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ORIGINAL PAPER

Gene analysis, optimized production and property of marine lipase from *Bacillus pumilus* B106 associated with South China Sea sponge *Halichondria rugosa*

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Abstract Gene cloning, optimized production and property of marine lipase from *Bacillus pumilus* B106 associated with South China Sea sponge *Halichondria rugosa* were investigated in this paper. A lipase gene with whole ORF encoding 215 amino acids was obtained by PCR, protein domain prediction suggested that the deduced lipase belongs to α/β hydrolases family. Based on single factor Seriatim-Factorial test and Plackett–Burman experimental design, the optimal medium consisted of (per l) 12.5 ml maize oil, 5.0 g beef extract, 2.0 g PO_4^{3-} (0.6 g KH_2PO_4, 1.4 g K_2HPO_4), 17.15 g Mg^{2+}, 5.0 g yeast extract, 2.282 g $CaCl_2$ and 5.0 ml Tween80 with artificial sea water. Using this optimum medium, lipase activity and cell concentration were increased by 3.54- and 1.31-fold over that of the basal medium, respectively. This lipase showed tolerance to high salinity, pH and temperature. About 10–20% methanol exhibited a stimulatory effect on the lipase activity, while activity was inhibited by 30–40% methanol, 2-propanol, DMSO, and ethanol. This study provides a valuable resource for marine lipase production and extends our understanding of the possible role of sponge-associated bacteria in the biotransformation of chemical compounds for the sponge host.

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Keywords *Halichondria rugosa* · *Bacillus pumilus* · Lipase · Gene cloning · Statistical optimization · Property

Introduction

Lipases have recently emerged as key enzymes in swiftly growing biotechnology, and are used in various industries like food, chemical, pharmaceutical, cosmetic and detergent production and leather processing (Gupta et al. 2004; Jaeger and Eggert 2002; Kulkarni and Gadre 2002), and especially in biodiesel production (Nelson et al. 1996). A number of lipases have been studied, and the majority of them originate from microorganisms such as *Achromobacter*, *Alcaligenes*, *Arthrobacter*, *Bacillus*, *Burkholderia*, *Chromobacterium* and *Pseudomonas* (Gaur et al. 2008; Gupta et al. 2004; Jaeger and Reetz 1998). However, only a few microbial lipases are commercially exploited (Palekar et al. 2000), for example the lipase produced by *Pseudomonas* sp. (Beisson et al. 2000). Thus, the finding of new producers and the optimized production strategy are very important.

Marine organisms such as microalgae with high fatty acid content and the waste from marine food industry have been suggested to be potential sources of biodiesel (Yusuf 2007). The characteristics of high pH and salinity tolerance of marine microbial lipases may greatly contribute to the related biotechnological field such as marine biodiesel. Cutignano et al. (2004) reported that lipase-mediated production of defensive toxins in the marine mollusc *Oxynoe olivacea*, suggesting the biochemical role of marine lipase in marine chemical ecology. Though marine lipases from *Staphylococcus epidermidis* associated with spoiled frozen marine fish samples (Joseph et al. 2006), marine mollusc

Oxynoe olivacea (Cutignano et al. 2004), red sea bream *Pagrus major* (Oku et al. 2002), Baltic Sea sediment bacteria (Hardeman and Sjoling 2007) have been investigated as well as cloning and expression of a marine microbial lipase gene (Karpushova et al. 2005; Zhang and Zeng 2008), the investigation of marine lipases still needs to be strengthened compared to terrestrial lipases.

It is known that medium components greatly affect the production of metabolites (Abdel-Fattah and Olama 2002; Aravindan and Viruthagiri 2007), therefore, it is important to evaluate the nutritional requirements of a microorganism when it is used as lipase producer. The conventional method for medium optimization such as the one-factor-per trial method is time-consuming, expensive and impractical when a lot of variables need to be investigated. In contrast, optimizing the medium components by statistical experimental design can be simultaneous, systematic and efficient (Aravindan and Viruthagiri 2007). For instance, Plackett–Burman (PB) experimental design can be used to identify significant variables as well as their significant level by two-level factorial designs.

Sponges harbor various microorganisms, but most of them are uncultured and therefore little is known about their roles for the sponge host (Hentschel et al. 2006). The symbiotic bacteria in sponge have been proposed to play roles in supplying nutrients, stabilizing sponge skeletons and protecting sponge from bio-fouling or predation (Lee et al. 2001). Microbial enzymes may play an important role in the compound transfer to sponge host. To our knowledge, the investigation of enzymes from sponge-associated microorganisms has been rarely reported (Graeber et al. 2008; Han et al. 2008, 2009; Karpushova et al. 2005) and no reports on the lipase from marine sponge-associated microorganisms have been found. Studies on the optimization of medium components of marine microbes for lipase production by statistical methods have been rarely reported either. In this study, the gene analysis, optimized production and property of marine lipase from *Bacillus pumilus* B106 associated with the South China Sea sponge *Halichondria rugosa* were investigated with the aim of finding a new producer of marine lipase and reveal the potential role of sponge-associated bacteria in compound transformation.

Materials and methods

Bacterial strain

Bacillus pumilus B106 with lipase activity was isolated from South China Sea sponge *Halichondria rugosa* and identified based on 16S rRNA gene sequence according to the method of Li et al. (2007). 16S rDNA sequence has been submitted to GenBank with accession numbers EU369175.

Cloning and bioinformatics analysis of lipase gene from *Bacillus pumilus* B106

Chromosomal DNA was prepared from *Bacillus pumilus* B106 by the method of Li et al. (2006). Primers BPUF/R [5′-GAGTCGTATAAGATGAATAAGGGGGAATG-3′ and 5′-TTAATTCGTATTTTG TCCTCCGCCGTTC-3′] (Bell et al. 2002) were used for lipase gene PCR according to the following protocol: initial denaturation at 94°C for 5 min, 35 cycles of 94°C for 1 min, 54°C for 1 min, 72°C for 2 min, and a final extension of 10 min at 72°C. The PCR product was purified by electrophoresis in 1.5% (wt/vol) agarose gel and recovered using gel purification kit (Shenergy Biocolor Bioscience & Technology Company, Shanghai, China). The purified DNA was cloned into pUCmT vector. Then the vector was transformed into $CaCl_2$-competent *Escherichia coli* DH5a and the positive recombinants were screened on X-Gal(5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside)-isopropyl-β-D-thiogalactopyranoside–ampicillin–tetracycline indicator plates by color-based recombinant selection. Positive clones were identified by PCR amplification with pUCmT vector primer pairs T7 (5′-TAATACGACTCACTATA GGG-3′) and M13 (5′-CAGGAAACAGCTATGACC-3′) using the same PCR program as described above. Computer analysis of the DNA sequence and the deduced amino acid sequence were performed with software including BLAST, ORF finder at NCBI (http://www.ncbi.nlm.nih.gov), the Expert Protein Analysis System (ExPASy) proteomics (http://cn.expasy.org), and PredictProtein (http://www.predictprotein.org). Multiple sequence alignment was performed with program DNAman. The lipase gene and deduced amino acid sequence of *Bacillus pumilus* B106 were deposited into GenBank under the accession numbers EU482152 and ACA60975, respectively.

Basal medium and bacterial fermentation

The first step was to select the best carbon and nitrogen sources because these two medium components were of great importance for the production of lipase. The second step was to screen the important factors using statistical method. The third step was to optimize the factors and obtain the optimal medium.

According to Kulkarni and Gadre (2002), the modified basal medium consisted of (/l) 5 ml olive oil, 5 g tryptone, 2.5 g yeast extract, 1 g PO_4^{3-} (0.3 g KH_2PO_4, 0.7 g K_2HPO_4). Media were prepared with artificial sea water (ASW; Li and Liu 2006) and pH was adjusted to an initial pH 7.0 using 2 M NH_4OH. A 5% inoculum was added to

100 ml of medium in 250-ml Erlenmeyer flasks and incubated at 180 rev/min at 28°C.

Cell growth was examined by measuring cell dry weight (CDW). Fermentation broth was put into a dried and weighed Eppendorf tube, after centrifugation at 8,000 rev/min, 4°C for 20 min, the supernatant was discarded. The Eppendorf tube with cells was placed into an oven at 60°C to constant weight.

Bioassay of lipase activity of broth

The cell-free broth supernatant collected by centrifugation at 8,000 rev/min, 4°C for 20 min was used as crude enzyme sample. Lipase activity was measured based on the hydrolysis of *p*-nitrophenylacetate (*p*NPA) by a modified method according to Kulkarni and Gadre (2002) and Kim et al. (1998). Sample (200 µl) was added to pre-warmed (35°C, 5 min) Tris–HCl buffer (50 mM, pH 8.0) containing 150 µl of *p*NPA (0.01 M, 45.2 mg of *p*NPA in 5 ml of acetonitrile and 20 ml of isopropanol) with a final volume of 3.0 ml. The mixture was incubated for 10 min at 35°C. The enzyme reaction was stopped by adding 3 ml of absolute ethanol, and lipase activity was determined by the rate of *p*-nitrophenol production (*p*NP). The *p*NP was released and measured at 410 nm using a UV spectrophotometer (V-2102PCS, Shanghai, China). One unit (U) of lipase activity is defined as the amount of enzyme that releases 1 µmol of *p*NP per min at 35°C.

Selection of carbon and nitrogen sources for lipase production by *Bacillus pumilus* B106

Single-factor optimization was used to select carbon and nitrogen sources, keeping other medium constituents and process conditions constant. To test the effect of different carbon sources on lipase production, 5 ml olive oil/l in the basal medium was replaced by the respective carbon source at equal carbon content. To test the influence of nitrogen sources on lipase production, 5 g tryptone/l in the basal medium with the optimum carbon source was replaced by different nitrogen sources on an equal nitrogen content basis.

Statistical optimization of medium components for lipase production by *Bacillus pumilus* B106

Plackett–Burman (PB) design, a two-level factorial design method, was used to identify the medium components that have significant effects on lipase production. Twelve experiments were performed for the 7 factors namely beef extract, yeast extract, PO_4^{3-}, maize oil, Tween-80, Mg^{2+}, and $CaCl_2$ with three dummy variables. The effect values, *P* values and confidence level were determined using statistical software MINITAB 15. The PB design was based on the first order model with no interaction among the factors. In the case of factor level optimization, one-factor test was used to optimize the significant factors. The parameters were tested at three levels coded −1, 0, +1 for the low, middle and high concentration, respectively.

Property investigation of lipase from *Bacillus pumilus* B106

The crude enzyme sample produced by *Bacillus pumilus* B106 in the optimal medium was used for the investigation of lipase property. The optimum pH was studied in the range of pH 4–12 at 35°C. Enzyme stability at different pH values was determined by incubating the crude enzyme in pH 4–12 buffers for 1 h at 35°C. The optimum temperature was determined in the range of 30–60°C at the optimum pH. Enzyme thermal stability was studied by incubating the crude enzyme at 30–60°C at the optimum pH for 1 h. The optimum salinity was also studied in the range of 0–150‰ using KCl as salt component. The influence of 0–40% (v/v) organic solvents was investigated with methanol, ethanol, 2-propanol and DMSO. Lipase activity was described by defining the relative activity and the maximum value as 100% except organic solvent test. For the test of organic solvent, 100% was defined as the value when no organic solvent was added.

Statistical analysis

All the experiments of lipase screening, bacterial cultivation, analysis of cell dry weight, lipase activity, and property investigation were conducted in triplicate and each value was an average of the three trials. In PB design, Design of Experiments (DOE) in MINITAB 15 software package was used to design the experiments and analyse the results.

Results

Cloning and bioinformatics analysis of the lipase gene from *Bacillus pumilus* B106

A lipase gene including a whole ORF of 648 bp was obtained by PCR from *Bacillus pumilus* B106. The lipase comprises 215 amino acids including 53.02% polar amino acids and 46.98% hydrophobic amino acids. According to BLAST analysis, the amino acid sequence is 98% identical to the lipase sequence from *Bacillus pumilus* (AAR84668). Figure 1 shows the multiple alignments of lipase amino acid sequences from *Bacillus pumilus* B106 (ACA60975),

Bacillus pumilus (AAR84668), *Bacillus pumilus* (ABC67449), *Bacillus licheniformis* (CAB95850), *Bacillus amyloliquefaciens* FZB42 (YP_001419931) and *Bacillus subtilis* (ABQ08591). Two typical features of lipase such as the catalytic triad and the oxyanion hole were identified in this lipase gene. The consensus sequence Ala-His-Ser-Met-Gly was found at positions 109–113 aa, which was the active site. Another homologous region at positions 44–45 aa was the oxyanion hole with a short conserved His-Gly (HG) dipeptide. By PredictProtein analysis, there are 25.58% α-helices and 23.72% β-sheets in the secondary structure of protein. Moreover, through PROSITE motif analysis, there are five potential patterns including one cAMP/cGMP-dependent protein kinase phosphorylation

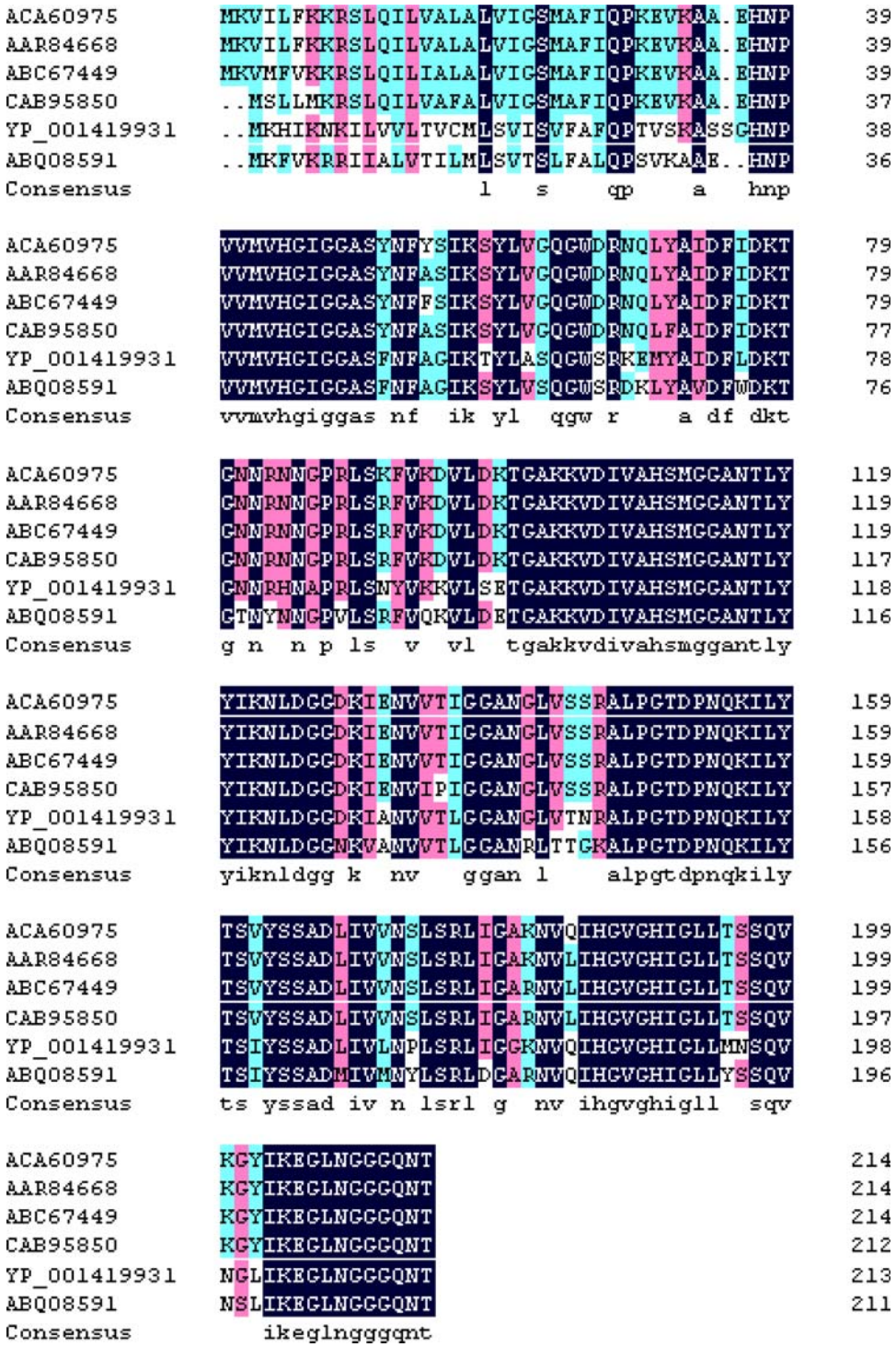

Fig. 1 Multiple alignments of lipase amino acid sequences from *Bacillus pumilus* B106 (ACA60975), *B. pumilus* (AAR84668), *B. pumilus* (ABC67449), *B. licheniformis* (CAB95850), *B. amyloliquefaciens* FZB42 (YP_001419931) and *B. subtilis* (ABQ08591)

site, two protein kinase C phosphorylation sites, one casein kinase phosphorylation site and one *N*-myristoylation site. Based on protein domain prediction, the lipase is suggested to belong to the α/β hydrolase family (Nardini and Dijkstra 1999).

Selection of carbon and nitrogen sources for lipase production by *Bacillus pumilus* B106

According to Table 1 showing the bacterial lipase activity at 60 h, the enzyme activity reached the maximum value 26.86 U/ml with maize oil as carbon source, and the highest lipase activity 31.07 U/ml was achieved at 60 h with beef extract as nitrogen source. Thus, maize oil and beef extract were selected as carbon and nitrogen sources, respectively, for lipase production by *Bacillus pumilus* B106.

Optimization of medium components and experimental validation

In the case of Plackett–Burman statistical experimental design used to identify the medium components having the greatest influence on lipase production, the (−) value and (+) value of the seven components are showed in Table 2. Table 3 represents the Plackett–Burman design for 12 trials with two levels of concentrations for each variable and the corresponding lipase activity at 60 h. The statistical analysis of the Plackett–Burman design using MINITAB package software clearly indicated that there was a considerable variation in the response, depending upon medium composition. According to Table 4, from the effect values that indicate the influence of each variable at the selected concentrations, the factor is positive for the lipase activity and production if the effect value is plus and vice versa. Maize oil, PO_4^{3-}, Mg^{2+}, yeast extract and $CaCl_2$ have positive effects while beef extract and Tween-80 have negative effects on lipase activity. Maize oil was proved to have the most profound influence on the lipase production with high confidence level of 99.8%, while the effects of other variables were negligible at the selected concentrations because the confidence levels were below 90%. The medium components with positive effect were kept at their higher levels and vice versa. So, according to Table 4, the concentrations of PO_4^{3-}, Mg^{2+}, yeast extract, $CaCl_2$ with positive effect and Tween-80, beef extract with negative effect were determined as the (+) values and (−) values in Table 2, respectively. In the case of maize oil, the coded values of the variable maize oil at three levels of 12.50, 10.00 and 7.50 ml/l and the corresponding lipase activity were tested. It was found that the high level concentration of 12.5 ml/l of maize oil was positive for lipase production.

In conclusion, the optimal medium for lipase production by *Bacillus pumilus* B106 is suggested to consist of 12.5 ml/l maize oil, 5 g/l beef extract, 2.0 g/l PO_4^{3-} (0.6 g/l KH_2PO_4, 1.4 g/l K_2HPO_4), 17.15 g/l Mg^{2+}, 5.0 g/l yeast extract, 2.282 g/l $CaCl_2$ and 5.0 ml/l Tween-80 with artificial sea water (ASW) and an initial pH 7.0. Based on Fig. 2, using this optimized medium, the maximum lipase activity 86.37 U/ml was achieved at 60 h and the maximum cell dry weight was 7.44 g/l at 55 h which were 3.54- and 1.31-fold above that of the basal medium, respectively.

Table 2 Values for the PB experiment

Variable	Medium component	+Value	−Value
X1	Maize oil (ml/l)	10.00	5.00
X2	Beef extract (g/l)	10.00	5.00
X3	Yeast extract (g/l)	5.00	2.50
X4	PO_4^{3-} (g/l)	2.00	1.00
X5	Tween80 (ml/l)	10.00	5.00
X6	Mg^{2+} (g/l)	17.15	8.575
X7	$CaCl_2$ (g/l)	2.282	1.141

Note: X4 was the mixture of KH_2PO_4 and K_2HPO_4 in proportion of 3:7 of mass concentration

Table 1 Cell concentration and lipase activity of *Bacillus pumilus* B106 at 60 h in the basal medium with different carbon and nitrogen sources

Carbon source[a]/ Nitrogen source[b]	Cell dry weight (g/l)	Lipase activity (U/ml)	Relative activity (%)
Olive oil[a]	6.25 ± 0.1583	24.98 ± 3.1423	93.00
Maize oil[a]	5.68 ± 0.0083	26.86 ± 1.7931	100
Soya oil[a]	6.41 ± 0.2292	25.76 ± 1.4045	95.90
Glucose[a]	3.22 ± 0.0502	3.66 ± 1.0912	13.63
Sucrose[a]	3.05 ± 0.0459	4.09 ± 0.5809	15.23
Peptone[b]	4.49 ± 0.5875	26.70 ± 2.3580	85.93
Yeast extract[b]	7.22 ± 0.1271	25.08 ± 2.9857	80.72
Beef extract[b]	6.55 ± 0.6750	31.07 ± 0.5185	100
Ammonium sulfate[b]	4.42 ± 0.5625	12.69 ± 1.0647	40.84
Tryptone[b]	6.22 ± 0.0500	23.62 ± 0.1705	76.02

Note: The results were presented as means ± SD, $n = 3$

[a] Different carbon sources

[b] Different nitrogen sources

Table 3 PB design matrix of 7 factors with lipase activity

Trial no.	Maize oil X1	Beef extract X2	Yeast extract X3	PO_4^{3-} X4	Tween80 X5	Mg^{2+} X6	$CaCl_2$ X7	Lipase yield (U/ml)
1	1	−1	1	−1	−1	−1	1	71.9542
2	1	1	−1	1	−1	−1	−1	55.7700
3	−1	1	1	−1	1	−1	−1	8.3615
4	1	−1	1	1	−1	1	−1	80.1207
5	1	1	−1	1	1	−1	1	68.4011
6	1	1	1	−1	1	1	−1	62.1693
7	−1	1	1	1	−1	1	1	28.1453
8	−1	−1	1	1	1	−1	1	23.3087
9	−1	−1	−1	1	1	1	−1	41.0182
10	1	−1	−1	−1	1	1	1	55.6398
11	−1	1	−1	−1	−1	1	1	24.7410
12	−1	−1	−1	−1	−1	−1	−1	13.2819

Note: PO_4^{3-} was the mixture of KH_2PO_4 and K_2HPO_4 in proportion of 3:7 of mass concentration

Table 4 Estimated effect and coefficient for lipase activity from the result of PB design

Factor	Term	Effect	SE	*T*	*P*	Confidence level (%)
X1	Maize oil	42.533	3.030	7.02	0.002	99.8
X2	Beef extract	−6.289	3.030	−1.04	0.358	64.2
X3	Yeast extract	2.535	3.030	0.42	0.697	30.3
X4	PO_4^{3-}	10.103	3.030	1.67	0.171	82.9
X5	Tween80	−2.519	3.030	−0.42	0.699	30.1
X6	Mg^{2+}	8.459	3.030	1.40	0.235	76.5
X7	$CaCl_2$	1.911	3.030	0.32	0.768	23.2

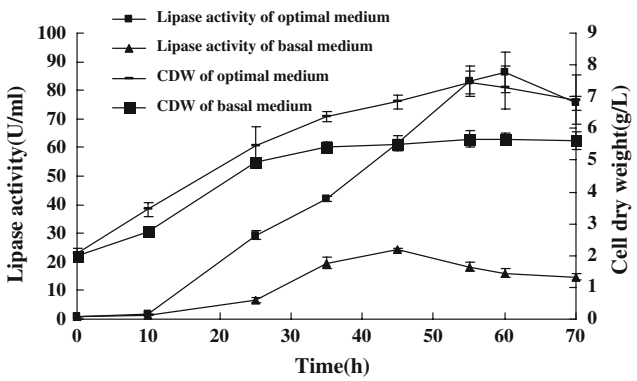

Fig. 2 The comparison of lipase activity and cell dry weight between the optimal medium and the basal medium. *CDW* is an abbreviation of cell dry weight

The property of lipase from *Bacillus pumilus* B106

The optimum temperature for lipase was found to be at 50°C (data not shown), and lipase retained more than 55% activity between 30 and 50°C for at least 1 h. The optimum pH for lipase was 8.0 (data not shown), while the enzyme was found to be stable over the range of pH 7–9 and retain more than 65% activity for at least 1 h. In the case of salinity tolerance, over 78% activity could be maintained in the range of 0–150‰ psu (data not shown). These results indicated the tolerance of lipase from *Bacillus pumilus* B106 to high salinity, pH and temperature.

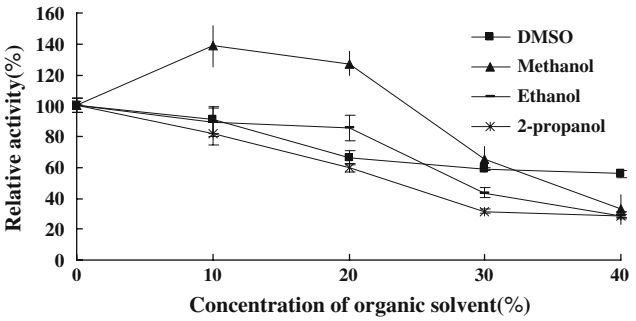

Fig. 3 Effect of organic solvent on lipase activity

The effects of DMSO, methanol, ethanol and 2-propanol on lipase activity are represented in Fig. 3. It is obvious that methanol exhibits a stimulated effect at 10–20% concentration and an inhibitory effect at 30–40% concentration on lipase activity. The lipase activity can be inhibited by 2-propanol, DMSO and ethanol as solvents based on this study.

Discussion

Most bacterial lipases are extracellular and inducible, and their production is strongly influenced by carbon sources. Generally, lipid sources such as triacylglycerols, fatty acids, hydrolysable esters, Tween, bile salts, glycerol and some other sugar carbon sources are inducers for lipase (Gupta et al. 2004). This study shows that lipid sources such as maize oil, soya oil and olive oil are better inducers than sugar sources such as glucose and sucrose. Among the

tested lipid sources, maize oil is an optimal factor for lipase production by *Bacillus pumilus* B106. The lipase production can be enhanced by increasing the concentration of maize oil in the test range from 5.00 to 12.50 ml/l.

As shown in this study, beef extract is an optimal nitrogen source for lipase production by *Bacillus pumilus* B106, but increasing the concentration of beef extract from 5.00 to 10.00 g/l will inhibit the lipase production. Tween-80 has been reported to be suitable for lipase production by *Bacillus* sp. RSJ1 (Sharma et al. 2002). However in this study, increasing the concentration of Tween-80 from 5.00 to 10.00 ml/l will inhibit the lipase production. So, in the optimal medium, both beef extract and Tween-80 were used at low concentration, which were 5.00 g/l and 5.00 ml/l respectively. As for alkaline lipase from *Burkholderia cepacia* RGP-10, Mg^{2+} (0.6 mM) in the presence of Ca^{2+} (0.4 mM) along with 1% w/v glucose enhanced lipase production up to three-fold (60 U/ml; Rathi et al. 2001). Sharma et al. (2002) found the stimulation of Ca^{2+} and Na^{+} on the lipase production by *Bacillus* sp. RSJ1. However, most other metal ions were found to inhibit lipase production by Gupta et al. (2004). In this study, Mg^{2+} and Ca^{2+} were not significant factors though they could promote lipase production to some extent in the test concentration range from 17.15 to 8.575 g/l and 2.282 to 1.414 g/l, respectively.

The factorial design of a limited set of variables is more advantageous than the conventional one-factor-per trial method. For example, lipase production by *Pseudomonas fluorescens* NS2 W was optimized in shake-flasks using a statistical experimental design (Kulkarni and Gadre 2002), where the optimized medium resulted in about a 5-fold increase in enzyme production compared to the control. A 2.4-fold increase in lipase production and a 1.8-fold increase in specific activity were obtained for *Burkholderia cepacia* (Rathi et al. 2002). In this study, a sequential optimization method was conducted to obtain an optimized medium for the lipase production by *Bacillus pumilus* B106 associated with sponge *Halichondria rugosa*. As a result, an overall 3.54-fold increase in enzyme activity and 1.31-fold increase in cell dry weight were obtained compared with that of the basal medium.

According to this study, the marine lipase from *Bacillus pumilus* B106 associated with the South China Sea sponge *Halichondria rugosa* displays stability in high salt concentration of psu 150‰. Analysis of optimum pH and pH stability of the lipase showed that the lipase exhibited maximum activity at pH 8.0 and the activity of the enzyme was stable between pH 7 and 9. The thermal stability of the enzyme was tested and the results showed that the enzyme exhibited high activity between 30 and 60°C. Therefore, the tolerance of this lipase to high salinity, pH and temperature will make it possible to be used in marine bioengineering field such as the production of marine organism-derived biodiesel. The influence of different organic solvents was tested because nearly all lipase-catalysed esterification reactions used in biotechnological applications are performed in non-aqueous systems. Among the tested organic solvents, methanol between 0 and 20% shows a positive influence on lipase activity. Whereas, the enzyme activity can be inhibited by 30–40% methanol, ethanol, 2-propanol, and DMSO, so, suitable solvent and concentration should be selected for the utilization of this lipase.

Lipase is an important group of biotechnologically relevant enzymes (Gupta et al. 2004). The challenges of the lipase industry include the discoveries of new lipases producers and novel lipase properties. Marine organism-derived lipases have application potential in related marine biotechnology field. Sponges with taxonomically diverse bacteria provide the potential for biotechnological utilization (Hentschel et al. 2006; Li et al. 2006). Based on this study and our previous studies (Han et al. 2008, 2009), sponge-associated microorganisms represent important sources for novel marine enzymes. According to the characteristics of the lipase mentioned above, this marine lipase from *Bacillus pumilus* B106 associated with South China Sea sponge *Halichondria rugosa* may have great potential in marine biotechnology industry. To our knowledge, this study is the first report on sponge-associated microbial lipase production, property and gene cloning. Practically, it provides us a valuable resource for marine lipase production. Meanwhile, in theory, it expends our understanding of the possible role of sponge-associated bacteria in the biotransformation of chemical compounds for the sponge host.

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