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Microbial Biosurfactants as Additives for Food Industries

Jenyffer Medeiros Campos and Tânia Lúcia Montenegro Stamford
Dept. de Nutrição, Universidade Federal de Pernambuco, Programa de Pós-graduação em Nutrição, Av. Prof. Moraes Rego, 1235, Cidade Universitária, Recife, CEP: 50670-901, PE, Brazil

Leonie Asfora Sarubbo, Juliana Moura de Luna, and Raquel Diniz Rufino
Centro de Ciências e Tecnologia, Universidade Católica de Pernambuco, Rua do Príncipe, 526, Boa Vista, Recife CEP: 50050-900, PE, Brazil

Ibrahim M. Banat
School of Biomedical Sciences, Faculty of Life and Health Sciences, University of Ulster, BT52 1SA, Northern Ireland, U.K.

DOI 10.1002/btpr.1796
Published online Month 00, 2013 in Wiley Online Library (wileyonlinelibrary.com)

Microbial biosurfactants with high ability to reduce surface and interfacial surface tension and conferring important properties such as emulsification, detergency, solubilization, lubrication and phase dispersion have a wide range of potential applications in many industries. Significant interest in these compounds has been demonstrated by environmental, bioremediation, oil, petroleum, food, beverage, cosmetic and pharmaceutical industries attracted by their low toxicity, biodegradability and sustainable production technologies. Despite having significant potentials associated with emulsion formation, stabilization, antiadhesive and antimicrobial activities, significantly less output and applications have been reported in food industry. This has been exacerbated by uneconomical or uncompetitive costing issues for their production when compared to plant or chemical counterparts. In this review, biosurfactants properties, present uses and potential future applications as food additives acting as thickening, emulsifying, dispersing or stabilising agents in addition to the use of sustainable economic processes utilising agro-industrial wastes as alternative substrates for their production are discussed. © 2013 American Institute of Chemical Engineers *Biotechnol. Prog.*, 000:000–000, 2013
Keywords: biosurfactants, food additives, bioemulsifiers, thickeners, solubilisers

Introduction

Surfactants are chemical compounds made up of amphipathic molecules containing hydrophilic and hydrophobic moieties that partition at the interface between liquid, gas, and solid phases such as oil–water or air–water interfaces, which have varying degrees of polarity and hydrogen bridges. The nonpolar portion is often a hydrocarbon chain, whereas the polar portion may be ionic (cationic or anionic), non-ionic or amphoteric.[1] Figure 1 shows the general surfactants structures.

Surfactants are characterized by their capacity to alter the surface and interfacial properties of a liquid, which allows the formation of microemulsions, in which oils can become solubilized in water or vice-versa.[2] Such properties enable a wide range of industrial applications, including emulsification, detergency, foaming capacity, lubrication, moisture retention, solubilization, and phase dispersion.[3,4] In recent years, surfactants produced by micro-organisms have attracted attention and as an ecologically adaptable, biologically safe, environmental friendly, and effective type of molecules.

Correspondence concerning this article should be addressed to L.A. Sarubbo at leonie@unicap.br

Surface tension which is an important parameter for surfactants effectiveness measures the force of attraction between the molecules of liquids, which significantly diminishes when the surfactant concentration in an aqueous medium is increased and micelles are formed. Micelles are aggregated amphipathic molecules with the hydrophilic and hydrophobic portion positioned toward the external on internal part of the micelle depending on whether the environment is water or oil, with hydrophilic moiety facing water and the hydrophobic moiety facing oil.[5] The critical micelle concentration (CMC) is the minimal concentration of surfactant needed to reduce surface tension to the maximal degree after which additional surfactants have no further effect. When the CMC is reached, a number of micelles are formed.[6] The CMC is the most frequently used measure for the assessment of surfactant activity and can also be defined as the solubility of a surfactant in the aqueous phase.[7] Efficiency and effectiveness are essential characteristics that determine a good surfactant; the former is usually determined by the CMC, whereas the latter is related to surface and interfacial tensions.[8]

Surfactant effectiveness is also determined by studying its hydrophilic–lipophilic balance (HLB), which indicates whether a biosurfactant is likely to form water-in-oil or oil-in-water emulsion. This is determined by calculating values for

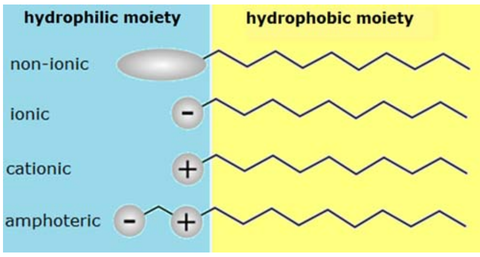

Figure 1. General surfactant structures according to the composition of their hydrophilic moieties and into nonionic, anionic, cationic, amphoteric compositions.

the different regions of the molecule and varies between 0 and 20. HLB values therefore are usually used to determine likely suitable applicability of surfactants. Emulsifiers with high HLB for example confers better solubility of oil in water, whereas those with low HLB value have the opposite effect and are more lipophilic in nature stabilizing water-in-oil.[6]

Most commercially available surfactants are chemically synthesized from the petrochemical industry and currently account for 70–75% of all surfactants used in the industrialized nations.[9] The total market value of surfactants in 2012 is ≈12 million tons when compared with 3.5 million tons for bio-based surfactants. The bio-based portion of the market has revenue of $ 6,588 million.[9] New environmental legislations however have led to the search for natural surfactants as an alternative to existing products.[10] This trend is fuelled by the search for environmentally friendlier products for the replacement of nonbiodegradable compounds (branched alkyl benzenes), as well as the enhancement of product specificity.[11]

There has been considerable interest in methods for obtaining surfactants from microorganisms.[12,13] A number of compounds with surfactant properties are synthesized by living organisms, such as plants (saponins), microorganisms (glycolipids) and even humans (biliary salts), which are considered natural surfactants.[14]

The term biosurfactant is used loosely in that it usually includes emulsifying and dispersing agents that are mainly metabolic byproducts from yeasts, bacteria and filamentous fungi. Compounds with these characteristics are useful to the petroleum, petrochemical, food, beverage, pharmaceutical,[15–18] cosmetic, mining, metallurgical, agrochemical, paper and other industries.[19,20] The use of surfactants has been mainly concentrated in areas of cleaning (soaps and detergents), environmental petroleum, and cosmetic industries.[21,22] During the past two decades worldwide use of surfactants has expanded a great deal, estimates of volume of ~9 million tonnes in 1995 increasing to 13 million tonnes in 2008 and 15.6 million in 2012 have been reported[9,23] with the majority used as raw material for the manufacturing of industrial and household detergents.

Most literature on biosurfactant applications have been concentrating on bioremediation of hydrocarbon pollution and pollutants in addition to microbial enhanced oil recovery.[24,25] These microbial compounds, however, exhibit a variety of useful properties and applications that are relevant in various fields.[26] In this review, we discuss the potential roles and applications of biosurfactants mainly focusing on areas such as food and food-related industries (as emulsifiers, foaming, wetting, solubilizers). The key features of the microbial biosynthesis of biosurfactants, their physicochemical and bioactive properties, and their food industry application potential are summarized. We also provide an overview of the emerging fields for their potential uses and considering the exploitation of agroindustrial wastes as alternative cost effective and sustainable substrates for biosurfactant production.

Biosurfactants Microbial Origin and Classification

Biosurfactants are mainly produced by aerobically growing microorganisms in aqueous media from a carbon source feedstock, for example carbohydrates, hydrocarbons, fats, oils or mixtures thereof. It is believed that biosurfactants are secreted into the culture medium to assist in growth of the microorganism through facilitating transport and translocation of insoluble substrates across cell membranes. All biosurfactants are of nonionic or anionic structures as there are no literature reports of cationic structures; however, in some instances the presence of nitrogen-containing groups imparts a degree of cationic feature to parts of the molecule altering adsorption on dispersed solids or their particle flocculation characteristics. Similar to all surface-active molecules, biosurfactants contain one or several lipophilic and hydrophilic moieties. The lipophilic moiety can be a protein or a peptide with a high proportion of hydrophobic side chains or can be a hydrocarbon chain of a fatty acid with 10–18 carbon atoms, although higher molecular weight fatty acids have been reported. The hydrophilic moiety can be an ester, a hydroxy, a phosphate or carboxylate group, or a sugar carbohydrate.

Chemically synthesized surfactants are normally classified according to their dissociation patterns in water, biosurfactants in comparison are categorized by their chemical composition, mode of action, molecular weight, physicochemical properties and microbial origin.[6] A fast and simple preliminary method of characterization of biosurfactants is by thin-layer chromatography with sequential staining.[27] The actual structure analysis is carried out by various spectrometric and classical chemical methods, which was outlined by Satpute et al.[28]

According to molecular weights, biosurfactants are divided into low-molecular-mass compounds including phospholipids, glycolipids and lipopeptides and into high-molecular-mass biosurfactants/bioemulsifiers containing amphipathic polysaccharides, proteins, lipoproteins, Lipopolysaccharides or complex mixtures of these biopolymers.[29,30] High-molecular-mass biosurfactants are more effective at stabilizing oil-in-water emulsions, whereas low-molecular-mass biosurfactants are efficient in lowering surface and interfacial tensions.[31]

A variety of microorganisms are capable of producing biosurfactants with different molecular structures. The following are among the most commonly reported microorganisms investigated for the production of biosurfactants: *Arthrobacter, Pseudomonas aeruginosa, Acinetobacter calcoaceticus, Bacillus subtilis, Candida lipolytica,* and *Torulopsis bombicola*. Most biosurfactants are produced from water-insoluble substrates, such as solid and liquid hydrocarbons, oils and fats, although many are obtained through soluble substrates or a combination of both types of substrates.[32]

Microbial biosurfactants attracted considerable attention because of their characteristics of biodegradability, low toxicity, ecological acceptability, and the ability to be produced

Table 1. Microbial Biosurfactants Main Functions Types and Applications

Functions	Types	Fields of Application
Emulsifier and dispersant	Liposan from *Candida lipolytica*	Cosmetics, paints, oils, foods
Solubilizer	Lipopeptide from *Pseudomonas aeruginosa*	Pharmaceuticals and hygiene products
Moisture retention and penetration agent	Polysaccharide-protein complex from *Curvularia lunata*	Pharmaceuticals, textiles and paints
Foaming agent	Glicolipids from *Torulopsis bombicola* KSM 35	Hygiene products and cosmetics
Thickener	Manoprotein from *Saccharomyces cerevisiae* Rhaminolipids W.O. 2004/040984 (patent)	Paints and foods
Metal sequestering agent	Rhaminolipids from *Pseudomonas aeruginosa*	Mining
Anti-microbial activity	Surfactin and Iturin from *Bacillus subtilis* Mannosylerythritol lipids from *Candida antarctica*Rufisan from *Candida lipolytica* and Lunasan from *Candida sphaerica*	Biological control of phytopathogens
De-emulsifier	Rhamnolipid from *Pseudomonas aeruginosa*	Waste treatment and oil recovery
Detergent, cleaner	Glycolipid from *Candida antarctica*	Detergent manufacturing
Oil mobilization	Sophorolipids from *Candida bombicola*	Enhanced Oil Recovery
Bioremediation agent	Rhamnolipids from *Pseudomonas aeruginosa*	Environmental restoration
Biofilms disruption/ penetration	Lipopeptide from *Bacillus licheniformis* PCT/IB2009/055334 (patent)	Pharmaceutical

Partly adapted from Banat et al.[21] and Muthusamy et al.[26]

from low-cost, renewable sources.[7,33–41] Bacteria account for the greatest production of biosurfactants, surfactin from *Bacillus subtilis*, and rhamnolipids form *Pseudomonas aeruginosa* considered some of the most powerful biosurfactants capable of efficiently reducing surface tension at concentrations below 0.005%. Among yeasts, representatives of the genus *Candida* (class *Deuteromycetes*, order *Saccharomycetales*) have been extensively reported able for the emulsification and solubilization of hydrocarbons and ability to produce biosurfactants. *Candida lipolytica* has been successfully used due to its potential for producing biopolymers with a high degree of emulsification activity using regional substrates as the carbon source.[33]

Besides, *Candida*, *Yarrowia* is also among the yeasts investigated for the production of emulsifiers and both genera have been successfully employed in the production of biosurfactants. Their status as "generally regarded as safe" (GRAS) is a considerable advantage to the use of yeasts and demonstrates that these microorganisms do not exhibit risks of toxicity or pathogenicity, thereby allowing their use in the food industry. *Yarrowia lipolytica, Saccharomyces cerevisiae,* and *Kluyveromyces lactis* are examples of such yeasts.[38]

Bacillus amyloliquefaciens and other members of the *Bacillus subtilis* group are also considered safe and classified with the GRAS status, with some cellular non biosurfactant products. A few notable examples are the enzymatic preparation pectate lyase from *Bacillus subtilis* (GRAS n° 114), α-amylase derived from *Bacillus licheniformis* (GRAS n° 79) and pullulanase from *Bacillus licheniformis* (GRAS n° 72).[8]

Biosurfactants Proprieties and Applications

Biosurfactants have varied applications in different industrial processes; and due to their diverse structures and properties it is likely that new applications for these biomolecules will be discovered. It is therefore believed that during the upcoming years biosurfactants will become known as "multifunctional materials."[22,26]

Biosurfactants are quite versatile chemical compounds as they have or can be applied in different fields, such as in enhancement of oil recovery as well as the biodegradation of water-insoluble pollutants and materials processed in the food industry.[42–44] They are most often employed in the petroleum industry in which they play a part in the oil extraction process or are incorporated in lubricant formulations. Owing to their action on the oil–water interface, they have been used in the bioremediation of contaminated water and soil for the emulsification and dispersal of hydrocarbons, thereby enhancing the degradation of such compounds in the environment.[13] Other applications include the dispersal of oil spills, the removal and mobilization of oil residues in storage tanks and microbial enhanced oil recovery.[43,45]

Lin et al.[46] employed biosurfactants for the partial solubilization of coal using a biosurfactant solution produced by *Candida bombicola,* whereas Mulligan[47] used sophorolipids, rhamnolipids, and surfactin in the removal of heavy metals from sediments. Other uses of biosurfactants have also been reported in the paper, textile and ceramic industries, as well as in uranium processing.[48] Similarly, a number of other industrial products require similar surfactants in their composition such as insect repellents, antacids, contact lens solutions, deodorants, fingernail products, toothpastes among many.[49]

Biosurfactants have also found applications in therapeutic, health, and biomedical fields. Their antiviral, antifungal, and antibacterial activities makes them suitable compounds/molecules for use as therapeutic agents, and owing to their biological origin, they are largely considered safer than synthetic pharmaceuticals.[18] Biosurfactants general abilities to disrupt membranes resulting in increased membrane permeability, metabolite leakage and cell lysis, and hence, antimicrobial activity are of relevance in these applications. Moreover, due to their ability to partition at the interfaces properties such as adhesion of cells/microorganisms on surfaces are also affected. Numerous literature describing such biomedical applications of biosurfactants have been published.[15,18,22,50,51]

Industrial and environmental processes are often associated with extreme conditions of temperature, pH, and ionic strength. Thus, stability studies under such conditions are important to determine the viability of possible use of some biosurfactants.[8,52] Table 1 lists the types of different industries using biosurfactant.

Despite the diversity in the chemical composition and properties of biosurfactants they exhibit a number of characteristics common to most of them and offer advantages over conventional surfactants including the following:

Surface and interfacial activity: which is the contractive tendency of the surface and or adhesive forces of a liquids that allows them to resist an external force; some biosurfactants are more efficient and effective than conventional surfactants, diminishing the surface tension of liquids at very low concentrations. Surfactin for example can lower the water's surface tension from 72 mN/m to 27 mN/m at a concentration as low as 20 μM.[53]

Tolerance to temperature, pH, and ionic strength: which is the ability to function under a wide range of each parameter showed many biosurfactants can be used under extreme conditions; glycolipids such as rhamnolipids, sophorolipids, and others are not affected by autoclaving at 120°C under pressure and remain effective within a wide range of pH values 5–10.

Biodegradability: which is the ability to be degraded by biological activities or enzymes shows biosurfactants are easily degraded by bacteria and other microorganisms in water and soil, which makes these molecules particularly suitable for bioremediation processes and waste treatment.

Low toxicity: measuring the degree to which a substance can damage living cells or organisms; biosurfactants have received considerable attention because of growing concerns regarding the allergic effects of artificial products and some preliminary testing showed low toxicity,[54] which favors the use of these substances in food products, cosmetics, and pharmaceuticals.

Availability: or the ease of obtaining the target product shows biosurfactants can be produced from widely available sustainable raw materials and can also be produced using industrial waste as substrate.

Specificity: as complex organic molecules with specific functional groups, biosurfactants also have specific actions, which are of considerable interest regarding the detoxification of specific pollutants or in particular applications in the pharmaceutical, cosmetic, and food industries.

Biocompatibility and digestibility: these properties allow the application of biosurfactants in the pharmaceutical, cosmetic, and food industries.

Emulsion formation and breaking: stable emulsions can be produced with a lifespan of months and years.[55] Biosurfactants may stabilize (emulsify) or destabilize (de-emulsifiy) the emulsion. Sophorolipids from Torulopsis bombicola have been shown to reduce surface and interfacial tension, but are not as good emulsifiers.[56] By contrast, liposan does not reduce surface tension, but has been used successfully to emulsify edible oils. Polymeric surfactants offer additional advantages because they coat droplets of oil, thereby forming stable emulsions. This property is especially useful for making oil/water emulsions for cosmetics and food industry uses.[26]

Many lipopeptide surfactants have potent antibiotic activity and have been studied for the discovery of new antibiotics and as antimicrobials.[15] Moreover, biosurfactants have physiological functions, such as the transport of hydrocarbons, cell adherence/release, and antibiotic activity, although the mechanisms of action remain not fully understood.[16,23,57]

Sustainable Biosurfactant Production

High quantity of organic waste materials are generally produced by the food, forestry, municipal, and agriculture related industries. Recent approaches toward enhancing sustainability and resource recovery has influenced waste management practices and resources production and utilization often supported by directives to reduce waste generation and promote recycling, recovery, reuse, and energy recapture from waste materials such as lignocellulosic materials.[58]

Although biosurfactants have a number of advantages over synthetic surfactants, they are not yet capable of competing cost-wise with chemical surfactants.[23] The increase in environmental awareness, however, has led to the need for the development of biosurfactants as an alternative to existing products.

The fermentation production process is key for reducing the cost of biosurfactant production, which can utilize different microorganisms and renewable substrates. Optimizing culturing conditions such as temperature, duration, agitation speed, pH, and added nutrients in addition to refining both upstream and downstream processes for product recovery allows the acquisition sufficient product with distinct structural characteristics and physical attributes. This makes biosurfactants comparable to synthetic surfactants in terms of efficiency; however, production costs, remain uncompetitive.[59]

The use of low-cost agro-industrial waste for alternative nutrients substrates would significantly improve the economic problem of biosurfactant production.[6] The main challenge using such waste is obtaining substrates with the proper balance of nutrients to support cell growth, as well as suitable and consistent product yields.[60]

In most agricultural countries the availability of agro-industrial by-products are quite significant; soap stock, a waste generated by the processing/refining soybean oil, palm oil and babassu oil and other vegetable oils refining products including sunflower, soybean, rapeseed, olive, groundnut, sesame, safflower, coconut, palm and mustard oils are all common.[61,62] Similar availabilities in the United States exist for soybean oil refining processes producing huge quantities of cheap soap stock, retailing at $1/10^{th}$ the cost of the refined vegetable oil.[63] Utilizing such wastes and reducing associated wastewaters generated from such industries are of great benefit to reduce waste disposal and associated environmental impact of these wastes.[64]

The literature describes many interesting researches with the use of industrial wastes for biosurfactant production. *Candida lipolytica* for example was grown on ground nut oil for production of a new biosurfactant yields up to 4.5 g/L and also grew well using soybean oil refinery residue as substrate.[7,37] The same group reported a low cost medium for biosurfactant production containing 5.0% groundnut oil refinery residue plus 2.5% corn steep liquor as substrates using *Candida sphaerica* and reported yields of 4.5 g/L low broth surface tension (26 mN/m) and CMC (0.08%).[39] Gusmão et al.[65] explored biosurfactant production using *Candida glabrata* on vegetable fat waste as substrate and reported on the effects and interactions of substrate, yeast extract, and glucose on surfactant production. Coimbra et al.[66] reported successful production of biosurfactant using six *Candida* strains cultivated in substrates for 144 h, including ground nut oil refinery residues and corn steep liquor, whereas Nawawi et al.[67] used sludge palm oil for biosurfactant production using locally isolated strains. Batista et al.[68] studied the influence of medium constituents on the production of biosurfactants by *Candida tropicalis* grown on waste frying oil. Their biosurfactant product showed emulsifications properties and significant surface tension reduction to 33.6 mN/m. Luna and group[69] characterized biosurfactant produced from industrial waste by *Candida sphaerica* UCP 0995 reporting surface tension reduction in the medium up to 25 mN/m. Fleurackers[70] also showed sophorolipids production by *Candida bombicola* ATCC 22214 using restaurant oil waste, whereas Shah et al.[71] reported yield of 34 g/L sophorolipids on restaurant oil waste using batch and fed batch fermentations.

Comparable observations were reported for rhamnolipids production using low-cost carbon substrates such as soybean, cottonseed, babassu, palm and corn oil, and strain *Pseudomonas aeruginosa* LBI. Soybean soap stock waste was reported best for this strain giving yields of 11.7 g/L rhamnolipids,[45] whereas Benicasa and Accorsini[72] reported using this strain in an integrated production process on wastes from refining sunflower oil. Similarly, Zhu et al.[73] reported 20 g/L rhamnolipids using *Pseudomonas aeruginosa* on restaurant waste oil in bioreactor configurations. Rocha and coworkers[74] reported the ability to produce biosurfactants from *Pseudomonas aeruginosa* using cashew apple juice and mineral media supplemented with peptone and nutritive broth. Glycerol, a by-product of biodiesel production processes, was also used to produce rhamnolipids using *Pseudomonas aeruginosa* UCP0992 achieving yields of 8.0 g/L with significant surface tension reduction (27.4 mN/m), emulsification index (E24) value of 80%, and CMC of 700 mg/L after 72 h[75].

Aparna and coworkers[76] evaluated five different low cost carbon substrates for biosurfactant production by *Pseudomonas sp*.2B. Using medium containing 1% molasses they reported biosurfactant yield of ≈5g/L and surface tension reduction to 30 mN/m. Cassava waste water + waste cooking oil were also successfully used to produce biosurfactant form *Pseudomonas aeruginosa* L2-1, which formed stable emulsions and low surface tension of 30 mN/m. Dubey et al[77] described the production of biosurfactants form crude whey by *Pseudomonas* PP2 and *Kokuria turfanesis* strain-j. The biosurfactants showed differences in their surface-active property and specificity to emulsify hydrophobic compounds under extreme conditions of temperature, pH, and salinity.

Another group based in Brazil have reported on the utilization of mineral medium containing clarified cashew apple juice by *Bacillus subtilis* LAMI008 strain.[78,79] Al-Bahry et al.[80] in Oman used date molasses for fermentation production of biosurfactants using *Bacillus subtilis* B20 and obtained a product yield of 2.3 g/L. The biosurfactant reduced the surface tension to 25 mN/m and showed significant stability under a wide range of temperatures, pH, and salt concentrations. Other agro-industrial wastes such as potato peels were also successfully used for biosurfactant production using two *Bacillus subtilis* strains in fermentation systems. The fermentation process resulted in biosurfactant (lipopeptides) with good surface activity and yield.[81]

Portilla-Rivera et al.[82] were the first to look in to the capability of *Lactobacillus* sp. to use hemicellulosic hydrolyzates from various agricultural residues for simultaneous production of biosurfactants and lactic acid. The use of such dual production strategy makes biosurfactant more economically viable.

Biosurfactant structure, concentration and recovery process determine the viability of large-scale production and technologies involved its extraction process. Thus, it is of fundamental importance to develop strategies that allow the production and consequent downstream processing on an industrial scale, such as the use of low-cost substrates, the selection of highly productive microorganisms, and suitable extraction and purification processes.[48,66]

One possible solution is the use of industrial by-products, such as vegetal oil residue. For example, large amounts of glycerol are generated by the biodiesel industry and can be used as a low-cost carbon source in the production of biosurfactants.[83] Glycerol, however, may be inhibitory for some microorganisms at high concentrations. Moreover, variations in the composition of the culture medium can also allow an increase in productivity through culture conditions optimization.[37] Another technology developing for biosurfactant production from agro industrial residuals is use of solid-state fermentation processes using filamentous fungi such as *Aspergillus fumigatus*.[84] Ohno et al (1995) reported the utilization of soybean residues for the simultaneous production of a lipopeptide surfactin and iturin in solid state fermentation (SSF) using *Bacillus subtilis* NB22.[85] Ghribi et al. also reported on the production of biosurfactants using another *B. subtilis* SPB1in solid state fermentation.[86]

Huge quantities of industrial waste are generated and discarded in the environment every year. Studies on the selection of adequate, low-cost substrates for the production of biosurfactants highlight the use of agro-industrial waste. Such waste include by-products from processing soybean, corn, coconut, peanut and canola oils and from animal fats, beet sugar, sorghum, soybean husks, sugarcane bagasse, fruit waste, and chicken fat.[87] Other agroindustrial products include dairy waste, whey, molasses and glycerine,[19] and cassava roots.[60] Substrate selection mainly depends on choosing waste with a balanced of nutrients for microorganism growth and for biosurfactant production such as a high carbohydrate and lipid content. Table 2 displays the different types of waste used by different microorganisms.

The production of biosurfactants by microorganisms using industrial waste as substrate represents an alternative for the production of petroleum based chemical surfactants. Maximum productivity can be achieved through the use of a factorial design aimed at minimizing costs and optimizing production.[37] A large number of studies are currently underway for the selection of suitable biosurfactant-producing microorganisms for the food and other industries. Moreover, studies are being carried out for the development of technologies aimed at improving strains and production processes.[111]

Food Additives

Food additives are ingredient with no nutritional value added to food to modify physical, chemical, biological, or sensory characteristics during the manufacturing, processing, preparation, treatment, packaging, storage, transport or handing. A fundamental principles of additive use is safety therefore before approval for use, an additive must comply with adequate toxicological evaluation, taking into account any accumulative, synergic, and protective effects stemming from its use.[112,113] Additives confer many properties such as thickening gelling, stabilization, and emulsifying. Table 3 lists the currently permitted additives and their properties in these categories. Glyceryl monostearate and carboxymethyl cellulose for example are synthetic emulsifiers that are widely used in the food industry. Despite being extremely

Table 2. The Main Organic Waste Products Used by Biosurfactant-Producing Microorganisms

Waste	Microorganism	Reference
Olive oil effluent	*Pseudomonas sp*	88
Bovine tallow	*Candida bombicola*	89
Frying oil	*Pseudomonas sp.*	90
Peanut oil residue	*Candida sphaerica* UCP 0995	39
Groundnut oil refinery residue and corn steep liquor	*Candida sphaerica* UCP 0995	69
Rice stalk	*Pseudomonas aeruginosa* BSZ07	91
Orange peels	*Pseudomonas aeruginosa* MTCC 2297	92
Soapstock	*Acinetobacter calcoaceticus* RGA-1 *Acinetobacter calcoaceticus* A2	93
Soapstock	*Pseudomonas aeruginosa*	94
Molasses	*Aureobasidium pullulans, Xanthomonas campestres ASpergillus niger Aureobasidium pullulans Bacillus subtilis* MTCC 2423 *Pseudomonas aeruginosa* GS3 *Gordonia polyisoprenivorans* CCT 7137	95–101
Whey	*Ganoderma lucidum Acetobacter xylinus Kluyveromyces fragilis Pseudmonas aeruginosa* BS2	102–105
Cassava roots	*Aureobasidium pullulans Rhizopus Rhizopus stolonifer* LAU 07	106–108
Potato processing waste	*Bacillus subtilis* ATCC 21332	109,110

Table 3. Some Additives Used in Foods[113–117]

INS*	Additive	Function
407 a	Processed Euchema seaweed	thickener/stabilizer/gelling agent
417	Tara gum	thickener/stabilizer
418	Gellan gum	thickener/stabilizer/gelling agent
440	Pectin	thickener/stabilizer/gelling agent
460 ii	Powdered cellulose	anti-humectant/emulsifier/thickener/stiffener
461	Methyl cellulose	thickener/stabilizer
462	Ethyl cellulose	Emulsifier/thickener/stabilizer/stiffener
464	Hydroxypropyl methyl cellulose	Emulsifier/thickener/stabilizer
466	Sodium carboxymethyl cellulose	Thickener
467	Ethyl hydroxyethyl cellulose	Emulsifier/thickener/stabilizer

*INS, International Numbering System.

efficient, these additives have been subject to restrictions particularly by consumers' demands for less use of "artificial" or chemically synthesized additives in favor of more natural ingredients.

Emulsifiers are essential ingredients in many foods particularly those containing oils and fats. They are surface active agents, which facilitate the formation of an emulsion due to their capacity to reduce interfacial tension between two immiscible phases and subsequently stabilize the emulsion formed[118] and improving texture and shelf life. For example, during foam fractionation gas bubbles are introduced into liquid containing surface active substances that leads to foam formation when surface active molecules attach to the gas–liquid interface of the introduced bubbles becoming stabilized.[119]

Application of Biosurfactants in Food Industry

Discovering new microbial polysaccharides and surfactants has been a highly sought after accomplishment in many industries to secure new ingredient additives with thickening and stabilizing abilities similar to xanthan gum or a new gelling emulsifier like emulsan. This combined with the desire to reduce dependency on plant emulsifiers produced by genetically modified (GM) crops such as GM modified soybean and availing of other favorable properties, including antioxidants, antiadhesives, antimicrobial, and biofilm disruption capacity has resulted in an increased interest in finding alternative natural sources for biosurfactant amphiphilic molecules suitable for used in new and advanced formulations in food and other industries.

Emulsification plays a role in consistency and texture as well as phase dispersion and the solubilization of aromas in most food industry products.[120] The function of an emulsifier is to stabilize the emulsion by controlling the clustering of globules and stabilizing aerated systems.[121,122] By definition, an emulsion is a heterogeneous system consisting of at least one immiscible liquid dispersed in another in the form of droplets. The stability of such systems can be enhanced by surfactants, which reduce the interfacial tension, thereby diminishing the surface energy between the two phases and preventing the coalescence of particles through the formation of steric and electrostatic barriers.[26] The emulsification index (E24) is a fast and qualitative method to determine the emulsifying properties of a surfactant.[3]

Emulsifying and dispersing agents used in food products do not necessarily need the ability to reduce the surface tension of water or of hydrocarbons. Cooper and Paddock[123] for example, analyzing some microbial emulsifiers such as the sophorolipids from *Turulopsis bombicola,* demonstrated that they have the power to reduce surface and interfacial tension even if they are not be classified as good emulsifiers. In contrast, liposan has been shown not to reduce the surface tension of water and yet has been used successfully to emulsify commercial edible oils.[124]

Biosurfactant as food emulsifiers

Food emulsions and colloids are intricate systems consisting of several components of food ingredients. Low molecular weight amphiphiles play and essential role in the stability of such liquid emulsions as (beverages, dressings, sauces and alcoholic emulsions and others). Food colloids therefore mainly consist of blends of monomeric and polymeric hydrocolloids and proteins. Food agencies and health authorities worldwide are constantly enforcing limitations and restrictions on the use of synthetic emulsifiers combined with the desire to reduce dependency on plant emulsifiers produced by genetically modified (GM)[125] crops has resulted in an increased interest in finding alternative natural sources for such amphiphilic molecules suitable for used in new and advanced food formulations.[126] In many food industries emulsifiers are used in order to obtain the right consistency and texture of food additives product process. The capacity of surface-active compounds to modifying the rheological characteristics of dough or the emulsification of partially broken fat tissue explains the need for their usage application in bakery and meat processing practices.[127]

A number of natural plant crop derived food emulsifiers, such as lecithin and gum Arabic, have traditionally been accepted by food industries. They, however, occasionally have some functional limitations in some modern process involving irradiation or use of microwaves. They also are increasingly being produced by genetically modified crops worldwide particularly soybean,[126] which creates some limitations for food industries reluctant to use such GM products due to biological and biomedical concerns.[128] An example is the use of lecithin in the food industry, which can be found in the production of cocoa powder and chocolates. Cocoa powder is one of the most popular ingredients in a number of dry mixes for desserts and drinks that are easy to prepare and to store. The advantages of dry mixes products include economical packaging and storage, longer shelf life, and easier transportation. However, one of the difficulties of using cocoa powders in such drinks is its low solubility in water, which resulted in the need to use emulsifiers and surfactants as an ingredient to enhance the ability to obtain such instant mixes. Cocoa powder tends to float on the surface of water because of its poor wettability caused by the presence of cocoa butter that repels water. Changing the hydrophobic (water-repelling) cocoa powder into hydrophilic powder is achieved by coating the cocoa powder particles with lecithin creating and outer surface that has a hydrophilic character enhancing wettability and dispersion.

Concern about the use of chemicals in food coupled with the avoidance to use GM crops such as soybean, which is the main source of lecithin, has intensified the search for natural alternatives that are able to provide wetting, dispersing and emulsifying properties. The production of microbial emulsifiers and biosurfactants offers an alternative to existing additives, some of which are more tailored to modern food processing demands. Mayonnaise, butters, cream, margarine, salad dressing, chocolates, and hotdogs are examples of foods processed from emulsions.[21,121] A bioemulsifier produced by *Candida utilis* has been reported used in salad dressings[124] and rhamnolipids have also been reported to improve the properties of croissants, butter, and frozen pastries.[26]

Saccharomyces cerevisiae mannorprotein produced through a low cost simple biotechnological fermentation process has been used to stabilize water-oil and creating emulsions suitable for the production of mayonnaise, cookies, and ice creams. In addition to stability at a broad pH range, its biomass and by-products can also be used in animal feeds. Most biosurfactant reported produced by yeasts and bacteria exhibits adequate stability at wide ranges of temperatures and pH values. The yeasts *Candida valida, Candida utilis, Hansenula anomala, Rrhodotorula graminis, Rhodospiridium diobovatum* and, the red alga *Porphiridium cruentum* and bacteria belonging to the *Klebsiella* sp and *Acinetobacter calcoaceticus* have been reported as producers of extracellular bioemulsifiers with better stabilizing activity than gum Arabic and carboxymethyl cellulose.[8]

In addition to the obvious role as surface and interfacial tension reducing agents enhancing stabilization of emulsion formation, microbial biosurfactants have other important uses in food including; controlling texture and shelf-life of starch-containing products, improving consistency and texture of fat-based products, agglomeration of fat globules, stabilizing aerated systems, and modifying rheological properties of wheat dough.[57] In bakery and ice cream formulations biosurfactants have been reported to improve consistency, delaying staling, and solubilizing flavor oils and as fat stabilizers and anti-spattering agents. Rhamnolipids in particular have been used to enhance dough texture, stability, volume and conservation of bakery products;[118,129] L-Rhamnose has considerable potential as a precursor for flavoring and it is already used industrially as a precursor of high-quality flavor components like furaneol (strawberry furanone).

Biosurfactants as antioxidant agents

Biosurfactants show some potential as antioxidant agents; Mannosylerythritol lipids (MELs) are versatile biosurfactants known for their versatile interfacial and biochemical properties. Using free-radical and superoxide anion-scavenging assay Takahashi et al.[130] reported antioxidant activity in vitro. They concluded that MEL-C has highest antioxidant and protective effects in cells and suggest potential use as anti-aging skin care ingredients. Similar observations were reported for a biosurfactant obtained from *B. subtilis* RW-I showing antioxidant capacity to scavenge free radicals and suggesting potentials use as alternative natural antioxidants.[131]

Some time ago a polysaccharide emulsifier from *Klebsiella* was also shown to have potent inhibition of the autooxidation of soybean oil.[132] The emulsifier suppressed soybean oil peroxidation by encapsulation, thereby isolating the oil from the surrounding medium. This polysaccharide was under development in France, as a source of rhamnose for the manufacture of furaneol, a flavor precursor.[124]

Biosurfactant as antiadhesives

Several biosurfactants display antiadhesive and antimicrobial activities.[133,134] Zeraik and Nitschke[135] recently reported antiadhesive activity against attachments of *Listeria monocytogenes, Staphylococcus aureus,* and *Micrococcus luteus* on polystyrene surfaces using rhamnolipids and surfactin. Lunasan produced by the yeast *Candida sphaerica* UCP0995 also completely inhibited the adhesion of several *Streptococcus, Staphylococcus, Pseudomonas, and Candida* strains on plastic tissue culture plates.[136] The same research group also described antiadhesive and antimicrobial activities of Rufisan, a biosurfactant produced by the yeast *Candida lipolytica*.[37] Similar observations were reported for biosurfactant produced by the strain *Lactobacillus strains* isolated from a Portuguese dairy plant[137] and from fresh cabbages[138] and other sources.[139] Such antiadhesive activity of biosurfactants against bacteria indicates their potential application either as coating agents for food related utensils and surfaces or to decrease antifouling rate or occurrence.

Potential applications have been suggested some time ago to decrease microbial fouling in dairy processing caused by microbial attachment to heat exchanger.[140] Such attachments are troublesome because they can cause the contamination of pasteurized milk. Lalande et al.[141] reported when biosurfactants are produced by cells adhering to surfaces act as anti-adhesive biological coating which reduces microbial fouling in dairy processing.

L. acidophilus biosurfactant were also reported to inhibit the adhesion of uropathogenic *enterococci*and inhibit the growth of uropathogens known to colonize the human body. Such application of dairy probiotic *L. acidophilus* strain to humans may therefore become possible as a strain suitable for oral delivery as a functional food with desirable effects.[142]

Role of biosurfactants in biofilm formations

Biofilms are aggregates of microorganisms in which microbial cells adhere to a surface and to each other. They include bacteria and a mixture of microbial extracellular material produced at the surface and any other material trapped within the resulting matrix. In the food industry bacterial biofilms present on surfaces mostly represent sources of contamination, which is often associated with disease transmission and food spoilage. Therefore, minimizing microbial adherence to surfaces in contact with food is an essential step in providing quality safe products to consumers. Biosurfactants involvement in microbial adhesion to, and detachment from surfaces, has been investigated and described above. Meylheuc et al.[143] reported on the preconditioning of stainless steel surfaces with a *Pseudomonas fluorescens* anionic biosurfactant as a method to reduce the number of *Listeria* cells adhering to the surfaces that favored disinfectants bactericidal activities. The bioconditioning of surfaces through the use of microbial surfactants has been suggested as a new strategy to reduce adhesion.

Nitschke et al.[144] also reported on the effect of rhamnolipids and surfactin biosurfactants use on the adhesion of several food pathogens to polypropylene and stainless steel surfaces. The use of surfactin in particular caused a reduction in the number of adhering cells on preconditioned stainless steel polypropylene and polystyrene surfaces and delayed bacterial adhesion of both growing and nongrowing cells. Rhamnolipids and other plant biosurfactants have also recently been reported to have some role in the inhibition of complex biofilms and as adjuvants to enhance some antibiotics microbial inhibitors.[145] These properties suggests that biosurfactants could be considered as suitable compounds for developing strategies to delay or prevent microbiological colonization of industrial plant surfaces used for foodstuffs preparation. Other possible future uses may be for biofilms prevention or reduction for oral cavities which are the main surfaces exposed to food stuffs within the human body. Whether such components may eventually become as a part of hygiene products or simply involved in food stuffs such as chewing gums remain to be seen.

Constraints for applications

Despite their potential applications in the food industry biosurfactants are not yet employed as additives on any significant scale. Many of the properties of biosurfactants, wide range of types and nature of the producing microorganisms, in addition to the availability of use food-approved downstream processing are all parameters that are constraining applications. Furthermore regulations governing new ingredients for foods require much research and testing to secure approvals for inclusion. Finally, in relation to use as food additive the lack of toxicity testing combined with the need to secure both consumers and concerned legislative bodies need also to be addressed.

Conclusions

Searching for novel emulsifiers/biosurfactants suitable for food industries has been steadily increasing driven by industries seeking to reduce dependency on plant emulsifiers produced by genetically modified crops. Unfavorable economic production costs and difficulties to produce sufficient quantities of biosurfactants, however, have hampered uptake by many industries. Using cheap sustainable resources, strains improvements, and optimizing production and extraction technologies to increase yields and productivities in addition to greater understanding of microorganisms' metabolic capabilities can bring the required breakthrough in production yields and costs. We conclude that through specialized cost-effective applications in the food industry and directed testing and investigations to establish toxicity we can look forward to biosurfactants as the molecules of the future.

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Manuscript received Apr. 17, 2013, and revision received Jul. 22, 2013.