

## Review Article

<https://doi.org/10.20546/ijcmas.2017.605.070>**Bacterial Biosurfactants - A Boon to Dairy Industry**

M.C. Sanjana\*, K. Shivalkar Yadav, L. Malashree and R. Prabha

Department of Dairy Microbiology, Dairy Science College, KVAFSU, Hebbal,  
Bengaluru -560 024, India

\*Corresponding author

**A B S T R A C T****Keywords**Bacteria,  
Synthetic  
Surfactants,  
Biosurfactants,  
Surfactin.**Article Info**Accepted:  
04 April 2017  
Available Online:  
10 May 2017

Bacteria are ubiquitous in nature. Based on their role bacteria may be grouped as beneficial, spoilage causing and pathogens. The saprophytic group, involved in causing the spoilage of milk and milk products, produce various kinds of surfactants to utilize the milk components. Synthetic surfactants dominate in the market especially in detergent formulations and considered as non-biodegradable and toxic. Biosurfactants are surface metabolites produced by bacteria and fungi having very different chemical structures and properties. Surfactin a lipopeptide biosurfactant is produced by various strains of *Bacillus subtilis*. Surfactin has the surface tension of 27 mN/m and active between pH 5 and 9. It has been preferentially considered for various commercial applications in the dairy industry as the emulsifier, antibacterial agent and in detergent formulations due to its characteristics. Generally used emulsifiers in the dairy industry are lecithin obtained from animal and plant sources having their own limitations. Surfactin is found to be more beneficial over lecithin and possibilities may be explored in the dairy industry.

**Introduction**

Bacteria are found everywhere including nook and corner of a room, on the human body and so on. Bacteria may be grouped as beneficial, spoilage causing and pathogens. Beneficial bacteria are used as starters in food fermentation like dahi, yoghurt, batters, lactic acid production etc. Spoilage causing or saprophytic group is involved in causing the spoilage of foods including milk and milk products. Pathogenic bacteria cause diseases to plant, animals and human beings.

Saprophytic bacteria enter the raw milk from various sources and liberate biosurfactants and extracellular enzymes that lead to defects reducing the shelf life. Biosurfactants or microbial surfactants are surface metabolites

produced by bacteria and fungi having very different chemical structures and properties. *Bacillus subtilis*, *Bacillus polymyxa*, *Bacillus licheniformis*, *Bacillus pumilus* and *Bacillus cereus* are biosurfactant producing bacteria in *Bacillus* sp. A large variety of *Bacillus subtilis* strains produces lipopeptide biosurfactants which possess a high surfactant activity such as surface active properties and antibacterial activity (Rashedi *et al.*, 2005).

Biosurfactants are amphiphilic compounds (water soluble and oil soluble) produced on microbial cell surfaces or excreted extracellularly and are hydrophobic (oil loving) and hydrophilic (water-loving) moieties that confer the ability to accumulate

between liquid phases, thus reducing surface and interfacial tension at the surface and interface respectively. They possess the characteristic property of reducing the surface and interfacial tension using the same mechanisms as chemicals surfactants (Singh *et al.*, 2007).

### **Classification of Biosurfactants**

Biosurfactants are generally categorized mainly by their chemical composition dictated by the different molecules forming the hydrophobic and hydrophilic moieties and microbial origin. Glycolipids are the most known biosurfactants. They are conjugates of carbohydrates and fatty acids. The linkage is by means of either ether or an ester group. Among the glycolipids, the best known are Rhamnolipid, Sophorolipids and Lipopeptides (Vijaya *et al.*, 2013).

### **Rhamnolipids**

Rhamnolipids is a group of biosurfactant that studied extensively. Rhamnolipid is a type of glycolipid biosurfactant that contains either one or two molecules of  $\beta$ -hydroxydecanoic acid. These are produced by many species of *Pseudomonas* and *Bacillus* species, have tremendous antimicrobial activity against several common microorganisms, which is an essential property of all cosmetics due to the daily contamination of the product by the human touch (Lourith and Kanlayavattanakul, 2009).

### **Sophorolipids (SL)**

Such glycolipids are synthesized by yeast and not by bacteria. These are the complex mixture of both free acid and lactone form. Generally, lactonic SL has better surface tension lowering property whereas the acidic SL have better potential to form foam and solubility properties (Nuneza *et al.*, 2003).

### **Lipopeptides**

Lipopeptide biosurfactants are cyclic compounds and they are mostly isolated from *Bacillus* and *Pseudomonas* type bacteria. Lipopeptides mainly consist of hydrophilic peptides, generally, they consist 7 and 10 amino acids long, linked to a hydrophobic fatty acid structure. Surfactin is the most commonly studied lipopeptide and it contains 7 amino acid cyclic sequences connected to a C<sub>13</sub>–C<sub>16</sub> fatty acid (Meena & Kanwar, 2015).

### **Biosurfactant producing bacteria**

Biosurfactant producing bacteria are *Pseudomonas putida*, *Pseudomonas aeruginosa*, *Pseudomonas stutzeri*, *Corynebacterium pilosum*, *Bacillus subtilis*, *Bacillus pumilus*, *Bacillus licheniformis*, *Bacillus laterosporus*, *Serratia marcescens*, *Bacillus cereus*, *Bacillus macerans*, *Alcaligenes faecalis* and *Lactobacillus* sp. (Ogunmola and Aboaba, 2016).

### **Screening of Biosurfactant producing bacteria**

Major species of *Bacillus* that produce biosurfactant are *Bacillus subtilis*, *Bacillus licheniformis*, *Bacillus pumilus* and *Bacillus cereus* (Sharma *et al.*, 2014). The species are isolated by subjecting the sample to 80°C for 10 min., serially diluting the sample and plating using sterile Butter fat agar (2% Nutrient agar and Butter fat). In order to confirm the production of surfactant, the isolated colonies were subjected to following three tests (Ogunmola and Aboaba, 2016).

### **Oil spreading technique**

The oil spreading assay was developed by Morikawa *et al.*, (2000). This was done by placing 10  $\mu$ l of butter oil on the surface of 40 ml of distilled water in a Petri dish to form a

thin oil layer. Culture supernatant (10 $\mu$ l of 10<sup>6</sup> cfu/ml) was gently placed in the center of the oil layer. If biosurfactant is present then oil displaces and forms a clear zone. Surfactant activity can be determined by measuring the diameter of the clear zone.

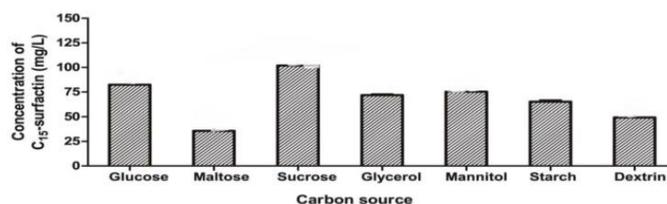
### Slide test

A wire loop was aseptically used to pick an inoculum from a 24 h old culture on Nutrient Agar. A droplet of Normal Saline (0.85% Sodium Chloride) was placed on it to make a wet preparation of the bacterial isolate on a grease free slide. The slide was slanted at 45°C and then observed visually for the flow of the wet preparation over the surface of the glass slide. The flow of the wet preparation of bacteria over the glass slide was recorded as a positive result (Olutola *et al.*, 2000).

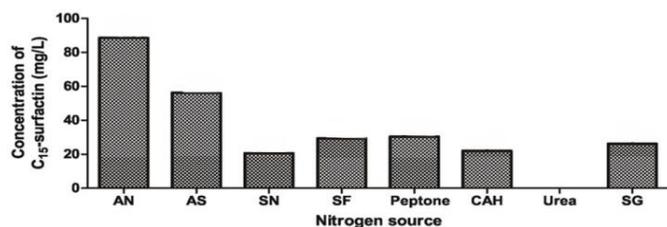
### Factors affecting biosurfactant production

The composition and emulsifying activity of the surfactin depends on the producer strain *Bacillus subtilis* but also on the culture conditions, thus the nature of the carbon source, the nitrogen source as well as the C: N ratio, parameters such as temperature, pH and time of incubation influence not only the amount of surfactin produced but also the type of polymer produced (Fakruddin, 2012).

**Carbon sources:** The quality and quantity of biosurfactant production are affected and influenced by the nature of the carbon substrate. Sucrose, Glucose, Mannitol, Starch, Maltose, Glycerol, and Dextrin have been reported to be a good source of carbon substrate for surfactin production. Optimum carbon source was found to be sucrose added about 21 g/L followed by glucose 10 g/L. Maximum yield of sucrose is 102 mg/L and glucose is 80 mg/L (Rahman and Gakpe, 2008).



**Nitrogen sources:** Nitrogen is important in the biosurfactant production medium because it is essential for microbial growth as protein and enzyme synthesis depends on it. Different nitrogen compounds have been used for the production of surfactin such as urea, peptone, yeast extract, ammonium sulfate, ammonium nitrate, sodium nitrate, Soybean Flour, Casein Acids Hydrolysate (CAH), Sodium Glutamate. Ammonium nitrate added about 2.5 g/L, surfactin yield is 90 mg/L (Adamczak and Bednarski, 2000).



**pH:** pH ranges between 6.3 to 6.7 was the optimal value for biosurfactant production with the highest rate of Surface tension reduction (38.5  $\pm$  2.1%) when compared with the treatment without inoculation.

**Temperature:** The maximum amount of biosurfactant at pH 6.5 when incubated at 37°C, which was significantly different from the production at other temperatures. The lowest Surface tension was produced at 20°C. Surface tension sharply increased when the temperature increased up to 37°C and then gradually decreased and remained constant at 46°C. Thus, temperature affects biosurfactant production (Dadrastia and Ismail, 2015).

**Time of Incubation:** At 72 hour or 3 days of incubation maximum biosurfactant produced

## **Production and Extraction of biosurfactant by Bacteria**

A pure culture of each of the biosurfactant producing isolates was inoculated into 100 ml minimal salt media, supplemented with hydrocarbon as the carbon source and incubated for 21 days. The culture broth was centrifuged at 5,000 rpm for 20 min at 4°C to obtain the cell-free supernatant. The biosurfactant was extracted by adding the equal volume of acetone to the supernatant and incubated at 4°C for 24 h. The mixture was centrifuged at 5,000 rpm for 20 min at 4°C and the pooled extracts evaporated to dryness over a water-bath at 45-50°C (Patil and Chopade, 2001).

## **Applications of biosurfactant in Dairy Industry**

Surfactin are biodegradable and non-toxic compounds so they can be used in dairy industry (Krishnaswamy, 2008) for the following properties.

As emulsifier  
Antibacterial property  
Antiadhesive property

Yeh *et al.*, (2005) demonstrated biosurfactant has the best emulsion stability and concentration of 0.16g/L when added to ice cream mix over lecithin (1g/L). It increases quality, improves overrun and organoleptic attributes.

Huang *et al.*, (2009) demonstrated that *Salmonella enteritidis* strain sensitive to 6.25 µg/mL of surfactin. This was done by Agar well diffusion test method 6.25 µg of surfactin added to wells it shows clear zone to *Salmonella enteritidis*. Then demonstrated that when surfactin was added to milk in the amount 6.25 µg/mL containing 7 log counts of *S. enteritidis*, counts reduced by 6 logs.

Nayak *et al.*, (2009) demonstrated the number of adhered cells of *L.monocytogenes* on the stainless steel reduced the two log units. Biosurfactant at 0.1% concentration added to the detergents reduced the pre-formed biofilms of *L.monocytogenes* by 95.9%, *S.enteritidis* by 35.5%. Meena and Kanwar (2015) showed surfactin gives anti adhesive activity by inhibiting the biofilm formation by two selected pathogenic strains of *S.aureus* and *E.coli* by 97% and 90% dairy cans and vats.

In conclusion, biosurfactants are produced by bacteria and fungi predominantly produced by bacteria. Biosurfactants are becoming an industrial reality over synthetic ones. Synthetic surfactants are Toxic and non-biodegradable so biosurfactants are used because it is non-toxic and biodegradable. Biosurfactants are produced by industrial wastes like cheese whey from the dairy industry. As process and production optimization is required. They possess dairy and other industrial applications to avoid environmental pollution.

## **References**

- Adamczak, M., and Bednarski, W. 2000. Influence of medium composition and aeration on the synthesis of biosurfactants produced by *Candida antarctica*. *Biotechnol. Lett.*, 22(4): 313-316.
- Dadrasnia, A., and Ismail, S. 2015. Biosurfactant Production by *Bacillus salmalaya* for Lubricating Oil Solubilization and Biodegradation. *Int. J. Environ. Res. Public Health*, 12(8): 9848–9863.
- Fakruddin, M.D. 2012. Biosurfactant: Production and Application. *Pet. Environ. Biotechnol.*, 3(4): 1-5.
- Huang, X.F., Yang, D.H., and Zhou, Q. 2009. Evaluation of screening methods for demulsifying bacteria and characterization of lipopeptide bio-demulsifier produced by

- Alcaligenes* sp. *Biores. Technol.*, 100(3): 1358–1365.
- Krishnaswamy, M., Subbuchettiar, G., Ravi, T.K., and Panchaksharam, S. 2008. Biosurfactants properties, commercial production, and application. *Curr Sci.*, 94: 736-747.
- Lourith, N., and Kanlayavattanakul, M. 2009. Natural surfactants used in cosmetics: glycolipids. *Int. J. Cosmet.*, 29: 225-261.
- Meena, K.J., and Kanwar S.J. 2015. Lipopeptides as the antifungal and antibacterial agents: applications in food safety and therapeutics. *Bio. Med. Res. Int.*, 1: 1-9.
- Morikawa, M., Hirata, Y., and Imanaka, T. 2000. A study on the structure-function relationship of lipopeptide biosurfactants. *Mol. Cell Biol. Lipids*, 1488(3): 211-218.
- Nayak, A.S., Vijaykumar, M.H., and Karegoudar, T.B. 2009. Characterization of biosurfactant produced by *Pseudoxanthomonas* sp. PNK-04 and its application in bioremediation. *Int. J. Bio. Biod.*, 63: 73–79.
- Nuneza, A., Foglia, T.A., and Ashby, R. 2003. Enzymatic Synthesis of galactopyranose Sophorolipid fatty acid ester. *Biotechnol. Lett.*, 25: 1291-1297.
- Ogunmola, C.O., and Aboaba, O. 2016. The emulsifying effect of biosurfactants produced by food spoilage organisms in Nigeria. *Int. J. Food Std.*, 5: 12-21.
- Olutola, P.O., Famurewa, O., and Sonntag, H.G. 2000. An introduction to general microbiology (a practical approach). Bolabay Publications Ltd Nigeria, 12: 169-180.
- Patil, J.R., and Chopade, B.A. 2001. Studies on bioemulsier production by *Acinetobacter* strains isolated from healthy human skin. *J. App. Microbiol.*, 91(2): 290-298.
- Rahman, K.S.M., Gakpe, E. 2008. Production, characterization, and application of Biosurfactants-Review. *Biotechnol.*, 7(2): 360-370.
- Rashedi, H., Assadi, M.M., Arpour, B., and Jamshidi, E. 2005. Environmental importance of rhamnolipid production from molasses as a carbon source. *Int. J. Environ. Sci. Technol.*, 2: 59 62.
- Sharma, A., Soni, J., Kaur, G., and Kaur. 2014. A Study on biosurfactant production in *Lactobacillus* and *Bacillus* sp. *Int. J. Curr. Microbiol. App. Sci.*, 3(11): 723-733.
- Singh, A., Van Hanne, J.D., and Ward, O.P. 2007. Surfactants in microbiology and biotechnology: Part 2. Application aspects. *Biotechnol. Adv.*, 25: 99-121.
- Vijaya, B., Jayalakshmi, N.R., and Manjunath, K. 2013. Enumeration of biosurfactant-producing microorganisms from oil contaminated soil in and around Bangalore (India). *Int. J. Curr. Sci.*, 5: 86-94.
- Yeh, M.S., Wei, Y.H., and Chang, J.S. 2005. Enhanced production of surfactin from *Bacillus subtilis* by the addition of solid carriers. *Biotechnol. Progress*, 2(1): 1329–1334.

**How to cite this article:**

Sanjana, M.C., K. Shivalkar Yadav, L. Malashree and Prabha, R. 2017. Bacterial Biosurfactants - A Boon to Dairy Industry. *Int.J.Curr.Microbiol.App.Sci.* 6(5): 608-612. doi: <https://doi.org/10.20546/ijcmas.2017.605.070>