Journal of
Chemical Engineering & Process Technology

Sinumvayo and Ishimwe, J Chem Eng Process Technol 2015, 6:2
DOI: 10.4172/2157-7048.1000223

Review Article Open Access

Agriculture and Food Applications of Rhamnolipids and its Production by *Pseudomonas Aeruginosa*

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Abstract

Rhamnolipids (RLs) are known as very efficient surface-active which are the most investigated biosurfactants usually produced by *Pseudomonas aeruginosa* strains. Other various bacterial species promising producers of rhamnolipids are shown herein. Researchers on rhamnolipids production concentrate their efforts on conventional mutagenesis and metabolic engineering of different strains especially *P. aeruginosa*. Several modifications of metabolic pathways in combination with optimization of fermentation strategies were suggested to increase the rhamnolipid yields. Unique properties of rhamnolipids, including sequestering, detergency, demulsifying, foaming, emulsifying, thickening, metal, solubilizing, wetting, vesicle forming and phase dispersion make them suitable to be used in a wide range of industrial applications such as cosmetics, food, pharmaceutical formulations and bioremediation of pollutants. The present review shows different rhamnolipid yields obtained and covers the potential applications of rhamnolipid, mainly focusing on food processing, storing, numerous applications in agriculture and farming.

Keywords: Agriculture; Biosurfactants; Food industry; *Pseudomonas aeruginosa;* Rhamnolipids

Introduction

Microorganisms showed a high capacity of synthesizing a wide range of surface-active compounds, generally called biosurfactants. These compounds are mainly classified according to their molecular weight, physico-chemical properties and mode of action [1,2].

Many surfactants including rhamnolipids alter the surface properties of liquids, even when present in small quantities [3]. Like other surfactants, rhamnolipids present a lyophilic group and a lyophobic group which facilitate the orientation of the surfactant to reduce the liquid surface free energy and increase surface viscosity [4,5].

Some chemical surfactants decontaminated and maintain membrane performance as well as membrane integrity under mild operation conditions [6]. Undefined cleaning activity together with its low biodegradability and high cost most possibly determine the much less frequent use of chemical surfactants in the industrial applications [7]. Rhamnolipids are potent natural glycolipid biosurfactants often biosynthesized by *Pseudomonas aeruginosa* strains through the fermentation process, with cheaper agro-based substrates and waste materials. They can be good substituents for chemical surfactants, in different industrial fields [8]. Rhamnolipids are classified as mono-rhamnolipids and di-rhamnolipids, depending on one or two rhamnose sugars linked to a dimer of β-hydroxyacids (primarily β-hydroxydecanoate) which can rise up to three hydroxyl fatty acids containing 8–14 carbons [9].

Non-toxic nature, excellent biodegradability, high surface/interfacial activity, low toxicity, high thermal/chemical stability, production from renewable resources and the ability to form microemulsions [10,11], make rhamnolipids to be potentially used as emulsifiers and stabilizers in a great number of food processing and from bioremediation to food additives [12]. The usefulness of rhamnolipids in cleaning ultrafiltration (UF) membrane fouled by protein is due to their high capability of binding the protein with high affinity as well as increasing the wettability of solid surface [6].

In the present review, we intend to summarize rhamnolipids production strategies by *Pseudomonas Aeruginosa*, its agriculture and food industrial applications are also discussed. Apart from their uses based on their special properties, rhamnolipids yields produced by *Pseudomonas Aeruginosa* in 15 years ago are reviewed and shown in Table 1.

Production of rhamnolipids

Rhamnolipids are well-studied glycolipids with high potential industrial applications [13]. *Pseudomonas* strains are the best producers of glycolipid containing rhamnose and 3-hydroxy fatty acids. However, the most promising bacteria strains, *P. aeruginosa,* was investigated and recommended as the best microorganism to produce two classes of rhamnolipids: monorhamnolipids and dirhamnolipids with excellent surface activity [14,15].

Therefore, their properties and potential application, have encouraged many researchers to improve their production and have become the most investigated biosurfactants [3].

The properties showed by rhamnolipids depend on their homologues composition and distribution that are determined by the bacterial strain, culture conditions and medium composition [14]. High prices of raw-material and low productivities have been found as potential inhibitors of rhamnolipids for industrial scale production. However, strain-engineering is now a promising tool for achieving accurate production so that large-scale production of rhamnolipids becomes economically feasible [16].

Rhamnolipids production by different *P. Aeruginosa* strains

Rhamnolipid are produced by fermentation process similar to

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Received March 03, 2015; **Accepted** April 17, 2015; **Published** April 24, 2015

Citation: Sinumvayo JP, Ishimwe N (2015) Agriculture and Food Applications of Rhamnolipids and its Production by *Pseudomonas Aeruginosa*. J Chem Eng Process Technol 6: 223. doi:10.4172/2157-7048.1000223

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Low cost or waste raw material	Producer microbial strain	Maximum yields (g/l)	Reference
Rapeseed oil	*Pseudomonas* species DSM 2874	45	[36]
Sunflower, soybean oil and glycerol	*Pseudomonas aeruginosa* DS10-129	4.31, 2.98 and 1.77	[37]
Waste frying oils (sunflower and olive oil)	*Pseudomonas aeruginosa* 47T2 NCIB 40044	2.7	[38]
Soybean soapstock waste	*Pseudomonas aeruginosa* LBI	11.72	[39]
Sunflower oil soapstock waste	*Pseudomonas aeruginosa* LBI	15.9	[40]
Soybean oil	*Pseudomonas aeruginosa* O-2-2	70.56	[33]
Corn steep liquor (10% (v/v)) and molasses (10% (w/v))	*Pseudomonas aeruginosa* strain #112	3.2	[22]
Waste frying oils(olive/ sunflower (50:50; v/v),)	*Pseudomonas. aeruginosa* 47 T2	8.1	[41]
Glycerol and ammonium nitrate	*Pseudomonas aeruginosa* DAUPE 614	3.9	[24]
Glucose and glycerol	*Pseudomonas aeruginosa* EM1	7.5 and 4.9	[26]
1% glucose+0.25% stearic acid (C18)	*Pseudomonas aeruginosa* ATCC 9027	2.1	[31]
Sunflower oil	*Pseudomonas aeruginosa* san-ai	3	[36]
Brazilian Nut (Bertholletia excelsa) and Passion Fruit oils	*Pseudomonas aeruginosa* LBI	9.9 and 9.2	[14]
Clarified blackstrap molasses	*Pseudomonas aeruginosa* mutant strain	*1.50*	[42]
Glucose and glycerol	*Pseudomonas aeruginosa* TMN	0.3 and 0.25	[43]
Soybean oil, safflower oil, and glycerol	**Pseudomonas aeruginosa** DS10-129	4.31, 2.98, and 1.7	[44]
Soybean oil	Pseudomonas aeruginosa MA01	12	[45]

Table 1: Rhamnolipids production in different *Pseudomonas aeruginosa* strains and their corresponding carbon sources.

those used to produce beer or other fermented products. Bacteria are added to a fermentation tank and provided nutrients source, and under properly controlled conditions the result will be rhamnolipids [17]. During biosurfactant production, the downstream processes are critical steps which involve recovery, concentration, and purification. The appropriate approach for downstream processing relies on the type and nature of the substrates, fermentation protocol (batch/fedbatch and growing/resting cells), and type and physicochemical properties of the resulted biosurfactants. Their high ratio of hydrophobic-to-hydrophilic characteristics, a number of traditional techniques such as precipitation, crystallization, centrifugation, and solvent extraction have been used to recover the most biosurfactants from the culture medium [18].

Most of the microorganisms investigated for the production of rhamnolipids are *P. aeruginosa strains*, however, by now, only a handful of strains are potentially relevant for industrial production processes, mainly due to vast differences in biosurfactant yields and achievable maximum concentrations [19].

The specific reason which makes *P. aeruginosa* a favorable biosurfactant-producing or oil-degrading strain may be found in its wide spread occurrence in contaminated environments,

rapid growth, easy for isolation and screening as well as the high biosurfactant production and crude oil degradation capacity [20].

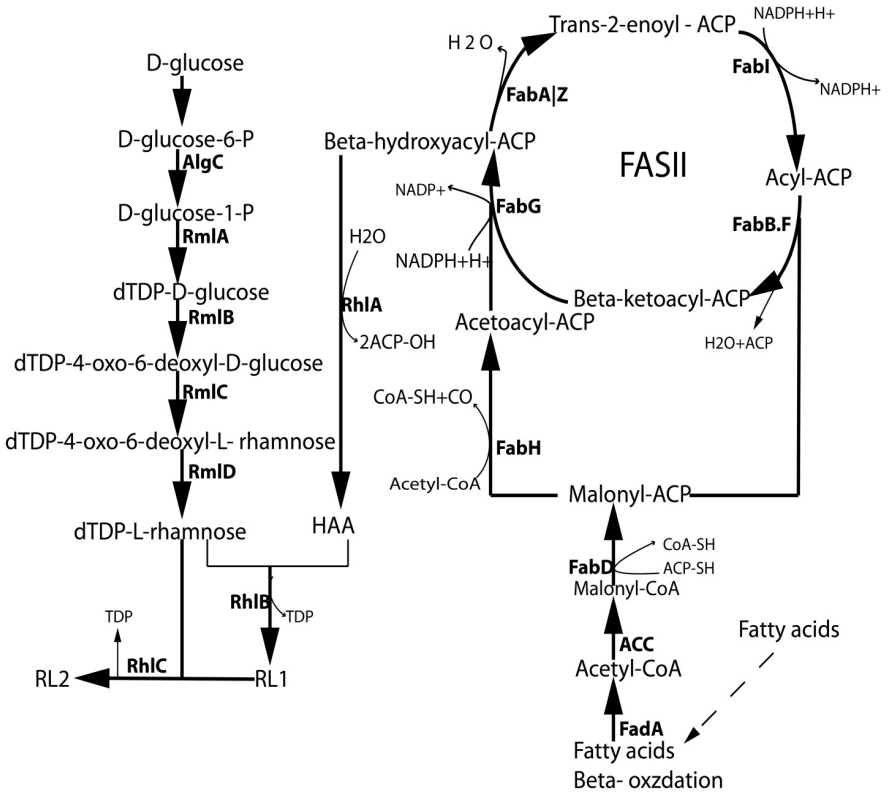
In *P. aeruginosa*, both mono- and di-rhamnolipids biosurfactants are the main products of the convergence of two metabolic pathways: the biosynthesis of dTDP-L-rhamnose and the diversion of the βhydroxydecanoyl-ACP intermediate from the FASII cycle by RhlA to synthesize the fatty acid dimer moiety of rhamnolipids and free 3-[3-hydroxyalkanoyloxy) alkanoic acid (HAA) (Figure1). The rhamnosyltransferases RhlB and RhlC catalyse the transfer of dTDP-L-rhamnose to either HAA, or a previously generated mono-rhamnolipid, respectively [21].

Recently, biosurfactant production by a *P. aeruginosa* with very low-cost substrates showed a highest biosurfactant production of (3.2 g/l) when a culture medium containing corn steep liquor (10% (v/v)) and molasses (10% (w/v)) is used [22]. By inserting rhlAB genes responsible for production of mono-rhamnolipids, into *P. aeruginosa* PA14 and *E. coli* TG2 has not showed any improvement for effective production of rhamnolipids [23]. *P. aeruginosa* DAUPE 614 produced rhamnolipids (3.9 g/l) in 216 h when cultivated on a medium containing glycerol as the sole carbon source and ammonium nitrate with a C:N ratio of 55:1 [24].

Sarachat et al. [18] confirmed the production of rhamnolipid biosurfactants by *P. aeruginosa* SP4 and its concentration using the foam fractionation technique. The *P. aeruginosa* strain BN10 [25], isolated indigenous strain *P. aeruginosa* EM1 originating from an oil-contaminated site [26] and *P. aeruginosa* IFO3924 [27] have been reported to produce rhamnolipid-type biosurfactants.

Two rhamnolipid biosurfactants, L-rhamnopyranosyl-L-rhamnopyranosyl-b-hydroxydecanoyl-b-hydroxydecanoate or Rha-Rha C10-C10 and L-rhamnopyranosyl-L-rhamnopyranosyl-b-hydroxydecanoyl-b-hydroxydodecanoate or Rha–Rha C10-C12 have been reported to be produced by *P. aeruginosa B189* strain [28].

The capacity of producing rhamnolipid biosurfactants from a single specific strain with variation of only one carbon source was emphasized by the usage of *P. aeruginosa* strain NY3. This strain appeared to be much more productive from other rhamnolipid-producing *Pseudomonas* strains due to its capability of producing many more minor components of rhamnolipid when grown with glycerol as the sole carbon source instead on glucose [29]. In 15 years ago, *P. aeruginosa* 57RP was isolated and its capability for rhamnolipid production was assessed on 2% (w/v) when mannitol or naphthalene as carbon sources. Results showed that in the mannitol culture, 2.311 g of purified rhamnolipid extract were obtained after 14 days of incubation, whereas about 180 mg of rhamnolipid extract were recovered from the naphthalene culture after 7 days of incubation [30]. *P. aeruginosa* ATCC 9027 have been demonstrated as other rhamnolipid-producing strain. It was reported that 1% glucose+0.25% stearic acid (C18) produced the greatest yield (2.1 g/l) compared to other glucose-fatty acid combinations (0.8–1.8 g/l) [31]. Evaluation of waste cooking oil (CWO) as low-cost substrate to produce rhamnolipids surfactants for industrials application have been performed by using another potential *P. aeruginosa* 12-1[32]. Other strain *Pseudomonas aeruginosa*



AlgC: phosphomannomutase, (RmlA, EC 2.7.7.24): glucose-1-phosphate thymidylyltransferase catalyzes the conversion of thymidylmonophosphate nucleotide to glucose-1-phosphate, (RmlB, EC 4.2.1.46): dTDP-D-glucose 4,6-dehydratase which catalyzes an oxidation of the C4 hydroxyl group of the D-glucose residue, then dehydration, ending to the formation of dTDP-4-oxo-6-deoxyl-D-glucose, (RmlC, EC 5.1.3.13) :dTDP-4-dehy-drorhamnose 3,5-epimerase which catalyzes a double epimerization reaction at the C3 and C5 positions of the 4-oxo-6-deoxyl-D-glucose ring, (RmlD, EC 1.1.1.133): dTDP-4-dehydrorhamnose reductase which reduces the C4 keto group of the 4-oxo-6-deoxyl-L-mannose moiety and leads to the formation of dTDP-L-rhamnose, FabD: malonyl-CoA:ACP transacylase, FabB, FabH, and FabF: β-ketoacyl-ACP synthetases, FabG: NADPH-dependent β-ketoacyl-ACP reductase, FabA, FabZ: β-hydroxyacyl-ACP dehydratases, FabI: The NADH-dependent enoyl-ACP reductase, HAA: 3-(3-hydroxyalkanoyloxy)alkanoic acid,: RhlA: 3-(3-hydroxyalkanoyloxy) alkanoate synthetases, RhlB: rhamnosyltransferase 1, and RhlC: rhamnosyltransferase 2. All required enzymatic steps are font bolded.

Figure 1: Biosynthesis pathway of RL1, monorhamnolipid; RL2, dirhamnolipid from fatty acids and glucose in *Pseudomonas Aeruginosa*.

O-2-2 has been used in order to improve the yield of rhamnolipid surfactants for reaching industrial requirements. The pH optimization led increased rhamnolipid production to 28.8 g/l, an improvement of 19.7%, and more substrate was converted to rhamnolipids rather than to biomass [32].

In *P. aeruginosa*, both direct and indirect factors like quorum sensing, nutritional status or stress response influence rhamnolipid production [33]. The nature of the carbon source and the nitrogen source as well as the C:N ratio, chemical and physical parameters such as temperature, nutritional limitations, dissolved oxygen, divalent cations and pH have been demonstrated to influence the amount of biosurfactants produced and the type of polymer formed [34]. Production of rhamnolipids can be inhibited by the presence of NH_4^+, glutamine, asparagine, and arginine as nitrogen source and contrary promoted by NO_3^-, glutamate and aspartate [35].

Biosynthesis of rhamnolipids in *P. aeruginosa*

P. aeruginosa produces a glycolipidic biosurfactants consisting of one or two hydrophilic L-rhamnose molecules (monorhamnolipids and dirhamnolipids,) and that of a hydrophobic fatty acid moiety [46]. Furthermore, *P. aeruginosa* produces extracellular rhamnolipid surfactants including the synthesis of rhamnolipids which is controlled by quorum sensing system [47].

The biosynthesis of biosurfactants, including rhamnolipids, can be either proceeded, (1) when both moieties are synthesized independently of the growth substrate (*de novo*), or (2) with a hydrophobic carbon source such as fatty acids and triglycerides, where the lipid moieties are directly derived from the carbon source, but the sugar is synthesized de novo. Another possibility (3) is when the sugar moiety is directly derived from the carbon source, but the lipid component is synthesized de novo [48].

It has been found that two genes rhlA and rhlB are organized in a single operon whereas another gene necessary for synthesis of di-rhamnolipids, *rhlC*, has also been identified and localized in another region of *P. aeruginosa* genome and which forms an operon with a gene of unknown function [13]. Those three kinds of genes *rhlA, rhlB* and *rhlC*, have been also investigated in various *Burkholderia* species where they are grouped within one putative operon, and have been shown to be required for rhamnolipid production [49]. The RhlC (rhamnosyltransferase II) catalyses the addition of the second rhamnose moiety to mono-rhamnolipids forming di-rhamnolipids [51]. Like *rhlA* and *rhlB*, *rhlC* is thought to be an ancestral gene controlled by the same quorum sensing system as *rhlA* and *rhlB*. The rhamnose moiety for mono- and di-rhamnolipids is derived from AlgC activity and the RmlABCD pathway, encoded on the *rml BCAD* operon [50]. In rhamnose synthesis, AlgC produces glucose-1-

phosphate which is converted to dTDP-D-glucose by RmlA followed by its conversion to dTDP-4-oxo-6-deoxyl-D-glucose by RmlB, the latter is converted to dTDP-6-deoxyl-L-deoxyl-4-rhamnose by RmlC. dTDP-6-deoxyl-L-deoxyl-4-rhamnose is converted to dTDP-L-rhamnose by RmlC [51]. Finally, dTDP-L-rhamnose, the key substrate of two rhamnosyltransferases RhlB and RhlC, is converted in one (monorhamnolipid) or two (di-rhamnolipid) molecules [52].

Genetic engineering for rhamnolipid production

Different direct and indirect factors such as nutritional status, quorum sensing and stress response can influence rhamnolipid production in *P. aeruginosa* [12]

Efforts have been concentrated to interfere with these regulatory mechanisms by introduction of genes responsible for rhamnolipid production into some microorganism's chromosome.

It has been proved that AlgR which controls various factors in *P. aeruginosa,* involves directly in rhlAB operon expression by binding to the promoter [53].

The deletion of AlgR PSL317 strain shows an increased rhamnolipid production compared with wild-type PAO1-strain [54]

The model for transcription activation of rhlAB (encoding rhamnosyltransferase 1) and rhlC (encoding rhamnosyltransferase 2) during biofilm-grown PAO1 and PSL317 increased the concentration of butanoyl-homoserine lactone (C4-HSL) to the transcription activator RhlR. The latter can work either as an activator or inhibitor for the operon transcription depending on concentration level of C4-HSL which is an autoinducer that binds to RhlR [46].

Therefore, the absence of C4-HSL influences RhlR to work as an inhibitor. Engineered strains by deleting lasR, essential gene for C4-HSL production did not produce any type of rhamnolipids [55].

Other regulators such as PtxR, RsaL, RsmA, DksA and various genes encoding sigma factors like RpoN and RpoS also influence the expression levels of the rhlAB operon [56,57], and several potential researches lie on manipulations of this complex regulatory system for rhamnolipids production.

Another strategy for enhancing rhamnolipid production was done by studying *P. aeruginosa* (NRRL B-771) and its mutant strain, PaJC, harboring the Vitreoscilla hemoglobin gene vgb, and it was reported as an effective strategy to increase rhamnolipid production where an increase up to 8.373 g/lwas obtained with PaJC mutant [58].

By knocking-out RhlC gene that is located in another operon with an upstream unknown gene (PA1131) in *P. aeruginosa* PAO1, and which is not organized with RhlAB, only resulted in production of monorhamnolipids [59].

A recombinant *E. coli* strain expressing *P. aeruginosa rhlAB* operon has been reported. The availability of dTDP-L-rhamnose a substrate of RhlB found in *E. coli* showed that metabolic engineering strategy leads to an increased monorhamnolipids production in heterologous host [60].

An insertion through transposome-mediated chromosome integration of the RhlAB gene into *P. aeruginosa* PAO1-rhlA^{-} and *Escherichia coli* BL21 (DE3) which could not produce rhamnolipid, showed a significant production of rhamnolipid in *P. aeruginosa* PEER02 and *E. coli* TnERAB recombinant [61]. Also, an introduction of rhlAB operon from *P. aeruginosa* PAO1 into *Burkholderia kururiensis* KP23, showed an increase of 6-fold [62].

Using *Burkholderia kururiensis* as expression platform of two *Pseudomonas aeruginosa* biosynthetic enzymes RhlA applied in fatty acid synthesis to generate the HAA and RhlB which catalyzes the transfer of dTDP-L-rhamnose to β-hydroxy fatty acids in the biosynthesis of rhamnolipids, an engineered *Burkholderia kururiensis* showed an increase over 600% comparing to the wild type [63].

An rhlABRI cassette contained genes for rhamnolipid synthesis was cloned and integrated into *P. putida* KT2440 chromosome random transposon vector with absence of antibiotic-resistance marker. This generate a genetically engineered microorganism named *P. putida* KT2440-rhlABRI, which showed a good expression of rhlABRI cassette and produced rhamnolipid at a yield of 1.68 g [64].

A recently engineered *P. stutzeri* Rhl was constructed for heterologous production of rhamnolipid under anaerobic conditions where the rhlABRI genes responsible for rhamnolipid biosynthesis were cloned into a facultative anaerobic strain *P. stutzeri* DQ1 and 1.61 g/l rhamnolipid was obtained [65].

The *rhlAB*-genes from *P. aeruginosa* PAO1 were expressed in *P. putida* KT2440 followed by removing of a competing pathway leads to the of formation polyhydroxyalkanoates (PHAs) and the highest yield of 0.15 $g/g_{glucose}$ was obtained [66].

Other producers of rhamnolipids

In last few decades, researchers concentrated their work on finding alternative strains for the production of rhamnolipids by using non-pathogenic bacteria, [67] demonstrated the potential use of mahua oil cake as substrate for the production of rhamnolipid using *S. rubidaea* SNAU02. Unlike *P. aeruginosa*, both *Acinetobacter* and *Enterobacter* non-pathogenic strains produced rhamnolipids that exhibited excellent emulsification activity with aromatic and aliphatic hydrocarbons. Several host strains such as *P. fluorescens*, *P. putida*, *P. oleovorans* and *E. coli* by introducing the rhlAB operon (rhamnosyltransferase), produced the highest rhamnolipid concentration of 60 mg/l for *P. putida* [68]. Compared to other rhamnolipids producers, P. *putida* can be considered as a potential rhamnolipid producer. *C* NCIM 2112 (Pd 2112) was screened for rhamnolipid production, however, the medium supplemented with sucrose, mannitol, or glycerol did not increase the yield. The medium supplemented with Mannitol and hexadecane produced more rhamnolipid compared to the medium supplemented with only mannitol [69]. The studies conducted on *P. chlororaphis* showed this strain as a potential non-pathogenic rhamnolipid producer due to its energy saving property [70]. The researchers have been carried out on several other bacterial species such as *Burkholderia mallei*, *B. pseudomallei, Serratia rubidea* and other non-pathogenic *B. thailandensis*, and by using optimized medium [71].

Potential industrial applications

Rhamnolipids, potential and current biosurfactants also exhibits many properties of, i.e. detergency, emulsifying, metal sequestering, demulsifying, solubilizing, wetting, thickening, foaming, environmental compatibility, and vesicle forming and phase dispersion. All of these properties are associated with the amphiphilic character of rhamnolipid molecules and confer upon them the ability to accumulate between fluid phases, therefore, reducing surface and interfacial tensions. The range of applications of rhamnolipids, including environmental biodegradation, food processing, agricultural, cosmetic and pharmaceutical uses, is potentially as extensive as their properties [19,72].

Applications of rhamnolipids in food processing and preservative

Nowadays, rhamnolipids produced by *P. aeruginosa* are the most promising class of biosurfactants, since the U.S. Environment Protection agency has approved their use in food products, and other industrials applications. Due to their high antimicrobial activity and physicochemical properties, some can be exploited by food industry particularly in increasing of food shelf life without concern to consumer health, excluding the need of adding synthetic comfits, which, in most of case, are harmful. In combination with niacin they extended shelf life and inhibited hemophilic spores in UHT soymilk. Niacin with rhamnolipids in salad has extended its shelf life and inhibited mold growth. Natamicin, nisin, and rhamnolipids combined together in cottage cheese have extended shelf life by inhibiting mold and bacterial growth, particularly gram-positive and spore-forming bacteria. Therefore, they can be used to avoid food contamination directly, as food additive, or indirectly, as a detergent formulation to clean surfaces that come in contact with the food [73].

Rhamnolipids can be used in bread, had rolls, soft rolls, hamburger buns, flat bread, baguettes, pizza, chinese steam breads, croissants, argentine breads, schnittbotchen, cake and sponge cake to improve their dough or batter stability ,volume and shape, structure, dough texture, width of the cut and microbiological conservation. Furthermore, they are used to improve the properties of butter cream, decoration cream and non-dairy cream filling for croissants, Danish pastries, and other fresh or frozen fine confectionery products [74]. They also serve as a source of rhamnose for the synthesis of food flavors [16].

Apart from their obvious role as agents that decrease surface and interfacial tension, rhamnolipids can have other several functions in food where they improve texture and shelf-life of starch-containing products, control the agglomeration of fat globules, stabilize aerated systems, modify rheological properties of wheat dough and improve stability, consistency and texture of oils and fat-based products and inhibits separation. They help in the general mixing of ingredients and can also slow the growth of molds and some bacteria in food [75]. In ice cream and bakery formulations, rhamnolipids can be used to control consistency, retard staling, solubilize flavor oils, stabilize fats, and reduce spattering [15]. It has been demonstrated that rhamnolipids can be explored to control the attachment and to disrupt biofilms of individual and mixed cultures of the food-borne pathogens [76].

Applications of rhamnolipids in agriculture and farming

Deep insight into the physiochemical effects of rhamnolipids and their biological importance would reveal new dimensions in the fields of research like agriculture. Rhamnolipids have the potential to be a part of alternative strategies in order to reduce or replace pesticides in agriculture. So, nowadays, they play a great importance for the efficiency of new biopesticides.

They are involved in non-specific immunity in plants, induce resistance in plants and are also active in other plant species. Rhamnolipids are capable of stimulating defense genes in tobacco, also potent protectors in monocotyledonous plants against biotrophic fungi [77]. Rhamnolipids are found to be useful in removal of polyaromatic hydrocarbons and pentachlorophenol from soil. They can facilitate the absorption of nutrients and fertilizers through the roots [78].

Stability constants were established by an ion-exchange resin technique. Due to the anionic nature of rhamnolipids, they are able to remove toxic metals from agriculture land and ions such as, lanthanum, cadmium, copper, lead and zinc due to their complexation ability. Cations of lowest to highest affinity for rhamnolipid are described as $K^{+}<Mg^{2+}<Mn^{2+}<Ni^{2+}<Co^{2+}<Ca^{2+}<Hg^{2+}<Fe^{3+}<Zn^{2+}<Cd^{2+}<Pb^{2+}<Cu^{2+}<Al^{3+}$. The affinities were approximately the same or higher than those of organic acids (acetic, citric, fulvic and oxalic acids) towards metals, thus indicating the potential of the rhamnolipid for metal remediation [79].

The success of rhamnolipids in increasing recovery of heavy metal contaminants from soils will also depend on the amount of rhamnolipid present in the aqueous phase [80]. Particularly, Rhamnolipid type I and type II, with surface tensions of 29 mN/m are suitable for heavy metals removal from contaminated agriculture areas [81].

Rhamnolipids with a fertilizer (Inipol EAp- 22) have a high capacity of enhancing the biodegradation of aromatic and aliphatic compounds in aqueous phase and soil reactors.

Those kinds of biosurfactants, have demonstrated inhibition of zoospore forming plant pathogens that have acquired resistance to commercial chemical pesticides and another investigation has shown that rhamnolipid can stimulate plant immunity which is considered as an alternative strategy to reduce the infection by plant pathogens.

Recent investigation has also established rhamnolipid as an insecticidal compound [82]. Agriculture land containing weathered petroleum hydrocarbons which are important land contaminants can be remedied by an introduction of rhamnolipids into soil due to their high solubilization and increased bioavailability properties on some inaccessible compounds [83].

As reviewed by [84], biosurfactants, such as rhamnolipids, are being widely used in soil remediation and also confirmed that the removal of phenanthrene from contaminated soil using rhamnolipid and short-chain organic acids (SCOA) gradually increased as the SCOA concentration increased up to a concentration of 300 mmol/l. They are also potential surfactants for removing dibenzothiophenes compound from soil [85]. Rhamnolipids, have been found to be able to remove chromium and arsenic oxyanions from spiked soils or mine tailings, conducted a series of dynamic column elution tests, suggested that rhamnolipids at a high concentration (5.0 g/l) could remove ~70% of the pyrene in soil. On the other side, polycyclic aromatic hydrocarbons (PAH) and chemical spills are known as most limiting factors to soil fertility and crop productivity; Therefore, their desorption from contaminated soils is assisted by rhamnolipids which improve the bioavailability of different pollutants [4]. Other application of rhamnolipid biosurfactants-enhanced soil washing coupled with activated carbon adsorption have been promised as alternative to remediate soils contaminated with hydrocarbon organic contaminants (HOC) [86].It has been reported that continuous application of 150 mg/l of rhamnolipid biosurfactant regulate 100% of disease caused by *P. capsici* (e.g. Phytophtora blight) in pepper plants (*Capsicum annuum*) [87].

Recently, researches indicated that integration of *R. glutinis* in combination with rhamnolipids at low concentrations might be an efficient and safe strategy in field application to control *A. alternate,* which is a serious pathogenic to certain cultivars of tomatoes, causing postharvest black rot at high frequencies [88]. Application of rhamnolipids have been suggested to be a good way for wastewater treatment and prevention of overwatering during irrigation due to their wettability properties which facilitates the breaking down of impenetrable barriers to water, allowing water to easily reach the soil and spread more evenly throughout the soil profile [89].

In addition, many techniques using rhamnolipids have been developed for cleaning up petroleum contaminated soil. Addition of rhamnolipids enhances the solubility and elimination of these contaminants by improving oil biodegradations rates [90].

Different comparisons of biosurfactants (aescin, saponin, lecithin, rhamnolipid and tannin) for washing crude oil contaminated soil was carried out where rhamnolipids showed a high removal capacity up to 80% oil [91]. Oil washing experiments done by a combination of 10 g/l NaCl, 5.0 g/l n-butyl alcohol and 2.0 g/l rhamnolipid provided a highest oil extraction rate of 74.55% [91]. Even if rhamnolipids are the best enhancers for petroleum hydrocarbon soil pollutants removing and their potential to facilitate the bioremediation of soil contaminated by hydrocarbons, Zheng et al. and Kaczorek et al. [91] suggested that their application must be evaluated carefully to reduce their exhibition on antimicrobial activity.

Outlook

This review outlined the successful development ways of the production and application of a better known rhamnolipid biosurfactant.

Nowadays, research on rhamnolipids biosurfactants is becoming easier due to the availability of several different strains screening methods and the ability to purify diverse amphiphilic molecules. Rhamnolipids have several applications in agriculture, agrochemical industries, food processing and food preservation relating to their potential properties as they increase solubility, foaming capacity and lower surface tensions. Regarding too many researches reviewed herein, we admire the *P. aeruginosa* strains to be the most potential microorganisms which can be involved in large-scale production of rhamnolipids. Most of the studies conducted on rhamnolipids production up to now, they confirm and suggest the exploitation of modern approach such as functional metagenomics which is the utmost essential and may even lead to discovery of novel green surfactants including rhamnolipids biosurfactants. Therefore, intense work on green surfactants is a priority to prevent the adverse effects of synthetic surfactants largely employed in many commercial sectors including agro- chemical industries. Considering the social and technological backgrounds, utilization of biosurfactants, which are environmentally friendly and highly functional, rhamnolipids have become more and more important. Therefore, their application in washing, bioremediation and microbial enhanced oil recovery basing on their physical chemical properties and environmental conditions must be considered. Like other surfactants, attention should be paid on rhamnolipids application on plants as they could be affected in many different ways. Thus, the improved study on their negative impact on crop yield or other important agronomical traits should be a crucial role to avoid any impact on plant growth or metabolism, while improving plant immunity.

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