REVIEW ARTICLE

# Production and functional characterization of food compatible biosurfactants

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**Sanchita K, Pritisnigdha P. Production and functional characterization of food compatible biosurfactants. Appl Food Sci J. 2019;3(1):1-4.**

**ABSTRACT:**

As food industries contribute the most in ascending the socio-economic status of the nation, it is mandatory as well as a necessity to uplift and maintain the food industry. But the major problem faced is spoiling of food which possibly will lead to reduced availability of food. Though, multiple precautions are being taken to reduce the loss due to spoilage, such as using chemical based preservatives, treatment with antimicrobial formulations, low temperature treatment. But they are accompanied with some disadvantages too, like increase in cost of the product, side effects, high maintenance, increase blood pressure etc. Hence, the potential solution for the food spoilage can be the use of biosurfactants, which are derived from edible food products (mainly focusing on food products containing Lactobacillus, and further it can be checked to exhibit the antimicrobial potential, due to which it can efficiently be used in food preservation, thereby proving as a bane to the food industry. Therefore, various tests for toxicity can also be implemented to check any toxic effects, if caused by the obtained biosurfactants. Thus, a negative toxicity test will render the biosurfactant "safe" for consumption.

**Key Words:** *Bio-surfactants; Ethylene; Methylcyclopropene; Sulphur dioxide; Bioemulsifiers*

Food industry contributes the most in ascending the socio-economic status of a nation. However, food spoilage is a bane in progress of its nation. Also, it is a problem which is continuing from the ancient times. Food spoilage is also responsible for lesser availability of food and eatables. The long distance transportation of food and eatables became a difficult task because 30% to 35% food gets spoiled during the transportation [1,2]. Some food spoiling microbial species are *Moniliasp* which is causing spoilage in grapes, cherry and peaches, *Phytophthora infestans* this is causing "late blight of potatoes" [3] then, *Penecilliumitalicum* causing spoilage of oranges, and *Rhizopus microsporous* this grows on maize [4], sunflower and rice and causes their spoilage. There many other strains, some are known while some are unknown which are causing spoilage in food and eatables. Presently some solutions are there to prevent this spoilage problem, such as, use of chemical based preservatives, storage at low temperature, and treatment with antimicrobial formulations and also, employing vacuum packages [5]. By the treatment of chemical preservatives such as "methylcyclopropene"[6] which is in gaseous state and "sulphur dioxide"[7] which is in liquid phase, apples and grapes can be preserved respectively [8]. Their purpose is to stop ethylene production in them, which delay their ripening. Sometimes food is carried at a low temperature because this delays the incubation period of the microbes in the food and eatables [9]. Most of the time transporting food or to increase the self-life of the food materials, treatment of food is done with antimicrobial formulations as they prevent the growth of food spoiling microorganisms in the eatables [10]. Widely and commonly used method amongst them all is the vacuum packaging, they are used for the storage and transportation of the chips and similar products, in them "nitrogen dioxide" ($NO_2$) is used which prevents the oxidation of the food inside the packets [11].

There are positive effects of these currently present methods but, there are certain loopholes in them as well such as, in using chemical based preservatives, there are certain side effects of the chemical on their direct consumption by an individual such as, breathing difficulties, heart damage, hormonal changes and sometimes cancer [12,13] While employing low temperature transportation [14], and treatment by antimicrobial formulations, it increases the cost of transportation and cost of production which in turn increases the price of the product also, there might be chances that the antimicrobial formulation used is not compatible for the consumption by the individuals [15]. And, in vacuum packaging, if there will be any casualty in the packaging, then there will be definite spoilage of the food which was been packed [16]. Potential solution for the control of food spoilage will be the use of the "biosurfactants" [17]. Biosurfactants are the Surface Active Compounds (SAC), which are group of structurally diverse molecules produced by microorganism [18]. They have hydrophilic moiety, which is comprised of an acid, peptide, cation, anions, mono along with di-polysaccharides and hydrophobic moiety of saturated hydrocarbon chain [19].

Depending on their molecular weight, SAC is divided into two classes, low-molecular weight, they are biosurfactant, and they can efficiently lower the tension at the surface or the interfaces of the two liquids [20]. Other is High-molecular weight, they are bio-emulsion or bio-emulsifiers, and they can effectively make the emulsion between oil and water but cannot reduce the tension between two interfacial surfaces [21].

Food and healthcare applications, when bio-surfactant is administrated in the heterogeneous systems (like human system), it aggregates at the boundaries and interfaces [22], which prevents the pathogen's attachment with the cell and also it causes cell lyses causing metabolic leakage [23]. Antimicrobial activity, there are many microbial recognized which produces biosufactants having antibiotic property [24]. They can be used for the synthesis of new SAC based antibiotics because the pathogens now days are reporting resistance for the pre-existing antibiotics [25]. Biosurfactants as anti-adhesives, bio films are the organic films which are produced by the microbes which are colonising on the surface. There are certain SACs reported which can completely remove the mature biofilm from the surface [26].

Considering the antibacterial and antifungal property of the bio surfactants it can be used in preventing the growth of food spoiling microbes on the surface of the food and eatables, hence contributing largely to the food industry [27].

## PROPERTIES OF BIOSURFACTANTS

To be used effectively in the food industry, the biosurfactants have a very unique property, so as to become potentially useful for their use in food processing [28]. These properties are related to their ionic strength, temperature variability, surface activity and its abilityto uphold the pH difference [29,30].

### Surface and interface activity

For a biosurfacatant to be used efficiently as a potential surfactant it should have the ability to lower the surface tension of water,also decrease the interfacial tension [31]. In previous studies, surfactin from

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Received: October 12, 2018, Accepted: January 05, 2019, Published: January 14, 2019

*B. subtilis* was found to reduce the surface tension of water whereas; the rhamnolipids from *P. aeruginosa* reduced the surface tension of water. The sophorolipids from *C. Bombicola* were reported to decrease the surface tension [32,33].

In general, it is found that the biosurfactants are comparatively more effective and their CMC (Critical Micelle Concentration) is also about 10-20 times lower than the chemical surfactants, hence, it can be stated that minimal surfactant is required to get a maximum decrease in surface tension [34]. It is also reported that the availability biosurfactants and their activity on surface are not much affected by factors like temperature and pH [35].

**Biodegradability**

Like other surfactants which are synthetic in nature, the compounds of microbial origin are easily degraded [36]. Whereas, the increasing difficulties being faced by the synthetic sources is becoming a matter of concern among people, also the rules imposed by the government for environmental safety is pressurising the industries for alternative products such as biosurfactants, due to which its demand is increasing rapidly [37].

**Low toxicity**

Although, not much research is being done on toxicity of the surfactants derived from microbes, but they are generally regarded as low or non-toxic products [38], thus, enabling its use in the pharmaceutical, cosmetic and other food industry. It has also been reported that Corexit, which is a synthetic anionic surfactant exhibit LC50 against *Photobacterium phosphoreum* 15 times lower than rhamnolipids [39]. Also, the toxicity of five biosurfactants is compared, which are three synthetic surfactants and two commercial dispersants, it was found that most biosurfactants were degraded at high rate, except for a synthetic based sucrose that was homologous to glycolipids and was degraded more rapidly [40].

**Emulsion forming and emulsion breaking**

An emulsion is a heterogeneous system consisting of at least one immiscible liquid dispersed in another in the form of droplets, whose diameter generally exceeds 0.1 mm [41,42]. There are generally two types of emulsion systems: Oil-in-water (o/w) or water-in-oil (w/o) emulsions [43]. These systems have minimal stability. Thus, emulsion system can be produced with time duration of months and years. Biosurfactants may stabilize or destabilize the emulsion [44]. It has also been reported that sophorolipids have also shown to decrease the surface as well as interfacial tension but are not promising to be a potential emulsifiers [45]. In contrast, liposan is able to reduce surface tension due to which, it is also used to emulsify edible oil [46]. In dairy products like cheese and ice creams, the incorporation of emulsifiers helps to improve the texture and consistency [47]. This potential is of great value for the evaluation of low fat products with emulsification ability.

**Antimicrobial activity**

From the studies, it has been stated that biosurfactants have the potential to exhibit antimicrobial action against various microbes like bacteria, yeast, fungi, and viruses. *B. Subtilis* have potentially proved its antifungal activity [48] as there was significant decrease in the micro flora present in stored grains of corn and cottonseeds with the final concentration of 50-100 ppm [49,50]. Whereas, a rhamnolipid mixture obtained from *P. Aeruginosa* illustrated inhibitory activity against the bacteria *Escherichia coli*, *Micrococcus luteus*, *Alcaligenes faecalis* [51].

## POTENTIAL FOOD APPLICATIONS

Due to its various advantages it has various applications; here its food applications will be discussed.

**Food formulation ingredients**

Other than their role in decreasing surface and interfacial tension, it can also promote the formation and stabilization of emulsions [52,53]. In the studies it is shown that its application has extended to control the agglomeration of fat globules, stabilize aerated systems also improving texture of the products [54]. In industries like, bakery and ice cream, formulation based biosurfactants act controlling consistency thereby lowering staling and also solubilising the flavour oils [55,56].

In the recent study, a bioemulsifier which was isolated from one of the marine strain of *Enterobacter cloacae* [57] was found to exhibit potential viscosity enhancement in the food industry, this good viscosity was observed at acidic pH, thus enabling its use in products which are comprised of citric or ascorbic acid.

**Antiadhesive agents**

A bio film can be described as a group of bacteria that have colonized a surface [58]. This biofilm not only involves microbes like bacteria, but it also inculcates all of the extracellular material which is being produced at the surface along with any material trapped within the resulting formed matrix [59]. Therefore, the firsts step in the biofilm formation is the bacterial adherence which may be affected by various factors including microorganism species [60], hydrophobicity of surface and electrical charges involved, environmental conditions and ability of microorganisms to produce extracellular polymers that help cells to anchor to surfaces [61]. Moreover, biofilms formed due to bacterial establishment might be the reason of contamination in certain food industries, which may also lead to disease transmittance [62].

**Bio surfactant production from food and agro industrial wastes**

While the synthetic medium is having more of the disadvantage, thus shifting to alternative sources for the purpose to extract biosurfactants, various by-products and residues of agro industrial waste should be utilized [63] This, method works well to stimulate and increase the production of bio surfactant, as an inexpensive medium [64]. Thus, at present the search on alternative low-cost substrates is primarily focused on agro industrial crops and residues [65].

**Production and screening of biosurfactant from food sample**

The screening of bacterial strain for the ability to possess biosurfactant potential is based on its ability to give positive interpretation in various tests like-a) Oil spread method, b) Drop collapsing method, c) Emulsification index. The following tests are performed by the samples broth which is preincubated for 72 hrs at 37°C. The tests for screening are performed as mentioned below [66].

**Oil spreading technique**

In this method the oil displacement activity of surfactants is checked as per the method given by. The principle of this method was based on the ability of biosurfactant to alter the contact angle at the oil-water interface. Thus, the pressure of biosurfactant at the surface displaced the oil. In this method Forty-eight hour old inoculum was allowed to grow in Lactobacillus MRS Agar and then used. Further, the petriplate was filled with 50 mL of distilled water, followed by 25 micro litre of crude oil (kerosene) was layered uniformly. Then, 10 micro litre of culture was added on the kerosene which is coated on the water surface. If the kerosene dispersed till the edge, and then the biosurfactant activity is present [67].

**Drop collapsing method**

For the qualitative screening of biosurfactant production the drop collapsing method is done. In which 2 µL of kerosene was applied to the wells of 96-well micro plates and were left to equilibrate for 24 hrs. Followed by transfer of 5 micro litres of the 48 h incubated culture this was centrifuged at 10,000 rpm for 10 min. After the transfer onto the oil coated wells, drop size was observed after 1 min [68-70]. The result will be considered as positive for the production of biosurfactant when the drop was flat, and if the culture gave rounded drops then it will be considered as a negative test.

**Emulsification assay**

For screening the emulsification activity, emulsification index (E24) is calculated. The culture was further screened on the basis of results obtained in the emulsification assay. Several colonies of the isolated pure culture were suspended in 2 ml *Lactobacillus* MRS Agar and incubated for 48 h, after which 2 mL of kerosene was added to each tube. Then the mixture was vortexed at high speed for 2 min, after which the tubes are allowed to stand for 24 hrs duration [63,71-74]. The emulsification index (E24) is calculated.

E24=Total height of the emulsion layer/height of the aqueous layer * 100

**Antimicrobial potential**

After performing the above tests, the samples are checked for their antimicrobial potential by coating the sample (which is expected to possess biosurfactant property) on the surface of fruit/vegetable, which is infected by its target pathogen. Therefore, its antimicrobial activity is checked after approx. 15 days. If there are no signs of patches or rots visible on its surface, then it can be stated that the organism present in the sample is possessing biosurfactant property.

**Antifungal activity on live samples**

Fruits and vegetables are selected carefully which are free from any disease or cuts along, on the basis of their size. The fruits and vegetables surface was disinfected by ethanol to eliminate the pathogen present on the surface and residual sodium hypochlorite. Then these were coated with the biosurfactant solution while the other one was kept uncoated. Then the spores of a fungal species were sprinkled and incubated at 28°C for 7 days [75-79]. The incubation was extended up to 15 days. After the incubation, the surface of the fruits and vegetables was observed for any abnormalities and fungal growth. Later these were sliced in two equal half and observed for the spread of fungal infection.

## CONCLUSION

The fruits and vegetables having the isolated biosurfactant coating when treated with the target pathogen, is less spoiled as compared to the one which is uncoated but infected with the pathogen. Thus, showing signs of spoilage by change in colour, odour, and increased rottenness with slimy appearance. Thereby from the above properties it can be state that the obtained biosurfactant can be rendered as compatible and safe for consumption.

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