เนื้อหางานวิจัย

Introduction

Proteinases are applied in many industrial activities, for example, wastewater treatment, food production and medicinal drugs industries. Although proteinases are produced by a wide range of microorganisms, plants, and animals, most proteinases used in our society are from bacteria. Bacteria produce various kinds of extracellular proteinases to degrade proteins in their habitats (Branden, C. and Tooze, J., 1999). The alkaline proteinases from the bacterial origin are the most important industrial enzymes, which contribute about 60% of the total world enzyme market (Ward, 1985; Kalisz, 1988; Outtrup, 1990). Bat faeces inside a limestone cave is one of unexpected habitats where can be screened bacteria producing alkaline proteinase. The bat faeces look like mouse dropping but are dry and powdery, containing urine, insect exoskeletons, the wings of butterflies, moths or other parts of their food chains (http://www. rics-foundation.org/publish/document.aspx?did=3039&f=y, 14/9/2004). These substances and parts act as protein sources to induce proteinase production of bacteria. There have been no reports on bacteria producing alkaline proteinase screened from bat cave. Wat Suwankuha cave in Phang Nga province is one of limestone caves in southern Thailand. Therefore, alkalophilic bacteria producing alkaline proteinases are possible to be screened. The bacteria or the enzyme can be applied in detergent industries, protein hydrolysates preparations or waste treatment industries.

The aim of this study was to screen and identify bacteria which produce proteinase outside the cell, and then to optimize the conditions for proteinase production by the bacteria, followed by purification and characterization of the enzyme.

Materials and methods

Materials

All purchased media and chemicals were analytical grades.

Screening

A total of 50 samples of bat faeces were collected from the Wat Suwankuha cave. NA plate containing 1% of skim milk at pH 8.0 was used for screening the bacteria producting proteinase outside the cells.

Cultivation of bacteria

Tryptic Soy medium was used for cultivating the bacteria. The bacteria were inoculated 10% (v/v) in a tube containing 5 ml of Tryptic Soy Broth (TSB) and incubated on a reciprocal shaker at 35°C for 24 hr. The culture was then transferred to Lee's medium (Lee and Chang, 1990) containing 2.0% of glucose, 2.0% of soybean meal, 0.04% of $CaCl_2$ and 0.02% of $MgCl_2$. The Lee's medium was used for proteinase production of the bacteria on a reciprocal shaker at 35°C for 24 hr. The culture broth was centrifuged at 12,000 rpm for 5 min. The supernatant was used for determining extracellular proteinase activity.

Proteinase assay

After cultivating the culture of bacteria, the culture broth was centrifuged at 12,000 rpm for 5 min. The supernatant was used for proteinase assay. Proteinase activity was measured by the casein Folin-Ciocalteau assay (Oda and Murao, 1974). Casein was dissolved in 0.1 M Tris-HCl buffer at pH 8.0. The reaction mixture was incubated at 35°C for 60 min. One unit of enzyme activity was defined as the enzyme quantity that liberates 1 μg of tyrosine per ml of the reaction mixture per min.

Identification of the bacteria

Some taxonomic characteristics of PN51 were identified according to the Bergey's Manual of Systematic Bacteriology (Sneath *et al.*, 1986): the following Gram staining, cell shape, endospore forming, motility, catalase production, NaCl and KCl requirement, and growth at acidic pHs and various temperatures.

16S rDNA sequence analysis

The strain PN51 was cultivated in nutrient broth under aerobic conditions at 35°C for 12 hr. Genomic DNA was extracted by using the standard method (Sambrook *et al.*, 1989) and then amplified by GeneAmp PCR System 9600. Universal primers were used; position 27 for forward and 1389 for reverse. The PCR product was 1.3 kb. The partial DNA sequencing was performed by starting at the region of 520 bp. The amplified DNA was sequenced by using API 377 DNA Sequencer.

Culture conditions for proteinase production

TSB medium was used for the growth and Lee's medium was used for proteinase production of strain PN51. The effect of peptone, yeast extract, pH, the speed of a reciprocal shaker, starter, temperature and time course on proteinase production were studied as follows.

1. Effect of peptone on proteinase production

PN51 was inoculated in TSB for 24 hr at 35°C on a reciprocal shaker. It was then transferred to modified Lee's medium containing 2% yeast extract, 0.04% $CaCl_2$, 0.02% $MgCl_2$ and various amount of peptone, 0.6, 0.8, 1.0, 1.2 and 1.4%, respectively. The proteinase activity was measured after cultivation for 24 hr.

2. Effect of yeast extract on proteinase production

The preculture of PN51 was prepared as mentioned above. It was then transferred to modified Lee's medium containing 1.0% peptone, 0.04% $CaCl_2$, 0.02% $MgCl_2$ and various amount of yeast extract, 0.3, 0.4, 0.5, 0.6, and 0.7%, respectively. The proteinase activity was measured after cultivation for 24 hr.

3. Effect of pH on proteinase production

The preculture of PN51 was transferred to modified Lee's medium containing 1.0% peptone, 0.5% yeast extract, 0.04% $CaCl_2$ and 0.02% $MgCl_2$ with pH at 6, 7, 8 and 9. The proteinase activity was measured after cultivation for 24 hr.

4. Effect of speed of shaker on proteinase production

The preculture was transferred to modified Lee's medium containing 1.0% peptone, 0.5% yeast extract, 0.04% $CaCl_2$ and 0.02% $MgCl_2$ at pH 8 on a reciprocal shaker at 160, 170, 180, 190 and 200 rpm. The proteinase activity was measured after cultivation for 24 hr.

5. Effect of temperature on proteinase production

After preparing the preculture of PN51, it was then transferred to modified Lee's medium containing 1.0% peptone, 0.5% yeast extract, 0.04% $CaCl_2$ and 0.02%

$MgCl_2$ at pH 8 on a reciprocal shaker at pH 8, at 180 rpm., with the temperature set at: 25, 30, 35, 40, 45 and 50°C. The proteinase activity was measured after cultivation for 24 hr.

6. Effect of starter on proteinase production

The strain was inoculated in TSB for 24 hr at 35°C on a reciprocal shaker. It was then transferred to modified Lee's medium containing 1.0% peptone, 0.5% yeast extract, 0.04% $CaCl_2$ and 0.02% $MgCl_2$ at pH 8 on a reciprocal shaker at 180 rpm with various amounts of starter, 0.3, 0.4, 0.5, 0.6 and 0.7%. The proteinase activity was measured after cultivation for 24 hr.

7. Time course of growth and proteinase production

PN51 was inoculated in TSB for 24 hr at 35°C on a reciprocal shaker. It was then transferred to modified Lee's medium containing 0.7% glucose, $CaCl_2$ and $MgCl_2$ at pH 8 on a reciprocal shaker at 180 rpm, 35° C. The growth and proteinase activity was measured after cultivation for 15, 18, 21, 22, 23, 24, 25, 26, 27, 45 and 48 hr.

Purification steps

All purification steps were carried out at 4°C except for Mono Q FPLC, which was performed at 25°C. Purification steps are $(NH_4)_2SO_4$ precipitation, DEAE-cellulose DE52 and Mono Q FPLC.

1. $(NH_4)_2SO_4$ precipitation

The culture supernatant was saturated overnight by 80% $(NH_4)_2SO_4$ in a cold room. The resultant precipitation was separated by centrifugation and then dissolved in 1/10-1/20 volume of 10 mM Tris-HCl at pH 8.0. The solution was then saturated by 20% $(NH_4)_2SO_4$ in the cold room. The precipitate was separated by centrifugation again and dissolved in a small volume of 10mM Tris-HCl at pH 7.5, followed by dialysis against the same buffer. The dialysate was centrifuged in order to get the supernatant. The volume, protein content, and proteinase activity of the supernatant were measured.

2. Anion exchange chromatography

2.1 DEAE-cellulose DE52 column chromatography

DEAE-cellulose DE52 column was first equilibrated with 10 mM Tris-HCl buffer at pH 8.0. The dialysate was loaded onto a DEAE-cellulose DE52 column and washed with 5 volume of the buffer at a flow rate of 1.0 ml/min. Proteinase was eluted with a linear gradient of NaCl from 0 to 1.0 M NaCl. Active fractions (3-ml each) were pooled and then dialyzed against the same buffer. The dialysate was concentrated by Centricon YM-30 (Amicon) and centrifuged to remove precipitate before loading onto a Mono Q column chromatography.

2.2 Mono Q column chromatography

Mono Q column was connected to a Fast Protein Liquid Chromatography (FPLC) system and equilibrated with 10 mM of Tris-HCl buffer at pH 8.6 containing 10 mM of $CaCl_2$. Active fractions from DEAE-cellulose DE52 column chromatography were loaded onto a Mono Q column which was then washed with the same buffer at a flow rate of 0.2 ml/min. A linear gradient of 1 M NaCl was used for elution of the proteinase. Active fractions (0.5-ml each) were pooled and concentrated by Centricon YM-30. The purity of the enzyme was checked by SDS-PAGE.

Protein determination

Protein concentration in the chromatography system was measured by an UV detector at an absorbance of 280 nm, whereas protein concentration of the purified

enzyme was determined using bicinchoninic acid (BCA) with bovine serum albumin as a protein standard.

Molecular mass and amino-terminal amino acid sequence analysis

The purified enzyme was loaded on a 12.5% polyacrylamide gel according to the method of Laemmli (1970). The gel was stained with Coomassie brilliant blue R-250 to observe the protein bands. The comparison of electrophoretic mobility of the purified proteins with that of marker proteins (Bio Rad, USA) was used for determining the molecular mass of the purified enzyme.

The purified proteinase was separated on a 12.5% SDS-PAGE. The protein was then transferred using a protein-blotting technique (Towbin *et al.*, 1979) with polyvinylidene difluoride (PVDF) as an immobilized membrane. The amino acid sequencer (Applied Biosystems, Procice HT492 Protein Sequencer, USA), and the program BLAST (Stephen *et al.*, 1997) were used.

Characterization of enzyme

Optimum pH

Casein solution was adjusted with 10 mM of acetate buffer, citrate-phosphate buffer, Tris-HCl buffer, or glycine-NaOH buffer to different pH values: 6, 7, 8, 9, 10, 10.5 and 12. Proteinase assay of the purified enzyme was carried out at each pH, and at a temperature of 35°C for 60 min.

Optimal temperature

Proteinase assay of the purified enzyme was carried out at pH 9.6, with the temperature set at 35, 40, 45, 50, 55 and 60°C, for a period of 60 min.

pH stability

The pH of the purified enzyme was adjusted to 6, 7, 8, 9, 9.5, 10, 10.5 and 12 with 0.1 N NaCl or 0.1 N HCl. The enzyme was incubated at 4°C for 20 hr. Residual proteinase activity was then assayed at 35°C for 1 hr.

Thermal stability

The purified enzyme was incubated at pH 10 and temperatures of 4, 35, 50, 55, 60, 65 and 70°C for 1 hr. Residual proteinase assay was carried out at pH 10 and 35°C for 1 hr.

Effect of inhibitors

In order to identify the type of proteinase, two combinations of proteinase inhibitors were used: 1) serine proteinase inhibitor: PMSF, TLCK, TPCK, chymostatin, leupeptin and elastatinal. 2) metalloproteinase inhibitor: EDTA-2Na and 1,10-phenanthroline. The enzyme plus inhibitor were mixed and incubated at 30°C for 1 hr. The proteinse activity was determined after at 35°C for 1 hr.

Results and conclusions

Screening

The agar plate assay was carried out for screening of bacteria producing proteinase. The results showed that each of the 75 strains gave a halo on NA-casein plate (data not shown). The casein Folin-Ciocalteau assay was carried out for the strains. Strain PN51 (Phang Nga 51) gave the highest proteinase activity and will be used for further study.

Identification of the bacteria

Taxonomic characteristics of strain PN51 were identified and showed that there were some characteristics similar to *Bacillus* sp. such as Gram staining, shape of cells (Fig. 1), endospore forming and catalase test, but we could not identify it belongs to which species of *Bacillus* (Table1). The 16S rDNA fragment of PN51 was amplified by PCR and its sequence was determined by an automatic DNA sequencer (Fig. 2). As shown in Fig. 3, the partial conserved region in 16S rDNA sequence of PN51 gave a significant similarity to 5 bacterial strains. Strain PN51 showed a homology of 100% correlation to that of *Bacillus* sp. CNJ904 PLO4 (Gontang *et al.*, 2006). The evolutionary tree between strain PN51 and other bacteria showed that PN51 is in this respect close to *Bacterium* sp. 47083 (96.5%), *Bacillus* sp. CNJ815 PLO4 (90.2%), *Bacterium* JL-74 (90.2%), and *Bacillus* sp. P01 (90.2%) (Fig. 3).

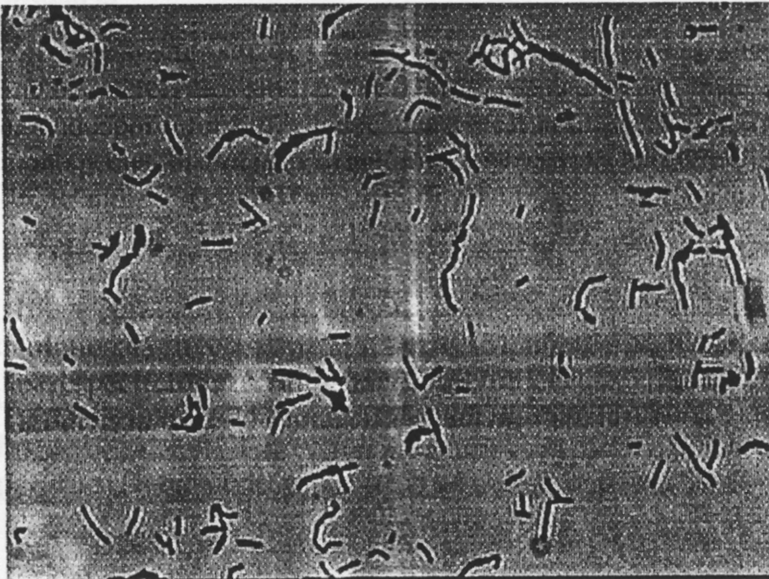

Fig. 1. Cell shape and Gram staining of PN51

Table 1. Characteristics of strain PN51

Characteristics	strain PN51
Gram staining	positive
Cell shape	rod
Rods or filaments curved shape	+[a]
Endospores forming	-
Motility	-
Catalase production	-
NaCl and KCl requirement	-
Growth at pH	
6.8, NB	+
5.7, NB	-
Growth in NaCl	
2%	+
5%	+
7%	-
10%	-
Growth at	
8°C	-
10°C	-
30°C	+
35°C	+
40°C	-
50°C	-
55°C	-
65°C	-
Isolation source	bat faeces

[a] Chains common

There was no published data of characteristics of *Bacillus* sp. CNJ904 PLO4 to compare the strain PN51 with.

```
1   ATTGGAAACT GGGAGACTTG AGTGCAGGAG AGAAAAGTGG AATTCCACGT
51  GTAGCGGTGA AATGCGTAGA GATGTGGAGG AACACCAGTG GCGAAGGCGG
101 CTTTTTGGCC TGTAACTGAC GCTGAGGCGC GAAAGCGTGG GGAGCAAACA
151 GGATTAGATA CCCTGGTAGT CCACGCCGTA AACGATGAGT GCTAGGTGTT
201 GGGGGGTTCC ACCCTCAGTG CTGAAGTTAA CACATTAAGC ACTCCGCCTG
251 GGGAGTACGA CCGCAAGGTT GAAACTCAAA GGAATTGACG GGGGCCCGCA
301 CAAGCAGTGG AGCATGTGGT TTAATTCGAA GCAACGCGAA GAACCTTACC
351 AGGTCTTGAC ATCCTCTGAC CACTCTAGAG ATAGAGCTTT CCCCTTCGGG
401 GGACAGAGTG ACAGGTGGTG CATGGTTGTC GTCAGCTCGT GTCGTGAGAT
451 GTTGGGTTAA GTCCCGCAAC GAGCGCAACC
```

Fig. 2. 16S rDNA sequence of strain PN51

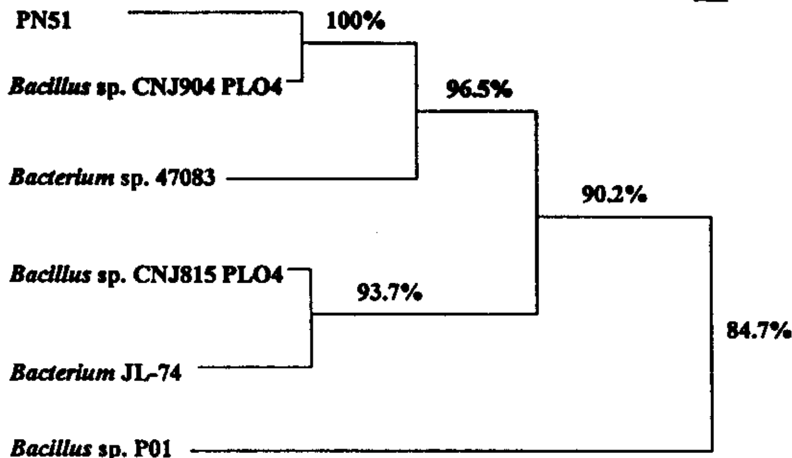

Fig. 3. The evolutionary tree between strain PN51 and other bacteria
The percentage indicates the similarity of 16S rDNA sequence of strain PN51 to those of others.

Culture conditions for proteinase production

1. Effect of peptone on proteinase production

The strain PN51 showed higher enzyme production in modified Lee's medium containing 1.0% peptone (Fig. 4).

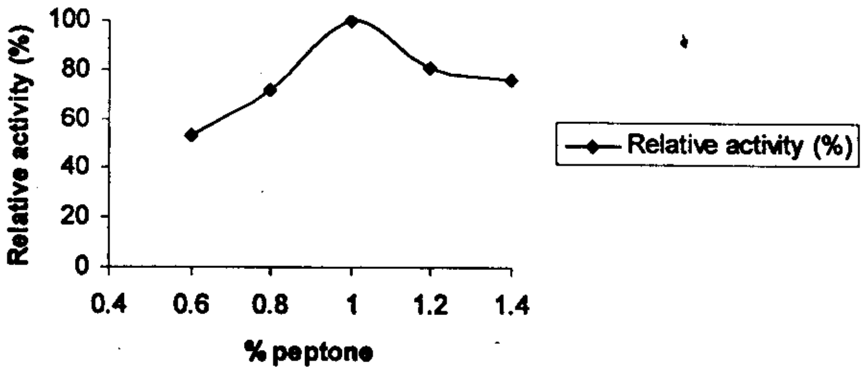

Fig. 4. The effect of peptone on proteinase production of strain PN51

2. Effect of yeast extract on proteinase production

The strain PN51 gave the highest proteinase production in modified Lee's medium containing 0.5% yeast extract (Fig. 5).

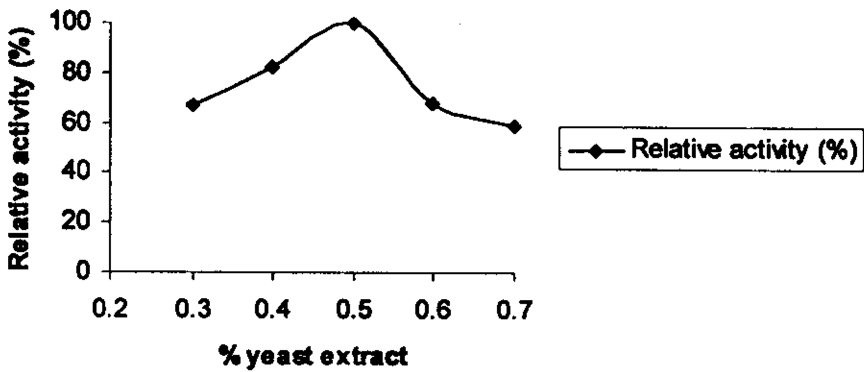

Fig. 5. The effect of yeast extract on proteinase production of strain PN51

3. Effect of pH on proteinase production

The most suitable pH in the medium for the proteinase production of strain PN51 was 8.0 (Fig. 6).

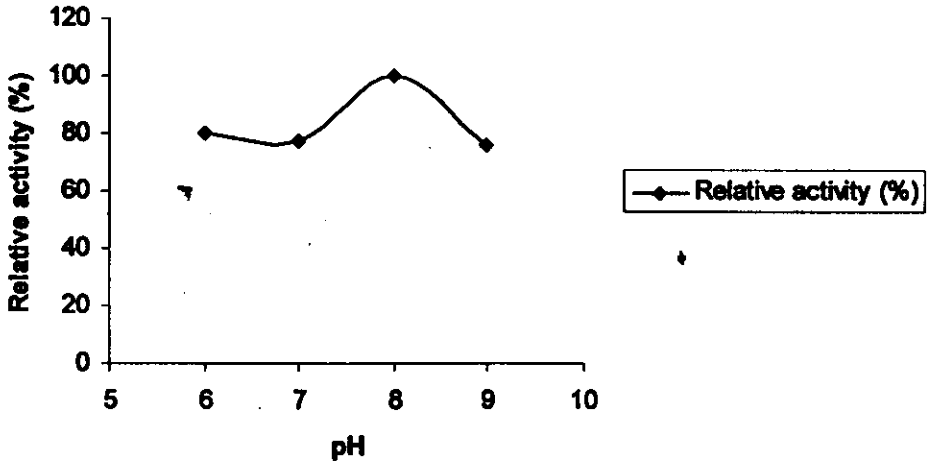

Fig. 6. The effect of pH on proteinase production of strain PN51

4. Effect of speed of shaker on proteinase production

The results show that strain PN51 gives the highest proteinase production on a reciprocal shaker at 180 rpm (Fig. 7).

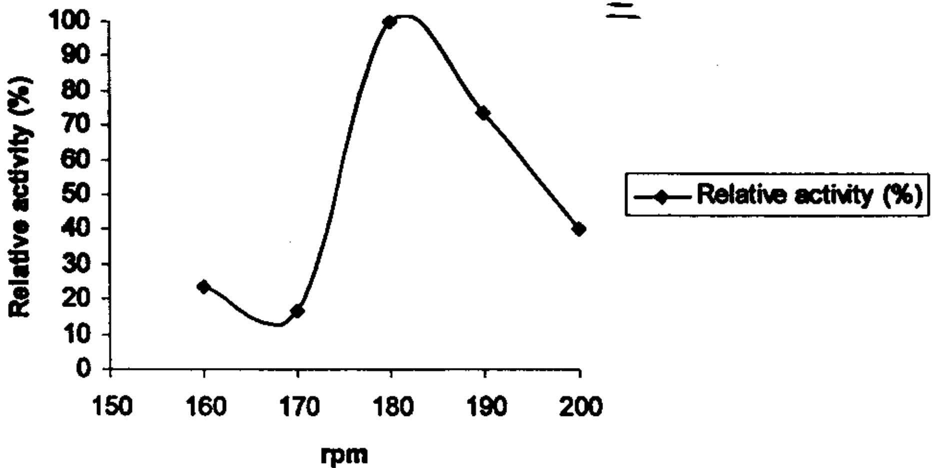

Fig. 7. The effect of the speed of a reciprocal shaker on proteinase production of strain PN51

5. Effect of temperature on proteinase production
The strain gives highest enzyme production at 35°C (Fig. 8).

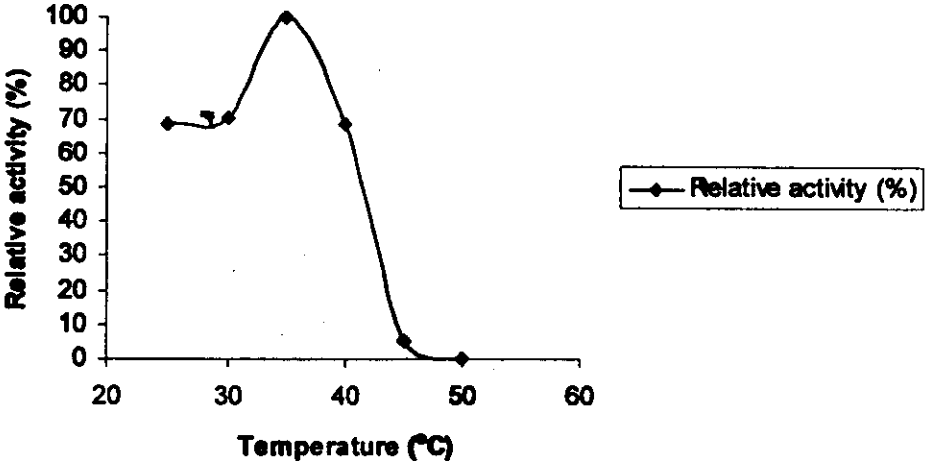

Fig. 8. The effect of temperature on proteinase production of strain PN51

6. Effect of starter on proteinase production
The results showed that 0.5-0.7% of starter gave the maximum of proteinase production (Fig. 9).

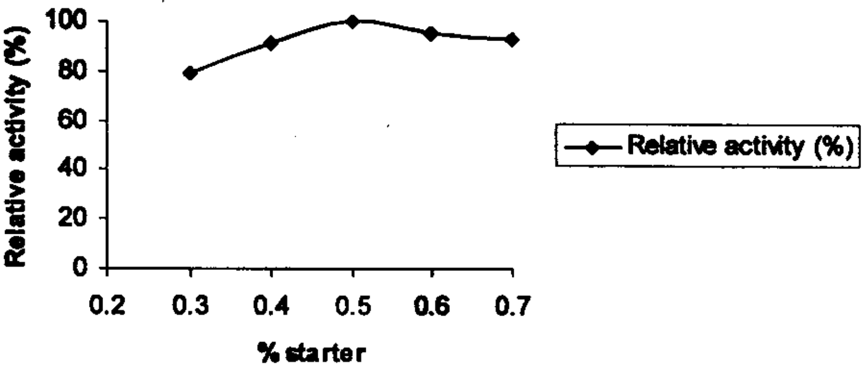

Fig. 9. The effect of %starter on proteinase production of strain PN51

7. Time course of growth and proteinase production

The 10% of PN51 was inoculated in modified Lee's medium containing 1.0% peptone, 0.5% yeast extract, 0.04% $CaCl_2$ and 0.02% $MgCl_2$ at 35°C, 180 rpm. The strain gave the highest proteinase production after cultivating for 22 hr (Fig. 10). pH is 7.3 up to about 30 hr and 7.2 from 45 to 48 hr.

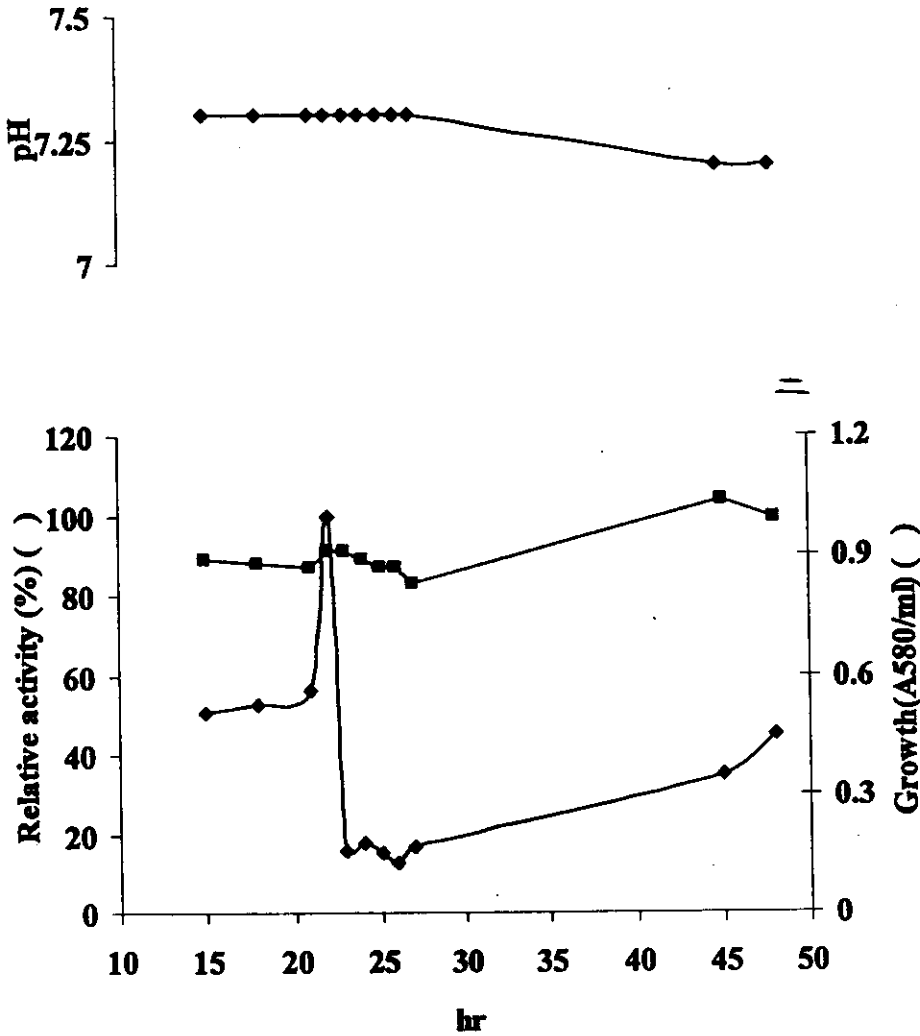

Fig. 10. Time course of proteinase production from strain PN51 in large scale culture (500 ml)

Enzyme purification

Culture filtrate from 1.9 litre-medium was precipitated with $(NH_4)_2SO_4$ at 20-80% saturation. The dialyzed crude enzyme was loaded onto a DEAE-cellulose DE52 column. Proteinase was eluted at the first peak of elution profile (Fig. 11). The active fractions were pooled and then loaded onto a Mono Q anion-exchange column. The active fractions were then eluted at 0.37-0.48 M NaCl (Fig. 12). The active fractions numbered 15, 16, and 17 came out at the resolution time, 41.2, 48.05, and 56.72 min, respectively. Fraction 16 showed the highest amount of proteinase activity, but the numbers 15 and 17 showed low proteinase activity. The purified enzyme from fraction 16 gave a single band on SDS-PAGE (Fig. 13).. As shown in Table 3, 12 mg of the purified enzyme from 1.9 liter of culture supernatant was obtained, with 2.5% proteinase activity yield, and a 28.4-fold purification (Table 3).

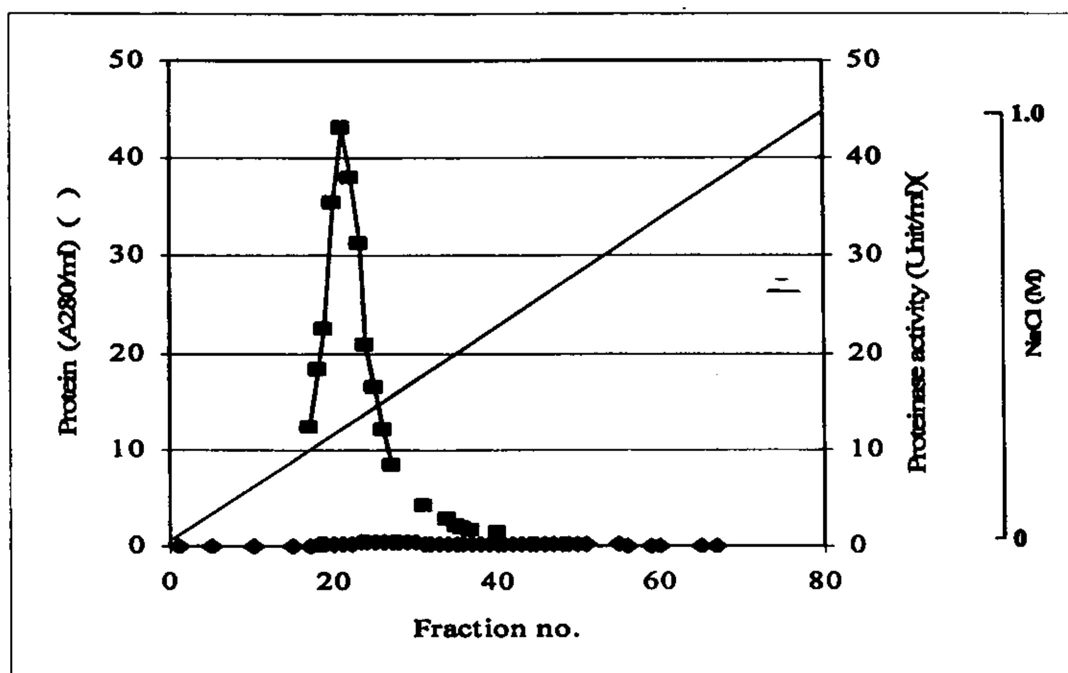

Fig. 11. Elution profile of proteinase from strain PN51 by DEAE-cellulose DE52

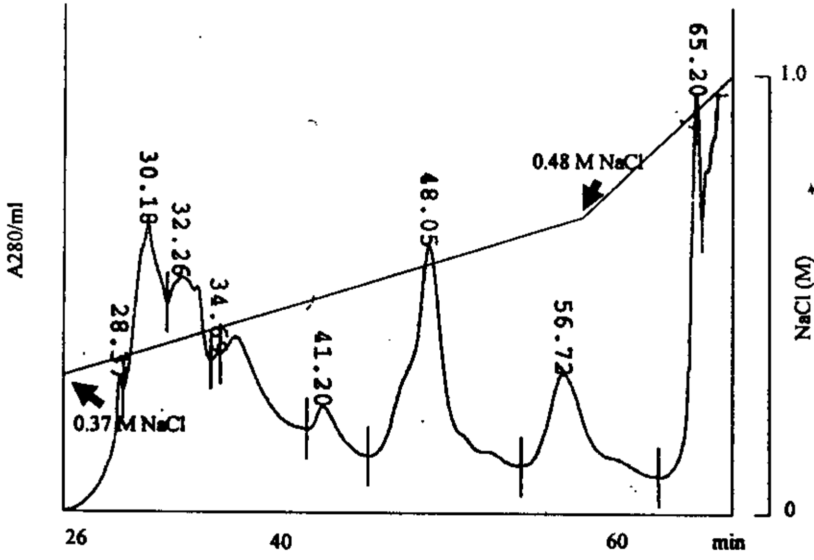

Fig. 12. Elution profile of proteinase from strain PN51 by Mono Q FPLC

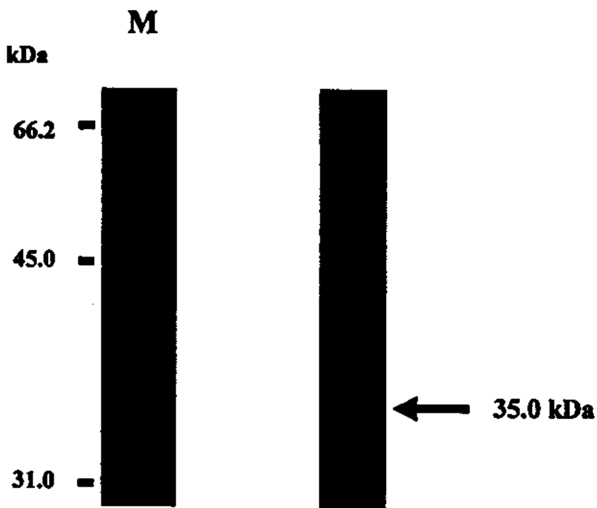

Fig. 13. SDS-PAGE analysis of the purified enzyme

Table 3. Purification of the extracellular proteinase from strain PN51

Step	Total proteinase act. (Unit)	Total Protein (mg)	Specific activity (Unit mg^{-1})	Yield (%)	Purification factor (fold)
1. Culture supernatant	24,356	13,870	1.76	100	1
2. 20-80% $(NH_4)_2SO_4$ ppt.	6,054	2,672	2.3	25	1.3
3. DEAE-cellulose DE52	3,040	120	25	12.5	14.4
4. Mono Q FPLC	600	12	50	2.5	28.4

Molecular mass and amino-terminal amino acid sequence analysis

The molecular mass of the purified enzyme was determined using SDS-PAGE with four different molecular weight marker proteins. Molecular mass of the purified enzyme was estimated to be 35 kDa. Regarding the protein database from FASTA program, the amino-terminal 25 amino acid sequence of the enzyme from PN51 showed identity to that of halolysin precursor from *Natrialba asiatica* (72%), halolysin-like extracellular serine protease from *Natrialba magadii* (72%) and serine protease halolysin R4 from *Haloferax mediterranei* (60%) (Fig. 14).

(1) Y V P N D P A Y K Q Q Y A P Q K V G T E Q A W D T
(2) P N D P Q Y G Q Q Y A P Q Q V N C E A A W D
(3) V P N D P M Y G Q Q Y A P Q Q V N C E G A W
(4) Y T A N D P K Y G S Q Y A P Q Q V N A D S A W D T

Fig. 14. Comparison of the amino-terminal amino acid sequence of the strain PN51 serine proteinase with those of other proteinases
1, PN51 serine proteinase; 2, *Natrialba asiatica* halolysin precursor; 3, *Natrialba magadii* halolysin-like extracellular serine protease; 4, *Haloferax mediterranei* serine protease halolysin R4

Characteristics of the enzyme

The effects of pH and temperature on the enzyme activity and stability were examined. The enzyme showed the maximum activity at pH 10 (Fig.15) and 60°C (Fig. 16). The enzyme was stable in the pH range from 6 to 10.5 for 20 hr (Fig. 17), and up to 60 °C at pH 10 for 1 hr (Fig. 18).

ฝ่ายหอสมุด
คุณหญิงหลง อรรถกระวีสุนทร

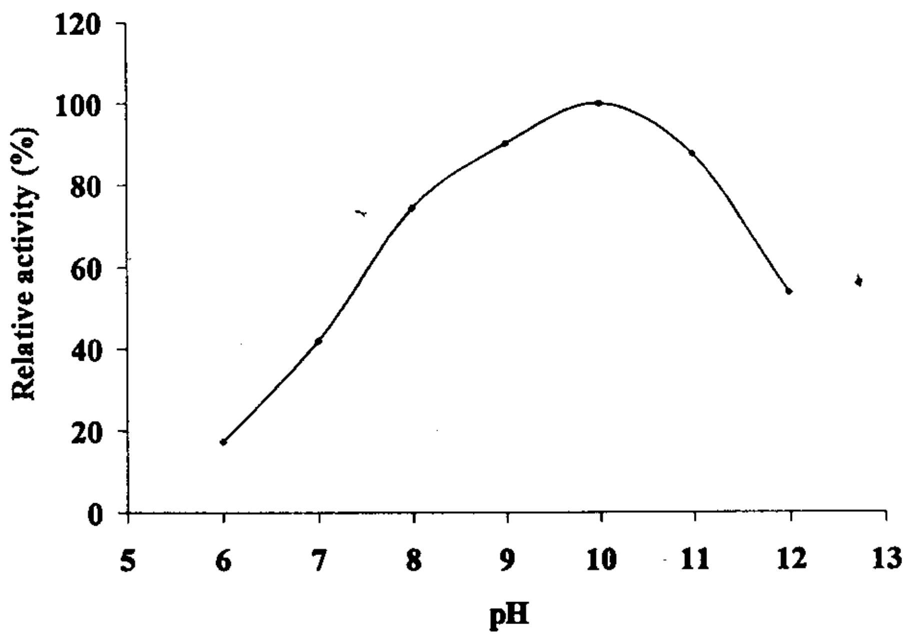

Fig. 15. Optimal pH of the proteinase from strain PN51

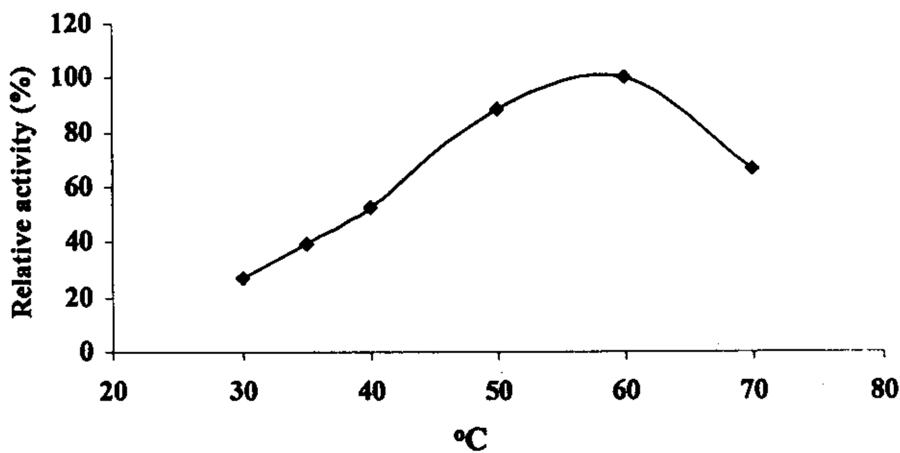

Fig. 16. Optimal temperature of the proteinase from strain PN51

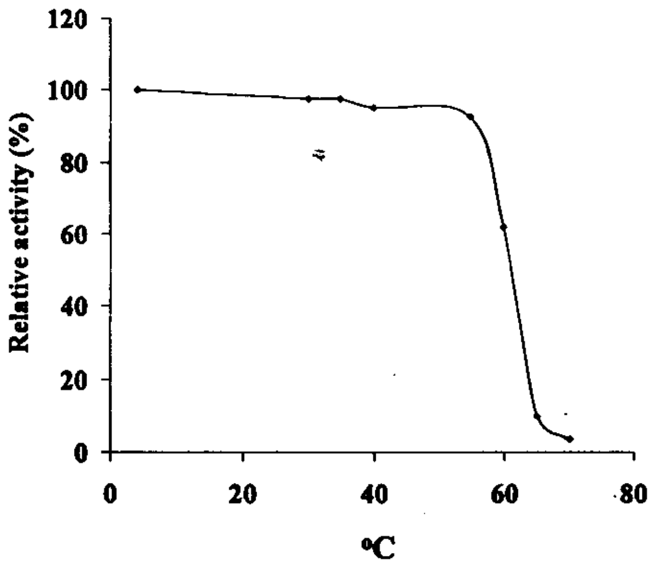

Fig. 17. Thermal stability of the proteinase from strain PN51

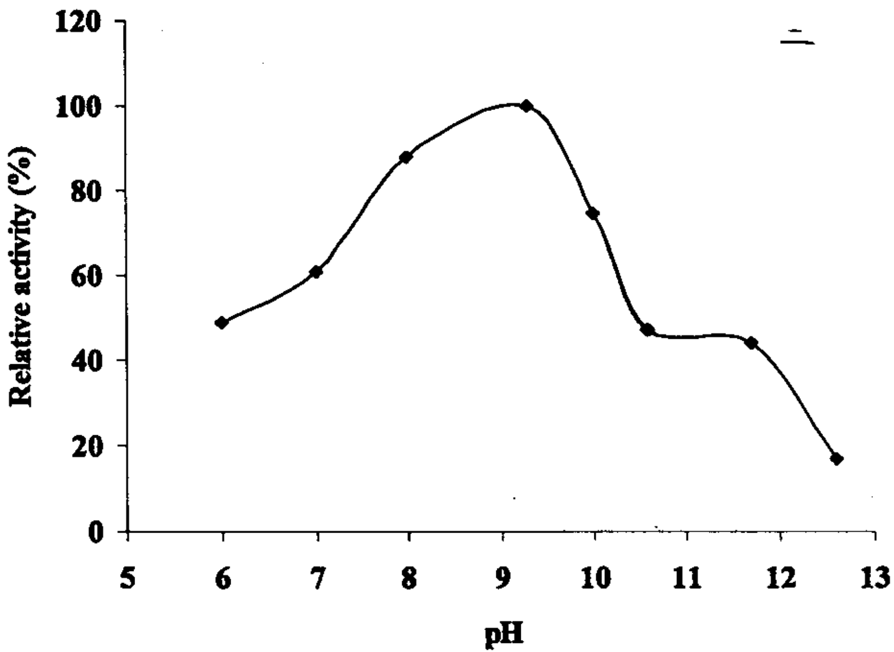

Fig. 18. pH stability of the proteinase from strain PN51

Effect of inhibitors

The proteinase from PN51 could be inhibited by PMSF and chymostatin which had relative activity 0 and 7%, respectively (Table 4). The enzyme was inhibited a little by elastatinal and leupeptin which retained 71 and 85% of its original activity, respectively. Other serine proteinase inhibitors and metalloproteinase inhibitors did not show any effect (Table 4).

Table 4. Effect of inhibitors on the proteinase activity from the strain PN51

Inhibitor	Concentration (mM)	Relative activity (%)
None	-	100
EDTA-2Na	10	100
1,10-phenanthroline	1	92
PMSF	1	0
TLCK	1	100
TPCK	1	100
Chymostatin	0.1	7
Elastatinal	0.1	71
Leupeptin	0.1	85

Discussion

The strain PN51 which gave the highest activity of proteinase was identified by taxonomic characteristics and 16S rDNA sequence analysis. The 16S rDNA sequence analysis of strain PN51 showed a homology of 100% correlation to that of *Bacillus* sp. strain CNJ904PLO4 isolated from tropical marine sediments (Gontang *et al.*, 2006 (unpublished data)). In the first report, Horikashi (1971) described an alkaline protease from alkaliphilic *Bacillus* sp. strain 221 screened from soil. Moreover, there are many reports on alkaline proteases of alkaliphilic *Bacillus* sp., but not screened from bat faeces (Fujiwara and Yamamoto, 1987; Fujiwara *et al.*, 1993; Kobayashi *et al.*, 1995; Takami *et al.*, 1989; Tsai *et al.*, 1983; Tsai *et al.*, 1984; Tsai *et al.*, 1986; Tsuchida *et al.*, 1986). Caves are opening in bedrock caused by solution or erosion by percolating water, flowing water or wave action (Ulukanli and Digrak, 2002). Some marine microorganisms might have enter the cave and establish populations similar to those that exist in marine sediments. Therefore, the strain PN51 or *Bacillus* sp. strain CNJ904PLO4 could be screened from the bat cave. However, there was no report on alkaline proteinase from *Bacillus* sp. strain CNJ904PLO4.

The strain PN51 produced its highest amount of proteinase at pH 8, meaning that the strain is an alkalophile. The growth did not require NaCl, which contrast to most of alkaliphilic microorganisms such as *Bacillus firmus* and *Exiguobacterium*

auranticum, which require NaCl for growth, because they use the Na^+/H^+ antiporter system in the range of pH from 7.0 to 9.0 (Ivey *et al.*, 1998). The strain PN51 produced the highest amount of proteinase in a modified Lee's medium containing 1.0% of peptone, 0.5% of yeast extract, 0.04% of $CaCl_2$, and 0.02% of $MgCl_2$, which is quite different from the Lee's medium containing 2.0% of glucose, 2.0% of soybean meal, 0.04% of $CaCl_2$, and 0.02% of $MgCl_2$ for alkaline protease production of *Bacillus licheniformis* (Lee and Chang, 1990). Regarding the growth of strain PN51 in a 500-ml of NB, modified NB, or Lee's medium, the proteinase activity was much lower than a small volume, 5- or 50-ml, of each medium (data not shown). The proteinase activity of strain PN51 was partially growth associated (Fig. 10).

There were three steps for purification of the enzyme, namely ammonium sulphate precipitation, DEAE cellulose DE 52, and Mono Q column chromatography. The results suggest that the last step, Mono Q column chromatography gave the lowest yield. The enzyme may not be stable at room temperature in this step. Molecular mass of the single band was estimated to be 35.0 kDa. The enzyme activity was completely inhibited by PMSF (1 mM). The activity lost 93% by adding chymostatin (0.1 mM), but not by supplying EDTA. The amino-terminal 25 amino acid sequence was NH_2-Y-V-P-N-D-P-A-Y-K-Q-Q-Y-A-P-Q-K-V-G-T-E-Q-A-W-D-T, which showed about 70% identity to those of halolysin precursor from *Natrialba asiatica* and halolysin-like extracellular serine protease from *Natrialba magadii*. Thus, the enzyme from the strain PN51 is thought to be a serine-type with chymotrypsin activity. The molecular mass of serine proteinase from PN51 was 35 kDa, whereas those of *N. magadii* and *H. mediterranei* were 45 and 41 kDa, respectively.

The enzyme showed the highest activity at 50°C and pH 10.0. In general, serine alkaline proteinases are active at pH around 10 (Rao *et al.*, 1998). Denizci *et al.* reported the maximum activity of alkaline protease from *Bacillus clausii* GMBAE 42 was at pH 11.0 and 60°C (Denizci et al., 2004). The proteinase from strain PN51 was stable in a pH range from 7 to 10 after treatment at 4°C for 20 hr and up to 60°C at pH 10 for 1 hr. A serine protease from *N. magadii* is much more sensitive to high temperature than that of strain PN51 (Giménez *et al.*, 2000). The protease from *N. magadii* incubated at 30-45°C had lost activity 20 and 60% after incubating for 2 and 5 hr, respectively, and incubated at 50-60°C was lost 50 to 70% of its activity after incubating for 2 hr (Giménez *et al.*, 2000). In addition, the enzyme from *N. magadii* showed the highest activity from pH 8 to 10 and was active over a range of pH from 6 to 12.

In general, proteinases from alkalophilic bacteria have high pH optima, broad pH activity spectra (pH 8-12), high temperature optima (60-65°C), and higher temperature stability (Durham *et al.*, 1987). These properties of the alkaline proteinases are suitable for use in detergent industry. The strain PN51 could therefore be used in commercial alkaline protease production and the enzyme may be considered as a candidate additive for detergent industry, exploited for protein hydrolysates preparation, e.g. constituents of dietic and health product, flavoring agents, and applied for waste treatment from food processing factories and household activities.

Output ที่ได้จากโครงการ

กำลังจัดเตรียมส่งตีพิมพ์

ภาคผนวก

-

รายงานการเงิน

ตามเอกสารที่แนบมา