



## Article **Production of Lipopeptide Biosurfactant by a Hydrocarbon-Degrading Antarctic** *Rhodococcus*

Syahir Habib <sup>1</sup>, Siti Aqlima Ahmad <sup>1</sup>, Wan Lutfi Wan Johari <sup>2</sup>, Mohd Yunus Abd Shukor <sup>1</sup>, Siti Aisyah Alias <sup>3</sup>, Jerzy Smykla <sup>4</sup>, Nurul Hani Saruni <sup>1</sup>, Nur Syafiqah Abdul Razak <sup>1</sup> and Nur Adeela Yasid <sup>1,\*</sup>

- <sup>1</sup> Department of Biochemistry, Faculty of Biotechnology and Biomolecular Sciences, Universiti Putra Malaysia, Serdang, Selangor 43400, Malaysia; syahirhabib@gmail.com (S.H.); aqlima@upm.edu.my (S.A.A.); mohdyunus@upm.edu.my (M.Y.A.S.); hanisaa17@gmail.com (N.H.S.); syaffy27@gmail.com (N.S.A.R.)
- <sup>2</sup> Department of Environment, Faculty of Forestry and Environment, Universiti Putra Malaysia, Serdang, Selangor 43400, Malaysia; wanlutfi@upm.edu.my
- <sup>3</sup> Institute of Ocean and Earth Sciences, C308 Institute of Postgraduate Studies, University of Malaya, Kuala Lumpur 50603, Malaysia; saa@um.edu.my
- <sup>4</sup> Institute of Nature Conservation, Polish Academy of Sciences, Mickiewicza 33, 31-120 Kraków, Poland; jerzysmykla@yahoo.com
- \* Correspondence: adeela@upm.edu.my; Tel.: +603-9769-8297

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**Figure S1.** The drop-collapse test of bacterial supernatant of ADL36. (**A**) The droplet shape of distilled water (control) remains stable while a near-complete collapse was observed for the supernatant (**B**).



**Figure S2.** The illustration of oil displacement activity for (**A**) distilled water; (**B**) bacterial supernatant of ADL36; (**C**) 1.0% sodium-dodecyl-sulphate (SDS); and (**D**) 1.0% Triton X-100.



**Figure S3.** The qualitative assessment of biosurfactant production by 96-well microplate assay for *Rhodococcus* sp. ADL36. The right well (**red circle**) filled with bacterial supernatant showed distortion and appeared smaller than the right well (distilled water) indicating the presence of surface-active agents. The control (distilled water) showed a flat image.



**Figure S4.** The emulsification of oil with cell-free supernatant using (**A**) used motor oil and (**B**) diesel oil. Distilled water (left) was used as a control in both experiments showed a distinct separation of two layers. Formation of emulsion layer (in both test tubes on the right) can be observed with the addition of supernatant from ADL36.



**Figure S5.** The formation of coloured spots on the thin-layer chromatography (TLC) plate through the exposure to ninhydrin (**A**) and iodine vapour (**B**).



**Figure S6.** The FTIR spectra for the biosurfactant produced by *Rhodococcus* sp. ADL36. The red line indicates the biosurfactant extracted using methyl-tert-butyl ether (MTBE) while the black line indicates the biosurfactant extracted by chloroform-methanol solvent system.



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