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Review Article

Biosurfactants and its Applications in Aquaculture

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Abstract

Aquatic environments harbour a wide diversity of microorganisms that produce several novel compounds. Biosurfactant is a compound synthesized by microorganisms which have distinct physiological and metabolic pathways. Structurally they are amphiphilic molecules with hydrophilic moiety (tail) and hydrophobic moiety (head) that accumulates between two fluid phases and reduces surface tension. The property of a particular biosurfactant molecule is dependent on the location as well as the size of the functional groups. These biosurfactants can form strong emulsions at wide ranges of pH and temperature. The physico-chemical and biological properties are unique which makes the biosurfactant widely applicable in different fields, including aquaculture. Most common applications include inhibition of quorum sensing mechanism of the bacterial communication and inhibiting the growth of pathogenic bacteria. In case of fish feed processing industries, the emulsions formed by the biosurfactant can be incorporated into diet. The micelle forming property of the biosurfactant can be exploited for developing targeted drug delivery techniques. The methodologies and techniques for screening potential microorganisms which produce biosurfactant from aquatic environment and its application in aquaculture sector assume great importance. This communication provides a detailed review on the developments in these areas.

Keywords: Aquaculture, antimicrobial activity, biosurfactant, quorum sensing, screening methods

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Introduction

Microorganisms harbouring the aquatic niche show great diversity in their metabolic pathways and physiological adaptations due to varied physical, chemical and biological conditions (Hamza et al., 2017). This leads to the production of various biomolecules such as vitamins, enzymes, drugs, biosurfactants and antibiotics (Gudina et al., 2016). Among them, the compounds with distinct surface-active properties are termed as “biosurfactant” (Satpute et al., 2017; Nitschke et al., 2018; Singh et al., 2019). The biosurfactant producing microorganisms are ubiquitous *viz.*, present in seawater, freshwater, soil, sludge and oil rich fields (Satpute et al., 2010).

In aqautic environment the main function of these compounds is to inhibit the growth of competing flora including prokaryotic and eukaryotic predators (Nasrolahi et al., 2012). The inhibitory action is mainly due to the presence of surface active compounds such as glycolipids, phospholipids, lipopeptides, polymeric compounds, mycolic acids and lipopolysaccharides (Anburajan et al., 2015). Researchers of late are focusing on the use of biosurfactants as an alternative to antimicrobial agents in aquaculture (Manivasagan et al., 2014). Certain microbial derived biosurfactants are able to act as biofilm distruptors by preventing the adhesion of biofilm forming cells (Dusane et al., 2011; Padmavathi & Pandian, 2014). Biosurfactants by virtue of vast diversity, find wide applications in public health, food, environmental bioremediation, health care and agriculture.

Based on the chemical structure, biosurfactants are classified as phospholipids, glycolipids, neutral lipids, lipopeptides and polysaccharide-protein complexes (Shekhar et al., 2015). The chemical nature of biosurfactants indicate that they are amphiphilic in nature and show affinity to organic and aqueous phase. The synthesis and assembling of both the hydrophilic and hydrophobic moieties occurs through specific pathways of biosynthesis subjected to the type of biosurfactant molecule produced (lipopeptides, phospholipids, hydroxy fatty acids, acylated serine-lactones, surface-active glycolipids and lipopeptides) and the microorganisms involved which precedes with the enzymes such as glycosyl-transferases, acyl-transferases, oxidoreductases, amino-transferases, and non-ribosomal peptide synthetases (Das et al., 2008).

Properties of biosurfactant

Biosurfactants have diverse physical, chemical and biological properties making them suitable for wide range of applications when compared to chemical surfactants.

Physicochemical properties

The common structure of the biosurfactant is having a hydrophobic head attached with the hydrophilic tail forming complex structures called "Micelle". This property allows the biosurfactants to respond with the hydrophobic and hydrophilic phases which reduces the surface tension/interfacial tension between two phases i.e. liquid-liquid or solid-liquid. The physical structures (Micelles) can form into aggregates of several monomers between two immiscible phases. After attaining sufficient concentration they start to form complex structure which is termed as critical micelle concentration (CMC). This phenomenon leads to the formation of emulsion in which one phase in the two liquid phases is dispersed into the other liquid phase forming two kinds of emulsions (water-in-oil and oil-in-water).

Biological Properties

The biological properties of the biosurfactant are mainly useful for the aquaculture and food industries. They are involved in several biological processes such as competition between microbes, quorum sensing for communication between cells, as a source of nutrition and sequestration of toxic and harmful compounds. At times they are employed as an alternative to the conventional therapies for treating diseases of animals and humans. In veiw of diverse physicochemical and biological properties, the biosurfactants are source for production of novel molecules for treating infections caused by bacteria. In case of fish pathogens such as *Vibrio anguillarum* and *Aeromonas salmonicida* the quorum sensing mechanism is mediated by signals that may affect the host severely. The mechanism of quorum sensing followed by the bacteria invading the host cells precedes in this manner: attachment to the host cells, formation of biofilm, invading the cells and finally ends with production of virulence factors (Miller & Bassler, 2001). The inhibition of the quorum sensing mechanism by the biosurfactant compounds provides as an alternative to antimicrobial agents in aquaculture usage.

Application of biosurfactant in aquaculture

Biosurfactant produced by the microorganisms, their applications, and their future potential was discussed in detail in different fields *i.e.* in agriculture as bio-pesticides (Banat et al., 2000; Banat et al., 2010) and in medical field as potential immunological molecules (Cameotra & Makkar, 2004); as antimicrobial, anti-cancer, anti-adhesive agents, immunological adjuvants, antiviral activity, and gene delivery (Fakruddin, 2012) and human medicine (Gudina et al., 2013). The applications in other sectors such as oil industry, bioremediation of oil-contaminated areas, food, agriculture, cosmetic and pharmaceutical industries are well documented (Desai & Banat, 1997; Gakpe et al., 2007). However, information on the applications of biosurfactants in aquaculture sector is scanty.

The detail applications of biosurfactant in aquaculture sector is depicted in Table 1 and Fig. 1. In aquaculture, the biosurfactant molecules can be effectively applied in controlling the pathogenic organisms, in preventing biofilm formation, as an ingredient in the aquatic animal diets, as a component of antifouling paints which are discussed below.

a) Inhibition of quorum sensing and antimicrobial activity

As discussed earlier, the peculiar physicochemical and biological properties of biosurfactant can be used in the inhibition of quorum sensing in bacteria and also as antimicrobial agents against the pathogens. For instance, lipopeptide (*Bacillus circulans*) and glycolipid (*Alcaligenes* sp.) have shown antimicrobial activity towards pathogens. Some of the biosurfactants with fatty acids as a component of structure can also be used for treating the bacterial diseases in fishes (Dinamarca et al., 2013). In case

Table 1. Applications of biosurfactant in aquaculture sector

Name of Microorganism	Type of biosurfactant	Applications	References
Alcaligenes sp.	Glucose-lipid	Growth inhibition of microalgae and microflagellates	Poremba et al., (1991b)
Pseudomonas sp. Strain GU 104	2-hydroxyquinolone	Metabolism of green mussel *Pernaviridis*	Coelho et al., (2003)
Candida bombicola ATCC22214	Sophorolipid	Control of algal (*Cochlodinium*) blooms	Lee et al., (2008)
B. circulans	Lipopeptide	Antimicrobial activity	Das et al., (2008)
B. circulans	Purified biosurfactant	Antimicrobial activity	Mukherjee et al., (2009)
Serratia marcescens	Glycolipid	Anti-biofilm formation	Dusane et al., (2011)
Planococcus maitriensis	Carboxylicacids	Antifungal agent	Sankar et al., (2013)
Cobetia sp. strain MM1IDA2H-1	3-hydroxy fatty acids	Quorum sensing of fish pathogens	Ibacache-Quiroga et al., (2013)
Pseudomonas aeruginosa DG2a	Fatty acid degrading biosurfactant	Contaminant fatty acid removal from aquaculture waste water	Pepi et al., (2013)
Serratia marcescens	Quorum sensing molecules	Diseased tilapia fish	Chan et al., (2013)
Bacillus sp. BS3	Lipopeptide	Antiviral activity against shrimp white spot syndrome virus (WSSV)	Donio et al., (2013)
Providencia rettgeri, Psychrobacter sp. *Bacillus flexus, Bacillus anthracis, Psychrobacter* sp. *and Bacillus pumilus*	Lipopeptide	Anti-biofilm formation	Padmavathi and Pandian (2014)

of human pathogenic bacteria (*Escherichia coli* and *Proteus mirabilis*) the fatty acid type of biosurfactant inhibits the growth and disturbs the cell to cell communication (Liaw et al., 2004; Widmer et al., 2007). This is also noticed in case of fish pathogens such as *A. salmonicida* where the hydroxyl fatty acids produced by *Cobetia* sp. can be used to control the bacterial infection in salmonid fish farming (Ibacache-Quiroga et al., 2013). Similarly, reports indicate the production of quorum sensing molecules by *Serratia marcescens* in diseased catfish (Chan et al., 2013). In quorum sensing, these compounds act on the receptors of the signaling molecules, they antagonize them breaking the cellular communication between the bacteria. Alternatively, they can inhibit the production of the signaling molecules. The biosurfactant molecules produced from marine sources are of particular interest in inhibition of quorum sensing of aquatic pathogens due to the differences in their chemical structures. Antimicrobial activity of lipopeptide biosurfactant produced by *B. circulans* observed against pathogenic bacteria (Das et al., 2008; Mukherjee et al., 2009). Similarly, the antifungal activity of biosurfactant produced by *Planococcus maitriensis* was also reported (Sankar et al., 2013). Studies also indicated the effect of biosurfactant produced by *Alcaligenes* sp. on growth inhibition of microalgae and microflagellates (Poremba et al., 1991b). Antiviral activity of the biosurfactant produced by *Bacillus* sp. BS3 against white spot syndrome virus (WSSV) was reported by Donio et al. (2013).

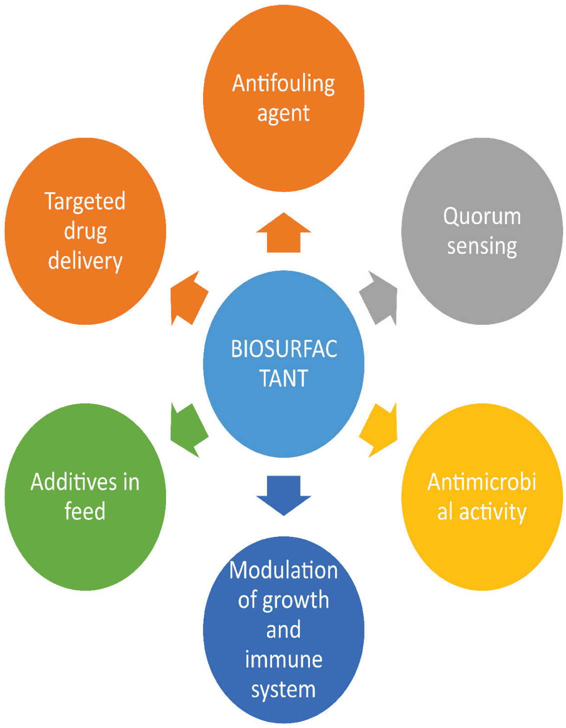

Fig. 1. Applications of biosurfactant in different areas of aquaculture sector

b) Modulation of growth and immune system

The biosurfactant molecules help the fish to grow faster with the help of enzymes and proteins secreted and inhibition of pathogenic bacterial virulence factors. The affectivity of the immune system depends on the growth of fish as the larval stages are vulnerable to bacterial infections (Bricknell & Dalmo, 2005). Fish harbour specific immune system, albeit innate immune responses play an important role in the bacterial infections (Magnadottir, 2006). These responses are not only activated by biological molecules such as lipoproteins, lipopolysaccharides and glycoproteins of microbial origin, but also by the enzymes produced by these bacteria (Arancibia, 2007). Reports indicate that the marine microbes are important sources of biosurfactant molecules that activate the innate immune responses in fishes (Cameotra & Makkar, 2004; Pastva et al., 2007).

c) Other applications

The significant advantage of microbial biosurfactants compared to synthetic surfactants is that they are less toxic, high stability, high biodegradability and can be produced from low cost sources (Van Hamme et al., 2006; Singh et al., 2007). The property of the biosurfactants of forming emulsions with high stability is useful in aquaculture practices. For instance, biosurfactant from *Cobetia* sp. can form stable emulsion at a different range of temperatures, strong force and pH (Ibacache-Quiroga et al., 2013). In fish feed manufacturing industry, these biosurfactant finds applicability as additives in feeds due to the emulsification property and stability at high temperature. The biosurfactants can be added into feeds during extrusion due to their stability at higher temperature. Also the emulsification property of the biosurfactant is useful in mixing the feed ingredient as polymeric food matrices for the preparation of fish diets. Another biological property *i.e.* micelle formation is very important feature of biosurfactant useful for the effective and targeted delivery of the drugs into aquaculture systems, thereby reducing the pollution from the antimicrobial agents. They are also useful for targeting aquatic pathogens by inhibiting the formation of biofilms on the water structures. Both the organic phase and aqueous phase can be incorporated into the feed during manufacturing. Important application of biosurfactant is due to their ability to exist both as water and oil emulsions as they can be frequently used for bioremediation of aquatic sources. In recent years, silver nanoparticles are employed for effective delivery systems for drugs and emulsions formed by the biosurfactants can be used for administering these inorganic particles (Xie et al., 2006). The accumulation of fouling organisms and biofilm formation on the fishing boat surfaces is major hindrance for fishing and aquaculture installations. Role of biosurfactant produced by several bacteria in inhibition of biofilm formation was reported (Dusane et al., 2011; Padmavathi & Pandian, 2014). The incorporation of these biosurfactant molecules into the antifouling and anti-biofilm paints can get rid of these problems because of the property of micelle formation. The biosurfactant produced by *Candida bombicola* were used to control algal blooms in aquatic environment (Lee et al., 2008).

Screening methods for biosurfactant producers from aquatic sources

There are several obstacles in finding suitable method for screening potential biosurfactant producing bacteria due to addition of varoius inputs for environmental management and also improving the growth performance of animals (Lang & Wagner,

Table 2. Screening methods employed for isolation of biosurfactant from aquatic sources

Screening methods	Source of isolation	Organism isolated	References
BATH assay	Marine environment	*Azotobacter chroococcum*	Thavasi et al., (2009)
Hemolytic activity, drop collapsing test, oil displacement method	Sponge-associated	*Nocardiopsis alba MSA10* (Actinomycetes)	Gandhimathi et al., (2009)
Hemolytic assay, BATH assay, drop collapse assay, oil spreading assay, emulsification assay and surface tension measurement	Sea water samples	*Branhamella catarrhalis, Citrobacter intermedius* and *Klebsiella ozaenae*	Thavasi et al., (2011)
Surface tension	Seawater	*Cobetia* sp. strain MM1IDA2H-1	Ibacache-Quiroga et al., (2013)
Hemolytic activity, Emulsification Index, Surface tension measurement	Sediment and water samples	*Pseudomonas aeruginosa*	Balan and Jayalakshmi (2013)
Hemolytic activity, Emulsification Index, Oil spreading assay	Water and sediment	*Planococcus maitriensis*	Sankar et al., (2013)
Emulsifying activity, oilspreading tests, haemolytic and cetyltrimethyl ammoniumbromide agar tests.	Aquaculture wastewaters	*Pseudomonas aeruginosa* DG2a	Pepi et al., (2013)
Surface tension measurement, Oil spreading method, Emulsification index	Sediment, matt and water samples	*Exiguobacterium* sp.	Tambekar et al., (2013)
Emulsification stability	Soil in fish processing industry	*B. subtilis*	Saranya et al., (2014)
Hemolytic activity, Drop collapsing test, Oil displacement test, Emulsification index	Marine sponge-associated	*Bacillus licheniformis* NIOT-AMKV06	Lawrance et al., (2014)
Oil displacement test, methylene blue method, Emulsification activity,	Solar salt works	*Kocuria marina* BS-15	Sarafin et al., (2014)
Blue agar plate (CTAB) Emulsification index, BATH assay, Oil spreading method	Aquaculture farm soil	*Pseudomonas aeruginosa*	Nadella et al., (2018)

1993). Hence, a multitude of screening methods for the identification of surface-active compounds produced by bacteria is need of the hour. Although there are several studies reported on biosurfactant production from aquaculture (Tambekar et al., 2013; Sankar et al., 2013; Padmavathi & Pandian, 2014; Nadella et al., 2018), systematic overview on the techniques followed for screening of biosurfactant production is not avaiable. Various methods used for screening and characterisation of biosurfactant producing bacteria are a) agar plate covered with hydrocarbons (Morikawa et al., 1992); b) shape analysis of liquid drop (Vegt et al., 1991). This is a useful method for slow-growing microorganisms; c) adhesion Bacterial cells to Hydrocarbons (BATH) assay (Rosenberg et al., 1980); d) Blue agar plate or CTAB method (Siegmund & Wagner, 1991), which is useful for the detection of the glycolipids and rhamnolipids (biosurfactant) produced by the Gram-negative organisms such as *Pseudomonas* sp; e) Lytic activity on blood agar plate (Mulligan et al., 1984); f) Drop collapse method (Jain et al., 1991); g) Oil spread method (Youssef et al., 2004); h) Tilted glass slide method (Persson & Molin, 1987); *i*) Emulsification assay (Patil & Chopade 2001b); j) Emulsification index (Cooper & Goldenberg, 1987); k) Turbidity assay (Rosenberg et al., 1980; Neu & Poralla, 1990); l) Measurement of surface tension (Dilmohamud et al., 2005) and m) Structural characterization of biosurfactant by Fourier Transform Infra-Red (FTIR) and GC-MS (Parthipan et al., 2017; Ibrahim 2018). The methods described above will be useful for screening, detecting and evaluating the potential biosurfactant producing microorganisms from aquatic sources. Studies pertaiming to different screening methods of biosurfactant from aquatic sources are listed in Table 2.

Advantages and disadvantages of biosurfactant

The biosurfactants compounds offers an alternative source for chemical surfactants. When compared to chemical surfactants, biosurfactants have several advantages *viz*., biodegradable, less toxic, better digestibility and biocompatible. In recent years, biosurfactants are being widely used in natural bioremediation process of contaminated soils (Fracchia et al., 2014). Other advantages include selective nature and specific activity at various environmental conditions (temperatures, salinity, pH). Biosurfactants can be produced cheaply from various low-cost raw materials. The major impediments are difficulty for large scale production and the expensive nature of up-scaling processes.

Conclusion

Microbes from aquatic sources produce diverse types of biosurfactants such as glycolipid, rhamnolipids Lipopeptide, and other types of biosurfactants, that show structural novelty, biological, physic-chemical properties. They exhibit wide range of activities *viz.,* antimicrobial, antiviral and adhesive properties that are useful for administering the drugs through dietary source to the aquatic animals for the proetction against pathogens. They can be employed as an alternative to broad-spectrum antibiotics which are used for treating diseases in aquaculture. The important advantage over the antibiotics is their stability at diverse conditions and non-persistent nature in the sediments or in animal tissues. The adhesive property of the biosurfactants can be used for administering medicines to aquatic animals. Therefore, the biosurfactants can act as safe vehicle in drug delivery. They can also be used in developing antifouling paints for the protection of the fishing vessels against fouling organisms. And hence, better understanding of the screening methods will help in isolating the microbes with novel biosurfactant producing capability and the scaling methods for the enhancing the production at pilot level to meet the demand of the aquatic industry. In addition, scaling up technologies employing substrates from aquatic sources are essential for economically viable production of biosurfactant.

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