ISSN 1682-296X (Print)
ISSN 1682-2978 (Online)

Bio Technology

ANSInet
Asian Network for Scientific Information
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan

Biotechnology 9 (2): 229-233, 2010
ISSN 1682-296X

Identification, Cloning and Sequence Analysis of Chitinase Gene in *Bacillus halodurans* Isolated from Salted Fish

[1]L.A. Rajan, [2]J. Dharini, [2]K.H.P. Singh, [1]S.N. Sivvaswaamy, [1]J.S. Sheela and [1]N. Sundar
[1]Department of Biotechnology, Shri Meera Biotech Pvt. Ltd.,
Ekkatuthangal, Chennai 600 078, Tamil Nadu, India
[2]Department of Bioinformatics, School of Biosciences, SRM University,
Ramapuram, Chennai 600 089, Tamil Nadu, India

Abstract: Chitin, a linear N-acetylglucosamine is a structural component of fungal cell wall and exoskeletons of invertibrates. Chitinases catalyses the conversion of chitin and it is produced by a wide range of bacteria. The gene for chitinase biosynthesis *Chi* was PCR amplified from genomic DNA of *Bacillus halodurans*. The amplified genes were cloned and nucleotide sequences were determined. The sequencing results showed that *Chi* gene contain 1800 bp long ORF encoding 599 amino acids respectively (GenBank accession No. GU481106). Computational sequence analysis of nucleotides and amino acids revealed that the *Chi* sequences of *Bacillus halodurans* were conserved in many eubacteria.

Key words: PCR, cloning, gene transformation, *in silico*, bioremediation

INTRODUCTION

Chitin, a linear b-(1, 4)-linked N-acetylglucosamine (Glc-NAc) polysaccharide (Gooday, 1999), is a main structural component of the fungal cell wall and the exoskeletons of invertebrates, such as insects and crustaceans (Wang and Hwang, 2000). It is one of the most abundant naturally occurring polysaccharides and has attracted tremendous attention in the fields of agriculture, pharmacology and biotechnology (Antranikian *et al*., 2005; Muzzarelli *et al*., 2005). Chitin can be hydrolysed by an enzyme chitinases. Chitinases catalyses the conversion of chitin and it is produced by a wide range of organisms, including bacteria (Kitamura and Kamei, 2003), plants (Masuda *et al*., 2001) and fungi (Ike *et al*., 2006). Chitin is a structural material in many marine invertebrates, such as cuttlefish, crab, lobster, fungi and algae (Li and Roseman, 2004). Marine bacteria possess the effective capability in chitin depolymerization (ZoBell and Rittenberg, 1938). Among eubacteria, marine bacteria are the excellent source for chitinases (Austin, 1988). Chitinase enzyme has been well characterized in several marine bacteria (Bassler *et al*., 1991; Tsujibo *et al*., 1993; Hirono *et al*., 1998; Aunpad and Panbangred, 2003; Howard *et al*., 2003). Chitinases can be used to treat fungal infections (Liu *et al*., 2002). Plants produce chitinases as defence agent against fungal pathogens (Taira *et al*., 2005). Chitin also has multiple applications mainly in the bioremediation of environmental pollutants like Pb^{2+}, Zn^{2+} and Bi^{3+} (Rae and Gibb, 2003). Chitinase enzyme possesses added value in food and pharmaceutical industries, anti-bacterial agents, elicitors, lysozyme inducers and immunoenhancers (Kato *et al*., 2003; Muzzarelli *et al*., 2005). Due to the massive biological applications, the characterization and synthesis of chitinase is highly anticipated in biotechnology industry. *Bacillus halodurans* is an alkaliphilic bacterium (Takami and Horikoshi, 1999) that can grow well at pH 7-10.5 in saline environments. Even though *B. halodurans* is well characterized physiologically, biochemically and genetically (Horikoshi, 1999; Takami and Horikoshi, 1999) the functional characterization of chitinase biosynthesis genes from *B. halodurans* has not been reported till date and ours is the first report on this subject. The aim of the present study is the identification, characterization and *in silico* analysis of the chitinase biosynthesis gene (*Chi*) in alkalophilic *Bacillus halodurans* isolated from fish.

MATERIALS AND METHODS

Bacterial strains and culture media: *Bacillus halodurans* was isolated from salted anchovies procured from local fish markets in Cochin, Kerala, India. Microbial identification and biochemical characterization of *B. haodurans* was carried out using conventional

Corresponding Author: L. Anbu Rajan, National Institute of Ocean Technology, Industrial Estate Road End, Dollygunj, P.O., Port Blair-744 103 Andaman and Nicobar Islands, India

method as per (Nielsen *et al*., 1995) as well as 16 S rDNA study. The 16 S rDNA sequence of the isolate showed 100% homology with that of *B. halodurans* C-125. Other bacterial strain used in this study includes *Escherichia coli* JM109. *Bacillus halodurans* was grown aerobically on alkali bacillus medium at 37°C. Chitinolytic activity of *Bacillus halodurans* was observed as the clear zone of chitin in alkali bacillus agar containing 1% chitin from shrimp exoskeleton. Genomic DNA was prepared as described by Ausubel *et al.* (1994).

Polymerase chain reaction: The chitinase gene, *Chi* was amplified by using gene specific primers. The PCR reaction was performed with the final volume of 50 μL that contained; 0.5 μM each of forward and reverse primers, 1.0 μL of crude genomic DNA, 200 μM of dNTPs, 1X *Taq* buffer, 2.5 mM $MgCl_2$, 1U *Taq* DNA polymerase (MBI Fermentas, Hanover, Maryland, USA) and autoclaved Millipore water. The PCR was performed using a Master cycler (PC-818, Astec Co., Ltd., Japan) with the following conditions; initial denaturation at 94°C for 3 min, followed by 30 repeated cycles of 94°C for 30 sec, 50°C for 1 min and 72°C for 2 min and final extension at 72°C for 5 min. The PCR amplified product was analyzed on 1.5% agarose gel along with DNA ladder (MBI Fermentas) and documented using a gel documentation system (Vilber Lourmat, France).

Cloning of PCR product: The PCR amplicon of *Chi* was purified by the use of Perfectprep Gel Cleanup Kit (Eppendorf, Germany) and cloned into the cloning vector, pTZ57R/T (MBI Fermentas). The cloned inserts were transformed into *E. coli* JM109 and plated on Luria Bertani (LB) agar containing ampicillin (100 μg mL^{-1}), IPTG (50 μM) and X-gal (80 μg mL^{-1}). The plates were incubated at 37°C and the transformants were selected and inoculated in 5 mL LB broth with the corresponding antibiotic. The recombinant plasmids were isolated from the overnight culture by alkaline lysis method (Sambrook and Russell, 2001).

Characterization of recombinant plasmid: The recombinant plasmid was double digested with *Bam*I and *Xba*I restriction enzymes. The reaction mixture contained recombinant plasmid 2 μL, Enzyme buffer (10X) 2 μL, each restriction enzyme (10U μL^{-1}) 0.5 μL and volume up to 20 mL with autoclaved Millipore water. The reaction mixture was incubated overnight at 37°C in a water bath. The digested products were analyzed on 1.5% agarose gel. The clone with the correct insert as judged by size was sequenced on an ABI PRISM 377 genetic analyzer (Applied Biosystems, Perkin Elmer Co., Foster City, CA, USA).

***In silico* sequence analysis:** The nucleotide sequences obtained were compared to the available database sequences by BLAST analysis using the NCBI (http://www.ncbi.nlm.nih.gov) database. The sequences were aligned and clustered using CLUSTAL-X version 1.81 (Thompson *et al*., 1997). The molecular masses and the theoretical pI values of the polypeptides were predicted using the ProtParam tool (http://www.expasy.org/tools/protparam.html).

RESULTS

The *Chi* gene was PCR amplified and is encoded by polynucleotides of 1800 bp (Fig. 1). The *Chi* encodes proteins of 599 amino acids with the pI value of 4.62. The molecular mass of the protein is 67059 Da, based on *in silico* estimates (Fig. 3). After PCR amplification, the products were purified from the agarose gel and cloned into pTZ57R/T cloning vector. The recombinant transformants with *Chi* gene was also confirmed by double digestion with restriction enzymes (Fig. 2). The nucleotide sequence of *Chi* gene was submitted to GenBank and have been given accession No. GU481106.

The search for homologous genes and deduced amino acid sequence were performed using BLAST. The nucleotide sequence of *Chi* gene matches significantly with the chitinase genes from other organisms. The *Chi* sequences from the *B. halodurans* isolate were compared with the reported nucleotide and amino acid sequences of other eubacteria viz., *Bacillus clausii* (GenBank accession No. AP006627), *Bacillus pumilus* (DQ859055), *Bacillus subtilis* (DQ661650), *Bacillus licheniformis* (GQ899144), *Bacillus amyloliquefaciens* (EF088513), *Bacillus circulans* (AF154827) and *Bacillus thuringiensis* (GQ183831) using Clustal W software. *In silico* nucleotide sequences analysis of *Chi* gene revealed a high degree of

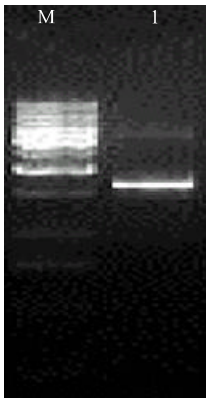

Fig. 1: Agarose gel electrophoresis of amplified product of *Chi* gene

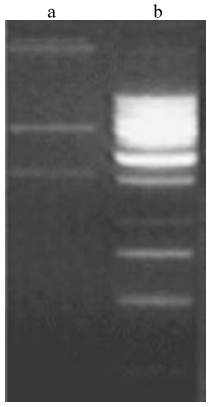

Fig. 2: Agarose gel electrophoresis of digested clones in plasmid pTZ57R/T (2886 bp): Lane a: *Chi* gene (1800 bp); Lane b: 1 kb DNA ladder

```
atgaggtttcgtaaacgaatggggaaactgctcatatggatcgtcactgttttactagtg
 M  R  F  R  K  R  M  G  K  L  L  I  W  I  V  T  V  L  L  V
gtcacatggatgccagctgatcaggttgtaggtgcagctgaaacgtcggacgaccaatac
 V  T  W  M  P  A  D  Q  V  V  G  A  A  E  T  S  D  D  Q  Y
aaaattgtcgcctactatccttcatggggagcttatggccgtgattaccaagtgtggggac
 K  I  V  A  Y  Y  P  S  W  G  A  Y  G  R  D  Y  Q  V  W  D
attgatgcgtctaagattagtcatataaactatgccttcgcaaatatttgctgggacggg
 I  D  A  S  K  I  S  H  I  N  Y  A  F  A  N  I  C  W  D  G
agcatggaaatccagatccggcaggtcctaatccgcaaacatggtcgtgtcaagatgaa
 R  H  G  N  P  D  P  A  G  P  N  P  Q  T  W  S  C  Q  D  E
aatggagtgattgatgttccaaatggttcgattggttatgggagatccttggattggatgca
 N  G  V  I  D  V  P  N  G  S  I  V  M  G  D  P  W  I  D  A
caaaagtcaaacccaggtgatacatgggacgagccacttcgtgggaatttaagcagctg
 Q  K  S  N  P  G  D  T  W  D  E  P  L  R  G  N  F  K  Q  L
aacaaattaaaagaggagcatcctcatttaaaaacgttgatttcagtcggtgggtggaca
 N  K  L  K  E  E  H  P  H  L  K  T  L  I  S  V  G  G  W  T
tggtctaaccgtttctctgacatggcggcaacgaaagagacgagagaaaacttcgcgaat
 W  S  N  R  F  S  D  M  A  A  T  K  E  T  R  E  N  F  A  N
tcagccgtcgagtttattcgtaaatacgggtttgacggtgtcgacgttgattgggagtat
 S  A  V  E  F  I  R  K  Y  G  F  D  G  V  D  V  D  W  E  Y
ccagtgagtggaggtcttccgaaatagccgtcgtccagaggataaagaaaatcacgtc
 P  V  S  G  G  L  P  G  N  S  R  R  P  E  D  K  E  N  H  V
ctgctcttacaagaggtgcgagacaaattagatggaggcggcggcaggaggacggcaaagac
 L  L  L  Q  E  V  R  D  K  L  D  E  A  G  Q  E  D  G  K  D
tatttattgacgatcgcttcaggtgctagccctggatatgtggaaaacaataagctcaat
 Y  L  L  T  I  A  S  G  A  S  P  G  Y  V  E  N  N  K  L  N
gaaattgctgagattgttgattggatcaacatcatgacctatgatttaacggtggctgg
 E  I  A  E  I  V  D  W  I  N  I  M  T  Y  D  F  N  G  G  W
caaaacattagcggtcataacgccccgctttactatgatccagctactgcaaatacggag
 Q  N  I  S  G  H  N  A  P  L  Y  Y  D  P  A  T  A  N  T  E
ttgccgacgccagaacatttaatgttgaaagcgctgttgaagggcacttacaagctggg
 L  P  T  P  E  H  F  N  V  E  S  A  V  E  G  H  L  Q  A  G
gtgccagaacataaactcgtattaggcatgccttttacggtagggatggagcaactgc
 V  P  E  H  K  L  V  I  G  M  P  F  Y  G  R  G  W  S  N  C
gatgggccatcaaggcgagtatccagcgctgcgctctctcgcgtgaaggaacatgggaa
 D  G  A  N  Q  G  E  Y  Q  R  C  A  P  P  R  E  G  T  W  E
aacggtgtcttcgatttttctgatcttgaggatcattatattaacaagaatggctaccag
 N  G  V  F  D  F  S  D  L  E  D  H  Y  I  N  K  N  G  Y  Q
cggtattggaacgatgtagcgaaggttcctttcttatataatgcaacaaatggtaacttt
 R  Y  W  N  D  V  A  K  V  P  F  L  Y  N  A  T  N  G  N  F
atcacctatgatgatgaagagtctttccgatacaagaccgattttattaaatctaataat
 I  T  Y  D  D  E  E  S  F  R  Y  K  T  D  F  I  K  S  N  N
ctagcaggctccatgttttgggatgtgacgcggtgatcgtaacggaacgctacttacggca
 L  A  G  S  M  F  W  D  V  S  G  D  R  N  G  T  L  L  T  A
ttagcggatcagcttggctttactccacatgaagggcaagaaccagaagagcctttcttca
 L  A  D  Q  L  G  F  T  P  H  E  G  Q  E  P  E  E  P  S  S
gcacgactaacattcaggcgactgaggttacgtcaacaactgttacccttacgtggcag
 A  P  T  N  I  Q  A  T  E  V  T  S  T  T  V  T  L  T  W  Q
gcaccgacccgaagagccaacgcaatactcggtagcctacgattcaaaagaaaaaacgacc
 A  P  T  E  E  P  T  Q  Y  S  V  A  Y  D  S  K  E  K  T  T
actcatacaacgattacgatcgaggatttgcagcctgaaacgacgtatacgtttgtcgtt
 T  H  T  T  I  T  I  E  D  L  Q  P  E  T  T  Y  T  F  V  V
tctgctgaacataaagacggaatccgtcatgcgggtcaagctctccaagtcacaacgaaa
 S  A  E  H  K  D  G  I  R  H  A  G  Q  A  L  Q  V  T  T  K
tctgaaactggcggtgacggtgtactgctccgacatggcaggcaaacaatgtgtatacа
 S  E  T  G  G  D  G  C  T  A  P  T  W  Q  A  N  N  V  Y  T
ggcggagaccaagttcagcatggaggaaagctgtatgaagcgaaatggtggacgacaggt
 G  G  D  Q  V  Q  H  G  G  K  L  Y  E  A  K  W  T  T  G
gaagagccgggaacaacaggtgagtgggcgtgtggaagcttattggcgattgtgaataa
 E  E  P  G  T  T  G  E  W  G  V  W  K  L  I  G  D  C  E  -
```

Fig. 3: Translated sequences of *Chi* gene

similarity with other eubacteria. The amino acids analysis divulged that the *Chi* gene encoded a protein belongs to the chitinase family. The protein illustrated partial homology with chitinase family from other bacteria as follows: *B. clausii*, 78% identity; *B. pumilus*, 64% identity; *B. subtilis*, 64% identity; *B. licheniformis*, 63% identity; *B. amyloliquefaciens*, 63% identity; *B. circulans*, 68% identity and *B. thuringiensis* with 50% identity. Various amino acid substitutions were observed in N-terminal, middle and C-terminal regions of all eubacterial chitinases.

Phylogenetic tree based on evolutionary distances was constructed for nucleotide and amino acid sequences of *Chi* with the MEGA software (Molecular Evolutionary Genetics Analysis, version 3.1), Kumar *et al*. (2004) using the neighbour-joining method. The tree at nucleotide and amino acid sequence of *Chi* (data not shown) reveals that, *B. halodurans* and *B. clausii* forms a single cluster with that of other eubacteria. Many bacterial species switched to different clusters for *Chi* gene at nucleotide and amino acid level indicating the divergence among the organisms and the degree of divergence in the sequences.

DISCUSSION

Chitinases (1, 4-beta-poly-N-acetylglucosaminidase**)** catalyses the conversion of chitin and is produced by a wide range of organisms, including bacteria, plants and fungi (Ike *et al*., 2006). Based on the enzyme activity, chitinase enzymes are classified into three types: endochitinases, exochitinases and N-acetylglucosaminases (Lee *et al*., 2007). Endochitinases cleave β-1, 4-glycosidic bonds of chitin randomly, while exochitinases cleave the nonreducing end of the chitin chain to form diacetyl chitobiose. N-acetylglucosaminases hydrolyzes diacetyl chitobiose into N-acetylglucosamine (Tanaka *et al*., 2001). Up to now, chitinases has been functionally characterized from various aerobic eubacteria: *Bacillus circurans* (Alam *et al*., 1995), *Serratia marcescens* (Burberg *et al*., 1996), *Aeromonas* sp. (Shiro *et al*., 1996), *Alteromonas* sp. (Tsujibo *et al*., 1992) and *Janthiobacterium lividum* (Gleave *et al*., 1995). Bacterial chitinases contains Chitin Binding Domain (CBD) and Wbronectin type III-like domain. The chitin binding domain mainly involves in the degradation of insoluble chitin (Morimoto *et al*., 1997). Chitinase enzyme tenders massive applications in food and pharmaceutical industries (Muzzarelli *et al*., 2005).

Based on the sequence analysis, it was previously reported that the *Chi* gene of *Bacillus* sp. codes for chitinase (Lee *et al*., 2007). To date, only least information on the characterization of *Chi* gene from marine source has been reported (Li and Roseman, 2004). In this study we cloned and analyzed the *Chi* gene from *B. halodurans* cells isolated from salted fish, as a first step towards the molecular characterization of chitinase. Evaluation of the deduced amino acid sequence of *Chi* gene with reported sequences in the database revealed a maximum similarity. However, the sequence analysis of *Chi* of present isolate showed several base substitutions with that of reported

sequences, resulting in the altered amino acid sequences of the translated proteins.

We conclude that, this study represents the first instance in which *Chi* gene from *B. halodurans* isolated from salted fish has been cloned and characterized in detail. Moreover, the determination of protein structure modification due to the nucleotide substitutions will certainly provide the basis for performing site-directed mutagenesis to improve the production and configuration of the chitinase of biotechnological interest.

ACKNOWLEDGMENTS

Authors are grateful to Mr. S.M. Chithambaram, CMD and other Directors of Shri Meera Biotech. Pvt. Ltd., Chennai for providing the necessary facilities to carry out this research. The research concept was developed by L.A.R and research experiments were performed by J.D., K.H.P.S. and J.S.S; S.N.S advised on enzymatic studies, while N.S. organized for and provided the necessary facilities.

REFERENCES

Alam, M.M., N. Nikaido, H. Tanaka and T. Watanabe, 1995. Cloning and sequencing of *chiC* gene of *Bacillus circulans* WL-12 and relationship of its product to some other chitinases and chitinase-like proteins. J. Ferment. Bioeng., 80: 454-461.

Antranikian, G., C.E. Vorgias and C. Bertoldo, 2005. Extreme environments as a resource for microorganisms and novel biocatalysts. Adv. Biochem. Eng. Biotechnol., 96: 219-262.

Aunpad, R. and W. Panbangred, 2003. Cloning and characterization of the constitutively expressed chitinase C gene from a marine bacterium, *Salinivibrio costicola* strain 5SM-1. J. Biosci. Bioeng., 96: 529-536.

Austin, B., 1988. Marine Microbiology. Cambridge University Press, Cambridge, pp: 24-25.

Ausubel, F.M., R. Brent, R.E. Kingston, D.D. Moore, J.G. Seidman, J.A. Smith and K. Struhl, 1994. Current Protocols in Molecular Biology. John Wiley and Sons, New York.

Bassler, B.L., C. Yu, Y.C. Lee and S. Roseman, 1991. Chitin utilization by marine bacteria: Degradation and catabolism of chitin oligosaccharides by *Vibrio furnissii*. J. Biol. Chem., 266: 24276-24286.

Burberg, M.B., I.F. Nes and V.G. Eijsink, 1996. Comparative studies of chitinases A and B from *Serratia marcescens*. Microbiology, 142: 1581-1589.

Gleave, A.P., R.K. Taylor, B.A. Morris and D.R. Greenwood, 1995. Cloning and sequencing of gene encoding the 69-kDa extracellular chitinase of *Janthinobacterium lividum*. FEMS Microbiol. Lett., 131: 279-288.

Gooday, G.W., 1999. Aggressive and defensive roles for chitinases. EXS., 87: 157-169.

Hirono, I., M. Yamashita and T. Aoki, 1998. Molecular cloning of chitinase genes from *Vibrio anguillarum* and *V. parahaemolyticus*. J. Appl. Microbiol., 84: 1175-1178.

Horikoshi, K., 1999. Alkaliphiles: Some applications of their products for Biotechnology. Microb. Mol. Biol. Rev., 63: 735-750.

Howard, M.B., N.A. Ekborg, L.E. Taylor, R.M. Weiner and S.W. Hutcheson, 2003. Genomic analysis and initial characterization of the chitinolytic system of *Microbulbifer degradans* strain 2-40. J. Bacteriol., 185: 3352-3360.

Ike, M., K. Nagamatsu, A. Shioya, M. Nogawa, W. Ogasawara, H. Okada and Y. Morikawa, 2006. Purification, characterization and gene cloning of 46 kDa chitinase (Chi46) from Trichoderma reesei PC-3-7 and its expression in *Escherichia coli*. Applied Microbiol. Biotechnol., 71: 294-303.

Kato, Y., H. Onishi and Y. Machida, 2003. Application of chitin and chitosan derivatives in the pharmaceutical field. Curr. Pharm. Biotechnol., 4: 303-309.

Kitamura, E. and Y. Kamei, 2003. Molecular cloning, sequencing and expression of the gene encoding a novel chitinase A from a marine bacterium, *Pseudomonas* sp. PE2 and its domain structure. Applied Microbiol. Biotechnol., 61: 140-149.

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

Lee, Y.S., I.H. Park, J.S. Yoo, S.Y. Chung and Y.C. Lee *et al*., 2007. Cloning, purification and characterization of chitinase from *Bacillus* sp. DAU101. Bioresource Tech., 98: 2734-2741.

Li, X. and S. Roseman, 2004. The chitinolytic cascade in Vibrios is regulated by chitin oligosaccharides and a two-component chitin catabolic sensor/kinase. Proc. Natl. Acad. Sci. USA., 101: 627-631.

Liu, M., Q.X. Cai, H.Z. Liu, B.H. Zhang, J.P. Yan and Z.M. Yuan, 2002. Chitinolytic activities in *Bacillus thuringiensis* and their synergistic effects on larvicidal activity. J. Applied Microbiol., 93: 374-379.

Masuda, S., H. Kamada and S. Satoh, 2001. Chitinase in cucumber xylem sap. Biosci. Biotechnol. Biochem., 65: 1883-1885.

Morimoto, K., S. Karita, T. Kimura, K. Sakka and K. Ohmiya, 1997. Cloning, sequencing and expression of the gene encoding *Clostridium paraputriWcum* chitinase ChiB and analysis of the functions of novel cadherin-like domains and a chitin-binding domain. J. Bacteriol., 179: 7306-7314.

Muzzarelli, R.A., M. Guerrieri, G. Goteri, C. Muzzarelli, T. Armeni, R. Ghiselli and M. Cornelissen, 2005. The biocompatibility of dibutyryl chitin in the context of wound dressings. Biomaterials, 26: 5844-5854.

Nielsen, P., D. Fritze and F. Priest, 1995. Phenetic diversity of alkaliphilic *Bacillus* strains: Proposal for nine new species. Microbiology, 141: 1745-1761.

Rae, I.B. and S.W. Gibb, 2003. Removal of metals from aqueous solutions using natural chitinous materials. Water Sci. Technol., 47: 189-196.

Sambrook, J. and D.W. Russell, 2001. Molecular Cloning: A Laboratory Manual. Cold Spring Harbor Laboratory Press, New York.

Shiro, M., M. Ueda, T. Kawaguchi and M. Arai, 1996. Cloning of a cluster of chitinase genes from *Aeromonas* sp. no. 10S-24. Biochim. Biophys. Acta., 1305: 44-48.

Taira, T., A. Ohdomari, N. Nakama, M. Shimoji and M. Ishihara, 2005. Characterization and antifungal activity of gazyumaru (*Ficus microcarpa*) latex chitinase: Both the chitin-binding and the antifungal activities of class 1 chitinase are reinforced with increasing ionic strength. Biosci. Biotechnol. Biochem., 69: 811-818.

Takami, H. and K. Horikoshi, 1999. Reidentification of facultatively alkaliphilic *Bacillus* sp. C-125 to *Bacillus halodurans*. Biosci. Biotech. Biochem., 63: 943-945.

Tanaka, T., T. Fukui and T. Imanaka, 2001. Different cleavage specificities of the dual catalytic domains in chitinase from the hyperthermophilic archaeon *Thermococcus kodakaraensis* KOD1. J. Biol. Chem., 276: 35629-35635.

Thompson, J.D., T.J. Gibson, F. Plewniak, F. Jeanmougin and V. Higgins, 1997. The clustal X windows interface: Flexible strategies for multiple sequence alignment aided by quality analysis tools. Nucl. Acids Res., 25: 4876-4882.

Tsujibo, H., Y. Yoshida, K. Miyamoto, C. Imada, Y. Lkami and Y. Inamori, 1992. Purification, properties, and partial amino acid sequence of chitinase from a marine Alteromonas sp. strain O-7. Can. J. Microbiol., 38: 891-897.

Tsujibo, H., H. Orikoshi, H. Tanno, K. Fujimoto and K. Miyamoto *et al*., 1993. Cloning, sequence and expression of a chitinase gene from a marine bacterium, *Altermonas* sp. strain O-7. J. Bacteriol., 175: 176-181.

Wang, S.L. and J.R. Hwang, 2000. Microbial reclamation of shellfish wastes for the production of chitinases. Enzyme. Microb. Technol., 28: 376-382.

ZoBell, C.E. and S.C. Rittenberg, 1938. The occurrence and characteristics of chitinoclastic bacteria in the sea. J. Bacteriol., 35: 275-287.