

Isolation, characterization and screening of rhizospheric bacteria of *Pittosferum resiniferum* Hemsl.

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Abstract - The bacterial rhizosphere species of host plant, Petroleum Nut (*Pittosferum resiniferum*) were isolated and characterized morphologically. The isolates were designated as, TSArp- Cr2, TSArp- Cr3, TSArp- Cr4, TSArp- Cr5, TSArp- Cr6 and TSArp- Cr7. All of the species were tested on three different concentration of phenol (1mM, 3mM and 5mM). Only species TSArp- Cr4 and TSArp- Cr6 growth were detected. The highest growth is 6Log₁₀CFU/ml in 1mM by TSA-Cr4. The lowest reading was 3.6 Log₁₀CFU/ml in 3mM by TSA-Cr6. Species TSArp- Cr4 has higher tolerance on phenol compared to TSArp- Cr6

1.Introduction

The existence of anthropogenic (man-made) organic compounds in the environment is a serious issue to the world residents. The man-made organic compounds such as BTEX (benzene, toluene, ethyl-benzene, *o*-, *m*-, *p*- xylene), and phenol are important industrial raw materials for paints, pesticides, resins, fiber, glass unit, varnish, phenolic resin manufacture, textile unit, making of organic dyes and as solvents for rubber and plastic as well [1] [2]. These aromatic compounds has a high toxicity level and carcinogenic that commonly found as contamination relatable to human activities [3][4][5] [6][7].

The variation of microorganism undergo biodegradation ranging from *Pseudomonads* [8] [9][10][11], fungi [12][13][14] [15] yeast [16][17][18]. The source of these bacteria can be found at oil effluent water [19], contaminated oil mousse from beach simulator tank [20], Crude oil-polluted river [21], natural lake and soil [22], soil contaminated with Crude oil spills [23] and rhizosphere soil [24][25]. Rhizospheric soil is the zone that is surrounded by the root of plant and the area of biological and chemicals activity are influenced by the compounds discharged by the root [26]. The most common rhizospheric bacterial can be found is *Pseudomonads*, *Bacillus* sp. and *Streptomyces* sp. [24] [25]. These isolated rhizospheric bacteria can degrade toxic compounds, especially petroleum compounds up until 13g/l [24] [25].

Metabolism of these compounds by microorganism is well known with physiological, biochemical and molecular research of the degradation report [27][28]. Microorganisms usually can be as pathogenic and harmful to human. But, apart from that, they can be beneficial to the environment. There are numerous type of pollutant that can be degraded by microorganism, phenol [29] n-alkane [30][31], TCE [32][33].

Since *Pittosferum resinisferum* (petroleum nut) can produce biofule, the hypothesis of rhizosheric bacteria of *Pittosferum resinisferum* has the potential to degrade hydrocarbon can be made. But currently there are no reports from the isolated *Pittosferum resinisferum* rhizospheric bacteria.

The aim of the study is to isolate and characterize the bacteria from rhizosphere soil and screening the bacteria with various concentration of phenol (1mM, 3mM and 5mM).



2. Material and methods

2.1 Sampling

Rhizospheric soil from *Pittosferum resiniferum* was collected in Kundasang, Sabah.

2.2. Physical properties of rhizospheric soil

The value of pH soil *Pittosferum resiniferum* was measured by using pH meter tool (Hanna Instrument, USA) (5.4). 1g of rhizospheric soil sample was mixed together with 3ml of distill water and the reading of the pH value was taken. Thermometer was used to determine the soil temperature (18°C).

2.3. Culture media preparation for isolation and screening of bacteria

For isolation of heterotrophic microorganism, Trypticase Soy Agar or TSA medium was prepared. The composition of TSA media consisting (g/L): 15.0g of Tryptone (Pancreatic Digest of Casein), 15.0g of Soytone (Papaic Digest of Soybean Meal), 5.0g of Sodium Chloride, 15.0g of Agar.

2.4. Isolation and Characterization of bacteria

10g of soil sample rhizospheric soil was taken for serial dilution series (10^{-1} until 10^{-9}) by using saline water (0.85%). The bacteria were originally isolated by direct technique on TSA agar and incubated for 24 hours at 37°C. The developed colonies was purified by streaking on nutrient agar for bacterial identification according to colony and cellular characteristics [34].

2.4.1. Colony morphological characteristics

The identification colony morphology based on colour, margin, elevation and configuration. The colony morphology was viewed and determined by under light microscope, Olympus 9800

2.4.2. Cellular morphological characteristics

Cellular morphology was done by doing Gram staining [35] and being viewed under light microscope, Olympus 9800.

2.5. Screening isolated rhizospheric bacteria with phenol.

The isolated bacteria were tested into 3 different concentration of phenol (1mM, 3mM and 5mM). The isolated rhizospheric bacteria were tested on 3 different concentration of phenol that is 1mM, 3mM and 5mM. The growth of isolated rhizospheric bacteria was cultivated in Ramsay broth for 24 hours under 37°C. After the incubation period, the dilution series was done and the incubation condition is the same as the inoculum preparation.

3. Result and Discussion

3.1 Isolation and characterization of bacteria.

The rhizospheric bacteria were isolated and colony morphology and cellular morphology were identified. There are 5 isolated bacteria from the soil sample taken at the area of Kundasang Sabah namely : TSARp- Cr2, TSARp- Cr3, TSARp- Cr4, TSARp- Cr5, TSARp- Cr6 and TSARp- Cr7. The colony morphology of the isolated strains are Cream with the margins figure of smooth, wavy and branching. The elevations of the strains are round with scalloped margin, filamentous and round gram staining for each strain is positive. Table 1 shows the characteristics of the isolated bacteria. All of the isolated bacteria comprises of gram positive and negative bacteria.

Compared to other research, of the isolated rhizospheric bacteria was gram negative done by reference [36]. Other than that, there were gram negative and gram positive bacteria was isolated from rhizosphere soil [37]. So, it was agreeable that rhizospheric bacteria dominated by both of gram negative and gram positive bacteria. Those positive and negative bacteria belongs to Proteobacteria, Actinobacteria and Firmicutes [37]

3.2 Physical and growth test

There were 6 species of isolated rhizospheric bacteria. Only 2 species were able to grow in three different concentration of phenol (1mM, 3mM and 5mM). The growth of the rest of the species were not detected. There were no colonies observed. Fig. 1 shows the trend of microbial growth ($\text{Log}_{10}\text{CFU/ml}$) of two different species.

Table 1 The colony morphology of selected isolates grown on TSA agar at 37°C after 24 hours incubation.

Strains	Colony characteristics				Gram Reaction
	Colour	Configuration	Margin	Elevation	
TSARp-Cr2	Cream	Round with scalloped margin	Branching	Hilly	Positive
TSARp-Cr3	Cream	Round	Smooth	Convex	Negative
TSARp-Cr4	Cream	Round	Smooth	Convex	Negative
TSARp-Cr5	Cream	Filementous	Wavy	Hilly	Positive
TSARp-Cr6	Cream	Round	Smooth	Convex	Positive
TSARp-Cr7	Cream	Round	Smooth	Convex	Positive

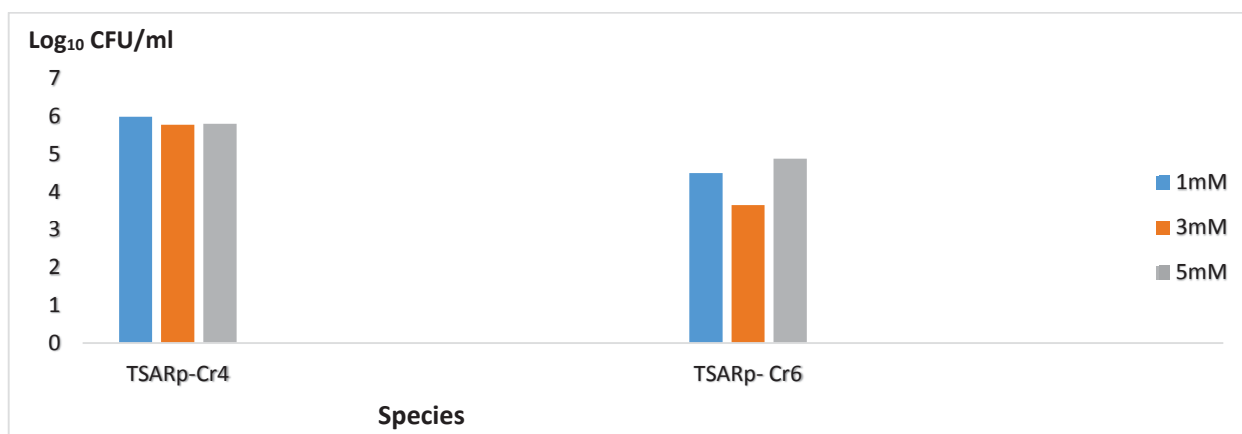


Figure 1 The growth pattern of isolated bacteria in different concentration of phenol.

The total number of culturable bacteria for TSARp-Cr4 ranging from 5.8Log₁₀CFU/ml to 6Log₁₀CFU/ml. While TSARp-Cr6 has the reading of total colony bacteria (Log₁₀CFU/ml) ranging from 3.6 Log₁₀CFU/ml to 4.9Log₁₀CFU/ml. Species TSARp-Cr4 growth on 3 different concentration did not vary significantly as the value of Log₁₀CFU/ml in 3mM and 5mM is approaching 6Log₁₀CFU/ml. Whereas species TSARp-Cr6 growth on 1mM, 3mM and 5mM of phenol concentration shows fluctuation result.

Species TSARp-Cr4 has the optimum growth value (Log₁₀CFU/ml) at 1mM of phenol while TSARp-Cr6 has the optimum growth at 5mM of phenol. The results of the growth species on 3 different concentration of phenol (1mM, 3mM and 5mM), TSARp-Cr4 able to grow greater than TSARp-Cr6.

4. Conclusion

Based on the screening result above, it illustrates that metabolically diverse and healthy community of microorganisms in the rhizosphere in different plants might be the 'hot spot' for hydrocarbon degrader [38]. The isolated rhizospheric bacteria could be highly potential of hydrocarbon degrader. This research could be continued for the biodegradation study on phenol by using these two species.

5. References

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