

Physical Characterization of *Rhodococcus zopfii* DSM 44108 Bacteria Isolated from Municipal Sludge

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Abstract. This paper presents physical characteristic of *Rhodococcus zopfii* through the use of Environment Scanning Electron Microscopy. This bacterium is very significant in enhancing degradation of polycyclic aromatic hydrocarbons (PAHs) in the presence of natural biofilm in waste water treatment system. *Rhodococcus zopfii* was isolated from municipal sludge at Universiti Teknologi MARA. The average size of *Rhodococcus zopfii* was measured in the range of 1.1–2.85 µm lengths and 0.55-0.80 µm diameters. The colour of this bacterium was slightly pink on agar plate and it had rod shaped. Experimental results obtained from confocal laser scanning microscopy showed that this bacterium can easily attach on the surface of biofilm. The experimental results provide a scientific knowledge of physical characteristic of *Rhodococcus zopfii* isolated from municipal sludge to grow on natural biofilm in wastewater treatment system.

Introduction

The increase in industrial effluents containing tons of pollutants is one of the major factor that creates environmental pollution. Industrial activities associated with petrochemical industries, petroleum refinery, pulp and paper mills contribute to severe discharge polycyclic aromatic hydrocarbons (PAHs) to the environment [1-2]. Due to this reason, PAHs were abundantly present in river, harbors and municipal sewage system [2-3]. Many studies have reported that PAHs are both toxic and persistent in the environment. PAHs can lead to serious diseases such as cancer, gene mutation and cause disorder to the respiratory system[3-4].

Several studies have demonstrated that biodegradation is an efficient method to transform toxic PAHs to non-toxic chemical compounds. Transformation of PAHs to catechol, carbon dioxide and water by the genus *Rhodococcus* at contaminated sites has been were reported [5]. However, the degradation kinetics of PAHs by the genus *Rhodococcus* required months and degradation pathways of PAHs were not completed [6]. Due to these reasons, toxic intermediate products of PAHs were present in the aquatic environment. Studies have also shown that under suspended growth condition, the *Rhodococcus* genus was not efficient to degrade PAHs as the bacteria was easily washed out from the system [7]. Therefore, degradation of PAHs by this bacterium in the presence of natural biofilm requires investigation as detail study on the capability of *Rhodococcus* bacteria to grow on biofilm remains unclear.

In this study, *Rhodococcus zopfii* has isolated from municipal sewage sludge taken from a wastewater treatment plant in Universiti Teknologi MARA Shah Alam. The aims of this study were to evaluate the physical characterization of *Rhodococcus zopfii* by using Environment Scanning Electron Microscopy (ESEM) and Confocal Laser Scanning Microscopy thus verify capability of this bacterium to grow in a system containing natural biofilm.

Material and Methods

Bacteria Culture. A pure culture of *Rhodococcus zopfii* was isolated from municipal sludge taken from Mawar wastewater treatment plant Universiti Teknologi MARA Shah Alam, Malaysia. A colony of pure culture *Rhodococcus zopfii* was transferred to a sterile nutrient broth (OXOID, England). It was grown in the nutrient broth for 7 days in the incubator at 30°C without shaking. 1mL of culture bacteria was used for analysis on the Confocal Laser Scanning Microscopy and ESEM.

Natural Biofilm Production System. Natural biofilm was developed in an aeration tank using bio-blocks purchased from Biokube Malaysia Sdn. Bhd. Biofilm-blocks containing wastewater was placed in suspension with oxygen supplied at the bottom of the tank. The bio-blocks were left for three months to achieve a mature biofilm production. The biofilm was taken out and cut into 2cm x 2cm and placed in a scotch bottle containing wastewater from the aeration tank. The collected sample was autoclave (Hirayama) at 121°C for 20 minutes. An autoclaved biofilm sample was ventilated at room temperature prior to further analysis.

Environmental Scanning Electron Microscopy (ESEM) Analysis. The pure culture of bacteria was centrifuged in suspension at 5000rpm for 10 minutes. An approximately 2.5% of decant was kept at 4°C for 4 to 6 hours after being subjected to vortex at 5000rpm for 5 minutes. The decant was washed three times with 0.1M sodium phosphate buffer (pH 7.2). For the post fixation of cell wall, sample was fixed with osmium tetroxide and stored at 4°C for 2 hours. The decant was washed again following of the same procedure previously described. For the dehydration cell, a series of acetone were used to soak the decant with 35%, 50%, 75% 95% (10 minutes each concentration) and 100% (15 minutes) prior viewed for ESEM.

Confocal Laser Scanning Microscopy. An autoclave biofilm sample (as described in section natural biofilm production system) was placed on the confocal laser scanning microscopy plate and 1mL of bacteria culture (as described in section bacteria culture) was poured onto the plate. Confocal laser scanning microscopy model Leica TSC SPE with 40 objectives and 3 light intensities were used to capture the image of bacteria. Lasaf software was used to capture images at every 5 minutes for 24 hours.

Results and Discussion

Environmental Scanning Electron Microscopy (ESEM) Analysis

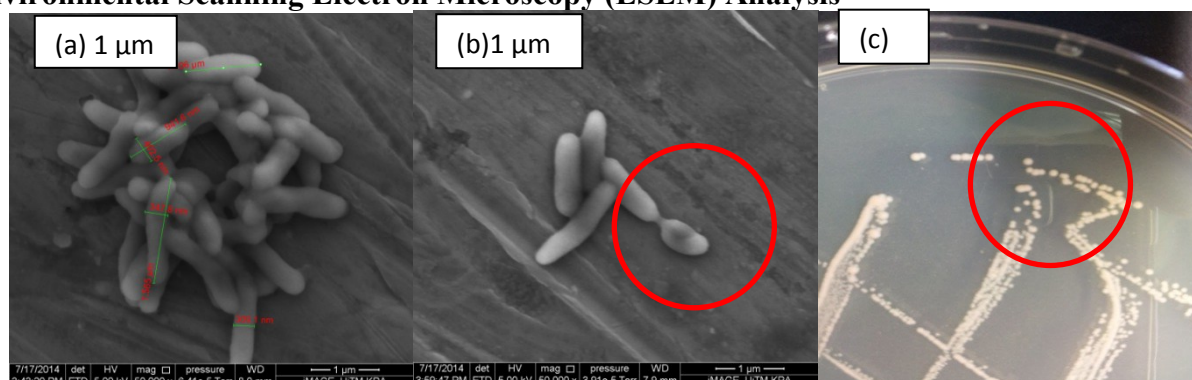


Figure 1 : ESEM Micrograph of *Rhodococcus zopfii* DSM 44108 Bacteria (a) Size of *Rhodococcus zopfii* (b) *Rhodococcus zopfii* started to multiply (c) *Rhodococcus zopfii* bacteria colony on agar plate

Figures 1 (a) and (b) show physical characteristic of *Rhodococcus zopfii* during cultivation period of 7 days in nutrient medium. Figure 1 (a) shows *Rhodococcus zopfii* has rod shaped image and its average size was in the range of 1.1 to 2.85 μm lengths and 0.55 to 0.80 μm diameters. This finding

is similar to reported physical characteristics of *Rhodococcus* genes isolated from soil [5,9 -10]. With this range size of bacteria, *Rhodococcus zopfii* may easily attach on the surface of natural biofilm and promote contact with PAHs in wastewater. Figure 1(b) demonstrates multiplying process of *Rhodococcus zopfii*. At this stage, new cells were formed and started to grow. This result indicates that within 7 days of incubation period is sufficient to initiate growth of *Rhodococcus zopfii* in this system. Figure 1(c) shows another physical characteristic study of *Rhodococcus zopfii*. It was streaked on nutrient agar plate after 7 days incubation in nutrient medium. The result shows *Rhodococcus zopfii* has light pink colour and its colony elevation was convex and raised. This finding is similar to findings from previous study on *Rhodococcus zopfii* isolated from soil [11-12].

Confocal Laser Scanning Microscopy

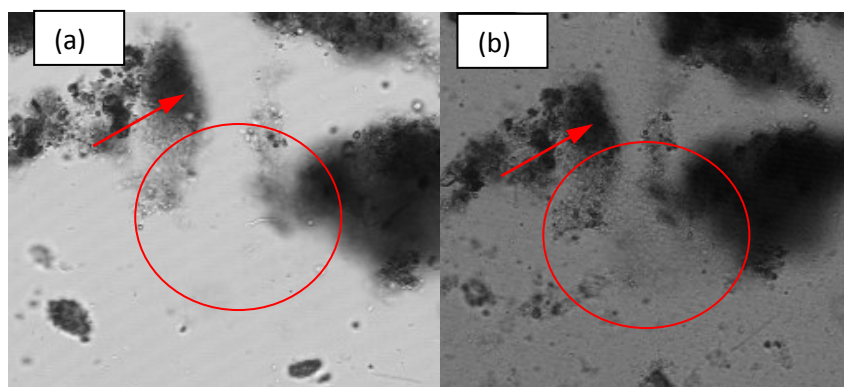


Figure 2 : Confocal Laser Scanning Micrograph image (a) 1 min (b) 12 hours

Figures 2(a) and 2(b) show images of *Rhodococcus zopfii* in wastewater with natural biofilm. Figure 2(a) shows that the image of *Rhodococcus zopfii* was not clearly observed by confocal laser scanning microscopy at 30 mins (Referring to bright area in the circle). This result suggests that *Rhodococcus zopfii* was remained suspended in the wastewater. However, image of *Rhodococcus zopfii* emerged after 12 hrs (Figure 2(b)), indicating that *Rhodococcus zopfii* was beginning to attach on the biofilm surface in the wastewater. This finding was similar with a previous study reported by Mohd-Towel (2014). Experimental results from this study demonstrates that *Rhodococcus zopfii* has ability to attach on the surface of natural biofilm. Literatures have reported that attachment of bacteria on the surface of biofilm was due to the existence of source of energy and carbon on its surface [14-15]. This results also suggest that *Rhodococcus zopfii* attached on the surface of natural biofilm will not easily washed out from the system.

Conclusions

This study demonstrates *Rhodococcus zopfii* has rod shape and has size range 1.1 to 2.85 μm lengths and 0.55 to 0.80 μm diameters. *Rhodococcus zopfii* has light pink colour. With the physical characteristic of *Rhodococcus zopfii* significantly can grow on biofilm which is advantages to remain in the system for a degradation process.

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References

- [1] L. Pizzul, M. Del Castillo, and J. Stenström, "Characterization of selected actinomycetes degrading polyaromatic hydrocarbons in liquid culture and spiked soil," *World J. Microbiol. Biotechnol.*, vol. 22, no. 7, pp. 745–752, Feb. 2006.
- [2] M. Palittapongarnpim, P. Pokethitiyook, E. S. Upatham, and L. Tangbanluekal, "Biodegradation of crude oil by soil microorganisms in the tropic.," *Biodegradation*, vol. 9, no. 2, pp. 83–90, Jan. 1998.
- [3] W. T. E. Ting, S. Y. Yuan, S. D. Wu, and B. V. Chang, "Biodegradation of phenanthrene and pyrene by *Ganoderma lucidum*," *Int. Biodeterior. Biodegradation*, vol. 65, no. 1, pp. 238–242, Jan. 2011.
- [4] F. Vincent-Hubert, K. Heas-Moisán, C. Munsch, and J. Tronczynski, "Mutagenicity and genotoxicity of suspended particulate matter in the Seine river estuary.," *Mutat. Res.*, vol. 741, no. 1–2, pp. 7–12, Jan. 2012.
- [5] L. Martínková, B. Uhnáková, M. Pátek, J. Nesvera, and V. Kren, "Biodegradation potential of the genus *Rhodococcus*," *Environ. Int.*, vol. 35, no. 1, pp. 162–77, Jan. 2009.
- [6] A. L. Juhasz and R. Naidu, "Bioremediation of high molecular weight polycyclic aromatic hydrocarbons: a review of the microbial degradation of benzo[a]pyrene," *Int. Biodeterior. Biodegradation*, vol. 45, no. 1, pp. 57–88, 2000.
- [7] F. Fatone, S. Di Fabio, D. Bolzonella, and F. Cecchi, "Fate of aromatic hydrocarbons in Italian municipal wastewater systems: an overview of wastewater treatment using conventional activated-sludge processes (CASP) and membrane bioreactors (MBRs).," *Water Res.*, vol. 45, no. 1, pp. 93–104, Jan. 2011.
- [8] D. Dean-ross, J. D. Moody, J. P. Freeman, D. R. Doerge, and C. E. Cerniglia, "Metabolism of anthracene by a *Rhodococcus* species," vol. 204, pp. 205–211, 2001.
- [9] S. Gaskin and R. Bentham, "Comparison of enrichment methods for the isolation of pyrene-degrading bacteria," *Int. Biodeterior. Biodegradation*, vol. 56, no. 2, pp. 80–85, Sep. 2005.
- [10] R. L. Stingley, A. a Khan, and C. E. Cerniglia, "Molecular characterization of a phenanthrene degradation pathway in *Mycobacterium vanbaalenii* PYR-1.," *Biochem. Biophys. Res. Commun.*, vol. 322, no. 1, pp. 133–46, Sep. 2004.
- [11] X.-Q. Tao, G.-N. Lu, Z. Dang, C. Yang, and X.-Y. Yi, "A phenanthrene-degrading strain *Sphingomonas* sp. GY2B isolated from contaminated soils," *Process Biochem.*, vol. 42, no. 3, pp. 401–408, Mar. 2007.
- [12] S. Hussain, M. Devers-Lamrani, N. El Azhari, and F. Martin-Laurent, "Isolation and characterization of an isoproturon mineralizing *Sphingomonas* sp. strain SH from a French agricultural soil," *Biodegradation*, vol. 22, no. 3, pp. 637–50, Jun. 2011.

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- [13] R. Mohd-Towel, A. Amir, and S. Abdul-Talib, "Characterization of Natural Biofilm in Wastewater for Enhanced Growth Rate of *Corynebacterium Uroalyticum* Bacteria," *4th Int. Malaysia-irel. Jt. Symp. Eng. Sci. Bus. 2014 IMiEJS2014 Penang Island, Malaysia 25th – 26th June 2014*, 2014.
- [14] C. Nicolella, M. C. van Loosdrecht, and J. J. Heijnen, "Wastewater treatment with particulate biofilm reactors.," *J. Biotechnol.*, vol. 80, no. 1, pp. 1–33, Jun. 2000.
- [15] E. M. Haase, T. Bonstein, R. J. Palmer, and F. A. Scannapieco, "Environmental influences on *Actinobacillus actinomycetemcomitans* biofilm formation," pp. 299–314, 2006.