

# High molecular weight bioemulsifiers, main properties and potential environmental and biomedical applications

Inès Mnif · Dhouha Ghribi

Received: 30 December 2014 / Accepted: 22 February 2015 / Published online: 5 March 2015  
© Springer Science+Business Media Dordrecht 2015

**Abstract** High molecular weight bioemulsifiers are amphipathic polysaccharides, proteins, lipopolysaccharides, lipoproteins, or complex mixtures of these biopolymers, produced by a wide variety of microorganisms. They are characterized by highly structural diversity and have the ability to decrease the surface and interfacial tension at the surface and interface respectively and/or emulsify hydrophobic compounds. Emulsan, fatty acids, phospholipids, neutral lipids, exopolysaccharides, vesicles and fimbriae are among the most popular high molecular weight bioemulsifiers. They have great physico-chemical properties like tolerance to extreme conditions of pH, temperature and salinity, low toxicity and biodegradability. Owing their emulsion forming and breaking capacities, solubilization, mobilization and dispersion activities and their viscosity reduction activity; they possess great environmental application as enhancer of hydrocarbon biodegradation and for microbial enhanced oil recovery. Besides, they are applied in biomedical fields for their antimicrobial and anti-adhesive activities and involvement in immune responses.

**Keywords** Polymeric bioemulsifiers · Emulsification · Surface activity · Environmental applications · Biomedical and therapeutic applications

## Introduction

Today, synthetic emulsifiers are among the most produced compounds in the world as they are used in various industries like textile, paper, polymer, plastic, cosmetics, pharmaceuticals, food and machinery manufacture and in bioremediation (Kitamoto et al. 2002). However, they are highly toxic to environment and living organisms. So, microbial derived emulsifiers have particular interest and became widespread for potential use in diverse fields. They were firstly discovered as extracellular amphiphilic compounds in research into hydrocarbon fermentation, which started in the late 1960s (Kitamoto et al. 2002). They attracted attention as “alternative to chemical emulsifiers” due to their high biodegradability and environmental safety. They are mainly classified into four categories, i.e., (1) glycolipid type (4), (2) fatty acid type (5), (3) lipopeptide type (6) and (4) polymer type (7), based on the structure of their hydrophilic part. Among them, polymeric bioemulsifiers have attracted a great attention for biotechnological applications.

Generally, they are amphipathic polysaccharides, proteins, lipopolysaccharides, lipoproteins, fatty acids or complex mixtures of these biopolymers. Also, some whole bacterial cells, vesicles and fimbriae with complex structure are recognized as emulsifying agents. They can be produced from inexpensive raw materials that are available in large quantities, such as industrial wastes (Panilaitis et al. 2007) and oily byproducts including hydrocarbons (Kaeppli and Finnery 1979; Leahy et al. 2003; Martinez-Checa et al. 2007; Arli et al. 2011) and vegetable oils (Liu et al. 2011). In many cases, they are produced by hydrocarbon degrading bacteria during their growth (Kaeppli and Finnery 1979; MacDonald et al. 1981; Pines and Gutnick 1986; Peng et al. 2007; Sadouk et al. 2009).

I. Mnif · D. Ghribi  
Higher Institute of Biotechnology, Sfax, Tunisia

I. Mnif (✉) · D. Ghribi  
Unit Enzymes and Bioconversion, National School of Engineers,  
ENIS, BP W 3038, Sfax, Tunisia  
e-mail: inesmnif2011@gmail.com

Furthermore, the production efficiency of the bioemulsifiers using microorganisms has been improved along with the progress of biotechnology by the amelioration of fermentation conditions (Doshi et al. 2010) and the optimization of production by means of the response surface methodology (Prapulla et al. 1992; Albuquerque et al. 2006; Su et al. 2009). Therefore, the yields of bioconversion are always so high that they permitted the retention of huge quantity of polymeric biosurfactants for diverse applications. They can be produced by a wide diversity of bacteria (Mousavian and Rahimi 2010; Burgos-Díaz et al. 2011; Franzetti et al. 2012), Actinomycete (Kokare et al. 2007; Maniyar et al. 2011), yeast (Walencka et al. 2007; Dikit et al. 2010) and fungus (Batrakov et al. 2001; Paraszkiwicz et al. 2002; Katemai et al. 2008). Generally, polymeric bioemulsifiers are released in the extracellular medium of the fermentation broth (Boyle and Reade 1983; Calvo et al. 1995). However, they can also be attached to the cell surface (Dikit et al. 2010; Huang et al. 2013).

Owing their structural diversity, interesting physico-chemical properties (great stability to drastic conditions of pH, salinity and temperature, low toxicity and biodegradability) and great functional properties (emulsification/de-emulsification, foaming, dispersing, hydrocarbon solubilizing ...), they are potential candidates for bioremediation of hydrocarbon contaminated sites. Also, polymeric bioemulsifiers can be recognized for their antimicrobial and anti-adhesive activities and can be involved in several immune responses allowing their use in biomedical and therapeutic fields. This review deals with the latest research and development in polymeric type bioemulsifiers, including their structural diversity, surface activity, functional properties and potential applications in environmental and biomedical fields.

### Polymeric bioemulsifiers: definition and classification

Numerous microorganisms are characterized by the synthesis of a wide variety of high- and low-molecular-weight bioemulsifiers. Generally, high molecular weight mass bioemulsifiers are proteins, polysaccharides, lipopolysaccharides, lipoproteins, or composite assortment of these biopolymers (Perfumo et al. 2010). The most studied polymeric bioemulsifiers are emulsan, alasan, liposan, mannoprotein and other polysaccharide protein complexes.

#### Emulsan family

Emulsan, an extracellular complex of acylated polysaccharide produced mainly by the gram-negative bacterium, *Acinetobacter* species, is among the most popular polymeric bioemulsifiers (Panilaitis et al. 2007). It's composed of a polysaccharide backbone with O-acyl and N-acyl

bound fatty acid side chains. The polysaccharide backbone consists of three amino sugars (D-galactosamine, D-galactosaminouronic acid, and a dideoxydiaminohexose) and the fatty acid side chains range in length from 10 to 20 carbons, and represent 5–23 % (w/w) of the polymer (Panilaitis et al. 2007). The emulsan amino groups are either acetylated or covalently linked by an amide bond to 3-hydroxybutyric acid (Panilaitis et al. 2007). The combination of hydrophilic anionic sugar main chain repeat units, along with the hydrophobic side groups leads to the amphipathic behavior of emulsan and, therefore, its ability to form stable oil-in-water emulsions (Panilaitis et al. 2007). In fact, emulsan is a very effective emulsifying agent for hydrocarbons in water even at concentration as low as 0.001–0.01 %.

Alasan, belonging to the emulsan family, is a high-molecular-weight complex of polysaccharide and protein synthesized generally by *Acinetobacter* species with a molecular weight of about 10<sup>3</sup> kDa (Navon-Venezia et al. 1998). However, the polysaccharide component of alasan is unusual in that it contains covalently bound alanine (Navon-Venezia et al. 1998). The purified polysaccharide and protein components have no emulsifying activity when used lonely. In fact, the protein component of alasan appears to play an important role in both the structure and the activity of the complex. However, the combination of the polysaccharide and the protein led to the reconstitution of the emulsifying activity (Toren et al. 2001).

In addition to emulsan family, a complex structure of polymeric bioemulsifiers consisting of a complex of lipids, proteins and polysaccharides, exopolysaccharides (EPS), proteins, glycoprotein compounds and glycolipoproteins can be found in the nature. Different examples of these compounds along with their producing strains and biological activities were presented in Table 1.

#### Fatty acids, phospholipids and neutral lipids

Large quantities of fatty acids, phospholipids or neutral lipids bioemulsifiers could be produced by several microorganisms when grown on n-alkanes. As suggested by Beeba and Umbreit (1971), the growth of *Thiobacillus thiooxidans* on sulfur particles is supported by the wetting of those particles by an heterogeneous mixture of neutral lipid and phospholipid, present in the medium, resulting from cellular disruption. Table 2 illustrated some examples of fatty acids, phospholipids and neutral lipids along with their microbial producing strains and biological activities.

#### Fatty acid biosurfactant

Generally, fatty acids produced by microorganisms might be simple straight-chain fatty acids or of complex nature

**Table 1** Major polymeric-surfactants sub-classes, biological activities and microbial sources

Sub-classes	Producing strain	Biological activities	References
Emulsan: a complex of acylated polysaccharide	<i>Acinetobacter venetianus</i> RAG-1	Emulsification activity	Su et al. (2009)
	<i>A. calcoaceticus</i> (PTCC 1318)	Emulsification activity	Dehghan–Noudeh et al. (2007)
	<i>Alcaligenes faecalis</i>	Potential candidate for microbial enhanced oil recovery	Salehizadeh and Mohammadizad (2009)
Alasan: complex of anionic heteropolysaccharid and protein	<i>A. radioresistens</i> KA53	Emulsifying activity	Toren et al. (2001)
	<i>A. venetianus</i> RAG-1	Coating agent	Castro et al. (2008)
	<i>Acinetobacter</i> sp.	Emulsifying activity	Jagtap et al. (2010)
Complex of polysaccharide and lipid	<i>Candida lipolytica</i> (Liposan)	Emulsification activity	Cirigliano and Carman (1985)
	<i>Burkholderia cepacia</i>	Complex lipid adjuvants	Kawai et al. (2002)
	<i>Gordonia</i> sp. strain JE-1058	Dispersing and washing activities	Saeki et al. (2009)
Yansan (lipid–carbohydrate–protein complex)	<i>C. lipolytica</i>	Emulsification activity	Albuquerque et al. (2006)
	<i>Yarrowia lipolytica</i> IMUFRJ 50682	Bioemulsifier	Trindade et al. (2008)
Complex of lipids, proteins and polysaccharids	<i>P. marginalis</i> PD-14B	Emulsifying activity and enhancement of hydrocarbons biodegradation	Burd and Ward (1996)
	<i>C. glabrata</i> UCP 1002	Bioemulsifier	Sarubbo et al. (2006)
	<i>C. lipolytica</i>	Bioemulsifier	Rufino et al. (2007)
	<i>B. licheniformis</i> K125	Potential candidate for microbial enhanced oil recovery	Suthar et al. (2008)
	<i>Alcaligenes</i> sp. S-XJ-1	Biodemulsifying activity	Huang et al. (2013)
Peptidoglycolipid bioemulsifer	<i>Streptomyces</i> sp. S22	Emulsification activity	Maniyar et al. (2011)
Biodispersane: anionic extracellular polysaccharide	<i>A. calcoaceticus</i> A2	Dispersing activity	Elkeles et al. (1994)
Exopolysaccharid	<i>P. putida</i> ML2	Bioemulsifier	Bonilla et al. (2005)
	<i>Halomonas eurihalina</i> , <i>B. subtilis</i>	Emulsification activity	Maneerat et al. (2006)
	<i>Alcaligenes faecalis</i> and <i>Enterobacter</i> sp.	Emulsification activity	Toledo et al. (2008)
	<i>A. calcoaceticus</i> CBS 962.97	Emulsifying activity	Cappello et al. (2011)
	<i>B. cereus</i> and <i>Brachybacterium</i> sp.	Emulsifying activity	Orsod et al. (2012)
	Protein	<i>Pseudomonas</i> PG-1	Enhancement of hydrocarbon biodegradation
<i>P. aeruginosa</i> S7B <sub>1</sub>		Emulsifying activity	Hisatsuka et al. (1977)
<i>Acinetobacter</i> sp. A3		Involved in hydrocarbon degradation	Hanson et al. (1994)
<i>Aeromonas salmonicida</i>		Immunomodulatory activity	Garduno et al. (1995)
<i>Lactobacillus fermentum</i> RC-14		Anti-adhesive activity	Heinemann et al. (2000)
<i>Brevibacillus brevis</i> (Gramicidin S)		Biocontrol activity (antifungal)	Edwards and Seddon (2001)
<i>Staphylococcus aureus</i>		Immunomodulatory activity	Haggar et al. (2003)
<i>B. mojavensis</i> XH-1		Biodemulsifying activity	Hou et al. (2013)
Glycolipoprotein	<i>Aspergillus ustus</i> MSF3	Antimicrobial activity and potential candidate for microbial enhanced oil recovery	Seghal Kiran et al.(2009)
	<i>Microbacterium</i> sp. MC3B-10 (Microbactan)	Emulsifying activity	Camacho-Chab et al.(2013)
Glycoprotein			

**Table 1** continued

Sub-classes	Producing strain	Biological activities	References
Glycoprotein	<i>Lactobacillus plantarum</i> KLAB21	Antimutagenic activity	Rhee and Park (2001)
Surlactin	<i>Lactococcus lactis</i> 53	Antimicrobial and anti-adhesive activity	Rodrigues et al. (2006)
	<i>Halomonas</i> species	Bioemulsifier	Gutiérrez et al. (2007)
	<i>Lactobacillus acidophilus</i> RC 1	Inhibition of biofilm formation	Smaeel et al. (2013)
Mannoprotein	<i>Kluyveromyces marxianus</i> FII 510700	Emulsification activity	Lukondeh et al. (2003)
	<i>S. cerevisiae</i>	Anti-adhesive and Anti-biofilm action	Walencka et al. (2007)
Glycoprotein	<i>Rhodotorula mucilaginosa</i> KUGPP-1	Astaxanthin-stabilizing activity	Kawahara et al. (2013)
Carbohydrate-protein complex	<i>Rhodotorula glutinis</i>	Bioemulsifying activity	Oloke and Glick (2005)
	<i>Streptomyces</i> species S1	Bioemulsifying activity	Kokare et al. (2007)
<i>Lipoprotein</i>			
Ornithine and taurine containing lipid	<i>Gluconobacter cerinus</i> IFO 3267	–	Tahara et al. 1976
Ornithine containing lipid	Different bacterial species	Strong stimulant for macrophages, adjuvant	Kawai et al. (1999)
	<i>S. paucimobilis</i> IAM 12576 <sup>T</sup>	Hemagglutinating activity	Kawai et al. (2002)
	<i>Myroides</i> sp. strain SM1	Bioemulsifier	Maneerat et al. (2006)
	<i>Agrobacterium tumefaciens</i>	–	Geske et al. (2013)
Ornithine and glutamine containing lipid	<i>Rhodobacter sphaeroides</i>	–	Zhang et al. (2009)
Emulcyan	<i>Phormidium</i> J-1	Dispersing activity	Fattom and Shilo (1985)

containing OH groups and alkyl branches (Rahman and Gakpe 2008). They resulted from the microbial oxidation of alkanes and have gained great attention as surfactants. They were also known as lowering the surface and interfacial tensions. Among them, we distinguish mycolic acids and corynomucolic acid. Mycolic acids are long-chain;  $\beta$ -hydroxy fatty acids substituted at the  $\alpha$ -carbon atom with a moderately long aliphatic chain and are primarily produced by *Mycobacterium*, *Nocardia*, *Rhodococcus*, and *Corynebacterium* species (Marrakchi et al. 2014). Corynomucolic acid is another example of a complex fatty acid containing OH groups and alkyl branches that are produced by *R. erythropolis* (Desai and Banat 1997). Fatty acids with chain length ranging from C<sub>9</sub> to C<sub>22</sub> (dicorynomucolates, monocorynomucolates and tetraester) produced by *R. Erythropolis* 3C-9 were shown to enhance the solubility of polycyclic aromatic hydrocarbons and the degradation rate of hexadecane (Peng et al. 2007).

#### Phospholipids biosurfactant

Phospholipids are known to be the key components of microbial membranes. When certain hydrocarbon-

degrading bacteria or yeast are grown on alkane substrates, the level of phospholipid increases greatly. For instance, using hexadecane-grown *Acinetobacter* sp. HO1-N, phospholipids (mainly phosphatidylethanolamine) loaded vesicles were produced (Kaeppli and Finnery 1979). Their effective surfactant properties are evidenced by their ability to generate optically clear micro-emulsions of alkanes in water (Ron and Rosenberg 2001). Moreover, they are recognized by their aptitude to reduce the surface and interfacial tension. Phosphatidyl ethanolamine produced by *R. erythropolis* grown on n-alkane resulted in the reduction of the interfacial tension between water and hexadecane at <1 mN/m with a Critical Micelle Concentration (CMC) of about 30 mg/L (Kretschmer et al. 1982).

#### Neutral lipids biosurfactant

Triacylglycerols found in all eukaryotic cells (yeasts, molds, plants and animals) and esters produced by *Acinetobacter* sp. related bacteria such as Moraxella and some eukaryotic algae are examples of neutral lipids biosurfactants (Desai and Banat 1997). They can be also produced

**Table 2** Fatty acids, phospholipids, vesicles, fimbriae and whole bacterial cells as polymeric surfactants

Sub-classes	Producing strain	Biological activities	References
Fatty acid	<i>Arthrobacter paraffineus</i>	Surface active-agent	Duvnjak et al. (1982)
	<i>Corynebacterium lepus</i>	Surface activity	Duvnjak and Kosaric (1985)
	<i>Mesorhizobium</i> spp.	–	Choma and Komaniecka (2002)
	<i>Candida ingens</i>	Surface activity	Amézcuca-Vega et al. (2007)
	<i>R. Erythropolis</i> 3C-	Surface active-agent	Peng et al. (2007)
	<i>C. ingens</i>	–	Amézcuca-Vega et al. (2007)
	<i>R. erythropolis</i> strain 3C-9	Enhancement of hydrocarbons solubility and degradation	Peng et al. (2007)
	<i>Issatchenkia orientalis</i> (Oleic acid)	–	Katamai et al. (2008)
	Different bacterial strain	Immunomodulatory activity	Seo et al. (2008)
	<i>P. citronellolis</i> KHA	Surface active and emulsifying agent	Sadouk et al. (2009)
Phospholipids	<i>Halomonas</i> sp. BS4	Antimicrobial and anticancer activity	Donio et al. (2013)
	<i>Acinetobacter</i> sp.	Surface active compounds	Kaeppli and Finnery (1979)
	<i>Thiobacillus thiooxidans</i>	Wetting elemental sulphur necessary for growth	Rosenberg and Ron (1999)
	<i>Absidia corymbifera</i>	–	Batrakov et al. (2001)
Phosphatidylethanolamine	<i>Mesorhizobium</i> spp.	–	Choma and Komaniecka (2002)
	<i>Sphingobacterium</i> sp.	Surface active agent	Burgos-Díaz et al. (2011)
	<i>Rhodococcus erythropolis</i>	Surface active-agent	Kretschmer et al. (1982)
Glycerophospholipids	<i>Absidia corymbifera</i>	–	Batrakov et al. (2001)
	<i>Absidia corymbifera</i>	–	Batrakov et al. (2001)
Fatty acid esters and phosphatidylethanolamine surfactants	<i>P. putida</i> BD2	Antiadhesive activity	Janek et al. (2013)
A monoglyceride, an ester, and a fatty acid	Consortium of marine bacteria	Emulsification and dispersion of petroleum hydrocarbons	Deshmukh et al. (2012)
	<i>Nocardia erythropolis</i> (ATCC 4277)	Surface-active lipids	MacDonald et al. (1981)
Polyol lipids	<i>Rhodotorula Glutinous</i>	Emulsifying activity	Johnson et al. (1992)
Triacylglycerols and diacylglycerols	<i>Absidia corymbifera</i>	–	Batrakov et al. (2001)
	<i>A. calcoaceticus</i> C42	–	Bashetti et al. (2012)
Extracellular lipid (mixture of phospholipid and neutral lipid, primarily free fatty acids)	<i>Thiobacillus thiooxidans</i>	Weeting agent	Beeba and umbreit (1971)
Diether phytanyl phosphatidyl glycerol phosphate lipid	<i>Halobacterium halobium</i> NRC 34003	Emulsifying activity	Post and Collins (1982)
Vesicles	<i>Xenorhabdus nematophilus</i>	Insecticidal activity	Patrick et al. (1996)
	<i>Acinetobacter</i> sp. strain HO1-N	Emulsification activity	Leahy et al. (2003)
	<i>Actinobacillus actinomycetemcomitans</i>	Anticancer activity	Demuth et al. (2003)
	<i>Porphyromonas gingivalis</i>	Platelet aggregation activity	Sharma et al. (2000)
	<i>Bacteroides fragilis</i>	Haemagglutinating and enzymic activities	Khandelwal and Banerjee-Bhatnagar (2003)
	<i>Brucella melitensis</i>	Induction of cell-mediated immunity in mice	Avila-Calderon et al. (2011)

**Table 2** continued

Sub-classes	Producing strain	Biological activities	References
Fimbriae	<i>A. calcoaceticus</i> RAG-1	Enhancing alkane uptake by microorganisms	Rosenberg et al. (1982)
	<i>P. fluorescens</i>	Hydrophobic attachment to polystyrene, hemagglutination, and attachment to corn roots	Vesper (1987)
	<i>Porphyromonas gingivalis</i>	Activate Human Gingival Epithelial Cells through Toll-Like Receptor 2	Asai et al. (2001)
Whole bacterial cells	<i>B. subtilis</i>	De-emulsification activity	Janiyani et al. (1994)
	<i>Nocardia amarae</i>	De-emulsification activity	Lee and Lee (2000)
	Aerial spores of a <i>Streptomyces</i> sp.	De-emulsification of oil-in-water emulsions	Park et al. (2000)
	<i>Micrococcus</i> sp.	De-emulsification capabilities	Das (2001)
	<i>Lactococcus lactis</i> subsp <i>lactis</i>	Control the stability of emulsion	Ly et al. (2006)
	<i>Micrococcus luteus</i> BN56	Emulsion-stabilizing agents	Tuleva et al. (2009)
	<i>Ochrobactrum. anthropi</i> strain RPI15-1	De-emulsifying activity	Mohebbali et al. (2012)

by *Nocardia erythropolis* (Desai and Banat 1997) and *A. calcoaceticus* C42 (Bashetti et al. 2012).

#### Exopolysaccharides bioemulsifiers

Exopolysaccharides complex active extracellular biopolymers, are high molecular weight carbohydrate polymers. They could be produced by various marine microorganisms form a layer surrounding the cells helping them to withstand or resist to adverse and extreme environmental conditions offering a high structural diversity (Satpute et al. 2010). These compounds are important in microbial interaction and emulsification of various hydrophobic substrates (Maki et al. 2000; Yim et al. 2005). They are also known to increase the viscosity of solutions at low pH value and emulsify several hydrocarbon compounds (Guezennec et al. 1994; Calvo et al. 1998).

#### Particulate biosurfactants and whole bacterial cells

Numerous microbial strain release extracellular membrane vesicles, called particulate biosurfactants, that partition to hydrocarbons to form microemulsion which plays an important role in alkane uptake by microbial cells. Vesicles of *Acinetobacter* sp. strain HO1-N, composed of protein, phospholipid, and lipopolysaccharide and those of *A. calcoaceticus* are some examples (Desai and Banat 1997). Moreover, some other particulate biosurfactants could be synthesized and remain adhered to the cell surfaces.

Therefore, the corresponding microbial cells are characterized by their highly hydrophobic surface having the capacity to adhere to hydrocarbons and they could be recognized as bio-emulsifying agents.

#### Main properties of polymeric bioemulsifiers

##### Emulsification capacity

Emulsification corresponds to a dispersion of one liquid into another (as microscopic droplets) leading to the mixing of two immiscible liquids. Generally, polymeric bioemulsifiers are recognized by their emulsification activity and are good emulsifiers for food and petrochemical industry.

A partially purified bioemulsifier produced by *Acinetobacter* sp. formed stable oil-in-water emulsions with plant oils with a maximum emulsification activity of about 400 EU/mL (Jagtap et al. 2010). Also, a *Streptomyces* sp. S22 strain produced a peptide glycol lipid bioemulsifier with a maximum emulsification activity of 320 EU/mL (Maniyar et al. 2011). A glycol lipoprotein bioemulsifier produced by *Microbacterium* sp. MC3B-10 microbactan emulsified aromatic hydrocarbons and oils to various extents with a higher emulsification index against motor oil of about 96 % with a higher stability (94 %) at 50 °C, pH 10 and 3.5 % NaCl content (Camacho-Chab et al. 2013). Moreover, mannoprotein extracted from the cell wall of the

yeast *Saccharomyces cerevisiae* exhibited emulsion with the vegetable oils tested and showed emulsion activity of 65 % towards palm oil as oil-in-water with a critical emulsifier concentration of 20 g/l with similar emulsifying properties to the commonly used food emulsifiers gum arabic and lecithin (Dikit et al. 2010). The emulsion was stable over a wide range of pH, temperature and salinity showing a great potential for application in salad dressing. A complex of lipids, proteins and polysaccharids bioemulsifier produced by *Candida lipolytica* formed stable emulsions with hydrophobic natural compounds (Rufino et al. 2007). In addition, fatty acid esters produced by *P. citronellolis* KHA showed greater emulsifying properties with an  $E_{24}$  of 75 % at the end of the exponential growth phase permitting its use in the cleanup of the sites contaminated with hydrocarbons (Sadouk et al. 2009). Besides that, whole bacterial cells of *Pseudomonas marginalis* PD-14B (Burd and ward 1996) and *Micrococcus luteus* BN56 (Tuleva et al. 2009) were reported as interesting emulsifying agents. Furthermore, EPS of *Alcaligenes faecalis* and *Enterobacter* sp. which were able to emulsify *n*-octane, toluene, xylene, mineral oils and crude oil, showed potential for bioremediation application (Toledo et al. 2008). Similarly, a *Sphingomonas paucimobilis* derived exopolysaccharide emulsified efficiently xylene, benzene, 2-methylnaphthalene, hexadecane, hexane, kerosene and paraffin oil as well as castor, coconut and olive oils when used at 1 mg/mL (Ashtaputre and Shah 1995). It stabilized the emulsions more efficiently than commercial gums such as arabic, tragacanth, karaya and xanthan and emulsions were stable for 6 months, from 4 to 40 °C and pH 4 to 10 and in the presence of NaCl up to 50 g/l (Ashtaputre and Shah 1995). A curd-like exopolysaccharide production by *Cellulomonas flavigena* UNP3 during growth on hydrocarbon substrates was able to emulsify hexadecane (Arli et al. 2011).

#### De-emulsification capacity

In addition to the emulsification capacity, polymeric bioemulsifiers are able to break down the formed emulsion; they are recognized as de-emulsifiers. De-emulsification is achieved by disturbing the thermodynamic conditions at the interface. It's very useful for the petrochemical industry. In fact, every year, millions of tons of crude oil emulsions are generated by petroleum exploration and water–oil emulsions occur throughout oil production, pipeline transportation and processing. Problems associated with the presence of water in oil include corrosion, scale formation and sludge accumulation in storage tanks. These altered the viscosity of oil and the flow properties and reduced distillation efficiency (Mohebbali et al. 2012). Before being transported and refined, crude oil emulsions should

be demulsified to reduce water content and recover crude oil (de Souza Sobrinho et al. 2013).

In this aim, a demulsifying strain, *Alcaligenes* sp. S-XJ-1, was investigated to synthesize a biodemulsifier using waste frying oils as carbon source (Liu et al. 2011). Hou et al. (2013) reported the production of biodemulsifier by *B. mojavensis* XH-1 identified as extracellular proteins attached to the cells or secreted into the culture medium. The de-emulsification mechanism was dogged as solubilization and replacement process (Hou et al. 2013). Moreover, a carbohydrate–protein–lipid complex isolated from the cell surface of *Alcaligenes* sp. S-XJ-1 exhibited 67.5 % of the de-emulsification ratio for water-in-kerosene emulsions when used at an amount of 356 mg/L providing demulsifying capability of the strain.

Regarding literature reviews and studies, many bacterial strains are investigated to have bio-demulsification competence. As suggested by Nadarajah et al. (2002), microorganisms are believed to modify emulsion properties by using hydrophobic cell surfaces or the dual hydrophobic/hydrophilic nature of biosurfactants to displace or alter the emulsifiers that are present at the oil–water interface. In fact, whole bacterial cells of *B. subtilis* (Janiyani et al. 1994), *Nocardia amarae* (Lee and Lee 2000), *Micrococcus* sp. (Das, 2001) and *Ochrobactrum anthropi* were reported as de-emulsifying agents (Mohebbali et al. 2012). Along with different experiments, mixed culture products were shown to exhibit high de-emulsifying activity as compared to the most effective pure culture of *A. calcoaceticus* (Nadarajah et al. 2002). In fact, a mixed culture of *Acinetobacter* sp., *Pseudomonas* sp. and *Alcaligenes latus* showed high de-emulsification activity with 96 % de-emulsification of a water-in-oil emulsion within 24 h. Mohebbali et al. (2012) isolated a new de-emulsifying bacterium, *O. anthropi* strain RIPI5-1. The potential activity of this strain was also explained using a complex oil field emulsion and suggested a biodemulsification capacity of about 63 and 72 % when using the whole culture and whole cells of the bacteria, respectively (Mohebbali et al. 2012). Also, the finding of Park et al. (2000) reported that aerial spores of *Streptomyces* sp. broke emulsions of low viscosity hydrocarbons such as kerosene and gasoline within 1 min, but after 3 min of contact they could demulsify <50 % of emulsion of high viscosity hydrocarbons diluted with kerosene.

#### Ability to reduce the surface and interfacial tension

Generally, polymeric bioemulsifiers are recognized by their emulsification activity. However, they were demonstrated to decrease the surface and interfacial tension and can be acknowledged as biosurfactants. According to Satpute et al. (2010), surfactant can have both surface tension

reduction and emulsification activity. Table 3 enumerates some polymeric biosurfactants along with their producing strains and corresponding surface and interfacial tensions values.

Fatty acids resulting from the microbial oxidation of alkanes have gained great attention as surfactants and were reported to lower the surface and interfacial tensions (Ron and Rosenberg 2001). Some of these complex fatty acids, e.g., those produced by *Candida ingensare* are potent surfactants able to reduce greatly the surface tension of culture media (Amézcuca-Vega et al. 2007). Also, emulsan and phospholipids were reported to reduce the surface and interfacial tension of the medium broth (Table 3).

The production of various polymeric surfactants was known to increase seriously when several hydrocarbon-degrading bacteria or yeast are grown on alkane substrates and were demonstrated to decrease the surface tension. However, the amount of the decrease of the surface tension can depend on the nature of the carbon source (Chamanrokh et al. 2010). When ethanol was used as a carbon source, the surface tension was about 30, 30 and 32 (mN/m) for three strains of *A. calcoaceticus* PTCC 1318, Pay-4 and IL-1, respectively. Whereas surface tension was 28.2, 29 and 30 (mN/m) for *A. calcoaceticus* PTCC 1318, PAY-4 and IL-1, respectively with soy oil as a sole source of carbon and energy. When Crude oil was used, the obtained surface tensions were 29, 29.2 and 31 (mN/m), respectively for the above-mentioned bacteria. Generally, this reduction of surface tension measurements indicated the production of surface-active compounds by the microbial culture, which has been shown to support the metabolism of the

substrate and stimulate microbial growth (Chamanrokh et al. 2010).

Tolerance to extreme conditions of pH, temperature and salinity

Biosurfactants are reported stable at various temperature, pH and salinity (Salihu et al. 2009; Augustin and Tene Hippolyte 2012). The polymeric bioemulsifier synthesized by *Microbacterium* sp. MC3B-10 produced a highly stable emulsion (94 %) at 50 °C, pH 10 and 3.5 % NaCl content (Camacho-Chab et al. 2013). Exopolysaccharide polymeric bioemulsifiers are well known by their great stability and activity at extreme pH values. *Halomonas eurihalina* EPS were found to increase effectively the viscosity of solutions at low pH value and emulsify several hydrocarbon compounds (Calvo et al. 1998). Similarly, an extracellular polysaccharide produced by *Volcaniella eurihalina* was found to be able to form high viscous solutions, like a gel, at low pH values even in the presence of inorganic salts (Calvo et al. 1995). Also, polysaccharides namely PS 3a24 and PS 3a35 produced by marine bacteria have been shown to have high specific viscosity, pseudoplasticity, and stability over a wide range of pH in the presence of a variety of salts (Boyle and Reade 1983). A high molecular weight polysaccharides bioemulsifiers produced by *Variovorax paradoxus* were able to produce a thick stable oil/water emulsion and maintained the emulsification activity after boiling and at low temperatures (Franzetti et al. 2012). An exopolysaccharide derived from *Paenibacillus macerans* demonstrated a great stability at 121 °C for 20 min, over a

**Table 3** Surface and interfacial tension of polymeric biosurfactants

Biosurfactants	Producing strain	Surface tension	Interfacial tension	References
Surface-active lipids	<i>Nocardia erythropolis</i>	34–37 mN/m	2 mN/m	MacDonald et al. (1981)
Corynomycolic acids and spiculisporic acids	<i>Arthrobacter paraffineus</i>	32 mN/m	2.5 mN/m	Duvnjak et al. (1982)
Phosphatidylethanolamine	<i>Rhodococcus erythropolis</i>	–	1 mN/m (CMC = 30 mg/L)	Kretschmer et al. (1982)
Corynomycolic acids	<i>Corynebacterium lepus</i>	31–33 mN/m	2–3 mN/m	Duvnjak and Kosaric (1985)
Corynomycolic acids	<i>R. erythropolis</i>	–	6 mN/m	Rosenberg and Ron (1999)
Trehalose onocorynomycolates	–	–	16 mN/m	
Trehalose dicorynomycolates	–	–	17 mN/m	
Fatty acid esters	<i>Pseudomonas citronellolis</i> KHA	35 mN/m (CMC = 60 mg/L)	–	Sadouk et al. (2009)
Emulsan	<i>Acinetobacter calcoaceticus</i>	30 mN/m	–	Mousavian and Rahimi (2010)
Phospholipid	<i>Sphingobacterium</i> sp.	33 mN/m	–	Burgos-Díaz et al. (2011)
Peptidoglycolipid	<i>Streptomyces</i> sp. S22	23.09 mN/m	–	Maniyar et al. (2011)



pH range from 3 to 11, and in saline conditions (Liang et al. 2014).

Kokare et al. (2007) showed a good stability at room temperature (28 °C) of the bioemulsifier derived from *Streptomyces* sp. S1. Navon-Venezia et al. (1995) reported a maximum emulsification activity of an *A. radioresistens* derived alasan at acidic pH value along with an activation at high temperature. The emulsifying activity of a mannoprotein derived from *S. cerevisiae* was stable over broad range of conditions, from pH 2 to 11, with up to 5 % sodium chloride or up to 50 % ethanol in the aqueous phase (Cameron et al. 1988). Moreover, in the presence of a low concentration of various solutes, emulsions were stable to three cycles of freezing and thawing. *Acinetobacter* bioemulsifiers created a highly stable oil-in-water emulsion that maintained 90 % of its stability up to 6 days at 37 °C (Patil and Chopade 2001).

These interesting properties offer the opportunities for the polymeric bioemulsifiers to be investigated in extreme environment for microbial enhanced oil recovery and in situ biodegradation of oil sludge (Sen 2008). Moreover, they enable their use in industrial processes for food and pharmaceuticals frequently involve exposure to extremes of temperature, pressure, pH and ionic strength.

#### Low toxicity and biodegradability

Owing their natural origins, microbial derived emulsifiers are recognized as low or non-toxic compounds. Munstermann et al. (1992) showed the reduced toxicity of microbial derived surface active compounds (Trehalose dicorynomycolate and Trehalose tetraester from *R. erythropoli*) towards different synthetic surfactants. Similarly, in a study conducted by Edwards et al. (2003) reporting a comparison of acute and chronic toxicity of three synthetic surfactants and three microbiological derived surfactants commonly used in oil spill remediation, emulsan was revealed among the least toxic. In another study, acute toxicity tests involving two species of marine larvae, *Mysidopsis bahia* (shrimp) and *Menidia beryllina* (fish), demonstrated low toxicity of the high molecular weight biosurfactant JE1058BS produced by *Gordonia* sp. (Saeki et al. 2009). Following an acute toxicity test conducted on mice, Ashtaputre and Shah (1995) confirmed the non-toxicity of an exopolysaccharide bioemulsifier produced by *S. paucimobilis*. Similarly, a mixture of high molecular weight polysaccharides produced by *V. paradoxus* did not show any toxic properties through different ecotoxicological tests (decrease in the bioluminescence of *Vibrio fischeri*; mortality of the crustacean *Daphnia magna*; seed germination and root elongation test was performed on *Cucumis sativus*, *Lepidium sativum* and *Sorghum saccharatum* and a contact acute toxicity test was performed on

earthworms *Eisenia foetida*) (Franzetti et al. 2012). Moreover, respirometric tests showed that moderate biodegradability of the bioemulsifier occurred by soil bacterial inoculum (Franzetti et al. 2012). Also, Camacho-Chab et al. (2013) reported the non-toxicity of a glycolipoprotein bioemulsifier produced by *Microbacterium* sp. MC3B-10. Thus, reduced toxicity towards synthetic surfactants recognized as highly toxic (Song et al. 2012) and biodegradability makes biosurfactants more suitable for industrial and environmental applications such as bioremediation. In fact, Cappello et al. (2011) recommended the biodegradability of an exopolysaccharide biosurfactant.

#### Foaming capacity

A foaming agent is a compound that facilitates formation of foam. A surfactant, which, when present in small amounts, reduces surface tension of a liquid (reduces the work needed to create the foam) or increases its colloidal stability by inhibiting coalescence of bubbles leading to the formation of foam. Also, stable foams can be generated by a selective enrichment of hydrophobic bacteria in them by a process of flotation (Petrovski et al. 2011). Studies suggested that there is a great correlation between cell surface hydrophobicity, biosurfactant production and foam formation (Stratton et al. 2002).

As suggested by Lang and Philp (1998), *Rhodococci* strains have been involved in foaming incidents due to their filamentous growth, although other actinomycetes are clearly implicated. A *R. rubra* strain and its crude lipidic biosurfactant preparation, generated foams in the laboratory which were similar to those produced in full-scale AS plants (Lang and Philp 1998).

#### Dispersion property

A dispersant is an agent that reduces the cohesive attraction between similar particles. With this, it keeps insoluble particles in suspension and prevents their aggregations. This property has application in oil field chemistry as it can lead desorption of hydrophobic molecules from rock surfaces enhancing mobility and recovery. The dispersants enhance the rate of natural dispersion of oil and its removal from the contaminated surface (Saeki et al. 2009). In addition, the dispersants amplify surface area of oil accordingly to its dispersion into small droplets that is expected to stimulate its biodegradation via the activity of naturally occurring microorganisms (Saeki et al. 2009). Besides, dispersion properties can be investigated in several other fields. In fact, it's suggested that production of emulcyan by *Phormidium* cells serves as a dispersal strategy by this non-hormogonia-producing cyanobacterium. As reported by Rosenberg et al. (1989) the use of biodispersan

produced by *Acinetobacter calcoaceticus* A2 in paper manufacturing industries is one of the interesting approaches. Also, Elkeles et al. (1994) reported that biodispersan is an extracellular anionic polysaccharide produced by *A. calcoaceticus* A2 that changes the surface properties of limestone and acts both as a dispersant and as a grinding support. Its addition as filler along with paper and limestone improves the quality of paper and significantly reduces the time required for the grinding process (Elkeles et al. 1994).

## Main application of polymeric biosurfactants

### Application in environment

#### *Viscosity reduction for cleaning potency and microbial enhanced oil recovery*

The viscosity of a fluid is a measure of its resistance to gradual deformation by shear stress or tensile stress. For liquids, it corresponds to the informal notion of “thickness”. Because of the existence of colloid and asphaltene, the viscosity of thick oil is mostly so high. Nevertheless, crude oil often needs to be transported over long distances from the extraction fields to the refineries. Such viscosity of heavy oils can be reduced by using surfactants to increase mobility and to facilitate its transport. Microbial derived surface active compounds can lower the dense oil viscosity, helping therefore their transportation. The bioemulsifier emulsan derived from *A. venetianus* RAG-1, is undoubtedly the most powerful, yet others such as alasan and biodispersan produced by different *Acinetobacter* strains, have been extensively studied to facilitate crude oil transportation in pipeline (Bryant 1987).

As described by Planckaert (2005), a reservoir rock is like a sponge that entraps and expels oil. Classical production technologies, namely primary and secondary recovery, can only partially recover the oil present in the field, with an efficiency estimated at one-third to half the overall amount of oil available. In this aim, the decrease of oil viscosity can stimulate the oil recovery. In fact, microbial enhanced oil recovery by using microbial derived surface active compounds is a promising technology. Previous studies described the use of polymeric bioemulsifiers for microbial enhanced oil recovery. A bioemulsifier produced by *B. licheniformis* and containing substantial amount of polysaccharide, protein and lipid gave  $43 \pm 3.3$  % additional oil recovery upon application to a sand pack column designed to simulate an oil reservoir (Suthar et al. 2008). Similarly, a polysaccharide-protein complex bioemulsifier produced by *B. licheniformis* increased the efficiency of the residual oil recovery increased

by 22 % in a sand-pack model saturated with liquid paraffin (Dastgheib et al. 2008). Salehizadeh and Mohammadzad (2009) reported the potential use of an *Alcaligenes faecalis* derived emulsan for microbial enhanced oil recovery. Equally, an *Aspergillus ustus* derived glycolipoprotein can be a potential candidate for microbial enhanced oil recovery (Seghal Kiran et al. 2009).

#### *Hydrocarbon dispersion, solubilization and mobilization capacities and enhancement of hydrocarbon biodegradation*

Generally, the biodegradation of hydrocarbons is limited by their low bioavailability to the microorganisms, which is due to their hydrophobicity, low aqueous solubility and strong adsorptive capacity in soil (Amodu et al. 2013). Addition of synthetic surfactants or biosurfactants can increase the aqueous solubility of water-insoluble compounds (Pacwa-Plociniczak et al. 2011). The mechanism proposed for improving solubility of hydrophobic organic compounds includes their encapsulation in the hydrophobic core of the surfactant micelles (Pacwa-Plociniczak et al. 2011). In fact, at concentrations above the CMC, surfactant molecules associate to form micelles able to encapsulate insoluble molecules into their core (Pacwa-Plociniczak et al. 2011). The effects of such a process are the reduction of surface and interfacial tension and the enhancement of the bio accessibility of the hydrophobic contaminants for microbial attack (Perfumo et al. 2010). Several studies reported the efficiency of low and high molecular weight biosurfactants as hydrocarbon solubilizing agents (Perfumo et al. 2009). Also, at concentrations below the biosurfactant CMC, the mobilization mechanism can occur. At such concentrations, biosurfactants reduce the surface and interfacial tension between air–water and soil–water systems. Due to the reduction of the interfacial force, contact of biosurfactants with a soil–oil system increases the contact angle and reduces the capillary force holding the oil and soil together leading to the release of oil into the aqueous phase (Perfumo et al. 2010). Therefore, biosurfactants increase the bioavailability of hydrophobic compounds resulting in enhanced growth of the degrading microorganism and the biodegradation of the contaminants. Numerous studies reported the enhancement of hydrocarbon biodegradation by the addition of high molecular weight biosurfactants.

High molecular weight biosurfactants are utilized as hydrophobic compounds solubilizers like alasan produced by *A. radioresistens* (Barkay et al. 1999) and an *A. calcoaceticus* derived bioemulsifier (Wong et al. 2010). Having the ability to solubilize hydrocarbons, alasan doubled the rate of fluoranthene mineralization and significantly increased the rate of phenanthrene mineralization

by *S. paucimobilis* EPA505 (Barkay et al. 1999). Also, similar studies showed their efficiency in hydrocarbon solubilization towards synthetic ones. A thermostable polymeric biosurfactants produced by *A. calcoaceticus* BU03 improved, significantly, the apparent aqueous solubility of phenanthrene, pyrene and benzo (a) pyrene at a concentration of 25 times their CMC (Zhao and Wong 2009). At concentrations of 0.5 CMC and 1 CMC, the biosurfactant enhanced the biodegradation of phenanthrene by a consortium of polyaromatic hydrocarbon-degrading microorganisms (Zhao and Wong 2009). Also, Burd and Ward (1996) reported the beneficial effect of a high molecular weight biosurfactant containing protein and lipopolysaccharide in the degradation of polycyclic aromatic hydrocarbons by *P. marginalis*. Similarly, a free fatty acid produced by *R. erythropolis* strain 3C-9 was reported to enhance the hydrocarbons solubility and degradation (Peng et al. 2007). Also, Rosenberg et al. (1982) discussed the enhancement of hydrocarbons solubility and degradation by fimbriae of *A. calcoaceticus* RAG-1. Hanson et al. (1994) reported the potential involvement of an *Acinetobacter* sp. A3 derived protein in hydrocarbon degradation.

Moreover, numerous polymeric surfactants were investigated for their washing capacity. In this aim, *Variovorax* bioemulsifiers of polysaccharides nature showed significant removal of crude oil from the sandy soil making these compounds potentially suitable for removal of crude oil spill from sandy shorelines (Franzetti et al. 2012). Also, extracellular biosurfactant derived from *A. calcoaceticus* RAG-1 was efficient for the recovery of hexachlorobiphenyl from soil slurries (41.9 %) (Van Dyke et al. 1993). A complex of fatty acid and saccharide produced by *Gordonia* sp. was demonstrated to stimulate the degradation of weathered crude oil via the activity of the indigenous marine bacteria and stimulate the removal of crude oil from the surface of contaminated sea sand (Saeki et al. 2009).

Dispersant agents reduced the cohesive attraction between similar particles. With this, they prevent insoluble particles to form aggregations with each other. This property has application in oil field chemistry as it can lead desorption of hydrophobic molecules from rock surfaces enhancing mobility and recovery. The dispersants enhance the rate of natural dispersion of oils and their removal from the contaminated surface (Saeki et al. 2009). In addition, the dispersants increase surface area of oil as a result of its dispersion into small droplets that is expected to stimulate its biodegradation via the activity of naturally occurring microorganisms (Saeki et al. 2009). In fact, earlier works have shown that high molecular weight biosurfactants can work as efficient biodispersants of hydrophobic compounds. Biodispersant is an extracellular anionic polysaccharide produced by *A. calcoaceticus* A2 that changes the

surface properties of limestone and acts both as a dispersant and as a grinding aid (Elkeles et al. 1994). Also, a complex of fatty acid and saccharide derived from *Gordonia* sp. showed great potential for application as an oil spill dispersant (Saeki et al. 2009). Pines and Gutnick (1986) reported the beneficial effect of emulsan in the dispersion of crude oil essential for the growth of the producing strain.

#### Use of biosurfactant for detergency and cleaning

A detergent is a surfactant or a mixture of surfactants with cleaning properties in dilute solutions. Owing diverse properties, biosurfactants and bioemulsifiers can act in similar way as that of detergents as well as in oil industry, laundry detergent formulation and cosmetic. According to Joshi-Navare et al. (2013), main properties enable the use of biosurfactants in detergent formulation, mainly, emulsifying, washing, wetting, foaming and dispersing capacities as well as the anti-microbial activity. This is specifically advantageous owing the biodegradability, ecofriendly, and nontoxic nature of biosurfactant.

In fact, biosurfactants are used as detergents in cleaning up hydrocarbon/crude oil storage tank. Chamanrokh et al. (2010) showed the efficiency of the bio-emulsifiers produced by autochthonous bacteria for cleaning hydrocarbonaceous residues at a like manner to emulsan produced by *A. calcoaceticus* PTCC 1318. Such cleanup process is highly desirable as it is economically rewarding and environmentally friendly. Moreover, a proteoglycan bioemulsifier with protein, polysaccharide and lipid moieties produced by *Acinetobacter* sp. formed stable emulsion oil-in-water emulsions with plant oils and displayed good cleaning property towards different oils (Jagtap et al. 2010). A high molecular weight, thermostable biopolymer produced by an alkaliphilic bacterium, *Klebsiella* sp. strain RJ-03, composed of sugar, uronic acid, protein and sulfate, showed an excellent oil removing efficiency from soil and cotton cloths as compared to chemical surfactants (Jain et al. 2012).

#### Biomedical and therapeutic applications

Biosurfactants are well known by their membrane permeabilization property as they can induce pore and ion channels formation in lipid bilayer membrane. So, they are able to destabilize membranes disturbing their integrity and permeability. Also, pore formation in membranes may cause trans-membrane ion influxes, including  $\text{Na}^+$  and  $\text{K}^+$ , which result in membrane disruption and cell death. These structural fluctuations acting therefore on biological membrane integrity can explain the primary mode of the antibiotic action resulting in the important biological

activities of biosurfactants including antibacterial; anti-fungal; antiviral; anti-mycoplasma and hemolytic activities (Fracchia et al. 2012). Therefore, high molecular weight bioemulsifiers are well known by their antibacterial activities against pathogenic bacteria. In fact, a novel biosurfactant isolated from *Lactobacillus paracasei* ssp. *paracasei* showed antimicrobial activity against several micro-organisms including the pathogenic *Candida albicans*, *E. coli*, *Staphylococcus aureus*, *Staphylococcus epidermidis* and *Streptococcus agalactiae* with a minimum inhibitory concentration and a minimum bactericidal concentration ranging from 25 to 50 mg/mL (Gudina et al. 2010). A fatty acid derivative produced by *Halomonas* sp. has a potent use as antimicrobial and anticancer drugs in biomedical field. It exhibited antimicrobial activity against a variety of human pathogenic bacteria (*S. aureus*, *Klebsiella pneumoniae*, *Streptococcus pyogenes* and *Salmonella typhi*) and fungi (*Trichophyton rubrum*, *Aspergillus niger*, *Aspergillus flavus* and *Fusarium* sp.). It could also suppress the proliferation of mammary epithelial carcinoma cell by 46.77 % at 2.5 µg concentration (Donio et al. 2013).

Rufino et al. (2011) have demonstrated the antimicrobial property of the crude biosurfactant isolated from *C. lipolytica* UCP0988 against several pathogenic bacteria suggesting the possibility of the use of this biosurfactant as an alternative antimicrobial agent in the medical field. Moreover, this biosurfactant showed anti-adhesive activity against most of the microorganisms tested (Rufino et al. 2011). Similarly, the surface-binding protein derived from *Lactobacillus fermentum* RC-14 inhibited adhesion of *Enterococcus faecalis* (Heinemann et al. 2000).

Having the ability to alter the cell surface hydrophobicity, adsorption of biosurfactant on solid surfaces can inhibit the bioadhesion of pathogenic bacteria (Cao et al. 2009). It's very interesting for medical and food applications to preserve food from pathogenic bacteria spoilage and to protect biomedical material from nosocomial infections and biofilm formation (Cao et al. 2009). A surface active agent, mannoprotein, derived from *S. cerevisiae* may be used as inhibitor of *S. aureus* and *S. epidermidis* biofilm development for potential application in pharmaceutical industry (Walenska et al. 2007). A *lactobacillus acidophilus* derived surlactin was proved to inhibit the adhesion of biofilm formed by *P. aeruginosa* to surfaces up to 60 %. So, it showed an ability to treat the infection in rabbits' eyes inoculated with *P. aeruginosa* (Ismaeel et al. 2013). Two homologs of phospholipids biosurfactants produced by *P. putida* were also shown to inhibit the bacterial adhesion by 23–72 % and that of *C. albicans* by 96–98 % (Janek et al. 2013).

In addition to the antibacterial potency and the anti-adhesive properties, polymeric biosurfactants are reported as useful as potent adjuvant and were also described as

involved in immune responses. The partially degraded lipopolysaccharides of *Burkholderia cepacia* and the ornithine-containing lipids were purified from some bacteria, having weak toxicity and a great ability to activate the immune systems of the living body, were developed as complex lipid adjuvants (Kawai et al. 2002). The typical bacterial ornithine-containing lipid, exhibited strong interleukin-1- and prostaglandin E<sub>2</sub>-inducing activities, and further, it induced the production of high IgG anti-tetanus toxoid antibodies in mice is expected to be utilized as a nontoxic, potent adjuvant (Kawai et al. 1999). In a study conducted by Panilaitis et al. (2002), it was demonstrated that emulsan activates macrophages in a dose-dependent manner and this significant immunopotential demonstrated by this complex polymer establishes emulsan as an exciting new candidate adjuvant. Seo et al. (2008) demonstrated the involvement of bacterial lipoteichoic acid in inducing immune responses to gram-positive bacteria and its role in inducing inflammatory responses. Moreover, bacterial fimbriae derived from *Porphyromonas gingivalis* and their peptides activate human gingival epithelial cells through Toll-Like Receptor 2 (Asai et al. 2001). Besides that, researchers developed the potential use of polymeric bioemulsifiers as anticancer agents such as the outer membrane vesicles of *Actinobacillus actinomycetemcomitans* (Demuth et al. 2003).

## Conclusion

In the past 30 years, several different polymeric biosurfactants have been discovered and numerous producing strains have been isolated and characterized. Microbial derived polymeric biosurfactants showed many interesting functional properties such as the ability to reduce the surface and interfacial tension, the emulsification and de-emulsification capacities, the foaming potency, the dispersing capacity, the solubilization and mobilization abilities and the pore-forming capacities. These permitted their applications in environmental field as great enhancers of hydrocarbons solubility, mobility and biodegradation. They are also potential candidates in biomedicine and therapeutics for their great antimicrobial, anti-adhesive and immune-modulating. Owing these fascinating properties and wide spectrum applications besides awareness of the importance of social development and environmental sustainability has aroused, polymeric biosurfactants became replacers of the synthetic surfactants. So, great efforts are needed in their production and purification at industrial scale.

**Acknowledgments** This work has been supported by grants from “Tunisian Ministry of Higher Education, Scientific Research and Technology”.

**Conflict of interest** The authors report no declaration of interest.

## References

- Albuquerque CDC, Filetti AMF, Campos-Takaki GM (2006) Optimizing the medium components in bioemulsifiers production by *Candida lipolytica* with response surface method. *Canad J Microbiol* 52:575–583
- Amézcuca-Vega C, Poggi-Varaldo HM, Esparza-García F, Ríos-Leal E, Rodríguez-Vázquez R (2007) Effect of culture conditions on fatty acids composition of a biosurfactant produced by *Candida ingens* and changes of surface tension of culture media. *Bioresour Technol* 98(1):237–240
- Amodu OS, Ojumu TV, Obed Ntwampe SK (2013) Bioavailability of high molecular weight polycyclic aromatic hydrocarbons using renewable resources. *Environmental biotechnology—new approaches and prospective applications*, Chapter 8 <http://dx.doi.org/10.5772/54727>
- Arli SD, Trivedi UB, Patel KC (2011) Curdlan-like exopolysaccharide production by *Cellulomonas flavigena* UNP3 during growth on hydrocarbon substrates. *World J Microbiol Biotechnol* 27:1415–1422
- Asai Y, Ohyama Y, Gen K, Ogawa T (2001) Bacterial fimbriae and their peptides activate human gingival epithelial cells through toll-like receptor 2. *Infect Immun* 69(12):7387–7395
- Ashtaputre AA, Shah AK (1995) Emulsifying property of a viscous exopolysaccharide from *Sphingomonas paucimobilis*. *World J Microbiol Biotechnol* 11:219–222
- Augustin M, Tene Hippolyte M (2012) Screening of biosurfactants properties of cell-free supernatants of cultures of *Lactobacillus* spp. isolated from a local fermented milk (Pendidam) of Ngaoundere (Cameroon). *Int J Eng Res Appn* 2(5):974–985
- Avila-Calderon ED, Lopez-Merino A, Jain N, Peralta H, Lopez-Villegas EO, Sriranganathan N, Boyle SM, Witonsky S, Contreras-Rodriguez A (2011) Characterization of outer membrane vesicles from *Brucella melitensis* and protection induced in mice. *Clin Develop Immunol*. doi:10.1155/2012/352493
- Barkay T, Navon-Venezia S, Ron EZ, Rosenberg E (1999) Enhancement of solubilization and biodegradation of polyaromatic hydrocarbons by the bioemulsifier alasan. *Appl Environ Microbiol* 65:2697–2702
- Bashetti SP, Palande PP, Mankar SG, Bhuyan SS, Chopade BA, Mujumdar SS (2012) Studies of bioemulsifier production by *Acinetobacter Calcoaceticus* C42 isolated from rhizosphere of corn. *Int J Inst Pharm Life Sci* 2(3):2249–6807
- Batrakov SG, Konova IV, Sheichenko VI, Esipov SE, Galanina LA (2001) Two unusual glycerophospholipids from a filamentous fungus, *Absidia corymbifera*. *Biochim Biophys Acta* 1531:169–177
- Beeba JL, Umbreit WW (1971) Extracellular lipid of *Thiobacillus thiooxidans*. *J Bacteriol* 108:612–615
- BonillaM Olivaro C, Corona M, Vazquez A, Soubes M (2005) Production and characterization of a new bioemulsifier from *Pseudomonas putida* ML2. *J Appl Microbiol* 98(2):456–463
- Boyle CD, Reade AE (1983) Characterization of two extracellular polysaccharides from marine bacteria. *Appl Environ Microbiol* 46:392–399
- Bryant R (1987) Potential use of microorganisms in petroleum recovery technology. *Proc Okla Acad Sci* 67:97–104
- Burd G, Ward OP (1996) Involvement of a surface-active high molecular weight factor in degradation of polycyclic aromatic hydrocarbons by *Pseudomonas marginalis*. *Can J Microbiol* 42:791–797
- Burgos-Díaz C, Pons R, Espuny MJ, Aranda FJ, Teruel JA, Manresa A, Ortiz A, Marqués AM (2011) Isolation and partial characterization of a biosurfactant mixture produced by *Sphingobacterium* sp. isolated from soil. *J Colloid Interf Sci* 361:195–204
- Calvo C, Ferrer MR, Martínez-Checa F, Be Jar B, Quesada E (1995) Some rheological properties of the extracellular polysaccharide produced by *Volcaniella eurihalina* F2-7. *Appl Biochem Biotechnol* 55:45–54
- Calvo C, Martínez-Checa F, Mota A, Bejar V, Quesada E (1998) Effect of cations, pH and sulfate content on the viscosity and emulsifying activity of the *Halomonas eurihalina* exopolysaccharide. *J Ind Microbiol Biotechnol* 20:205–209
- Camacho-Chab JC, Guézenne J, Chan-Baca MJ, Ríos-Leal E, Sinquin C, Muñoz-Salazar R, De la Rosa-García S, Del C, Reyes-Estebanez M, Ortega-Morales BO (2013) Emulsifying activity and stability of a non-toxic bioemulsifier synthesized by *Microbacterium* sp. MC3B-10. *Int J Mol Sci* 14:18959–18972
- Cameron DR, Cooper DG, Neufeld RJ (1988) The mannoprotein of *Saccharomyces cerevisiae* is an effective bioemulsifier. *Appl Environ Microbiol* 54(6):1420–1425
- Cappello S, Crisari A, Denaro R, Crescenzi F, Porcelli F, Yakimov MM (2011) Biodegradation of a bioemulsificant exopolysaccharide (EPS2003) by marine bacteria. *Wat Air Soil Poll* 214:645–652
- Castro GR, Panilaitis B, Kaplan DL (2008) Emulsan, a tailorable biopolymer for controlled release. *Bioresour Technol* 99:4566–4571
- Cao X-H, Liao Z-Y, Wang C-L, Yang W-Y, Lu M-F (2009) Evaluation of a lipopeptide biosurfactant from *Bacillus natto* TK-1 as a potential source of anti-adhesive, antimicrobial and antitumor activities. *Braz J Microbiol* 40:373–379
- Chamanrokh P, Mazaheri Assadi M, Amoabediny Gh, Rashedi H (2010) Cleaning oil-contaminates vessel by emulsan producers (autochthonous bacteria). *Iran J Environ Health Sci Eng* 7(3):209–222
- Choma A, Komaniecka I (2002) Analysis of phospholipids and ornithine-containing lipids from *Mesorhizobium* spp. *Syst Appl Microbiol* 25:326–331
- Cirigliano MC, Carman GM (1985) Purification and characterization of liposan, a bioemulsifier from *Candida lipolytica*. *Appl Environ Microbiol* 50:846–850
- Das M (2001) Characterization of de-emulsification capabilities of a *Micrococcus* species. *Bioresour Technol* 79:15–22
- Dastgheib SMM, Amoozegar A, Elahi E, Asad Sand Banat M (2008) Bioemulsifier production by a halothermophilic *Bacillus* strain with potential applications in microbially enhanced oil recovery. *Biotechnol Lett* 30:263–270
- de Souza Sobrinho HB, de Luna JM, Rufino RD, Lúcia A, Porto F, Sarubbo LA (2013) Assessment of toxicity of a biosurfactant from *Candida sphaerica* UCP 0995 cultivated with industrial residues in a bioreactor. *Elect J Biotechnol*. doi:10.2225/vol16-issue4-fulltext-4
- Dehghan-Noudeh G, Moshafi MH, Shariffar F, Masoumi MA (2007) Studies of biosurfactant production by *Acinetobacter calcoaceticus* (PTCC 1318). *Jundish J Nat Pharm Prod* 2(2):116–123
- Demuth DR, James D, Kowashi Y, Kato S (2003) Interaction of *Actinobacillus actinomycetemcomitans* outer membrane vesicles with HL60 cells does not require leukotoxin. *Cell Microbiol* 5(2):111–121
- Desai JD, Banat IM (1997) Microbial production of surfactants and their commercial potential. *Am Soc Microbiol* 61:47–64
- Deshmukh C, Jagtap CB, Titus S, Kumar P (2012) Isolation and characterization of fatty acid esters and phosphatidylethanolamine surfactants from a consortium of marine bacteria. *Ind J Geo-Mar Sci* 41(5):398–404

- Dikit P, Maneerat S, Musikasang H, H-kittikun A (2010) Emulsifier properties of the mannoprotein extract from yeast isolated from sugar palm wine. *Sci Asia* 36:312–318
- Donio MBS, Ronica FA, Viji VT, Velmurugan S, Adlin Jenifer JSC, Michaelbabu M, Dhar P, Citarasu T (2013) *Halomonas* sp. BS4, a biosurfactant producing halophilic bacterium isolated from solar salt works in India and their biomedical importance. *Springer Plus* 2:149
- Doshi DV, Maniyar JP, Bhuyan SS, Mujumdar SS (2010) Studies on bioemulsifier production *Actinopolyspora* sp. A18 isolated from garden soil. *Ind J Biotechnol* 9:391–396
- Duvnjak Z, Kosaric N (1985) Production and release of surfactant by *Corynebacterium lepus* in hydrocarbon and glucose media. *Biotechnol Lett* 7:793–796
- Duvnjak Z, Cooper DG, Kosaric N (1982) Production of surfactant by *Arthrobacter paraffineus* ATCC19558. *Biotechnol Bioeng* 24:165–175
- Edwards SG, Seddon B (2001) Mode of antagonism of *Brevibacillus brevis* against *Botrytis cinerea* in vitro. *J Appl Microbiol* 91(4):652–659
- Edwards KR, Lepo JE, Lewis MA (2003) Toxicity comparison of biosurfactants and synthetic surfactants used in oil spill remediation to two estuarine species. *Mar Pollut Bull* 46(10):1309–1316
- Elkeles A, Rosenberg E, Ron EZ (1994) Production and secretion of the polysaccharide biodispersant of *Acinetobacter calcoaceticus* A2 in protein secretion mutants. *Appl Environ Microbiol* 60:4642–4645
- Fattom A, Shilo M (1985) Production of emulcyan by *Phormidium* J-1: its activity and function. *FEMS Microbiol Lett* 31(1):3–9
- Fracchia L, Cavallo M, Martinotti MG, Banat IM (2012) Biosurfactants and bioemulsifiers biomedical and related applications—present status and future potentials, biomedical science, engineering and technology, Prof. Dhanjoo N. Ghista (Ed.), ISBN: 978-953-307-471-9
- Franzetti A, Gandolfi I, Raimondi C, Bestetti G, Banat IM, Smyth TJ, Papacchini M, Cavallo Fracchia ML (2012) Environmental fate, toxicity, characteristics and potential applications of novel bioemulsifiers produced by *Variovorax paradoxus* 7bCT5. *Bioresour Technol* 108:245–251
- Garduno RA, Phipps BM, Kay WW (1995) Physical and functional s-layer reconstitution in *Aeromonas salmonicida*. *J Bacteriol* 177:2684–2694
- Geske T, Vom Dorp K, Dörmann P, Hölzl G (2013) Accumulation of glycolipids and other non-phosphorous lipids in *Agrobacterium tumefaciens* grown under phosphate deprivation. *Glycobiol* 23:69–80
- Gudina EJ, Rocha V, Teixeira JA, Rodrigues LR (2010) Antimicrobial and antiadhesive properties of a biosurfactant isolated from *Lactobacillus paracasei* ssp. *paracasei* A20. *Lett Appl Microbiol* 50:419–424
- Guezennec JG, Pignet P, Raguene G, Deslandes E, Lijour Y, Gentric E (1994) Preliminary chemical characterization of unusual eubacterial exopolysaccharides of deep-sea origin. *Carbohydr Polym* 24:287–294
- Gutiérrez T, Mulloy B, Black K, Green DH (2007) Glycoprotein emulsifiers from two marine *Halomonas* species: chemical and physical characterization. *J Appl Microbiol* 103:1716–1727
- Haggar A, Hussain M, Lönnes H, Herrmann M, Norrby-Teglund A, Flock JI (2003) Extracellular adherence protein from *Staphylococcus aureus* enhances internalization into eukaryotic cells. *Infect Immun* 71(5):2310–2317
- Hanson KG, Kale VC, Desai AJ (1994) The possible involvement of cell surface and outer membrane proteins of *Acinetobacter* sp. A3 in crude oil degradation. *FEMS Microbiol Lett* 122:275–280
- Heinemann C, van Hylckama Vlieg JET, Janssen DB, Busscher HJ, van der Mei HC, Reid G (2000) Purification and characterization of a surface-binding protein from *Lactobacillus fermentum* RC-14 that inhibits adhesion of *Enterococcus faecalis* 1131. *FEMS Microbiol Lett* 190(1):177–180
- Hisatsuka K, Nakahara T, Yamada K (1972) Protein-like activator for n-alkane oxidation by *Pseudomonas aeruginosa* S7B1. *Agric Biol Chem* 36:1361–1369
- Hisatsuka K, Nakahara T, Minoda Y, Yamada K (1977) Formation of protein-like activator for n-alkane oxidation and its properties. *Agric Biol Chem* 41:445–450
- Hou N, Li D, Ma F, Zhang J, Xu Y, Wang J, Li C (2013) Effective bioemulsifier components secreted by *Bacillus mojavensis* XH-1 and analysis of the demulsification process. *Biodegr*. doi:10.1007/s10532-013-9679-5
- Ismael M Ch, Ibrahim KM, Al-Malikey M Kh (2013) The effect of surfactin produced by *Lactobacillus acidophilus* on eye infectious bacteria in rabbits. *J Baghdad Sci* 10(1):133–143
- Jagtap S, Yavankar S, Pardesi K, Chopade B (2010) Production of bioemulsifier by *Acinetobacter* species isolated from healthy human skin. *Ind J Exp Biol* 48:70–76
- Jain RM, Mody K, Mishra A, Jha B (2012) Physicochemical characterization of biosurfactant and its potential to remove oil from soil and cotton cloth. *Carbohydr Polym* 89:1110–1116
- Janek T, Łukaszewicz M, Krasowska A (2013) Identification and characterization of biosurfactants produced by the Arctic bacterium *Pseudomonas putida* BD2. *Colloids Surf B Biointerf*. doi:10.1016/j.colsurfb.2013.05.008
- Janiyani KL, Purohit HJ, Shanker R, Khanna P (1994) Deemulsification of oil-in-water emulsions by *Bacillus subtilis*. *World J Microbiol Biotechnol* 10:445–452
- Johnson V, Singh M, Saini VS, Adhikari DK, Sista V, Yadav NK (1992) Bioemulsifier production by an oleaginous yeast *Rhodotorula glutinis* IIP-30. *Biotechnol Lett* 14(6):487–490
- Joshi-Navare K, Khanvilkar P, Prabhune A (2013) Jatropa oil derived sophorolipids: production and characterization as laundry detergent additive. *Biochem Res Int*. doi:10.1155/2013/169797
- Kaeppli O, Finney WR (1979) Partition of alkane by an extracellular vesicle derived from haexdecane-grown *Acinetobacter*. *J Bacteriol* 140(2):707–712
- Katamai W, Maneerat S, Kawai F, Kanzaki H, Nitoda T, H-Kittikun A (2008) Purification and characterization of a biosurfactant produced by *Issatchenkia orientalis* SR4. *J Gen Appl Microbiol* 54:79–82
- Kawahara H, Hirai A, Minabe T, Obata H (2013) Stabilization of astaxanthin by a novel biosurfactant produced by *Rhodotorula mucilaginosa* KUGPP-1. *Biocontrol Sci* 18(1):21–28
- Kawai Y, Nakagawa Y, Matuyama T, Akagawa K, Itagawa K, Fukase K, Kusumoto S, Nishijima M, Yano I (1999) A typical bacterial ornithine-containing lipid N<sup>2</sup>-(D)-3-(hexadecanoyloxy)hexadecanoyl-ornithine is a strong stimulant for macrophages and a useful adjuvant. *FEMS Immunol Med Microbiol* 23:67–73
- Kawai Y, Watanabe M, Matsuura M, Nishijima M, Kawahara K (2002) The partially degraded lipopolysaccharide of *Burkholderia cepacia* and ornithine-containing lipids derived from some gram-negative bacteria are useful complex lipid adjuvants. *EMS Immunol Medical Microbiol* 34:173–179
- Khandelwal P, Banerjee-Bhatnagar N (2003) Insecticidal activity associated with the outer membrane vesicles of *Xenorhabdus nematophilus*. *Appl Environ Microbiol* 169(4):2032–2037
- Kitamoto D, Isoda H, Nakahara T (2002) Functions and potential applications of glycolipids biosurfactants—from energy-saving materials to gene delivery carriers. *J Biosci Bioeng* 94:187–201
- Kokare CR, Kadam SS, Mahadik KR, Chopade BA (2007) Studies on bioemulsifier production from marine *Streptomyces* sp. S1. *Ind J Biotechnol* 6:78–84
- Kretschmer A, Bock H, Wagner F (1982) Chemical and physical characterisation of interfacial-active lipids from *Rhodococcus*

- erythropolis* grown on n-alkanes. Appl Environ Microbiol 44:864
- Lang S, Philip JC (1998) Surface-active lipids in rhodococci. Antonie Van Leeuwenhoek 74:59–70
- Leahy JG, Khalid ZM, Quintero EJ, Jones-Meehan JM, Heidelberg JF, Batchelor PJ, Colwell RR (2003) The concentrations of hexadecane and inorganic nutrients modulate the production of extracellular membrane-bound vesicles, soluble protein, and bioemulsifier by *Acinetobacter venetianus* RAG1 and *Acinetobacter* sp. strain HO1-N. Canad J Microbiol 49(9):569–575
- Lee JC, Lee KY (2000) Emulsification using environmental compatible emulsifiers and de-emulsification using DC field and immobilized *Nocardia amarae*. Biotechnol Lett 22:1157–1163
- Liang T-W, Wu C-C, Cheng W-T, Chen Y-C, Wang C-L, Wang I-L, Wang S-L (2014) Exopolysaccharides and antimicrobial biosurfactants produced by *Paenibacillus macerans* TKU029. Appl Biochem Biotechnol 172:933–950
- Liu J, Peng J, Huang X, Lu L, Cheng H, Yang D, Zhou Q, Deng H (2011) Application of waste frying oils in the biosynthesis of biodemulsifier by a demulsifying strain *Alcaligenes* sp. S-XJ-1. J Environ Sci 23(6):1020–1026
- Lukondeh T, Ashbolt NJ, Rogers PL (2003) Evaluation of *Kluyveromyces marxianus* FII 510700 grown on a lactose-based medium as a source of a natural bioemulsifier. J Ind Microbiol Biotechnol 30:715–720
- Ly MH, Naïtali-Bouchez M, Meylheuc T, Bellon-Fontaine M-N, Le Mai T, Belin J-M, Waché Y (2006) Importance of bacterial surface properties to control the stability of emulsions. Int J Food Microbiol 112:26–34
- Macdonald CR, Cooper DG, Zajic JE (1981) Surface-active lipids from *Nocardia erythropolis* grown on hydrocarbons. Appl Environ Microbiol 41(1):117–123
- Maki JS, Ding L, Stokes J, Kavouras JH, Rittschof D (2000) Substratum/bacterial interactions and larval attachment: films and exopolysaccharides of *Halomonas marina* (ATCC 25374) and their effect on barnacle cyprid larvae, *Balanus amphitrite* Darwin. Biofouling 16:159–170
- Maneerat S, Bamba T, Harada K, Kobayashi A, Yamada H, Kawai F (2006) A novel crude oil emulsifier excreted in the culture supernatant of a marine bacterium *Myroides* sp. Strain SM1. Appl Microbiol Biotechnol 70:254–259
- Maniyar JP, Doshi DV, Bhuyan SS, Mujumdar SS (2011) Bioemulsifier production by *Streptomyces* sp. S22 isolated from garden soil. Ind J Exp Biol 49:293–297
- Marrakchi H, Lanéelle M-A, Daffé M (2014) Mycolic acids: structures, biosynthesis, and beyond. Chem Biol 21(1):67–85
- Martinez-Checa F, Toledo FL, El Mabrouki K, Quesada E, Calvo C (2007) Characteristics of bioemulsifier V2-7 synthesized in culture media added of hydrocarbons: chemical composition, emulsifying activity and rheological properties. Bioresour Technol 98:3130–3135
- Mohebbali G, Kaytash A, Etemadi N (2012) Efficient breaking of water/oil emulsions by a newly isolated de-emulsifying bacterium, *Ochrobactrum anthropi* strain RIPI5-1. Colloids Surf B Biointerf 98:120–128
- Mousavian SS, Rahimi KY (2010) Eumsan production by *Acinetobacter calcoaceticus* RAG-1 ATCC-31012. Iran J Food Sci Technol 7(3):117–125
- Munstermann B, Poremba K, Lang S, Wagner F (1992) Studies on environmental compatibility: influence of (bio) surfactants on marine microbial and enzymatic systems. In Proceedings of the international symposium on soil decontamination using biological processes p. 414–420, 6–9, Karlsruhe, Germany. Frankfurt: Dechema
- Nadarajah N, Singh A, Owen P (2002) Evaluation of a mixed bacterial culture for de-emulsification of water-in-petroleum oil emulsions. World J Microbiol Biotechnol 18:435–440
- Navon-Venezia S, Zosim Z, Gottlibe A, Leggman R, Carmeli S, Ron EZ, Rosenberg E (1995) Alsan, a new bioemulsifier from *Acinetobacter redioresistens*. Appl Environ Microbiol 61:3240–3244
- Navon-Venezia S, Banin E, Ron EZ, Rosenberg E (1998) The bioemulsifier alsan: role of protein in maintaining structure and activity. Appl Microbiol Biotechnol 49:382–384
- Oloke JK, Glick BR (2005) Production of bioemulsifier by an unusual isolate of salmon/red melanin containing *Rhodotorula glutinis*. Afr J Biotechnol 4(2):164–171
- Orsod M, Joseph M, Huyop F (2012) Characterization of exopolysaccharides produced by *Bacillus cereus* and *Brachybacterium* sp. Isolated from Asian Sea Bass (*Lates calcarifer*). Malays. J Microbiol 8(3):170–174
- Pacwa-Plociniczak M, Plaza GA, Piotrowska-Seget Z, Cameotra SS (2011) Environmental applications of biosurfactants: recent advances. Int J Mol Sci 12:633–654
- Panilaitis B, Johri A, Blank W, Kaplan DL, Fuhrman J (2002) Adjuvant activity of emulsan, a secreted lipopolysaccharide from *Acinetobacter calcoaceticus*. Clin Diagn Lab Immunol 9:1240–1247
- Panilaitis B, Castro GR, Solaiman D, Kaplan DL (2007) Biosynthesis of emulsan biopolymers from agro-based feedstocks. J Appl Microbiol 102:531–537
- Paraszkiewicz K, Kanwal A, Długonski J (2002) Emulsifier production by steroid transforming filamentous fungus *Curularia lunata*. Growth and product characterization. J Biotechnol 92:287–294
- Park SH, Lee J-H, Ko S-H, Lee D-S, Lee HK (2000) Demulsification of oil-in-water emulsions by aerial spores of a *Streptomyces* sp. Biotechnol Lett 22:1389–1395
- Patil JR, Chopade BA (2001) Studies on bioemulsifier production by *Acinetobacter* strains isolated from healthy human skin. J Appl Microbiol 91:290–298
- Patrick S, McKenna JP, O'Hagan S, Dermott E (1996) A comparison of the haemagglutinating and enzymic activities of *Bacteroides fragilis* whole cells and outer membrane vesicles. Microb Pathog 20:191–202
- Peng F, Liu Z, Wang L, Shao Z (2007) An oil-degrading bacterium: *Rhodococcus erythropolis* strain 3C-9 and its biosurfactants. J Appl Microbiol 102(6):1603–1611
- Perfumo A, Smyth TJP, Marchant R, Banat IM (2009) Production and roles of biosurfactants and bioemulsifiers in accessing hydrophobic substrates: microbiology of hydrocarbons, oils, lipids, and derived compounds. In: Timmis KN (ed). Springer, UK (in press)
- Perfumo AS, Smyth TJP, Marchant R, Banat I (2010) Production and roles of biosurfactants and bioemulsifiers in accessing hydrophobic substrates: In handbook of hydrocarbon and lipid microbiology. In: Timmis K.N (ed). Chap. 47. 2(7):1501–1512
- Petrovski S, Dyson ZA, Quill ES, McIlroy SJ, Tillett D, Seviour RJ (2011) An examination of the mechanisms for stable foam formation in activated sludge systems. Water Res 45:2146–2154
- Pines O, Gutnick D (1986) Role for emulsan in growth of *Acinetobacter calcoaceticus* RAG-1 on crude oil. Appl Environ Microbiol 51(3):661–663
- Planckaert M (2005) Oil reservoirs and oil production. Petroleum microbiology. ASM Press, Washington
- Post FJ, Collins NF (1982) A preliminary investigation of the membrane lipid of *Halobacterium halobium* as a food additive. J Food Biochem 6(1):25–38
- Prapulla SG, Jacob Z, Nagin C, Rajalakshmi R, Karanth NG (1992) Maximization of lipid production by *Rhodotorula gracilis* CFR-1 using response surface methodology. Biotechnol Bioeng 40:965–970
- Rahman KSM, Gakpe E (2008) Production characterization and applications of biosurfactants-review. Biotechnol 7(2):360–370

- Rhee CH, Park HD (2001) Three glycoproteins with antimutagenic activity Identified in *Lactobacillus plantarum* KLAB21. *Appl Environ Microbiol* 67(8):3445–3449
- Rodrigues RL, Teixeira AJ, van der Mei CH, Oliveira R (2006) Physicochemical and functional characterization of a biosurfactant produced by *Lactococcus lactis* 53. *Colloids Surf B Biointerf* 49:79–86
- Ron E, Rosenberg E (2001) Natural roles of biosurfactants. *Environ Microbiol* 3:229–236
- Rosenberg E, Ron EZ (1999) High- and low-molecular-mass microbial surfactants. *Appl Microbiol Biotechnol* 52:154–162
- Rosenberg M, Bayer EA, Delarea J, Rosenberg E (1982) Role of thin fimbriae in adherence and growth of acinetobacter calcoaceticus RAG-1 on hexadecane. *Appl Environ Microbiol* 44(4):929–937
- Rosenberg E, Schwartz Z, Tenenbaum A, Rubinovitz C, Legmann R, Ron EZ (1989) Microbial polymer that changes the surface properties of limestone: effect of biodispersant in grinding limestone and making paper. *J Dispers Sci Technol* 10:241–250
- Rufino RD, Sarubbo LA, Campos-Takaki GM (2007) Enhancement of stability of biosurfactant produced by *Candida lipolytica* using industrial residue as substrate. *World J Microbiol Biotechnol* 23(5):729–734
- Rufino RD, de Luna JM, Sarubbo LA, Marona Rodrigues LR, Teixeira JAC, de Campos-Takaki GM (2011) Antimicrobial and anti-adhesive potential of a biosurfactants produced by *Candida* Species. Practical applications in biomedical engineering; Chapter 10; doi: [10.5772/52578](https://doi.org/10.5772/52578)
- Sadouk Z, Tazerouti A, Hacene H (2009) Biodegradation of diesel oil and production of fatty acid esters by a newly isolated *Pseudomonas citronellolis* KHA. *World J Microbiol Biotechnol* 25:65–70
- Saeki H, Sasaki M, Komatsu K, Miura Matsuda H (2009) Oil spill remediation by using the remediation agent JE1058BS that contains a biosurfactant produced by *Gordonia* sp. strain JE-1058. *Bioresour Technol* 100:572–577
- Salehizadeh H, Mohammadzad S (2009) Microbial enhanced oil recovery using biosurfactant produced by *Alcaligenes faecalis*. *Iran J Biotechnol* 7(4):216–223
- Salihu A, Abdulkadir I, Almstapha MN (2009) An investigation for potential development on biosurfactants. *Biotechnol Mol Biol Rev* 3(5):111–117
- Sarubbo LA, de Luna JM, de Campos-Takaki GM (2006) Production and stability studies of the bioemulsifier obtained from a new strain of *Candida glabrata* UCP 1002. *Elect J Biotechnol* 9(4):400–406
- Satpute SK, Banat IM, Dhakephalkar PK, Banpurkar AG, Chopade BA (2010) Biosurfactants, bioemulsifiers and exopolysaccharides from marine microorganisms. *Biotechnol Adv* 28:436–450
- Seghal Kiran G, Hema TA, Gandhimathi R, Selvin J, Anto Thomas T, Rajeetha Ravji T, Natarajaseenivasan K (2009) Optimization and production of a biosurfactant from the sponge-associated marine fungus *Aspergillus ustus* MSF3. *Colloids Surf B Biointerf* 73(2):250–256
- Sen R (2008) Biotechnology in petroleum recovery: the microbial EOR. *Prog Energy Combust Sci* 34:714–724
- Seo HS, Michalek SM, Nahm MH (2008) Lipoteichoic Acid Is Important in Innate Immune Responses to Gram-Positive Bacteria. *Infect Immun* 76(1):206–213
- Sharma A, Novak EK, Sojar HT, Swank RT, Kuramitsu HK, Genco RJ (2000) *Porphyromonas gingivalis* platelet aggregation activity: outer membrane vesicles are potent activators of murine platelets. *Oral Microbiol Immunol* 15:393–396
- Song H-Y, Kim Y-H, Seok S-J, Gil H-W, Yang J-O, Lee E-Y, Hong S-Y (2012) Cellular toxicity of surfactants used as herbicide additives. *J Korean Med Sci* 27:3–9
- Stratton HM, Brooks PR, Griffiths PC, Seviour RJ (2002) Cell surface hydrophobicity and mycolic acid composition of *Rhodococcus* strains isolated from activated sludge foam. *J Ind Microbiol Biotechnol* 28:264–267
- Su W-T, Chen W-J, Lin Y-F (2009) Optimizing emulsan production of *A. venetianus* RAG-1 using response surface methodology. *Appl Microbiol Biotechnol* 84:271–279
- Suthar H, Hingurao K, Desai A, Nerurkar A (2008) Evaluation of bioemulsifier mediated microbial enhanced oil recovery using sand pack column. *J Microbiol Methods* 75:225–230
- Tahara Y, Yamada Y, Kondo K (1976) A new lipid; the ornithine and taurine-containing ‘cerilipin’. *Agric Biol Chem* 40:243–244
- Toledo FL, Gonzalez-Lopez J, Calvo C (2008) Production of bioemulsifier by *Bacillus subtilis*, *Alcaligenes faecalis* and *Enterobacter* species in liquid culture. *Bioresour Technol* 99:8470–8475
- Toren A, Navon-Venezia S, Ron EZ, Rosenberg E (2001) Emulsifying activities of purified alasan proteins from *Acinetobacter radioresistens* KA53. *Appl Environ Microbiol* 67:1102–1106
- Trindade JR, Freire MG, Amaral PFF, Coelho MAZ, Coutinho JAP, Marrucho IM (2008) Aging mechanisms of oil-in-water emulsions based on a bioemulsifier produced by *Yarrowia lipolytica*. *Colloids Surf A Physicochem Eng Aspects* 324:149–154
- Tuleva B, Christova N, Cohen R, Antonova D, Todorov T, Stoineva I (2009) Isolation and characterization of trehalose tetraester biosurfactants from a soil strain *Micrococcus luteus* BN56. *Process Biochem* 44:135–141
- Van Dyke M, Gulley S, Lee H, Trevors J (1993) Evaluation of microbial surfactants for recovery of hydrophobic compounds in soil. *J Ind Microbiol Biotechnol* 11:163–170
- Vesper SJ (1987) Production of pili (fimbriae) by *Pseudomonas fluorescens* and correlation with attachment to corn roots. *Appl Environ Microbiol* 53(7):1397–1405
- Walencza E, Wieckowska-Szakiel M, Rozalska S, Sadowska B, Rozalska B (2007) A surface-active agent from *Saccharomyces cerevisiae* influences staphylococcal adhesion and biofilm development. *Z Naturforsch C* 62(5–6):433–438
- Wong JWC, Zhao Z, Zheng G (2010) Biosurfactants from *Acinetobacter calcoaceticus* BU03 enhance the bioavailability and biodegradation of polycyclic aromatic hydrocarbons. Proceedings of annual international conference on soils, sediments, water and energy
- Huang X-f, Peng K, Feng Y, Liu J, Lu L (2013) Separation and characterization of effective demulsifying substances from surface of *Alcaligenes* sp. S-XJ-1 and its application in water-in-kerosene emulsion. *Bioresour Technol* 13:257–264
- Yim JH, Ahn SH, Kim SJ, Lee YK, Park KJ, Lee HK (2005) Production of novel exopolysaccharide with emulsifying ability from marine microorganism, *Alteromonas* sp. strain 00SS11568. *Key Eng Mat* 277(279):155–161
- Zhang Q, He G, Wang J, Cai W, Xu Y (2009) Mechanisms of the stimulatory effects of rhamnolipid biosurfactant on rice straw hydrolysis. *Appl Energy* 86:233–237
- Zhao Z, Wong JWC (2009) Biosurfactants from *Acinetobacter calcoaceticus* BU03 enhance the solubility and biodegradation of phenanthrene. *Environ Technol* 30(3):291–299