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ISOLATION AND SELECTION OF LIPASE - PRODUCING BACTERIA IN VIETNAM

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Abstract

From deferent sources of Vietnam such as petrol product reservoirs, oil polluted sand, airplane fuel, food wastewater were isolated decades of lipase-producing bacteria. Six strains with high lipase-producing activity were selected. The effect of olive oil on lipase production was investigated. Olive oil concentration was optimal for lipase producing from 4% to 7%. In particularly, all strain was capable to produce lipase on medium containing 10% olive oil. Using API 20E kit identified three of strains. They belong to *Pseudomonas* genus. One of which is *Pseudomonas aeruginosa* N9. This strain produced high lipase (2.53 u/ml) therefore it could have great potential for application in the detergent industry.

Key words: API 20E kit, Characterization, Lipase activities, Lipase - producing bacteria, *Pseudomonas* sp., Olive oil, RhodaminB.

Introduction

A large number of surface-active agents are used in the detergent industry. In particular, lipases that catalyze syntheses of a wide variety of esters have been studied by a large number of researcher [7].

Lipases can be obtained from many species of plants, animals and microorganisms. However, most attention has been paid to microbial source [3].

Lipases are a class of triglyceride-hydrolyzing enzymes whose act at oil-water interfaces to catalyze the hydrolysis of long-chain triglycerides. Lipases not only hydrolyze ester bonds, trans-esterify triglycerides, and resolve racemic mixture, but also, in the reverse mode, to synthesise ester and peptide bonds depending on their sources with respect to positional, specificity, fatty acid specificity, thermo-stability, pH and temperature optimum, etc. Since lipases can evidently catalyze numerous different reactions, they have been widely used in industrial applications such as in food, chemical, pharmaceutical, and detergent industries [5,6,8,9,11].

In this paper we report the production of lipase by some bacteria strains. The aim of work is isolation, selection high lipase-producing bacteria, investigation the effect of olive oil concentration on lipase production, and identification some species producing high lipase.

Materials and methods

Microorganisms

Some lipase-producing strains: TL-N₁A, TL-N₁B, TL-N₁C were isolated from Thuongly petrol product reservoirs. Strain ASB was isolated from S7-A320 airplane fuel. Another strain, P.HQV4 was isolated from food oil wastewater. The other strain, *Pseudomonas aeruginosa* N9 was isolated from expanse of stand containing oil polluted on Nhatrang beach.

Medium and cultivation

Medium A for isolation and selection of the strains contained 0.5%(w/v) peptone; 0.3%(w/v) yeast extract; 1% (v/v) olive oil; and 0.2%(v/v) TritonX-100 in running water. The pH was initially adjusted to 6.0 by

PBS buffer 0.1M(pH 6.0). For plate cultivation, 1.5%(w/v) agar and 0.01%(v/v) RhodaminB were added to this medium. Cultivation on agar plate for isolation of strains was carried out at 30⁰C for 3 days [8].

Isolation of strains

All strains were pre-cultivated with shaking (140 rpm) in medium A for 1 day, and then were inoculated on agar plates. After 3 days, the strains growing on the plates were picked up, and single-colony isolation on medium agar A was done and observed on fluorescence microscope.

Characterization of bacteria

The morphological properties and taxonomical characteristics of bacteria were determined by Gram staining methods, were observed on JEM 1010 electron microscope and was identified by using API 20E kit.

Preparation of extracellular lipases

Medium B for lipase production is the same to medium A without agar. However, ASB strain was inoculated in mineral medium supplemented 1% NaCl, 0.5% yeast extract, and 0.2% TritonX-100 and olive oil. For determination of extracellular lipase activity, shake culture were carried out in 20 ml of medium B with shaking (140 rpm) in 100 ml flasks at 30⁰C. After 2 days, the cultures were centrifuged at 10000 rpm for 10 min at 4⁰C, and the supernatants were used as extracellular lipase fractions.

Assay of lipase activity

The reaction mixture contained 1ml olive oil, 8ml PBS buffer (100 mM) pH 7.6, 1ml CaCl₂ 0.1M and 1ml enzyme solution. The reaction was carried out at 37⁰C for 15 min with stirring at 500 rpm. Amount of liberated fatty acid was titrated with 10mM KOH by a pH meter. One unit of lipase activity was defined as the amount of enzyme required to release 1μmol fatty acid per min at 37⁰C and pH7.6 [1,3,5,8,9,].

Results and discussion

Isolation of bacteria strains

We isolated decade's strains from water samples from various environmental sites that were screened as potential sources of lipase-producing bacteria. Among 10 strains, we selected 6 strains showing better produce lipase than the other did.

Characterization of strains

6 strains were Gram negative, rod shapes, flagella. 4 of strains were observed on electron microscope (Fig1). TL-N₁C strain had 0.55μm× 1.15μm, rod shape, and polar flagella. ASB strain had 0.5μm×1μm, short, rod shape, polar flagella. *Pseudomonas aeruginosa* N9 had 0.83μm×3.08μm, rod shape, and single polar flagella. P.HQV4 had 0.6μm ×1.25μm, polar flagella. After 3 days incubating on Rhodamin B agar medium, ASB and *Pseudomonas aeruginosa* N9 were observed on fluorescence microscope. Both two strains tinted fluorescence (Fig 2).

Dentification of strains

3 of strains were identified using API 20E test as *Pseudomonas*. One strain was *Pseudomonas aeruginosa* N9. TL-N₁B and P.HQV4 strains were *Pseudomonas* sp.

Fig. 1. Cells of lipase-producing bacteria on electron microscope ($\times 20000$): A - cell of TL-N₁C; B - cell of ASB; C - cell of *Pseudomonas aeruginosa* N9.

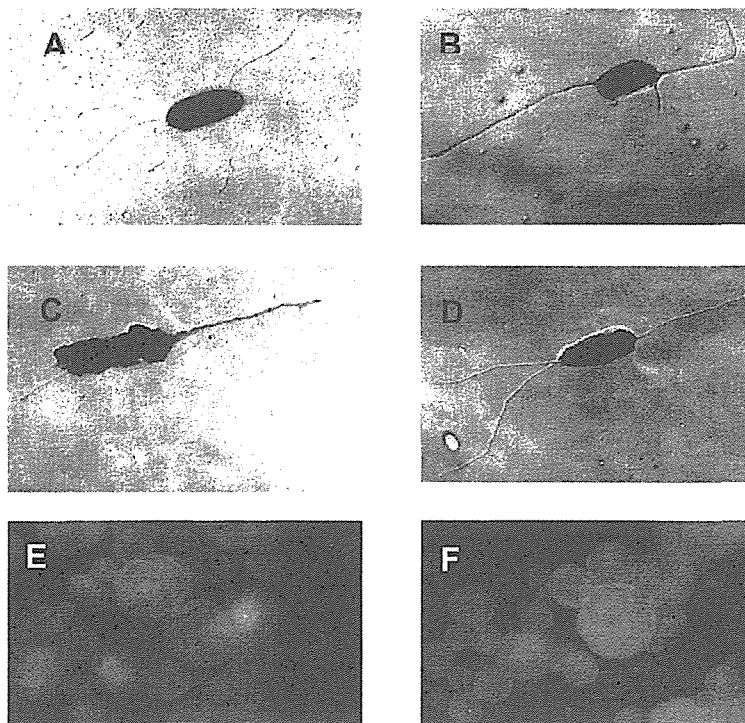


Fig 2. Colony of lipase producing bacteria on fluorescence microscope ($\times 330$): E - ASB; F - *Pseudomonas aeruginosa* N9.

Effect of olive oil on the production of lipase

Because olive oil is suitable natural substrate for lipase producing bacteria. It is a limiting factor in lipase production. In this work, we cultivated 6 strains in medium B containing different olive oil concentration such as 1%, 4%, 7% and 10%. The result was showed on Fig 3 to Fig 8.

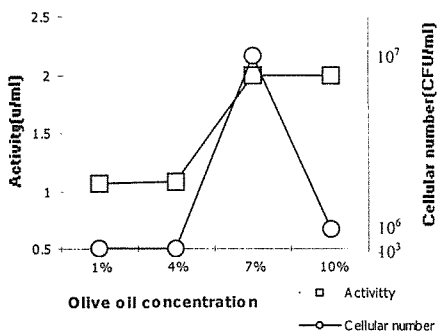


Fig. 3. Production of lipase by TL-N₁A

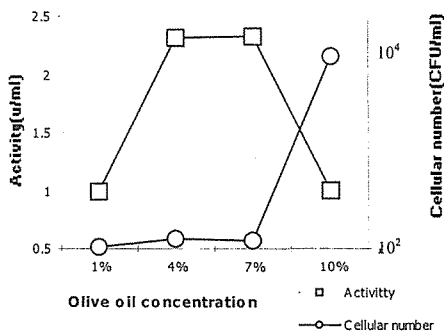


Fig. 4. Production of lipase by TL-N₁B

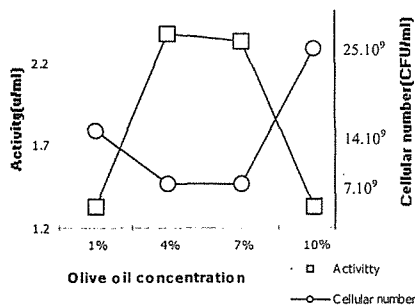


Fig. 5. Production of lipase by TL-N₁C

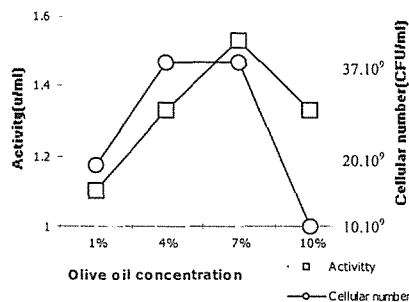


Fig. 6. Production of lipase by ASB

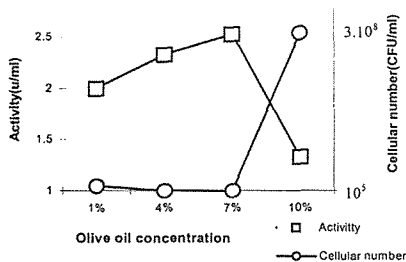


Fig. 7. Production of lipase by Pseudomonas

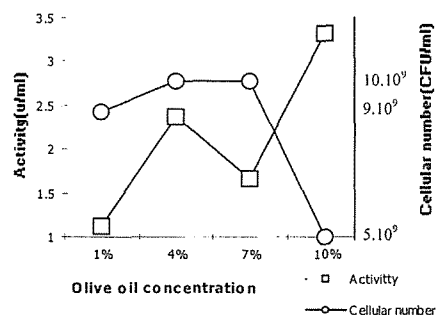


Fig. 8. Production of lipase by P.HQV4

The result showed that all strains were able to produce lipase in 10% olive oil. In particular, each strain produced lipase optimal in different olive oil concentration. 4% olive oil was optimal for TL-N₁C. 7% olive oil was optimal for *Pseudomonas aeruginosa* N9, TL-N₁A, TL-N₁B, ASB strains. 10% olive oil was optimal for P.HQV4 strain and this strain was capable to produce lipase in high oil concentration. It was very interesting that *Pseudomonas aeruginosa* N9 produced high lipase in all different olive oil concentration. *Pseudomonas* strain may be applied in detergent industry.

Conclusion

From different sources of Vietnam, 6 strains of lipase producing bacteria were isolated and selected. The highest lipase activity was produced by *Pseudomonas aeruginosa* N9 (2,53 u/ml). The lipase activity of this strain could be applied in detergent industry.

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