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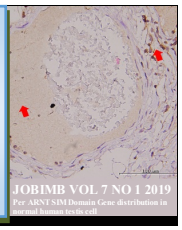

Screening of Xenobiotics-degrading Microorganisms from Sumatera's Soil

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ABSTRACT

Microorganisms from thirty soil samples from various locations in West Sumatera was screened for their ability to grow on xenobiotics such as acrylamide, propionamide, polyethylene glycol (PEG) 1000, Sodium dodecyl Sulfate (SDS), Sodium dodecylbenzene sulfonates (SDBS), diesel and phenol. The screening media contain all of these xenobiotics as carbon sources, ammonium sulphate as nitrogen sources and trace elements. The growth of these microorganisms on the xenobiotics was monitored at A600 nm on a microplate. Of the isolates screened, the number of bacterial isolates than can grow on acrylamide, propionamide, polyethylene glycol (PEG) 1000, SDS, SDBS, diesel and phenol were 23, 22, 13, 17, 3, 17 and 17, respectively, all as carbon sources. These xenobiotics-degrading isolates can be further utilized in the future as bioremediation agent.

INTRODUCTION

West Sumatera especially the province of Padang is an intense industry and agricultural area. These activities have created recurrent problems of pollutions that have affected the livelihood and the environment [1–3]. Common xenobiotics such as acrylamide, propionamide, polyethylene glycol (PEG) 1000, Sodium dodecyl Sulfate (SDS), Sodium dodecylbenzene sulfonates (SDBS), diesel and phenol are the top pollutants in developing countries due to industrial activities.

An incredible number of tonnes of xenobiotics are made annually with a tremendous amount discovered damaging the surroundings. Xenobiotic for example diesel, is a complex mixture of hydrocarbons, is important in our everyday life as the primary principle of energy resources for travel, energy and electricity. The overpowering use of diesel in our lives also has a crucial disadvantage; diesel is also a pollutant found in the environmental [4–7]. Detergents are one of the main water pollutants globally. Aquatic life are most affected by detergents [8]. Anionic surfactants from concentration as low as 0.0025 to 300 mg/L exhibited toxic effects to aquatic life. Daily used detergent include compounds such as SDS and SDBS [9]. Toxicity towards invertebrates and crustaceans has been documented in vivo and in vitro due to the massive amount of anionic surfactants continuously released into water bodies. Phenol, a toxic compound, is not only toxic to humans but to many other organisms as well [10]. Its vapours are corrosive to the mucous membranes, skin, eyes, and the respiratory tract. Prolonged skin contact causes dermatitis with a possibility of a third-degree burn. Chronic exposure has harmful effects on the liver and kidneys. Toxicity is due to its hydrophobicity and to a certain extent the formation of phenoxyl radicals [11]. Its global pollution is well known.

The removal of heavy metals and organic pollutants through bioremediation is the most economical approach in the long term especially at low concentrations, where other methods such as physical or chemical methods would not be effective [12]. In order to realize this goal, local xenobiotics-degrading bacteria need to be isolated to prepare for the current and future bioremediation works. In this study, bacterial isolates from soil samples from various localities in the province of Padang in West

Sumatera were screened for their ability to grow on xenobiotics as carbon sources.

MATERIALS AND METHODS

Growth and isolation of bacteria from soils

Soils were sampled from West Sumatera especially in the province of Padang specifically in Pagaruyung, Tanah Datar, Danau Maninjau, Padang Panjang, Padang City, Pariaman, Sawahlunto and Bukittinggi. The soil was sampled about 5 cm from the topsoil and placed in sterile polypropylene container and brought to the laboratory at room temperature. About 1 gram of soil sample was diluted in 10 mL of sterile tap water and 0.1 mL was spread plated on nutrient agar. Unique colonies were purified by re-streaking on nutrient agar several times. Unique colonies were grown overnight in nutrient broth (Oxoid).

The nutrient broth was made by first adding 2.5 g of Oxoid Nutrient Broth powder to 100 mL of sterile distilled water in a conical flask followed by swirling and mixing the mixture until the broth is fully dissolved. About 0.5 mL of this overnight grow culture was then mixed with 500 µL of 50% glycerol in a 2 mL screw top container and gently mix. The freezing of this glycerol stock at -80°C allowed long term storage. For short term storage, the bacteria were streaked onto nutrient agar and stored at 5 °C for a maximum of two weeks. Cycloheximide was added to the final concentration of 100 mg/L to inhibit fungal and yeast growth and preliminary partial identification of the bacterium was made using Gram staining [13].

Preparation of resting cells for molybdenum reduction characterization

The screening work was carried out statically using resting cells in a microplate or microtiter format as previously developed [14]. Cells from a 10 mL overnight culture from each isolate was grown in nutrient broth at room temperature on an orbital shaker (150 rpm. Cells were harvested by centrifugation at 15,000 x g for 10 minutes and the pellet was washed several times and resuspended in 2 mL of sterile distilled water.

Xenobiotics as carbon sources for growth

The ability of the isolated bacteria to grow on xenobiotics was tested using the microplate format using the xenobiotics such as acrylamide, propionamide, polyethylene glycol (PEG) 1000, Sodium dodecyl Sulfate (SDS), Sodium dodecylbenzene sulfonates (SDBS), diesel and phenol at the final concentration of 500 mg/L (0.05% w/v or v/v for diesel). The growth media (pH 7.5) was as follows: $(NH_4)_2.SO_4$ (0.3%), $NaNO_3$ (0.2%), $MgSO_4.7H_2O$ (0.05%), yeast extract (0.001%), NaCl (0.5%), Na_2HPO_4 (0.705% or 50 mM) and 1 mL of trace elements solution. The trace elements solution composition (mg/L) was as follows: $CaCl_2$ (40), $FeSO_4 \cdot 7H_2O$ (40), $MnSO_4 \cdot 4H_2O$ (40), $ZnSO_4 \cdot 7H_2O$ (20), $CuSO_4 \cdot 5H_2O$ (5), $CoCl_2 \cdot 6H_2O$ (5), $Na_2MoO_4 \cdot 2H_2O$ (5). Exactly 200 uL of the media was added into the microplate wells followed by the addition of 50 uL of the washed overnight bacterial cell suspension. A sterile sealing tape that allows gas exchange (Corning® microplate) was used for sealing the tape. The microplate was incubated at room temperature for three days. The final absorbance at 600 nm was subtracted from the initial absorbance at the start of the incubation time and then the plate was read in a BioRad (Richmond, CA) Microtiter Plate reader (Model No. 680).

RESULTS AND DISCUSSION

After three days of incubation it was discovered that out of the 132 bacterial isolates from these soils, the number of bacterial isolates than can grow on acrylamide, propionamide, polyethylene glycol (PEG) 1000, Sodium dodecyl Sulfate (SDS), Sodium dodecylbenzene sulfonates (SDBS), diesel and phenol were 23, 22, 13, 17, 3, 17 and 17, respectively (**Table 1**).

Table 1. Growth of soil isolates on various xenobiotics. Growth was determined to be a minimum increase of about D 0.050 unit of absorbance after three days of incubation at room temperature.

Xenobiotics	n	Acm	Ppm	PEG 1000	SDS	SDBS	diesel	phenol
Pagaruyung	10	PR1, PR2 and PR3	PR1, PR2 and PR3	PR5	PR1, PR2 and PR3	-nil-	PR4, PR9	PR4, PR9
Tanah Datar	10	TD2	TD2	-nil-	-nil-	-nil-	TD1	TD1
Bukittinggi	21	BT1, BT2, BT4	BT1, BT2, BT4	BT5	BT4, BT5	-nil-	BT23	BT23
Danau Maninjau	23	DM1, DM 2	DM 1, DM2	-nil-	DM1	-nil-	DM17	DM7
Padang Panjang	21	PP11, PP12, PP15	PP11, PP12, PP15	PP1, PP2,	PP1, PP2, PP4, PP5	-nil-	PP20, PP22	PP20, P22
Padang City	25	PC1, PC2, PC3, PC4, PC5	PC1, PC2, PC3, PC4, PC5	PC10, PC11	PC4, PC5	PC24, PC25	PC1, PC2, PC13, PC14, PC19	PC1, PC2, PC13, PC14, PC19
Pariaman	12	PR1, PR2, PR4, PR5	PR1, PR2, PR4, PR5	PR1, PR2, PR4, PR5, PR6, PR12	PR1, PR2, PR4, PR5	-nil-	PR10, PR12, PR13, PR15	PR10, PR12, PR13, PR15
Sawahlunto	10	S3	S3	S1	S4	S4	S4	S4
Total isolates	132	23	22	13	17	3	17	17

Note
N No of unique isolate
Acm Acrylamide
Ppm Propionamide
The abbreviation PR, TD, B, DM, PP, PC, Pn and S indicates bacteria isolated from Pagaruyung, Tanah Datar, Bukittinggi, Danau Maninjau, Padang Panjang, Padang City, Pariaman and Sawahlunto, respectively, and the Arabic numerals proceeding the abbreviated names indicate unique isolate.

Out of the 132 bacterial isolates from these soils, the number of bacterial isolates than can grow on acrylamide, propionamide, polyethylene glycol (PEG) 1000, Sodium dodecyl Sulfate (SDS), Sodium dodecylbenzene sulfonates (SDBS), diesel and phenol were 23, 22, 13, 17, 3, 17 and 17, respectively.

The number of unique isolates from the soils from the province of Padang was 132. Generally, the isolates that can grow on acrylamide can also grow on propionamide due to the similar structure between the two. Isolates that can grow on diesel can also grow on phenol while there are only 1 SDBS-degrading bacteria isolated suggesting that this detergent is recalcitrant to degradation, perhaps due to the stable benzene ring in the stucture [15,16]. A greater number of xenobiotics-degrading bacteria was isolated from Padang Panjang, Padang City and Pariaman, probably because of the high antrhopogenic activities in this area.

The ability of bacteria isolated from these areas to degrade xenobiotics have been reported before and include molybdenum reduction [17], dye-degrading [18], SDS-degrading [19] and acrylamide [17]. However, there are very few reports on other xenobiotics-degrading bacteria from these areas and it is high time that more xenobiotics-degrading microorganisms need to be isolated from these areas due to the intense anthropogenic activities in these areas [1–3]. These bacteria can be utilized for bioremediation activity to remediate polluted sites.

CONCLUSION

Soil samples from various areas in the province of Padang including Pagaruyung, Tanah Datar, Bukittinggi, Danau Maninjau, Padang Panjang, Padang City, Pariaman and Sawahlunto yield 132 bacterial isolates. These isolates were then screened for their ability to grow on various xenobiotics such as such as acrylamide, propionamide, PEG 1000, SDS, SDBS, diesel and phenol and it was observed that isolates that can grow on acrylamide can also grow on propionamide and isolates that can grow on diesel can also grow on phenol. Only one SDBS-degrading bacteria was isolated. In the future, the characterization and optimization of these xenobiotics-degrading bacteria will be carried out and it is suggested that more xenobiotics-degrading microorganisms need to be isolated from these areas for the purpose of bioremediation due to the intense anthropogenic activities in these areas.

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