

Screening of Isolated Rhizospheric Bacteria of *Pittosporum Resiniferum* Hemsl With Toluene

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Abstract

Toluene are hazardous man-made hydrocarbon. This hydrocarbon can be degraded using biological approach. In the past research, the bacteria were isolated and characterized in terms of morphology. Based on screening on screening result, TSArp- Cr3, TSArp- Cr4, TSArp- Cr5, TSArp- Cr6 able to grow on 3 different concentration of toluene. Strain TSArp- Cr6 shows the best reading as it has ascending number bacterial population as the concentration of toluene increased compared to the rest three strains.

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Introduction

Toluene (toluol : methylbenzene ; phenylmethane) is an aromatic hydrocarbon which is related to benzene. Toluene has major usage (92%) of gasoline component which contained 5 to 7% of toluene by weight (U.S.EPA, 1979). Toluene is one of the important component of numerous chemical products as well as plastics article paints, dyes, cosmetics, pharmaceuticals and various chemicals (ASTDR, 2000 ; ASTDR 1989). Toluene is one of the element of petroleum which the major source of benzene, toluene ethylbenzene and xylene pollution to the surrounding (ASTDR, 2000 ; ASTDR 1989).

Toluene has a sweet pungent and benzene smell- like odour. The lowest concentration of toluene on human inhalation ranging from 0.64 to 139 mg/m³ (Van der Heijden,, 1988). The threshold of the odour in water is 0.024 - 0.17 mg/l (Alexander *et al.*, 1982). Toluene spillage can cause huge problems to the world resident including suppressed colour vision, kidney damaged, sleeping disorder, depression, attention disorders and reproductive anomalies (ASTDR, 2000 ;

ASTDR 1989). Furthermore, toluene is a priority pollutant classified by US Environmental Protection Agency (USEPA) (ASTDR, 2000 ; ASTDR 1989).

Toluene exposure may take place from breathing ambient or indoor surrounding affected by several factors. The central nervous system (CNS) is the major target of toluene toxicity in both animals and human for acute (short term) or chronic (long term) exposures (U.S.EPA, 2011). Dysfunction of CNS and narcosis has been commonly detected in human acutely exposed to the elevation of airborne level of toluene including fatigue, sleepiness, headache and nausea (U.S.EPA, 2011). Table 2.4 shows the health hazards of toluene. Since toluene is hazardous to human being, numerous biodegradation studies of toluene had been done by researchers to reduce the risk towards world residents. The definition of degradation is disintegration of certain component by the action of enzyme or microorganisms. Biodegradation is the process where toxic compounds transformed into non- toxic components by biological approach. Biodegradation defined as decomposition of organic component in the environment by microorganism reaction such as bacteria, actinomycete and fungi (Wackett and Hershberger, 2001). Those microorganisms gained the energy from decomposition process that will increase the biomass production (Riser- Roberts, 1998).

The biodegradation studies usually conducted in aerobic condition as it is widely known regarding aerobic and facultative aerobic bacteria. Microorganisms known as *Pseudomonas* sp. and *Escherichia coli* were usually being tested with certain chemical because they are easily being grown as well as highly produced cell density within a night. Aerobic biodegradation is easier compared to anaerobic biodegradation because it involves oxidation metabolism of organic compounds into carbon dioxide and water. Aerobic process produced high energy in the form of ATP (Adenosine Triphosphate) and biomass that helps in biodegradation process. It is important for oil fuel that contained alkane, benzene, toluene, xylene and octane. Those components can be decomposed in soil that has good aeration system and also can be degraded quickly in the laboratory by using aerobic culture (Wackett and Hershberger, 2001). The aim of the study is to screen the isolated bacteria with various concentration of and toluene (1mM, 3mM and 5mM).

Methodology

Media preparation

Sterile Ramsay agar was prepared according to Ramsay *et al.*, (1983).

Screening growth test

Inoculum of 6 strains of bacteria was incubated for 24 hours at 37°C. Next, inoculum was inoculated into dilution series of 10^{-1} to 10^{-9} contained saline water (0.85%) then direct and streaking technique were done on Ramsay Agar with various concentration of toluene. After that, the streaked agar was incubated for 24 hours at 37°C.

Colony counting

The colony formed was counted on colony counter. Then, the growth were quantified by doing Colony Forming Unit/ Volume (CFU/ml) calculation.

Result and discussion

The rhizospheric bacteria were isolated and colony morphology and cellular morphology were identified (Hanirah *et al.*, 2014).

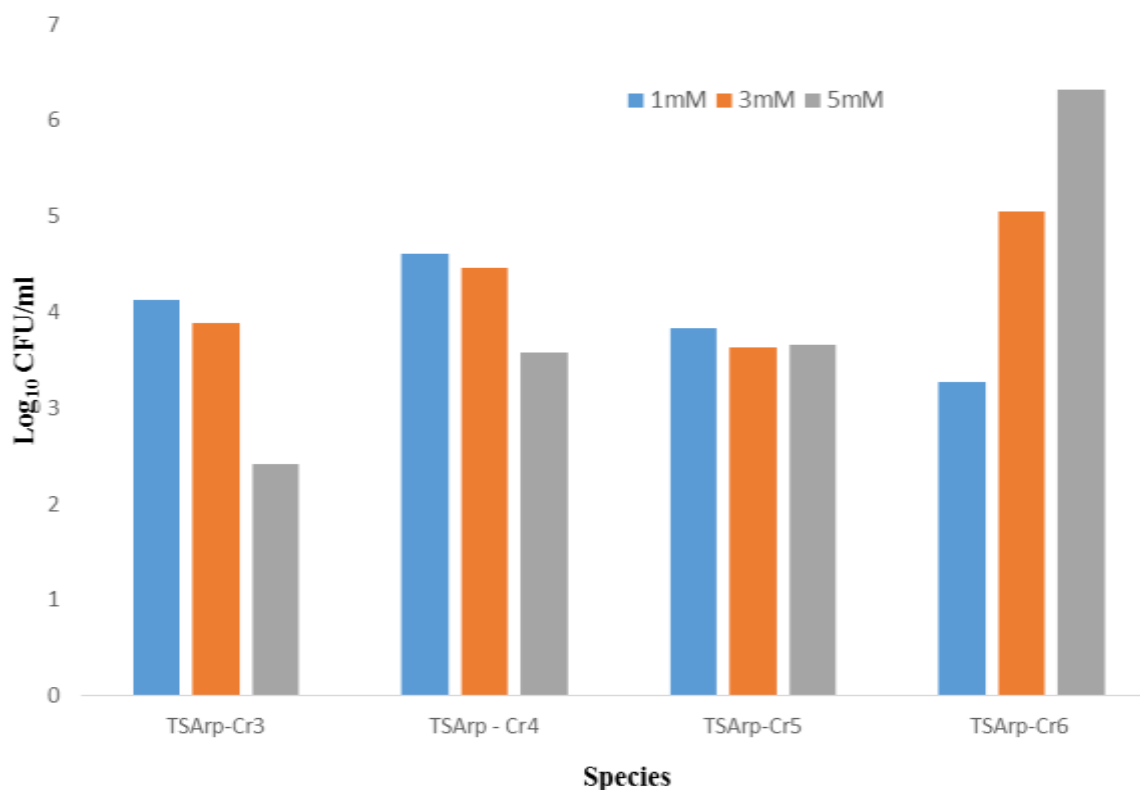


Figure 1. Colony forming unit of isolated bacteria in different concentration of toluene.

The bacterial capacity of isolated strains on various concentration of toluene illustrated in Figure 1. Species TSArp – Cr4 has the highest and the lowest reading of bacterial population. The difference were shown to be the 2 fold. Where the bacterial population on 1mM and 3mM were \log_{10} CFU/ml 5.5 and \log_{10} CFU/ml 2.4. Figure 1 shows the different of bacterial capacity of the strain TSArp – Cr5 was 7.9%. The highest reading of bacterial capacity was \log_{10} CFU/ml 3.8 and the lowest was \log_{10} CFU/ml 3.5. Displays the growth of TSArp-Cr6 increased as the concentration of toluene increased. The difference between the highest reading and the lowest reading of bacterial capacity was almost 2 fold. Compared to Aimon *et al.*, (2011), the isolated bacteria was tested on 0.5mM and 1mM of toluene and only 1 type of species has growth on 1mM of toluene.

Strain TSARp- Cr6 shows the best reading as it has ascending number bacterial population. Based on the screening result above, it illustrate that metabolically diverse and healthy community of microorganisms in the rhizosphere in different plants might be the 'hot spot' for hydrocarbon degrader (Wejjian & Zhang, 2007)

Conclusion

This research could be continued for the bidegradation study on toluene by using the selected species.

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References

- [1] Agency for Toxic Substances and Disease Registry (ASTDR). 1989. Toxicological profile for toluene. ATSDR/TP-89/23 ed. Atlanta, GA: US Department of Health and Human Services, Public Health Service.
- [2] Agency for Toxic Substances and Disease Registry (ASTDR). 2000. Toxicological profile for toluene. Atlanta, GA: U.S. Department of Health and Human Services, Public Health Service.
- [3] Ainon, H., Arezoo, T. & Amir, R. (2011). Detection of Toluene degradation in bacteria isolated from oil contamination. *Sains Malaysia*, **40** (11), 1232-1235.
- [4] Alexander, H. C., McCarty, W. M., Bartlett, E. A. & Syverud, A. N. (1982). Aqueous odor and taste threshold values for industrial chemicals. *J. Am. Water Works Assoc.*, **74**(1982), 595-599
- [5] Hanirah, R., Piakong, M. T. & Syaufi, L. 2015. Isolation, characterization and screening of rhizospheric bacteria of *Pittosferum resiniferum* Hemsl. *Materials Science and Engineering*, **78**(2015), 1-6
- [6] Ramsay, D. J., Thrasher, T. N. & Keil, L. (1983). The organumvasculosum laminae terminalis: A critical area for osmoreception.. *Program Brain Resolution*, **196**, 117- 122
- [7] Riser-Roberts, E. (1998). Remediation of Petroleum Contaminated Soils: Biological, Physical, and Chemical Processes. Florida: CRC Press LLC.
- [8] U.S. EPA. 1979. Source assessment: Manufacture of acetone and phenol from cumene. Research Triangle Park, NC: U.S. Environmental Protection Agency.
- [9] U.S. EPA (2011). Analytical method approved for drinking water compliance monitoring of organic contaminants. U.S. Environmental Protection Agency, Washington, DC. Available at: http://water.epa.gov/scitech/drinkingwater/labcert/upload/methods_organic.pdf. U.S.
- [10] Van der Heijden (1988). Integrated criteria document toluene — effects. Appendix. Bilthoven, National Institute of Public Health and Environmental Protection (Report No. 758473010).
- [11] Wackett, L. P. & Hershberger, C. D. (2001). Biocatalysis and Biodegradation: Microbial Transformation of Organic Compounds. Sterling: ASM Press.

- [12] Weijian, C., Li, J. & Zhang, Z. (2007). The characteristics and mechanisms of phenol biodegradation by *Fusarium* sp.. *Journal. Hazard. Materials.*, **148** (1-2), 38 -42.