4 Utilization of Bio Surfactants in Food Technology

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Abstract

Several microorganisms are capable of producing a wide range of amphipathic compounds that carry both hydrophilic and hydrophobic moieties in the same molecule, and this allows them to exhibit surface activities at the interfaces; such molecules are termed as bio surfactants. Bio surfactants are the surface active compounds that are classified mainly on the basis of their mode of action, physiochemical properties and also their molecular weight. Bio surfactants include the low molecular weight compounds that reduce interfacial tension and surface at the gas-liquid-solid interface. Even though a large population of microorganisms is responsible for the production of bio surfactants, their significant role is not clear, but due to their diverse chemical structure and properties, these surface active agents might have different natural roles in the growth of microorganism producing them and also positively affect the ecological niche. Due to their diverse characteristic features, bio surfactants are compounds of keen interest and are being studied and used widely for different purposes. Such detailed studies have revealed their potential to be used as various agents and additives in different industries including medical, agriculture, fermentation and food industries. One of their major and important contributions is in the food processing industries where they find their application as food formulation ingredient, anti-adhesive agent and antimicrobial agent. These compounds are responsible for promoting the formation and stabilization of emulsion. They are also used as agents to control the agglomerations of fat globules and effectively enhance the food texture and shelf life of starch-containing products. As anti-

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adhesive and antimicrobial agents, they potentially inhibit the colonization of pathogenic food spoilage microorganism and also inhibit the formation of biofilm by these pathogens on the surface of food processing materials.

Keywords

Bio surfactants · Bioemulsifiers · Antimicrobial agents · Antiviral agents

4.1 Introduction to Bio Surfactants

Bio surfactants by nature are secondary metabolites developed from living or static cells of bacteria, fungi and yeast which have huge promising applications. Surfactants usually are compounds that confer the presence of both hydrophobic and hydrophilic moieties which possess the ability to accumulate in between the molecules of oil in combination with water as well as air and water and thus possess prodigious applications. Bio surfactants can be regarded to contain both moieties which means having hydrophobic and hydrophilic natures. They are amphiphilic in nature and are excavated from unicellular organisms, namely, bacteria, fungi and yeast. Because of the above-mentioned properties, these are also known to possess emulsifying activities (Ahimou et al. 2001). Chemical nature possess by Biosurfactants are glycolipids, lipopeptides, protein complexes (Polysaccharide in nature), Phospholipids and fatty acids.

Biosurfactants have several industrial applications, one of which well used is removal of petrochemical wastes. Biosurfactants have a market size of around 1.6 billion CGAR from 2020 to 2026. It is gauged that its demand will increase by 5.5% in the pursuit of removing petrochemicals, but it has been estimated that demands in the fields of food processing and application will emerge at a very faster pace (Fig. 4.1).

The intercontinental market of bio surfactants has evolved to nine million tonnes. Bio surfactants are extricated out from non-replenishing energy sources which possess immediate feedback mechanism towards oil sources.

The very common products which have been formed are sophorolipids, methyl ethyl sulphonates [MES], sorbitan esters and lipopeptides as well as rhamnolipids.

The industry supporting usage of biosurfactants possess variety of it because of majority of customers have emerged a knowledge that the products employed to their personal care or food supplements should be derived from organic sources and should completely lack synthetics.

As a matter of fact, the lavish quantity of bio surfactants requires a huge focus on down-streaming of products; the process of better endowment requires optimization of certain conditions which include flexible ranges of pH, temperature, speed of agitation and suitable duration provision of air; rates of dissolution are also implemented for better mixing and amalgamation. These criteria employ huge-scale machinery setup and manual handling, thus providing employment to several people.

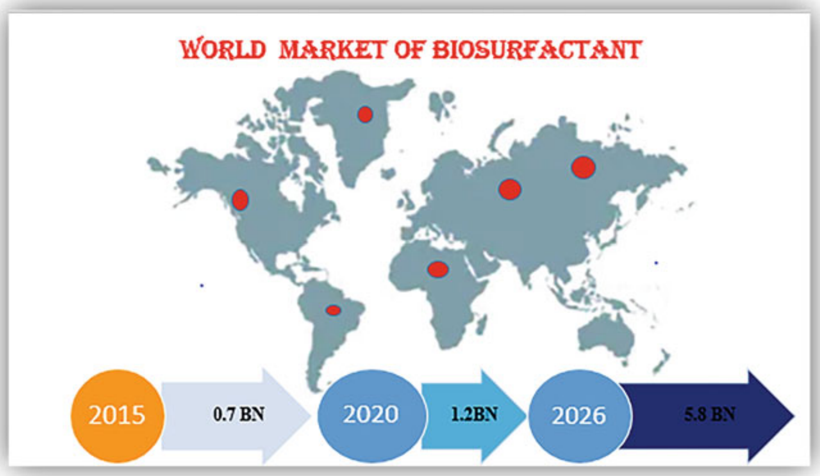

Fig. 4.1 Bio surfactant market in the span of 2020–2026

This chapter will deal with certain properties of bio surfactants which will demonstrate its helpfulness in the food industry and related co-operations.

4.2 Antibacterial, Antifungal and Antiviral Actions as Supreme Actions of Bio Surfactants

4.2.1 Antimicrobial/Antifungal and the Low Toxic Nature of Bio Surfactants

A range of antimicrobial activities of bio surfactants has been described in several literatures against bacteria, fungi and algae as well as viruses (Nitschke and Costa 2007). Most of the literature report that lipopeptides as well as glycolipids are the most widely studied bio surfactants which portray a huge spectrum of antimicrobial effects (Hayes et al. 2019). Sophorolipids, rhamnolipids and trehalolipids are the major forms of glycolipid-based bio surfactants; also, a category of mannosylerythritol lipids is included in lipopeptide bio surfactant (Adcox et al. 2005).

Poisoning of food and development of food-borne pathogens have been a major hitch in public health worldwide. In a detail provided by the WHO, for the people of about 500, 1 among each of 10 dies from eating contaminated food. Remediation of food-borne pathogenicity by utilization of bio surfactants as natural antimicrobial agents has become a revolution. Our environment comprises several pollutants which when take their way into the body cause several problems including the toxicity caused by microorganisms, namely, the species of *Vibrio parahaemolyticus, Clostridium perfringens, Listeria monocytogenes, Escherichia coli, Pseudomonas aeruginosa* and so on.

Among bio surfactants, lipopeptides form the widely reported class having antimicrobial action. The genus *Bacillus* is responsible for producing the most well-known lipopeptide bio surfactants. *B. subtilis* produces the first bio surfactant that presents antimicrobial property, that is, surfactin (Cawoy et al. 2015). It also produces other lipopeptides with the same property: fengycin, iturin, bacillomycin and mycosubtilin (Cawoy et al. 2015).

There has been wide range of bio surfactants potentially existing to combat against several pathogenic eukaryotes as reported by Nitschke and Costa (2007). The lipopeptides which are usually derived from bacterial sources are known to carry antibacterial properties.

The antibacterial efficacy, antifungal potential and antiviral activity of bio surfactants make them eligible to be prospective treatment of several diseases; they are not only therapeutic, but they could also be incorporated into food products which somehow manages the prevention of any further intoxication (Cameotra et al. 2004). The lipopeptide category of surfactants contains hydrophobic aptitude related to hydrophilic stream which together becomes capable of exhibiting various biological activities which confers the actions on surfaces or internal mechanism of enzymatic activities (Roongsawang et al. 2011).

In a study carried out by Yuliani et al. (2018), they mentioned microorganisms, namely, *Staphylococcus aureus, Escherichia coli, Salmonella typhi and Listeria monocytogenes* as well as fungi *Candida albicans* are causdal of several food borne infections and found out that bio surfactants produced by gram-positive *Bacillus subtilis* C19 is hazardous for above mentioned microbes (Table 4.1).

4.2.2 Bio Surfactants as Antiviral Agent

The antiviral efficacy of the bio surfactant has been reported in the literature; as reported by Muthusamy et al. (2008), the number of incidence of HIV in women is increasing, and this major issue has led to the need to develop agents with higher efficacy that could act as a safe vaginal topical microbicide agent. The sophorolipid surfactants obtained form *C. bombicola* as well as the analogues of this compound for example the sophorolipid diacetate ethyl ester carry a potential spermicidal and veridical agent, and the reports have also shown that this substance carry the veridical activity similar to the potential of nonxynol-9 against the human semen. The surfactant and its analogues have also been reported to have a good antiviral activity (Naruse et al. 1990) against enveloped viruses than the non-enveloped viruses, and this fact suggests that the physiochemical interaction between the surfactants and the virus envelope protein might be the reason behind the inhibitory action (Vollenbroich et al. 1997). The studies suggest that the formation of ion

Table 4.1 Microorganisms derived from various sources having susceptibility against bio surfactants

Food material	Microorganism	Antimicrobial effect	Reference
Milk, milk-derived products	*E. coli, B. subtilis, P. aeruginosa*	Bio surfactant derived from *B. subtilis* C19	Yuliani et al. (2018)
Packaged food material	*S. cerevisiae, Cladosporium herbarum, Aspergillus niger, A. cinnamomeus*	Glycolipids and other bio surfactants usually derived from *Pseudomonas* species	Sobrinho et al. (2008)
Probiotics, health drinks	*Cladosporium, Aspergillus, Penicillium, Rhizopus, Lactobacillus paracasei*, all LAB microorganisms, *L. brevis, L. buchneri, L. plantarum, L. perolens* and yeast *Zygosaccharomyces bailii*	Bio surfactants derived from varied microorganisms, bio surfactants made from lactic acid bacteria	Schuller et al. (2000), Rodriguez et al. (2013)
Food sale by street vendors	*Shigella flexneri, Salmonella typhimurium, Vibrio cholerae*	Bio surfactant derived from *Bacillus cereus, Staphylococcus aureus*	Fakruddin et al. (2017)
Food served by family restaurants	*Salmonella* spp., *Shigella* spp., *Bacillus cereus*	*Sophorolipids, rhamnolipids*	Anibijuwon et al. (2012)
Raw materials Cereals pulses, wheat, rice	*Clostridium botulinum, E. coli, Clostridium perfringens, Staphylococcus aureus*	Sophorolipids, rhamnolipids, bio surfactants extracted from the materials of lactobacillus bacteria	Diaz et al. (2016)

channel that causes viral proteins to leak out and disturb the normal metabolism process of the virus could be the mechanism of antiviral potential of some of the lipopeptides (Seydlova and Svobodova 2008). Two major productions from *Bacillus subtilis* fmbj, that is, surfactin and fengycin, under in vitro studies have shown the potential activity to inactivate the cell-free virus stocks of different viruses like Newcastle disease virus and porcine parvovirus. Both of these compounds could also potentially resist the infections as well as replication mechanisms of these viruses (Huang et al. 2006). A study reported that the trehalose lipids, namely, TDM and trehalose dimycolate, are able to confer resistance against intranasal infection-causing influenza virus in mice models; they induced the proliferation of T-lymphocytes that carry the gamma/delta T-cell receptors (Franzetti et al. 2010). The complex of rhamnolipid with alginate has shown a good antiviral activity against type 1 and 2 herpes virus by inhibiting the cytopathic effects in the Madin-Darby bovine kidney cell line (Remichkova et al. 2008).

4.3 Physical Applications of Bio Surfactants

4.3.1 Bio Surfactants as Bioemulsifiers

There are numerous food contents which comprise a combination of water and oil; it is a matter of fact whenever liquids with two different densities get mixed with each other. In a solution of two different densities, the liquid of lighter density gets separated, and there is formation of air bubbles. To resist this formation of air bubbles, emulsifiers are added in the food product to bring stability to it. The food materials that usually contain emulsifiers are margarine, ice creams and usually milk and other products that constitute them. Surfactants as clear from the name are the agents that act on the surfaces of two different compounds (Hasenhuettl and Hartel 2008).

Bio polysaccharides are heavy molecular weight compounds consisting hetero- and lipo-polysaccharides as well as proteins and lipoprotein in nature. They are heavy in molecular containment than bio surfactants. Emulsion of oil and water is generally of two different categories; one among them comprises oil in water which forms bubbles in water, while the emulsion of oil in water creates suspension (Calvo et al. 2004). The application of adding emulsifier is that it is responsible in reducing surface tension between the molecules of different densities, thereby causing their miscibility (Haften 1979). In the sector of food industry, the addition of emulsifier is the major cause to deploy solubilization of fat, vitamin stabilization amino acid equalization.

There are a huge number of applications of an emulsifier in the industry of food and packaged food materials:

1. The first and foremost application of emulsifier in food industry requires the formation of pellucid solution. The pellucid or clear solution can only be observed if the two solutions of liquid phases get contacted to each other; the colours and their type require dissolution (Rosenberg et al. 1979).
2. Mitigation of tissue bonds in the case of starch to break them into simpler ones in order to obtain good entities of Pazzta, spaghetti and noodles (Sharma and Sharma 2018).
3. Starch response: Several emulsifiers possess an angulate category of fatty acid layer in their entity which amalgamates mixture of amylose. The attribute is very significant to delay or rupture the stalling as well as they are also responsible to reduce any sort of binding and adjoining between such products such as tomato puree and white sauce pasta (Lukondesh et al. 2003; Cirigliano et al. 1985).
4. Developing interconnectivity with proteins and peptides: The emulsifiers contain a specific interaction with protein molecules and thus are responsible to generate an interchangeable structure which provides flexibility and instructiveness amongst them (Ericson and Le 1975).
5. The creation and subtleness of foam are also very important in the food industry: In food products there are several served materials which are better with frothing; this is done by incorporating emulsifiers in the bottom of the material so that there is no hindrances left by the presence of saturated fatty acids. This type of action of emulsifier is required for the improvisation in the volume of the material (Haferburg et al. 1986).

6. Removal of oiliness from the surface of cookies and other related stuffs: Emulsifiers are added to the products having sugar crystals and oils which when coagulated together join together and give oiliness-free appearances; this makes the food look good and shows sincerity in servings (Mnif and Ghribi 2016).

It has been a well-known fact that chemical emulsifiers create problems like acidity indigestions or other stuff. Due to its toxicity and expensiveness in the market, researchers have stated that availability of any material will depend upon the mixing. According to several researchers, there are several microorganisms which produce byproducts that represent emulsifying properties (Torabizadeh et al. 1996). A varied number of bioemulsifiers have been processed, and they have been approved by several international health organizations including the World Health Organization, but several studies have been taken out to transfer. A varied number of metabolites extracted from the microorganisms have nutritional values as well. A huge number of biomolecules are employed in the production of materials used as supplementary in the food industry (Campus et al. 2015).

4.3.1.1 Extraction and Employment of Bio Surfactant-Derived Bioemulsifiers

With the help of pasteurization, yeast and fungi could be brought down to dormant stage, and the bioemulsifiers could be extracted out from them. Mannoproteins are common glycoproteins that are extracted out from fungi or yeast. It is a form of glycoprotein. Mannoprotein molecules are developed at pH 3–11 in which the yeast and fungi are suitably placed. Beta 1 and 3 glucanases are the enzymes which can be suitably employed; further, mannoproteins could be removed. When the protein segment gets subtracted from the main chain, the emulsifier group of mannoproteins expresses itself (Luna et al. 2015; Pinto et al. 2018). *Candida tropicalis* have been reported to produce mannan fatty acids (Siporin et al. 1975). *Candida lipolytica* have been known to produce sophorolipid (Shekhar et al. 2015); *Candida tropicalis* are known to produce liposan (Ruffino et al. 2008; Dilarri et al. 2016). *Penicillium chrysogenum* is known to develop polyketide derivative (Luna et al. 2015).

4.3.2 Bio Surfactants as Enzyme Inhibitors and Activators

Microorganisms and the extracellular material provided by them have been employed in fermentations and food processing from time immemorial (Tunga and Tunga 2003). Microbial enzymes contained in bio surfactants are more stable than enzymes of flora and fauna. Microbial enzyme alpha-amylases are responsible for varied actions that include clarification of fruit juices which means rupturing of any fibre present in the liquid (Sharma et al. 2014), formation of rice cakes (Sivaramakrishnan et al. 2006) and bread quality improvement (Gupta et al. 2003). Glucoamylase enzyme is used in the beer production (Mohan et al. 2014), and brewing is also one of its applications (Monfort et al. 1996). Beta galactosidase is added in food materials to suppress the action of lactose intolerance (Gibson and Wang 1994). Although these enzymes are not directly considered as bio surfactants, attached entities with them show considerate effects and are responsible to perform these actions.

4.3.3 Employment of Bio Surfactants in Flotation or Froth Formation

In the last 80–90 years, several different structural contents of bio surfactants have been investigated. Several strains which are capable of developing bio surfactants have been investigated and studied in several researches. The property of bio surfactants depicts the low toxicity suppression in wastes (biodegradability) and their counterresponses towards chemical surfactants. The varied number of bio surfactant characteristics makes them suitable for further processes in industries where produced materials are of importance (Li et al. 2014).

4.3.4 Anti-Adhesive Agent

The materials' surfaces used for the food processing environment are pre-treated with bio surfactants. The pathogenic microorganisms that are responsible for food-borne illness outbreak easily cause the biofilm formation on the food contact surfaces, and for these kinds of pathogenic microbes, the sanitation process is ineffective, affecting the free-living cells (Kim et al. 2006; Stepanovic et al. 2004). The preconditioning of the surface with surface active agents like bio surfactants can be the most interesting and effective strategy for the prevention of food-borne pathogens that adhere to the solid surfaces. As demonstrated by Meylheu et al. (2006b), when the surface of stainless steel is preconditioned with the bio surfactant produced by the bacterium *Pseudomonas fluorescens*, it carried the reduction in the number of *Listeria monocytogenes* LO28 adhering cells; along with this it also promoted disinfectants like hydrogen peroxide and sodium hypochlorite for their bactericidal activities. They reported the similar findings with the bio surfactant obtained from *Lactobacillus* helveticus which inhibited the adhesion potential of four strains of *Listeria* on the stainless steel surface (Meylheu et al. 2006a). A study reports that the bio surfactant fraction obtained from the bacterium *Lactobacillus plantarum* shows a potential, more than 50%, anti-adhesive activity in the range of 4–25 mg/mL against food-borne pathogens like *Escherichia coli, Staphylococcus aureus, Salmonella typhi* and *Yersinia enterocolitica* and completely inhibits their growth at the maximum concentration (Madhu and Prapulla 2014). Another work was reported to assess the anti-adhesive effects of the rhamnolipids and surfactant bio surfactants on stainless steel and polypropylene surface against food pathogens like *L. monocytogenes, S. enteritidis* and *E. sakazakii* (Nitschke et al. 2009). The findings from their work showed that the preconditioning with surfactin bio surfactant leads to the reduced number of the adhering cells and that mostly of

L. monocytogenes. The surfactin bio surfactant was capable of delaying the adhesion of the bacterial cells within a short period for the non-growing cells and a long period for the growing cells. Bio surfactants obtained from the bacterium *Lactobacillus paracasei* ssp. *Paracasei* A20 obtained from the Portuguese dairy plant carry a good antimicrobial property as well as a promising anti-adhesive potential against several pathogenic microbes (Gudina et al. 2010). The attributes depicted by the bio surfactants clearly suggest that these bioactive surface agents can be considered as an alternate or a new tool for the development of strategies that could help in the prevention and delaying of the microbiological colonization on the surface of industrial equipment that are used for the preparation and processing of food stuff.

4.3.5 Probiotics Bio Surfactants Activity

The mechanisms of action used by probiotics vary widely, but some of them include the production of certain agents that could impart antimicrobial activity like organic acids, hydrogen peroxides, bacteriocin and diacetyl (Merck et al. 2005). Probiotics are also known to create interference with the adherence of cells for the biofilm formations by pathogenic microbes on the epithelial cells lining the urogenital and intestinal tracts (Reid et al. 2001). This interference is caused by the release of some surface active molecules like bio surfactants (Gudina et al. 2010). As reported by Hong et al. (2005), the antimicrobial lipopeptides produced by the bacterium *Bacillus* probiotics carry an antimicrobial potential, and this product inhibits pathogenic microorganisms that are present in the gastrointestinal tract from growing. The *Lactobacillus* probiotics are very well known for interfering with vaginal pathogens by producing bio surfactants or by competing with them for adherence (Barrons and Tassone 2008). Investigations have reported that the nosocomial pathogens can be antagonized by the bio surfactants from the probiotic-type microorganisms (Walencka et al. 2008). Silicone rubber adsorbed with the bio surfactant layer obtained from the probiotic bacterium *Lactococcus lactis* potentially inhibits the adhesion of pathogenic bacteria and yeast. Similar authors also demonstrated that the probiotic bio surfactants also reduce the microbial load on the voice prostheses (Rodrigues et al. 2004). Several discoveries overall conclude that both the probiotics and their bio surfactants are potentially capable of antagonizing the growth and development of the major pathogenic microbes like *Streptococcus spp. S. aureus, E. faecalis* and *C. albicans* (Van Hoogmoed et al. 2000).

4.4 Limitations of Bio Surfactants

One of the major limitations for the use of bio surfactants is the high cost of their production which can be somewhat decreased by the use of agro-industrial waste as the raw material for its production on large scale. The major restriction of bio surfactant use in the food industry could be because of safety reasons as these compounds are derived from microbes and some of the bio surfactants are produced by opportunistic bacteria, and hence there is a need to look for non-pathogenic source of bio surfactants like yeast and probiotic bacteria, or the strategies could be implemented to modify them structurally to reduce the toxicity strength. As the microbial diversity is considerably vast, there is a great opportunity for the exploration of new surfactants especially from non-pathogenic microorganisms and also from extremophiles. The emerging resistance to bio surfactants by food-borne pathogenic microorganisms is also an unexplored field that needs to be considered prominently to devise them as antimicrobial agents in food and feed industries.

4.5 Conclusion and Future Prospects

The diverse functional properties of bio surfactants have made them to be one of the most important compounds to be considered in the current era for various strategies and applications. These compounds of microorganism origin carry an excellent detergency property and emulsifying, foaming and dispersive traits and are therefore considered as versatile chemical process. The different properties of bio surfactants can be used in food industries some of which are being highly exploited for the betterment of food processing strategies. The recent anti-adhesive potential of these compounds has driven the attention towards and are now used as alternate tools for the inhibition and disruption of biofilm formation in the food and food processing surfaces made by major pathogenic food spoilage microorganisms like *Listeria monocytogenes*. These compounds are also good bioemulsifiers, stabilize the emulsion formation and carry potential antimicrobial attribute and hence inhibit the colonization of pathogenic and spoilage-causing bacteria and fungi. These combined attributes of bio surfactants have made them as potential and effective multipurpose food additive. As their different biological and functional potentials are known, they are being addressed as the active component for food formulations; hence, the efforts could be made to discover some of their more effective attributes to broaden and diversify their application in these industries. These natural compounds are tolerant to common processing methods and can also effectively compete with synthetic surfactants for their ability to reduce interfacial tensions and surface and due to their low toxicity and biodegradability. The studies have shown that the bio surfactant-producing microbes like some yeast impart no toxicity or pathogenicity for the consumer, and this makes them as the ideal agent for food formulations. The research approaches made to determine the potential and biological properties of these compounds have depicted that along with its attributes suitable for the food industry, these compounds also carry valuable biological properties including antioxidant, anti-tumour, anti-inflammatory and antimicrobial, and these attributes enable them to be considered in the products for the avoidance of contaminations both during and after processing. All the characteristic features and attributes of bio surfactants allow them to be used as additives and versatile ingredients for the processing of food products.

Bio surfactants are one of the potential metabolites of microbial origin to have an extended spectrum of functional and biological properties and applications in various industries including food manufacturing and processing industries. The accurate use of these compounds can be implemented in the food and feed industries only when the exact knowledge of their toxicity is known. The ongoing potential applications of these compounds in the food processing industries encourage researches to consider and determine their better efficacy, toxicity, stability and more diversification. Even though the current knowledge on the application of bio surfactants and their biological potential is vivid, its application and usage in the food industry are limited, and hence research needs to focus on extending this for the betterment of food processing strategies.

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