Screening and Molecular Identification of a Bacterial Strain with Higher Lipase Productivity

Yan-Qiong GU, Xiao-Han Zhang, Bin-Bin LI, Wei-Qing CHEN*

College of Biological and Environmental Engineering, Zhejiang Shuren University, Hangzhou 310015, China
cwq135790@sina.com
*Corresponding author

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Abstract. The objective of this study was to screen lipase-producing strain from rich oil soil, then complete molecular identification and some culture characteristics of the isolated strain. One out of 10 strains isolated from different samples was obtained with higher lipase production, which was identified as Proteusbacillus vulgaris according to its 16S rDNA sequence and analysis phenotypic characterization together with its morphological and physiological characterization, it was nominated as Proteusbacillus vulgaris Cz-6. Some conditions of Proteusbacillus vulgaris Cz-6 for lipase production were studied in detail. The results showed that the optimal carbon source and nitrogen source in culture medium were glucose and peptone. The optimal initial pH and culture temperature were pH 5.5 and 37°C respectively. Under these conditions, after cultivated for 72 h, the measured strain’s enzyme activity grew up to 15.36 U•m L^{-1}, which increased by 12% than the initial one before optimization.

Introduction

Lipases (triacylglycerol acylhydrolases, EC 3.1.1.3) can catalyze the hydrolysis of natural oil, release free fatty acids and glycerol. They were found in animals, plants, microorganisms (such as fungi, bacteria), etc [1]. Due to the ability to catalyze not only the hydrolysis of triacylglycerides in aqueous solutions, but also enantioselective synthetic reactions in organic media, lipases are widely used in biocatalysis. In recent years, the prospect of lipase catalysis in organic solvents with its associated advantages received wide attention [2]. Although people found the large number of lipases, only several species had adequate stability and biosynthetic capabilities to allow normal use in organic reactions, and most of them were produced by microorganisms [3]. The study of different members of lipases can provide an insight to their potential applications in biocatalysis and biotransformation.

Materials and Methods

Sample Collection and the Primary Screening

The samples were collected from the rich oil soil located in the campus of Zhejiang Shuren University, during March and October of 2014, respectively. The temperature on the sampling sites varied between 20 and 37°C. The pH of samples was 6.0-8.2. Samples were collected in sterile plastic containers and were cultured not later than 2 h after collection.

The primary screening for the strains with lipolytic activity was carried out on rhodamine B agar. The growth medium containing 1.0% (w/v) of peptone, 0.2% (w/v) of (NH₄)₂SO₄, 0.1% (w/v) of
K$_2$HPO$_4$, 0.15% (w/v) of MgSO$_4$, 1.5% (w/v) of agar, was adjusted to pH 7.2, autoclaved and cooled to 60°C. Then, filter-sterilized rhodamine B stock solution (1.0 mg/mL) in distilled water was added to a substrate lipoidal emulsion to yield a final concentration of 0.004% (w/v). The substrate lipoidal emulsion consisted of 1.2% (w/v) olive oil with 0.2% (v/v) Tween 80 in distilled water that was sterilized by autoclaving. Cultures were incubated at 30°C for 2-6 d. The plates with visible growth were UV irradiated (350 nm). Lipase production was identified as orange fluorescence under UV light.

**Screening in Submerged Fermentation Broth**

For further screening, submerged cultivation was applied. The fermentation medium containing 2.0% (w/v) of peptone, 0.5% (w/v) of sucrose, 0.1% (w/v) of (NH$_4$)$_2$SO$_4$, 0.1% (w/v) of K$_2$HPO$_4$, 0.15% (w/v) of MgSO$_4$, 1.5% (w/v) olive oil with 0.2% (v/v) Tween 80, pH 7.2. 50 mL of basal medium in 250 ml Erlenmeyer flasks was inoculated with 2.5 mL seed culture. After 72 h, samples were treated for lipase activity assay. 1 mL of culture was centrifuged at 4°C, 10,000g for 10 min to obtain the supernatant.

**Determination of Lipase Activity**

To choose the analysis method for lipase activity, we compared p-nitrophenyl palmitate (p-NPP) as substrate[5] and classic polyvinyl alcohol emulsion method of olive oil[6], and selected the latter. Extracellular lipase activity was quantitatively assayed in cell-free supernatants using

**Taxonomic Characterization of Strains**

Phenotypic characterization of selected strains were performed after 24 h incubation on nutrient agar. Cell morphology was observed with a light microscope (Leica DMLB2, Germany). Some conventional physiological and biochemical assays were carried out.

Genomic DNA of the screened strains was extracted by DNA extraction kit (Fermentas, China), according to the manufacturer’s recommended procedure. DNA was precipitated by ethanol and sodium acetate, resuspended in 50 μL of TE buffer (pH 8.0) and stored at −20°C.

The 16S rDNA gene was amplified with the universal primers (F: 5’-AGAGTTTGATCC TGGCTCAG-3’ and R: 5’-ACGGCTACCTTGTTACGACTT -3’). Bio-Rad PCR cycler (PTC-200) was used for this amplification. Amplitication reactions contained template DNA 1 μL, 1.0 μL of each primer(50 μM), MgCl$_2$ (50 mM) 1.5 μL, dNTP (10 mM) 1.0 μL, PCR buffer 5.0 μL, taq DNA polymerase (Fermentas, China) 1.0 μL, and ddH$_2$O 37.5 μL, in a total volume of 50 μL. The following conditions were used in the amplification of 16S rRNA gene: 94°C for 5 min, followed by 30 cycles of 94°C for 1 min, 55°C for 45 s and 72°C for 60 s, with final 10 min extension at 72°C. The PCR products were then checked on agarose gel with SYBR Green I staining. PCR product purification was conducted using PCR purification kit (UNIQ-10, China). The purified PCR product was sequenced in both directions using an automated sequencer by Shanghai Shenggong Co., Ltd (China).

In order to identify the strain, the sequence was compared with available data from GenBank databases (National Centre for Biotechnology Information website; http://www.ncbi.nlm.nih.gov/) using the BLASTN program. The phylogenetic relationship of the isolates was determined by comparing the sequencing data with the related 16S rDNA gene sequences in the GenBank database of the National Center for Biotechnology Information, via BLAST search. Phylogenetic analysis was performed using the software Molecular Evolutionary Genetics Analysis version 5 (MEGA5).

**Culture Conditions Optimization**

On the basis of the initial culture conditions, incubation temperature, pH, carbon source and nitrogen source were optimized. Lipase activity was analyzed for every sample.
Results and Discussion

Screening of Bacteria with Lipolytic Activity

A total of 10 bacterial isolates able to produce lipase, selected from hundreds of strains screened. A strain numbered CZ-6, turned out to be the most powerful and used for further studies (Table 1).

Table 1. Isolation Results of Lipase-producing Strains.

<table>
<thead>
<tr>
<th>No.</th>
<th>Lipase activity (U.mL⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cz-1</td>
<td>11.10</td>
</tr>
<tr>
<td>Cz-2</td>
<td>8.45</td>
</tr>
<tr>
<td>Cz-3</td>
<td>9.21</td>
</tr>
<tr>
<td>Cz-4</td>
<td>12.33</td>
</tr>
<tr>
<td>Cz-5</td>
<td>11.89</td>
</tr>
<tr>
<td>Cz-6</td>
<td>13.72</td>
</tr>
<tr>
<td>Cz-7</td>
<td>7.32</td>
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<tr>
<td>Cz-8</td>
<td>9.42</td>
</tr>
<tr>
<td>Cz-9</td>
<td>8.82</td>
</tr>
<tr>
<td>Cz-10</td>
<td>10.02</td>
</tr>
</tbody>
</table>

Identification of Strain CZ-6

16S rDNA sequence determination and phylogenetic analysis: Fig.1 revealed PCR products of 16S rDNA of strain Cz-6. The partial 16S rDNA sequence from strain Cz-6 was determined, and compared with those of some most closely related to *Proteusbacillus vulgaris* present in the GenBank database with a sequence identity of more than 98%, by the use of MEGA5, it showed the closest relationship between strain Cz-6 and *Proteusbacillus vulgaris*. This newly isolated bacterium was nominated as *Proteusbacillus vulgaris* Cz-6.

Morphological and Physiological Characterization

Strain was tested for morphological and physiological characterization. The data showed that strain was facultative anaerobic, motile, Gram-negative bacterium, nonspore-forming morphology. Colonies on nutrient agar are smooth, wettish and milk white. It fermented glucose but couldn’t ferment lactose.

![Figure 1. PCR Products of 16S rDNA of Strain Cz-6.](image)
Effect of Incubation Conditions of Proteusbacillus Vulgari Cz-6

Various culture parameters were investigated on the basis of initial culture conditions to improve lipase production. We found that carbon and nitrogen source could strongly influence culture growth and lipase activity. Lipase production and biomass was found to be highest with glucose as carbon source (Fig. 3) and peptone as organic nitrogen source (Fig. 4). Meanwhile, pH 5.5 was the optimal initial pH (Fig. 5) Under the optimal conditions, after cultivated for 72 hours, the strain’s enzyme activity grew up to 15.36 U·m L⁻¹, increased by 12% than the initial one before optimization.
Conclusions

In the current work, a wild bacterial strain with lipase activity was screened from rich oil soil. Subsequently, its molecular identification and some culture characteristics were carried out. It was identified as *Proteusbacillus vulgaris* according to its 16S rDNA sequence and analysis phenotypic characterization together with its morphological and physiological characterization, and nominated as *Proteusbacillus vulgaris* Cz-6. Some conditions of *Proteusbacillus vulgaris* Cz-6 for lipase production were studied in detail. The results showed that the optimal carbon source and nitrogen source in culture medium were glucose and peptone. The optimal initial pH and culture temperature were pH 5.5 and 37°C respectively. Under these conditions, after cultivated for 72 hours, the measured strain’s enzyme activity grew up to 15.36 U•m L\(^{-1}\), which increased by 12% than the initial one before optimization.

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References


