

**PRODUCTION OF BIOSURFACTANT STABILIZED NANOPARTICLES****POOJA SINGH, SELVAN RAVINDRAN<sup>\*</sup>, JITENDRA KUMAR SUTHAR, POOJA DESHPANDE,  
RUTUJA ROKADE AND VINAYKUMAR RALE***Symbiosis School of Biomedical Sciences, Symbiosis International University, Pune, India.***ABSTRACT**

Biosurfactants belong to a well known class of microbial compounds possessing interfacial and biological activities as well as environment friendly characteristics. Recently extensive research work has been carried out by scientific community; and various novel applications of biosurfactants are being probed by industrial communities to a greater extent. This paper reviews one of the most recent applications of biosurfactants and synthesis of nanoparticles especially with respect to its application in the biomedical field. This review also emphasize the interdependent production of biosurfactants and nanoparticles.

**KEYWORDS:** Nanoparticles, Biosurfactants, Production, Microbial products, GreenSynthesis**Dr. SELVAN RAVINDRAN<sup>\*</sup>**

Symbiosis School of Biomedical Sciences, Symbiosis International University, Pune, India.

E-mail: [selvan\\_ravindran@yahoo.com](mailto:selvan_ravindran@yahoo.com); [selvan.ravindran@ssbs.edu.in](mailto:selvan.ravindran@ssbs.edu.in)

Ph: +91 9049480567.

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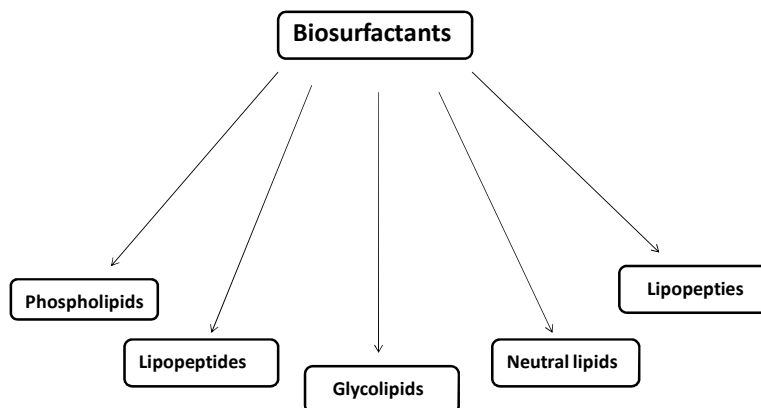
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## INTRODUCTION

Biosurfactants are amphipathic compounds produced by microorganisms as a means to reduce interfacial tension in the utilization of various substrates<sup>1</sup>. Biosurfactants from microbial origin are primarily composed of five different types of chemical structures such as glycolipids, phospholipids, neutral lipids, polymeric compounds and lipopeptides (Table 1). Microorganisms such as *Pseudomonas*, *Burkholderia*, *Acinetobacter*,

*Mycobacterium*, *Rhodococcus*, *Nocardia*, *Candida*, *Arthrobacter* and *Corynebacterium* have been reported to produce mainly glycolipids. Phospholipids and fatty acids have been reported to be produced mostly by organisms such as *Aspergillus*, *Acinetobacter*, *Arthrobacter*, *Corynebacterium* and *Nocardia*. Microorganisms like *Bacillus*, *Actinoplanes*, *Aspergillus*, *Serratia* and *Streptomyces* have been reported to produce lipopeptides majority of which are cyclic in nature<sup>2,3</sup>.



**Figure 1**  
Classification of biosurfactants

Thus the compounds produced from microorganisms have varied chemical nature (Figure 1) but have significant biological importance and can be utilized for eco friendly production for a number of industrial compounds, including nanomaterials. The focus of this review is on the interdependent commercial production of nanomaterials and biosurfactants.

### APPLICATION OF BIOSURFACTANTS

Biosurfactants are amphipathic molecules that lower the surface tension at water oil interface. They have been found to play important roles in the life cycles of the producing organisms as well. These include helping in the formation of biofilm and quorum sensing among

others<sup>4</sup>. However, due to their lower toxicity, greater biodegradability, biocompatibility, functionality under extreme conditions and specificity in action, biosurfactants have been widely used in numerous industries in the place of their synthetic counterparts. They have been used extensively in bioremediation of hydrocarbons / metal contaminated soils, petroleum industry, agriculture, waste treatment besides various other household and industrial applications<sup>5,6</sup> (Table 1). Due to their greater biodegradability and biocompatibility, the usages of biosurfactants have increased in food industry and in the field of biomedical sciences<sup>7,8</sup> (Table 1).

**Table 1**  
Properties and Application of Biosurfactants

Property of Biosurfactants	Industry	Application	Reference
Solubilizer, Demulsifier, Emulsifier	Food	Emulsification and de-emulsification	6, 8, 56
Dissolving of oil	Petroleum	Increased Oil recovery	1, 2, 3
Lowering of interfacial tension	Environmental	Bioremediation	1, 2, 3
Foaming agents, solubilizers and cleansers	Cosmetic	Cosmetic products for dermal applications	5, 6, 56
Microemulsions, Biotransformation	Bioprocessing	Downstream Processing	6, 50, 51
Parasitism and antibiosis	Agricultural	Biocontrol	5, 6, 56
Antibacterial and Antifungal	Pharmaceutical	Cure for diseases	1, 7, 48
Plant and animal pathogenesis	Microbiological	Biological applications	5, 6

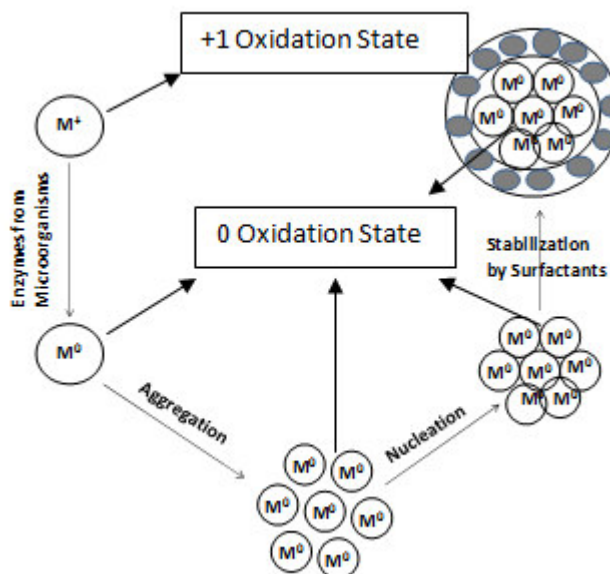
### BIOSURFACTANTS FOR GREEN SYNTHESIS OF NANOPARTICLES (NPs)

A fast emerging novel application of biosurfactants is the production and stabilization of nanomaterials (Figure 2). Nanoemulsions are widely being used in the food, beverage, personal care and pharmaceutical industries to provide desirable and customer compatible texture, stability and delivery<sup>9</sup>. Surface active agents help in emulsion formation and stabilization by reducing the

surface tension at the interface, thereby enhancing droplet disruption thus preventing aggregation during storage<sup>10</sup>. Biosurfactants are an ecofriendly, sustainable, biocompatible and safer alternative to the use of synthetic surfactants for nanoemulsion formation. Biosurfactants also exhibit additional advantage over other natural biopolymers like whey, casein and gum arabic among others by being more ecofriendly and having ability to function at varied pH and in varied sizes<sup>11</sup>. There are reports of number of microorganisms

being implicated in the synthesis of various nanoparticles. This mode of synthesis is referred to as "green synthesis"<sup>12, 13, 14</sup>. However, using a microbial product like biosurfactant for the nanoparticle synthesis and emulsion stabilization holds significance in terms of enhanced biocompatibility, stability, flexibility in

bioprocess optimization, easier and shorter downstream processing and nanoparticle (NP) size variation<sup>15</sup>. Hence, microbially derived surfactants or biosurfactants are emerging as a promising option for nanoparticle green synthesis.



**Figure 2**  
**Bisurfactant assisted synthesis of nanoparticles**  
( $M^0$  – nanoparticle in Zero Oxidation State and  $M^+$  - nanoparticle in +1 Oxidation State)

### GLYCOLIPIDS AS BIOSURFACTANTS TO SYNTHESIZE NANOPARTICLES

Glycolipids are the most common biosurfactants widely implicated in the synthesis and stabilization of nanoparticles. Rhamnolipids were used to synthesize and stabilize spherical and uniform rhamnolipid reverse micelle silver NPs average diameter of 11 nm. This microemulsion technique was found to be temperature and pH dependent and is a promising approach for synthesis of green nanoparticle with specific size and shape<sup>16</sup>. Rhamnolipids at a concentration of 25% were used to synthesize Nickel Oxide nanorods of 150-200 nm length and 22 nm diameter in a simple experimental setup. It was also established that difference in morphology of rhamnolipids at different pH is an attributed advantage for the ecofriendly synthesis of nanomaterials with different dimensions and morphology<sup>17</sup>. In a recent study, rhamnolipid biosurfactant was successfully tested to form and stabilize medium and long chain triglyceride nanoemulsion (droplets <150 nm) at biosurfactant to oil ratio of less than 1:10. The droplets formed were found to be stable over temperatures from 30-90°C and pH range of 5-9<sup>18</sup>.

### P.aERUGINOSA AND GLYCOLIPIDS TO PRODUCE SILVER NANOPARTICLES

The production of silver nanoparticles were carried out using glycolipid and *Pseudomonas sp.* Glycolipid from marine bacterium *Brevibacterium casei* MSA19 had been reported to be used for synthesizing and stabilizing silver nanoparticles under solid state fermentation conditions. Treated agro industrial wastes like molasses, tannery sludge, oil seed cake and wheat bran were used

for biosurfactant production by *B. casei*, which was elucidated to be a glycolipid type of biosurfactant. Use of cheap waste substrate as raw material would further reduce the economical challenge involved in nanoparticle production<sup>19</sup>. In another report on the use of biosurfactants produced from cheap substrates, biosurfactant producing strain of *P. aeruginosa* was used to synthesize and stabilize silver nanoparticles of size 1.13nm, from a medium supplemented with 2.5% vegetable oil refinery waste and 2.5 % corn steep liquor<sup>20</sup>. Such ecofriendly microemulsion techniques for nanoparticle synthesis and stabilization coupled with the use of cheap agro industrial waste substrate would be a promising strategy in not only decreasing the nanoparticle production cost but also in mitigating the unprecedented waste generated across the globe. Culture supernatant of *P. aeruginosa* was used for the green synthesis of Ag NPs of average size around 13nm which also exhibited good antimicrobial activity against gram positive and gram negative bacteria as well as few *Candida* species when tested. Such an approach would further cut down the cost of nanoparticle synthesis and make this approach more economical<sup>21</sup>. Rhamnolipid was also reported to be acting as a stabilizing and protecting agent for silver NPs in high salt concentrations<sup>22</sup>. Not much literature is available on the use of biosurfactant for the production of gold nanoparticles. A recent strategy in this field is the use of biosurfactant producing microbial strain instead of isolated biosurfactant from the same. Biosurfactant producing strain of *Pseudomonas aeruginosa* was used to synthesize gold nanoparticles wherein the biosurfactant acted as a capping agent for nanoparticle stabilization<sup>23</sup>. Hence, a lot of work needs to be done in

this aspect to make the production of gold nanoparticles an economical and ecofriendly process. Gold nanoparticles are finding huge potential to be used in biomedical field including drug delivery, antimalarial activity, anti-HIV activity, anti-angiogenesis and anti-arthritis activity<sup>24</sup>. Hence an economical and ecofriendly production of these particles would greatly benefit the biomedical sector.

### **SOPHROLIPID CAPPED NANOPARTICLES**

There have been reports on the use of surfactin and sophorolipid biosurfactants for nanoparticle synthesis as well. Sophorolipid biosurfactant was reported to be used as an effective capping and reducing agent for the synthesis of silver and gold NPs (15 nm average size range) which exhibited good aqueous re-dispersibility with no cytotoxicity and genotoxicity activity<sup>25</sup>. Oleic acid derived sophorolipids, produced by *Starmarella bombicola*, was used to make sophorolipid capped cobalt NP for biomedical applications<sup>26</sup>. Biosurfactants

have acted as capping agents for NPs thus making the process more safe and ecofriendly.

### **LIPOPEPTIDE AND NANOPARTICLES**

Limited work had been done on successful use of lipopeptides for nanoparticle synthesis and stabilization. In a study on the production of Au and Ag NPs by *Bacillus subtilis*, proteins of a molecular weight between 66 and 116 kDa were found to be responsible for the stabilization of the synthesized bacterial NPs. These were speculated to be surfactin molecules released by the bacteria *Bacillus subtilis*<sup>27</sup>. Use of surfactin as a templating agent and stabilizer led to the formation of rose like Zinc Oxide (ZnO) nanostructures of petals with average thickness between 10-13nm. The thickness of petals was found to decrease with increasing surfactin concentration. These structures were also found to have better photocatalytic activities<sup>28</sup>. Table 2 summarizes the different biosurfactants used for the synthesis of various types of nanomaterials.

**Table 2**  
**Biosurfactants for nanoparticle synthesis / stabilization**

Sr No.	Nanomaterial	Dimensions	Biosurfactant	Additional Information (if any)	Reference
1.	Au NP	4.70 nm	Surfactin	4 <sup>0</sup> C; pH 7.0	29
2	FeO NP	8 nm	Surfactin		30
3	Fe NP	100 nm	Rhamnolipid	Enhanced stabilization and mobility of Pd-NZVI	31
4	FeO NP	10-30 nm	Sophorolipid	Biosurfactant as surface complexing agent	32
5	Ba Titanate NP	20-30 nm	Low molecular weight microbial surfactant		33
6	Ag NP	6.9+/- 1.5 nm	Surfactin	4 <sup>0</sup> C; pH 7.0	34
7	Ag NP	OASL: 5.5 nm LASL: 11 nm	Oleic Acid Sophorolipid (OASL) & Linoleic Acid Sophorolipid (LASL)	90 <sup>0</sup> C	35
8	Ag NP	15.1 nm	Rhamnolipid	RT	36
9	Ag NP	15 nm	Rhamnolipid		37
10	Ag NP	OASL: 5.5 nm SASL: 5-9 nm	Oleic Acid Sophorolipid (OASL) & Stearic Acid Sophorolipid (SASL)	90 <sup>0</sup> C	38
11	Brushite NP	16-200 nm	Surfactin		39
12	ZnS NP	4.5 nm	Rhamnolipid		40
13	ZnS NP	10-15nm	Rhamnolipid		41
14	CdS NP	3-4nm	Surfactin	Biosurfactant as a stabilizing and protecting agent	42
15	ZnO NP	35-80 nm	Rhamnolipid		43
16	ZnO NP	6.55 nm	Sophorolipid	Biosurfactant as a stabilizing and biofunctionalizing agent	44
17	Silica NP	4-30 nm	Polymeric biosurfactants		45
18	Polyaniline nanorods (NR) and nanotubes (NT)	NR: 121+/- 12 nm NT: 152+/- 13 nm (average diameter)	Rhamnolipid	Biosurfactant used as a template	46
19	NiO nanospheres	47-86 nm	Rhamnolipid	pH 12.5-11.6	47

### **BIOSURFACTANTS AND NANOPARTICLES AS DRUG DELIVERY SYSTEMS**

Applications of biosurfactants have greatly contributed to the growth of Biomedical Sciences. The size and shape of nanoparticles predominantly affects its biological activity. Hence, flexibility and specificity of nanoparticle size imparted by the use of biosurfactants gains importance in the field of nanobiotechnology. Capping of nanomaterials by biosurfactants greatly alters their transport potential and hence assumes

importance for the use of nanomaterials as drug delivery agents<sup>48</sup>. Microemulsions are emerging as a novel drug delivery system for drug administration by various routes<sup>49</sup>. Much work needs to be done on the effective use of biosurfactant capped nanoparticles as drug delivery agents<sup>50</sup>. There have been reports of patenting rhamnolipid liposomes as drug delivery systems which were predicted to be safe, biocompatible, long lasting and stable<sup>51</sup>. Rhamnolipids nanoparticles loaded with drug were found to be successful and safe for use as

dermal drug delivery agent<sup>52</sup>. In a study by Cheow and Hadinoto (2012), rhamnolipid- polymer coated drug nanoparticles were found to be effective for target specific release of drugs at the biofilm site formed during lung infections<sup>53</sup>. Hence biosurfactant-nanoparticles have immense potential for use as targeted drug delivery agents.

### **PRODUCTION OF BIOSURFACTANTS BY NANOMATERIALS**

Not only biosurfactants are known to enhance nanoparticle biosynthesis but also biosurfactant synthesised by microorganisms had been found to be enhanced by nanoparticles as well. Fe and Au nanoparticles, at a concentration of 1mg/L were found to enhance biosurfactant production with the help of *Serratia* species by 63 percent<sup>54</sup>. This enhancement was found to be dependent on nanomaterial size, concentration and duration of contact. Presence of 10 mg/l of iron NPs was found to increase the production of biosurfactant by *Nocardiosis* MSA13A by around 80 percent<sup>55</sup>. Therefore, Industrial applications of nanoparticles and biosurfactants derived from natural sources<sup>56, 57</sup> are an emerging area of interest in various fields of Science and Technology.

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### **CONCLUSION**

Organic contents of microorganisms promote green synthesis of nanoparticles which find variety of applications such as in food industry, health care and catalysis. Recently it has been discovered that nanoparticles also assist in the economical enhancement of biosurfactant production. It is an interdependent synthesis wherein nanoparticles and biosurfactants can be produced from one another. Hence nanoparticle and biosurfactant bioprocesses are interlinked. Optimization and economization of any one production process affects the economy of the other compound as well as greatly reduces the cost of novel strategies of treatment being adopted in the biomedical field. However, this is a new area of integrated research which needs to be explored fully in order to create a sustainable industrial development in biomedical field.

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### **CONFLICT OF INTEREST**

Conflict of interest declared none.

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