to identify the biosurfactants from *Enterobacter* sp. N18. They reported the presence of surfactin homologues such as n-C12, iso-C13, anteiso-C13, iso-C14, n-C14, iso-C15, anteiso-C15, iso-C16, n-C16 and iso-C17 b-OH fatty acids. Korenblum *et al.* (2012) identified surfactin analogues as biosurfactants produced by the *Bacillus* sp. H2O-1 using TLC, GC-MS, ESI-MS and Tandem-MS. These compounds contained a similar heptapeptide chain of surfactin (Glu-Leu-Leu-Val-Asp-Leu-Leu) as the hydrophilic moiety, and C13, C14, C15, C16 β-hydroxy-fatty acids as the hydrophobic moiety. TLC, GC-MS and MALDI TOF-MS/MS was used to identify biosurfactants derived from *P. fluorescens* BD5 (Janek *et al.*, 2010). The authors reported two cyclic peptides namely pseudofactin I (palmitoyl-Gly–Ser–Thr–Leu–Leu–Ser–Leu–Val-O-) and pseudofactin II (palmitoyl-Gly–Ser–Thr–Leu–Leu–Ser–Leu–Leu-O-).

The biosurfactants from *B. subtilis* 309, *B. subtilis* 311 and *B. subtilis* 573 were identified by Pereira *et al.* (2013) using FTIR-ATR, ^{1}H NMR and MALDI-TOF MS/MS. They found that

509 the biosurfactants were made of a heptapeptide (L-Glu-L-Leu-D-Leu-L-Val-L-Asp-D-Leu-L-
510 Leu) linked to a C13, C14 or C15 hydroxy fatty acid chain. Lichenysin–A, is a compound
511 made of a small peptide (Gln, Leu, Leu, Val, Asp, Leu, and Ile) that was linked to 3-hydroxy
512 fatty acid residue (3-hydroxylated tri, tetra, penta, or hexadecanoic acids) with amide and
513 lactone bonds forming a cyclic structure, was identified as the biosurfactants produced by
514 *Bacillus licheniformis* W16 while using FTIR, HPTLC–ESI–MS and MALDI-TOF-MS, ^{1}H
515 and ^{13}C NMR as analytical techniques. In the study conducted by Oluwaseun *et al.* (2017), L-
516 rhamnosyl-L-rhamnosyl-3-b-hydroxydodecenoate was identified as the biosurfactants from
517 *Pseudomonas aeruginosa* C1501 using LC-ESI-MS and ^{1}H and ^{13}C NMR.

518 Saravanakumari and Mani (2010) used GC-MS and ^{1}H NMR to identify O-methyl-β-D-
519 xylopyranoside and octadecanoic acid was the major constitutive elements of the
520 biosurfactants from *L. lactis*. The final structure proposed by the authors was 2-methyl-O-
521 methyl-β-D-xylopyranosyl octadecanoic acid. The combination of TLC, GC-MS, ESI-MS and
522 NMR (^{1}H and ^{13}C) at one and two dimensions (COSY, HMBC and HSQC) was used in the
523 characterization process of biosurfactants from *L. plantarum* (Sauvageau *et al.*, 2012). The
524 authors identified four fractions including α-D-Glucopyranosyl-diglyceride (GL1), α-D-
525 Galactopyranosyl-(1→2)-α-D-Glucopyranosyl-diglyceride (GL2a), β-D-Glucopyranosyl-
526 (1→6)-β-D-Galactopyranosyl-(1→2)-6-O-acyl-α-D-Glucopyranosyl-diglyceride (GL2b) and
527 β-DGlucopyranosyl-(1→6)-α-D-Galactopyranosyl-(1→2)-α-D-Glucopyranosyl-diglyceride
528 (GL3). Xylopyranosyl linked to octadecanoic acid was identified as the biosurfactants from *L.*
529 *helveticus* MRTL91 while using TLC, UPLC-ESI-MS, GC-MS, FTIR and NMR (^{1}H and ^{13}C)
530 (Sharma *et al*., 2014). Using the same techniques latter, the authors identified xylopyranosyl
531 β-hydroxydecanoic acid as the biosurfactants produced by *E. faecium* MRTL9 (Sharma *et al.*,
532 2015). Mouafo *et al.* (2020a) identified 2,5-O-methyl-rhamnofuranosyl-palmitate in

biosurfactants from *L. casei* TM1B using elemental analysis, FTIR, GC-MS, and NMR (^{1}H and ^{13}C).

Conclusion

This review presents the different techniques of purification of biosurfactants. It suggests that, the choice of the purification techniques of biosurfactants depends on the producing strain, the culture media composition and the extraction method. Chromatographic, spectrometric and mass spectrometry methods are suitable for fully characterization at structural level of biosurfactants. However, biosurfactants which are known as complex mixture of compounds must be fractionated before being analyzed. They are in the food industry to enhance the shelf live of food, remove biofilms from food processing surfaces, stabilize emulsions, improve dough stability and texture of bakery products. In agricultural field, they are used as biopesticides. In environmental field, they are used for bioremediation, to enhance oil recovery and remove heavy metals from contaminated soils. They are also used in cosmetic, detergency and painting. The full structural characterization of biosurfactants will improve their added values and their application in some highly pointed industrial fields such as medicine and pharmacy.

Author Contributions:

Conceptualization, A.M. and H.T.M.; methodology, investigation, data curation, writing—original draft preparation, formal analysis, H.T.M., M.M.P., and A.T.S.; review and editing, A.J.M.M., G.M.V., K.Z.S., K.A.A.; resource and supervision, D.S., and A.M. All authors have read and agreed to the published this version of the manuscript.

Funding:

This research was not funded.

Data Availability Statement:

Upon request, the data will be made available from the corresponding author.

Conflicts of Interest:

The authors declare no conflict of interest.

References

Abdalsadiq, N., Hassan, Z., Lani, M. and Bulgasem, B. 2018. Identification of biosurfactant produced by *Lactobacillus* spp. using mass spectrometry. Res. J. Life Sci. Bioinformatics, Pharma. Chem Sci. 4(4), 247–258. https://doi.org/10.26479/2018.0404.21

Abdel-Mawgoud, A.M., Hausmann, R., Lepine, F., Muller, M.M. and Deziel, E. 2011. Rhamnolipids: detection, analysis, biosynthesis, genetic regulation, and bioengineering of production. *In* G. Soberon□Chavez (ed.), Biosurfactants, Microbiology Monographs 20, Springer-Verlag Berlin Heidelberg 2011. https://doi.org/10.1007/978-3-642-14490-5_2

Adu, S.A., Twigg, M.S., Naughton, P.J., Marchant, R. and Bant, I.M. 2023. Characterisation of cytotoxicity and immunomodulatory effects of glycolipid biosurfactants on human keratinocytes. Appl. Microbiol. Biotechnol. 107, 137–152. https://doi.org/10.1007/s00253-022-12302-5

AOAC 1990. Official Methods of Analysis of AOAC International, Method 960.52 (Surplus Method), The Association of Analytical Chemists, Arlington, VA, USA, 15th edition, 1990.

Atta, H.M., Abul-hamd, A.T. and Radwan, H.G. 2009. Production of Destomycine - A antibiotic by *Streptomyces* sp. using rice straw as fermented substrate. *Communication in Applied Biology and Science*, Ghent University. 74, 879–897.

Banerjee, S. and Ghosh, U. 2021. Production, purification and characterization of

580 biosurfactant isolated from *Bacillus oceanisediminis* H2. Materials Today: Proceedings.
581 https://doi.org/10.1016/j.matpr.2021.04.366

582 Behzadnia, A., Moosavi-nasab, M., Tiwari, B.K. and Setoodeh, P. 2020. *Lactobacillus*
583 *plantarum*-derived biosurfactant : Ultrasound-induced production and characterization.
584 Ultrasonics – Sonochem. 65, 105037. https://doi.org/10.1016/j.ultsonch.2020.105037

585 Bergevin, M., Marion, A., Farber, D., Golding, G.R. and Levesque, S. 2017. Severe MRSA
586 enterocolitis caused by a strain harboring enterotoxins D, G, and I. Emerg. Infect. Dis. 23,
587 865.

588 Bhosale, S.S., Rohiwal, S.S., Chaudhary, L.S., Pawar, K.D., Patil, P.S. and Tiwari, A.P. 2019.
589 Photocatalytic decolorization of methyl violet dye using rhamnolipid biosurfactant modified
590 iron oxide nanoparticles for wastewater treatment. J. Mat. Sci. Mat. Elec. 30, 4590–4598.
591 https://doi.org/10.1007/s10854-019-00751-0

592 Bjerk, T.R., Severino, P., Jain, S., Marques, C., Silva, A.M., Pashirova, T., and Souto, E.B.
593 2021. Biosurfactants: Properties and Applications in Drug Delivery, Biotechnology and
594 Ecotoxicology. Bioengineering (Basel). 8(8), 115.
595 https://doi.org/10.3390/bioengineering8080115

596 Bradford, M.M. 1976. A rapid and sensitive method for the quantitation of microgram
597 quantities of protein utilizing the principle of protein-dye binding. Anal. Biochem. 72, 248–
598 254.

599 Dabaghi, S., Ataei, S.A. and Taheri, A. 2023. Production of rhamnolipid biosurfactants in
600 solid-state fermentation: process optimization and characterization studies. BMC Biotechnol.
601 23, 2. https://doi.org/10.1186/s12896-022-00772-4

602 de Faria, A.F., Stefani, D., Vaz, B.G., Silva, I.S., Garcia, J.S., Eberlin, M.N., Grossman, M.J.,
603 Alves, O.L. and Durrant, L.R. 2021. Purification and structural characterization of fengycin

604 homologues produced by *Bacillus subtilis* LSFM-05 grown on raw glycerol. J. Ind. Microbiol.
605 Biotechnol. 38, 863–871. https://doi.org/10.1007/s10295-011-0980-1

606 de Faria, A.F., Teodoro-Martinez, D.S., Barbosa, G.N.O., Vaz, B.G., Silva, I.S., Garcia, J.S.,
607 Tótola, M.R., Eberlin, M.N., Grossman, M., Alves, O.L. and Durrant, L.R. 2011. Production
608 and structural characterization of surfactin (C14/Leu7) produced by *Bacillus subtilis* isolate
609 LSFM-05 grown on raw glycerol from the biodiesel industry. Process Biochem. 46, 1951–
610 1957. https://doi.org/10.1016/j.procbio.2011.07.001

611 De Gregorio, P.R., Parolin, C., Abruzzo, A., Luppi, B., Protti, M., Mercolini, L., Silva, J.A.,
612 Giordani, B., Marangoni, A., Nader-Macias, E.M.F. and Vitali, B. 2020. Biosurfactant from
613 vaginal *Lactobacillus crispatus* BC1 as a promising agent to interfere with *Candida* adhesion.
614 Microbial Cell Fact. 19(133), 1–16. https://doi.org/10.1186/s12934-020-01390-5

615 Dehghan-Noudeh, G., Housaindokht, M. and Bazzaz, B.S.F. 2005. Isolation, characterization,
616 and investigation of surface and hemolytic activities of a lipopeptide biosurfactant produced
617 by *Bacillus subtilis* ATTC 6633. J. Microbiol. 43, 272–276.

618 Desai, J.D. and Banat, I.M. 1997. Microbial production of surfactants and their commercial
619 potential. Microbiol. Mol. Biol. Rev. 61(1), 47–64. https://doi.org/10.1128/mmbr.61.1.47-
620 64.1997

621 Devale, A., Sawant, R., Pardesi, K., Perveen, K., Khanam, M.N., Shouche, Y. and Mujumdar,
622 S. 2023. Production and characterization of bioemulsifier by *Parapedobacter indicus*.
623 Frontiers Microbiol. 14, 1111135. https://doi.org/10.3389/fmicb.2023.1111135

624 Dubois, M., Gilles, K.A., Hamilton, J.K., Rebers, P.A. and Smith, F. 1956. Colorimetric
625 method for determination of sugars and related substances. Anal. Chem. 28, 350–356.

626 Faisal, Z.G., Mahdi, M.S. and Alobaidi, K.H. 2023. Optimization and chemical
627 characterization of biosurfactant produced from a novel *Pseudomonas guguanensis* strain

628 Iraqi ZG.K.M. Int. J. Microbiol. 1–16. https://doi.org/10.1155/2023/1571991

629 Fernandes, N.d.A.T., Simões, L.A. and Dias, D.R. 2023. Biosurfactants produced by yeasts:
630 fermentation, screening, recovery, purification, characterization, and applications.
631 Fermentation. 9, 207. https://doi.org/10.3390/fermentation9030207

632 Ferreira, A., Vecino, X., Ferreira, D., Cruz, J.M., Moldes, A.B. and Rodrigues, L.R. 2017.
633 Novel cosmetic formulations containing a biosurfactant from *Lactobacillus paracasei*. Coll.
634 Surf. B: Biointerfaces. 155, 522–529. https://doi.org/10.1016/j.colsurfb.2017.04.026

635 Folch, J., Lees, M. and Stanley, S.G. 1957. A simple method for the isolation and purifcation
636 of total lipides from animal tissues. J. Biol. Chem. 226, 497–509.

637 Fookao, A.N., Mbawala, A., Nganou, N.D., Nguimbou, R.M. and Mouafo, T.H. 2022.
638 Improvement of the texture and dough stability of milk bread using
639 bioemulsifiers/biosurfactants produced by lactobacilli isolated from indigenous fermented
640 milk (*pendidam*). LWT–Food Sci. Technol. 163, 113609.
641 https://doi.org/10.1016/j.lwt.2022.113609

642 Fookao, A.N., Mbawala, A., Nganou, N.D., Nguimbou, R.M., and Mouafo, T.H. 2022.
643 Improvement of the texture and dough stability of milk bread using
644 bioemulsifiers/biosurfactants produced by lactobacilli isolated from indigenous fermented
645 milk (*pendidam*). LWT–Food Science and Technology. 163, 113609.
646 https://doi.org/10.1016/j.lwt.2022.113609

647 Fouad, H. K., Khanaqa, H. H. and Munira, I. 2010. Purification and characterization of
648 surlactin produced by *Lactobacillus acidophilus.* Al-Anbar Med. J. 8(1), 34–39.

649 Ghasemi, A., Moosavi-Nasab, M., Behzadnia, A. and Rezaei, M. 2018. Enhanced
650 biosurfactant production with low-quality date syrup by *Lactobacillus rhamnosus* using a fed-
651 batch fermentation. Food Sci. Biotechnol. 27, 1137–1144. https://doi.org/10.1007/s10068-

018-0366-5

Ghasemi, A., Moosavi-nasab, M., Setoodeh, P., Mesbahi, G. and Yousefi, G. 2019. Biosurfactant production by lactic acid bacterium *Pediococcus dextrinicus* SHU1593 grown on different carbon sources: strain screening followed by product characterization. Sci. Rep. 9, 5287. https://doi.org/10.1038/s41598-019-41589-0

Habib, S., Ahmed, A., Haque, M.F.U. and Ejaz, R. 2023. Efficacious use of potential biosurfactant producing plant growth promoting rhizobacteria to combat petrol toxicity in *Zea mays* L. plants. Env. Sci. Poll. Res. https://doi.org/10.1007/s11356-023-25925-6

Hamerly, T., Tripet, B., Wurch, L., Hettich, R.L., Podar, M., Bothner, B. and Copié, V. 2015. Characterization of fatty acids in Crenarchaeota by GC-MS and NMR. Archaea. 2015, 1–9. http://dx.doi.org/10.1155/2015/472726

Inamuddin and Adetunji C.O. 2023. Applications of Next Generation Biosurfactants in the Food Sector. Academic Press, Elsevier Inc. 545p. https://doi.org/10.1016/C2019-0-05486-6.

Ismail, W., Al-Rowaihi, I.S., Al-Humam, A.A., Hamza, R.Y., El Nayal, A.M. and Bououdina, M. 2013. Characterization of a lipopeptide biosurfactant produced by a crude-oil-emulsifying *Bacillus* sp. I-15. Int. Biodeterioration Biodegradation. 84, 168–178. http://dx.doi.org/10.1016/j.ibiod.2012.04.017

Janek, T., Łukaszewicz, M. and Krasowska, A. 2013. Identification and characterization of biosurfactants produced by the Arctic bacterium *Pseudomonas putida* BD2. Coll. Surf. B: Biointerfaces. 110, 379–386. http://dx.doi.org/10.1016/j.colsurfb.2013.05.008

Janek, T., Łukaszewicz, M., Rezanka, T. and Krasowska, A. 2010. Isolation and characterization of two new lipopeptide biosurfactants produced by *Pseudomonas fluorescens* BD5 isolated from water from the Arctic Archipelago of Svalbard. Biores. Technol. 101, 6118–6123. https://doi.org/10.1016/j.biortech.2010.02.109

676 Jemil, N., Ben Ayed, H., Manresa, A., Nasri, M. and Hmidet, N. 2017. Antioxidant properties,
677 antimicrobial and anti-adhesive activities of DCS1 lipopeptides from *Bacillus*
678 *methylotrophicus* DCS1. BMC Microbiol. 17(144), 1–11. https://doi.org/10.1186/s12866-017-
679 1050-2

680 Joshi, S.J., Al-wahaibi, Y.M., Al-bahry, S.N., Elshafie, A.E. and Ennis, C.J. 2016 Production,
681 characterization, and application of *Bacillus licheniformis* W16 biosurfactant in enhancing oil
682 recovery. Frontier Microbiol. **7**, 1–14. https://doi.org/10.3389/fmicb.2016.01853

683 Korenblum, E., de Araujo, L.V., Guimarães, C.R., de Souza, L.M., Sassaki, G., Abreu, F.,
684 Nitschke, M., Lins, U., Freire, D.M.G., Bergter, E.B. and Seldin, L. 2012. Purification and
685 characterization of a surfactin-like molecule produced by *Bacillus* sp. H2O-1 and its
686 antagonistic effect against sulfate reducing bacteria. BMC Microbiol. 12(252), 1–13.
687 https://doi.org/10.1186/1471-2180-12-252

688 Kurozuka, A., Onishi, S., Nagano, T., Yamaguchi, K., Suzuki, T., Minami, H. 2017.
689 Emulsion Polymerization with a Biosurfactant. Langmuir ACS Journal of Surface Colloids.
690 33, 5814–5818. https://doi.org/10.1021/acs.langmuir.7b00851.

691 Lara, V.M., Vallejo, M., Parada, R., Ossa, J.S.H., Gliemmo, M.F. and Campos, C.A. 2020.
692 Characterization of the emulsifying activity of biosurfactants produced by lactic acid bacteria
693 isolated from the Argentinian Patagonia. J. Disp. Sci. Technol. 1–8.
694 https://doi.org/10.1080/01932691.2020.1845961

695 Liu, J.F., Mbadinga, S.M., Yang, S.Z., Gu, J.D. and Mu, B.Z. 2015. Chemical structure,
696 property and potential applications of biosurfactants produced by *Bacillus subtilis* in
697 petroleum recovery and spill mitigation. Int. J. Mol. Sci. 16, 4814–4837.

698 Lowry, O.H., Rosenbrough, N.J., Farr, A.L. and Randall, R.J. 1951. Protein measurement
699 with the Folin phenol regent. J. Biol. Chem. 193, 265–275.

700 Madhu, A.N. and Paprulla, S.G. 2013. Evaluation and Functional Characterization of a
701 Biosurfactant Produced by *Lactobacillus plantarum* CFR 2194. Appl. Biochem. Biotechnol.
702 1–13. https://doi.org/10.1007/s12010-013-0649-5

703 Marchant, R. and Banat, I.M. 2012a. Microbial biosurfactants: challenges and opportunities
704 for future exploitation. Trends Biotechnol. 30, 558–565. https://doi.org/10.
705 1016/j.tibtech.2012.07.003

706 Marchant, R. and Banat, I.M. 2012b. Biosurfactants: a sustainable replacement for chemical
707 surfactants? Biotechnol. Lett. 34, 1597–1605. https://doi.org/10.1007/s10529-012-0956-x

708 Meena, K.R., Dhiman, R., Singh, K., Kumar, S., Sharma, A., Kanwar, S.S., Mondal, R., Das,
709 S., Franco, O.L. and Mandal, A.K. 2021. Purification and identification of a surfactin
710 biosurfactnat and engine oil degradation by *Bacillus velezensis* KLP2016. Microbial Cell
711 Fact. 20(26), 1–12. https://doi.org/10.1186/s12934-021-01519-0

712 Monteiro, S.A., Sassaki, G.L., de Souza, L.M., Meira, J.A., de Araújo, J.M., Mitchell, D.A.,
713 Ramos, L.P. and Krieger, N. 2007. Molecular and structural characterization of the
714 biosurfactant produced by *Pseudomonas aeruginosa* DAUPE 614. Chem. Physic Lipids. 147,
715 1–13.

716 Morais, I.M.C., Cordeiro, A.L., Teixeira, G.S., Domingues, V.S., Nardi, R.M.D., Monteiro,
717 A.S., Alves, R.J., Siqueira, E.P. and Santos, V.L. 2017. Biological and physicochemical
718 properties of biosurfactants produced by *Lactobacillus jensenii* - P 6A and *Lactobacillus*
719 *gasseri* - P 65. Microbial Cell Fact. 1–15. https://doi.org/10.1186/s12934-017-0769-7

720 Mouafo, H.T., Mbawala, A., Tanaji, K., Somashekar, D., & Ndjouenkeu, R. 2020b.
721 Improvement of the shelf life of raw ground goat meat by using biosurfactants produced by
722 lactobacilli strains as biopreservatives. LWT-Food Science and Technology. 133, 110071.
723 https://doi.org/10.1016/j.lwt.2020.110071

724 Mouafo, H.T., Mbawala, A., Tchougang, M.H., Ndjouenkeu, R., and Somashekar, D. 2018b.
725 Application of response surface methodology to improve the production of antimicrobial
726 biosurfactants by *Lactobacillus paracasei* subsp. tolerans N2 using sugar cane molasses as
727 substrate. Biores. Bioprocess. 5(48), 1–16. https://doi.org/10.1186/s40643-018-0234-4

728 Mouafo, T.H. 2019. Production of biosurfactants by *Lactobacillus* spp. strains using sugar
729 cane molasses as substrate: Application to the biopreservation of fresh ground beef. Ph.D
730 thesis. National School of Agro-Industrial Sciences (ENSAI), University of Ngaoundéré,
731 Cameroon. 325p.

732 Mouafo, T.H. and Somashekar, D. 2020. Biosurfactants and its application in the food
733 industry. Indian Food Industry Mag. 4(1), 49–57.

734 Mouafo, T.H., Mbawala, A. and Ndjouenkeu, R. 2018a. Effect of different carbon sources on
735 biosurfactants' production by three strains of *Lactobacillus* spp. Biomed Res. Int. 2018, 1–15.
736 https://doi.org/10.1155/2018/5034783

737 Mouafo, T.H., Mbawala, A., Somashekar, D., Tchougang, H.M., Harohally, N.V. and
738 Ndjouenkeu, R. 2020a. Biological properties and structural characterization of a novel
739 rhamnolipid like-biosurfactants produced by *Lactobacillus casei* subsp. casei TM1B.
740 Biotechnol. Appl. Biochem. 1–12. https://doi.org/10.1002/bab.1966

741 Mouafo, T.H., Sokamte, T.A., Mbawala, A., Ndjouenkeu, R. and Somashekar, D. 2022.
742 Biosurfactants from lactic acid bacteria: a critical review on production, extraction, structural
743 characterization and food application. Food Biosci. 46, 101598.
744 https://doi.org/10.1016/j.bio.2022.101598

745 Mulligan, C. N., Sharma, S. K. and Mudhoo, A. 2014. Biosurfactants research trends and
746 applications. Boca Raton, London, New-York: Taylor & Francis Group, LLC. 346p.

747 Nataraj, B.H., Ramesh, C. and Mallappa, R.H. 2021a. Characterization of biosurfactants
748 derived from probiotic lactic acid bacteria against methicillin-resistant and sensitive
749 *Staphylococcus aureus* isolates. LWT - Food Sci. Technol. 151, 112195.
750 https://doi.org/10.1016/j.lwt.2021.112195

751 Nataraj, B.H., Ramesh, C. and Mallappa, R.H. 2021b. Functional group characterization of
752 lactic bacterial biosurfactants and evaluation of antagonistic actions against clinical isolates of
753 Methicillin-Resistant *Staphylococcus aureus* (MRSA). Lett. Appl. Microbiol. 73(3), 372–382.
754 https://doi.org/10.1111/lam.13523

755 Nelson, J., El-Gendy, A.O., Mansy, M.S., Ramadan, M.A. and Aziz, R.K. 2020. The
756 biosurfactants iturin, lichenysin and surfactin, from vaginally isolated lactobacilli, prevent
757 biofilm formation by pathogenic Candida. FEMS Microbiol. Lett. 367(15), 1–9.
758 https://doi.org/10.1093/femsle/fnaa126

759 Nitschke, M.N. and Silva, S.S. 2018. Recent food applications of microbial surfactants.
760 Critical Rev. Food Sci. Nutr. 58(4), 631–638.
761 https://doi.org/10.1080/10408398.2016.1208635.

762 Ohadi, M., Shahravan, A., Dehghannoudeh, N., Eslaminejad, T., Banat, I.M.,
763 Dehghannoudeh, G. 2020. Potential Use of Microbial Surfactant in Microemulsion Drug
764 Delivery System: A Systematic Review. Drug Des. Devel. Ther. 14, 541–550.
765 https://doi.org/10.2147/DDDT.S232325.

766 Okpokwasili, G.C. and Ibiene, A.A. 2006. Enhancement of recovery of residual oil using a
767 biosurfactant slug. Afr. J. Biotechnol. 5(5), 453–456.

768 Oluwaseuna, A.C., Kola, O.J., Mishra, P., Singh, J.R., Singh, A.K., Cameotra, S.S. and
769 Micheal, A.O. 2017. Characterization and optimization of a rhamnolipid from *Pseudomonas*
770 *aeruginosa* C1501 with novel biosurfactant activities. Sustainable Chem. Pharm. 6, 26–36.

https://doi.org/10.1016/j.scp.2017.07.001

Patel, M., Siddiqui, A.J., Hamadou, W.S., Surti, M., Awadelkareem, A.M., Ashraf, S.A., Alreshidi, M., Snoussi, M., Rizvi, S.M.D., Bardakci, F., Jamal, A., Sachidanandan, M. and Adnan, M. 2021. Inhibition of bacterial adhesion and antibiofilm activities of a glycolipid biosurfactant from *Lactobacillus rhamnosus* with Its physicochemical and functional properties. Antibiotics. 10, 1546. https://doi.org/10.3390/antibiotics10121546

Pereira, J.F.B., Gudiña, E.J., Costa, R., Vitorino, R., Teixeira, J.A., Coutinho, J.A.P. and Rodrigues, L.R. 2023. Optimization and characterization of biosurfactant production by *Bacillus subtilis* isolates towards microbial enhanced oil recovery applications. Fuel. 111, 259–268. https://doi.org/10.1016/j.fuel.2013.04.040

Rahaman, S.M., Bhattarai, A., Kumar, D., Singh, B., Saha, B. 2023. Application of biosurfactants as emulsifiers in the processing of food products with diverse utilization in the baked goods. *In* Inamuddin and Adetunji C.O. 2023. Applications of Next Generation Biosurfactants in the Food Sector. Academic Press, Elsevier Inc., pp. 203-237. https://doi.org/10.1016/B978-0-12-824283-4.00021-6

Randhawa, K.K.S. and Rahman, P.K.S.M. 2014. Rhamnolipid biosurfactants – past, present, and future scenario of global market. Frontiers Microbiol., *5*, 454.

Reddy, K.S., Khan, M.Y., Archana, K., Reddy, M.G. and Hameeda, B. 2016. Utilization of mango kernel oil for the rhamnolipid production by *Pseudomonas aeruginosa* DR1 towards its application as biocontrol agent. Biores. Technol. 221, 291–299. https://doi.org/10.1016/j.biortech.2016.09.041

Reshmy, R., Philip, E., Madhavan, A., Binod, P., Awasthi, M.K., Pandey, A., Raveendran Sindhu, R. 2023. Applications of biosurfactant as an antioxidant in foods. *In* Inamuddin and Adetunji C.O. 2023. Applications of Next Generation Biosurfactants in the Food Sector.

795 Academic Press, Elsevier Inc., pp. 391-401. https://doi.org/10.1016/B978-0-12-824283-
796 4.00008-3

797 Rodrigues, L.R., Teixeira, J.A., van der Mei, H.C. and Oliveira, R. 2006. Isolation and partial
798 characterization of a biosurfactant produced by *Streptococcus thermophilus* A. Coll. Surf. B:
799 Biointerfaces. 53(1), 105–112. https://doi.org/10.1016/j.colsurfb.2006.08.009

800 Sakr, E.A.E., Ahmed, H.A.E. and Saif, F.A.A.A. 2021. Characterization of low-cost
801 glycolipoprotein biosurfactant produced by *Lactobacillus plantarum* 60 FHE isolated from
802 cheese samples using food wastes through response surface methodology and its potential as
803 antimicrobial, antiviral, and anticancer activities. Int. J. Biol. Macromol. 170, 94–106.
804 https://doi.org/10.1016/j.ijbiomac.2020.12.140

805 Santoso, S.P., Angkawijaya, A.E., Kurniawan, A., Cheng, K.C., Chang Wei Hsieh, C.W.
806 2023. The role of biosurfactants in the improvement of texture and shelf life of starch-
807 containing products. *In* Inamuddin and Adetunji C.O. 2023. Applications of Next Generation
808 Biosurfactants in the Food Sector. Academic Press, Elsevier Inc., pp. 149-169.
809 https://doi.org/10.1016/B978-0-12-824283-4.00018-6

810 Saravanakumari, P. and Mani, K. 2010. Structural characterization of a novel xylolipid
811 biosurfactant from *Lactococcus lactis* and analysis of antibacterial activity against multi-drug
812 resistant pathogens. Biores. Technol. 101(22), 8851–8854.
813 https://doi.org/10.1016/j.biortech.2010.06.104

814 Satpute, S.K., Banpurkar, A.G., Dhakephalkar, P.K., Banat, I.M. and Chopade, B.A. 2010.
815 Methods for investigating biosurfactants and bioemulsifiers: a review. Critical Rev.
816 Biotechnol. 30(2), 127–144. https://doi.org/10.3109/07388550903427280

817 Satpute, S.K., Bhawsar, B.D., Dhakephalkar, P.K. and Chopade, B.A. 2008. Assessment of
818 different screening methods for selecting biosurfactant producing marine bacteria. Indian J.

819 Marine Sci. 37(3), 243–250.

820 Satpute, S.K., Mone, N.S., Das, P., Banat, I.M. and Banpurkar, A.G. 2019. Inhibition of
821 pathogenic bacterial biofilms on PDMS based implants by *L. acidophilus* derived
822 biosurfactant. BMC Microbiol. 19(39), 1–15. https://doi.org/10.1186/s12866-019-1412-z

823 Sauvageau, J., Ryan, J., Lagutin, K., Sims, I.M., Stocker, B.L. and Timmer, M.S.M. 2012.
824 Isolation and structural characterisation of the major glycolipids from *Lactobacillus*
825 *plantarum*. Carbohydrate Res. 357, 151–156. https://doi.org/10.1016/j.carres.2012.05.011

826 Sekon, K.K. and Rahman, P.K. 2014. Rhamnolipid biosurfactants–past, present and future
827 scenario of global market. Frontier Microbiol. 5, 454.
828 https://doi.org/10.3389/fmicb.2014.00454

829 Sen, S., Borah, S.N., Arijit, Bora A. and Deka, S. 2017. Production, characterization, and
830 antifungal activity of a biosurfactant produced by *Rhodotorula babjevae* YS3. Microbial Cell
831 Fact. 16(95), 1–14. https://doi.org/10.1186/s12934-017-0711-z

832 Sharma, A., and Arya, S.K. 2023. The application of biosurfactant for the modification of the
833 rheological properties of wheat dough. *In* Inamuddin and Adetunji C.O. 2023. Applications of
834 Next Generation Biosurfactants in the Food Sector. Academic Press, Elsevier Inc., 171-186.
835 https://doi.org/10.1016/B978-0-12-824283-4.00016-2

836 Sharma, D. and Saharan, B.S. 2016. Functional characterization of biomedical potential of
837 biosurfactant produced by *Lactobacillus helveticus*. Biotechnol. Rep. 11, 27–35.
838 https://doi.org/10.1016/j.btre.2016.05.001

839 Sharma, D., Saharan, B. S., Chauhan, N., Procha, S. and Lal, S. 2015. Isolation and functional
840 characterization of novel biosurfactant produced by *Enterococcus faecium*. SpringerPlus.
841 4(4), 1–14. https://doi.org/10.1186/2193-1801-4-4

842 Sharma, D., Saharan, B.S. and Kapil, S. 2016. Biosurfactants of lactic acid bacteria. Springer

Briefs in Microbiology. Springer International Publishing AG Switzerland. 96p. https://doi.org/10.1007/978-3-319-26215-4

Sharma, D., Saharan, B.S., Chauhan, N., Bansal, A. and Procha, S. 2014. Production and structural characterization of *Lactobacillus helveticus* derived biosurfactant. Scientific World J. 2014, 1–9. https://doi.org/10.1155/2014/493548

Silva, I.A., Veras, B.O., Ribeiro, B.G., Aguiar, J.S., Guerra, J.M.C., Luna, J.M. and Sarubbo, L.A. 2020. Production of cupcake-like dessert containing microbial biosurfactant as an emulsifier. PeerJ. 8, e9064. https://doi.org/10.7717/peerj.9064

Smyth, T.J., Rudden, M., Tsaousi, K., Marchant, R. and Banat, I.M. 2014. Protocols for the Isolation and Analysis of Lipopeptides and Bioemulsifiers. *In* T.J. McGenity et al. (eds.), Hydrocarbon and Lipid Microbiology Protocols, Springer Protocols Handbooks, Springer-Verlag Berlin Heidelberg. https://doi.org/10.1007/8623_2014_29

Smyth, T.J.P., Perfumo, A., McClean, S., Marchant, R. and Banat, I.M. 2010. Isolation and Analysis of Lipopeptides and High Molecular Weight Biosurfactants. *In*, Timmis, K.N. (ed), *Handbook of Hydrocarbon and Lipid Microbiology*. Springer-Verlag, Berlin Heidelberg, pp. 3687–3704. https://doi.org/10.1007/978-3-540-77587-4_290

Sun, W., Zhu, B., Yang, F., Dai, M., Sehar, S., Peng, C., Ali, I., Naz, I. 2021. Optimization of biosurfactant production from Pseudomonas sp. CQ2 and its application for remediation of heavy metal contaminated soil. Chemosphere. 265, 129090. https://doi.org/10.1016/j.chemosphere.2020.129090.

Syldatk, C. and Wagner, F. 1987. Production of biosurfactants. In *Biosurfactants and Biotechnology*; Kosaric, N., Cairns, W.L., Gray, N.C.C., Eds.; Marcel Dekker: New York, NY, USA, pp. 89–120.

Thavasi, R., Jayalakshmi, S. and Banat, I.M. 2011. Application of biosurfactant produced

867 from peanut oil cake by *Lactobacillus delbrueckii* in biodegradation of crude oil. Biores.
868 Technol. 102, 3366–3372. https://doi.org/10.1016/j.biortech.2010.11.071

869 Tokumoto, Y., Nomura, N., Uchiyama, H., Imura, T., Morita, T., Fukuoka, T. and Kitamoto,
870 D. 2009. Structural characterization and surface-active properties of a succinoyl trehalose
871 lipid produced by *Rhodococcus* sp. SD-74. J. Oleo Sci. 58(2), 97-102.
872 https://doi.org/10.5650/jos.58.97

873 Toren, A., Navon-Venezia, S., Ron, E.Z. and Rosenberg, E. 2001. Emulsifying activities of
874 purified alasan proteins from *Acinetobacter radioresisten* HA53. Appl. Env. Microbiol. 67,
875 1102–1106.

876 Transparency Market Research. 2013. Specialty surfactants market and biosurfactants market:
877 Global scenario, raw material and consumption trends, industry analysis, size, share and
878 forecast. 2010–2018. Available from http://www.transparencymarketresearch.com/specialty-
879 and-biosurfactants-market.html. Accessed November 15, 2015.

880 Vallejo, C.M., Restrepo, M.A.F., Duque, F.L.G. and Díaz, J.C.Q. 2021. Production,
881 characterization and kinetic model of biosurfactant produced by lactic acid bacteria. Elec. J.
882 Biotechnol. https://doi.org/10.1016/j.ejbt.2021.06.001

883 Vecino, X., Rodríguez-López, L., Gudiña, E.J., Cruz, J. M., Moldes, A.B. and Rodrigues,
884 L.R. 2017. Vineyard pruning waste as an alternative carbon source to produce novel
885 biosurfactants by *Lactobacillus paracasei*. J. Ind. Eng. Chem. 55, 40–49.
886 https://doi.org/10.1016/j.jiec.2017.06.014

887 Vigneshwaran, C., Vasantharaj, K., Krishnanand, N. and Sivasubramanian, V. 2021.
888 Production Optimization, Purification and characterization of lipopeptide biosurfactant
889 obtained from *Brevibacillus* sp. AVN13. J. Env. Chem. Eng. 9(1), 104867.
890 https://doi.org/10.1016/j.jece.2020.104867.

Wattoo, A.G., Yaseen, S., Kanwal, M., Zaidi, S.M.M., Alamgeer, M., Saeed, D., Shabir, F., Ikram, K., Khushnood, R.A., Buzdar, S.A., Song, Z. 2023. Application of biosurfactants in juice industry. *In* Inamuddin and Adetunji C.O. 2023. Applications of Next Generation Biosurfactants in the Food Sector. Academic Press, Elsevier Inc., pp. 527-536. https://doi.org/10.1016/B978-0-12-824283-4.00013-7

Wilcox, C.F. and Wilcox, M.F. 1995. Visible and ultraviolet spectroscopy. *In*: Experimental Organic Chemistry, second Ed. Prentice-Hall, New Jersey, pp. 254–262.

Yang, S., Wei, D. and Mu, B. 2006. Determination of the amino acid sequence in a cyclic lipopeptide using MS with DHT mechanism. J. Biochem. Biophys. Methods. 68, 69–74.

You, J., Yang, S. and Mu, B.Z. 2015. Structural characterization of lipopeptides from *Enterobacter* sp. strain N18 reveals production of surfactin homologues. Eur. J. Lipid Sci. Technol. 117, 890–898. https://doi.org/10.1002/ejlt.201400386

Zachara, N.E. and Gooley, A.A. 2000. Identification of glycosylation sites in mucin peptides by Edman degradation. Methods Mol. Biol. 125, 121–128. https://doi.org/10.1385/1-59259-048-9:121

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Table 1. Purification methods and nature of biosurfactants produced by some microorganisms

Microorganisms	**Method of purification**	**Nature of biosurfactants**	**References**
L. casei subsp. casei TM1B	- Centrifugation (13,000×g, 4°C, 20 min) - Filtration (0.22 µm) - RP-HPLC	Glycolipid	(Mouafo *et al*., 2020)
L. plantarum CFR 2194	- Centrifugation (10,000×g, 4 °C, 15 min) - Precipitation (pH 2)	Glycoprotein	(Nelson *et al*., 2020)
B. velezensis KLP2016	- TLC - Size exclusion chromatography - Ion exchange chromatography	Lipopeptide	(Dabaghi *et al*., 2023)
B. oceanisediminis H2	- TLC - Column chromatography	Lipopeptide	(Meena *et al*., 2021)
B. subtilis LSFM-05	- Column chromatography	Lipopeptide	(Satpute et al., 2010)
S. thermophilus A	- Filtration (0.22 µm) - Dialysis (6000–8000 Da) - Hydrophobic interaction chromatography	Glycolipid	(Sauvageau *et al*., 2012)
L. plantarum IRL-560	- TLC - Column chromatography - Size exclusion chromatography	Glycolipid	(Banerjee and Ghosh, 2021)
L. delbrueckii	- Column chromatography - Dialysis (6000–8000 Da)	Glycolipid	(Sharma and Saharan, 2016)
L. helveticus MRTL91	- Dialysis (6000–8000 Da) - Column chromatography	Glycolipid	(de Faria *et al*., 2021)
L. lactis	- Precipitation (pH 2) - Centrifugation (6000×g, 15 min, 4°C)	Glycolipid	(Sharma *et al*., 2015)
L. rhamnosus PTCC 1637	- Filtration (0.22 µm) - Dialysis (6000–8000 Da)	Phosphoglycoprotein	(Satpute *et al*., 2008)
L. paracasei ssp. *paracasei* A20	- Filtration (0.22 µm) - Dialysis (6000–8000 Da)	Glycolipoprotein	(Ghasemi *et al*., 2018)
L. acidophilus NCIM 2903	- Filtration (0.22 µm) - Dialysis (6000–8000 Da)	Glycolipoprotein	(Vecino *et al*., 2017)

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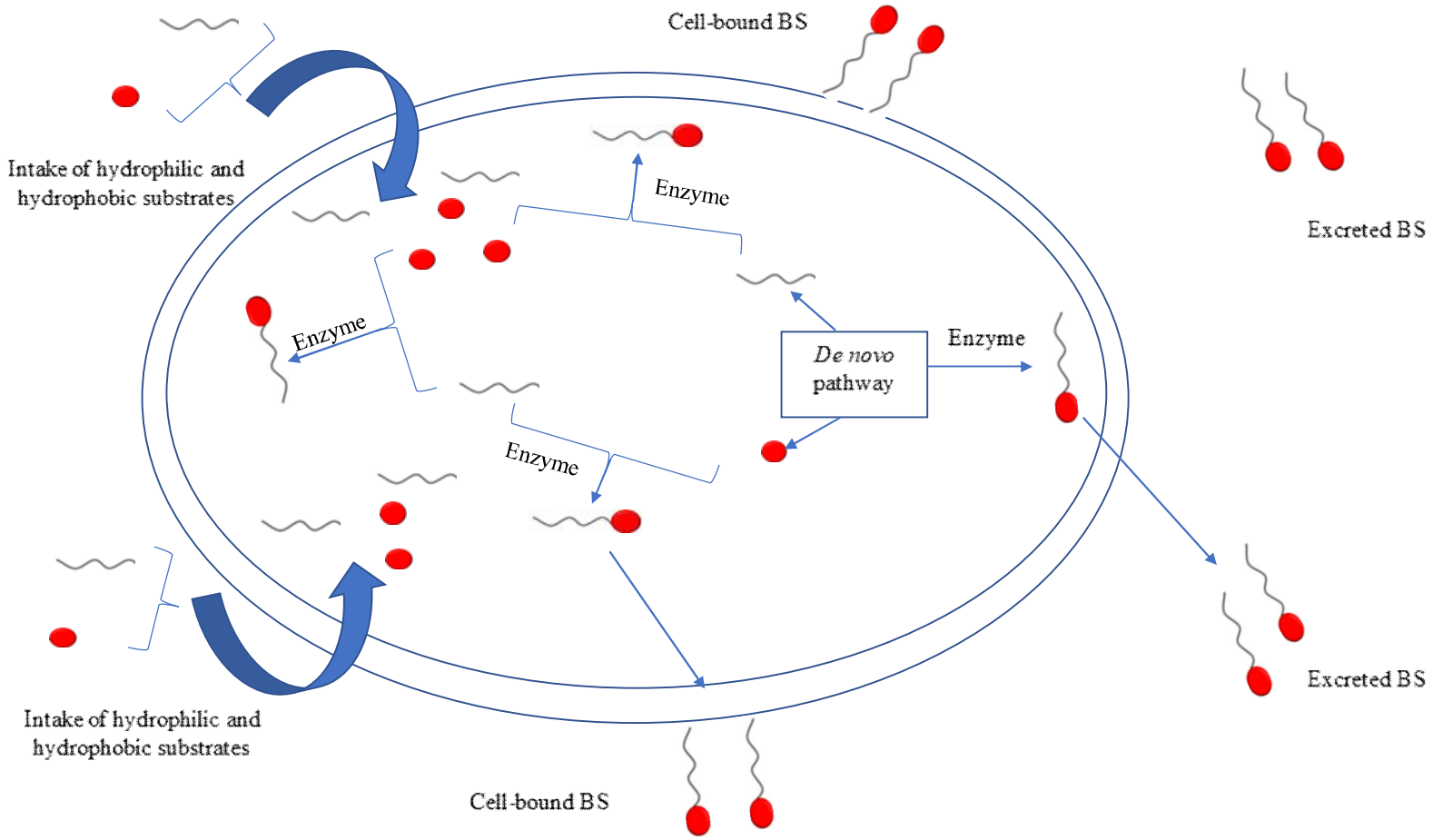

Fig. 1. The different metabolism routes of biosurfactants by microorganisms.

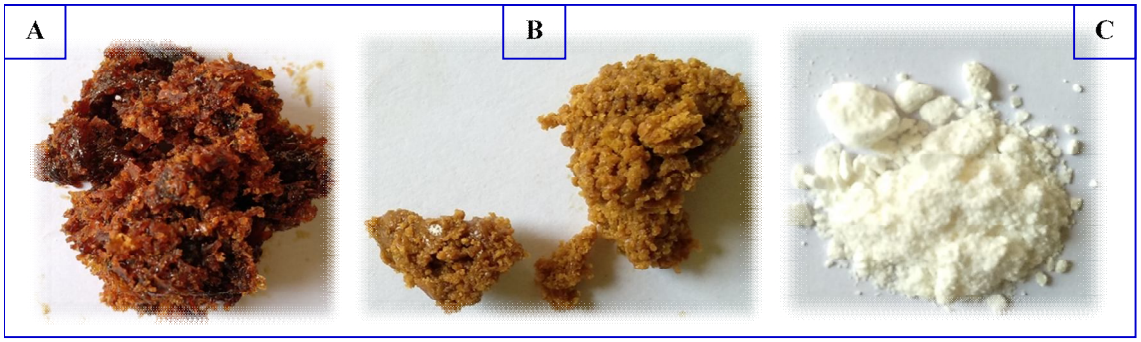

Fig. 2. Pictures showing color of biosurfactants produced by *Lactobacillus paracasei* subsp. tolerans N2 with sugar cane molasses as substrate (A), *Lactobacillus casei* subsp. casei TM1B with sugar cane molasses as substrate (B) and *Lactobacillus rhamnosus* G88 with glycerol as substrate (C) (Mouafo, 2019).