Environmental and Microbial Biotechnology

Inamuddin
Mohd Imran Ahamed
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Microbial Biosurfactants

Preparation, Properties and Applications

Springer

Environmental and Microbial Biotechnology

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Ram Prasad, Department of Botany, Mahatma Gandhi Central University, Motihari, Bihar, India

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Inamuddin • Mohd Imran Ahamed •
Ram Prasad
Editors

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Editors
Inamuddin
Department of Applied Chemistry, Zakir Husain College of Engineering and Technology, Faculty of Engineering and Technology
Aligarh Muslim University
Aligarh, India

Mohd Imran Ahamed
Department of Chemistry, Facualty of Science
Aligarh Muslim University
Aligarh, Uttar Pradesh, India

Ram Prasad
Department of Botany
Mahatma Gandhi Central University
Motihari, Bihar, India

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Preface

Microbial biosurfactants are biomolecules obtained from bacteria, yeast, fungi, as well as animals and plants. Biosurfactants owing to their biocompatible nature can be used as emulsifying, defoaming, anti-adhesive, antioxidant, antimicrobial, antibiofilm, and antioxidant agents. Microbial biosurfactants have a wide range of applications starting from household detergents to cosmetics, environmental biotechnology to agriculture, and food processing to biomedical industries. This book reviews the applications of microbial biosurfactants in the food industry, such as antioxidant, environmental biotechnology, biomedicine, energy, and household detergents. It is written by experts having considerable experience in the area of preparation and characterization of biosurfactants. It aims to cater to the needs of people from a wide variety of disciplines from food industry to household applications as well as from industries to biomedicine, energy, and environmental biotechnology. It is an in-depth resource for graduate and postgraduate students, researchers, biotechnologists, industrialist, material scientists, and R&D professionals of food industries working in the area of biosurfactants. It contains 14 chapters. The summaries of the chapters are given below.

Chapter 1 discusses the characteristics of biosurfactants advantageous for the food industry. Also, it addresses the concept of food additive and how the multiple functions of biosurfactants can be used, such as emulsifier, antimicrobial, antibiofilm, and antioxidant agent.

Chapter 2 presents a brief description of the main contaminants in food processing and the biosurfactants applied to avoid them. It focuses on the application of glycolipids and lipopeptides as the major microbial biosurfactants, such as preservatives, and antimicrobial, antioxidant, and antibiofilm agents in food processing.

Chapter 3 discusses several aspects of biosurfactants such as sources, structure, isolation, and potential role and applications of biosurfactants. The microbial-derived surfactants can replace synthetic surfactants in a variety of industrial applications as detergents, emulsifiers, solubilizers, and foaming and wetting agents. This chapter discusses the antioxidant property of biosurfactants with examples.

Chapter 4 discusses the classification and physical and chemical properties of biosurfactants. Furthermore, it highlights the factors affecting the production of biosurfactants and the methods of cultivation in the laboratory and industrial scale.

Chapter 5 focuses on various classes of microbial biosurfactants, their basic chemical properties, and the details of genes encoding various types of biosurfactants. It also elaborates the potential industrial and pharmaceutical applications of biosurfactants.

Chapter 6 reviews the biodegradation pathway of PAHs involving enzymes and microorganisms. The production of biosurfactants by microorganisms and their contribution toward the degradation of insoluble PAHs are properly discussed.

Chapter 7 discusses the different types of biosurfactants and the structure of surfactin, along with its membrane interaction, synthesis, and regulation. Breast cancer is a global issue. Surfactin, which is a biosurfactant, can act against breast cancer.

Chapter 8 describes the current research and knowledge of microbial biosurfactants with anticancer potential. Information on the structure and production of biosurfactants is detailed. The main emphasis is given to the anticancer activity in the treatment of breast cancer, lung cancer, leukemia, melanoma, colon cancer, and drug delivery systems.

Chapter 9 discusses the various sources of oil and petroleum pollutants and technologies for their remediation using biosurfactants. A major focus is given on the mode of action of biosurfactant and biosurfactant producing microorganisms for the removal of oil pollutants from soil and water.

Chapter 10 deals with the main classes of biosurfactants with anti-adhesive action. It also discusses the process of microbial adhesion for the formation of biofilms and the studies involving the applications of microbial biosurfactants as disruptive agents on different surfaces.

Chapter 11 discusses the applications of surfactants, especially biosurfactants, on the treatment of waste-activated sludge. Recent developments on value-added biometabolite production, bioenergy recovery, dewatering, decontamination of organic contaminants, and heavy metal removal are covered. Besides, state-of-the-art processes to promote biotransformation of organics from sludge are presented.

Chapter 12 emphasizes the role of biosurfactants in the medical and pharmaceutical industries. Important physicochemical properties of biosurfactants are included. Potential applications in cancer treatment, drug delivery, wound healing, and antimicrobial therapy are described in detail. Moreover, future perspectives are also included.

Chapter 13 discusses different types of biosurfactants and their production. Various applications of biosurfactants are reported especially emphasizing their antibacterial property.

Chapter 14 describes the chemical nature of biosurfactants and media composition required for microbial growth. Even genetic regulation and biosynthesis of surfactants are also discussed with a diverse group of genes. Additionally, the applications of biosurfactants in different industries like textile, leather, petroleum, cosmetic, household detergents, and washing soaps are also discussed.

Aligarh, India Inamuddin
Aligarh, India Mohd Imran Ahamed
Motihari, Bihar, India Ram Prasad

Contents

Application of Microbial Biosurfactants in the Food Industry

1

Italo José Batista Durval, Ivison Amaro da Silva,
and Leonie Asfora Sarubbo

Abstract

The food industry has evolved over the centuries, accompanying changes in dietary habits. Technologies have emerged to improve both the flavor and useful life of food products. The quest for efficient additives that do not affect the health of consumers, increase durability, offer nutraceutical advantages, and satisfy market niches has led to increasing research into natural alternatives for the replacement of synthetic additives. Biosurfactants emerge as a biocompatible solution with multiple functions that can be used as emulsifying, antimicrobial, antibiofilm, and antioxidant agents.

Keywords

Food additives · Food preservatives · Emulsifier · Antimicrobial · Antibiofilm · Antioxidant

1.1 Surfactants in the Food Industry

Technological advances in the nineteenth century enabled the manipulation of food products through the use of additives, favoring the mass production of foods with a pleasant flavor. This led to the further development and ever-increasing use of such

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I. J. B. Durval (✉) · I. A. da Silva
Northeast Biotechnology Network (RENORBIO), Federal Rural University of Pernambuco, Recife, Pernambuco, Brazil

Advanced Institute of Technology and Innovation (IATI), Recife, Pernambuco, Brazil

L. A. Sarubbo
Advanced Institute of Technology and Innovation (IATI), Recife, Pernambuco, Brazil

Centre of Science and Technology, Catholic University of Pernambuco, Recife, Pernambuco, Brazil

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additives. Moreover, changes in lifestyle in recent decades have transformed eating habits, with the increased incorporation of added ingredients to food products (Onaolapo and Onaolapo 2018).

Chemically synthesized surfactants are used in numerous food formulations. Biosurfactants have also been used for this purpose, such as lecithin and some proteins used in salad dressings and cake frosting. However, chemically synthesized surfactants are toxic, and, therefore, biosurfactants have gained ground due to their biodegradable nature and low toxicity, making these natural compounds more attractive as novel functional additives for use in the food industry (Sharma 2016).

There is a growing demand on the part of consumers for the replacement of more harmful synthetic products with less harmful natural products that perform the same functions. In this context, biosurfactants emerge as a biocompatible solution that can be used as emulsifying, antimicrobial, antibiofilm, and antioxidant agents (Table 1.1) with applications in the formulation of food products (Ranasalva et al. 2014).

Biosurfactants can be used in baked goods and ice creams during the cooking of fats and oils and for the control of consistency, extending the useful life of the product and solubilizing aromatic oils. Rhamnolipids improve the stability of dough as well as the volume, texture, and conservation of baked goods (Vijayakumar and Saravanan 2015).

1.1.1 Food Additives

According to the World Health Organization (2018), food additives are substances added to maintain or improve the safety, freshness, flavor, texture, and/or appearance of foods. Numerous additives have been developed over the years to fulfill the needs of food products, as large-scale food production is very different from making products on a small scale at home. Additives are needed to ensure that processed foods remain in a good state and safe to consume throughout their entire journey from factories or industrial kitchens and during transportation to warehouses and stores until finally reaching the consumer. Food additives are derived from plants, animals, or minerals or may be synthetic. There are thousands of additives—all of which are designed for a specific purpose, making foods safer or more attractive.

Among the fundamental principles of the use of additives, safety is paramount, and the adoption of the procedures necessary for the acquisition of innocuous, healthy foods is indispensable. Therefore, previous to authorization usage, an additive must be submitted to a satisfactory toxicological assessment, considering possible secondary effects such as accumulation, synergisms, or even protectiveness (Brazil 1997, 2002).

Authorization for the use of additives in the food industry and the inspection of these foods follow different standards depending on the country. This responsibility falls to the Food and Drug Administration (FDA) in the United States and the *Agência Nacional de Vigilância Sanitária* (Anvisa [National Health Surveillance

Table 1.1 Biosurfactants with uses in the food industry

Microorganism	Biosurfactant type	Function	Target	References
Bacillus sp. MTCC 5877	Glycolipid	Antiadhesive, antimicrobial	*E. coli*	Anjun et al. (2016)
Candida utilis	Carbohydrate-lipid-protein complex	Emulsifier	–	Campos et al. (2019)
A. piechaudii CC-ESB2	–	Emulsifier, antioxidant	–	Chen et al. (2015)
Pseudomonas aeruginosa ATCC-10145	Rhamnolipid	Antimicrobial	*S. lutea*, *M. luteus*, *B. pumilus*, *P. chrysogenum*, *C. albicans*	El-Sheshtawy and Doheim (2019)
Candida albicans SC5314	Sophorolipid	Emulsifier, antimicrobial	*Pseudomonas aeruginosa* (MTCC 424), *Escherichia coli* (MTCC 723), *Bacillus subtilis* (MTCC 441), and *Staphylococcus aureus* (MTCC 9886)	Gaur et al. (2019)
Candida glabrata CBS138	Sophorolipid	Emulsifier, antimicrobial	*Pseudomonas aeruginosa* (MTCC 424), *Escherichia coli* (MTCC 723), *Bacillus subtilis* (MTCC 441), and *Staphylococcus aureus* (MTCC 9886)	Gaur et al. (2019)
Bacillus licheniformis VS-16	Phospho-lipopeptide	Antibiofilm	*E. coli*	Giri et al. (2017)
Bacillus spp.	Surfactin	Antimicrobial	*Bacillus cereus*, *Listeria monocytogenes*, *Staphylococcus aureus*, *Streptococcus pneumoniae*, *Salmonella typhimurium*, *Serratia marcescens*, and *Klebsiella pneumoniae*	Isa et al. (2020)
Acinetobacter indicus M6	Glycolipoprotein	Antibiofilm, antimicrobial	*P. aeruginosa* ATCC 9027, *Staphylococcus aureus* ATCC 6538	Karlapudi et al. (2020)

(continued)

Table 1.1 (continued)

Microorganism	Biosurfactant type	Function	Target	References
Nesterenkonia sp.	Lipopeptide	Emulsifier, antioxidant, antibiofilm, antimicrobial	*Staphylococcus aureus*	Kiran et al. (2017)
Lactobacillus casei ATCC 393	–	Antioxidant, antibiofilm, antimicrobial	*S. aureus ATCC 6538, S. aureus* 9P, *S. aureus* 29P	Merghni et al. (2017)
Candida lipolytica UCP 0988	Rufisan	Antiadhesive, antimicrobial	*S. agalactiae*, *S. mutans*, *S. mutans* NS, *S. mutans* HG, *S. sanguis* 12, *S. oralis* J22	Rufino et al. (2011)
Pseudomonas spp.	Glycolipid	Antibiofilm	*Staphylococcus aureus*	Silva et al. (2017)
Lactobacillus pentosus	Glycolipopeptide	Emulsifier	–	Vecino et al. (2015)
Bacillus subtilis C19	Lipopeptide	Antimicrobial	*C. albicans*	Yuliani et al. (2018)
Bacillus subtilis SPB1	Lipopeptide	Antioxidant	–	Zouari et al. (2016)

Agency]) in Brazil. Countries belonging to economic blocks, such as the European Union, adopt norms determined by the union health board.

1.1.2 Biosurfactants as Food Preservatives

1.1.2.1 Emulsifying Agents

An emulsion is a mixture of different systems consisting of one or more immiscible liquids, which are spread in another in the form of droplets (Santos et al. 2016). These types of systems are characterized by low stability, which can be magnified by surfactants, thus reducing interfacial tension, consequently lessening the surface energy between the two phases, and forestalling the union of the particles by the formation of hysteric and electrostatic barriers (McClements and Gumus 2016).

In food, the emulsifier acts promoting the stability of the formed emulsion. The reduction of surface tension at the oil-water interface is the key point and results in the control of developed droplets, as well as in the stabilization of aerated systems. As a result, the emulsifiers work by improving the consistency and texture of the formulated food, promoting the solubilization of aromas as well. Another function is the increasing of shelf life. Therefore, emulsifiers are essential for food industry, in which water-oil foams and emulsions are often used (Satpute et al. 2018; Radhakrishnan et al. 2011; Patino et al. 2008).

Natural food emulsifying agents derived from plants, such as lecithin and gum arabic, already enjoy considerable participation and acceptance in the market. However, lecithin has functional limitations when employed in products submitted to modern processing conditions, such as microwave cooking and irradiation. Cream, butter, margarine, and mayonnaise are examples of emulsions.

The production of emulsifiers from microbial cultures is an alternative to existing additives, enabling the acquisition of more resistant products that meet the requirements of modern food processing technologies (Nitschke and Costa 2007). There are reports of the use of biosurfactants as emulsifiers for the processing of raw materials with applications in baked goods (affecting the rheological characteristics of dough) and processed meats (emulsification of fat).

Biosurfactants can be used as emulsifiers to control the clustering of fat globules, stabilize aerated systems, and improve the consistency of fatty products. Studies report the use of rhamnolipids to improve the properties of butter, croissants, and frozen pastries (Muthusamy et al. 2008). A bioemulsifier produced by *Candida utilis* was used in salad dressings (Campos et al. 2014, 2015), and a biosurfactant produced by *Bacillus subtilis* was used in the formulation of cookies (Zouari et al. 2016).

Microorganisms such as *Candida utilis*, *Candida valida*, *Hansenula anomala*, *Rhodospiridium diobovatum*, *Rhodotorula graminis*, *Klebsiella* sp., and *Acinetobacter calcoaceticus* and the alga *Porphyridium cruentum* were identified as robust producers of bioemulsifiers, presenting stability superior to commercial emulsifiers (Barros et al. 2007).

1.1.2.2 Antibiofilm Agents

A biofilm is a set of entangled microorganisms that reside within an extracellular polymer matrix adhered to a surface. About 5 to 35% of the biofilm is comprised of microorganisms, and the rest is extracellular matrix (Jamal et al. 2018).

In the food industry, bacterial biofilms are a potential source of contamination, the transmission of disease and the deterioration of food products. Thus, reducing the formation of biofilm on the surface of foods is of extreme importance to providing quality products to consumers (Campos et al. 2013). Methods for the prevention or eradication of biofilm encompass physical, chemical, or biological processes as well as the development of novel or modified packaging materials (Silva et al. 2017). Due to their considerable surface activity, biosurfactants are effective at avoiding the formation of biofilm (Sharma 2016).

Biosurfactants are suggested to reduce hydrophobic interactions, which diminish the hydrophobicity of the surface and impede the adherence of microbes (Mnif and Ghribi 2015). Therefore, biosurfactants have the ability to interrupt the formation of biofilm by controlling microbial interactions with interfaces and altering the chemical and physical conditions of the environment of the developing biofilm (Kiran et al. 2010).

A biosurfactant isolated from the bacterium *Lactobacillus paracasei* exhibited antibiofilm activity against *Candida albicans*, *Staphylococcus aureus*, *Staphylococcus epidermidis*, and *Streptococcus agalactiae*, which are all well-known food pathogens (Gudiña et al. 2010). A biosurfactant derived from *Bacillus licheniformis* reduced formation of *E. coli* biofilm by 54% (Giri et al. 2017). A biosurfactant produced by *Nocardiopsis* sp. MSA13 significantly interrupted the formation of biofilm by *Vibrio alginolyticus* (Kiran et al. 2014). A glycolipid produced by *Brevibacterium casei* significantly inhibited the production of biofilm by *Vibrio* spp., *E. coli*, and *Pseudomonas* spp. (Kiran et al. 2010).

1.1.2.3 Antimicrobial Agents

Numerous secondary metabolites derived from microorganisms have been described as antimicrobial agents. The quest for compounds with action against pathogens emerges from the need to inhibit the activity of these microorganisms, especially in the food industry. Conventional antibiotics no longer serve this purpose due to the occurrence of increasingly resistant pathogens (Sharma 2016).

Biosurfactants have been successfully used to inhibit or retard the development of common microorganisms in food products. The mechanism of action depends on the physicochemical characteristics of the bioactive compound. Some of the mechanisms described include a change in permeability, destabilization and rupture of the cell membrane, or destruction of protein conformations, with the alteration of vital functions, including the generation and transport of energy (Fracchia et al. 2015).

Anjun et al. (2016) performed tests with effective results using a biosurfactant produced by *Bacillus* sp. to inhibit the growth of *E. coli.* Yuliani et al. (2018) investigated a biosurfactant produced by *Bacillus subtilis* C19 and found activity against five pathogens (*Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Salmonella enterica typhi* and *Listeria monocytogenes*). Biosurfactants isolated from *Pediococcus acidilactici* and *Lactobacillus plantarum* demonstrated antimicrobial activity against *Staphylococcus aureus* CMCC26003 (Yan et al. 2019). Previously, some food-related pathogens, such as *Bacillus cereus*, *Staphylococcus aureus*, and *Micrococcus luteus* decreased their proliferation when rhamnolipids derived from *P. aeruginosa* were added in the culture (Costa et al. 2010).

1.1.2.4 Antioxidant Agents

Antioxidants are a class of food additive used to avoid lipid oxidation, thereby increasing the useful life of food products. The generation of toxic compounds and the development of rancidness and undesirable flavors are the negative effects of lipid peroxidation, leading to a reduction in the quality and safety of the product (Nitschke and Silva 2017).

The necessity of synthetic antioxidants replacement in food industry led the search of natural compounds with antioxidant potential. Biosurfactants have significant antioxidant activity and therefore have the potential to fulfill this purpose (Sharma 2016). Biosurfactants isolated from strains of *Lactobacillus casei* demonstrated satisfactory activity regarding the sequestering of DPPH free radicals, with a greater effect achieved when the concentration of the biosurfactant was increased (Merghni et al. 2017).

Yalçin and Çavuşoğlu (2010) suggest that a lipopeptide produced by *Bacillus subtilis* RWI could be used as a natural alternative antioxidant. The authors evaluated its antioxidant activity based on its redox power, the sequestration of DPPH, and the chelation of iron ions, concluding that the biocompound has good antioxidant capacity for the elimination of free radicals.

1.1.3 Industrial Prospects

The properties of versatility, biocompatibility, and sustainability have led to growing interest in biosurfactants, especially in the food industry, where there is a quest to discover novel compounds for use as gelling, emulsifying, or dispersing agents, such as xanthan gum and emulsan (McClements and Gumus 2016). In addition, increasing of shelf life and additional nutraceutical benefits can be achieved by the use of biosurfactants.

High production costs result primarily from inefficient bioprocessing methods as well as the use of expensive substrates, which account for up to 50% of final cost of the product. In order for biosurfactants to gain a significant portion of the market, there is a need for the use of inexpensive substrates that provide high yields, improvements in processing technologies to facilitate the recovery of the product, greater knowledge in manipulating the metabolism of biosurfactant-producing microorganisms, and the selection of biosurfactants for specific applications (Campos et al. 2013). The development of fermentation technologies will also increase the possibility of modifying the structure and function of biopolymers in a controllable manner, enabling the development of “designer biopolymers.”

New ingredients will be developed that can tolerate modern food processing techniques, such as ultrahigh temperatures, extrusion by microwave heating, etc., and can function adequately in new formulations with low salt, fat, and calorie contents. However, the success of food products and ingredients produced by biotechnology also depends on consumer acceptance. There is no doubt that new discoveries in biotechnology will offer solutions to the challenges faced by the food industry (Campos et al. 2013).

Despite the potential applications, the food industry does not yet employ biosurfactants as additives on a large scale. Moreover, the use of biosurfactants as novel ingredients in foods requires the approval of regulatory agencies.

References

Anjun F, Gautam G, Edgard G, Negi S (2016) Biosurfactant production through *Bacillus sp.* MTCC 5877 and its multifarious applications in food industry. Bioresour Technol 212:262–269. https://doi.org/10.1016/j.biortech.2016.02.091.

Barros FFC, Quadros CP, Maróstica Júnior MR, Pastore GM (2007) Surfactina: propriedades químicas, tecnológicas e funcionais para aplicações em alimentos. Quim Nova 30:409–414. https://doi.org/10.1590/S0100-40422007000200031

Brazil (1997) Ministry of Health by National Health Surveillance Agency. Resolution RDC no. 540. Accessed 27 Oct 1997

Brazil (2002) Ministry of Health by National Health Surveillance Agency. Resolution RDC no. 234. Accessed 19 Aug 2002

Campos JM, Stamford TLM, Sarubbo LA, Luna JM, Rufino RD, Banat IM (2013) Microbial biosurfactants as additives for food industries. Biotechnol Prog 29:1097–1108. https://doi.org/10.1002/btpr.1796.

Campos JM, Stamford TLM, Sarubbo LA (2014) Production of a bioemulsifier with potential application in the food industry. Appl Biochem Biotechnol 172:3234–3252. https://doi.org/10.1007/s12010-014-0761-1

Campos JM, Stamford TLM, Rufino RD, Luna JM, Stamford TCM, Sarubbo LA (2015) Formulation of mayonnaise with the addition of a bioemulsifier isolated from Candida utilis. Toxicol Rep 2:1164–1170. https://doi.org/10.1016/j.toxrep.2015.08.009

Campos JM, Stamford TLM, Sarubbo LA (2019) Characterization and application of a biosurfactant isolated from *Candida utilis* in salad dressings. Biodegradation 30:313–324. https://doi.org/10.1007/s10532-019-09877-8

Chen C, Lin T, Shieh Y (2015) Emulsification and antioxidation of biosurfactant extracts from Chinese medicinal herbs fermentation in vitro. J Biosci Bioeng 120:387–395. https://doi.org/10.1016/j.jbiosc.2015.02.010.

Costa SGVA, Nitschke M, Lépine F, Déziel E, Contiero J (2010) Structure, properties and applications of rhamnolipids produced by *Pseudomonas aeruginosa* L2-1 from cassava wastewater. Process Biochem 45:1511–1516. https://doi.org/10.1016/j.procbio.2010.05.033

El-Sheshtawy HS, Doheim MM (2019) Selection of *Pseudomonas aeruginosa* for biosurfactant production and studies of its antimicrobial activity. Egypt J Petrol 23:1–6. https://doi.org/10.1016/j.ejpe.2014.02.001

Fracchia L, Banat JJ, Cavallo M, Ceresa C, Banat IM (2015) Potential therapeutic applications of microbial surface-active compounds. AIMS Bioeng 2:144–162. https://doi.org/10.3934/bioeng.2015.3.144

Gaur VK, Regar RK, Dhiman N, Gautam K, Srivastava JK, Patnaik S, Kamthan M, Manickam N (2019) Biosynthesis and characterization of sophorolipid biosurfactant by *Candida* spp.: application as food emulsifier and antibacterial agent. Bioresour Technol 285:121314. https://doi.org/10.1016/j.biortech.2019.121314

Giri SS, Sen SS, Jun JW, Sukumaran V, Park SC (2017) Role of Bacillus licheniformis VS16-derived biosurfactant in mediating immune responses in carp rohu and its application to the food industry. Front Microbiol 8:2–13. https://doi.org/10.3389/fmicb.2017.00514

Gudiña EJ, Teixeira JA, Rodrigues LR (2010) Isolation and functional characterization of a biosurfactant produced by *Lactobacillus paracasei*. Colloids Surf 76:298–304. https://doi.org/10.1016/j.colsurfb.2009.11.008

Isa MHM, Shamsudin NH, Al-Shorgani NKN, Alsharjabi FA, Sl Kalil M (2020) Evaluation of antibacterial potential of biosurfactant produced by surfactin-producing *Bacillus* isolated from selected Malaysian fermented foods. Food Biotechnol 34:1–24. https://doi.org/10.1080/08905436.2019.1710843

Jamal M, Ahmad W, Andleeb S, Jalil F, Imran M, Nawaz MA, Hussain T, Ali M, Rafiq M, Kamil MA (2018) Bacterial biofilm and associated infections. J Chin Med Assoc 81:7–11. https://doi.org/10.1016/j.jcma.2017.07.012

Karlapudi AP, Venkateswarulu TC, Srirama K, Kota RK, Mikkili I, Kodali VP (2020) Evaluation of anti-cancer, anti-microbial and anti-biofilm potential of biosurfactant extracted from an A*cinetobacter* M6 strain. J King Saud Univ Sci 32:223–227. https://doi.org/10.1016/j.jksus.2018.04.007

Kiran GS, Sabarathnam B, Selvin J (2010) Biofilm disruption potential of a glycolipid biosurfactant from marine *Brevibacterium casei*. FEMS Immunol Med Microbiol 59:43–438. https://doi.org/10.1111/j.1574-695X.2010.00698.x

Kiran GS, Nishanth LA, Priyadharshini S, Anitha K, Selvin J (2014) Effect of Fe nanoparticle on growth and glycolipid biosurfactant production under solid state culture by marine *Nocardiopsis sp.* MSA13A. BMC Biotechnol 14:48. https://doi.org/10.1186/1472-6750-14-48

Kiran GS, Priyadharshini S, Sajayan A, Priyadharsini GB, Poulose N, Selvin J (2017) Production of lipopeptide biosurfactant by a marine *Nesterenkonia* sp. and its application in food industry. Front Microbiol 8:1138. https://doi.org/10.3389/fmicb.2017.01138.

McClements DJ, Gumus CE (2016) Natural emulsifiers—biosurfactants, phospholipids, biopolymers, and colloidal particles: molecular and physicochemical basis of functional performance. Adv Colloid Interface 234:3–26. https://doi.org/10.1016/j.cis.2016.03.002

Merghni A, Dallel I, Noumi E, Kadmi Y, Hentati H, Tobji S, Amor A, Mastouri M (2017) Antioxidant and antiproliferative potential of biosurfactants isolated from *Lactobacillus casei* and their anti-biofilm effect in oral *Staphylococcus aureus* strains. Microb Pathog 104:84–89. https://doi.org/10.1016/j.micpath.2017.01.017

Mnif I, Ghribi D (2015) Review lipopeptides biosurfactants: mean classes and new insights for industrial, biomedical, and environmental applications. Biopolymers 104:129–147. https://doi.org/10.1002/bip.22630

Muthusamy K, Gopalakrishnan S, Ravi TK, Sivachidambaram P (2008) Biosurfactants: properties, commercial production, and application. Curr Sci 94:736–747

Nitschke M, Costa S (2007) Biosurfactants in food industry. Trends Food Sci Technol 18:252–259. https://doi.org/10.1016/j.tifs.2007.01.002.

Nitschke M, Silva SS (2017) Recent food applications of microbial surfactants. Crit Rev Food Sci Nutr 58:631–638. https://doi.org/10.1080/10408398.2016.1208635

Onaolapo AY, Onaolapo OJ (2018) Food additives, food and the concept of 'food addiction': is stimulation of the brain reward circuit by food sufficient to trigger addiction? Pathophysiology 25:263–276. https://doi.org/10.1016/j.pathophys.2018.04.002

Patino JMR, Sánchez CC, Niño MMR (2008) Implications of interfacial characteristics of food foaming agents in foam formulations. Adv Colloid Interface 140:95–113. https://doi.org/10.1016/j.cis.2007.12.007

Radhakrishnan N, Kavitha V, Madhavacharyulu E, Gnanamani A, Mandal AB (2011) Isolation, production and characterization of bioemulsifiers of marine bacteria of coastal Tamil Nadu. Indian J Mar Sci 40:76–82

Ranasalva N, Sunil R, Poovarasan G (2014) Importance of biosurfactant in food industry. IOSR JAVS 7(5):6–9. e-ISSN: 2319-2380

Rufino RD, Luna JM, Sarubbo LA, Rodrigues LRM, Teixeira JAC, Campos-Takaki GM (2011) Antimicrobial and anti-adhesive potential of a biosurfactant Rufisan produced by *Candida lipolytica* UCP 0988. Colloids Surf B Biointerfaces 84:1–5. https://doi.org/10.1016/j.colsurfb.2010.10.045

Santos DKF, Luna JM, Rufino RD, Santos VA, Sarubbo LA (2016) Biosurfactants: multifunctional biomolecules of the 21st century. Int J Mol 17:401–430. https://doi.org/10.3390/ijms17030401

Satpute SK, Zinjarde SS, Banat IM (2018) Recent updates on biosurfactants in the food industry. In: Microbial cell factories. CRC, Boca Raton, FL, pp 1–20. https://doi.org/10.1201/b22219-1

Sharma D (2016) Applications of biosurfactants in food. In: Sharma D (ed) Biosurfactants in food. Springerbriefs in food, health, and nutrition. Springer, Berlin, pp 43–80. https://doi.org/10.1007/978-3-319-39415-2_3

Silva SSE, Carvalho JWP, Aires CP, Nitschke M (2017) Disruption of *Staphylococcus aureus* biofilms using rhamnolipid biosurfactants. J Dairy Sci 100:7864–7873. https://doi.org/10.3168/jds.2017-13012

Vecino X, Barbosa-Pereira L, Devesa-Rey R, Cruz JM, Moldes AB (2015) Optimization of extraction conditions and fatty acid characterization of *Lactobacillus pentosus* cell-bound biosurfactant/bioemulsifier. J Sci Food Agric 95:313–320. https://doi.org/10.1002/jsfa.6720

Vijayakumar S, Saravanan V (2015) Biosurfactants-types, sources and applications. Res J Microbiol 10:181–192. https://doi.org/10.3923/jm.2015.181.192

World Health Organization (WHO) (2018) Food additives. https://www.who.int/news-room/fact-sheets/detail/food-additives. Accessed 27 Nov 2018

Yalçin E, Çavuşoğlu K (2010) Structural analysis and antioxidant activity of a biosurfactant obtained from *Bacillus subtilis* RW-I. Turk J Biochem 35:243–247

Yan X, Gu S, Cui X, Shi Y, Wen S, Chen H, Ge J (2019) Antimicrobial, anti-adhesive and anti-biofilm potential of biosurfactants isolated from *Pediococcus acidilactici* and *Lactobacillus* plantarum against *Staphylococcus aureus* CMCC26003. Microb Pathog 127:12–20. https://doi.org/10.1016/j.micpath.2018.11.039

Yuliani H, Perdani MS, Savitri I, Manurung M, Sahlan M, Wijanarko A, Hermansyah H (2018) Antimicrobial activity of biosurfactant derived from *Bacillus subtilis* C19. Energy Procedia 153:274–278. https://doi.org/10.1016/j.egypro.2018.10.043

Zouari R, Besbes S, Ellouze-Chaabouni S, Ghribi-Aydi D (2016) Cookies from composite wheat–sesame peels flours: dough quality and effect of Bacillus subtilis SPB1 biosurfactant addition. Food Chem 194:758–769. https://doi.org/10.1016/j.foodchem.2015.08.064

Microbial Biosurfactants for Contamination of Food Processing

2

Isadora Ferreira da Silva, Nathália Andrade Neves,
Bianca Marise Pereira e Silveira, Kele Aparecida Costa Vespermann,
and Maria Emília Rodrigues Valente

Abstract

Food security is one of the biggest concerns in food processing. In recent decades, the effects of contaminants in food crops are currently compromising food security and human health. Food contamination can be microbiological, chemical and physical. It can occur on different steps of food processing, such as transport, storage and packaging of raw or processed food, as well as during heating processes. Therefore, substances including the additives can help to maintain the food security during food processing until it reaches the consumers. They can provide food preservation, maintaining freshness and preventing bacterial contamination, among many others. The additives from natural sources have been receiving more attention from food manufacturers when compared to the synthetic ones, due to their higher quality and safety. An example of natural additives are biosurfactants, which are derived from microorganisms. Interests in the use of biosurfactants have been increasing in the food market, as a result of their capacity to replace synthetic additives in the food industry. The objective of this chapter is to present the main microbial biosurfactants used to avoid contamination during food processing. We briefly discuss their potential applications as food preservatives, presenting antimicrobial, antioxidant and antibiofilm activities against food pathogens.

I. Ferreira da Silva (✉)
Department of Applied Biochemistry, Federal University of Viçosa—UFV, Viçosa, MG, Brazil

N. Andrade Neves · B. M. Pereira e Silveira · K. A. Costa Vespermann
Institute of Science and Technology, Federal University of Valleys of Jequitinhonha and Mucuri—UFVJM, Diamantina, MG, Brazil

M. E. Rodrigues Valente
Department of Food Engineering, Federal University of Espírito Santo—UFES, Alegre, ES, Brazil

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Biosurfactants · Microorganisms · Contamination · Additives · Food processing · Biofilms

Summary This chapter presents a brief description of the main contaminants in food processing and the biosurfactants applied in avoiding them. It is focused on the application of glycolipids and lipopeptides as the main classes of microbial biosurfactants, as preservatives, antimicrobial, antioxidant and antibiofilm agents during food processing.

2.1 Introduction

2.1.1 Food Contamination

The origins of food contaminants can be microbiological, physical or chemical. Data from the World Health Organization informs around 600 million people get sick after eating contaminated food, causing 420,000 deaths per year (World Health Organization 2019). Understanding the origin and when it happens during food processing can contribute to a more effective control and avoid contamination.

Chemical contaminants are one of the serious sources of food contamination. They include the environmental contaminants (organic pollutants, heavy metals, pesticides, disinfectants, detergents, deodorants, veterinary products); food processing contaminants that are formed during heating, cooking or packaging (such as furanes, polycyclic aromatic hydrocarbons and acrylamide) and processing contaminants that are released during cooking, processing or packaging (McKay and Scharman 2015).

In a microbiological contamination, bacteria, virus and parasites can spread in food and cause harm to humans. The most common sources of microbiological contamination include *Salmonella* spp., *Bacillus cereus*, *C. perfringens*, *E. coli*, *Shigella* spp., *Listeria monocytogenes*, *Clostridium botulinum*, *Yersinia enterocolitica* and *Vibrio cholera* (Hussain 2016). There is also the physical contamination, which happens when any foreign matter enters food and causes illness or injury when consumed. Examples of physical hazards could include glass, metal and plastic fragments, hair, fingernails, dirt and insects (Echavarri-Bravo et al. 2017; Aguiar et al. 2018).

The contamination could occur naturally or be introduced by humans. A significant amount of food contamination can occur during food processing steps, packaging, transportation and storage of raw or processed foods (Rather et al. 2017).

2.1.2 Contamination in Food Processing

Food processing involves activities for the conversion of raw food into more stable final products. Its goal is to increase the quality, nutritive value, taste and shelf life. The main stages of food processing include heating, packaging, storage, distribution and transport (Sethu and Ananth Viramuthu 2008). In order to avoid contamination in food processing, it is important to identify the most likely contaminants from each of these stages.

The environment of food processing inside the industry is usually one of the main sources of contamination. The exposure to contaminated surfaces plays a major role in possible food contamination in the industries. Equipment, utensils, hallways, workbenches and pipes are the main sources of contamination in industries. These contaminants can be in contact with the food or can be transferred to the food by air, other materials and people. Employees can contaminate the food during its processing through the direct transfer of microorganisms from their body to the food or by carrying them from a contaminated area to another (Masotti et al. 2019). The equipment design is also important in avoiding contamination as it influences the cleaning and rinsing operations and therefore the removal of contaminants (Faille et al. 2018). Good hygiene practices can limit the microbial contamination, decreasing cross-contamination and formation of biofilms. Once it is formed, the biofilm becomes a source of contamination, and it is considered one of the main concerns for the food industry (Carrasco et al. 2012).

In addition to the processing of food in industries, food contamination can also take place during transportation, including the transportation of raw food to the industries and the processed food from the industries to the stores. Around 200 billion metric tons of food is transported in the world every year; 35% is transported by land, 60% by sea and 5% by air. The possibility of contamination during transport and storage of food is highlighted by the large amount of food transported, along with the handling requirements for each food product (Ackerley et al. 2010). Food contamination during transport can occur from vehicle exhausts of fuel or due to cross-contamination. This contamination can often happen in long-distance ships, through the use of chemicals present in cleaning products or other sources (Nerín et al. 2016). The packages used in food are often not tested to resist and protect the food for transportation across long distances, leading to food contamination (Rather et al. 2017). A good example of cross-contamination happened in 1994 when around a quarter of a million people got gastroenteritis after eating Schwan's ice cream. The ice cream contained *Salmonella* that came from eggs that were earlier transported using the same trucks (Hennessy et al. 1996).

Heating treatment is the most recognized and used approach for food process in the industry or at home. The heating combined with external factors during cooking causes the release of toxic substances (Bordin et al. 2013). These compounds including acrylamide, furanes, nitrosamines and chloropropanols are generated during heating, cooking, fermentation, canning and backing (Nerín et al. 2016). For example, when using a microwave, the packaging material used such as plastics, paperboard and composites can have their components migrated to the food during heating. Fasano et al. (2015) detected significant amounts of plastic components (phthalates, bisphenol A (BPA), polybrominated diphenyl ethers (PBDE) and tetrabromobisphenol A (TBBPA)) after using a microwave to heat food in a polyethylene packaging during 3 min at 800 W.

The contamination during food storage is mainly due to changes in the storage conditions (high temperature and humidity) that can affect the packaging material properties (Nerín et al. 2016). Direct sunlight and packaging can accelerate the deterioration of food, causing an adsorption of unwanted off odours (Rather et al. 2017). Another contamination factor is the moisture, which can increase the susceptibility of the food to microbial contamination, leading to modifications of its texture and decreasing shelf life (Gaikwad et al. 2019). The contamination also depends on the type of food. For example, dry and canned foods usually present a long shelf life; however, they may deteriorate in colour, flavour and nutritive value over time (McCurdy et al. 2009). Fresh meat, such as seafood, beef and poultry should be maintained at low temperatures in a freezer and refrigerator. Choi et al. (2020) demonstrated that storing beef at 4 °C was essential for the maintenance of food quality, reducing significantly the growth of pathogens such as *E. coli* when compared to storage at 25 °C.

These changes during storage also depend on the packaging material, as it should exhibit very good barrier properties. The packaging needs to provide physical protection and helps with the increase in the shelf life of the product. The use of stabilizers, plasticizers, antioxidants and shipping agents are common in packaging processes to enhance the characteristics of the packaging material (Conte et al. 2013). However, some substances from the packaging material can be transferred to the food and can cause health risks to the consumers if they have toxic effects (Lau and Wong 2000). Therefore, strict legislation is applied worldwide for the use of substances in packaging materials. Adverse effects can be also caused by substances that were not intentionally added to the food packaging and are present in food. They can come from the packaging under a heating process as mentioned before and be generated from reactions of substances present in the packaging or by the reaction of substances with foodstuffs. Bauer et al. (2019) identified 50, in which 8 were NIAS, in baby food from contact of the food with the polyurethane layer of the plastic multilayer packaging.

The use of preservatives in foodstuff is crucial to prevent contamination and deterioration of food. These substances can be used during transportation, processing, storage and packaging of food. Benzoates, sulphites, sorbates, propionates, nitrites and parabens are the most used antimicrobials used in food. Regarding the synthetic antioxidants, *tert*-butylhydroquinone (TBHQ), butylated hydroxytoluene (BHT), propyl gallate and butylated hydroxyanisole (BHA) are considered as the most common (Carocho et al. 2015).

Although studied for decades, the use of these synthetic preservatives to avoid food contamination and deterioration can cause health problems and negatively affect the environment (Botterweck et al. 2000; Iammarino et al. 2013; Vandghanooni et al. 2013). For this reason, the interest in natural, safe and environmentally friendly preservatives has increased. Among these, the microbial surfactants have been extensively studied during the last few years. From this point on, we will discuss the use and relevance of the microbial biosurfactants in preventing contamination during food processing.

2.2 Microbial Biosurfactants Use in Food Processing

Surfactants are compounds that create micelles in a solution and adsorb to the interfaces between a solution and a different phase (gases-liquids, liquid-liquid) leading to reductions in the tension surface. This is possible because of the two different functional groups with different affinity within their molecule. The surfactants are composed of amphiphilic molecules, with a chemical structure consisting of a hydrophilic group (carbohydrates or amino acids) and a hydrophobic one (fatty acids). In food processing, the microbial surfactants can be used as emulsifying, antimicrobial, anti-adhesive, antibiofilm and antioxidant agents (Sharma et al. 2018).

The microbial surfactants present a broad variety of compounds produced by bacteria, yeast or fungi. They are favoured over the synthetic ones in this industry due to the non-toxicity nature, excellent biodegradability, high surface/interfacial activity, biocompatibility, stability under extreme conditions of pH, temperature and sanity (Nitschke and Silva 2018; Parthasarathi and Subha 2018).

The production of biosurfactants has been extensively studied. Inexpensive raw substrates available in high amounts can be used to produce biosurfactants. Agricultural and industrial wastes, by-products including hydrocarbons and oil waste can be used for the production of these compounds, enhancing the cost-effectiveness of the biosurfactant production process. The biosurfactants are generally extracellularly produced (Inès and Dhouha 2015a). Downstream and recovery process has also been widely investigated, since the most methods used increase significantly the costs of biosurfactant production process (Jimoh and Lin 2019). Improvement of microbial strain by using genetic strategies, optimization of media composition and development of scaled-up methods, among others, can also help in achieving a viable commercial production of high quantities of biosurfactants.

In food processing compounds like phospholipids, fatty acids, lipoheteropolysaccharides and protein-sugar-lipid complex molecules are used as biosurfactants (Nitschke and Silva 2018). They can be divided in classes according to their chemical composition, molecular weight, physicochemical properties and microbial source (Naughton et al. 2019). The high molecular weights are amphipathic polysaccharides, proteins, lipopolysaccharides and lipoproteins. The main group is comprised of compounds with lower molecular weights, including fatty acids, glycolipids, lipopeptides and phospholipids. Among these, glycolipids and lipopeptides are the most widely applied to avoid contamination in food processing.

2.2.1 Glycolipids

Glycolipids are the most popular group of biosurfactants. It presents carbohydrate and a fatty acid as the hydrolytic and hydrophobic portions, respectively (Abdel-Mawgoud and Stephanopoulos 2018). When compared with synthetic surfactants, the natural glycolipids present better surfactant activity. According to Liu et al. (2020), the natural glycolipids present a higher molecular richness than the synthetic ones. They present a distinctive distribution of the polarity groups over the glycolipid molecule and more branched structures in comparison with the synthetic glycolipids (Abdel-Mawgoud and Stephanopoulos 2018). However, the industrial production and application in large scale of these natural surfactants are not viable yet. As it is hard to separate and purify these natural surfactants, it is still not possible to fully understand their structure-activity relationships (Liu et al. 2020).

The glycolipids can be categorized into subclasses according to the carbohydrates and lipid portions, as follows: rhamnose lipids, trehalose lipids, sophorose lipids, cellobiose lipids, mannosylerythritol lipids (MEL), lipomannosyl-mannitols, lipoarabinomannans, lipomannans, diglycosyl diglycerides, monoacylglycerol and galactosyl diglyceride (Mnif and Ghribi 2016). Among these, rhamnolipids, sophorolipids and trehalolipids are the best known subclasses of glycolipids.

Rhamnolipids are an extensively studied biosurfactant. They are characterized by rhamnose molecules linked to β-hydroxydecanoic acid molecules (Inès and Dhouha 2015a). Their production was first described using *P. aeruginosa* in 1949. The fatty acids present in the rhamnolipid molecules ranges from 8 to 16 carbons. The β-hydroxydecanoic acid is predominant in the rhamnolipid produced by *P. aeruginosa*, whereas β-hydroxytetradecanoic acid is mostly found in the molecules produced by *Burkholderia* sp. (Henkel et al. 2012).

Due to the several advantages of the biosurfactants such as rhamnolipids, many companies have shown interests in exploiting the production of this biosurfactant as follows: Jeneil biosurfactant (USA), Paradigm Biomedical Inc. (USA), GlycoSurf LLC (USA), AGAE Technologies LLC (USA) Logos Technologies LLC (USA), Rhamnolipids Companies Inc. (USA), TeeGene Biotech Ltd. (United Kingdom) and Urumqi Unite Bio-Technology Co., Ltd. (China).

Regarding the substrates used for rhamnolipids, the most commonly ones are plant oils, sugars and glycerol. It has been found on literature, the utilization of oil wastes such as vegetable oil, palm oil, mango kernel oil, glycerol and glycerin, among others (Table 2.1).

Investigations to optimize the costs and the production of these compounds are important to enable bioprocesses, considering the potential associated with rhamnolipid. Genetic engineering has been used in order to improve its synthesis. Boles et al. (2005) used *Pseudomonas aeruginosa*, and strains with the insertion/exclusion of rhlAB, for the production of rhamnolipid using a complex medium (Na_2HPO_4; KH_2PO_4; NaCl; $CaCl_2$; $MgSO_4$; glucose, glutamate), for 48 h at 37 °C. The rhamnolipid yield improved from 0.1 mg/mL to 0.5 mg/mL when using a genetically modified strain in comparison with the wild strain. Zheng et al. (2020) also achieved an increase of 32.63% on the rhamnolipid yield by *Pseudomonas*

Table 2.1 Conditions of fermentation for production of glycolipids by microorganisms

Biosurfactant	Microorganism	Process conditions	Yield	Reference
Sophorolipids	*Rhodotorula babjevae* YS3	10% glucose, 19 °C, 200 rpm, 72 h, 5% inoculum	19.0 g/L	Sen et al. (2017)
Sophorolipids	*Candida albicans*	30 °C, 72 h, 2% glucose, 150 rpm	1320 mg/L	Gaur et al. (2019)
Trehalose	*Rhodococcus fascians*	28 °C, 24 h, Davis minimal media	0.14 mg/mL	Janek et al. (2018)
Mannosylerythritol lipid	*Pseudozyma aphidis*	28 °C, 180 rpm, soybean oil, 240 h	61.50 g/L	Niu et al. (2019)
Sophorolipids	*C. Bombicola*	20 g/L residual oleic acid, 30 °C, 450 rpm, 288 h, 10% inoculum	69.83 g/L, 0.24 g/L/h	Silveira et al. (2019)
Sophorolipid	*C. Bombicola*	200–800 rpm, 50 g/L rapeseed oil, 25 °C, 1023 h	20.22 g/g 1.07 g/L/h	Dolman et al. (2017)
Sophorolipids	*C. Bombicola*	25 °C, 200 rpm, 48 h, 168 h, 1–5 g/L rapeseed oil, 20–60 g/L glucose	11.4 g/g 1.45 g/L/h	Liu et al. (2019)
Sophorolipids	*C. Bombicola*	5 mL food waste hydrolysate, 100–120 g/L glucose 24 h, 30 °C, 150 rpm, 9% inoculum	0.26 g/g 0.39 g/L/h	Kaur et al. (2019)
Rhamnolipid	*Pseudomonas stutzeri*	46.55 g/1 glycerol, 150 rpm, 30 °C, 144 h	4.78 g/L	Sheikh et al. (2019)
Rhamnolipid	*P. aeruginosa*	25.4–116.0 g/L glycerin, 30 °C, 170 rpm, 288 h	17.6 g/L	Dobler et al. (2020)
Rhamnolipid	*P. aeruginosa*	12% rice-based distillers' dried grains with solubles, 48 h, 35 °C, 180 rpm	14.87 g/L	Borah et al. (2019)
Rhamnolipid	*P. guguanensis*	Vegetable oil, peptone + yeast extract 150 rpm, 30 °C, 168 h,	12.5 mg/mL	Ramya Devi et al. (2018)
Rhamnolipid	*P. aeruginosa*	10 g/L palm oil waste, glucose, 37 °C, 36 h	3.4 g/L	Radzuan et al. (2018)
Rhamnolipid	*P. aeruginosa*	Mango kernel oil, glucose, 120 h, 30 °C	2.8 g/L	Reddy et al. (2016)

aeruginosa after performing the fermentation in a 1.5 L bioreactor through a new continuous production process, based on a cyclic fermentation coupled with the fractionation of foam when compared to the process performed in a decoupled system.

Sophorolipid structure comprises a hydroxyl fatty acid and a disaccharide sophorose linked by β-1, 2 bond (Varjani and Upasani 2017). This biosurfactant can present an acidic structure, when the fatty acids' carboxylic end is free, or a lactonic ring structure, when this end is esterified (Sen et al. 2017). The composition of the sophorolipids such as the length of carbon chain and the fatty acid structure and the proportion of the acidic and lactonic forms depend on factors such as medium composition, environment conditions (pH, temperature, aeration) and strain used for their production (Díaz De Rienzo et al. 2015; Oliveira et al. 2015).

Different from rhamnolipids, sophorolipids are synthesized by non-pathogen yeast strains. The most common ones used are *Centrolene petrophilum*, *Candida bombicola*, *Rhodotorula bogoriensis* and *Candida apicola* (Banat et al. 2010). Díaz De Rienzo et al. (2015) obtained a mixture of 45% (v/v) of acidic and lactonic congeners of sophorolipids using the strain *Candida bombicola* ATCC 22214, in a process conducted at 30 °C, using 10% (w/v) glucose, 1% (w/v) yeast extract and 0.1% (w/v) urea (GYU medium). The same strain was also used in a process conducted with a working volume of 10 L, using *Candida* growth media for 7 days at 26 °C, achieving around 90% of acidic and lactonic congeners of sophorolipids (Zhang et al. 2016).

The sophorolipids usually present antimicrobial and antibiofilm activities important to avoid contamination in food processing (Sharma et al. 2018; Jimoh and Lin 2019). The production of this surfactant has shown greater interest from the companies when compared to the rhamnolipids (Sen et al. 2017). The current main companies exploring the production of this biosurfactant are Saraya (Japan),

Ecover Eco-Surfactant (Belgium), Groupe Soliance (France), MG Intobio Co., Ltd. (South Korea) and SyntheZyme LLC (USA).

Trehalolipids is another glycolipid biosurfactant, which is composed of a disaccharide trehalose connected at C_6 to long-chain α-spread and β-hydroxy unsaturated fats (Varjani and Upasani 2017). This subclass of biosurfactants is considered chemically stable. Changes on pH values, salt concentrations and temperature usually do not cause any modification in their surface activities (Kitamoto et al. 2009). The first biosurfactant of this subclass was a trehalose dimycolate (TDM) described in 1930s and obtained by *Mycobacterium tuberculosis*. It plays a major role in infections caused by this pathogen (Kuyukina et al. 2015). The TDM was later found to be also produced by the genus *Nocardia* and *Corynebacterium*.

Similar to sophorolipids, the structure of the trehalolipids such as the size and structure of fatty acids, the quantity of carbon molecules and the degree of unsaturation depend on the strain and growth conditions used (Roy 2017). This subclass presents a high variety of structure. In addition to trehalose dimycolate (TDM), the trehalose trimycolates, mono-, tetra- and octa-acylated derivatives also represent anionic trehalose-type molecules (Niescher et al. 2006).

Trehalolipids can be produced by different microorganisms, including *Nocardia*, *Williamsia*, *Mycobacterium*, *Corynebacterium, Dietzia, Gordonia*, *Tsukamurella*, *Skermania* and *Rhodococcus*. The most widely studied subclass is trehalose dimycolates obtained from *Rhodococcus erythropolis* (Banat et al. 2010). The trehalolipids obtained from *Rhodococcus* are commonly involved in adhesion to surfaces and increase solubility of hydrophobic compounds. They assist the cells in accessing hydrophobic substrates, by promoting a contact between the cells and the substrates or by indirect contact through the adhesion to the emulsified oil (Bages-estopa et al. 2018).

Table 2.1 presents the microorganisms used, cultivation conditions and fermentation strategies in the production of glycolipids.

2.2.2 Lipopeptides

Lipopeptides are consisted of a fatty acid portion connected to a peptide chain. They can be divided into three different subclasses, depending on the sequence of amino acids presented: surfactins (cyclic lipoheptapeptide linked to a β-hydroxy-fatty acid group), iturins (heptapeptides cyclized by amide bond formed between the α-COO group of the seventh amino acid and the β-NH_2 group of the β-fatty acid) and fengycins (β-hydroxy fatty acid connected to a peptide domain, composing of 10 amino acids. 8 of them are presented in a cyclic structure) (Hentati et al. 2019).

Surfactin is the most widely studied natural lipopeptides. It is characterized as highly surface active and water soluble, and it is consisted of four isomers (surfactin A–D). This biosurfactant is considered as cyclic lipopeptides, comprising of a cyclic heptapeptide structure connected with a fatty acid containing 13–15 carbons. The type of microorganism and culture conditions during its production will influence on the amino acids composition and fatty acids presented in its molecule. The Asp and Glu residues are generally placed in the heptapeptide ring. The ring then presents a saddle shape containing two negative residues on each top end (Liu et al. 2020). Surfactin can be produced by a variety of gram-positive strains of endospore-producing, *Bacillus subtilis* (de Araujo et al. 2011).

A concentration of 20 μM of surfactin can cause a decrease in surface tension of water from 72 to 27 mN/m, which is significantly lower when compared to the reductions in surface tensions of most biosurfactants found in literature. Surfactin also presents a critical micelle concentration (CMC) of 23 mg/L in water, which is significant below the CMC of other biosurfactants (Chen et al. 2015). Surfactin has been considered as antiviral, antibacterial, antifungal, anti-mycoplasma and antibiofilm on metallic and polypropylene surfaces and also has haemolytic properties, presenting cation-carrier and pore-forming effects (Banat et al. 2010; Chen et al. 2015; Sharma et al. 2018; Meena et al. 2020).

The commercial surfactin produced by Kaneka Corporation (Japan) and Soft Chemical Laboratories (South Africa) are used for contamination in food processing.

Table 2.2 Conditions of fermentation for production of lipopeptides by microorganisms

Biosurfactant	Microorganism	Process conditions	Yield	Reference
Surfactin	*Bacillus subtilis*	30 °C, Luria-Bertani broth, 200 rpm, 72 h	547.0 mg/L	Meena et al. (2020)
Surfactin	*B. tequilensis*	30 g/L sucrose, 30 °C, 200 rpm, 48 h	1879.43 ± 30.4 mg/L	Singh and Sharma (2020)
Fengycin	*B. subtilis*	30 °C, 200 rpm, 16 h, 26.2 g/L mannitol, 21.9 g/L soybean meal	3.5 g/L	Wei et al. (2010)
Iturin A	*B. amyloliquefaciens*	30 g/L corn starch, 70 g/L soybean meal, 28 °C, 230 rpm, 72 h	2013.43 ± 32.86 mg/L	Xu et al. (2020)
Surfactin	*B. amyloliquefaciens and B. subtilis*	200 g/L distillers' grains, 30 °C, 160 rpm, 96 h	3.4 g/L	Zhi et al. (2017)
Iturin	*B. amyloliquefaciens*	4% sunflower oil cake, 1% inoculum, 37 °C, 48 h, 180 rpm	819 mg/L	Kumar et al. (2017)
Surfactin	*B. subtilis*	2 g/L mg-Al-layered double hydride, 10 g/L sucrose, 200 rpm, 30 °C 120 h	3.8 g/L	Kan et al. (2017)
Surfactin	*B. subtilis*	Trypticase soy broth 32 °C, 170 rpm, 96 h	99.6 ± 1.38 mg/L	Alvarez et al. (2020)

The lipopeptides are generally produced by bacteria, moulds or yeast (Inès and Dhouha 2015b). *Bacillus* species are the most known microorganism used in the production of surfactin, iturin and fengycin (Table 2.2).

2.3 Application of Microbial Surfactants in Food Processing

Food security is one of the biggest concerns in food processing. Therefore, the main goal of food processing is to obtain products that are safe and have good organoleptic properties. The use of natural additives instead of synthetic ones, as well as an increase in environmental requirements and health concerns, has raised the need for natural additives in food (Nitschke and Silva 2018). Therefore, the antioxidant, antimicrobial and anti-adhesive properties of the microbial biosurfactants make them possible to be used as additives in the food industry to prevent contamination during food processing.

2.3.1 Biofilm Control

Biofilms are highly organized multicellular communities composed of microorganisms enclosed within an extracellular polysaccharide matrix. The dynamic process of biofilms is an important strategy for the microbes as a mechanism of resistance and survival against antibiotics and host defence mechanisms (Gebreyohannes et al. 2019). During the formation of biofilms, microorganisms can adhere to the surfaces that come into contact with the food, thus leading to undesirable alterations in the sensory properties of the final product (Zeraik and Nitschke 2010; Sharma et al. 2018).

Biofilm formation is composed of three phases: adherence, maturation and dispersion. For example, in *P. aeruginosa*, they are (1) formation of a layer and irreversible adhesion of microorganisms to its surface, (2) microcolony formation with the appearance of multilayers and (3) dispersal of bacteria cells which may then colonize other areas (Gebreyohannes et al. 2019).

The presence of biofilms in food contact or food processing surfaces can cause transmission of food-borne diseases, contamination by non-starter cultures, advanced food deterioration and metal loss with the deterioration of pipelines used for food transport and tanks for food storage (Sharma et al. 2018). Its formation can be avoided by using biochemical and physical cleaning strategies. However, once it is formed, biofilm is resistant to antimicrobial agents and mechanical removal (Gomes and Nitschke 2012). Therefore, efficient measures are urgently needed. One example that has shown to be effective in preventing and removing biofilms is the use of biosurfactants (Sharma 2016).

Several studies have demonstrated that the prior adhesion of biosurfactants to solid surfaces decreases the amount of bacterial cells attached on a surface of stainless steel and reduces the number of bacterial cells attaching to polystyrene surfaces, as a result of their anti-adhesive properties against food-borne pathogens, such as *Listeria innocua*, *Salmonella enteritidis*, *Listeria monocytogenes* and *Enterobacter sakazakii* (Sharma 2016; Nitschke and Silva 2018).

The biosurfactant can also change the physico-chemical properties of the surfaces directly involved in the adhesion, at the beginning of the biofilm formation (Nitschke and Silva 2018). It specifically modifies the hydrophobicity of the surface, affecting the adhesion of the bacteria cells on the surface (Gomes and Nitschke 2012; Harshada 2014). De Araujo et al. (2016) has demonstrated that surfactin changes the stainless steel surfaces to become more hydrophilic by increasing the electron acceptor components. Rhamnolipids can promote the same modification in polystyrene surfaces (de Araujo et al. 2016).