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PURIFICATION OF NOVEL ENZYMES FROM NEWLY ISOLATED INDIGENOUS SOIL BACTERIAL

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ABSTRACT

The culturability of bacteria in the bulk soil of an Malaysia palm oil plantation was investigated by using nutrient agar at of its normal concentration (dilute nutrient agar) as the growth medium. Some newly enzymes were purified from these microorganisms. We collected soil samples and isolated microorganism from it and screened in incubator. so far, six isolates obtained from plate and completed a series of culture conditions including temperature ,ph and so on. But identification by comparative analysis of partial 16S rRNA gene sequences and their biochemical test by API kit are still in the process. Two crude enzymes purified from microorganisms were assayed by using crude palm oil as substrate, the crude enzymes have activity in transestefication reaction as biocatalyst.

Keywords: Microorganisms; identification; transeterification; enzyme; purification; 16S rRNA.

INTRODUCTION

The release of microorganisms from soil samples is routine in the microbiological laboratory. The literature is extensive(Riis, 1997). It has been established that the genetic diversity of soil bacteria is high and that soil containing many bacterial species of lineages for which no known cultivated isolated are available. Many soil bacteria are referred to as uncultured or even nonculturable. A range of methods have been developed to study these organisms directly in their habitats and are extremely useful for studying their characteristics[Janssen, 2002]. Currently, the identification of bacterial can be difficult and time consuming. Compared with the traditional methods, amplification of bacterial DNA using the polymerase chain reaction (PCR) is both rapid and sensitive (McCabe and Zhang, 1999). The basic strategic approaches to PCR amplification of bacterial DNA could be carried out in a ordinary setting. The species-specific amplification has been shown to be effective for the identification of a variety of organisms. The use of universal PCR primers targeting DNA regions conserved in bacteria for the purpose of DNA sequencing or probe design has been described (Senda and Arakawa, 1996). PCR primers and probes were designed for the detedtion of bacteria in blood and other normally sterile body fluids. The universal bacterial primers were tested against DNA from more than 100 bacteria strains (Greisen et al., 1994).

Lipase catalyses the hydrolysis of triglycerides at the oil-water interface. This reaction is reversible and the enzyme also catalyses the synthesis of esters and transestrification in microaqueous conditions. Lipases are presently xonsidered as choice tools of chemists owing to their ability to catalyse various types of synthetic reactions in non-aqueous environment (Saxena and Davidson, 2003). Microbial enzyme are diversified in their properties and substrate specificities, which improve their biotechnological importance and justify the search for novel enzymes possessing entirely new properties and specific substarte specificities depending on their applications. Lipases enzyme have been proven to be efficient and selective biocatalysts in many industrial applications such as biodiesel, pharmaceutical, food, cosmetics (Mhetras et al., 2009). The present study focus on the isolation and identification of some microorganisms which were collected from palm oil plantation, apart from this, we also focus on purification of some newly enzymes.

MATERIALS AND METHODS

Soil and sampling

The soil used in this study was collected from a palm oil plantation , some simple characteristics of the soil have been tested. A 20-cm-spatula was used to obtain soil from a depth between 10 and 30 cm. The external 3 mm of each core was discarded, after that, swiftly collected about 30 g soil into prepared sterile bottles. Using a thermometer to test the temperature of the around soil and tested its PH using PH meter.

Nutrient medium

Suspend 20 g in 1 liter demineralized water by heating in a boiling water bath and autoclave (15min. at 121℃). Pour to plates. Store at 4 ℃ refrigerator.

Cultivation experiments

Accurately weighed samples consisting of about 10 g freshly sieved soil were added to 90-ml prepared sterile distilled water in 150-ml conical flasks and dispersed by stirring with Teflon-coated magnetic bars (6 mm in diameter , 30 mm long) for 20 min at approximately 200 rpm, and then diluted the mixed solution according to the following concentration from 10-1until10-8 . From each dilution tube (but not the 10-2) place 0.1 ml of dilution fluid into each of two sterile Petri plates. Placed all Petri dish in the incubator or at 37℃ for at least 3 days. Find several different surface colonies that are well isolated from other colonies.

Identification of isolates

For each colony, perform the Gram Stain to observe the cellular morphology and Gram reaction of the bacteria which make up each colony, meanwhile, perform the biochemical test and compared with Bergy Manual. On the other hand, the cultures from agar slants were then transferred in the 250 ml shake flask containing 50 ml nutrient broth to extract DNA of selected bacteria, then run PCR consisted of a series of reagent and designed primer, following blast the sequence in GenBank.

Enzyme assay and purification

Enzyme activity was assayed by using crude palm oil and methanol as substrates, normally, one mole of palm oil, three moles of methanol, and a desired amout of catalyst were placed in a 300 ml glass flask. The reactants were stirred at 150 rpm, which was sufficient to keep system uniform temperature and suspension. The system temperature was set at 40 ℃. The time of the system were lasting for 48 h. The biodiesel products were analyzed by GC. The biodiesel samples were diluted with hexane (GC grade) and injection volume was 1ul. Ammonium sulphate was added to the supernatant after fermentation to give a concentration at 90% of saturation at 4 ℃. Dissolved the precipitated protein in minimum volume of 10 mM glycine-NaOH buffer PH 9.0. The partially enzyme was applied to a phenyl-sepharose column(2.5×16.5 cm) pre-equilibrated with 10 mM glycine-NaOH buffer PH 9.0. The bound enzyme was eluted with the same buffer till the flow-through fractions showed no lipase activity.

RESULTS AND DISCUSSION

Study site and sample collection

All the samples collected from palm oil plantation, totally collected 10 bottles form two different componential places, From picture 1 and 2, the two groups samples only have different color, but also have different degree of organisms. When they were carrying from the bottom to top under strictly sterile operation as much as possible, each group collected from top 10 cm and follow interval distance.

Figure 1: The first group

Figure 2: The second group

The optimum diluted concentration

The use of dilution culture techniques to obtain bacterial isolates which are significant in, dilution cultures select for organisms present in high numbers in the sample, given they are able to grow in the media used. Diluted the samples follow the protocol of Fig 3. After incubation for 3 days, 10-3 and 10-4 are the best concentration for the bacteria, we could get superior microorganisms.

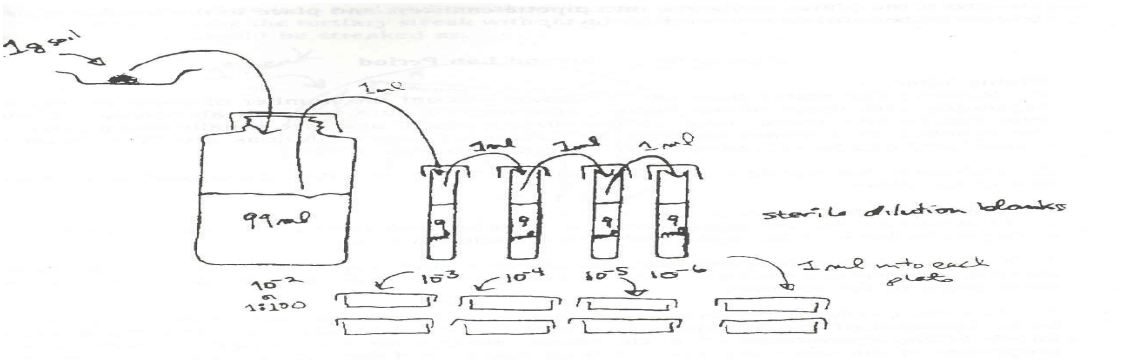

Figure 3: The method of dilution

Characteristics

The new isolates resemble each other physiologically. So far we have get at least 10 type of bacteria by the shape and Gram-stain, as currently we lack of a serial of reagents, the identification has not stated yet, so we temporarily named them using letter from A to K. The Gram-stain of all the bacteria were already carried out, from the Table 1.

Table 1: Characteristics of bacteria

Number and items	Shape	Color	State	Gram-stain
A	Irregular	White	Foliation	Negative
B	Rod	Violet	Concatenate	Negative
C	Oval	Light brown	Concatenate	Negative
D	Rod	hoariness	Foliation	Negative
E	Rotundity	Light brown	Foliation	Negative
F	Rotundity	White	Dispersion	Negative
G	Rod	White	Dispersion	Negative
H	Oval	White	Concatenate	Positive
I	Rotundity	White	Foliation	Negative
G	Rod	White	Foliation	Negative
K	Rod	White	Concatenate	Positive

Identification of isolates

Owing to the purchase order of the identification reagent is still under delivery, so we do not have related results about this part.

Enzyme assay and purification

The crude enzymes assay were already carried out for several superior isolates. Normally, mixed methanol and crude palm oil as a ratio of 1:3, needed less than 1% crude enzyme to catalyze the reaction. The product was detected by GC. In fact, the methanol had better added three times, because the exceed methanol might influence activity of enzyme. Though till now, the ratio of conversion is not good as expected, but at least it certified the enzyme has activity, and then we can conclude that the bacterial we have could dissolve crude palm oil.

CONCLUSION

After long period of preparation, we found the fertilized soil is the best place for current research, of course, to find an ideal bacteria is seriously difficult, on the other hand, for bacteria, they were easy to survive from one environment to another one. Bacterial cultures in this study had different proportions. Till now, several bacteria were isolated and used superior bacteria to catalyze trasesetefication reaction.

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