# Rhamnolipids and Trichosporon cutaneum Biofilm

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Rhamnolipids are a class of glycolipids, and their molecule is formed by one or two (l)-rhamnose molecules, with a glycosidic linkage to the hydrophobic group made up of one or two β-hydroxy fatty acids. These amphiphilic compounds belong to biosurfactants, produced mainly by microorganisms. The best-studied producer of rhamnolipids is the Gram-negative bacteria Pseudomonas aeruginosa .

rhamnolipids biofilm Trichosporon cutaneum

## 1. Introduction

The function of rhamnolipids is also connected with the formation of transport channels in biofilm and cell release. Their antimicrobial nature helps the producer to compete for the colonization of the environment. The mechanism of action is believed to be based on the solubilization of cell membranes [1][2]. Therefore, rhamnolipids are intensively studied as potential antimicrobial and antibiofilm substances to be used for the treatment of surfaces against drug-resistant pathogens, the development of new pharmaceuticals [3] or the modulation of hydrophobic substrates intake [4].

Biofilm is defined as a highly structured community of microorganisms established in a three-dimensional structure that is irreversibly attached to a surface, an interface or each other. Biofilm cells are enclosed in a matrix of extracellular polymeric substances and exhibit altered phenotypes in comparison with planktonic cells [5]. Biofilms in nature have unique architectural features, including interstitial voids between macro- and microcolonies. The voids allow the diffusion of nutrients, gasses, signal molecules and other substances. The main advantages of biofilm formation include protection from the environment and resistance to physical and chemical stress and thus better resistance and adaptability to various conditions [6]. Therefore, biofilm formation by microbial strains, which are resistant to treatment by antibiotic or antimicrobial substances, represents serious complications in many fields, particularly in the medicine and food industries [7].

There are several experimental platforms suitable for the study of biofilms. Each method has advantages and limitations that must be considered. Experiments performed in static systems (typically microtiter plates or Petri dishes), which employ static cultivation followed by a rinsing step and evaluation of the attached biomass, is the most commonly used method [8]. However, these static systems have several problems, which must be taken into account when the method is used, e.g., definition of magnitude of the rinsing forces applied, definition of loosely adhering cells, and percentage estimation of the total adhering cells that were removed by rinsing [9]. In addition,

microtiter experiments are in a batch formation, which results in nutrient exhaustion, unless the media are not replaced. Therefore, this organization is rather suitable for screening experiments [8].

To overcome the above disadvantages, several continuous systems were designed, e.g., rotating disk reactor or modified Robbins device [8]. For the study of cell adhesion under specific hydrodynamic conditions, a flow chamber system was developed. In these devices, the biofilm is cultivated in a closed reactor (flow cell chamber), usually with a window for real-time microscopic analysis. Other advantages of this system are rapid mass transfer, a reduction in mixing time and processing time, the minimization of expensive substrates and a minimal sample volume [10]. Continuous laminar flow also ensures that all planktonic-growing and detached cells are taken away with the medium, and therefore, they do not interfere with the microscopic analysis.

## 2. Rhamnolipids as a Tool for Eradication of *Trichosporon cutaneum* Biofilm

Rhamnolipids have many advantageous properties, which are used in solving problems of environmental pollution; in the food, cosmetics and agricultural industries; and finally, in pharmacy or medicine. In these industries, emulsifying, solubilizing or wetting properties are mainly used, as well as metal sequestration [11] and possibly antimicrobial activity in medicine [12]. These properties are underlined by the environmental friendliness of rhamnolipids. Rhamnolipids are biodegradable substances and, therefore, when applied, we do not encounter toxicity and accumulation in the environment, and thus have huge potential for use in bioremediation [13].

The antimicrobial and antibiofilm activity of rhamnolipids has been reported in many publications [14][15][16][17]. The rhamnolipid antibacterial activity against a wide variety of microorganisms has been reported in many studies [12][17][18]. The mechanism of action of rhamnolipids is complex and mainly involves interactions with cell surface structures such as lipopolysaccharides, phospholipids and proteins [19][20][21]. Rhamnolipids interact with lipopolysaccharides [20], the phospholipid membrane and protein structures [19]. The action of rhamnolipids results in a change in the character of the cell surface, for example, a change in hydrophobicity [20] and / or the surface charge of the cell [22].

Additionally, the effectiveness of action is dependent on the composition of the rhamnolipid mixture, as well as on the type of exposed microorganism [20]. Studied rhamnolipid mixtures produced by four different strains of *P. aeruginosa* showed significantly different representations of congeners, comprising RhaFA, RhaFAFA, RhaRhaFA and RhaRhaFAFA. The presence of unsaturated FA was also determined for the properties of rhamnolipid mixtures. In studied mixtures, the abundance of unsaturated FA varied from 5.4% to 21.6%. Rooney et al. [23] reported similar differences between rhamnolipid mixtures produced by several *P. aeruginosa* strains. The content of unsaturated FA in these mixtures varied between 0% to 12.7%.

The composition of rhamnolipids is crucial for the value of the critical micelle concentration (CMC). Among other factors, CMC increases with the amount of unsaturated FA [24]. On the other hand, the higher content of congeners containing only one molecule of rhamnose (RhaFA and RhaFAFA) results in a decrease in CMC [25]. This correlates

with the results, in which the lowest content of unsaturated FA (5.4%) was found in rh3776, as well the lowest value of CMC (15 mg $L^{-1}$). On the contrary, the highest content of unsaturated FA was found in rh3777 (21.6%), but the value of CMC (55.4 mg $L^{-1}$) was lower than in the rh3774 mixture (unsaturated FA 18.8%, CMC 75.5 mg $L^{-1}$). This was probably influenced by the higher abundance of mono-rhamnolipid congeners in rh3777 in comparison with rh3774.

From the screening experiments under static conditions, it is obvious that the composition of rhamnolipids influenced the response of *T. cutaneum* biofilm to the treatment. Interactions between cell surface structures and rhamnolipids are probably a crucial step for the mechanism of action. In addition, the production or presence of rhamnolipids has an important role in the development of biofilm, including the maintenance of open channels and void spaces, as well as the facilitation of cell detachment from the biofilm structure [26]. It was found that a concentration higher than CMC must be used to produce a significant decrease in the colonized area. When the biofilm was exposed to the highest concentration of surfactants (1000 mg $L^{-1}$), removal of more than 90% was achieved. However, the effectiveness of concentrations between CMC and 1000 mg $L^{-1}$ varied. Obviously, the suitability of rhamnolipid mixtures and the used concentration must be studied before concrete application. For example, rh3777 was proven to have a significant impact already at CMC concentration (55.4 mg $L^{-1}$), and the biofilm was reduced by almost 33%. Moreover, the treatment by 250 mg $L^{-1}$ resulted in a decrease of almost 80%. Conversely, rh3776 showed the same effect after biofilm exposition to 100 mg $L^{-1}$, and from this point of view, rh3776 seemed to be the most effective mixture. However, at the CMC, it had a very low impact (9–18%), which may be attributed to the low CMC value (15 mg $L^{-1}$), showing that the absolute concentration value was a more important factor than the CMC. Singh et al. [27] also showed the dependency of rhamnolipid action on concentration when biofilm of *Candida albicans* was treated by rhamnolipids produced by *P. aeruginosa* in a concentration range of 40–5000 mg $L^{-1}$.

Kim et al. [28] reported that the CMC (240 µg $mL^{-1}$) of used rhamnolipids demonstrated efficacy on *P. aeruginosa* biofilm. The anti-adhesive activity of rhamnolipid produced by *P. aeruginosa* against several bacterial and yeast strains isolated from voice prostheses was evaluated in [29]. The experiments were performed under dynamic conditions in a parallel plate flow chamber. The best results for the reduction in the adhesion rate occurred for *Streptococcus salivarius* GB 24/9 and *Candida tropicalis* GB 9/9 (an average of 66%). The potential of rhamnolipids to prevent biofilm formation was reported by Gomes and Nitschke [30]. The treatment by rhamnolipids (1.0% solution) reduced adhesion to the polystyrene of *Listeria monocytogenes* (by 57.8%) and *Staphylococcus aureus* by (67.8%). Dusane et al. [15] showed that rhamnolipid disrupted the pre-formed biofilm of *Yarrowia lipolytica* in a more effective manner than chemical surfactants (cetyl-trimethyl ammonium bromide and SDS).

The conduction of pilot experiments under static conditions was chosen due to its simplicity, cost efficiency and multiplicity. On the other hand, these conditions also have several limitations, including problems with the separation of attached and loosely attached cells, the definition of the washing process and the quantification of washed-off cells [9]. Concurrently, it must be taken into account that the process of biofilm development is very stochastic; therefore, the independent repetition of biofilm cultivation may vary, even if the cultivation conditions are kept constant [31]. In addition, culture conditions are changed over the duration of the experiment (substrate

utilization and cell metabolism). These factors could have a significant effect on biofilm stability and further eradication. Therefore, the antibiofilm activity of rhamnolipids was also investigated under dynamic conditions conducted in a single-channel flow cell. The behavior of rhamnolipids and synthetic surfactants did not correspond with those obtained from static conditions in all cases. Rh3774, rh3775 and rh3777 had the same ability to reduce biofilm colonization, up to 95% (16 h). The same effect on biofilm eradication was found after SDS treatment, almost of 86% (16 h). However, rh3776 and Tween 80 showed a different effect; the colonized area was reduced by only 41% and 59%, respectively. These differences in surfactant effectiveness support the necessity to perform experiments under both static and dynamic conditions and highlight the importance of rhamnolipid mixture composition determination and characterization in relation to their intended application. Performed experiments showed that dynamic conditions had no impact on the biological activity of rhamnolipids with higher CMC as well SDS, in contrast to rh3776 and Tween 80, with very low CMC (15 and 13 mg $L^{-1}$, respectively), which were very effective in biofilm eradication.

Rhamnolipids with low CMC have hydrophobic characteristics, as their molecules are formed predominantly by mono-rhamnolipid congeners with a low abundance of unsaturated FA [20]. The amphiphilicity of rhamnolipid molecules is crucial for the interactions with the structures of the cell surface or colonized area. Adsorbed rhamnolipids thus change the surface charge, resulting in varied microbial adhesion ability [15]. In addition, interactions with proteins or lipids forming the surface of cells can lead to the alteration of cell permeability [32], resulting in a direct impact on cell viability. The treatment of *T. cutaneum* by rhamnolipids caused a decrease in the hydrophobicity of cells. Chrzanowski et al. [33] reported a decrease in the hydrophobicity of yeast *Candida maltosa* after treatment by rhamnolipids (150 mg $L^{-1}$). The same effect of rhamnolipids on food pathogenic bacteria was found by Gomes and Nitschke [30]. Similarly, the conditioning of a glass microscope slide led to a significant decrease in surface hydrophobicity depending on rhamnolipid CMC, and thus rhamnolipid composition.

Differences between results obtained under static and dynamic conditions suggest that biofilm eradication under static conditions is mostly a function of rhamnolipid properties (composition), whereas under the dynamic condition, the eradication is influenced by rhamnolipid properties and medium flow rate. The flow rate of the medium can detach weakly bound cells, which are able to adhere under static condition.

## 3. Conclusions

Many studies have reported the possibility of using rhamnolipids as antibiofilm agents. However, rhamnolipid composition and properties must be studied in detail with respect to their potential application, with a focus on the target microorganism, surface properties and environmental conditions. The results indicate that rhamnolipid may be used for *Trichosporon* biofilm disruption. After treatment with rhamnolipids at a concentration of 1000 mg $L^{-1}$, the removal of more than 90% of the colonized area was reached. It was also found that rhamnolipids significantly change the hydrophobicity of the microbial cell surface and the glass carrier. From the results, it is obvious that the effect of rhamnolipids in static conditions could differ significantly in comparison with dynamic conditions. This finding supports the necessity to take into account the character of the application area, and thus an appropriate experiment design is crucial in biosurfactant studies. Confirmation of this conclusion would allow the wider use of

rhamnolipids, not only as substances promoting hydrophobic substance bioavailability, but also as compounds directly influencing biofilm formation.

## References


1. Soberón-Chávez, G.; Lépine, F.; Déziel, E. Production of rhamnolipids by Pseudomonas aeruginosa. Appl. Microbiol. Biotechnol. 2005, 68, 718–725.
2. Chrzanowski, Ł.; Ławniczak, Ł.; Czaczyk, K. Why do microorganisms produce rhamnolipids? World J. Microbiol. Biotechnol. 2012, 28, 401–419.
3. Banat, I.M.; Franzetti, A.; Gandolfi, I.; Bestetti, G.; Martinotti, M.G.; Fracchia, L.; Smyth, T.J.; Marchant, R. Microbial biosurfactants production, applications and future potential. Appl. Microbiol. Biotechnol. 2010, 87, 427–444.
4. Matatkova, O.; Gharwalova, L.; Zimola, M.; Rezanka, T.; Masak, J.; Kolouchova, I. Using odd-alkanes as a carbon source to increase the content of nutritionally important fatty acids in Candida krusei, Trichosporon cutaneum, and Yarrowia lipolytica. Int. J. Anal. Chem. 2017, 2017, 9.
5. Shunmugaperumal, T. Biofilm eradication and prevention: A pharmaceutical approach to medical device infections; John Wiley & Sons Inc.: Hoboken, NJ, USA, 2010.
6. Singh, S.; Singh, S.K.; Chowdhury, I.; Singh, R. Understanding the mechanism of bacterial biofilms resistance to antimicrobial agents. Open Microbiol. J. 2017, 11, 53.
7. Ramage, G.; Rajendran, R.; Sherry, L.; Williams, C. Fungal biofilm resistance. Int. J. Microbiol. 2012, 2012, 14.
8. Azeredo, J.; Azevedo, N.F.; Briandet, R.; Cerca, N.; Coenye, T.; Costa, A.R.; Desvaux, M.; Di Bonaventura, G.; Hébraud, M.; Jaglic, Z. Critical review on biofilm methods. Crit. Rev. Microbiol. 2017, 43, 313–351.
9. Busscher, H.J.; van der Mei, H.C. Microbial adhesion in flow displacement systems. Clin. Microbiol. Rev. 2006, 19, 127–141.
10. Wagner, K.; Friedrich, S.; Stang, C.; Bley, T.; Schilling, N.; Bieda, M.; Lasagni, A.; Boschke, E. Initial phases of microbial biofilm formation on opaque, innovative anti-adhesive surfaces using a modular microfluidic system. Eng. Life Sci. 2014, 14, 76–84.
11. Nitschke, M.; Costa, S.G.; Contiero, J. Rhamnolipids and PHAs: Recent reports on Pseudomonas-derived molecules of increasing industrial interest. Process. Biochem. 2011, 46, 621–630.

12. Banat, I.; Franzetti, A.; Gandolfi, I.; Bestetti, G.; Martinotti, M.; Fracchia, L.; Smyth, T.; Marchant, R. Microorganism in environmental management: Microbes and environment. Appl. Microbiol. Biotechnol. 2010, 87, 427–444.
13. Sim, L.; Ward, O.; Li, Z. Production and characterisation of a biosurfactant isolated from Pseudomonas aeruginosa UW-1. J. Ind. Microbiol. Biotechnol. 1997, 19, 232–238.
14. De Araujo, L.V.; Guimarães, C.R.; da Silva Marquita, R.L.; Santiago, V.M.; de Souza, M.P.; Nitschke, M.; Freire, D.M.G. Rhamnolipid and surfactin: Anti-adhesion/antibiofilm and antimicrobial effects. Food Control. 2016, 63, 171–178.
15. Dusane, D.H.; Dam, S.; Nancharaiah, Y.V.; Kumar, A.R.; Venugopalan, V.P.; Zinjarde, S.S. Disruption of Yarrowia lipolytica biofilms by rhamnolipid biosurfactant. Aquat. Biosyst. 2012, 8, 1–7.
16. E Silva, S.; Carvalho, J.; Aires, C.; Nitschke, M. Disruption of Staphylococcus aureus biofilms using rhamnolipid biosurfactants. J. Dairy Sci. 2017, 100, 7864–7873.
17. Maťátková, O.; Kolouchová, I.; Kvasničková, E.; Ježdík, R.; Masák, J.; Čejková, A. Synergistic action of amphotericin B and rhamnolipid in combination on Candida parapsilosis and Trichosporon cutaneum. Chem. Pap. 2017, 71, 1471–1480.
18. Abdel-Mawgoud, A.M.; Lépine, F.; Déziel, E. Rhamnolipids: Diversity of structures, microbial origins and roles. Appl. Microbiol. Biotechnol. 2010, 86, 1323–1336.
19. Davey, M.E.; Caiazza, N.C.; O'Toole, G.A. Rhamnolipid surfactant production affects biofilm architecture in Pseudomonas aeruginosa PAO1. J. Bacteriol. 2003, 185, 1027–1036.
20. Nitschke, M.; Costa, S.G.; Contiero, J. Rhamnolipid surfactants: An update on the general aspects of these remarkable biomolecules. Biotechnol. Prog. 2005, 21, 1593–1600.
21. Rodrigues, L.; Van Der Mei, H.; Banat, I.M.; Teixeira, J.; Oliveira, R. Inhibition of microbial adhesion to silicone rubber treated with biosurfactant from Streptococcus thermophilus A. FEMS Immunol. Med. Microbiol. 2006, 46, 107–112.
22. Kaczorek, E. Effect of External Addition of Rhamnolipids Biosurfactant on the Modification of Gram Positive and Gram Negative Bacteria Cell Surfaces during Biodegradation of Hydrocarbon Fuel Contamination. Pol. J. Environ. Stud. 2012, 21, 901–909.
23. Rooney, A.P.; Price, N.P.; Ray, K.J.; Kuo, T.-M. Isolation and characterization of rhamnolipid-producing bacterial strains from a biodiesel facility. FEMS Microbiol. Lett. 2009, 295, 82–87.
24. Guo, Y.-P.; Hu, Y.-Y.; Gu, R.R.; Lin, H. Characterization and micellization of rhamnolipidic fractions and crude extracts produced by Pseudomonas aeruginosa mutant MIG-N146. J. Colloid Interface Sci. 2009, 331, 356–363.

25. Abdel-Mawgoud, A.M.; Aboulwafa, M.M.; Hassouna, N.A.-H. Characterization of rhamnolipid produced by Pseudomonas aeruginosa isolate Bs20. Appl. Biochem. Biotechnol. 2009, 157, 329–345.
26. Raya, A.; Sodagari, M.; Pinzon, N.M.; He, X.; Newby, B.-m.Z.; Ju, L.-K. Effects of rhamnolipids and shear on initial attachment of Pseudomonas aeruginosa PAO1 in glass flow chambers. Environ. Sci. Pollut. Res. 2010, 17, 1529–1538.
27. Singh, N.; Pemmaraju, S.C.; Pruthi, P.A.; Cameotra, S.S.; Pruthi, V. Candida biofilm disrupting ability of di-rhamnolipid (RL-2) produced from Pseudomonas aeruginosa DSVP20. Appl. Biochem. Biotechnol. 2013, 169, 2374–2391.
28. Kim, L.H.; Jung, Y.; Yu, H.-W.; Chae, K.-J.; Kim, I.S. Physicochemical interactions between rhamnolipids and Pseudomonas aeruginosa biofilm layers. Environ. Sci. Technol. 2015, 49, 3718–3726.
29. Rodrigues, L.; Banat, I.M.; Van der Mei, H.; Teixeira, J.; Oliveira, R. Interference in adhesion of bacteria and yeasts isolated from explanted voice prostheses to silicone rubber by rhamnolipid biosurfactants. J. Appl. Microbiol. 2006, 100, 470–480.
30. Do Valle Gomes, M.Z.; Nitschke, M. Evaluation of rhamnolipid and surfactin to reduce the adhesion and remove biofilms of individual and mixed cultures of food pathogenic bacteria. Food Control. 2012, 25, 441–447.
31. Heydorn, A.; Nielsen, A.T.; Hentzer, M.; Sternberg, C.; Givskov, M.; Ersbøll, B.K.; Molin, S. Quantification of biofilm structures by the novel computer program COMSTAT. Microbiology 2000, 146, 2395–2407.
32. De Freitas Ferreira, J.; Vieira, E.A.; Nitschke, M. The antibacterial activity of rhamnolipid biosurfactant is pH dependent. Food Res. Int. 2019, 116, 737–744.
33. Chrzanowski, Ł.; Kaczorek, E.; Olszanowski, A. Relation between Candida maltosa hydrophobicity and hydrocarbon biodegradation. World J. Microbiol. Biotechnol. 2005, 21, 1273–1277.