Recent updates on biosurfactant/s in Food industry

Surekha K. Satpute[1*], Smita S. Zinjarde[1,2*], Ibrahim M. Banat[3]

[1]Department of Microbiology, [1,2]Institute of Bioinformatics & Biotechnology Savitribai Phule Pune University, Pune, Maharashtra, India[3:] School of Biomedical Sciences, University of Ulster, Coleraine, N. Ireland, UK

Abstract :

Biological surfactants particularly of microbial origin have recently been gaining increased interest in the surfactants markets. Diverse functional properties viz., surface, interfacial tension, foaming detergency and wettability of biosurfactant (BS) / bioemulsifier (BE) facilitate their wide application potentials in the industrial sector. Petrochemical based surfactant / emulsifiers do exhibit similar properties however, their toxic and non-biodegradable characteristics limit their uses or application in many industries particularly food related. The opportunities offered by BS/BE are encouraging for commercial exploitation particularly due to other beneficial properties to food industries such as antimicrobial, antibiofilm and antiadhesive, non-fouling utilities. Safety and freshness are essential for ingredients/components used in food/feed industries and BS properties mentioned above makes them highly applicable to such industries. This chapter deals with role of BS/BE in various food industries. We briefly discuss certain food and food wastes utilized for BS production process. The article also presents information of BS/BE mediated synthesis and stabilization of nanoparticles. We also highlight different formulations based on BS/BE reported in food industry.

Key words: Biosuractant, biolemulsifier, biofilms, food, antimicrobial, lactic acid bacteria.

Correspondence:

Dr. Surekha K. Satpute, Department of Microbiology, Savitribai Phule Pune University, Pune 411007, Maharashtra, India

E-mail: drsurekhasatpute@gmail.com

Introduction:

Surfactants and emulsifiers have a large market share during the past few decades that seems to be ever growing with a compound annual growth rate estimate of 6% (Markets and Markets, 2016). Along with synthetic surfactant, biosurfactant (BS) and bioemulsifiers (BE) are also beginning to create their own commercial demand with a compound annual growth rate forecast of 8-9% (Markets and Markets, 2016). In the industry terms, it is crucial to accentuate that the use of renewable substrates tender immense competition with other markets (Satpute et al., 2017). Nature offers us number of different BS/BE from diverse origins having varied structural and functional diversity. For example Saponin obtained from soap nuts (*Sapindus mukorosi*) (Ghagi et al., 2010), cereals (soya, wheat, oats) lecithin from egg yolk and other proteins, casein, gelatin, wax, cholesterol etc. are some representative examples. Among the different plant based surfactants, lecithin has been a widely explored natural low-molecular-weight biosurfactant for industrial purposes (Dickinson 1993). Our own lungs alveoli cells (type II) produces phospholipoprotein based surfactant to facilitate breathing and gaseous exchange. Infants lacking the ability to produce surfactant result in respiratory distress syndrome (Xu et al., 2011). In addition to plants and animals produced BS/BE; microorganisms do represent one of the most suitable candidates for production of diverse forms of surface active compounds.

When we consider the microbial originated BS/BE; the available global literature scenario reflects the great diversity, with respect to structure, composition and properties. This wide diversity among BS/BE, therefore offers huge applications not only in food industry (Mnif and Ghribi 2016; Sharma et al., 2016; Kralova and Sjöblom 2009) but also in bioremediation (Satpute et al., 2005; Sáenz-Marta et al., 2015), agriculture (Sachdev and Cameotra 2013), medical (Santos et al., 2016; Rodrigues et al., 2006), cosmetics and pharmaceutical sectors (Fracchia et al., 2010). In addition, to the naturally available BS/BE, manmade synthetic surfactants namely sodium dodecyl sulphate (SDS), aerosol – OT (AOT), cetytrimethyl bromide (CTAB), Triton derivatives, Sorbitan esters (also known as Spans, Tween, etc.) have been exploited extensively for various commercial applications. However considering their toxic, non – biodegradable nature i.e. not ecofriendly nature, synthetic surfactants are not the preferred choice for biological based applications and or green sustainable credential (Campos et al 2013; Kourkoutas and Banat 2004).

BS/BE have been utilized in variety of food formulations, preparations and dressings as food additives. BS like rhamnolipids (RHL), surfactin, sophorolipids (SL) has been exploited in various food preparations. Presently, BS based products are frequently seen in the market. For example JBRR products coming from Jeneil Biosurfactant Co. US, sell RHL in different aqueous solutions of different purity levels as Bio-fungicide. The RHL products have been proved with great potential for numerous uses. Understanding the promising implication of RHL, the United States Environmental Protection Agency (EPA / USEPA) has permitted the broad use of RHL in or on all food merchandises. RHL is anticipated to avoid and regulate zoosporic, pathogenic fungi found on horticultural and agricultural harvests (ZONIX, EPA Reg. No 72431-1, 2012; Nitschke and Costa 2007). Literature equivalently depicts the frequent use of lactic acid bacterial (LAB) originated BS/BE from the genus *Lactobacillus* genus due to their benefits in the food industries. This chapter deals with different properties of BS/BE in addition to their other interesting features such viz., antimicrobial, antibiofilm, antiadhesive and non-fouling which are finding special services and application for the food industrial sector. A brief description of actual and potential uses of different BS/BE in various food industries and inclusion in different food formulations available in the market are detailed. In addition discussion on utilization of certain food and food wastes for BS production is also included.

Diverse biological-functional allied properties of biosurfactants (BS) / bioemulsifiers (BE):

The amphiphilic (hydrophilic and hydrophobic) nature of BS/BE confers on unique properties such as the ability to reduce surface and interfacial tension. Other interesting properties viz., aggregation, cleansing, emulsification, foaming, wetting, phase separation, surface activity and reduction in oil viscosity permit their exploitation in various industries. The diversity of their microbial origin for example fungi (Rufino et al 2014; Zinjarde & Pant 2002), bacteria (Satpute et al. 2016), actinomycetes (Zambry et al 2017) gives BS/BE wide structural, compositional and as well as functional properties Fig. 1 shows the main characteristics most BS/BE may have to be considered as **‘surfactant or emulsifier’**. However, it is not suggested that all the properties mentioned in the Fig. 1 are shared by all surfactant or emulsifier’ type compounds. The fact is that their basic structural organization is the main reason for their differences. The molecules armed with such diverse properties definitely find broad range of applications and are therefore motivating researchers worldwide.

Role of additives in food preparations / dressings / formulations:

The use of flavoring and preserving substances in food has been a routine practice for maintaining good quality of foods since ancient time. Good flavor, nutritionally rich, safe to eat and appealing appearances were always minimum criteria that need to be fulfilled in food products. In addition, cost and affordability are always main concerns for food. There are many additives / ingredients in use by the food industries, and customer nowadays have become quite demanding in their current food requirements and constituents. We have become much more conscious of food products with regards to safety and originality. Some additives like pentosanases, hydrocolloids, enzymes (amylases, lipases, hemicellulases etc.) are being used intensively to improve texture and consistency of food. Other additional benefits from additives include enhancing freshness and increased shelf life (Munif et al., 2012). Following are some of the important points should be considered during formulating any preparations in food industry.

1. **Maintaining freshness:** We are aware of the hazardous effect of foodborne diseases where botulism is one of those life-threatening toxins of microbial origconditionsin. The use of antioxidants as preservatives is quiet common for preventing oxidation of oils and fats containing food to delay or reduce the development of bad flavors.
2. **Safety maintenance:** Food products are subject to spoilage caused the presence of various microorganisms like bacteria, yeast, fungi, molds and actinomycetes. Air is an important source and facilitator of microbial growth in food products. Therefore, retaining the desired quality of the food is quite challenging making food safe is also a major concern for all food products used for human and animal consumption.

3. **Improvement and maintenance of nutritional value:** Most food products contain several minerals, vitamins, fibres, sugars, fats and proteins which ultimately affects its utilization and nutritive value. Under certain circumstances, additional nutritional values components may have to be added to enrich the nutritional value of the food products; however while performing such alterations; retaining the quality and taste of food is highly critical.
4. **Enhancing the texture and appearance:** The addition of naturally available flavoring spices and sweeteners is often carried out to improve food products' taste while colouring agents are generally included to improve the appearance and appeal to consumers. In addition to all these regular components, emulsifiers, stabilizers and thickeners are used to achieve the desired homogeneity, rheological behavior, appearance, texture, acidity and alkalinity of food (Kourkoutas and Banat 2004).

Use of Surfactants / Emulsifiers in food industry:

Emulsifiers and surfactants compounds are not new to the food industry and have been routinely used in the formulation of numerous food products over the centuries. Dairy, fermented products, bakery, breweries regularly use synthetic and natural emulsifiers and surfactants. In most of the dairy based products, like milk, curd, cheese, creams formulations food grade surfactant/emulsifiers are always permissible. Other products like salad, dressings, mayonnaise, deserts, etc. are often supplemented with such compounds to improve their flavor, appearance, storage rather than as nutritional aids. Other properties that are conferred by BS are stabilization of flavor oils, property improvement in bakery and dairy formulations (Kosaric and Sukan 2014; Kosaric 2001). Monoglycerides for examples are currently utilized as emulsifiers for numerous food products while synthetic surfactants like sorbitan esters and their ethoxylate derivatives have materialized in many food products (Tadros 2016; 2013; Hasenhuettl, 2008).

Understanding the various properties of surfactant/emulsifier is essential to exploit them for wider industrial applications. Low molecular weight compounds like monoglycerides, lecithins, glycolipids and fatty alcohols effectively reduce surface and also interfacial tension. Whereas, high molecular weight compounds mostly composed of protein, polysaccharide type molecules facilitate stabilization of emulsions (Satpute et al 2010a; 2010b). Under these circumstances, electrostatic interactions promote effective penetrating power. Different kind of foods represent a colloidal systems having various forms of aggregations made up of particles and drops giving rise to the appearance of ‘‘gels.’’ Surfactant and polymer molecules aggregate due to number of interactions including van der Waals forces and repulsive forces. The mechanisms are absolutely suitable / fit for the food having oil and fat content. Reduction in surface tension aids formation of emulsions between immiscible phases and improves the texture. Similar mechanisms are also seen in case of foam formation in liquids system having surface active molecules (Campos et al 2013). A food formulation determines various phases among particles (Kralova and Sjöblom 2009). Basically three major types of emulsions are important in variety of foods as shown in Fig. 2. This precise structural organization of surfactant molecules empowers surface active agents / emulsifiers to quintessence at the oil-water (O/W) interphase leading to increasing the thermodynamic stability of an unstable system (Berton-carabin et al., 2014). Emulsifiers yields high emulsifying abilities due to their amphiphilic nature making it feasible to mould with starchy and proteins fractions of food products. BS/BE competently emulsifies / homogenizes the partially digested fatty fractions. The emulsifier gets associated with protein fractions of food ingredients leading to their aggregations (Munif et al., 2012). Mannorprotein producing *Saccharomyces cerevisiae* facilitates the stabilization of W/O emulsions for products like ice creams and mayonnaise (Cameron, et al 1988; Moreira et al., 2016). A more complex type duplex emulsions (multiple) viz., water in oil in water (W/O/W) and oil in water in oil (O/W/O) are also achievable (Fig. 2).

The purpose of addition is mainly to alter or retain certain chemical (pH, temperature, and taste), biological (safe for consumption), and physical (constancy, appearance) uniqueness to food product that undergoes several procedures like preparation, processing dressings, manufacturing, storage, packaging, handling and finally transportation. Extensive use of carboxymethyl cellulose and glyceryl monostearate is of regular practice. BS/BE chiefly used as thickening, stabilizing gelling agent however, their emulsification property cannot be ignored while considering them for food applications. These dramatically affect the texture and consistency of foods. At the same time other interesting parameters including phase dispersion and aroma solubilization are also influenced by the emulsification phenomena and characteristics of emulsifiers. The main objective of emulsion stabilization is achieved by the aggregation of the fat globules by the emulsifier and the stabilizing aerated systems. Thus two heterogenous systems get homogenized and the SFT reduces energy between the two phases preventing particles coalescence (Berton-Carabin, 2014).

Cream, margarine, mayonnaise, butters, chocolate, salad dressing requires extensive usage of emulsifiers (Nitschke and Costa 2007). Best example can be cited as RHL in the preparation of frozen pastries, cream filling for Danish pastries by adding in sufficient amount. L- Rhamnose is already in the picture for its usage as high quality flavor compound (Van Haesendonck and Emmanuel 2002). In addition to bakery products, RHL also helps in improving the properties of dairy products like butter cream and frozen products (Van Haesendonck and Vanzeveren 2004). BE from *Candida utilis* is used in salad dressings and is proving to be highly useful for innovative texture modifications. The BE (for example liposan) having good emulsification properties can be successfully utilized for emulsification of edible oils commercially (Shepherd et al., 1995). Most of the BE particularly of high molecular weight exhibit superior stabilizing behavior than carboxymethlyl cellulose and Arabic acid. Excellent alterations are displateed due to addition of BS/BE in food (Marchant and Banat 2012). BE isolated from *Enterobacter cloaceae* works in the capacity of viscosity enhancer which gives the way to use them in low pH acid containing products like ascorbic and citric acid (Iyer et al., 2006). Even though several reports discuss on BS/BE production, a small number of BS/BE have been studied distinctively for food products at commercial scale.

Diminution of adhesion and eradication of biofilms formers from food products with aid of biosurfactant / bioemulsifiers:

Well established applications are seen for microbial surfactants in a range of food formulations, dressings and food processing. In addition to surface-active properties, other properties like antiadhesive, antimicrobial, antibiofilm make BS extra special molecules. Table 1 summarizes different BS exhibiting potential activities against pathogens.It is important to highlight that BS/BE represents a new generation of food additives as well as antiadhesive agents. The noteworthy application of BS/BE in food formulations are as agglomeration of fat molecules which ultimately upgrades the shelf life of food. Other properties like rheological behavior, texture of dough are also improved in oil / fat-based food dressings and formulations (Guerra-Santos et al., 1984). Well explored glycolipid type BS namely RHL and sophorolipids (SL) have enriched the properties of salad dressings and sweet / confectionary preparations

(Guerra-Santos et al., 1986). In case of meat products, BS proficiently emulsifies the partially digested fatty molecules. Another interesting role played by BS/BE for food material is impeding the growth of harmful microbial biofilms. Thus the microbial colonization has been successfully prevented and/or removed through application of various kinds of BS/BE. The production of microbial biofilms through secreting extracellular polysaccharides (EPS) leads severe spoilage of food (Sharma and Malik 2012). Therefore, defensive procedures are important to reduce the adherence and establishment of pathogens in and on food surfaces. The role of microbial originated surfactants in biofilm disruption and or prevention has become an important topic related to food and pharmaceutical applications.

Pre-conditioning of inanimate surfaces with popular BS like surfactin and RHL successfully prevents the adhesion of food spoiling and pathogenic bacteria. Both types of BSs effectively disrupt pre-formed biofilms (Gomes et al., 2012). RHL and surfactin has been proved to inhibit the adhesion of *Listeria monocytogenes* on polystyrene surfaces. The effective role of surfactin in reducing the adhesion of *L. monocytogenes* on stainless steel and polypropylene is well accepted (Nitschke et al., 2009). Polystyrene is extensively used in various food industries and its surfaces frequently exposed to food; hence, development of biofilm on such surfaces raises the risk of food contamination. Like polystyrene, metallic surfaces have been tested to prevent microbial colonization of pathogens. Meylheuc et al., (2006) tested two types of BS namely Pf (*P. fluorescens*) and Lh (*L. helveticus*) against biofilms formed by *L. monocytogenes* on stainless steel surface. Such experimental design supports antiadhesive biological coating abilities of BS on different surfaces. Research contributed by Zeraik and Nitschke (2010) describes the effects of surfactin and RHL against attachment of *Staphylococcus aureus*, *Micrococcus luteus* and *L. monocytogenes* on polystyrene surfaces. Even after surface conditioning tests including high temperature treatment; surfactin displays an anti-adhesive activity and therefore is suitable as anti-adhesive agent to protect various surfaces from pathogens. De Araujo et al., (2011) investigated the adhesion profiles of biofilm forming strains and reported that both BSs effectively reduce the attachment of biofilm formers. In fact RHL strongly impedes the adherence of *L. monocytogenes*. This work demonstrated the usefulness and effectiveness of this approach in controlling the growth of bacterial populations formed during biofilm formation.

Food, fermentation, medical and environmental industries are all concerned with the attachment of bacteria to surfaces and consequent biofilm formation. The occurrence of biofilm in food processing areas can lead to food spoilage and transmission of dreaded diseases resulting in health concerns and greater risks. *Salmonella enteritidis* and *Staphylococcus aureus* are known as prominent food-borne pathogens. Various platforms/surfaces like plastics, glass, stainless steel, rubber are generally affected due to growth of biofilm forming microorganisms. The economic losses resulting from such circumstances are very severe to food industry (Simões, Simões, and Vieira, 2010).

Like medical industries, various food industries are severely affected due to colonization of pathogenic organisms. Since food is directly consumed by human, it has serious health implications. Since food materials are rich with carbon, nitrogen, vitamins, minerals, wide variety of microorganisms can easily grow in and on food surfaces. It is highly impossible to eradicate well-established microbial biofilms. This is the way microbial biofilms proves to be one of the foremost sources of food contamination (Zhang et al., 2008). Researchers have concentrated on tackling this challenging situation through use of LAB or probiotic microorganism and their BS/BE to fight against microbial growth on inert surfaces (Satpute et al., 2016; Sharma et al., 2015). LAB derived BS/BE have been cherished as antimicrobial, antiadhesive, antibiofilm agents to eliminate the colonization of dangerous organisms. This issue is further discussed in detail in the next section.

The use of BS/BE on any surfaces does alters its hydrophobicity and inturn interferes with adhesion of microbial mats. Recent research illustrates active role of LAB derived BS for antibiofilm properties (Sharma and Saharan 2014; 2016; Sharma et al., 2014; 2015). Interestingly, BS obtained from microbes of dairy origin is found to be useful in removal of established biofilms through change in the morphology of developed microbial mats. A xylolipid type BS produced by *Lactococcus lactis* exhibits remarkable antibacterial activity against several clinical pathogens. The organisms *L. lactis* was isolated from a fermented dairy preparation by Saravanakumari and Mani (2010), Thus such BS are completely safe for human and animal health for being nontoxic in nature. The BSs obtained from LAB appear to be very effective against multidrug resistant microorganisms (Falagas and Makris 2009). The unique properties of BS/BE thus offers exceptional characteristics viz., antimicrobial, antiadhesive, emulsifying, antibiofilm. Thus BS/BE create possibilities for further improvisation in the food products in more positive ways.

Use of lactic acid bacteria for biosurfactant/ bioemulsifier production

Lactic acid bacteria (LAB) symbolize a noteworthy group of organisms that contribute towards natural microbiota of human’s genitourinary and gastrointestinal tracts. Consequently they play a key role in maintaining the homeostasis within those habitats by preventing colonization of pathogenic microorganisms. LAB are represented as probiotic agents suggesting that the consumption of live microbial preparations in sufficient quantity confers enumerable a health benefits to the consumer. LABS successfully impede the growth of pathogens through production of various antibacterial compounds including bacteriocins, lactic acid, and hydrogen peroxide. In addition to all those molecules, LAB also secretes cell bound or cell associated BS/BE (Satpute et al., 2016). Among several metabolites, the food based industries have comprehensively explored lactic acid producing strains of Lactobacilli. Global literature scenario depicts BS producing LAB as useful strains exploited commercially in various formulations (Sharma and Saharan 2014; Sharma et al., 2015)

Streptococcus thermophilus releases BS that detaches previously existing adhered cells and makes anti-adhesive coating on a substratum. In addition BSs has the capacity to get adsorbed to heat exchanger plates in pasteurizers and impedes aggregation of microorganisms. Thermo-resistant microorganisms are known to form heavy deposits in different sections of pasteurizer plants which lead the development of fouling. The growth of thermo-resistant microbes ultimately affects the quality, texture and appearance of dairy products. At the same time it also leads depletes nutritional value. The fouling deposits are also responsible to diminish the efficiency of heat transfer in pasteurizing plants. Therefore, it is essential to control fouling in heat exchangers pasteurizing systems (Busscher, et al., 1996). *S. thermophilus is* one of the well identified fouling forming Thermo-resistant bacterium. Heavy biofilm formation is observed in pasteurized milk than in raw milk (may contain some inhibitory compounds). As the growth of *S. thermophilus* continues the well established cells are progressively detached giving the appearance of clean surface. Further newly cultured organisms cannot adhere to the surface. This might be happening due to production of BS by adhering *S. thermophilus* which does not allow the organisms to deposit and develop the colonization. In fact *S. thermophiIus* cells are well known for their own detachment, through release and further adsorption of BSs (Busscher et al., 1990)

Among LAB, several species of *Lactobacillus* are used in combination with *Streptococcus* for formulation of range of dairy based products. Due to their acid and flavor production capabilities they are the preferred bacteria among LAB. Lactobacilli species have been explored thoroughly for production of BS. Generally *L. acidophilus, L. brevis L. plantarum, L ruteri, L. rhamnosus, L. acidophilus, L. pentosus, L. fermentum* and *L. casei* are known for BS/BE production. Although Lactobacilli species have been known for some time for BS production, complete characterization of their BS appears to be challenging as they basically appear to be a multi component mixture containing various percentages of protein and polysaccharides. Therefore it has been very tedious to predict the complete structure of BS produced by *Lactobacillus* spp. BS produced by lactobacilli especially reduces the adherence abilities of pathogens on surfaces and thus prevents their proliferation and biofilm formation (Satpute et al., 2016b). Due to the presence of antimicrobial activities, BS interfere with the adhesion mechanisms of pathogens to urogenital and intestinal tracts epithelial cells. As a result BS derived from *Lactobacillus* spp. can function as antibiofilm agents. About 46 articles have been reported on production of BS from Lactobacilli spp. which can be broadly classified as cell free and cell associated or cell bound. Among all 46 reports, 40 came from cell associated BS type where merely half of those have been discussed for their detailed composition. Literature survey display glycolipidic, proteinaceous, gycoproteins, or glycolipopeptides type BS from several Lactobacilli spp. A majority of BS are of proteinaceous type and are therefore generally termed as surlactin. Majority of cell associated / bound BS type are specially known for their antibiofilm and anti-adhesive properties (Rodrigues et al., 2006; Walencka et al., 2008). We are frequently responsive with harmless nature of the genus *Lactobacillus*; however certain strains may prove to be pathogenic under certain circumstances.

It is important to highlight that the protein and polysaccharide components of BS obtained from *Lactobacilli* spp. are altered due to change in the composition of fermentation medium, pH, temperature and time of incubation, inoculums volume, as well as the growth phase of bacteria (Fouad et al., 2010). Yeast extract is responsible for growth of bacteria used in fermentation process, at the same time peptone is essential for synthesis of BS. Gudiña et al., (2011) showed that the use of peptone and meat extract can result for high amount of BS production in comparison with De-Man, Rogosa, and Sharpe medium which is used regularly used for cultivation, production and purification of BS from Lactobacilli spp. (De-Man et al., 1960). The addition of manganese and magnesium has been proved to be supportive for bacterial growth and production of surlactin, protein rich BS. (Fracchia, et al., 2010). In addition to growth supplements, environmental parameters like pH, temperature also establish the type and activity of BS (Gudiña, et al., 2010).

Role of food and food waste in production of biosurfactant / bioemulsifiers

The routine use of various cheap and renewable waste substrates from dairy, distillery, agriculture, animal fat processing, food processing industry, oil processing mills, fruit processing industry represents rich sources for several oil, sugars, minerals, vitamins. In spite of these facts being true, lower yield of BS at commercial scale is a major concern. High monetary inputs are indispensable in order to drive large scale fermentations processes. To some extent, use of renewable substrates has provided some relief against the cost related issues (Banat et al., 2014).

Among different dairy based products, cheese whey seems to be very popular alternative substrates. Maximum work documents on use of cheese whey for BS production at industrial scale. Rodrigues et al., (2008) reported *L. pentosus* CECT-4023 as strong BS producing strain on whey cheese. Gudiña, et al., (2015) demonstrated BS production from different *Lactobacillus* strains on conventional MRS medium which is well known for growth and production of BS from lactic acid bacteria. Glycoprotein type BS produced by *L. agilis* reduces SFT upto 42.5 mN/m, with an emulsification activity (E24) of 60% with the utilization of cheese whey as a culture medium, BS production by *L. agilis* was enhanced from 84 to 960 mg/l. BS does exhibit substantial anti-adhesive activity against *S. aureus*. Same BS also possesses antimicrobial activity against bacterial pathogens like *S. aureus*, *S. agalactiae* and *P. aeruginosa*. Such studies are applicable for inhibition of the adherence of pathogens on biomedical devices. Abundant availability of agricultural residues draws attention of several researchers to use them in BS production processes. However agriculture residues frequently need prior treatments including acid hydrolysis and thermal treatment before their actual use in fermentation industries. These steps provide predigested substrates, which can be utilized efficiently by organisms considered in the different fermentation processes.

Surfactin production from *Bacillus* spp. is widespread; where newer substrates have been tested by several investigators. Portilla-Rivera et al., (2007a, b; 2008) and Paradelo et al., (2009) reported the use of agriculture based digested substrates for growing this bacterial system efficiently and found them suitable for the same. *L. pentosus* grows and produce BS on grape marc hydrolysates and is efficient in reduction of water repellence of hydrophobic material, which is very superior in comparison to synthetic surfactants. In addition, sugars from vineyard pruning waste have been tested for large-scale production of BS from *Lactobacillus* spp. In this way it also facilitates towards reducing environmental load for disposal of waste material (Moldes et al., 2013).

Biosurfactant / bioemulsifier based food formulations and other applications

Diverse emulsifiers have been previously tested to improve texture of crumbs, bread volume and dough rheological proprieties. Basically edible grade emulsifiers do provide strength and softness to crumbs. BS is continuously utilized by bread makers. Thus emulsifier greatly affects the functional properties of wheat bread. BS obtained from *B. subtilis* SPB1 improves the quality and shelf-life of bread (Mnif et al., 2012). The authors have claimed that results are quite interesting with respect to improvement in shape and also superior specific volume and voided fraction of loaves in comparison to soya lecithin, a well-known commercial surfactant. The BE SPB1 noticeably improves texture profiles of bread when a concentration of 0.075% (w/w) is applied. In addition it also leads decreased chewiness, firmness along with adhesion values. The BE SPB1 increases cohesion for bread as compared with soya lecithin. The emulsifier results in strong protein network and enhances gas retention ability of dough during fermentation thereby increasing the volume of bread. Hydrophilic emulsifiers make easy the formation of lamellar liquid-crystalline phases in water. Van Haesendonck and Vanzeveren (2002) have filed a patent on the use of RHL to enhance dough or batter stability and dough texture of bakery products. RHL also gives positive effect on other properties of butter cream, fresh or frozen sweet, decoration cream etc. The composition of liquid, powder or emulsion having RHL works synergistically to lengthen the emulsion stability of the dough / batter. Lactic acid esters of mono, di, glycerides of fatty acids can be substituted by RHL for various dairy and non-dairy products.

In current years BS have been used for synthesis and or capping agents for green nanoparticles (NPs) (Kiran et al., 2010a, b; Ganesh et al., 2010). The appearance of such reports encourages budding researchers towards this area. *P. aeruginosa* produces RHL mediated silver NPs (Ganesh et a. 2010). Such preparations are facilitated in a water-in-oil micro-emulsion system (Xie et al., 2006). Another *P. aeruginosa* strain namely NaBH 4 demonstrates the synthesis of RHL reverse micelles. Glance at global literature scenario depicts maximum work is reported on RHL mediated NP synthesis. NiO NP has been synthesized by using micro-emulsion system in heptane (Palanisamy et al., 2009). Rods of ZnS nanoparticles, are formulated by using the capping agents (Narayanan et al., 2010). Other microbial systems like *Brevibacterium casei* has been used for glycolipid based formulations in combinations with Ag NP. Like RHL; surfactin can stabilize gold and silver NPs (Reddy et al., 2009) and cadmium sulfide NPs (Singh et al., 2011). Other type of BS like mannosylerythritol lipids (MELs) exhibitself- assembling capacity and are therefore suitable candidates for diverse properties (Kitamoto et al., 2009). Thus, BS represents as a “green” alternates towards synthesis as well as stabilization of metal NPs and proved to be effective for various applications.

Removal of heavy metal from food by using biosurfactants

Presence of heavy metals in food products is extremely hazardous when health related issues are concerned. Variety of plant, their growth phase, soil condition, presence of heavy metals and surrounding environment are the parameters in determining the uptake of heavy metals. Therefore it is essential to keep a close look and consideration towards their presence and accumulation in and on food surfaces to prevent the damages that could be caused by these heavy metals. To date efforts have been directed towards treating the waste water plants, food industries. Newer technologies are definitely trying to tackle the heavy metal contamination related issues. Nevertheless no assured solutions have been proposed to eradicate the heavy metal contamination from foods. The available methodologies are incompetent and expensive (Hidayati et al., 2014).

Ionic surfactants bind to heavy metals via ion exchange phenomena and are precipitated. Thus the metals are removed in the form of aggregates (Wang and Mulligan 2004) which is shown in Fig 3. Amalgamation procedure by using foam technology and BS also seems to be interesting. The capacity of RHL in formation of microemulsion results in efficient removal of heavy metals in comparison with plain distilled water and / or surfactant solution (Mulligan and Wang 2006). Based on the literature it can be proposed that heavy metals forms complexes with BS on food surfaces similar to the soil surface and finally are separated from food and come in the surrounding solution. BS predominantly of anionic type (RHL) can efficiently remove positively charged metals because of surface activity between BS and metal (Xu et al. 2011).

Work carried out by Anjum et al., (2016) is significant as it reports successfu removal of cadmium Cd) (up to 70%) from various vegetables like potato, radish, garlic and onion by using surfactin isolated from *Bacillus* sp. MTCC 5877. The BSs are capable of removing heavy metals from contaminated food material. In addition BS has capacity to reduce biofilm formation also and therefore, can prevent the adherence of microbes from various surfaces. Another recent work published by Giri et al (2017) demonstrated BS derived from *B. licheniformis* VS16 is capable of removing Cd from carrot, ginger, radish, and potato in addition it can also inhibiting biofilms of various pathogenic organisms. BS finds definite applications in food industry at commercial level. Thus surface active molecules definitely assist in cleaning the environmental pollutants.

Role of biosurfactants in food processing sanitation

Well known facts about food spoilage caused by microorganisms compel explorations for new techniques to tackle the food spoilage challenges. Fruits, vegetables, food products however should retain the nutritional value and safety till they are consumed. The use of chlorine compounds, organic acids, tri-sodium phosphate, iodine solutions and ammonia compounds are common in day today life to overcome to the food spoilages (Hricova et al., 2008). On the other hand these conventional techniques have some drawback in many ways. These techniques often fall short in maintaining the integrity of products with respect to taste, colour, and appearances. Therefore search of new methodologies becomes indispensable to eliminate microbes in food (Dilarri et al., 2016). Considerable studies contributed towards reducing the food susceptibility against microbial contamination.

Many microbial systems are well accustomed to survive under variety of surrounding environments and it is also important to note that BS production by microbial cells proves to be advantageous to endure in foods (Mellor et al., 2011). These authors suggested that bacterial count can increase in the stored food in presence of BS. Their work gave the evidence of increase in the total bacterial count of *P. fluorescens* on chicken which is stored aerobically up to three days. BS affects the bioavailability of nutrients for the bacteria and making them aggressive to sustain and improving the decomposition of food materials. At the same time, it should be noted that the exact physiological role of BS is not known completely (Jirku et al., 2015). One of the reports from Lima et al., (2013) suggested that a food borne pathogen namely *S. enteritidis* possess a natural propensity of adhering to the surfaces lettuce leaves. Recent studies demonstrated by Rossi et al., (2016) revealed that *Salmonella enteritidis* SE86 c produce BS that affects its adherence to lettuce leaves and confers resistance to sanitizers. The studies are evident through scanning electron microscopy (SEM) indicating the formation of lumps by organisms and BS produced by this bacterium favors invasion of stomata. The studies are remarkable in understanding the role of BS affecting the adherence capacity and therefore enhancing the resistance power of organism against sanitizers.

A germicidal composition having SDS and sophorolipid was developed by Pierce and Heilman (2001 US 6262038) for sanitization of fruits, vegetables. The composition is extremely suitable for 100% killing of dangerous pathogens like *E. coli*, S*almonella*, and *Shigella*. Foliage of agricultural plants are also cleaned with the help of BE in combination with acids and alkylsulphonate. Not only the food surfaces but the containers like milk tanks are also covered by biofilm formed by *B. cereus* where surfactin, a well known BS produced by *Bacillus* spp. facilitates their survival (Shaheen et al., 2010). We need to put efforts towards understanding the possible function of BS produced by microorganisms while in contact with food surfaces. BS do not necessarily have role in enhancing the adherence of organisms to the surfaces. There are exactly differing reports suggesting that the BS produced by *P. aeruginosa* NBIMCC 1390 increasing the cell hydrophobicity leading to alter the cell surfaces (Sotirova and Vasileva-Tonkova, 2009).

RHL has been evaluated for fruit washing / sanitation purposes. The studies included tap water along with electrolyzed water in addition to RHL solution for examination of impeding effect on microbial growth. RHL are very efficient for preventing the growth of microorganisms and also for increasing shelf life of the fruits. Hence RHL mediated fruits sanitation is one of the most recommended methods (Dilarri et al., 2016). The RHL are influential compound to inhibit various bacteria as well as fungi (Murray et al., 2006). RHL type BS although important role in improving the food textures, owing to human safety concerns, use is not practically feasible. In spite of all these facts, glycolipid BS have achieved significant place in food processing technologies (Mnif and Ghribi 2016).

Future prospects

BS/BE represents potential metabolites with broad spectrum of functional / biological properties in various food industries. Accurate utilization of BS/BE needs knowledge about toxicity prior to their applications to the food industry. Pioneering applications of BS/BE in food manufacturing, processing are encouraging researchers towards these surface active molecules. Although there is ample knowledge on diverse functional and biological properties of BS/BE the food related applications are few on the usage of such formulations. We need to reveal probable role / interactions exhibited by organisms when they are in contact with variety of food surfaces. Novel strategies used in medical and pharmaceutical can be extended to the food industries. The consistently faced problem, for BS/BE production engineering are the large-scale production, structural characterization and the monetary inputs. Further work in these proposed areas may facilitate us in commercial exploitation of BS/BE for public domain.

Conclusions

Different biological and functional properties of BS/BE address their uses as active component in various food formulations / preparations. Some newer approaches can be tried out to broaden their applications in food industries. The ever increasing requirement of BS/BE from the market; is raising the curiosity among researchers to investigate newer microbial cell systems with innovative structural and functional diversity. One of the recent approaches namely amalgamation of BS with NPs finds valuable applications to design newer food formulations. Along with use in different food formulations, packing of food material is also shared by metal NPs. Currently unreachable applications of BS/BE for food industries can be achieved with innovative modifications which was never possible with conventional emulsifiers. The gigantic objectives of designing innovative BS/BE based formulations can be achieved with aid of recent advanced technologies.

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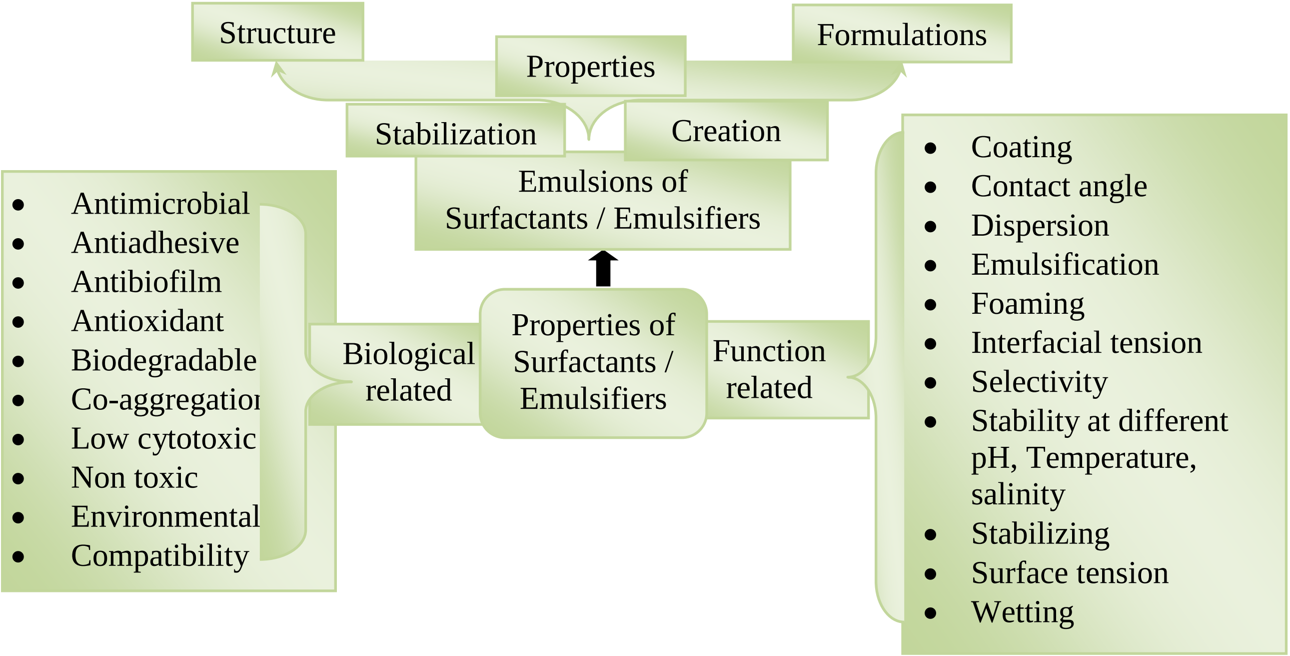
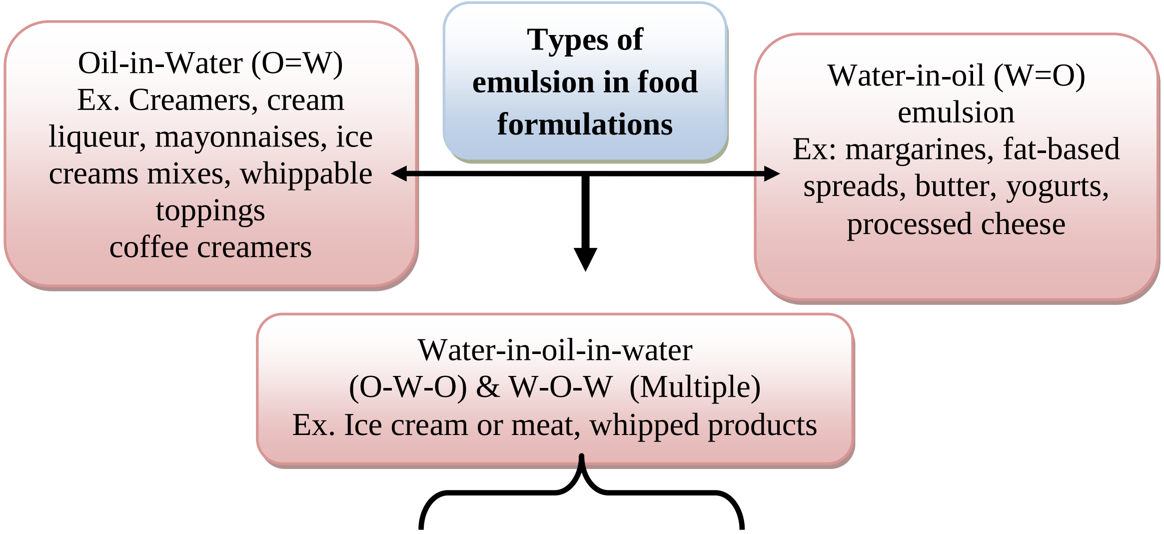
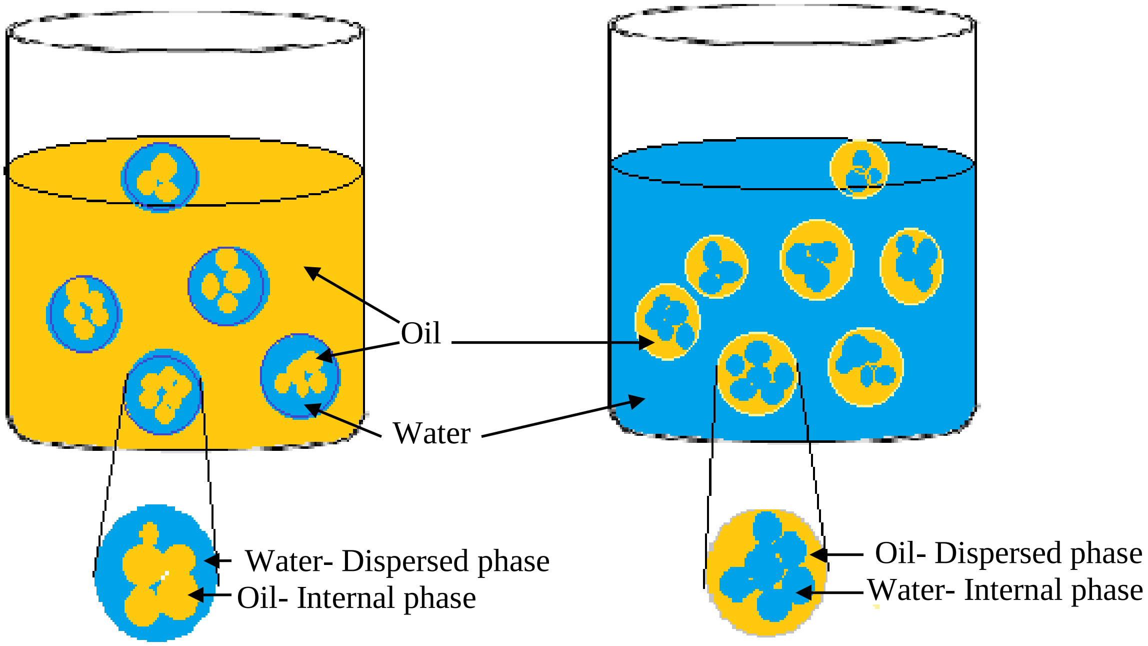


Fig. 1 Representation of various biological and functional properties of biosurfactant (BS) / bioemulsifiers (BE) to be considered for potential applications


Types of emulsion in food formulations
Oil-in-Water (O=W)
Ex. Creamers, cream liqueur, mayonnaises, ice creams mixes, whippable toppings
coffee creamers
Water-in-oil (W=O) emulsion
Ex: margarines, fat-based spreads, butter, yogurts, processed cheese
Water-in-oil-in-water
(O-W-O) & W-O-W (Multiple)
Ex. Ice cream or meat, whipped products



Oil-in-Water-in-Oil emulsion
Oil- Continuous phase

Water-in-Oil-in-Water emulsion
Water- Continuous phase

Fig. 2 Three major types of emulsions important in variety of foods.

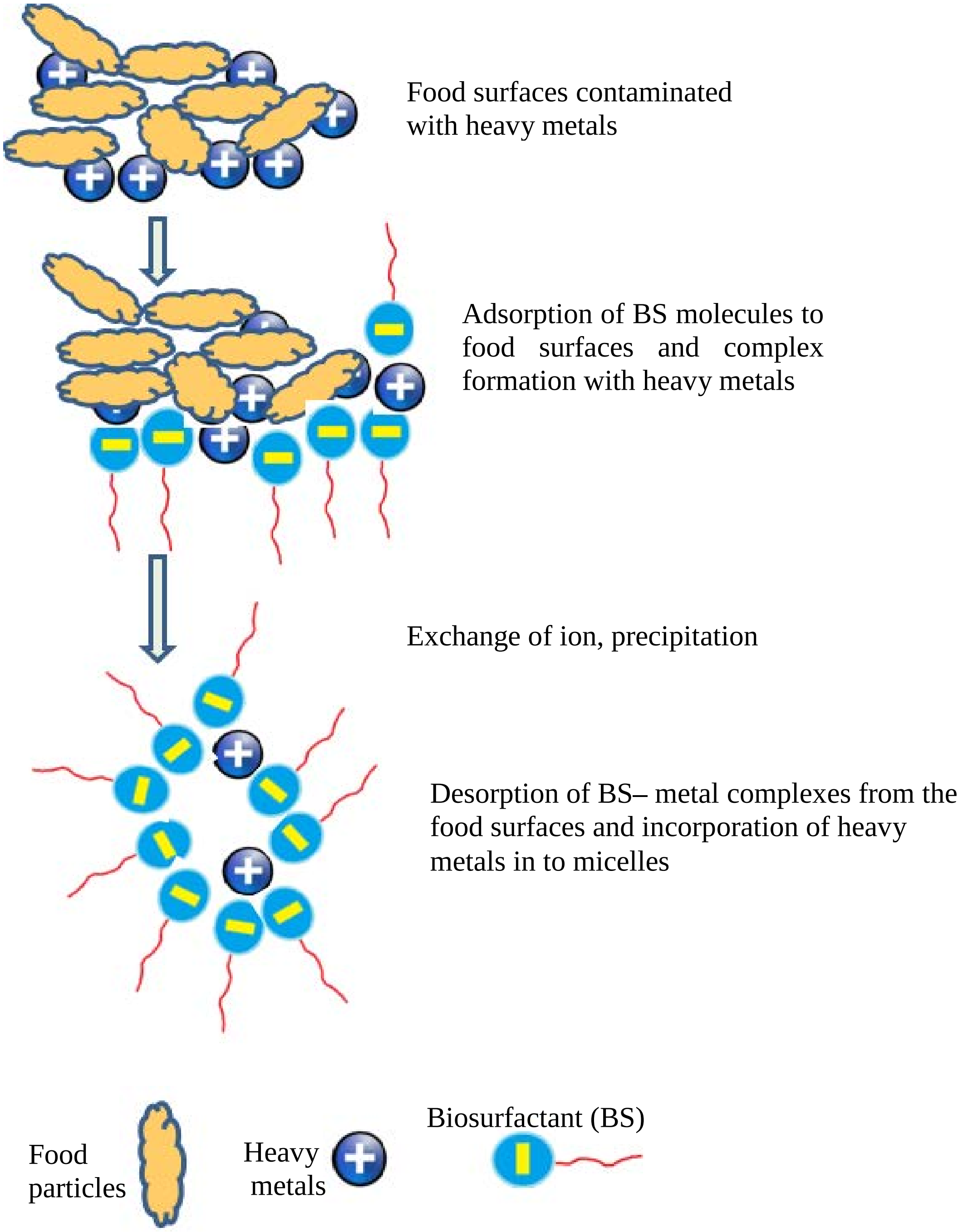


Fig. 3 Mechanism of heavy metal removal by biosurfactant from food surfaces

Note : The different properties of BS like hydrophobicity, molecular size, solubility, flexibility, and surface charge do impart during interaction all above interactions with heavy metals.

Table 1.Summary for biosurfactant / bioemulsifier exhibiting potential activity against pathogens

Biosurfactant / Bioemulsifier	**Potential activity**	**Used against**	**Reference**
Rhamnolipid	• Increasing shelf life of fruit	Inhibiting bacteria, molds, fungi growth	Dilarri et al., Food 2016
Rhamnolipid	• Antimicrobial activity	Inhibiting the growth of Gram positive and Gram negative bacteria and yeasts	Al-Asady. et al., 2016
Glycolipid from marine actinobacterium *Brachybacterium paraconglomeratum*	• Antibacterial activities	*S. aureus*, *B. subtilis*, *C. albicans*, *K. pneumonia*, *Mi. luteus*, *S. epidermidis*, *E. faecalis*, *P. aeruginosa*, *E. coli*, *P. mirabilis*,	Kiran et al 2014
Glycolipid *Streptomyces*	• Antimicrobial • activities	*B. megaterium*, *B. cereus*, *S. aureus*, *E. faecalis*, *Salmonella shigalla*, *S. dysenteriae*, *S. boydii*, *C. albicans*, *A. niger*	Haba et al 2014
Surfactin and Rhamnolipid	• Removing biofilms, • Modifying surface properties	*S. aureus*, *L. monocytogenes*, *S. Enteritidis*	Gomes and Nitschke 2012
Rhamnolipid	• Antimicrobial • activity	*C. albicans* and *S. aureus*	Manivasagan et al 2011
Rhamnolipid	• Antifungal activity	*Botrytis sp*, *Rhizoctonia* sp, *Pythium* sp, *Phytophtora* sp. *Plasmopara* sp	Vatsa et al., 2010
Rhamnolipids	• Inhibits spore germination and mycelial growth Protection of vines	*Botrytis cinerea*	Varnier et al., 2009
Bacillus licheniformis VS16	• Antibacterial activities • Inhibiting biofilm formation • Removal of cadmium (Cd) from vegetables	*Brevibacterium casei*, *Nocardiopsis*, *Vibrio alginolyticus*	Giri et al 2017