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Maximization of Biodiesel Production from *Proteus* sp. SW1 Recombinant Lipase and Palm Oil

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Biodiesel is a fuel, produced by transesterification process of oil and alcohol. The enzymatic biodiesel production is a green technology which can overcome drawbacks of conventional alkaline catalysis. Our previous study successfully isolated organic solvent resistant lipase enzyme from *Proteus* sp. SW1. The lipase gene was cloned, and the recombinant lipase was overproduced and purified. The establishment of biodiesel reaction conditions to obtain the highest yield of biodiesel products was investigated in this study. The single step addition of 15% ethanol at 40°C with rotatory shaking provides optimum conditions with highest yield. Linoleic and oleic ethyl esters are the most prominent fatty acid ethyl esters found in the biodiesel reaction analyzed by GC-MS.

Keywords: Biodiesel, lipase, organic solvent resistant, transesterification process

Biodiesel is an environmental friendly fatty acid ester compound, produced from vegetable oil, alcohol and lipase (Fig. 1). However, the reaction involves methanol or ethanol which inactivates enzyme. Therefore, additional of alcohol in biodiesel reaction was normally performed as a 3 stepwise in order to maintain activity and avoid high concentration of alcohol (Ketsara *et al*., 2010). To solve this problem, the alcohol resistant lipase "made in Thailand" is an urgent need to reduce the production cost and make the biodiesel industry economically possible. Our previous study successfully cloned the organic solvent resistant lipase gene (LipA) from *Proteus* SW1. The LipA lipase was used to develop the efficient biodiesel production and the most suitable conditions was investigated in this study.

Figure 1. Transesterification of triglyceride and alcohol

Biodiesel reaction

The enzymatic bioconversion of palm oil to biodiesel was conducted as follows: Palm oil 220 µl, 20 mM glycine buffer pH 10, 100 mM $ZnSO_4$, LipA enzyme 50U and ethanol were added in a microtube for 2-5 h at 28°C with rotatory shaking (175 rpm). The Maximization of biodiesel reaction was performed by varying the following conditions which are the stepwise ethanol addition (1-step and 3-step (Mahabubur *et al*., 2010)), % ethanol (5%, 10%, 15%, 20%, 25%, 30% and 35%), the temperature (30°C, 40°C and 50°C) and the different acyl accepters (ethanol, methanol, butanol, isopropanol and ethyl acetate (Wei *et al*., 2004)).

1. Addition of organic solvent to biodiesel reaction can be reduced to single step

The stability of microbial lipases in high concentration of organic solvent are quite low, therefore the stepwise addition of alcohol has been employed to overcome this limitation. From our previous study, LipA was stable in high ethanol concentration. In this study LipA demonstrated the ability in biodiesel production under the condition of 1simple step ethanol addition. Moreover, it gave the higher biodiesel products than the conventional 3 steps ethanol addition.

2. 15 % Ethanol gave the highest biodiesel synthesis

The reaction with varied alcohol concentration was performed and found that LipA was stabled and active at 15% ethanol addition (single step ethanol addition) (Figure 2).

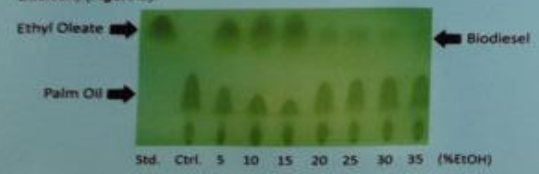

Figure 2. Effect of % ethanol in biodiesel reaction at 1 h analyzed by Thin Layer Chromatography

3. The optimal temperature for biodiesel reaction is at 40°C

Temperature is one important parameter that limits biodiesel product yield by inactivate enzyme. LipA showed the highest ability in biodiesel production when the reaction was conducted at 40°C with rotatory shaking (175 rpm).

4. Ethanol is the preferred acyl acceptor

The different acyl acceptors give the different efficiency in biodiesel production. For LipA, ethanol and methanol gave the highest yield of biodiesel. Moreover, butanol and isopronol also showed the moderate ability as acyl acceptor while ethyl acetate gave the lowest yield.

5. Gas chromatography-Mass spectrometry analysis of biodiesel products

To confirm that fatty acid ethyl ester were synthesized, GC-MS analysis was performed and found that the major fatty acid ethyl esters are linoleic and oleic, respectively (Figure 3.)

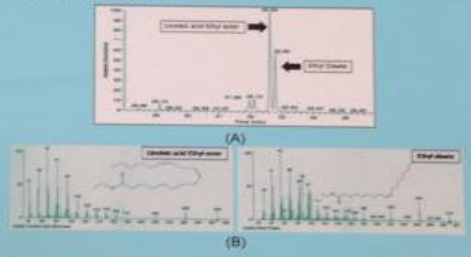

Figure 3. Chromatogram of the enzymatic reaction of LipA in biodiesel reaction analyzed by Gas chromatography (A) and Mass spectrometry (B)

Summary

1. Single step addition of 15% ethanol at 40 °C with rotatory shaking gave the highest yield of biodiesel products.
2. Linoleic ethyl ester and oleic ethyl ester are the most prominent fatty acid ethyl esters found in the biodiesel reaction.

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References

1. Kyung SY, Jung HS, and Hyung KK. Catalytic Properties of a Lipase from *Photobaterium lipoliticum* for Biodiesel Production Containing a High Metha nol Concentration. J. Biosci. Bioeng. 2009;107:599-604.
2. Md. Mahabubur RT, Jin CW, Ng MF, Yeo LSM. Two-step lipase catalysis for production of biodiesel. Biochem. Eng. J. 2010;49:207–212.

Biodegradation of Toxic Endocrine Disruptor: Isolation of Dibutyl Phthalate Degrading *Sphingobium* sp. SM42

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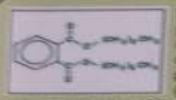
Bacteria capable of using toxic dibutyl phthalate (DBP) as the sole carbon and energy source were isolated from the soil samples collected from gasoline stations. A Gram negative bacterium identified as *Sphingobium* sp. SM42 showed the highest capability to degrade DBP. The *E. coli* expressing DBP-degrading enzyme(s) was successfully isolated from genomic library of this bacterium and designated as *E. coli* pSM42. The degradation of DBP by *E. coli* pSM42 into non-detectable level within 18 h at 37 °C was demonstrated by HPLC.

Keywords: Dibutyl phthalate; Endocrine disruptor; Plasticizer; Biodegradation; Esterase



Dibutyl phthalate (DBP) is the most abundant phthalate esters commonly used in plastic industries as a plasticizer. Release of this compound into the ecosystem occurs during production, use and disposal of plastic products (Wang et al. 2003). DBP is classified as priority pollutant by the US Environmental Protection Agency (EPA) and considered as current environmental threat. DBP is known as an endocrine-disrupting chemical that interferes with reproductive systems and behavior in humans and wildlife. However, there is very limited information available on DBP-biodegradation as an environmental friendly technology. The objectives of this study were to isolate and characterize DBP-degrading bacterium.

1. Isolation and identification of DBP-degrading *Sphingobium* sp. SM42

Among the bacteria isolated from the soil samples, the strain SM42 grow rapidly in the minimum medium (MM) containing DBP and capable of utilizing DBP as a sole carbon and energy source (Fig.1). It is a Gram negative bacterium and the result of 16S rRNA sequence compared to those in the GenBank suggested the isolate belongs to a *Sphingobium* sp.

Figure 1. (A) The soil bacterium, *Sphingobium* sp. SM42 growing in the minimum medium supplemented with 10 mM DBP compared to (B) uninoculated control. (C) Pure culture of *Sphingobium* sp. SM42 on LA plate.

2. Biodegradation of DBP by *Sphingobium* sp. SM42

3. Fosmid library construction and screening of esterase producing clone

Sphingobium sp. SM42 fosmid library was constructed and the clones which produced lipolytic zones on tributyrin agar plate were isolated (Fig. 3A). The DBP ester-degrading ability of the positive clones were confirmed by HPLC. It was exhibited that DBP level decreased from 100 uM initial level to almost undetectable level in the culture of *E. coli* pSM42 compared to vector control within 18 h at 37 °C. (Fig. 3B)

Figure 3. (A) Esterase producing *E. coli* clones showing clear zones on tributyrin agar plate. (B) HPLC chromatogram demonstrating the biodegradation of DBP by recombinant *E. coli*. The upper panel revealed the decreased level of DBP by *E. coli* pSM42 compared to the vector control in the lower panel.

Summary

1. The DBP-degrading bacterium, *Sphingobium* sp. SM42, was isolated from soil samples collected from gasoline stations.
2. The *E. coli* expressing DBP-degrading enzyme(s) was successfully isolated from genomic library of this bacterium. The recombinant *E. coli* efficiently degraded DBP to an undetectable level.
3. The cloned DBP-degrading gene is being sequenced and characterized.

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References

Wang Y, Fan Y, Gu JD. 2003. Microbial degradation of the endocrine-disrupting chemicals phthalic acid and dimethyl phthalate ester under aerobic conditions. Bull Environ Contam Toxicol 71: 810-818.

Xu X-R, Li H-B, Gu J-D. 2005. Biodegradation of an endocrine-disrupting chemical di-n-butyl phthalate ester by *Pseudomonas fluorescens* B-1. International Biodeterioration & Biodegradation 55: 9-15.

Xu XR, Li HB, Gu JD. 2007. Photocatalytic reduction of hexavalent chromium and degradation of di-n-butyl phthalate in aqueous TiO2 suspensions under ultraviolet light irradiation. Environ ... 061.


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