Ann Microbiol (2010) 60:429–437
DOI 10.1007/s13213-010-0060-1

ORIGINAL ARTICLE

Screening and identification of a highly lipolytic bacterial strain from barbecue sites in Hainan and characterization of its lipase

Qiongli Ma · Xuepiao Sun · Shufeng Gong · Jiaming Zhang

Received: 2 February 2010 / Accepted: 19 April 2010 / Published online: 19 May 2010

Abstract A cheap and rapid screening method for isolation of lipolytic bacteria was established. A total of 145 lipolytic strains were isolated from oil-contaminated soil samples at barbeque sites in Haikou, China. Highly lipolytic strains were screened based on the formation of clearance zones on turbid solid media supplemented with emulsified peanut oil. One strain, C737-11, had the highest lipolytic activity and was further analyzed. This strain had physiological and biochemical characteristics that were similar to the genus *Burkholderia*. Phylogenetic analysis based on the ferric uptake regulator (*fur*) gene sequences located the strain to the clade of *Burkholderia cepacia*, Genomovar I, while resolution of the phylogeny based on 16S rRNA sequences was not good enough to distinguish *Burkholderia* species. The fermentation conditions were optimized and the optimal medium inducing or supporting lipase production was 0.5% glucose, 2% peanut oil, 2% peptone, 0.1% $(NH_4)_2SO_4$, 0.1% $K_2HPO_4{\cdot}3H_2O$ and 0.05% $MgSO_4{\cdot}7H_2O$ at pH 8.0. The optimal fermentation temperature was 37°C, and the best fermentation time was 72 h. Under these optimal conditions, lipolytic activity reached 10.5 U mL^{-1}. The lipase produced by C737-11 was thermally stable and had an optimal reaction pH of 8.0 and an optimal reaction temperature of 37°C.

Keywords *Burkholderia cepacia* · Ferric uptake regulator · Lipase · Lipolytic bacteria · Plate screening

Q. Ma · X. Sun · S. Gong · J. Zhang (✉)
Institute of Tropical Bioscience and Biotechnology,
Chinese Academy of Tropical Agricultural Sciences (CATAS),
Haikou, Hainan 571101, China
e-mail: lab.zhang@hotmail.com

J. Zhang
Spice and Beverage Research Institute, CATAS,
Xinglong, Hainan 571533, China

Q. Ma
College of Agriculture, Hainan University,
Haikou, Hainan 571101, China

Introduction

Lipases (EC3.1.1.3) form a family of triglyceride ester hydrolases widely distributed among plants, animals, and microorganisms. They act on the ester bonds in triacylglycerol at the ester–water interface, resulting in the reversible hydrolysis of glycerol ester bonds and, thereby also catalyzing the synthesis of glycerol esters and alkyl esters (transesterification; Jaeger et al. 1994, 1999; Arpigny and Jaeger 1999). Lipases are well known in many industrial applications including their addition to detergents, the manufacture of food ingredients, pitch control in the pulp and paper industry, and biocatalysis of stereoselective transformations (Jaeger and Reetz 1998). The potential utilization of lipases in biodiesel production from oils and fats has received much attention (Hsu et al. 2002; Noureddini et al. 2005; Ying and Chen 2007). Lipases from different sources may have different catalytic characteristics and enantio-, chemo-, and stereo-selectivities towards their substrates (Holmquist 1998; Gupta et al. 2003), so that the characterization of these lipases may provide choices for different experimental and industrial applications.

In this paper, we describe direct screening of a highly lipolytic bacterial strain isolated from barbeque sites in Hainan and the properties of its lipase.

Materials and methods

Sample preparation

Six soil samples were collected from barbecue sites in Haikou, Hainan Province, China, where the ground soil has been contaminated continuously by oils and fats over several years and is rich in lipase-active bacteria and fungi. Soil suspensions were prepared by vigorously shaking approximately 10 g soil sample in 100 mL sterile water in a flask containing glass beads.

Preparation of emulsified oil

One volume of peanut oil or palm oil was mixed with four volumes of 5% polyvinyl alcohol solution (w/v), and the mixture was emulsified by shaking at a speed of 250 rpm overnight. The emulsion was autoclaved for 20 min at 121°C. The emulsion is stable for at least half a year at room temperature.

Enrichment of lipase-producing bacteria

The soil samples contained numerous microorganisms, both lipolytic and non-lipolytic, thus enrichment of the lipolytic microorganisms was necessary before screening. A series of media using oil as the main carbon source were tested. A medium including 0.05% peptone (w/v), 0.15% KH_2PO_4 (w/v), 0.1% Na_2HPO_4 (w/v), 0.05% $MgSO_4$ (w/v), 0.5% $(NH_4)_2SO_4$ (w/v), 0.05% NaCl (w/v) and 12% sterilized peanut oil emulsion (v/v), was found to be efficient. The pH was adjusted to 6.0, 7.0 or 7.8 for enrichment of different strains. The sterilized emulsified oil was added to the medium after autoclaving at 121°C for 20 min. A 2 mL sample of each soil suspension was added to 50 mL enrichment medium in 150 mL flasks. The flasks were incubated on a shaker at 200 rpm and 28°C or 37°C for 3 days. A 0.5 mL aliquot of the suspension was subcultured in 50 mL fresh liquid medium for a second 3 days of enrichment.

Screening of lipase-producing bacteria

Screening of lipolytic bacteria was based on the formation of clearance zones around the colonies grown on semi-solid turbid media. The medium contained 0.4% peptone, 0.1% $(NH_4)_2SO_4$, 0.15% KH_2PO_4, 0.05% $MgSO_4{\cdot}7H_2O$, 0.05% NaCl, 12% peanut oil emulsion, and 2% agar. The pH was adjusted to 6.0, 7.0 or 7.8. The screening medium was autoclaved at 121°C for 20 min. The plates were poured so that the medium was 2 mm thick.

The suspensions enriched in lipolytic organisms were diluted with sterilized water and plated on the screening media. The plates were incubated at 28°C or 37°C for 3 days. Lipase-producing colonies were indicated by the presence of clearance zones. Colonies with large clearance zones were selected for further purification by subculturing on the same medium several times until the morphology of colonies was uniform.

Taxonomical identification of lipolytic bacteria

Representative strains were identified according to their morphological, physiological and biochemical characteristics using standard procedures as described in the literature (Bergey et al. 1984; Boone et al. 2001)

The 16S rDNA of representative strains was amplified from genomic DNA by polymerase chain reaction (PCR) with the primers 5′-AGA GTT TGA TCC TGG CTC AG-3′ and 5′-AAG GAG GTG ATC CAG CCG CA-3′ (Wu and Shi 2008).The fragments were sequenced at Shanghai Sangon Biological Engineering Technology & Services (Shanghai, PR China). The sequences were analyzed with MacVector software (Oxford Molecular, Oxford, UK). BLAST searches using the 16S rDNA sequences as queries were performed against GenBank databases.

To further genotype the *Burkholderia* strain, 5′ ATG ACC AAT CCG ACC GAT CTC AA 3′ and 5′ TCA GTG CTT GCG ITN IGG GCA GTT 3′ were used to amplify the ferric uptake regulator (*fur*) gene fragment in bacteria (Lynch and Dennis 2008).

For phylogenetic analysis, 16S rRNA or the *fur* gene sequences of related taxa were obtained from GenBank databases and aligned with ClustalX 2.0 (Chenna et al. 2003). The alignment result was exported to Mega3.1 (Kumar et al. 2004). Phylogenetic trees were generated with Neighbor-Joining (NJ), Minimum Evolution (ME), and Maximum Parsimony (MP) methods with 1,000 bootstrap replicates. The trees were rooted with a 16S rDNA sequence from *Burkholderia gladioli* (X67038) for the 16S phylogeny, or with two *fur* gene sequences from *B. gladioli* (EU090888 and EU090889).

Optimization of fermentation conditions

The bacterial strain was inoculated in the growth medium overnight and subcultured in the lipase-induction medium supplemented with different combinations of carbon sources and nitrogen sources. The cultures were inoculated at 37°C for 72 h with shaking at 300 rpm. The growth medium contained 2% peptone, 0.1% $(NH_4)_2SO_4$, 0.1% $K_2HPO_4{\cdot}3H_2O$, and 0.05% $MgSO_4{\cdot}7H_2O$, at pH 7.0. The medium was autoclaved at 121°C for 20 min. The basal lipase-induction medium contained 2% peptone, 0.1% $(NH_4)_2SO_4$, 0.1% $K_2HPO_4{\cdot}3H_2O$, 0.05% $MgSO_4{\cdot}7H_2O$ and 2% emulsified peanut oil, at pH 7.0. The medium was autoclaved at 121°C for 20 min.

Lipase assay

Lipolytic activity was measured as described previously (Hamid et al. 2003), with minor modifications. Emulsified peanut oil mixture (4 mL) and 4 mL 0.1 M Tris-HCl (pH 7.8) were added to a 150 mL flask and mixed thoroughly; the mixture was incubated in a water bath at 37°C for 5 min, then 2 mL crude lipase (supernatant of culture medium) was added to the reaction mixture and incubated at 37°C for 20 min; 10 mL 95% ethanol was added to terminate the reaction. Phenolphthalein (1%) was then added to the mixture, which was titrated with 0.05 M NaOH until the end point was reached. One unit of lipase activity (U) is defined as the release of 1 μmol fatty acid per minute under the above conditions. The amount of fatty acid liberated and lipase activity were calculated accordingly.

Optimization of lipase assay conditions

Lipase activity was analyzed over a pH range of 5.0–11.0 and a temperature range of 20°C–80°C. The thermal stability of the lipase was assayed by incubating the supernatant of the culture medium containing lipase secreted by C737–11 at 50°C, 60°C and/or 70°C for 1 h; an aliquot was removed every 20 min to measure the remaining enzyme activity. pH stability experiments were carried out by incubating the supernatant of the culture medium in 0.1 M Tris-HCl (pH 6.0–11.0) at 37°C for 1 h, followed by measuring the residual lipase activity as described above at pH 7.8 and 37°C.

Data analysis

Single factor ANOVA was used to compare the effects of different carbon and nitrogen sources. To test the effects of different carbon sources on lipase induction from C737–11, 0.5% (w/v) sucrose, glucose, maltose, galactose, and mannitol were used as supplements to the basal lipase-induction medium. Nine replicates were performed. Peptone, soybean powder, yeast extract, beef extract, and corn powder were used to test the effects of different nitrogen sources on lipase induction: 2% (w/v) of each nitrogen source was used; four replicates were performed. Data were analyzed using SAS (Release 9.1.3). Duncan's test was performed to determine the significance of the differences between the group means.

Results and discussion

Isolation of lipase-producing bacteria

The method most commonly used to screen lipolytic bacteria is based on the formation of clear halos around colonies grown on tributyrin- or triolein-containing agar plates (Jaeger et al. 1994; Bora and Kalita 2007; Ertugrul et al. 2007). We developed a simple procedure that requires only household peanut oil or palm oil. Oils and fats are insoluble in water, so that a fine and stable oil emulsion is important to the subsequent medium preparation and enzyme activity assay. Some formulations and protocols have been tried for preparing an oil emulsion of good quality (San Clemente and Vadehra 1967; Sorokin and Jones 2009). The best formulation was achieved by suspending one volume of analytical pure olive oil in four volumes of 5% gellan gum (an emulsifier and stabilizer) by sonication on ice (Sorokin and Jones 2009). We used polyvinyl alcohol, which is more commonly available, as the emulsifier instead. One volume of peanut oil or palm oil was emulsified in four volumes of 5% polyvinyl alcohol solution (w/v) by shaking at a speed of 250 rpm overnight. The autoclaved peanut oil emulsion is stable for at least half a year at room temperature.

The turbid agar plates plus the enrichment medium supplemented with emulsified peanut oil or palm oil were effective in the enriching and screening of lipase-producing bacterial. After enrichment, most bacteria clones formed clearance zones on the turbid agar plates (Fig. 1a). The lipolytic bacterial strains were further purified by subculturing on turbid solid medium until the morphology of the clones was uniform. In total, 145 lipolytic bacterial strains were isolated from oil-contaminated soil samples.

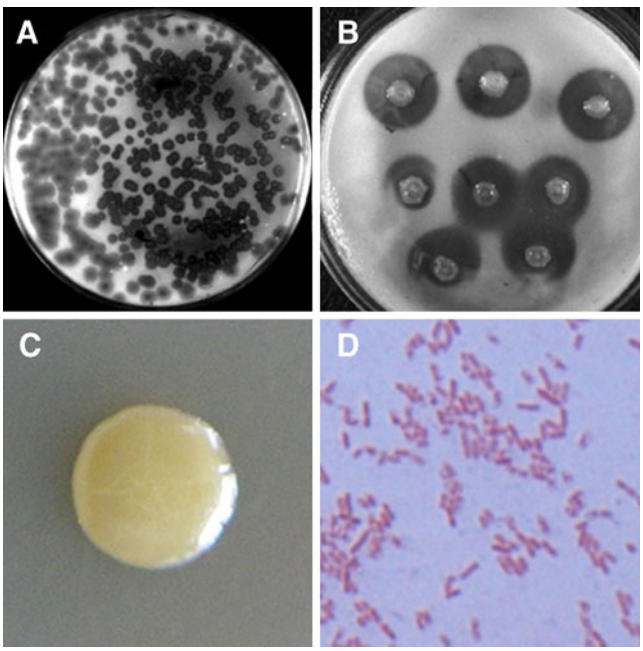

Fig. 1a–d Screening of lipolytic strains and morphology of C737-11. **a** A representative plate showing the screening method for lipolytic bacteria. **b** Clearance rings of strain C737-11 on solid turbid medium containing peanut oil. **c** Colonial morphology of C737-11. **d** Cell morphology of C737-11 after Gram staining

Table 1 Comparison of lipolytic activity of the representative bacteria strains

Strain	C7828-1	C737-11	H728-11	C7837-1	E7837-2	H728-4	F7828-1
Lipolytic activity (U mL^{-1})	1.19	3.01	1.30	0.67	1.20	1.75	1.07
Diameter of clearance zone (cm)	0.3	1.7	0.3	0.1	0.5	0.33	0.2

The lipolytic activity of the purified strains was determined by dotting the strains on solid turbid plates. Strain C737-11 produced the largest clearance zones among the strains tested (Fig. 1b). Representative strains were chosen for preliminary lipase production. All of the cultures showed good lipolytic activities (Table 1). Strain C737-11 exhibited a lipolytic activity as high as 3.01 U mL^{-1}, followed by strain H728-4 with an activity of 1.75 U mL^{-1}. Strains H728-11, E7837-2, C7828-1, and F7828-1 had moderate lipolytic activities of 1.30, 1.20, 1.19, and 1.07 U mL^{-1}, respectively. Strain C737-11 was selected for further analysis.

Classical and phylogenetic identification of strain C737–11

Colonies of C737-11 grown on Luria-Bertani agar plates were round, yellow and opaque with a wet, convex and smooth surface (Fig. 1c). No secreted pigments were observed in the medium. Conventional physiological and biochemical examinations showed that the cells were motile, short rods, Gram negative (Fig. 1d), aerobic, oxidase-negative, catalase-positive, methylred-positive, V-P-negative, indole-negative, denitrification-negative and positive to assay of gelatin liquefaction. C737-11 utilized glucose, sucrose, maltose, mannitol, soluble starch, or galactose as the sole carbon source for cell growth and lipase production. It also utilized various organic and inorganic nitrogen sources including ammonium sulfate, beef extract, peptone, yeast extract, soybean powder, and corn powder as the sole nitrogen source for growth and lipase production. It grew well at temperatures between 20 and 40°C. Based on these features, strain C737-11 was identified as *Burkholderia cepacia* complex species.

Burkholderia cepacia complex (BCC) is a group of Gram-negative bacteria composed of at least nine different species, including *B. cepacia*, *Burkholderia multivorans*, *Burkholderia cenocepacia*, *Burkholderia vietnamiensis*, *Burkholderia stabilis*, *Burkholderia ambifaria*, *Burkholderia dolosa*, *Burkholderia anthina*, and *Burkholderia pyrrocinia* (Lipuma 2005). Although BCC species are capable of causing life-threatening respiratory tract infections in persons with cystic fibrosis (Coenye et al. 2001; Mahenthiralingam et al. 2001; Magalhaes et al. 2002), studies from several countries reiterate that two species, *B. cenocepacia* and *B. multivorans*, account for most BCC infections in cystic fibrosis (Smalley et al. 2003; Lipuma 2005), while *B. dolosa* and *B. vietnamiensis* were occasionally isolated in cystic fibrosis (Coenye et al. 2001; Lipuma 2005). The BCC species are also of ecological importance and some species have been isolated from diverse environments

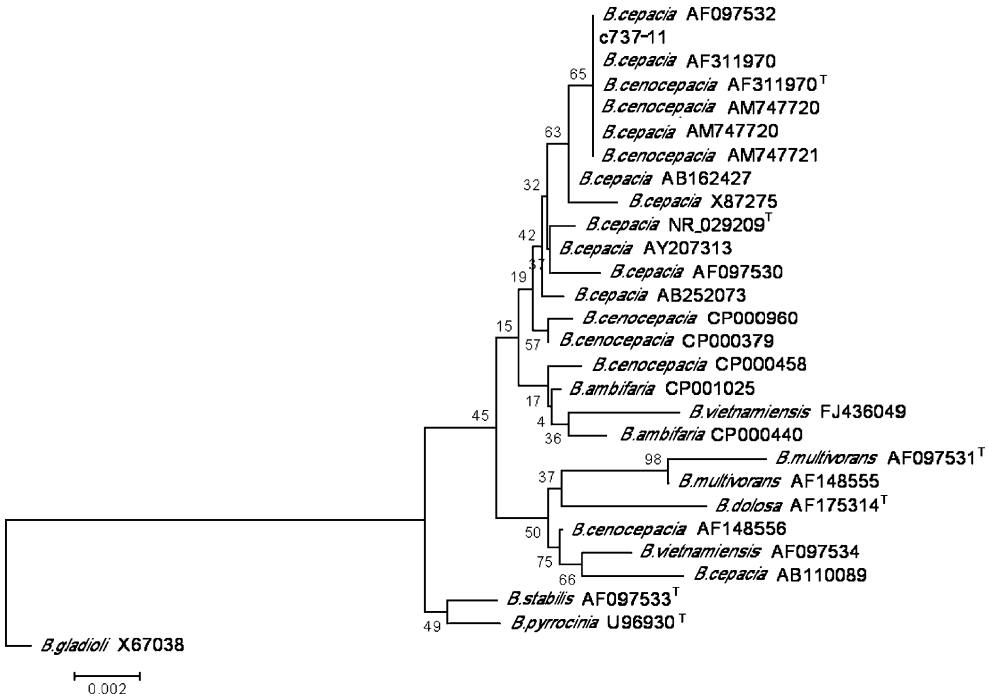

Fig. 2 Phylogenetic analysis of C737-11 based on 16S rDNA sequences. The tree was rooted with a 16S rDNA sequence of *Burkholderia gladioli* (X67038). The sequences were aligned with ClustalX 2.0, using the Blosum 30 matrix. The alignment result was exported to Mega3.1. Phylogenetic trees were calculated with Neighbour-Joining (NJ), Minimum Evolution (ME) and Maximum Parsimony (MP) methods. Only the phylogeny calculated with the NJ method is presented in this figure. The bootstrap values provided on the branches were calculated using the NJ method with 1,000 replicates

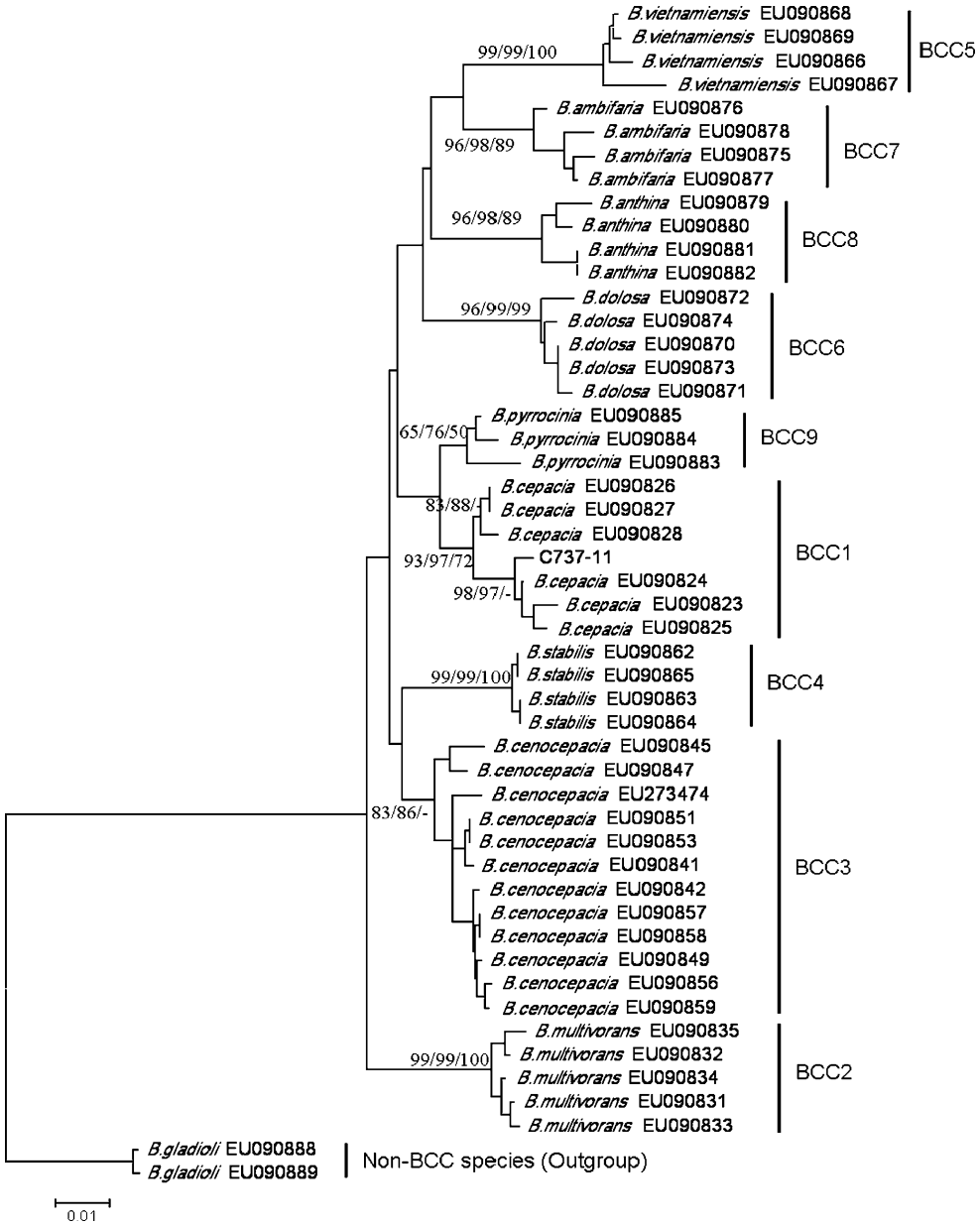

Fig. 3 Phylogenetic analysis of C737-11 based on ferric uptake regulator (*fur*) gene sequences. The tree was rooted with (*fur*) gene sequences from *Burkholderia gladioli* (EU090888, EU090889). The sequences were aligned with ClustalX 2.0, using the Blosum 30 matrix. The alignment result was exported to Mega3.1. Phylogenetic trees were calculated with NJ, ME and MP methods. The topologies of trees generated with different methods were similar. The topology presented in this figure was generated with the NJ method. The bootstrap values provided on the branches were calculated using the NJ, ME, and MP methods with 1,000 replicates, respectively

Table 2 Effect of carbon and nitrogen sources on lipase production. Data presented as mean ± standard derivation of percentage activity compared to the basal lipase-induction medium

Carbon source	Relative activity (%, n=9)	Nitrogen source	Relative activity (%, n=4)
BC + maltose	350±69*	Peptone	100±4
BC + glucose	342±58*	Soybean powder	71±8***
BC + galactose	211±20*	Yeast extract	62±9***
BC + mannitol	186±14**	Soybean and corn powder	38±2***
BC + sucrose	154±10	Beef extract	35±6***
BC[a]	100±4	Corn powder	18±1***

*Significant vs BC by Duncan's test, P<0.01, **Significant vs BC, P<0.05, ***Significant vs peptone by Duncan's test, P<0.01

[a] Basal carbon source (2% peanut oil)

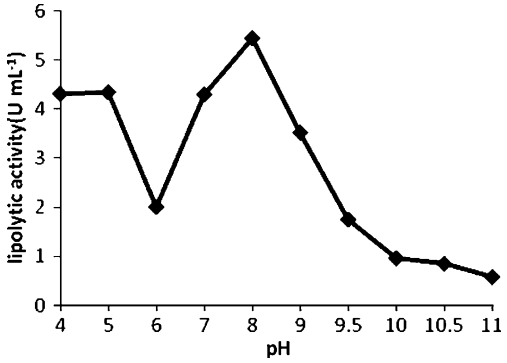

Fig. 4 Effect of initial pH on lipase production

including soil, water, and plants (Singh et al. 2006; Bartholdson et al. 2008; Jacobs et al. 2008).

To identify which species strain C737-11 belongs to, the 16S rDNA fragment was amplified from its genomic DNA and sequenced. BLAST searches in the GenBank databases revealed 99.9% identities with BCC Genomovar III, *B. cenocepacia* strain LMG 12614 (AF311970.1), and BCC Genomovar I, *B. cenocepacia* strains ATCC700070 (AY741360) and ATCC17759 (AY741334). Phylogenetic analysis based on the 16S rRNA sequences showed that C737-11 was closely related to *B. cepacia* and *B. cenocepacia* (Fig. 2). Unfortunately, the resolution of the phylogeny was not good enough to distinguish *B. cepacia* and *B. cenocepacia*, and both *B. cepacia* and *B. cenocepacia* were dispersed in several clades in the phylogenetic tree, with very low bootstrap supports, similar to previous reports (LiPuma et al. 1999; Mahenthiralingam et al. 2000; Brown and Govan 2007; Lynch and Dennis 2008).

The *fur* gene sequence was reported to be more effective in rapid differentiation of BCC species (Lynch and Dennis 2008). Therefore, the *fur* gene fragment from C737-11 was amplified and sequenced. The *fur* gene in the *Burkholderia* genus is more divergent than the 16S gene. BLAST searches with the *fur* gene sequence against the GenBank databases resulted in the highest identities of 99% with *B. cepacia* type strain ATCC 25416. Phylogenetic trees calculated with NJ, ME and MP methods clearly separated the BCC species into nine clades (Fig. 3), and C737-11 was located in the clade of *B. cepacia* (Genomovar I, BCC1) with bootstrap support of 93%, 97% and 72% as calculated with NJ, ME and MP methods, respectively. Thus, we concluded C737-11 is a *Burkholder cepacia* strain.

Burkholderia cepacia strains have been isolated from a variety of habitats, including naturally growing surface-sterilized roots (Singh et al. 2006; Mendes et al. 2007), stems (Mendes et al. 2007), field soil (Jacobs et al. 2008), water (Koenig et al. 1995), and even machines (Vaisanen et al. 1998). They are not only conditionally pathogenic to plants and animals, but also promote biomass accumulation and grain yield in greenhouse-grown rice plants inoculated with some isolates (Singh et al. 2006), and/or serve as antagonistic bacteria (Mu et al. 2008).

Optimization of lipase-induction conditions for C737-11

Lipases from different *B. cepacia* strains may have different enzymatic properties that are useful for different applications (Yang et al. 2002; Noureddini et al. 2005; Turner et al. 2006). To induce lipase production from C737-11 for

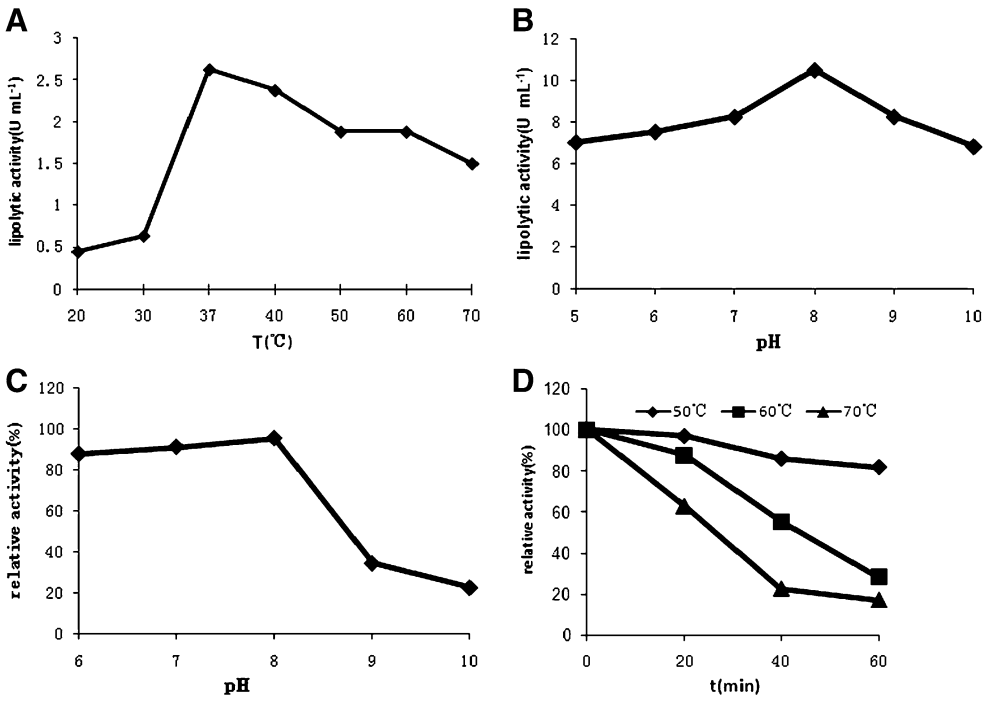

Fig. 5a–d Properties of the lipase produced by C737-11. **a** Effect of reaction temperature on enzyme activity. **b** Effect of pH on enzyme activity. **c** Stability of the enzyme at different pH values. **d** Stability of the enzyme at different temperatures

enzymatic analysis, the fermentation conditions were optimized.

The production of extracellular lipases is often promoted by the presence of lipids in the growth medium (Gupta et al. 2004; Kiran et al. 2008; Yan and Yan 2008). When 2% peanut oil was added to the basal growth medium, the lipolytic activity of the culture medium increased by 284%. A readily available carbon source in the medium may repress lipase production (Kiran et al. 2008). However, when 0.5% sucrose was added to the basal lipase-induction medium containing 2% peanut oil, the lipolytic activity of C737-11 increased by 154%. The addition of 0.5% maltose, glucose, manitol, or galactose enhanced lipase production by 350%, 342%, 186%, or 221%, respectively (Table 2). Glucose was then used to promote lipase production in further experiments.

A nitrogen source is an indispensable nutritional component for microbial growth. Peptone was used as the nitrogen source in both growth and lipase-induction media generally in this research. Other nitrogen sources were also tested, however, and when peptone was replaced by yeast extract, beef extract, soybean powder, or corn powder, lipase production decreased significantly (Table 2). Therefore, peptone was selected as the optimal nitrogen source.

The pH of the medium is also an important factor influencing lipase production (Papon and Talon 1988; Ghosh et al. 1996; Shariff et al. 2007). The optimal initial pH for C737-11 was 8.0 (Fig. 4), which is relatively high when compared to other lipase producing *Pseudomonas* sp. (pH 5.5; Sarkar et al. 1998), and low when compared to *Burkholderia* sp. C20 (pH 9.0; Liu et al. 2006) and HY-10 (pH 8.5; Park et al. 2007).

In summary, the optimal lipase producing medium was 0.5 % glucose, 2% peanut oil, 2% peptone, 0.1% $(NH_4)_2SO_4$, 0.1% $K_2HPO_4{\cdot}3H_2O$, and 0.05% $MgSO_4{\cdot}7H_2O$, at pH 8.0. Under optimal conditions, which included a fermentation temperature of 37°C and fermentation time of 72 h, lipase production reached 10.5 U mL^{-1}.

Properties of the lipase produced by C737-11

Different *Burkholderia* strains produce lipases with different enzymatic properties. The lipase produced by C737-11 had its highest activity at 37°C. It was sensitive to low temperatures; at 30°C it had only 20% of its highest activity. This enzyme was relatively active at higher temperatures, and maintained about 70%, 65%, and 50% of its highest activity at 50°C, 60°C, and 70°C, respectively (Fig. 5a). The optimal reaction temperature for one previously reported *Burkholderia* strain, G63 was 37°C (Yang et al. 2007), and for strains HY-10 and C20, it was 60°C and 55°C, respectively (Liu et al. 2006; Park et al. 2007), while the lipase from another *Burkholderia* strain had a low optimal reaction temperature of 25°C (Liu et al. 2009).

Most bacterial lipases have neutral or alkaline pH optima (Gupta et al. 2004). The optimal reaction pH for lipases produced by different *Burkholderia* strains was similar with slight variations. The optimal pH for the lipase produced by C737-11 was pH 8.0 (Fig. 5b), similar to a previously reported *Burkholderia* strain GXU56 (Wei and Wu 2008). The optimal pH for *Burkholderia* sp. strains HY-10, C20, and 34–5 was 8.5, 9.0 and 9.6, respectively (Liu et al. 2006, 2009; Park et al. 2007).

Generally, bacterial lipases are stable over a wide pH range (Yadav et al, 1998; Dong et al. 1999; Gupta et al. 2004; Hasan et al. 2009). Lipase produced by C737-11 was stable between pH 6.0 and 8.0 (Fig. 5c), and the activity dropped sharply after incubation at pH 9.0 and 10.0 (Fig. 5c). The lipase retained 97%, 88%, and 63 % of its original activity after incubation at 50, 60, and 70°C, respectively for 20 min (Fig. 5d), and 86%, 55%, and 23 % of its original lipase activity was retained after being incubated at 50, 60 and 70°C, respectively, for 40 min, suggesting that the lipase produced by C737-11 was moderately thermally stable. Little data on the thermal stability of other *Burkholderia* strains are available. The lipase from a *Burkholderia* strain RGP-10 was stable at 50°C (Rathi et al. 2001). More stable lipases were reported from the thermophilic *Bacillus* sp. J33, which was stable for 12 h at 60°C (Nawani and Kaur 2000) and from *Aspergillus terreus*, which retained 100% activity after incubating at 60°C for 24 h (Yadav et al. 1998). An extremely thermostable lipase was reported from *Bacillus stearothermophilus* SB-1, which had a half-life of 25 min at pH 3.0 and 15 min at pH 6.0 at 100°C (Bradoo et al. 1999).

Acknowledgment This work was supported by a National Nonprofit Institute Research Grant of CATAS-ITBB (Grant No. ITBBZX0843 and ITBBZX0943) and the International Science and Technology Cooperation Program of China (2010DFA62040).

References

Arpigny JL, Jaeger KE (1999) Bacterial lipolytic enzymes: classification and properties. Biochem J 343:177–183

Bartholdson SJ, Brown AR, Mewburn BR, Clarke DJ, Fry SC, Campopiano DJ, Govan JR (2008) Plant host and sugar alcohol induced exopolysaccharide biosynthesis in the *Burkholderia cepacia* complex. Microbiology 154:2513–2521

Bergey DH, Krieg NR, Holt JG (1984) Bergey's manual of systematic bacteriology. Williams & Wilkins, Baltimore

Boone DR, Castenholz RW, Garrity GM (eds) (2001) Bergey's manual of systematic bacteriology, 2nd edn, vol 1. The Archaea and the deeply branching and phototrophic bacteria. Springer, New York

Bora L, Kalita MC (2007) Production and optimization of thermostable lipase from a thermophilic *Bacillus* sp LBN 4 The Internet Journal of Microbiology, 4. http://www.ispub.com/ostia/index.php?xmlFilePath=journals/ijmb/vol4n1/bacillus.xml

Bradoo S, Saxena PK, Gupta R (1999) Two acidothermotolerant lipases from new variants of *Bacillus* spp. World J Microbiol Biotechnol 15:87–91

Brown AR, Govan JR (2007) Assessment of fluorescent in situ hybridization and PCR-based methods for rapid identification of *Burkholderia cepacia* complex organisms directly from sputum samples. J Clin Microbiol 45:1920–1926

Chenna R, Sugawara H, Koike T, Lopez R, Gibson TJ, Higgins DG, Thompson JD (2003) Multiple sequence alignment with the Clustal series of programs. Nucleic Acids Res 31:3497–3500

Coenye T, LiPuma JJ, Henry D, Hoste B, Vandemeulebroecke K, Gillis M, Speert DP, Vandamme P (2001) *Burkholderia cepacia* genomovar VI, a new member of the *Burkholderia cepacia* complex isolated from cystic fibrosis patients. Int J Syst Evol Microbiol 51:271–279

Dong H, Gao S, Han S, Cao S (1999) Purification and characterization of a *Pseudomonas* sp. lipase and its properties in non-aqueous media. Biotechnol Appl Biochem 30:251–256

Ertugrul S, Donmez G, Takac S (2007) Isolation of lipase producing *Bacillus* sp. from olive mill wastewater and improving its enzyme activity. J Hazard Mater 149:720–724

Ghosh PK, Saxena RK, Gupta R, Yadav RP, Davidson S (1996) Microbial lipases: production and applications. Sci Prog 79:119–157

Gupta R, Rathi P, Gupta N, Bradoo S (2003) Lipase assays for conventional and molecular screening: an overview. Biotechnol Appl Biochem 37:63–71

Gupta R, Gupta N, Rathi P (2004) Bacterial lipases: an overview of production, purification and biochemical properties. Appl Microbiol Biotechnol 64:763–781

Hamid SA, Zen HB, Tein OB, Halifah YM, Saari N, Bakar FA (2003) Screening and identification of extracellular lipase-producing thermophilic bacteria from a Malaysian hot spring. World J Microbiol Biotechnol 19:961–968

Hasan F, Shah AA, Hameed A (2009) Methods for detection and characterization of lipases: a comprehensive review. Biotechnol Adv 27:782–798

Holmquist M (1998) Insights into the molecular basis for fatty acyl specificities of lipases from *Geotrichum candidum* and *Candida rugosa*. Chem Phys Lipids 93:57–66

Hsu AF, Jones K, Foglia TA, Marmer WN (2002) Immobilized lipase-catalysed production of alkyl esters of restaurant grease as biodiesel. Biotechnol Appl Biochem 36:181–186

Jacobs JL, Fasi AC, Ramette A, Smith JJ, Hammerschmidt R, Sundin GW (2008) Identification and onion pathogenicity of *Burkholderia cepacia* complex isolates from the onion rhizosphere and onion field soil. Appl Environ Microbiol 74:3121–3129

Jaeger KE, Reetz MT (1998) Microbial lipases form versatile tools for biotechnology. Trends Biotechnol 16:396–403

Jaeger KE, Ransac S, Dijkstra BW, Colson C, van Heuvel M, Misset O (1994) Bacterial lipases. FEMS Microbiol Rev 15:29–63

Jaeger KE, Dijkstra BW, Reetz MT (1999) Bacterial biocatalysts: molecular biology, three-dimensional structures, and biotechnological applications of lipases. Annu Rev Microbiol 53:315–351

Kiran GS, Shanmughapriya S, Jayalakshmi J, Selvin J, Gandhimathi R, Sivaramakrishnan S, Arunkumar M, Thangavelu T, Natarajaseenivasan K (2008) Optimization of extracellular psychrophilic alkaline lipase produced by marine *Pseudomonas* sp. (MSI057). Bioprocess Biosyst Eng 31:483–492

Koenig DW, Mishra SK, Pierson DL (1995) Removal of *Burkholderia cepacia* biofilms with oxidants. Biofouling 9:51–62

Kumar S, Tamura K, Nei M (2004) MEGA3: integrated software for molecular evolutionary genetics analysis and sequence alignment. Brief Bioinform 5:150–163

LiPuma JJ (2005) Update on the *Burkholderia cepacia* complex. Curr Opin Pulm Med 11:528–533

LiPuma JJ, Dulaney BJ, McMenamin JD, Whitby PW, Stull TL, Coenye T, Vandamme P (1999) Development of rRNA-based PCR assays for identification of *Burkholderia cepacia* complex isolates recovered from cystic fibrosis patients. J Clin Microbiol 37:3167–3170

Liu CH, Lu WB, Chang JS (2006) Optimizing lipase production of *Burkholderia* sp. by response surface methodology. Process Biochem 41:1940–1944

Liu RJ, Wang HK, Lu FP, Qi W (2009) Screening and optimization of fermentation medium of bacterium producing cold-adapted and alkaline lipase. J Tianjin Univ Sci Technol 24:6–10

Lynch KH, Dennis JJ (2008) Development of a species-specific fur gene-based method for identification of the *Burkholderia cepacia* complex. J Clin Microbiol 46:447–455

Magalhaes M, de Britto MC, Vandamme P (2002) *Burkholderia cepacia* genomovar III and *Burkholderia vietnamiensis* double infection in a cystic fibrosis child. J Cyst Fibros 1:292–294

Mahenthiralingam E, Bischof J, Byrne SK, Radomski C, Davies JE, Av-Gay Y, Vandamme P (2000) DNA-Based diagnostic approaches for identification of *Burkholderia cepacia* complex, *Burkholderia vietnamiensis*, *Burkholderia multivorans*, *Burkholderia stabilis*, and *Burkholderia cepacia* genomovars I and III. J Clin Microbiol 38:3165–3173

Mahenthiralingam E, Vandamme P, Campbell ME, Henry DA, Gravelle AM, Wong LT, Davidson AG, Wilcox PG, Nakielna B, Speert DP (2001) Infection with *Burkholderia cepacia* complex genomovars in patients with cystic fibrosis: virulent transmissible strains of genomovar III can replace *Burkholderia multivorans*. Clin Infect Dis 33:1469–1475

Mendes R, Pizzirani-Kleiner AA, Araujo WL, Raaijmakers JM (2007) Diversity of cultivated endophytic bacteria from sugarcane: genetic and biochemical characterization of *Burkholderia cepacia* complex isolates. Appl Environ Microbiol 73:7259–7267

Mu Z, Lu G, Ji X, Gai Y, Wang Y, Gao H, Cha C (2008) Identification and colonization of an antagonistic endophytic *Burkholderia cepacia* Lu10–1 isolated from mulberry. Wei Sheng Wu Xue Bao 48:623–630

Nawani N, Kaur J (2000) Purification, characterization and thermostability of lipase from a thermophilic *Bacillus* sp. J33. Mol Cell Biochem 206:91–96

Noureddini H, Gao X, Philkana RS (2005) Immobilized *Pseudomonas cepacia* lipase for biodiesel fuel production from soybean oil. Bioresour Technol 96:769–777

Papon M, Talon R (1988) Factors affecting growth and lipase production by meat lactobacilli strains and *Brochothrix thermosphacta*. J Appl Bacteriol 64:107–115

Park DS, Oh HW, Heo SY, Jeong WJ, Shin DH, Bae KS, Park HY (2007) Characterization of an extracellular lipase in *Burkholderia* sp. HY-10 isolated from a longicorn beetle. J Microbiol 45:409–417

Rathi P, Saxena RK, Gupta R (2001) A novel alkaline lipase from Burkholderia cepacia for detergent formulation. Process Biochem 37:187–192

San Clemente CL, Vadehra DV (1967) Instrumental assay of microbial lipase at constant pH. Appl Microbiol 15:110–113

Sarkar S, Sreekanth B, Kant S, Banerjee R, Bhattacharyya BC (1998) Production and optimization of microbial lipase. Bioprocess Biosyst Eng 19:29–32

Shariff FM, Leow TC, Mukred AD, Salleh AB, Basri M, Rahman RN (2007) Production of L2 lipase by *Bacillus* sp. strain L2: nutritional and physical factors. J Basic Microbiol 47:406–412

Singh RK, Mishra RP, Jaiswal HK, Kumar V, Pandey SP, Rao SB, Annapurna K (2006) Isolation and identification of

natural endophytic rhizobia from rice (*Oryza sativa* L.) through rDNA PCR-RFLP and sequence analysis. Curr Microbiol 52:117–122

Smalley JW, Charalabous P, Hart CA, Silver J (2003) Transmissible *Burkholderia cepacia* genomovar IIIa strains bind and convert monomeric iron(III) protoporphyrin IX into the mu-oxo oligomeric form. Microbiology 149:843–853

Sorokin DY, Jones BE (2009) Improved method for direct screening of true lipase-producing microorganisms with particular emphasis on alkaline conditions. Mikrobiologiia 78:144–149

Turner C, Wani S, Wong R, Lin JT, McKeon T (2006) Lipase-catalyzed esterification of 2-monoricinolein for 1, 2 (2, 3)-diricinolein synthesis. Lipids 41:77–83

Vaisanen OM, Weber A, Bennasar A, Rainey FA, Busse HJ, Salkinoja-Salonen MS (1998) Microbial communities of printing paper machines. J Appl Microbiol 84:1069–1084

Wei HN, Wu B (2008) Screening and immobilization Burkholderia sp. GXU56 lipase for enantioselective resolution of (R, S)-methyl mandelate. Appl Biochem Biotechnol 149:79–88

Wu W, Shi B (2008) Isolation and screening of thermostable lipase-producing bacterium FS1403 and analysis of its 16S rDNA gene sequence. Pharm Biotechnol 15:6–10

Yadav RP, Saxena RK, Gupta R, Davidson WS (1998) Purification and characterization of a regiospecific lipase from *Aspergillus terreus*. Biotechnol Appl Biochem 28:243–249

Yan JY, Yan YJ (2008) Optimization for producing cell-bound lipase from *Geotrichum* sp. and synthesis of methyl oleate in micro-aqueous solvent. Appl Microbiol Biotechnol 78:431–439

Yang J, Koga Y, Nakano H, Yamane T (2002) Modifying the chain-length selectivity of the lipase from *Burkholderia cepacia* KWI-56 through in vitro combinatorial mutagenesis in the substrate-binding site. Protein Eng 15:147–152

Yang J, Guo D, Yan Y (2007) Cloning, expression and characterization of a novel thermal stable and short-chain alcohol tolerant lipase from *Burkholderia cepacia* strain G63. J Mol Catal, B Enzym 45:91–96

Ying M, Chen G (2007) Study on the production of biodiesel by magnetic cell biocatalyst based on lipase-producing *Bacillus subtilis*. Appl Biochem Biotechnol 137–140:793–803