African Journal of Biotechnology Vol. 10(20), pp. 4147-4156, 16 May, 2011
Available online at http://www.academicjournals.org/AJB
DOI: 10.5897/AJB10.1905
ISSN 1684–5315 © 2011 Academic Journals

Full Length Research Paper

Screening and optimization of extracellular lipases by *Acinetobacter* species isolated from oil-contaminated soil in South Korea

Periasamy Anbu#*, Myoung-Ju Noh#, Da-Hye Kim, Jun-Seok Seo, Byung-Ki Hur* and Kyeong Ho Min

Department of Biological Engineering, College of Engineering, Inha University, Incheon 402-751, Republic of Korea.

Accepted 6 December, 2010

A total of 53 strains of bacteria were isolated from oil contaminated soil collected in South Korea. The isolated bacteria were screened using spirit blue agar and Rhodamine-B agar media. Two of the isolated strains exhibited a greater clear zone than the others, indicating higher lipase activity. Therefore, these two strains (BK43 and BK44) were selected and identified based on their morphological and physiological characteristics. The 16S rRNA gene sequencing was also implemented. Phylogenetic analyses based on the results of 16S rRNA gene sequencing revealed that BK43 and BK44 were close in identity to *Acinetobacter junii*. The optimum pH and temperature for lipase production by BK43 were found to be 6.0 at 30°C, after 24 h of incubation, while BK44 were found to be 6.0 at 25°C, after 12 h of incubation. In addition, increased enzymatic production was obtained when the organisms were cultured in medium supplemented with 1% sucrose as the carbon source. Among the different lipase inducers tested, both strains utilized Tween 80 and produced a great level of extracellular lipase. Overall, the results of the present study demonstrate that the genus *Acinetobacter* is good for extracellular lipase production under acidic conditions.

Key words: *Acinetobacter,* lipase, optimization, screening.

INTRODUCTION

Lipases are triacylglycerol acylhydrolases (EC 3.1.1.3) that catalyze the hydrolysis of triacylglycerol to glycerol and fatty acids (Sharma et al., 2001). Lipases are ubiquitous in nature and produced by animals, plants and microorganisms. Currently, microbial lipases are receiving a great deal of attention due to their potential for use in industrial processes. Most commercial extracellular products are obtained from microorganisms and have the ability to catalyze a wide variety of reactions in aqueous and non-aqueous phases (Saxena et al., 2003). Microbial lipases have also received more attention due to their selectivity, stability and substrate specificity (Treichel et al., 2010). Particularly, extracellular bacterial lipases are of commercial importance in the food, detergent, cosmetic and pharmaceutical industries, as well as inprocesses involving organic synthesis and fat/oil degradation (Jaeger et al., 1994).

Extracellular lipase production by bacteria are influenced by the composition of the growth medium, cultivation conditions and many physico-chemical (pH and temperature) and nutritional factors (carbon, nitrogen and lipid sources) (Jaeger et al., 1994). Most bacterial lipases are generally induced in medium that contains the proper fatty acids and oils (Joseph et al., 2006; Immanuel et al., 2008; Kiran et al., 2008). However, a few investigators have reported that the activity of lipase produced by *Pseudomonas aeruginosa* EF2 and *Acinetobacter calcoaceticus* was repressed by the presence of a lipid source in the medium (Gilbert et al., 1991; Mahler et al., 2000). In addition, Lin et al. (2006) have found that

*Corresponding author. E-mail: anbu25@yahoo.com, biosys@inha.ac.kr. Tel: +82-32-860-7512. Fax: +82-32-872-4046.

#These authors contributed equally to this work.

vitamins influenced lipase production by the edible Basidiomycetes, *Antrodia cinnamomea*.

A variety of extracellular lipases of bacterial origin with different properties and specificities have been described and characterized. Extracellular lipase was isolated from many different bacterial species, including *Bacillus* (Ertugrul et al., 2007) and *Pseudomonas* (Kiran et al., 2008; Wang et al., 2009). Additionally, many studies have been con-ducted to evaluate lipase production under alkaline condi-tions (Chen et al., 1998; Kasana et al., 2008; Kiran et al., 2008; Wang et al., 2009) but few studies have focused on acidic lipases (Bradoo et al., 1999; Liu et al., 2007).

Lipase production by *Acinetobacter radioresistens* under alkaline conditions in the presence of n-hexadecane was evaluated (Chen et al., 1998). In recent years, most studies conducted to improve lipase production have focused on their production by one important genus, *Acinetobacter* (Chen et al., 1998; Dharmsthiti et al., 1998). *Acinetobacter* strains have been isolated from a variety of sources, including soil (Bompensieri et al., 1996) and water (Blaise and Armstrong, 1973). However, industries are still seeking strains of bacteria that produce a high yield of potent lipase with excellent properties using cost-effective methods. Therefore, the present study was conducted to isolate novel lipase producing bacteria, after having the isolated strains identified and the culture conditions for optimal production of extracellular lipase determined.

MATERIALS AND METHODS

Materials

Gum arabic, p-nitrophenyl palmitate, sodium deoxycholate and tributyrin were purchased from Sigma (USA). Tween 80 was obtained from Duchefa, Biochemica (The Netherlands). All other chemicals used in this study were of analytical grade.

Isolation and screening of lipase-producing microorganisms

Fifty three strains of bacteria were isolated from oil contaminated soil collected in South Korea using serial dilution. The isolates were maintained on LB agar plates at 4°C and stored at -80°C in glycerol. The isolated bacteria were screened for the production of lipase using spirit blue agar containing lipase reagent as Marshall (1992) method. The lipolytic activities of all isolates were then compared by measuring the width of the areas of clearing or areas of deep blue color around the colonies. In addition, the same strains were screened using the Rhodamine-B method (Kouker and Jaeger, 1987). The bacterial strains were inoculated on the agar plates, after which the lipolytic activity was determined by the formation of an orange fluorescent zone around the fungal colonies that was visible upon UV irradiation (350 nm).

The liquid culture medium (Tryptic soy broth) used for lipase production contained the following (g/L): Pancreatic casein, 17; enzymatic digest soybean, 3; NaCl, 5; dipotassium phosphate, 2.5; glucose, 2.5; pH 7.5. To produce the lipase, an Erlenmeyer flask (250 ml) containing 50 ml of medium was inoculated with an aliquot of approximately 1% of the preculture prepared in LB broth (g/L): Tryptone, 10; yeast extract, 5; NaCl, 10; pH 7.0. The inoculated flasks were then incubated at room temperature with constant shaking at 180 rpm. Then, the cell-free supernatant was recovered by centrifugation (10,000 rpm, 10 min at 4°C) and the clear supernatant was used to determine the lipase activity. The growth of the microorganisms was then determined by measuring the absorption at 600 nm.

Identification of the microorganisms

The lipase producing bacteria was identified by morphological and biochemical characterizations. A gram (positive/negative) stain reaction was conducted using the Biomerieux system according to the manufacturer's instructions. The biochemical tests were conducted using the API 20NE system according to the manufacturer's protocols (Biomerieux). The identification was further confirmed by the 16S rRNA gene sequencing method. Briefly, genomic DNA was extracted using a genomic purification kit (Promega, USA). The DNA was then amplified by PCR using the following universal 16S rRNA gene primers, 8-27F: 5'-AGAGTTTGATCCTGGCTCAG-3' and 1472R: 5'-TACGGYTACCTTGTTACGACTT-3'. PCR was conducted by subjecting a reaction mixture to initial denaturation at 94°C for 5 min, followed by 35 cycles of 94°C for 45 s, 55°C for 1 min, 72°C for 1 min and a final extension step at 72°C for 10 min.

Phylogenetic analysis

The 16S rRNA gene sequence was compared with sequences available in the nucleotide database using the BLAST algorithm at the NCBI. Phylogenetic tree was constructed using the neighbor joining method (http://www.phylogeny.fr).

Fatty acid analysis

To analyze total cellular fatty acid content, the bacterial cells were cultured at 30°C for 24 h, after which the fatty acid profiles were determined based on the method described by Lepage and Roy (1984) using a gas chromatograph (Hewlett Packard 6890, USA) equipped with a flame-ionized detector (FID) and a DB23 (30 m × 0.25 mm × 0.26 µm, Agilent Technologies, USA) capillary column.

Lipase assay

Lipase activity was assayed quantitatively using 4-nitrophenyl palmitate as the substrate according to the method described by Winkler and Stuckmann (1979). Briefly, 10 ml of isopropanol containing 30 mg of 4-nitrophenyl palmitate (pNPP) was mixed with 90 ml of 0.05 M phosphate buffer (pH 8.0) containing 207 mg of sodium deoxycholate and 100 mg of gum arabic. A total of 2.4 ml of freshly prepared substrate solution was then pre-warmed at 37°C and mixed with 0.1 ml of enzyme solution. After incubation at 37°C for 15 min, the absorbance was measured at 410 nm against an enzyme free control. One enzyme unit was defined as the amount of enzyme that liberated 1 µmol of 4-nitrophenol per minute under the assay conditions.

Optimization of lipase production

Initially, the lipase production was determined after culture in tryptic soy broth from 6 to 48 h at 28°C. The lipase production was then evaluated at different pHs ranging from 5.0 to 10.0 and at tempera-

Table 1. Screening isolated bacterial strains for extracellular lipase.

Isolates No.	Sprit blue agar (mm)	Rhodamine-B agar (mm)	Isolate No.	Sprit blue agar (mm)	Rhodamine-B agar (mm)
BK01	-	++	BK28	-	-
BK02	++	-	BK29	+	-
BK03	++	+	BK30	++	+
BK04	-	-	BK31	+	+++
BK05	-	++	BK32	++	+
BK06	+	++	BK33	-	+
BK07	++	++	BK34	-	++
BK08	-	+	BK35	-	-
BK09	-	++	BK36	++	+++
BK10	+	+	BK37	-	-
BK11	-	-	BK38	++	+
BK12	-	-	BK39	-	++
BK13	-	-	BK40	-	-
BK14	-	+	BK41	++	+
BK15	-	-	BK42	+	-
BK16	-	-	BK43	+++	+++
BK17	-	+	BK44	+++	+++
BK18	++	+	BK45	-	-
BK19	-	-	BK46	-	-
BK 20	++	-	BK47	-	-
BK21	-	-	BK48	+	-
BK22	+	-	BK49	++	+
BK23	+	-	BK50	-	-
BK24	+	-	BK51	+	-
BK25	++	+	BK52	++	++
BK26	-	++	BK53	-	+
BK27	++	++			

+++, High activity (above 50 mm); ++, moderate activity (above 25 to below 50 mm); +, low activity (below 25 mm); -, no activity.

tures of 20, 25, 30, 37 and 45°C. The pH of the medium was adjusted prior to autoclaving. Further, the changes in lipase production in response to the following carbon sources (1%) were evaluated: glucose (control), fructose, xylose, maltose, lactose, sucrose, mannitol and starch. The carbon sources were sterilized separately and then aseptically added to the autoclaved medium. The following substances were used as lipase inducers (1%): olive oil, sesame oil, soybean oil, tributyrin and Tween 80. Medium that contained no lipase inducer was used as a control.

RESULTS AND DISCUSSION

Isolation, screening and identification of lipase producing bacteria

A total of 53 distinct morphological bacterial strains were isolated from oil-contaminated soil in South Korea. The isolated strains were screened for extracellular lipase using spirit blue agar and Rhodamine-B agar media. Two of the isolates produced a larger clear zone than the others, indicating higher lipase activity. These two strains that produced blue color around bacterial colonies, were grown on spirit blue agar medium due to the hydrolysis of tributyrin and Tween 80. Furthermore, a fluorescent zone on Rhodamine-B agar medium was observed under UV irradiation (350 nm), indicating that the two strains were able to hydrolyze olive oil (Table 1). The earlier results confirmed that the two strains were potent to produce lipase and also indicate that lipolytic bacteria are widespread in the oil-contaminated environments. The selected strains (BK43 and BK44) were then identified based on morphological, physiological and biochemical characterizations. The physiological and biochemical characteristics are presented in Table 2. The results showed that both strains are gram negative, aerobic and coccoid rod shape. A biochemical test was conducted using an API identification kit. Both strains were oxidase negative and capable of assimilating caprate and malate (Table 2), which indicates that strains BK43 and BK44 were closely related to *Acinetobacter junii*. Taken together, these characteristics indicated that both strains belong to the genus *Acinetobacter.*

Table 2. The morphological, biochemical and physiological characteristics of strains BK43 and BK44.

Tests	Reactions	BK43	BK44
Gram staining	-	Negative	Negative
Shape	-	Coccoid rod	Coccoid rod
NO3	Nitrate/nitrite reduction	-	-
TRP	indole production	-	-
GLU	Acid production from glucose	-	-
ADH	arginine dihydrolase	-	-
URE	Urease	-	-
ESC	β-glucosidase	-	-
GEL	Protease (gelatin hydrolysis)	-	-
PNPG	β-galactosidase	-	-
GLU	assimilation of glucose	-	-
ARA	assimilation of arabinose	-	-
MNE	assimilation of mannose	-	-
MAN	assimilation of mannitol	-	-
NAG	assimilation of N-acetyl-glucosamine	-	-
MAL	assimilation of maltose	-	-
GNT	assimilation of gluconate	-	-
CAP	assimilation of caprate	+	+
ADI	assimilation of adipate	-	-
MLT	assimilation of malate	+	+
CIT	assimilation of citrate	-	-
PAC	assimilation of phenyl-acetate	-	-
OX	cytochrome oxidase	-	-

+; Positive result, -; negative result.

Sequencing and phylogenetic analysis

The identities of the bacteria were further confirmed by 16S rRNA sequencing. Approximately 1381 and 1383 bp sequences were obtained from BK43 and BK44, respectively, and then aligned with other 16S rRNA sequences available in the GenBank database. A phylogenetic tree was then constructed using the neighbor-joining method by only culturable *Acinetobacter* species (Figure 1). The phylogenetic analysis indicated that the sequences of strain BK43 and BK44 were highly homologous (99 and 100%, respectively) with the sequence of *A. junii* (NCBI accession No. AM184300) followed by *Acinetobacter* sp. (NCBI accession No. AM412159). The 16S rRNA sequences of strains BK43 and BK44 were deposited in GenBank under accession numbers GQ202270 and GQ202271, respectively. Based on these results, BK43 and BK44 are *Acinetobacter* sp.

Fatty acid analysis

The fatty acid profiles of the *Acinetobacter* species isolated in this study are presented in Table 3. Although the patterns of fatty acid compositions of both strains were similar, the fatty acid contents differed. The fatty acid profiles were more similar to *A. junii* (Yoon et al., 2004) than to other *Acinetobacter* species such as *Acinetobacter antiviralis* (Lee et al., 2009) and *A. radioresistens* (Nishimura et al., 1988). The major fatty acids produced by the strains were C16:0, C16:1 (n-7) and C18:1 (n-9). However, the C18:1 (n-9) accumulated more than 50% in both strains. These results further suggest that the isolated strains BK43 and BK44 are *Acinetobacter* sp. The identified bacterial strains BK43 and BK44 were used for further studies to optimize their extracellular lipase production.

Production of extracellular lipase

Most extracellular bacterial lipases are influenced by nutritional and physiological factors such as pH, temperature and carbon sources (Immanuel et al., 2008; Kiran et al., 2008; Wang et al., 2009). The lipase activity of both isolates was evaluated from 6 to 48 h. The maximum lipase production was observed after 12 h of incubation for BK44 and after 24 h for BK43 (Figure 2). Furthermore, the enzyme activity was gradually decreased after 12 and 24 h, respectively. Therefore, the optimum incubation

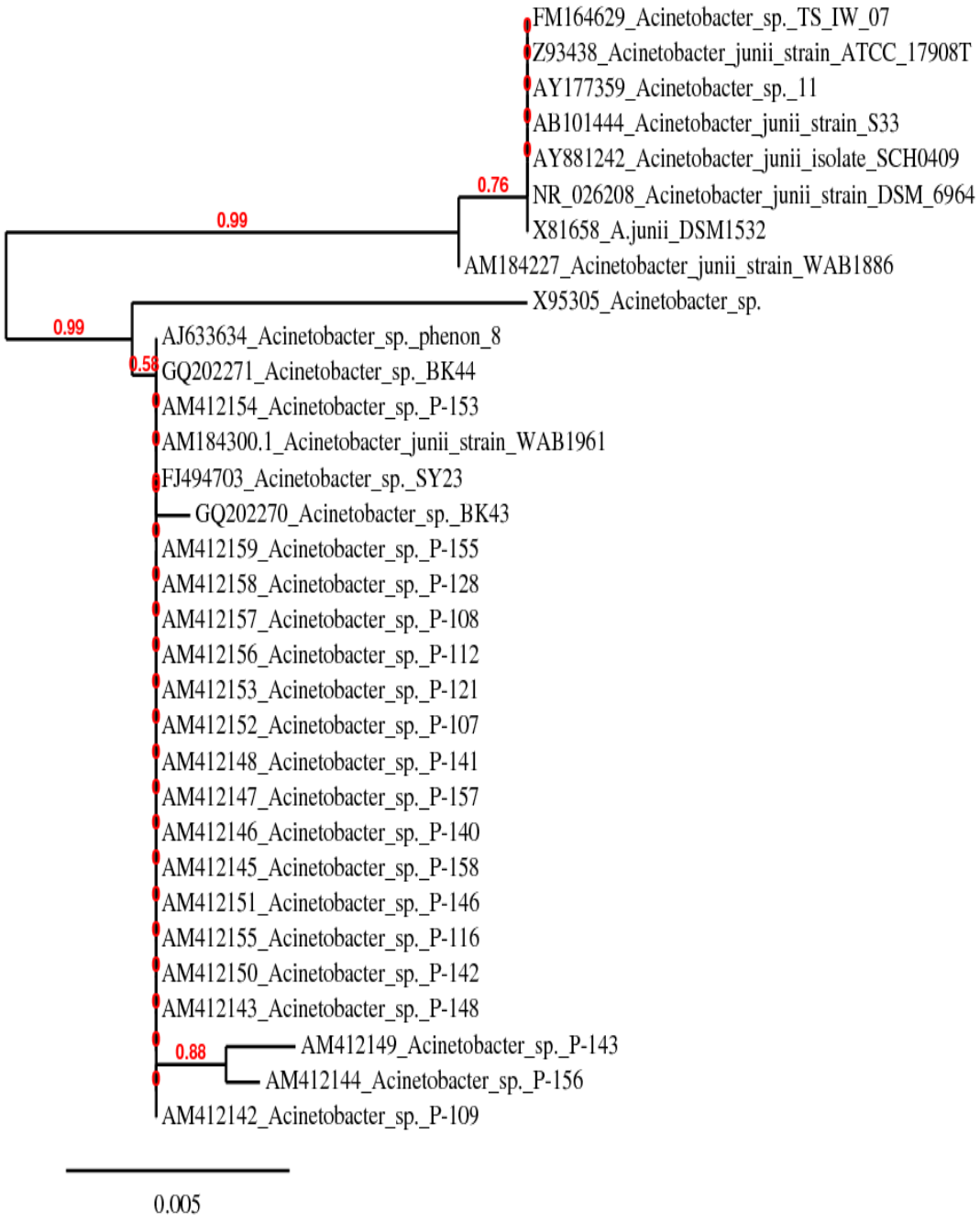

Figure 1. Neighbor-joining phylogenetic tree based on 16S rRNA gene sequences showing the relationships between strains BK43, BK44 and related strains. Bar, 0.005 changes per nucleotide position.

periods for each strain were maintained throughout the studies. The results of the present study are similar to those of several other studies in which the optimum incubation period for lipase production was found to be 12 to 24 h (Chen et al., 1998; Dharmsthiti et al., 1998). However, some bacterial species required more than 2 days for the maximum lipase activity to occur (Joseph et al., 2006; Kiran et al., 2008; Wang et al., 2009). Theresults of the present study indicate that the organisms isolated here can be used to produce large quantities of lipase within a short period of time.

Both strains were able to produce a high level of lipase at pH 6.0. However, the enzyme activity decreased rapidly at alkaline pHs. Figure 3A shows that both strains prefer an acidic pH. These findings differ from the results of other studies in that the optimum lipase production

Table 3. Fatty acid profiles of *Acinetobacter* species.

Fatty acids	Contents in BK43 (%)	Contents in BK44 (%)
C10:0	1.504	1.864
C12:0	1.602	1.497
C14:0	trace	trace
C15:1	3.6	3.09
C16:0	19.2	20.81
C16:1 (n-9)	trace	1.506
C16:1 (n-7)	16.31	16.86
C17:0	trace	trace
C17:1	trace	trace
C18:0	trace	trace
C18:1(n-9)	54.2	51.524

Trace: fatty acids present in amounts less than 1%.

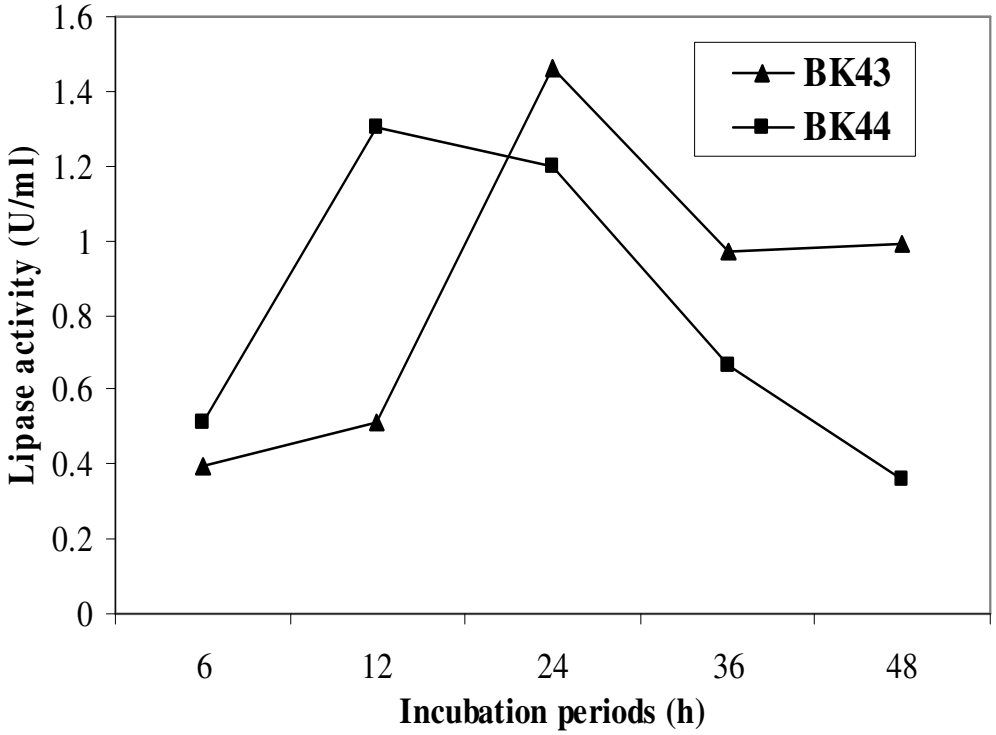

Figure 2. Effect of different incubation periods on lipase production. Each point represents the mean ± SEM of three independent experiments.

occurred at neutral pH (Joseph et al., 2006) or alkaline pH (Chen et al., 1998; Kiran et al., 2008; Wang et al., 2009), but similar at pH 6.0 (Ertugrul et al., 2007). Most bacterial species are able to produce greater amounts of lipase at pH 6.5 to 7.0 (Dharmsthiti et al., 1998; Gao et al., 2004; Joseph et al., 2006). Most reports, available regarding the production of lipases by fungi, involved studies conducted under acidic conditions (Cihangir and Sarikaya, 2004; Mhetras et al., 2009).

Among different temperatures tested (20 to 37°C), the highest production by BK43 was obtained at 30°C, while the highest production by BK44 was obtained at 25°C (Figure 3B). In the case of BK44, approximately 25% of the enzyme activity was lost when the temperature increased from 25 to 30°C. In addition, the enzyme production by both strains has decreased dramatically at 37°C, which indicates that these strains are unable to grow and produce lipase at higher temperatures. Many species of *Acinetobacter* have been reported to be psychrophilic and psychrotrophic (Kasana et al., 2008;

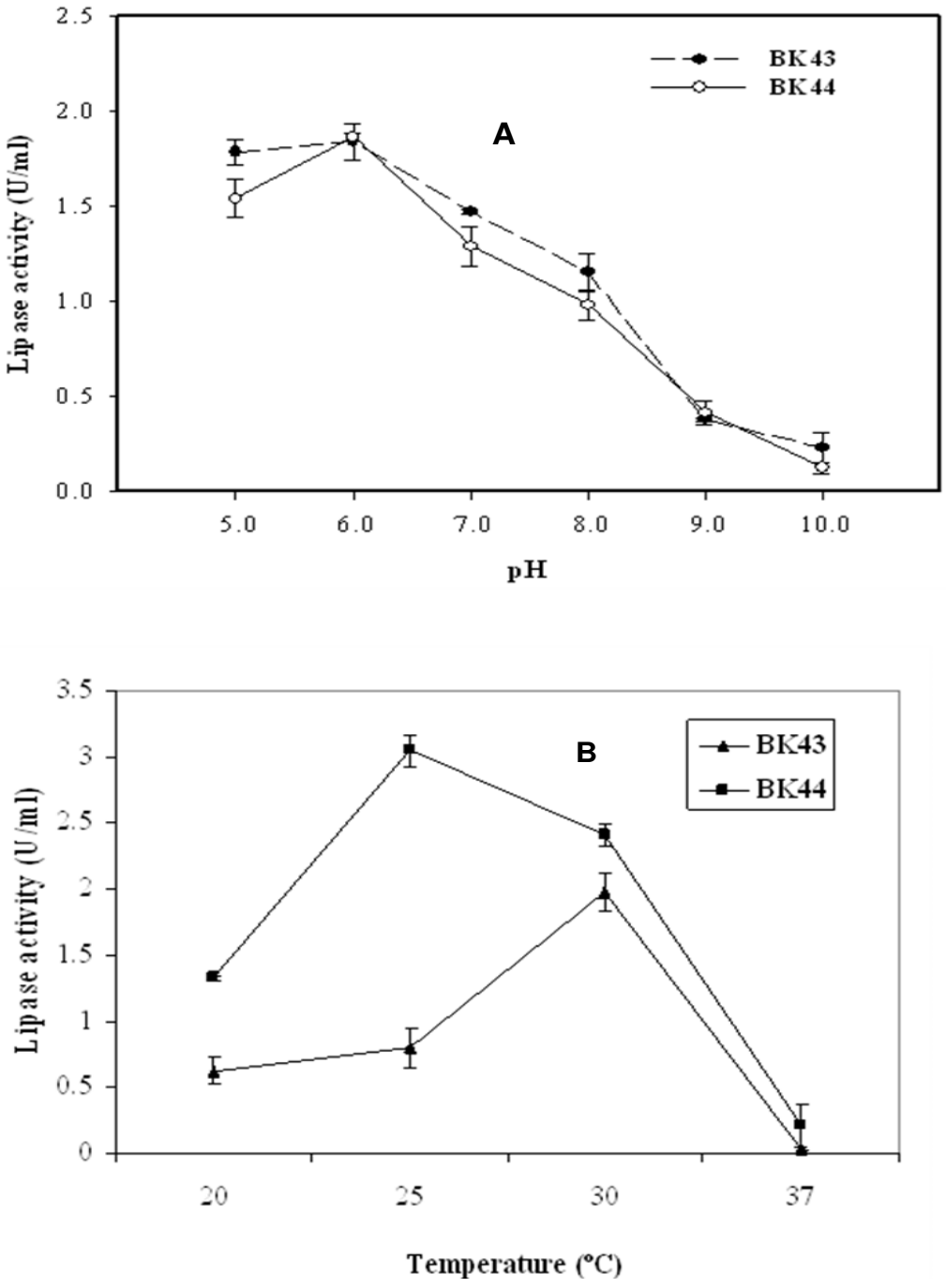

Figure 3. Effect of different pHs (A) and temperatures (B) on lipase production. Each point represents the mean ± SEM of three independent experiments.

Park et al., 2009), however, the strains isolated here failed to grow and produce lipase at low temperatures even after 1 week of incubation. The optimum temperature for lipase production was 25 to 30°C, which agrees with the production of lipase by many microorganisms, including *A. radioresistens* and *A. calcoaceticus* LP009 (Chen et al., 1998; Dharmsthiti et al., 1998; Kiran et al., 2008).

Several studies have shown that lipase production was influenced by different carbon sources present in media (Mahler et al., 2000; Immanuel et al., 2008). In general, the required specific carbon source and its concentration differ among organisms. Therefore, in this study, various carbon sources (1%) were added to the culture medium and their effects on lipase production, by both strains, were evaluated. The greatest increase in lipase production

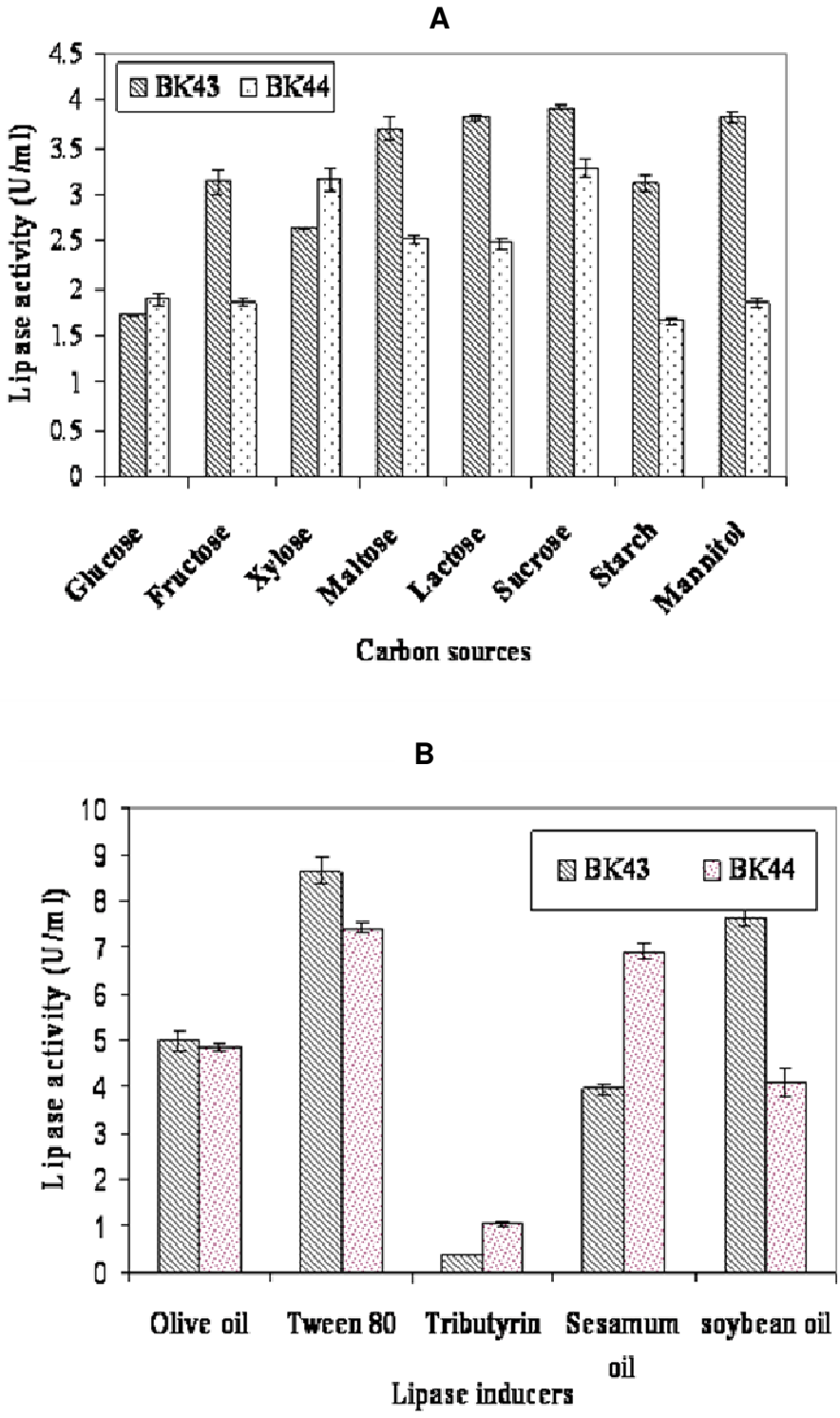

Figure 4. Effect of various carbon sources (A) and lipase inducers (B) on lipase production. Each point represents the mean ± SEM of three independent experiments.

by both strains was observed in response to supplementation of the culture medium with sucrose as the carbon source, followed by lactose in the case of BK43 and xylose in the case of BK44 (Figure 4A) compared with glucose (control). Sucrose has been reported to have similar effects on lipase production by *Bacillus pumilus* SG2 (Sangeetha et al., 2008). The other carbon sources evaluated in this study, such as fructose, starch

and mannitol, led to a decrease in lipase production by BK44 of more than 50%. These results suggest that the above carbon sources act as inhibitors of lipase production and indicate that the carbon source has the potential to increase the lipase production significantly.

Lipase inducers were evaluated as shown in Figure 4B, lipase production by both strains increased dramatically (2-fold) when 1% Tween 80 was added to the media. However, a moderate level of lipase production by BK43 and BK44 was obtained when soybean and sesame oil, respectively, were used as the lipase inducers (Figure 4B). These findings agree with the results of other studies in which other lipids induced lipase production (Gilbert et al., 1991; Joseph et al., 2006; Immanuel et al., 2008; Kiran et al., 2008). Similarly, Dharmsthiti et al. (1998) and Gao et al. (2004) reported that lipase production by *A. calcoaceticus* LP009 and *Serratia marcescens* ECU1010 was induced by 1 and 0.5% Tween 80, respectively. Conversely, Joseph et al. (2006) reported that Tween 20 and 80 induced a poor level of lipase production by *Staphylococcus epidermis*. In the present study, other lipase inducer such as tributyrin resulted in very low enzyme production by both strains.

The results of the present study provide useful information for the optimization of culture conditions such as carbon sources and lipase inducers, and physico-chemical properties such as pH and temperature to provide the best lipase production by *Acinetobacter* species. These results clearly demonstrated that lipase-producing bacteria are widespread in oil contaminated soil. The optimized growth conditions developed in this study can be used for a large scale in industrial purposes. Furthermore, the majority of studies conducted to date have evaluated lipase production under alkaline conditions; therefore, the results of the present study will be useful for development of methods of lipase production under acidic conditions. To our knowledge, this is the first report on the highest lipase production in *Acinetobacter* species under acidic conditions. The final optimized medium resulted to about 5.9 fold (BK43) and 5.7 fold (BK44) more lipase production when compared with lipase obtained in the original medium. More studies will be conducted to purify and characterize the acidic lipase produced by the two strains isolated here under optimized culture and physico-chemical conditions.

ACKNOWLEDGEMENT

This work was supported by Inha University Grant, Inha University, Republic of Korea.

REFERENCES

Blaise CR, Armstrong JB (1973). Lipolytic Bacteria in the Ottawa River. Appl. Microbiol. 26: 733-740.

Bompensieri S, Gonzalez R, Kok R, Miranda MV, Nutgeren-Roodzant I, Hellingwerf KJ, Cascone O, Nudel BC (1996). Purification of a lipase from *Acinetobacter calcoaceticus* AAC323-1 by hydrophobic-interaction methods. Biotechnol. Appl. Biochem. 23: 77-81.

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

Chen SJ, Cheng CY, Chen TL (1998). Production of an alkaline lipase by *Acinetobacter radioresistens*. J. Ferment. Bioeng. 86: 308-312.

Cihangir N, Sarikaya E (2004). Investigation of lipase production by a new isolate of *Aspergillus* sp. World J. Microbiol. Biotechnol. 20: 193-197.

Dharmsthiti S, Pratuangdejkul J, Theeragool G, Luchai S (1998). Lipase activity and gene cloning of *Acinetobacter calcoaceticus* LP009. J. Gen. Appl. Microbiol. 44: 139-145.

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.

Gao L, Xu JH, Li XJ, Liu ZZ (2004). Optimization of *Serratia marcescens* lipase production for enantioselective hydrolysis of 3-phenylglycidic acid ester. J. Ind. Microbiol. Biotechnol. 31: 525-530.

Gilbert EJ, Drozd JW, Jones CW (1991). Physiological regulation and optimization of lipase activity in *Pseudomonas aeruginosa* EF2. J. Gen. Microbiol. 137: 2215-2221.

Immanuel G, Esakkiraj P, Jebadhas A, Iyapparaj P, Palavesam A (2008). Investigation of lipase production by milk isolate *Serratia rubidaea*. Food Technol. Biotechnol. 46(1): 60-65.

Jaeger KE, Ransac S, Dijkstra BW, Colson C, Hauvel MV, Misset O (1994). Bacterial lipases. FEMS Microbiol. Rev. 15: 29-63.

Joseph B, Ramteke PW, Kumar PA (2006). Studies on the enhanced production of extracellular lipase by *Staphylococcus epidermidis*. J. Gen. Appl. Microbiol. 52: 315-320.

Kasana RC, Kaur B, Yadav SK (2008). Isolation and identification of a psychrotrophic *Acinetobacter* sp. CR9 and characterization of its alkaline lipase. J. Basic Microbiol. 48: 207-212.

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.

Kouker G, Jaeger KE (1987). Specific and sensitive plate assay for bacterial lipases. Appl. Environ. Microbiol. 53: 211-213.

Lee JS, Lee KC, Kim KK, Hwang IC, Jang C, Kim NG, Yeo WH, Kim BS, Yu YM, Ahn JS (2009). *Acinetobacter antiviralis* sp. nov., from Tobacco Plant Roots. J. Microbiol. Biotechnol. 19: 250-256.

Lepage G, Roy CC (1984). Improved recovery of fatty acid through direct transesterification without prior extraction or purification. J. Lipid Res. 25: 1391-1396.

Lin ES, Wang CC, Sung SC (2006). Cultivating conditions influence lipase production by the edible Basidiomycete *Antrodia cinnamomea* in submerged culture. Enzyme Microb. Technol. 39: 98-102.

Liu CH, Chen WM, Chang JS (2007). Methods for rapid screening and isolation of bacteria producing acidic lipase: feasibility studies and novel activity assay protocols. World J. Microbiol. Biotechnol. 23: 633-640.

Mahler GF, Kok RG, Cordenons A, Hellingwerf KJ, Nudel BC (2000). Effects of carbon sources on extracellular lipase production and *lipA* transcription in *Acinetobacter calcoaceticus*. J. Ind. Microbiol. Biotechnol. 24: 25-30.

Marshall RT (1992). Standard methods for the examination of dairy products. 16th ed. Am. Publ. Health Assoc., Washington, DC.

Mhetras NC, Bastawde KB, Gokhale DV (2009). Purification and characterization of acidic lipase from *Aspergillus niger* NCIM 1207. Bioresour. Technol. 100: 1486-1490.

Nishimura Y, Ino T, Iizuka H (1988). *Acinetobacter radioresistens* sp. nov. isolated from cotton and soil. Int. J. Syst. Bacteriol. 38: 209-211.

Park IH, Kim SH, Lee YS, Lee SC, Zhou Y, Kim CM, Ahn SC, Choi YL (2009). Gene cloning, purification, and characterization of a cold-adapted lipase produced by *Acinetobacter baumannii* BD5. J. Microbiol. Biotechnol. 19: 128-135.

Sangeetha R, Geetha A, Arulpandi I (2008). Optimization of protease and lipase production by *Bacillus pumilus* SG2 isolated from an industrial effluent. Internet J. Microbiol. 5(2).

Saxena RK, Sheoran A, Giri B, Davidson SW (2003). Purification

strategies for microbial lipases. J. Microbiol. Method, 52: 1-18.
Sharma R, Chisti Y, Banerjee UC (2001). Production, purification, characterization and applications of lipases. Biotechnol. Adv. 19: 627-662.
Treichel H, Oliveira DD, Mazutti MA, Luccio MD, Oliveira JV (2010). A Review on Microbial Lipases Production. Food Bioprocess Technol. 3: 182-196.
Wang SL, Lin YT, Liang TW, Chio SH, Ming LJ, Wu PC (2009). Purification and characterization of extracellular lipases from *Pseudomonas monteilii* TKU009 by the use of soybeans as the substrate. J. Ind. Microbiol. Biotechnol. 36: 65-73.
Winkler UK, Stuckmann M (1979). Glycogen, hyaluronate, and some other polysaccharides greatly enhance the formation of exolipase by *Serratia marcescens*. J. Bacteriol. 138: 663-670.
Yoon MY, Shin PK, Han YS, Lee SH, Park JK, Cheong CS (2004). Isolation of an *Acinetobacter junii* SY-01 strain producing an extracellular lipase enantioselectively hydrolyzing itraconazole precursor and some properties of the lipase. J. Microbiol. Biotechnol. 14: 97-104.