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Review Article

Molecular Genetics of Rhamnolipid Biosurfactant Production (A Mini-Review)

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ABSTRACT

Rhamnolipid is a well characterized lipid having a broad spectrum of application from clinics to industry. It has been considered as one of the most vital molecules because of biological origin and surface activity. Rhamnolipid is composed of β-hydroxy fatty acid connected by the carboxyl end to a rhamnose sugar molecule which in nature are being produced by Pseudomonas aeruginosa. It was first discovered in 1984 and until 2018, hundreds and thousands of papers and patents have been published indicating the huge interest of biotechnologists all over the world. Almost all countries of the world, have worked on it and because of its diversity in structure, there are two types of rhamnolipids, mono- and di-rhamnolipids. Di-rhamnolipids are of most importance and enzymes have been prepared in labs to convert the mono-rhamnolipids to di-rhamnolipids. During the last few years, work has been done on the enhanced production of rhamnolipid which could fulfilled the industrial demand and can substitute the current chemical surfactants. Understand the molecular biology of rhamnolipid production will help its enhanced production and would explore the novel chapters of its applications.

Key words: Rhamnolipids, Biosurfactants, *Pseudomonas aeruginosa*, Molecular biology of Rhamnolipid

Introduction

Rhamnolipids, biosurfactants were first isolated from *Pseudomonas aeruginosa* and described by Jarvis and Johnson in 1949. These compounds are predominantly constructed from the union of one or two rhamnose sugar molecules and one or two β-hydroxy (3-hydroxy) fatty acids (1). Rhamnolipids with one sugar molecule are referred to as mono-rhamnolipid, while those with two sugar molecules are di –rhamnolipids. The length of the carbon chains found on the β-hydroxyacyl portion of the rhamnolipid can vary significantly. However in the case of *Pseudomonas aeruginosa* 10-C molecule chain is predominantly formed (2). *Pseudomonas aeruginosa* is a facultative anaerobe and non-spore forming bacterium. *Pseudomonas aeruginosa* involves secretion of Pyoverdin (florescence- a flourish yellow-green siderophores under iron limited conditions, such other siderophores are Pyocyanin (blue-green), Pyorubin (Red-brown) pigments. *Pseudomonas aeruginosa* produces exopolysacharides (Slime Layers). Secretion of exopolysacharide makes it difficult for *Pseudomonas aeruginosa* to be

phagocytosed by human white blood cells. Rhamnolipid from *Pseudomonas aeruginosa* decrease the surface tension of the water to 26 mN/m and the interfacial tension of water / hexadecane to < 1mN/m (3). Some biosurfactants are stable even after autoclave (121 ˚C /20 min) and after 6 months at -18˚C, the surface activity did not change from pH 5 to 11 (4). Microbial products like surfactants are easily degradable and particularly suited for environmental applications such as bioremediation and dispersion of oil spills (5).

Rhamnolipids have recently emerged as promising bioactive molecules due to their novel structures, diverse and versatile biological functions, lower toxicity, higher biodegradability, as well as production from renewable resources. The advantages of rhamnolipids make them attractive targets for research in a wide variety of applications, including cosmetics, agriculture, biomedicine etc (6-8).

Rhamnolipids are well-characterized biomolecules and gaining significance with passing every day. As uptill now, 2019, it has witnessed the highest number of patents and publications when compared with any other biosurfactants. However, cost-competitiveness and difficulty in its production is the only remaining challenging which is stopping Rhamnolipids becoming the king of biosurfactants. Research needs to be focused on suitable vigorous production strains, cheap substrates and nominal bioreactor technology. Currently priced as $200-225/10mg (AGAE technologies, USA & Sigma-Aldrich, Germany, respectively) it has the potential of reaching the market value of $2.8 billion in 2023 (Grand View Research Inc., 2014) substituting the global synthetic surfactant molecules (9). This mini-review has been focused to study the molecular genetics of rhamnolipids biosurfactant production which will help the biotechnologists to understand the synthesis process of rhamnolipids.

Synthesis of Rhamnolipid

Synthesis of rhamnolipid occurs with the help of enzymes that sequentially add sugars to the lipid. When the lipids are required to be broken down, enzymes in the lysosome of the cell help to remove the sugar subunits. This is important medically, because a deficiency of any of the enzymes involved in these processes cause an accumulation of a particular glycolipid that cannot be further broken down. When this accumulation occurs, the excess lipid remains trapped in the plasma or the cells and deposits in various organ/tissue systems and unfortunately, damages them.

Precursors of rhamnolipid synthesis

Polyhydroxyalkanoates (PHAs) are bacterial storage compounds, which are synthesized by the polymerization of b-hydroxyacids by the PHA synthases (PhaC), with the coenzyme A (CoA)-linked fatty acids as substrates (10).

The NADPH-dependent b-ketoacyl-CoA reductase (PhaB) is responsible for the reduction step in the production of the b-hydroxyacids. These storage compounds are intracellularly deposited as granules in many species. *P. aeruginosa* mainly produces PHAs consisting of medium-chain-length polymers, mainly poly-b-hydroxydecanoate (11).

Metabolic Synthesis:

Two primary metabolic precursors namely hydrocarbon and carbohydrate are involved in the synthesis of their hydrophobic and hydrophilic moieties, respectively. The pathways for the synthesis of these two groups of precursors are diverse and utilize specific sets of enzymes. In many cases the enzymes for the synthesis of these two precursors are regulatory enzymes, therefore in spite of the diversity, there are some common features of their synthesis and regulation. The detailed biosynthetic pathway for the major hydrophobic and hydrophilic moieties has been extensively investigated and are well documented (12).

According to Syldatk and Wagner (1985), there are four possibilities exist for the synthesis of different moieties of biosurfactants and their linkage (13).

i. The hydrophobic and hydrophilic moieties are synthesized de novo by two independent pathways.

ii. The hydrophilic moiety is synthesized de novo while the synthesis of the hydrophobic moiety is induced by the substrate.

iii. The hydrophobic moiety is synthesized de novo while the hydrophilic moiety is substrate dependent.

iv. The synthesis of both hydrophobic and hydrophilic moieties are substrate dependent.

Rhamnolipid synthesis occurs through two sequential trans- ference reactions of deoxy-thymidine-diphosphate-l-rhamnose (dTDP-l-rhamnose), catalyzed by the enzymatic complexes rhamnosyltransferase I (RhlA/RhlB) (14) and rhamnosyltransferase II (RhlC) (15), to-hydroxydecanoil--hydroxydecanoate or rhamnosyl--hydroxydecanoil--hydroxydecanoate, respectively (16). The synthesis of the hydrophobic portion occurs through a not well known deviation pathway in the de novo synthesis of fatty acids (17). The synthesis of the rhamnosil group is a ubiquitous pathway carried out by the rmlBDAC operon (18).

Genetics of Rhamnolipid Synthesis:

Ochsner *et al* in 1994, have extensively studied the genetics of rhamnolipid biosynthesis in *Pseudomonas aeruginosa* the rhlABR gene cluster found to be responsible for the synthesis of RhlR regulatory protein and a rhamnosyltransferase, both are essential for rhamnolipid synthesis. The synthesis of mono-rhamnolipid is catalyzed by RhlAB (rhamnosyltransferase) and proceeds by a glycol transfer reaction in which dTDP–L-rhamnose is transferred to the fatty acids acceptor molecules (β-hydroxydecanoyl-β-hysroxy decanoate). The gene encoding rhamnosyltransferase 1 are organized as an operon. The rhlA consist of 888 nucleotides (295 amino acids and encode a protein with a molecular mass of 32.5 kDa. Although rhlA seems to play an important role in the synthesis of rhamnolipids it exact function is unknown (19).

Ochsner suggests that rhlA protein is necessary for the stabilization of the rhlB in the cytoplasm membrane. rhlB is hypothesized to be the cateretic subunit of the rhamnosyltransferase 1 (Ochsner *et al*, 1994). Several evidences indicateed that rhlA is probably involve in the synthesis of 3-hydroxyacy-ACP precursors that are common intermediates in the biosynthesis of polyhydroxyalkanoic acid (20).

The active rhamnosyltransferase complex is located in the cytoplasmic membrane, with the 32.5kDa. rhlA protein harboring a putative signals while the 47kDa rhlB protein is located in the periplasmic region and contains at least two putative spanning domains (19).

The rhlR gene encodes a transcriptional activator, the 28 kDa rhlR protein belonging to the luxR family, which positively regulate rhamnolipid biosynthesis. Recently identified another regulatory gene rhlL located downstream of the rhlABR operon. After it was also found that the regulation of rhamnolipid production in *Pseudomonas aeruginosa* is mediated by rhlL-rhlR systems involving an autoinducer (19).

The binding of activated rhlR protein upstream of the rhlA promoter, enhances the transcription of the rhlAB operon encoding rhamnosyltransferase 1. The expression of the rhlAB genes in heterologous hosts has also been studied. In *Pseudomonas fluorescences* and *Pseudomonas putida*, these genes were expressed and produced rhamnolipid. However in *E.coli*, active rhamnosyltransferase was synthesized but rhamnolipids were not produced. According to the biosynthetic pathway proposed by Burger *et al*, (1963) rhamnolipid synthesis proceeds by two

sequential glycosyl transfer reactions, each catalyzed by a different rhamnosyltransferase. The first rhamnosyltransferase which catalyses the transfer of TDP-L-rhamnose to 3-(3-hydroxyalkanoyloxy) alkanoic acid (HAA) is encoded by the rhlAB operon (19).

Both genes co-expressed from the same promoter, are essential for rhamnolipid synthesis but whereas rhlB is known to encode the catalytic subunit of the rhamnosyltransferase, rhlA is probably an inner –membrane-bound protein (15).

Ochsner reported in 1994 that rhlA involved in the synthesis or transport of rhamnosyltransferase precursor substrates or in the stabilization of rhlB protein (21). Furthermore Cell-to-cell signaling regulates the expression of rhlAB operon. This quorum sensing system is composed of rhlI, the N-butyrylhomoserine lactone auto inducer synthase gene, and rhlR which encode the transcriptional activator. RhlR positively regulates the expression of genes involved in rhamnolipid production

The second rhamnosyltransferase encoded by rhlC, has been and its expression shown to be coordinately regulated with rhlAB by the same quorum sensing system (15).

HAA are extra cellular compounds produced by the *Pseudomonas aeruginosa* are the intermediates in the rhamnolipid synthesis and rhlA is required for the production of HAA (14).

Steps in genetics based synthesis:

On the genetic level, biosynthesis of RLs in *P. aeruginosa* occurs through three sequential steps (22):

1- RhlA (encoded by rhlA gene) is involved in the synthesis of the fatty acid dimer (HAAs) moiety of RLs from 3-hydroxyfatty acid precursors (23).

2- The membrane-bound RhlB rhamnosyltransferase (encoded by rhlB gene) uses dTDP-l-rhamnose and a HAA molecule as precursors, yielding mono-RL (19).

3- These mono-RLs are in turn the substrates, together with dTDP-l-rhamnose, of the RhlC rhamnosyltransferase (encoded by rhlC gene) to produce di-RLs (15). In *P. aeruginosa*, a bicistronic rhlAB operon encodes the first two enzymes while rhlC is elsewhere on the genome. While *P. aeruginosa* produces a mixture of mono- and di-RLs.

It is hypothesized that since *P. aeruginosa* harbors rhlA and rhlB in one operon and rhlC in another, this may result in different levels and timing of expression of the first and second rhamnosyltransferases (RhlB and RhlC, respectively), thus decoupling both activities.

For the synthesis of monorhamnolipid, the enzyme rhamnosyltransferase 1 (Rt 1) catalyzes the rhamnose transfer to b-hydroxydecanoylb-hydroxydecanoate, while Rt 2 synthesizes dirhamnolipid from TDP–L-rhamnose and monorhamnolipid. Genes coding for biosynthesis, regulation, and induction of Rt 1 enzyme are organized in tandem in the rhlABRI gene cluster around min 38 of the *P. aeruginosa* chromosome (24).

rhlG gene is involved in rhamnolipid biosynthesis. Complementation studies and measurement of the rhlG mRNA suggest that the RhlG protein is required for rhamnolipid biosynthesis and can be used in PHA production (25).

RhlR protein is necessary for the efficient expression of the rhlA gene. RhlR protein is necessary for the transcriptional activation of the rhlAB genes encoding the rhamnosyltransferase (19). Biosynthetic pathway of rhamnolipid has been well investigated to the genetic level (26).

Regulation of Rhamnolipid Synthesis:

Rhamnolipids are biosurfactants produced by Pseudomonas aeruginosa (26) that are regulated by the quorum-sensing response dependent on the transcription activator RhlR (19) and the autoinducer (Al) N-butyryl-homnoserine lavtone (C4-HSL) synthesized by RhlI (27).

The rhlAB operon encodes rhamnosyltransferase 1, which is responsible for monorhamnolipid production from TDP-L-rhamnose and β-hydroxy-fatty acids (19) and rhlC encodes rhamnosyltransferase 2, which produces dirhamnolipid by using monorhamnolipid and TDP-L-rhamnose as substrates (28).

The model for the transcription activation of rhlAB and rhlC is that with increasing bacterial cell density the concentration of C4-HSL reaches a threshold level and then attaches to the transcription activator RhlR (19)

The RhlR-C4-HSL complex activates the transcription of rhlAB operon (29) and rhlC (15).

P. aeruginosa contains a second quorum-sensing regulon, consisting of the transcription regulator LasR, which is activated by the Al N-3-oxodoecanoyl homoserine lactone (3-o-C12-HSL). The transcription of several genes encoding virulence-associated traits is activated by the Las system (30) and it has a central role in the transcription of rhlR (19).

Conclusion:

Rhamnolipid is a valuable biomolecule in surfactant industry gained the top most position based on the significance in multi-billion dollars' market of the world but its cost-effectiveness and industrial scale production remained the biggest challenge for being the most valuable biomolecules in industry. Its pathogenic origin also remained a biggest challenge, but with the discovery of several non-pathogenic strains producing Rhamnolipids, have given a big push to enhance its production capacity and scaling up to fulfil the biosurfactants market demand (31). Downstream processing is being applied for the enhanced production and cost-effectiveness of production of other biomolecules. Focusing more research on the genetics basis of its bio-synthesis and implementing these strategies in the bioreactor for its molecular production could fulfilled the promising industrial applications (32).

Conflict of Interest:

This manuscript is not having any conflict of interest with anybody or any organization.

References:

1. Chong H, Li Q. Microbial production of rhamnolipids: opportunities, challenges and strategies. Microbial cell factories. 2017;16(1):137.

2. Edwards JR, Hayashi JA. Structure of a rhamnolipid from Pseudomonas aeruginosa. Archives of biochemistry and biophysics. 1965;111(2):415-21.

3. Nguyen TT, Sabatini DA. Formulating alcohol-free microemulsions using rhamnolipid biosurfactant and rhamnolipid mixtures. Journal of surfactants and detergents. 2009;12(2):109-15.

4. Bharali P, Singh SP, Bashir Y, Dutta N, Konwar BK, Singh CB. Characterization and assessment of biosurfactant producing indigenous hydrocarbonoclastic bacteria: potential application in bioremediation. Nova Biotechnologica et Chimica. 2018;17(2):103-14.

5. Fenibo EO, Ijoma GN, Selvarajan R, Chikere CB. Microbial Surfactants: The Next Generation Multifunctional Biomolecules for Applications in the Petroleum Industry and Its Associated Environmental Remediation. Microorganisms. 2019;7(11):581.

6. Chen J, Wu Q, Hua Y, Chen J, Zhang H, Wang H. Potential applications of biosurfactant rhamnolipids in agriculture and biomedicine. Applied microbiology and biotechnology. 2017;101(23-24):8309-19.

7. Kumar R, Das AJ. Industrial Applications of Rhamnolipid: An Innovative Green Technology for Industry. Rhamnolipid Biosurfactant: Springer; 2018. p. 65-77.

8. Ahmadi-Ashtiani H-R, Baldisserotto A, Cesa E, Manfredini S, Zadeh HS, Gorab MG, Khanahmadi M, Zakizadeh S, Buso P, Vertuani S. Microbial Biosurfactants as Key Multifunctional Ingredients for Sustainable Cosmetics. Cosmetics. 2020;7(2):46.

9. Sekhon Randhawa KK, Rahman PK. Rhamnolipid biosurfactants—past, present, and future scenario of global market. Frontiers in microbiology. 2014;5:454.

10. TIMM A, STEINBÜCHEL A. Cloning and molecular analysis of the poly (3-hydroxyalkanoic acid) gene locus of Pseudomonas aeruginosa PAO1. European journal of biochemistry. 1992;209(1):15-30.

11. Timm A, Steinbüchel A. Formation of polyesters consisting of medium-chain-length 3-hydroxyalkanoic acids from gluconate by Pseudomonas aeruginosa and other fluorescent pseudomonads. Applied and environmental microbiology. 1990;56(11):3360-7.

12. Hommel RK. Formation and function of biosurfactants for degradation of water-insoluble substrates. Biochemistry of microbial degradation: Springer; 1994. p. 63-87.

13. Syldatk C, Lang S, Wagner F, Wray V, Witte L. Chemical and physical characterization of four interfacial-active rhamnolipids from Pseudomonas spec. DSM 2874 grown on n-alkanes. Zeitschrift für Naturforschung C. 1985;40(1-2):51-60.

14. Burger MM, Glaser L, Burton RM. The enzymatic synthesis of a rhamnose-containing glycolipid by extracts of Pseudomonas aeruginosa. Journal of Biological Chemistry. 1963;238(8):2595-602.

15. Rahim R, Ochsner UA, Olvera C, Graninger M, Messner P, Lam JS, Soberón-Chávez G. Cloning and functional characterization of the Pseudomonas aeruginosa rhlC gene that encodes rhamnosyltransferase 2, an enzyme responsible for di-rhamnolipid biosynthesis. Molecular microbiology. 2001;40(3):708-18.

16. Campos-García J, Caro AD, Nájera R, Miller-Maier RM, Al-Tahhan RA, Soberón-Chávez G. The Pseudomonas aeruginosa rhlG gene encodes an NADPH-dependent β-ketoacyl reductase which is specifically involved in rhamnolipid synthesis. Journal of bacteriology. 1998;180(17):4442-51.

17. Lyczak JB, Cannon CL, Pier GB. Establishment of Pseudomonas aeruginosa infection: lessons from a versatile opportunist. Microbes and infection. 2000;2(9):1051-60.

18. Köplin R, Arnold W, Hötte B, Simon R, Wang G, Pühler A. Genetics of xanthan production in Xanthomonas campestris: the xanA and xanB genes are involved in UDP-glucose and GDP-mannose biosynthesis. Journal of bacteriology. 1992;174(1):191-9.

19. Ochsner UA, Fiechter A, Reiser J. Isolation, characterization, and expression in Escherichia coli of the Pseudomonas aeruginosa rhlAB genes encoding a rhamnosyltransferase involved in rhamnolipid biosurfactant synthesis. Journal of Biological Chemistry. 1994;269(31):19787-95.

20. Rehm B, Kruger N, Steinbuchel A. A new metabolic link between fatty acid de novo synthesis

and other proteins required for PHA synthesis. J Biol Chem. 1998;273:24044-51.

21. Soberon-Chavez G, Aguirre-Ramírez M, Sanchez R. The Pseudomonas aeruginosa RhlA enzyme is involved in rhamnolipid and polyhydroxyalkanoate production. Journal of Industrial Microbiology and Biotechnology. 2005;32(11-12):675.

22. Gunther NW, Nunez A, Fett W, Solaiman DK. Production of rhamnolipids by Pseudomonas chlororaphis, a nonpathogenic bacterium. Applied and environmental microbiology. 2005;71(5):2288-93.

23. Cabrera-Valladares N, Richardson A-P, Olvera C, Treviño LG, Déziel E, Lépine F, Soberón-Chávez G. Monorhamnolipids and 3-(3-hydroxyalkanoyloxy) alkanoic acids (HAAs) production using Escherichia coli as a heterologous host. Applied microbiology and biotechnology. 2006;73(1):187-94.

24. Ochsner UA, Reiser J. Autoinducer-mediated regulation of rhamnolipid biosurfactant synthesis in Pseudomonas aeruginosa. Proceedings of the National Academy of Sciences. 1995;92(14):6424-8.

25. Soberón-Chávez G. Biosynthesis of rhamnolipids. Pseudomonas: Springer; 2004. p. 173-89.

26. Sandrin TR, Chech AM, Maier RM. A rhamnolipid biosurfactant reduces cadmium toxicity during naphthalene biodegradation. Applied and Environmental Microbiology. 2000;66(10):4585-8.

27. Ochsner UA, Reiser J, Fiechter A, Witholt B. Production of Pseudomonas aeruginosa Rhamnolipid Biosurfactants in Heterologous Hosts. Applied and environmental microbiology. 1995;61(9):3503-6.

28. Pessi G, Williams F, Hindle Z, Heurlier K, Holden MT, Cámara M, Haas D, Williams P. The global posttranscriptional regulator RsmA modulates production of virulence determinants andN-acylhomoserine lactones in Pseudomonas aeruginosa. Journal of bacteriology. 2001;183(22):6676-83.

29. Pearson JP, Pesci EC, Iglewski BH. Roles of Pseudomonas aeruginosa las and rhl quorum-sensing systems in control of elastase and rhamnolipid biosynthesis genes. Journal of bacteriology. 1997;179(18):5756-67.

30. Withers H, Swift S, Williams P. Quorum sensing as an integral component of gene regulatory networks in Gram-negative bacteria. Current opinion in microbiology. 2001;4(2):186-93.

31. Sekhon Randhawa KK, Rahman PKSM. Rhamnolipid biosurfactants—past, present, and future scenario of global market. Frontiers in Microbiology. 2014 2014-September-02;5(454). DOI: 10.3389/fmicb.2014.00454.

32. Invally K, Sancheti A, Ju L-K. A new approach for downstream purification of rhamnolipid biosurfactants. Food and Bioproducts Processing. 2019;114:122-31.

