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Synergism between Rhizosphere Bacteria Isolates from *Scleria* sp., *Clidemia* sp., and *Panicum* sp. to Increase the Effectiveness of Mixed Cultures in Hydrocarbon Biodegradation

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Abstract

The purpose of this research is to obtain hydrocarbon degrading bacteria that work synergistically in a consortium. Consortium microorganisms is mixture of microbial populations in the form of communities that have mutualistic relationships and doesn't inhibition the growth of other microbes. In this study, isolates were obtained from the rhizosphere of soil contaminated with petroleum. The isolates obtained were tested for synergism to determine the relationship between bacterial isolates. Synergism testing was carried out using the spread plate method on agar media. The results of this study showed that isolate number one showed antagonistic properties to other bacterial isolates by forming a clear zone around the disc paper. A total of eight bacterial isolates showed the greatest percentage of synergism, namely ≥ 80% so that the eight rhizosphere bacterial isolates could be used as materials for mixed culture.

Keywords: Hydrocarbon, Synergism, Rhizosphere

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1. Introduction

The location of petroleum mining is an area that is very vulnerable to environmental pollution. Petroleum-contaminated soil is found around oil wells and oil collection sites. This pollution occurs as a result of oil spills during the mining and transfer of petroleum. This causes the ecosystem around the location of petroleum mining to become damaged, there are less and less plants and soil fauna that can live. Based on the type of organism that is able to live around petroleum-polluted soil, it only allows a few microorganisms such as fungi and bacteria. Bacteria that are around petroleum contaminated soil can have the potential to degrade hydrocarbon compounds because they are able to utilize element C as its energy source. Generally, bacteria will be abundant if they are found around the roots of plants that also grow around oil-contaminated soil. Biological handling of pollution caused by petroleum hydrocarbons requires microbes that can actively degrade petroleum hydrocarbons.

Petroleum degrading bacteria are widespread in several ecosystems, both those found in environments that are directly contaminated with petroleum, in forests, plant rhizosphere, in fields, even in grasses [1]. Rhizosphere bacteria are bacteria found in plant root areas which are known to have high diversity. Rhizosphere bacteria have various roles such as providing nutrients for plants, protecting plants from infection with pathogenic bacteria, producing growth hormones such as indole acetic acid, phosphate solvents, and nitrogen-fixing [2].

Naturally, the soil microbes in the nature can be degrade hydrocarbons that pollute the soil. The short-chain group of hydrocarbons is the most quickly degraded by soil microbes because they have fewer carbon chains. Meanwhile, complex hydrocarbons such as hydrocarbons in the form of aromatic microbes require a longer time to

degrade due to their cyclic structure like benzene. Based on the group of hydrocarbon compounds, certain types of microbes can only degrade certain hydrocarbons, so that in natural conditions various types of microbes work together in the form of a consortium to degrade hydrocarbons in oil-polluted ecosystems [3].

The ability of microbes to degrade hydrocarbons has been exploited since the 70s and 80s on agricultural land where oil is being dumped. The microbes used can be in the form of a single culture or a mixed culture which can degrade petroleum. The microbes used in degrading the oil waste usually have a higher degradability if used as consortium or mixed cultures [4]. The consortium is a group of microbes that mutually benefit from one another and carry out a process that each organism cannot do separately [5]. The consortium of bacteria both naturally and artificially formed has the advantage of having complementary metabolic functions in an ecosystem [6].

The use of a microbial consortium tends to give better results than using a single isolate, because it is expected that the enzymes work of each type of microbe can complement each other to survive using the nutrient source available in the carrier media [7]. Microbial consortiums are often made by considering that the microbes that are members of the consortium isn't complete in carrying out a certain process, but it is hoped that the members of the consortium will have a synergistic work [5].

Synergism is an association or living relationship between the two species which carry out activities that doesn't disturb each other. However, each activity is a mutually beneficial sequence. Bacterial synergism is an interaction between genus or species of bacteria with each other in synergy, and sharing the same source of nutrition in the same living medium [6].

2. Materials and Methods

2.1. Rhizosphere Bacteria Isolate Retrieval

Rhizosphere bacterial isolates were obtained from plant root areas that were able to grow in oil-contaminated soil. Soil sampling was carried out at 4 root points of the rhizosphere. A total of 0.5-1 kg of soil samples from each point of collection are composited. Soil samples were taken from the closest distance to the roots of the plant, namely at a depth of 15 cm [8].

2.2. Preparation of Medium

Soil samples from the rhizosphere were enriched using BHMS medium and incubated at room temperature. The rhizosphere bacterial samples were then isolated and purified so that they could be separated from one isolate to another. To determine the rhizosphere bacteria that can survive in an environment containing oil, pure isolates were selected using a medium containing sterile crude oil.

2.3. Synergism Test Between Bacterial Isolates

Bacterial isolates that passed the second selection were subjected to synergistic testing between each bacterial isolate obtained. Synergic testing is carried out based on the modification of the methods that have been carried out [9]. Each bacterial isolate was made inoculum by means of an ose culture of bacteria taken using a loop needle then put into a liquid medium (Nutrient broth) and incubated at 37°C for 24 hours. Solid media (Nutrient agar) was prepared to test bacterial isolates in a petri dish by applying 0.1 ml of bacterial isolate one inoculum using drygal sky on the surface of NA media with the spread plate method (Figure 1). Meanwhile, the inoculum of other bacterial isolates was placed on the surface in order to use a paper disk which was dipped first into the inoculum that had been made. Each isolate did the same thing as bacterial isolate 1 so that it could be seen which bacteria were synergistic and antagonistic. Then incubated and observed the growth of each isolate whether it inhibited the growth of other bacterial isolates or not. Isolates that have the potential for synergism, are indicated by the absence of an inhibition zone. The % synergy of each isolate with one another can be found using the formula:

$$Percentage\ of\ synergism = \frac{\sum bacterial\ isolates\ that\ can\ synergism}{the\ total\ \ of\ tested\ bacterial\ isolates}\ x\ 100\ \%$$

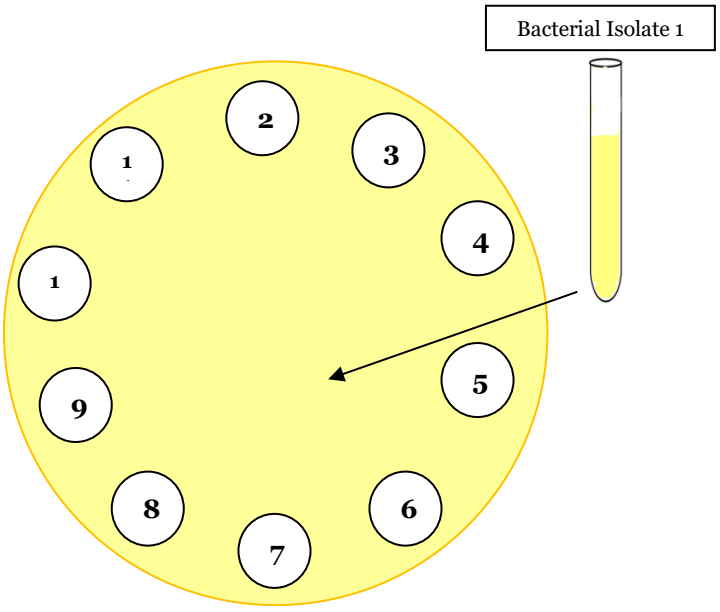

Figure 1. Synergism Testing Scheme

2.4. Preparation of Mixed Culture

Each bacterial isolate that is synergistic and shows potential as a hydrocarbon degrading agent is made a growth curve. Determination of bacterial growth curve is done by inoculating each selected bacterial isolate with a density of 10^8, then the inoculum is inserted as much as 1 ml into 50

ml of medium minerals and crude oil as much as 2%. The cultures were incubated at room temperature and the number of cells of each bacteria was counted every 3 hours until the numbers decreased. The data on the number of each bacteria at each observation time obtained were graphed so that the growth phases were known. Each growth curve of the obtained bacteria was determined by the shortest generation time in the exponential growth phase. The shortest generation time obtained from each bacterium is used as the basis for making a starter containing mixed cultures [10].

3. Results and Discussion

Based on the synergy test between rhizosphere bacterial isolates obtained from petroleum contaminated soil, from 11 isolates cultured together on nutrient agar, 8 bacterial isolates were found to synergize with each other, namely isolate A.5.6, isolate C.4.1, isolate A.4.10, isolate A .5.1, isolate A.6.8, isolate A.5.8, isolate C.6.7, and isolate A.6.3. Meanwhile, 3 other bacterial isolates (isolate B.5.1, isolate A.5.4, and isolate A.5.3 showed an antagonistic relationship characterized by the formation of a clear zone around the paper disk (Figure 2). The results of synergism testing between rhizosphere bacterial isolates can be seen in Figure 1.

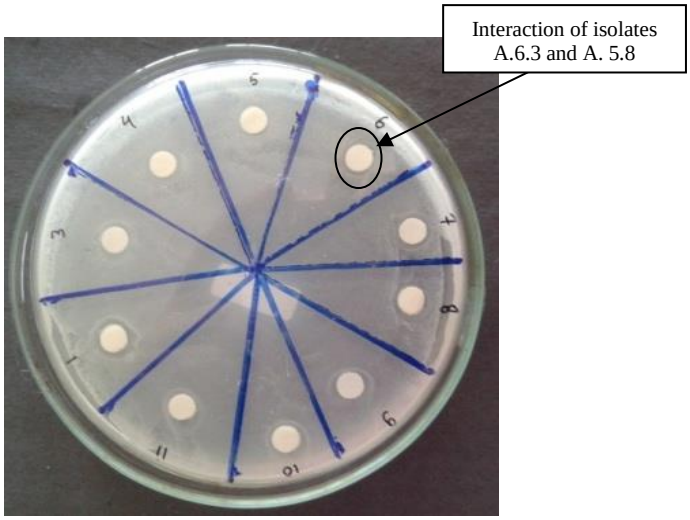

Figure 1. Testing Synergism between Rhizosphere Bacterial Isolates

The bacterial isolates that were flattened on the agar surface with the spead plate method were isolate 1 (B.5.1), while the other isolates were found on the paper disk that had been inserted first into each bacterial inoculum. In the figure, it can be seen that isolate 2 to isolate 11 inhibits the growth of isolate 1 by forming a clear zone. This means that isolate 1 has antagonistic properties to other isolates so that isolate 1 cannot be used in making mixed cultures (Figure 2). Hydrocarbon compounds can be more easily degraded by bacteria if each bacterium works synergistically. The existence of compatibility or compatibility between bacteria with one another is very necessary to be used as a mixed culture [11]. The synergistic nature of two or more bacteria is a factor that is indispensable for these bacteria to work well together. Moreover, if in mixed cultures, synergy properties are needed to support bacterial growth without antagonism in the mixed culture, because mixed cultures will produce compounds that support and cooperate with one another [12].

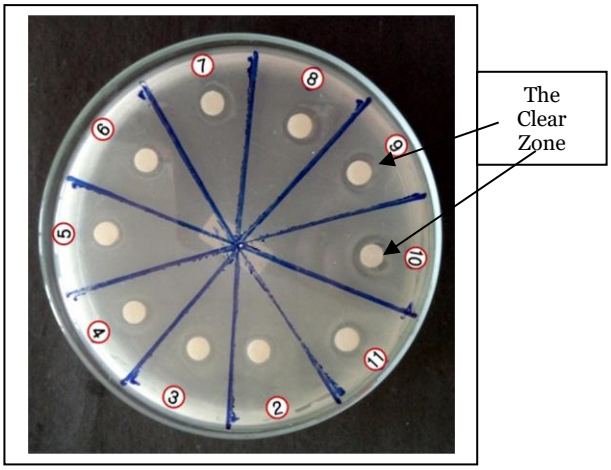

Figure 2. Antagonic Interactions

The synergism percentage of 8 rhizosphere bacterial isolates showed a large percentage, namely ≥ 80% (Table 1), so that the 8 isolates made it possible to make mixed cultures. The synergistic between isolates can accelerate the biodegradation of hydrocarbons that pollute the environment. The rhizosphere bacterial isolate will work effectively as a mixed culture because of its synergistic nature in the utilization of hydrocarbon compounds. Synergistic interactions between bacterial isolates and other bacteria will show better growth if made in the form of mixed cultures (consortium). In synergistic interactions, the rate of degradation of a compound is slow by each single bacterial population, but when different bacterial populations are mixed, the rate of degradation will increase. The same need for bacteria to utilize hydrocarbon compounds for growth will cause the degradation process to occur faster [13].

The synergistic relationship between one bacteria and another bacteria is very beneficial in the process of pollutant degradation. The role of bacteria with specific properties in utilizing pollutant compounds can increase bacterial growth as well. Meanwhile, the relationship between bacteria which is actually antagonistic can lead to competition between bacteria, so that the growth and degradation process of pollutant compounds will be low. The synergistic process among bacterial isolates will have different influences, such as bacteria of different genera can provide nutrients to other bacterial genera, can even protect other bacteria from toxic compounds in the environment

because certain bacterial genera can use these toxic compounds for their growth activities [14]. Hydrocarbon compounds have a complex structure so that a single species of microorganism cannot degrade all of the components that make up petroleum, because each species of bacteria requires a specific substrate. Several bacteria that can interact synergistically in the form of a consortium are very important during the degradation process of petroleum [15].

Table 1. The results of Synergism Testing between Rhizosphere Bacteria Isolates

Isolat are Spread On The Surface of the media \ Isolat on a paper disk	1	2	3	4	5	6	7	8	9	10	11	% Synergism
1		+	+	+	+	+	+	+	+	+	+	0 %
2	+		-	-	-	-	-	-	-	-	-	90 %
3	+	-		-	-	-	-	-	-	-	-	90 %
4	+	-	-		-	-	-	-	+	-	-	80 %
5	+	-	-	-		-	+	+	+	-	-	60 %
6	+	-	-	-	-		-	-	-	-	-	90 %
7	+	-	-	-	+	-		-	-	-	-	80 %
8	+	-	-	-	+	-	-		-	-	-	80 %
9	+	-	-	+	+	+	+	+		-	-	40 %
10	+	-	-	-	-	-	+	-	-		-	80 %
11	+	-	-	-	-	-	-	-	-	-		90 %

Note : Isolate Code 1 : B.5.1, 2 : A.6.3, 3 : C.6.7, 4 : A.5.1, 5 : A.5.4, 6 : A.5.8, 7 : A.4.10, 8 : C.4.1, 9 : A.5.3, 10 : A.5.6, 11 : A.6.8

Result Test : + : An Inhibition zone is formed
- : An Inhibition zone is not formed

4. Conclusion

From the results and discussion above, it can be concluded as follows :

1. In the rhizosphere of plants *Scleria* sp., *Clidemia* sp., and *Panicum* sp. found 11 bacterial isolates capable of living on petroleum-contaminated soil
2. Eight rhizosphere bacterial isolates which synergize with each other can be used as compatible bacteria in the consortium
3. The synergistic percentage of large bacterial isolates of ≥ 70% will have a positive influence in lowering hydrocarbon compounds.
4. This research can be used as a basis in the manufacture of mixed cultures that act as bioremediation agents.

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