Screening and Culture Condition of Lipase Strains from Biodiesel

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Abstract. Biodiesel, a biodegradable, sustainable and clean energy has attracted growing interest in recent years, mainly due to development in bio-diesel fuel and ecological pressures including climatic changes. In this study, lipase strains FS14 extracted from soil which is polluted by oil and fat were analyzed .The highest enzyme activity strain was screened in culture of bromcresol purple. It was gram positive and short rod. The result of identification shows that it respectively belongs to *Serratia* sp. through the shape of the colony, determination physiology and biochemistry. The optimal culture conditions were determined as follows: initial pH 7.0 , at olive oil concentration of 8 to10 mg/L, incubation at 30 ℃ for 3d.

1 Introduction

Increased demand for energy, price hike of crude oil, global warming due to emission of green house gases, environmental pollution, and fast diminishing supply of fossil fuels are major key factors leading to search for alternative sources of energy. Some of the most notable alternative sources of energy capable of replacing fuels include water, solar and wind energy, and biofuels. Currently, 86% of the energy being consumed worldwide and nearly 100% of energy desired in the transportation sector is provided by non-renewable fossil fuels[1]. Biofuels production is being supported by the European Union (EU) with the objective of increasing fuel supply sources, decreasing hazardous gaseous emission which causes global warming effects, providing more earning opportunities in rural communities and developing long term plan for finite fossil fuels replacement. Currently, several countries such as United States, Australia, Italy, and Austria are already using biofuels such as biodiesel and bioethanol. This trend is expected to continue worldwide with more countries joining in to use biofuels as brand source of energy[2].

Bio-diesel, a substitute to diesel fuel, is produced from renewable natural sources such as vegetable oils and animal fats. It is biodegradable, sustainable, and also environmentally beneficial, thereby providing lower gas emission profile. Bio-diesel is considered to be carbon neutral, as biodiesel yielding plants such as rape plant and palm trees absorb carbon-dioxide to a greater extent than that contributed to the atmosphere when used as fuel in diesel engines. Also, biodiesel has similar physicochemical properties to that of diesel produced from crude oil and can be used directly to run existing diesel engines without major modifications or as a mixture with petroleum diesel and produces less harmful gas emission such as sulfur oxide[3].

Lipase, derived from biodisel, was widely used in catalytic synthesis. However, the cost itself has not been solved that limited its wide application. Therefore, screening strain to modify current lipase producing strain could provide insight for its industrial production.

2 Material and methods

2.1 Strain: All strains were selected from polluted soil.

2.2 Culture medium:

Isolation medium: Inorganic medium with 1% olive oil.

Screening medium(L-1): Inorganic salt culture medium: 960 mL, olive oil and polyvinyl alcohol,

40 mL bromocresol

Fermentation medium(L-1): soybean powder 30 g, sucrose 5 g, peptone 15 g , KH2PO4 1.5 g , MgSO4 • 7H2O 0 .75g , (NH4)2SO4 3 .75g, olive oil 10 ml, pH value of 7.

2.3 Methods

2.3.1 Screening and purification of lipase producing Strain: soil water solution containing bacteria was shaken at 180 rpm, 30℃ for 3d before dilution. 0.1 mL dilution was coated on peptone medium plate for 48h to select the single colony.

2.3.2 Identification of lipase producing strain: According to Bergy's Manual of Systematic Bacteriology,observation and physiological and biochemical tests were used to identify the isolated strain.

2.3.3 Lipase activity assay: The purified bacteria was cultured at 30 ℃, 180 r min for 3 d before collecting the supernatant at low temperature. Ammonium sulfate was used to precipitate in order to obtain crude lipase. Lipase activity assay was detected according to reference of olive oil emulsion method-olive oil emulsification method[4]. A unit of the lipase was determined as the quantity of released per minute 1 mol fatty acids required for lipase.

3 Result and discussion

3.1 The colony and cell morphology: The bacteria was gram positive bacteria. rod shaped, single or short chain arrangement, with high starch hydrolysis and low nitrate reduction ability. According to

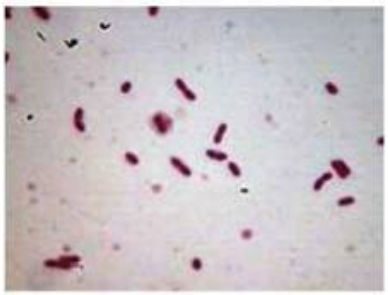

Fig.1 Gram staining of isolated strain FS14

3.2 16S rDNA identification

According to colony cultured on LB solid plate, 1500 bp was collected from 16S rDNA sequenceing technology, showing in Fig 2.

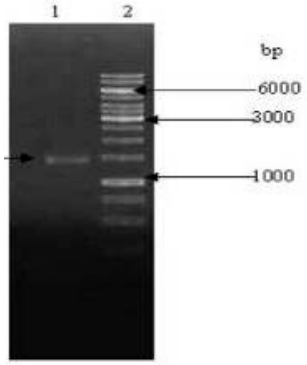

Fig.2 Colony PCR results of bacterial 16S rDNA
1：16S rDNA PCR product； 2：1kb DNA ladder

The targeted PCR product was ligated into PMD-19 T vector, transformed into E.coli DH5 α , the enzyme digestion result was illustrated in Fig.3.

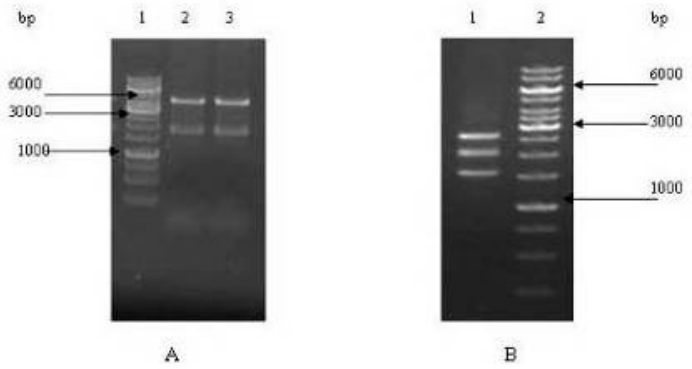

Fig.3 Enzyme digestion of the recombinant plasmids

A：1 1kb Marker； 2 Hind III digestion； 3: BamH I digestion； B: 1 BamHI+HindIII digestion； 2: 1kb Marker

According to Fig.3A, 4500 bp fragment was collected, 3 kbp and 1.5 kbp was harvested digested by BamHI and HindIII (Fig 3B). The right vector was delivered for sequencing to draw phylogenetic tree (Fig.4). In this tree, FS14 was clearer to Serratia KRED-AB61685, the homology of which was 97.476%, consequently, FS was named Serratia sp. FS14.

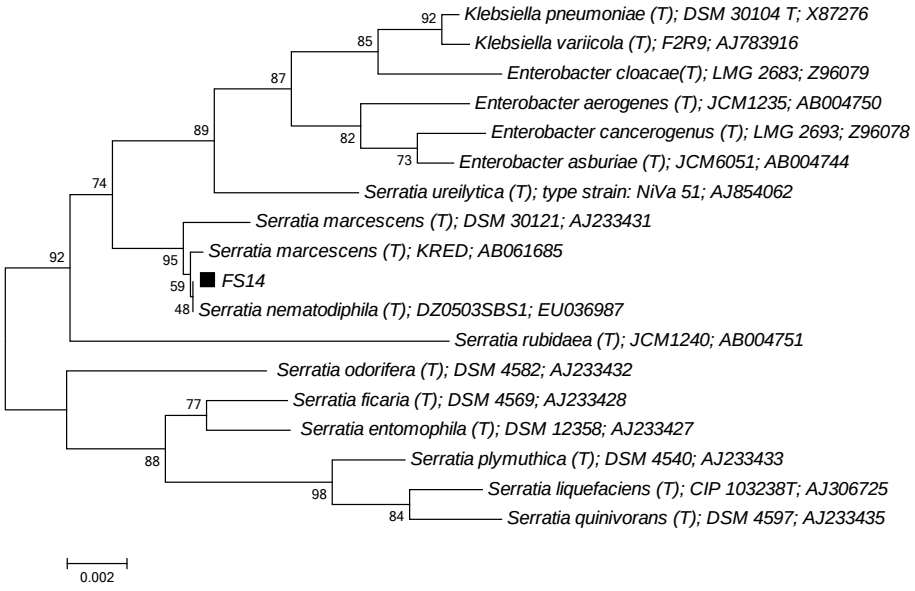

Fig.4 Phylogenetic analysis of Serratia sp. FS14 and related species by the neighbor-jointing approach. Bootstrap values obtained with 1,000 resamplings are indicated as percentages at all branches.

3.3 Initial pH on lipase production

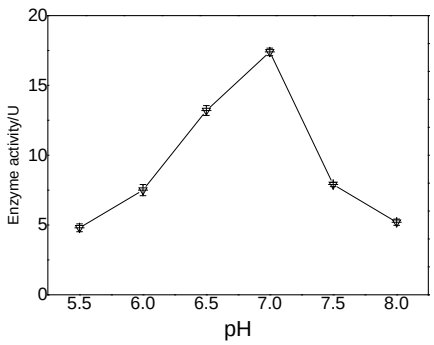

Fig.5 pH on lipase activity

In Fig.5, initial pH was a significant factor. pH 7.0 was its optimum pH, indicating the great

influence off olive oil content on enzyme activity and bacterial growth.

3.4 Olive content on lipase activity

Maia have found olive oil was benefitial ro lipase production[5], Hence, olive was used as the sole carbon source to isolated. In Fig.6, lipase was 18.2 mg/L at its maximum.

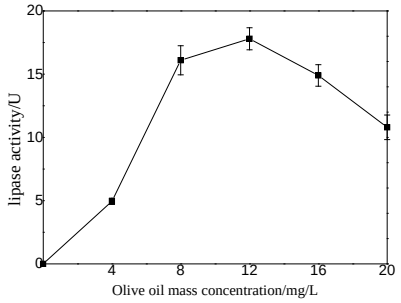

Fig.6 Olive oil on lipase activity

5 Conclusion

1. Serratia sp. FS14 was isolated from oilve contaminated soil.
2. The optimal culture conditions were determined as follows: initial pH 7.0 , at olive oil concentration of 8 to10 mg/L, incubation at 30 ℃ for 3d.
3. The maximum lipase activity was 19.2 U in 12mg/L olive oil concentration.

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