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ISOLATION AND ANALYSIS OF *RHLC* and *RHLR* FROM RHAMNOLIPID PRODUCING BACTERIUM, *PSEUDOMONAS AERUGINOSA* strain S5

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DEPARTMENT OF MICROBIOLOGY
FACULTY BIOTECHNOLOGY AND BIOMOLECULAR SCIENCES
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PENGESAHAN

Dengan ini adalah disahkan bahawa projek yang bertajuk *Isolation and analysis of rhlC and rhlR from rhamnolipid producing bacterium, Pseudomonas aeruginosa strain S5* telah disiapkan serta dikemukakan kepada Jabatan Mikrobiologi oleh Noor Hayati binti Mohd Sidik (161604) sebagai syarat untuk kursus BMY 4999 projek.

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ABSTRACT

Rhamnolipids are biomolecules with a huge potential in leading global surfactant market, which find applications in various kind of industry in demand for emulsifying and solubilizing agent including food industry, pharmaceutical industry, and petroleum industry. Rhamnolipids are being majorly produced by microorganism, particularly, *Pseudomonas aeruginosa.* This study aims to test rhamnolipid production in *Pseudomonas aeruginosa* strain S5 and to analyze the genes involve for the rhamnolipids biosynthesis which are *rhlC* and *rhlR* that code for rhamnosyltransferase 2 and transcription regulator respectively. Qualitative test on cetyltrimethyl ammonium bromide agar and quantitative tests by emulsification index valued 37.44 %, display capabilities of *Pseudomonas aeruginosa* strain S5 in producing rhamnolipid. In molecular detection of rhamnolipid production, BLAST result shows high similarity between both *rhlC* and *rhlR* sequences, and sequence available in database. The information from the gene analysis could facilitate the understanding on the rhamnolipid biosynthesis in *Pseudomonas aeruginosa* strain S5, hence can be further used in genetic modulation in order to meet future prospects.

ABSTRAK

Rhamnolipid merupakan biomolekul yang berpotensi tinggi dalam menerajui pasaran surfaktan dunia, seiring dengan kemampuannya untuk diaplikasikan dalam pelbagai industri yang mempunyai permintaan tinggi terhadap bahan emulsifikasi dan bahan pelarut, termasuk industri makanan, industri farmasi, dan juga industri petroleum. Rhamnolipid secara utamanya dihasilkan oleh mikroorganisma, khususnya *Pseudomonas aeruginosa*. Kajian ini bertujuan untuk menguji penghasilan rhamnolipid oleh *Pseudomonas aeruginosa* strain S5 dan untuk menganalisis gen-gen yang terlibat dalam penghasilan rhamnolipid iaitu gen *rhlC* dan *rhlR*, masing-masing mengekod *rhamnosyltransferase 2* dan pengawalatur transkripsi. Ujian kualitatif terhadap agar cetiltrimetilammonium bromida dan ujian kuantitatif oleh indek emulsifikasi yang bernilai 37.44 % menunjukkan keupayaan *Pseudomonas aeruginosa* strain S5 menghasilkan rhamnolipid. Dalam pengesanan molekular, keputusan BLAST merekodkan persamaan yang tinggi di antara kedua- dua gen *rhlC* dan *rhlR* dengan jujukan yang terdapat dalam pengkalan data. Informasi analisis gen yang diperolehi dapat membantu kefahaman tentang penghasilan rhamnolipid oleh *Pseudomonas aeruginosa* strain S5, sekaligus dapat diguna pakai dalam modulasi genetik bagi menepati prospek masa hadapan.

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CHAPTER 1

INTRODUCTION

Rhamnolipid is a biosurfactant that serves an excellence surface and interfacial activities, hence shaping its way to be commercially exploited by many industries in demand for emulsifying, detergency, and surface activity agents. Besides, biosurfactants are well- known to dissolve hydrocarbons in water and vice versa. Throughout the time, intensive studies have been conducted in order to replace the available synthethic surfactant with this biomolecules due to its sustainability sources, less toxicity, and high environmental compatibility when compared to the synthetic surfactant.

It is long known that most *Pseudomonas aeruginosa* is a rhamnolipid producer and vast research have been administered in elucidating the genetic mechanism of the rhamnolipid biosynthesis in order to enchance its production in bigger scale. As for this study, *Pseudomonas aeruginosa* strain S5 was to be identified whether or not this strain is producing rhamnolipid. *Pseudomonas aeruginosa* strain S5 as discussed by Rahman et al. (2006), was originally isolated in the capabilities to degrade benzene, toluene, ethyl-benzene, and xylene (BTEX) and to produce an organic solvent- tolerant lipase. Therefore, it becomes an interesting subject to figure out whether this organism also produces a surface active agent, particularly rhamnolipids.

The objectives of this study are;

1. To determine the rhamnolipids production of *Pseudomonas aeruginosa* strain S5
2. To isolate the *rhlC* and *rhlR* from *Pseudomonas aeruginosa* strain S5
3. To analyze the sequence of *rhlC* and *rhlR* that are responsible for rhamnolipids production in *Pseudomonas aeruginosa* strain S5

REFERENCES

Abdel-Mawgoud, A. M., Hausmann, R., Lepine, F., Muller, M. M., & Déziel, E. (2011). Rhamnolipids: detection, analysis, biosynthesis, genetic regulation, and bioengineering of production. In *Biosurfactants* (pp 13-55). Berlin Heidelberg: Springer.

Abouseoud, M., Maachi, R., & Amrane, A. (2007). Biosurfactant production from olive oil by *Pseudomonas fluorescens*. *Trends in Applied Microbiology*, 340-347.

Aguirre-Ramírez, M., Medina, G., González-Valdez, A., Grosso-Becerra, V., & Soberón-Chávez, G. (2012). The *Pseudomonas aeruginosa* rmlBDAC operon, encoding dTDP-l-rhamnose biosynthetic enzymes, is regulated by the quorum-sensing transcriptional regulator RhlR and the alternative sigma factor σ. *Microbiology*, *158* (4), 908-916.

Al-Araji, L. I. Y., Rahman, R. N. Z. R. A., Basri, M., & Salleh, A. B. (2007). Optimisation of rhamnolipids produced by *Pseudomonas aeruginosa* 181 using Response Surface Modeling. *Annals of Microbiology*, *57*(4), 571-575.

Baharum, S. N., Salleh, A. B., Razak, C. N. A., Basri, M., Rahman, M. B. A., & Rahman, R. N. Z. R. A. (2003). Organic solvent tolerant lipase by *Pseudomonas* sp. strain S5: stability of enzyme in organic solvent and physical factors effecting its production. *Annals of Microbiology*, *53*(1), 75-84.

Bhardwaj, G., Cameotra, S. S., & Chopra, H. K. (2013). Biosurfactants from Fungi: A Review. *Journal of Petroleum & Environmental. Biotechnology*, *4*, 160-166.

Burgos-Diaz, C., Pique, I. C. N., Manresa, P. M. A. M. A., & Marques, V. A. M. (2012). Advances in the research of new biosurfactants and their potential use in the biomedical and pharmaceutical industry. *Recent Advances in Pharmaceutical Sciences II, 2012, Editor: Diego Muñoz- Torrero, Diego Haro and Joan Vallès, Chapter 9*, 151-167.

Cappuccino, J. G., & Sherman, N. (2011). In *Microbiology – A Laboratory Manual* (69- 72). New York, NY: Benjamin Cummings Publishing Company.

Croda-García, G., Grosso-Becerra, V., Gonzalez-Valdez, A., Servín-González, L., & Soberón-Chávez, G. (2011). Transcriptional regulation of *Pseudomonas aeruginosa* rhlR: role of the CRP orthologue Vfr (virulence factor regulator) and quorum-sensing regulators LasR and RhlR. *Microbiology*, *157*(9), 2545-2555.

Desai, J. D., & Banat, I. M. (1997). Microbial production of surfactants and their commercial potential. *Microbiology and Molecular Biology Reviews*, *61*(1), 47-64.

Fakruddin, M. D. (2012). Biosurfactant: production and application. *Journal of Petroleum & Environmental Biotechnology*,*3*(124), 47-64.

Gharaei-Fathabad, E. (2011). Biosurfactants in pharmaceutical industry: A mini-review. *American Journal of Drug Discovery and Development*, *1*(1), 58-69.

Gunther, N. W., Nunez, A., Fett, W., & Solaiman, D. K. (2005). Production of rhamnolipids by *Pseudomonas chlororaphis*, a nonpathogenic bacterium. *Applied and Environmental Microbiology*, *71*(5), 2288-2293.

Kaloorazi, N. A., & Choobari, M. F. S. (2013). Biosurfactants: Properties and Applications. *Journal of Biology*, *2*(5), 235-241.

Kadhim, D., & Ali, M. I. R. (2014). Prevalence study of quorum sensing groups among clinical isolates of *Pseudomonas aeruginosa*. *International. Journal of Current Microbiology & Applied Sci*ence, *3*(11), 204-215.

Khoshdast, H., Sam, A., Vali, H., & Noghabi, K. A. (2011). Effect of rhamnolipid biosurfactants on performance of coal and mineral flotation. *International Biodeterioration & Biodegradation*, *65*(8), 1238-1243.

Leitermann, F., Walter, V., Syldatk, C., & Hausmann, R. (2010). Rhamnolipids. In *Handbook of Hydrocarbon and Lipid Microbiology* (3037-3051). Berlin Heidelberg: Springer.

Medina, G., Juarez, K., Diaz, R., & Soberon-Chavez, G. (2003). Transcriptional regulation of *Pseudomonas aeruginosa* rhlR, encoding a quorum-sensing regulatory protein. *Microbiology*, *149*(11), 3073-3081.

Muller, M. M., Hormann, B., Syldatk, C., & Hausmann, R. (2010). *Pseudomonas aeruginosa* PAO1 as a model for rhamnolipid production in bioreactor systems. *Applied Microbiology and Biotechnology*, *87*(1), 167-174.

Muller, M. M., Kugler, J. H., Henkel, M., Gerlitzki, M., Hormann, B., Pohnlein, M., Syldatk, C., & Hausmann, R. (2012). Rhamnolipids—next generation surfactants?. *Journal of Biotechnology*, *162*(4), 366-380.

Nitschke, M., & Costa, S. G. V. A. O. (2007). Biosurfactants in food industry.*Trends in Food Science & Technology*, *18*(5), 252-259.

Ochsner, U. A., Reiser, J., Fiechter, A., & Witholt, B. (1995). Production of *Pseudomonas aeruginosa* rhamnolipid biosurfactants in heterologous hosts. *Applied and Environmental Microbiology*, *61*(9), 3503-3506.

Pacwa-Plociniczak, M., Płaza, G. A., Piotrowska-Seget, Z., & Cameotra, S. S. (2011). Environmental applications of biosurfactants: recent advances. *International Journal of Molecular Sciences*, *12*(1), 633-654.

Pearson, J. P., Pesci, E. C., & Iglewski, B. H. (1997). Roles of *Pseudomonas aeruginosa* las and rhl quorum-sensing systems in control of elastase and rhamnolipid biosynthesis genes. *Journal of Bacteriology*, *179*(18), 5756-5767

Pornsunthorntawee, O., Wongpanit, P., & Rujiravanit, R. (2010). Rhamnolipid biosurfactants: Production and their potential in environmental biotechnology. In *Biosurfactants* (211-221). New York, NY: Springer.

Randhawa, K. K. S., & Rahman, P. K. (2014). Rhamnolipid biosurfactants- past, present, and future scenario of global market. *Frontiers in Microbiology*, *5*, 454.

Rahim, R., Ochsner, U. A., Olvera, C., Graninger, M., Messner, P., Lam, J. S., & Soberon-Chavez, G. (2001). Cloning and functional characterization of the *Pseudomonas aeruginosa* rhlC gene that encodes rhamnosyltransferase 2, an enzyme responsible for di-rhamnolipid biosynthesis. *Molecular Microbiology*, *40*(3), 708-718.

Rahman, R. N. Z. R. A., Baharum, S. N., Salleh, A. B., & Basri, M. (2006). S5 lipase: An organic solvent tolerant enzyme. *The Journal of Microbiology*, 583-590.

Reis, R. S., Pacheco, G. J., Pereira, A. G., & Freire, D. M. G. (2013). Biosurfactants: production and applications. *Biodegradation—Life of Science*, 31-61.

Rebello, S., Asok, A. K., Joseph, S. V., Joseph, B. V., Jose, L., Mundayoor, S., & Jisha, M. S. (2013). Bioconversion of sodium dodecyl sulphate to rhamnolipid by *Pseudomonas aeruginosa*: a novel and cost-effective production strategy. *Applied Biochemistry and Biotechnology*, *169*(2), 418-430.

Sachdev, D. P., & Cameotra, S. S. (2013). Biosurfactants in agriculture. *Applied Microbiology and Biotechnology*, *97*(3), 1005-1016.

Salager, J. L. (2002). Surfactant's types and uses. *FIRP Booklet-E300- A Teaching Aid in Surfactant Science and Engineering in English.*

Siegmund I. and Wagner F. (1991). New method for detecting rhamnolipids excreted by *Pseudomonas* species during growth on mineral agar. *Biotechnology Techniques*, *5*(4), 265-268.

Shoeb, E., Akhlaq, F., Badar, U., Akhter, J., & Imtiaz, S. (2013). Classification and industrial applications of biosurfactants. *Academic Research International*, *4*(3), 243-252.

Soberon-Chavez, G., Lépine, F., & Deziel, E. (2005). Production of rhamnolipids by *Pseudomonas aeruginosa*. *Applied Microbiology and Biotechnology*, *68*(6), 718-725.

Souza, E. C., Vessoni-Penna, T. C., & de Souza Oliveira, R. P. (2014). Biosurfactant-enhanced hydrocarbon bioremediation: An overview. *International Biodeterioration & Biodegradation*, *89*, 88-94.

Schmidberger, A., Henkel, M., Hausmann, R., & Schwartz, T. (2013). Expression of genes involved in rhamnolipid synthesis in *Pseudomonas aeruginosa* PAO1 in bioreactor cultivation. *Applied Microbiology and Biotechnology*, *97*(13), 5779-5791.

Schramm, L. L., Stasiuk, E. N., & Marangoni, D. G. (2003). 2 Surfactants and their applications. *Annual Reports Section" C"(Physical Chemistry)*, *99*, 3-48.

Sumiardi, A., Mangunwardoyo, W., Hudiyono, S., & Susilaningsih, D. (2012). Biosurfactant characterization of bacterial consortium from soil contaminated hydrocarbon in Cepu Area, Central Java, Indonesia. *International Journal of Scientific Reasearch Publications*, *2*(7), 1-7.

Thavasi, R. (2011). Microbial biosurfactants: from an environmental application point of view. *Journal of Bioremediation & Biodegradation*, *2*(5).

Toribio, J., Escalante, A. E., & Soberon-Chavez, G. (2010). Rhamnolipids: Production in bacteria other than *Pseudomonas aeruginosa*. *European Journal of Lipid Science and Technology*, *112*(10), 1082-1087.

Wittgens, A., Tiso, T., Arndt, T. T., Wenk, P., Hemmerich, J., Muller, C., Wichmann, R., Kupper, B., Zwick, M., Wilhelm, S., Hausmann, R., Syldatk, C., Rosenau, F., & Blank, L. M. (2011). Growth independent rhamnolipid production from glucose using the non-pathogenic *Pseudomonas putida* KT2440. *Microbial Cell Factories*, *10*(1), 80.

Zhang, G., Wu, Y., Qian, X., & Meng, Q. (2005). Biodegradation of crude oil by *Pseudomonas aeruginosa* in the presence of rhamnolipids. *Journal of Zhejiang University Science B*, *6*(8), 725-730.

Zhu, K., & Rock, C. O. (2008). RhlA converts β-hydroxyacyl-acyl carrier protein intermediates in fatty acid synthesis to the β-hydroxydecanoyl-β-hydroxydecanoate component of rhamnolipids in *Pseudomonas aeruginosa*. *Journal of Bacteriology*, *190*(9), 3147-3154.