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ORIGINAL ARTICLE

Purification and characterization of a pectin lyase produced by *Geobacillus stearothermophilus* Ah22 and its application in fruit juice production

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Abstract Extracellular pectin lyase (PL) (EC 4.2.2.10) was produced by *Geobacillus stearothermophilus* Ah22 in solid state fermentation. The PL enzyme was purified 40.8-fold by DEAE-cellulose anion exchange column chromatography and characterized. The molecular weight of the enzyme was determined as 36 kDa by Sephadex G-100 gel filtration chromatography. Purification of the enzyme was verified by SDS-PAGE. The optimum pH and temperature of the enzyme were determined as pH 6.0 and 60°C, respectively. The PL was mostly stable at 40°C. Its activity deceased by 50% after 2 h at 60°C and by 60% after 6 h at 50°C. The V_{max} and K_m were calculated as 0.47 mg/mL and 355.3 μmol/L·min, respectively. The presence of 10 mM Ca^{2+}, Cu^{2+}, Mn^{2+}, Mg^{2+}, Zn^{2+}, Hg^{2+}, Fe^{2+} and EDTA, L-cysteine and ascorbic acid significantly enhanced enzyme activity. The purified PL enzyme was used in the production of fruit juices; yields of fruits juice improved significantly compared with controls.

Keywords Pectin lyase · Purification · *Geobacillus stearothermophilus* Ah22 · Pectin · Juice clarification

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Introduction

The molecular structure of pectin is a linear backbone comprised of α-1,4-linked D-galacturonic acid residues, which may be methylated and substituted with L-rhamnose, arabinose, galactose and xylose (Gadre et al. 2003; Kashyap et al. 2001; Nakagawa et al. 2004). Hydrolysis of the pectin backbone is achieved by the synergistic action of several enzymes, including pectin methylesterase (E.C.3.1.11.1), endo-polygalacturonase (E.C. 3.2.1.15), exopolygalacturonase (E.C. 3.2.1.67), pectate lyase (E.C.4.2.2.2) exo-pectate lyase (E.C. 4.2.2.9) and endopectin lyase (E.C. 4.2.2.10) (Gummadi and Panda 2003; Soares et al. 2001). The pectin esterase removes the methoxyl group from pectin ,and depolymerizing enzymes (polygalacturonase and lyase) cleave the of β-1,4 bonds between two α-galacturonic acid residues in the pectin main-chain polysaccharide by β-elimination (lyase) or hydrolysis (polygalacturonase) (Parisot et al. 2003; Shevchik et al. 1999).

Pectinolytic enzymes have widespread biotechnological applications. Among such applications, there are a good number of reports on the use of these enzymes in the fruit juice, textile and papermaking industries, fermentation of coffee and tea, oil extractions and treatment of pectic waste water (Kashyap et al. 2001; Gummadi and Panda 2003; Olsson et al. 2003: Celestino et al. 2006).

Pectinases are produced by many organisms, such as bacteria (Horikoshi 1972; Karbassi and Vaughn 1980), fungi (Aguilar and Huitron 1990) and yeasts (Gainvors and Belarbi 1995). In the industrial sector, acidic pectinases are used in the extraction and clarification of fruit juices (Rombouts and Pilnik 1986), whereas alkalophilic pectinases are used extensively in the degumming of ramie fibers (Cao et al. 1992), retting of flax (Sharma 1987), plant protoplast formation and treatment of effluents discharged from fruit processing units (Tanabe et al. 1987). Although the major source of acidic pectinases is fungi, alkaline pectinases are produced from alkalophilic bacteria, mainly *Bacillus* spp.

In recent years, thermophilic bacteria have generated great interest due to their thermostable enzymes and industrial importance (Maugeri et al. 2001; Rahman et al. 2004). Most of the identified thermophilic microorganisms belonging to the *Bacillus* genus are aerobic, spore-forming microorganisms containing genetic groups 1 and 5 (Banat et al. 2004). On the basis of 16S rRNA sequence analysis data of the *Bacillus* genus, many thermophilic microorganisms that are members of *Bacillus* rRNA group 5 have been gathered together in the genus *Geobacillus* by a recent reclassification: *G. stearothermophilus*, *G. thermoglucosidasius*, *G. thermodenitrificans, G. thermocatenulatus*, *G. thermoleovorans*, *G. kaustophilus*, *G. pallidus* (Maugeri et al. 2001; Rahman et al. 2004; Banat et al. 2004; Nazina, 2001). The type species of this new genus is *G. stearothermophilus* and many species of this type exhibit thermophilic, acidophilic, alkalophilic and halophilic properties (Canakci et al. 2007). Members of this genus are widespread in various thermophilic and mesophilic geographic areas on Earth, such as hydrothermal vents or soils, hay compost, and oilfields (Zaliha et al. 2007).

In this paper, extracellular pectin lyase (PL) (E.C. 4.2.2.10) was produced from *G. stearothermophilus* Ah22 isolated from Ilica Hot Spring, Erzurum, Turkey. The enzyme was purified and characterized, and we also determined the action of PL in fruit juice. This is the first report on the production, purification and characterization, and application in fruit juice processing of a PL from *G. stearothermophilus* Ah22.

Materials and methods

Materials

Pectin (ED 9.4%) from citrus fruit, DEAE-cellulose, phenol, dithiothreitol (DTT), thiobarbituric acid (TBA), Sephadex G100 and G150, the electrophoresis equipment and reagents were purchased from Sigma (St. Louis, MO). The pGEM-T vector system was obtained from Promega (Southampton, UK), and the ABI PRISM cycle sequencing kit was from Macrogen (Seoul, Korea). The other reagents were of analytical grade.

Isolation of strains

The water sample was taken from the Ilica hot springs in the province of Erzurum, Turkey, with water temperature values of 40°C and/or above. After collection, the water sample was placed immediately in nutrient broth (NB) at 60–65°C. After 1 day, this enrichment culture was transferred to agar plates and purified into different colony forms. Cell and colony morphology of the purified isolate was evaluated by microscopy, and the colonies were stored in glycerol containing NB at −86°C until use.

Physiological characterization of isolates

The temperature range for growth was determined by incubating the isolate from 30 to 85°C. The effect of NaCl on the thermophilic bacterial growth was studied in NB medium containing 2.0, 3.0, 4.0, 5.0, 8.0, 10.0% (w/v) NaCl. The pH dependence of growth was tested in the pH range 4.0–11.0 in NB medium. Gram character, motility, oxidase, catalase and nitrate reduction were investigated according to the methods described by Harley and Prescott (2002).

DNA extraction from pure culture

Total genomic DNA was extracted from bacterial samples using the method described by Adiguzel (2006).

Amplification, cloning and sequencing of 16S rRNA

The 16S rRNA genes were amplified selectively from purified genomic DNA using oligonucleotide primers designed to anneal to conserved positions in the 3′ and 5′ regions of bacterial 16S rRNA genes. The forward primer, UNI16S-L (5′-ATTCTAGAGTTTGATCATGGCTCA-3′), corresponded to positions 11–26 of *Escherichia coli* 16S rRNA, and the reverse primer, UNI16S-R (5′-ATGG TACCGTGTGACGGGCGGTGTGTA-3′), corresponded to the complement of positions 1411 to 1393 of *E. coli* 16S rRNA (Adiguzel et al. 2009). PCR reaction conditions were as described by Beffa et al. (1996), and the PCR product was cloned in a pGEM-T vector system (Promega, UK).

Production of pectic enzyme by solid-state fermentation

Solid-state fermentation was carried out in 250 ml Erlenmeyer flasks containing 5 g wheat bran, 1% citrus pectin (DE 9.4%, Sigma) and 10 mL salt mixture composed of 0.14%

$(NH_4)_2SO_4$, 0.02% $MgCl_2$ and 0.02% K_2HPO_4. The final moisture content of the medium was approximately 67%. The flasks were sterilized for 20 min at 115°C, inoculated, and then incubated at 27°C for 5 days. The fermented material was mixed with 60 mL of 1% NaCl and centrifuged (4°C; 10,000 rpm; 30 min). The supernatant was used to measure enzyme activity (Taragano et al. 1999).

Pectin lyase purification

The crude extract was produced from *G. stearothermophilus* Ah22 and dialysed against the sodium phosphate buffer, (50 mM, pH 8.0).

Ion exchange chromatography

The dialyzed solution was subjected to an anion exchange chromatography using a DEAE-cellulose fast flow column. The bound proteins were eluted with a sodium phosphate buffer, (50 mM, pH 8.0) using a linear gradient of 0–0.4 M NaCl. Protein elution was monitored spectrophotometrically by measuring the absorbance at 280 nm. Activity was measured by using pectin from citrus fruits as the assay substrate (see below). After dialyzing the eluents against 50 mM phosphate buffer (pH 8), the eluents were collected. The fractions showing pectinase activity (Fig. 1) were pooled, concentrated and saved for further analysis (Kashyap et al. 2000).

Gel filtration chromatography

A glass column was packed with Sephadex G 150 (35×1.5 cm, bed volume 60 mL). The concentrated sample was loaded onto this column and proteins were eluted using Tris-HCl buffer (50 mM, pH 8). Fractions were collected, and the protein content and PL activity were determined as described above. Pectinase-positive fractions (Fig. 2) were pooled, concentrated and saved for further analysis (Kashyap et al. 2000).

Protein concentration was determined according to Bradford's method using bovine serum albumin as a standard (Bradford 1976).

Pectin lyase activity

PL activity was assayed by the colorimetric method of Nedjma et al. (2001). Briefly, 250 µL enzyme solution was mixed with 250 µL pectin from citrus fruits (1%, w/v) previously prepared in 50 mM sodium phosphate buffer, pH 8.0 at 60°C, for 30 min. After addition of 50 µL NaOH (1 *N*), the mixture was shaken briefly. The solution was heated at 80°C in a water bath for 5 min and cooled. After addition of 600 µL HCl (1 *N*) to acidify the medium, the yellow color disappeared. The solution was then shaken, and 500 µL of a solution (0.04 M) of TBA was finally added. The tube was incubated a second time at 80°C for 5 min in a water bath. The solution was briefly cooled in an ice-water bath before measuring the absorbance at 550 nm in a Spekol 13000 spectrophotometer (Analytic Jena, Germany) with 1-cm quartz cells.

SDS polyacrylamide gel electrophoresis

The purified enzyme was subjected to SDS polyacrylamide gel electrophoresis (SDS-PAGE), using 3% and 10% acrylamide concentrations for the stacking and running gels, respectively, each containing 0.1% SDS (Laemmli 1970). The sample (20 µg) was applied to the electrophoresis medium. Bromothymol blue was used as a tracking dye. Gels were stained in 0.1% Coomassie Brilliant Blue R-250 in 50% methanol, 10% acetic acid and 40% distilled water for 1.5 h, and destained by washing with 50% methanol, 10% acetic acid and 40%

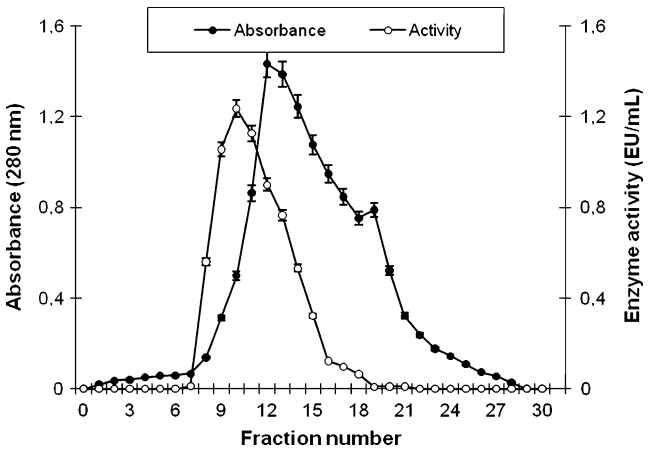

Fig. 1 Purification of pectin lyase (PL) by ion exchange chromatography using DEAE-cellulose

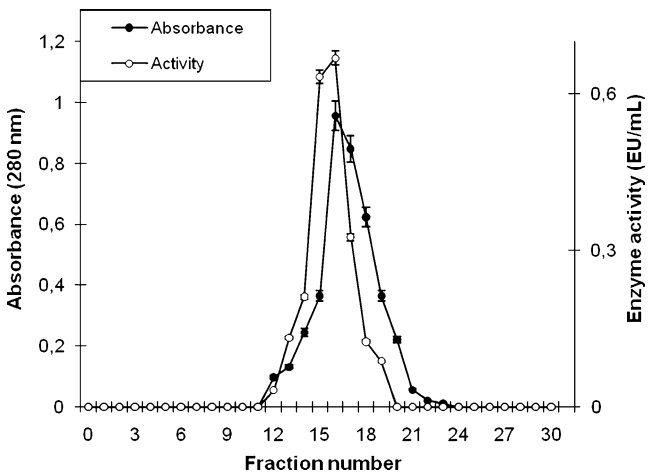

Fig. 2 Elution profile of protein and PL activity on Sephadex G150

Table 1 Purification of pectin lyase (PL) from *Geobacillus stearothermophilus* Ah22

Enzyme fraction	Volume (mL)	Activity (EU/mL)	Total activity		Protein (mg/mL)	Specific activity (EU/mg)	Purification fold
			EU	%			
Crude extract	100	0.912	91.2	100	0.865	1.039	-
DEAE-Cellulose	50	1. 466	73.3	80.4	0.243	6.03	5.80
Sephadex G 150	30	1.822	54.66	59.9	0.043	42.37	40.77

distilled water several times (Laemmli 1970). The electrophoretic pattern was photographed.

Molecular weight determination by gel filtration

A column (3×70 cm) of Sephacryl S-100 was prepared. The column was equilibrated with buffer (0.05 M Na_2HPO_4, 1 mM dithioerythretol, pH 7) until the absorbance at 280 nm was zero. The standard protein solution (bovine serum albumin, 66 kDa; egg ovalbumin, 45 kDa; pepsin, 34 kDa; trypsinogen, 24 kDa; β-lactoglobulin and lysozyme, 14 kDa) was added to the column. The purified PL enzyme was added to the column separately and then eluted under the same conditions. The flow rate through the column was 20 mL/ h. The elution volume was compared with standard proteins (Whitaker 1963).

Effect of pH and temperature on the activity of PL

The optimum temperature for PL activity was determined by carrying out the standard assay in 50 mM sodium phosphate buffer, pH 8 at temperatures ranging from 0 to 90°C. In each case, the substrate was preincubated at the desired temperature for 5 min.

The pH optimum of the PL was measured at a fixed assay temperature of 60°C at various pH values between pH 4 and 11, using different buffers. Buffers used were 0.05 M sodium acetate (pH 4–5), 0.05 M sodium phosphate (pH 6–8) and 0.05 M sodium carbonate (pH 9–11).

Effect of temperature on the enzyme stability

In order to study the thermal stability of the enzyme, buffered enzyme samples (50 mM sodium phosphate buffer, pH 8) were incubated for time periods of 1–24 h at 40, 50, 60, 70 and 80°C. Enzyme samples were taken periodically and assayed to determine the residual enzyme activity as described above.

Carbohydrate content

Carbohydrate content of the purified PL enzyme was determined by the phenol sulfuric acid method (Hounsell et al. 1997). Different amounts of purified PL (1–10 μg in a volume 10 μL buffer) and 25 μL 4% aqueous phenol was added to each tube. After 5 min, 200 μL concentrated H_2SO_4 was added and the increase in absorbance was measured at 492 nm. The carbohydrate content of the enzyme was determined by comparing absorbance with that of a galactose standard.

Effect of various metal ions on protease activity

The effect of various metal ions (10 mM Cu^{2+}, Mg^{2+}, Hg^{2+}, Fe^{2+} and Zn^{2+}) on PL activity was investigated. Each inhibitor solution was prepared at 100 mM concentration and 50, 100, 150, 200 or 250 μL of each solution was added in a cuvette containing 250 μL enzyme. The total volume was adjusted to 750 μL with a buffer solution. The activity of a control assay of enzyme activity determined without inhibitors was taken as 100%. The effect of each agent was determined by measuring the enzyme activity using pectin from citrus fruits as the assay substrate (Nedjma et al. 2001).

Fruit juice

Apple, orange, carrot, banana were used to prepare fruit juice. The fruits were carefully washed, dried in air and

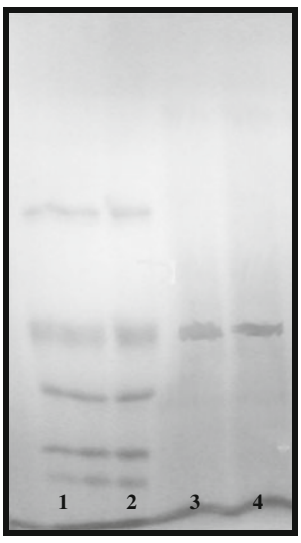

Fig. 3 SDS-polyacrylamide gel electrophoresis of the PL purified from *G. stearothermophilus* (Ah22). Lanes: *1*, *2* homogenate; *3*, *4* PL enzyme purified by gel filtration chromatography (Sephadex G 150)

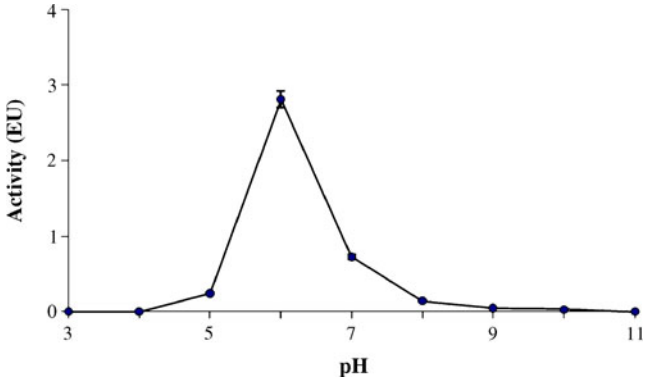

Fig. 4 The effect of pH on the activity of purified PL from *G. stearothermophilus* Ah22

homogenized. To 10 g apple homogenate, 2 mL enzyme solution was added (in control experiment 2 mL distiled water was added). The same experiment was done using 2 mL crude homogenate. Fruit homogenates were treated for 4 h at 60°C at a natural pH value. In the course of the experiment, a volume of the juice was filtered through a paper filter for 15 min, and the volume of fruit juice was calculated (Perry and Staley 1997).

Result and discussion

The isolate (Ah22) from the water sample of Ilica's hot springs in the provinces of Erzurum, Turkey was Gram-, catalase-, oxidase- and nitrate-positive, was a motile rod, and spore-forming. The strain grew well at 40–70°C, with optimum growth at 57°C, and exhibited significant growth at a pH 5.5–10.0, with optimum growth at pH 7–8.5. These characteristics meet the criteria of thermophilic bacteria, which grow at temperatures above 50°C (Perry and Staley 1997). The strain was able to grow in the salt concentration range of 2–8%.

To verify the systematic position of this bacterium, 16S rRNA analysis was undertaken. The 16S rRNA gene

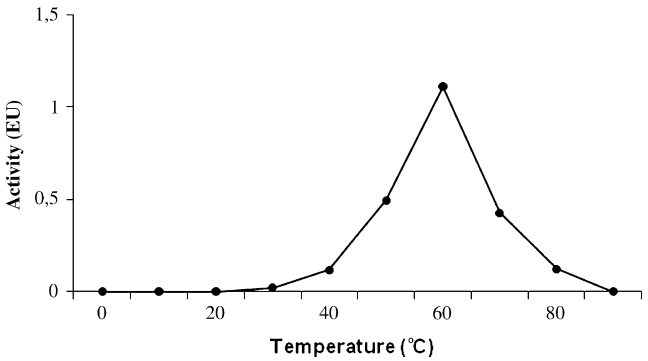

Fig. 5 The effect of temperature on the activity of purified PL from *G. stearothermophilus* Ah22

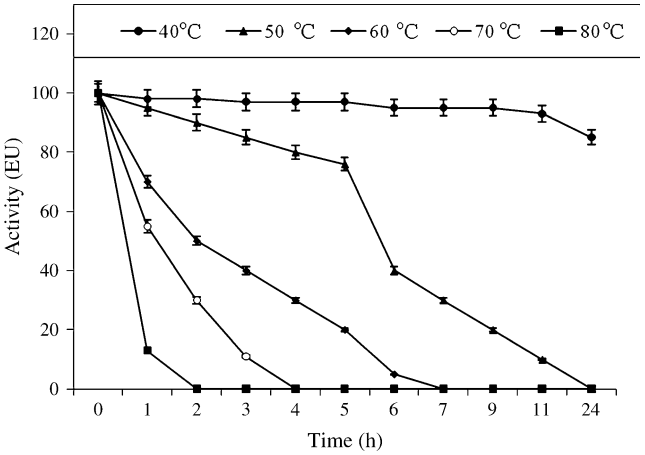

Fig. 6 The temperature stability of the purified PL from *G. stearothermophilus* Ah22

sequence determined for strain Ah22 was about 1,400 nucleotides long. The sequence of strain Ah22 was most similar to that of *G. stearothermophilus* Ah22, having 99% sequence similarity. The GenBank accession number for strain Ah22 is FJ808712.

In this study, PL was produced by *G. stearothermophilus* Ah22 using solid state fermentation. PL activity produced per gram of wheat bran was 1,755 EU. By using DEAE-cellulose anion exchange chromatography, PL was purified 40.7-fold from bacteria extract (Table 1). The purified enzyme was characterized.

After purification of the enzyme from *G. stearothermophilus* Ah22 by gel filtration chromatography, the sample was checked with SDS-PAGE (Fig. 3). A single band was obtained on 10% SDS–PAGE, indicating purity of the protein.

V_{max} and K_m values of purified PL were calculated using Lineweaver-Burk graphs at 0.47 mg/mL and 355.3 μmol/L·min, respectively. The molecular weight of

Table 2 The effect of some metal ions on PL from *G. stearothermophilus* Ah22 activity

Chemical compound	Concentration	PL activity (%)
Control	-	100
$CaCl_2$	10 mM	112.5
$MgCl_2$	10 mM	179.2
$Hg(NO_3)_2$	10 mM	0
$MnCl_2$	10 mM	0
$ZnSO_4$	10 mM	43.1
$Cu(NO_3)_2$	10 mM	62.2
$FeCl_2$	10 mM	70.5
EDTA	10 mM	0
β-Mercaptoethanol	10 mM	0
SDS	10 mM	0

Table 3 Increase in yield (expressed as % of control) in juice yield from different fruit pulps treated with purified PL from *G. stearothermophilus* Ah22 crude extract or Pectinex 100 L Plus for 5 h

	Apple (5 g)		Orange (5 g)		Peach (5 g)		Banana (5 g)	
	Juice (mL)	Yield (% of control)	Juice (mL)	Yield (% of control)	Juice (mL)	Yield (% of control)	Juice (mL)	Yield (% of control)
Control	17.5	100	20	100	22.0	100	13.0	100
Purified PL	19.0	108.6	26.5	132.5	23.0	104.5	14.5	111.5
Crude extract	18.9	108	23.8	119	23.9	108.7	15.2	116.9
Pectinex 100 L Plus	24.8	141.7	37.5	187.5	26.8	121.8	17.5	134.6

the enzyme was determined by Sephadex G 100 gel filtration chromatography. The molecular weight of the purified PL was 36 kDa. Compared to other studies, this value is approximately similar to the molecular weight of PL (52 kDa monomer) from *Bacillus* sp. PN33 (24 kDa), and higher than that from *Penicillium italicum* (34 kDa; Alana et al. 1991) and *Aspergillus niger* (30,900 Da; Obi and Moneke 1985).

Some properties (optimum pH, optimum temperature and stability) of the purified enzyme from *G. stearothermophilus* Ah22 were determined. In addition, the effects of some substances on enzyme activity were examined. Enzyme inhibition and stability are considered to be the major constraints to the rapid development of biotechnological processes. Stability studies also provide valuable information about the structure and function of enzymes. The stability of PL is affected by both physical (pH and temperature) and chemical (inhibitors or activators) parameters. The enzymatic hydrolysis of pectic substances also depends on several physicochemical factors, e.g., contact time, enzyme concentration, temperature of incubation and pH.

Similarly, optimal pH for the purified PL was investigated in 1 pH unit increments between 4 and 11 (Fig. 4). The optimal reaction pH for PL was 6, but it was active between pH 5 and 10. The optimum pH of our PL was identical to that of PL from *Bacillus* sp. DT7 and *B. pumilus* (P9) (Kashyap et al. 2000; Nadaroglu et al. 2010), but higher than that of the PLs produced from *Curvularia inaequalis* NRRL 13884 (pH 5; Afifi et al. 2002) and *Aspergillus niger* (pH 5; Obi and Moneke 1985). However, it is less than the optimum pH of PL from *Moniliella* SB9 (pH 9) and *Penicillium* sp. EGC5 (pH 10; Martin et al. 2004).

The effect of temperature was investigated between 0°C and 90°C in 10 degree increments (Fig. 5) and the optimum temperature was found to be 60°C. The enzyme has activity between 20°C and 80°C. The optimum temperature of PL was similar to PLs from *Bacillus* sp. DT7 and *B. pumilus* (P9) (Kashyap et al. 2000; Dosanjh and Hoondal 1996; Nadaroglu et al. 2010) but was higher than that of PLs from *Rhizopus oryzae* (Hamdy 2005), *Curvularia inaequalis* NRRL 13884 (Afifi et al. 2002), and *Bacillus* sp. PN33 (Kim et al. 1998).

The thermostability study of PL was tested at temperatures ranging from 40°C to 80°C (Fig 6). The purified enzyme appeared to be stable and retained full activity after 1 h incubation from 40°C to 50°C, but activity was reduced to 20% after 1 h at 60°C. PL activity decreased dramatically when the temperature increased above 70°C, with only 13% and 8% activity remaining at 70°C and 80°C, respectively. The PL was thermostable at 40°C, because it remained nearly full active at 40°C for at least 24 h. The thermostability of PL from *Aspergillus niger* was determined as 40–50°C (Obi and Moneke 1985). It was determined that PL from *Rhizopus oryzae* is inactivated

Table 4 Percent decrease in dry weight solid residue (DWSR) from different fruit pulps treated with purified PL from *G. stearothermophilus* Ah22, crude extract or Pectinex 100 L Plus for 5 h

	Apple (5 g)		Orange (5 g)		Peach (5 g)		Banana (5 g)	
	DWSR (g)	% Decrease	DWSR (g)	% Decrease	DWSR (g)	% Decrease	DWSR (g)	% Decrease
Control	2.6	-	4.5	-	2.6	-	8.5	-
Purified PL	1.8	30.8	2.1	53.3	1.7	34.6	2.4	71.8
Crude extract	1.7	34.6	2,3	58.9	2.1	19.2	3.3	61.2
Pectinex 100 L Plus	1.6	38.5	2.2	51.1	1.8	30.8	2.6	69.4

after 45 min at 70°C (Hamdy 2005). It was found that PL from *Pythium splendens* was stable at 4–50°C, but its activity decreased rapidly beyond 50°C (Chen et al. 1998).

The effect of various metal ions on PL activity was tested at 10 mM (Table 2). Purified PL was completely inhibited by 10 mM of Hg^{2+}, Mn^{2+}, EDTA, β-mercaptoethanol and SDS. A slight activation effect on the purified PL was observed in the presence of 10 mM Ca^{2+}. While Mg^{2+} (10 mM) stimulated the activity of PL, 10 mM Zn^{2+}, Cu^{2+} and Fe^{2+} has an inhibitory effect. In the presence of 10 mM Ca^{2+}, a decrease in activity was observed.

Removing solid substances and improving productivity were assessed separately with purified enzyme, crude extract and Pectinex 100 L Plus, and the results are presented in Tables 3 and 4. Treatment of fruit pulps with purified enzyme, crude extract and Pectinex 100 L Plus led to an increase in the volume of juice extracted from apple, orange, peach, and banana compared to the control (Table 3). The results obtained for purified enzyme, crude extract and Pectinex 100 L Plus were similar for fruit juice except for apple. The most acceptable result for peach was achieved with commercial Pectinex 100 L Plus, which also produced the highest overall yield, giving extraction levels in orange of 187.5% of the control.

The material was pressed more easily than the control and the residual dry weight of solid residue decreased from 19 to 72% (Table 4). As a result, the productivity yield of the fruit juice was increased. Banana, which has a high level of soluble pectin (Pilnik and Voragen 1993), resulted in a semi gelled mass that was very difficult to press after maceration. The juice obtained by enzymatic treatment had lower viscosity compared to non-treated samples, possibly due to the reduction in the pectin content.

In this paper, we report the production, purification and characterization of extracellular PL (E.C. 4.2.2.10) produced by *G. stearothermophilus* Ah22 in solid state fermentation. This is the first report on the production of PL from *G. stearothermophilus* Ah22. We also determined the action of PL in fruit juice processing. It was concluded that purified PL from *G. stearothermophilus* Ah22 can be used successfully in the production of fruit juice.

Conclusion

This is the first report on the production, purification and characterization of extra cellular PL (E.C. 4.2.2.10) produced by *G. stearothermophilus* Ah22. We also determined the action of PL in fruit juice production. It was concluded that purified PL from *G. stearothermophilus* Ah22 can be used in the process of obtaining fruit juice from fruit pulp.

References

Adiguzel A (2006) Molecular characterization of thermophilic bacteria isolated from water samples taken from various thermal plants. PhD Thesis, Ataturk University, Graduate School at Natural and Applied Sciences, Erzurum, Turkey

Afifi AF, Fawzi EM, Foaad MA (2002) Purification and characterization of a pectin lyase produced by *Curvularia inaequalis* NRRL 13884 on orange peels waste, solid state culture. Ann Microbiol 52:287–297

Adiguzel A, Ozkan H, Baris O, Inan K, Gulluce M, Sahin F (2009) Identification and characterization of thermophilic bacteria isolated from hot springs in Turkey. J Microbiol Methods 79 (3):321–328

Aguillar G, Huitron C (1990) Constitutive exopectinase produced by *Aspergillus* sp. CH-Y-1043 on different carbon sources. Biotechnol Lett 12:655–660

Alana A, Llama MJ, Serra JL (1991) Purification and some properties of the pectin lyase from *Penicillium italicum*. FEBS Lett 280:335–340

Banat IM, Marchant R, Rahman TJ (2004) *Geobacillus debilis* sp. nov., a novel obligately thermophilic bacterium isolated from a cool soil environment, and reassignment of *Bacillus pallidus* to *Geobacillus pallidus* comb. nov. Int J Syst Evol Microbiol 4(Pt 6):2197–2201

Beffa T, Blanc M, Lyon PF, Vogt G, Marchiani M, Fischer JL, Aragno M (1996) Isolation of *Thermus* strains from hot composts (60 to 80°C). Appl Environ Microbiol 62:1723–1727

Bradford MM (1976) Rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal Biochem 72:248–253

Canakci S, Inan K, Kacagan M, Belduz AO (2007) Evoluation of arabinofuranosidase and xylanase activities of *Geobacillus* spp. isolated from some hot springs in Turkey. J Microbiol Biotechnol 17(8):1262–1770

Cao J, Zheng L, Chen S (1992) Screening of pectinase producer from alkalophilic bacteria and study on its potential application in degumming of rammie. Enzyme Microb Technol 14:1013–1016

Celestino SMC, Freitas SM, Medranoc FJ, Sousa MV, Filho EXF (2006) Purification and characterization of a novel pectinase from *Acrophialophora nainiana* with emphasis on its physicochemical properties. J Biotechnol 123:33–42

Chen WC, Hsieh HJ, Tseng TC (1998) Purification and characterization of a pectin lyase from *Pythium splendens*-infected cucumber fruits. Bot Bull Acad Sin 39:181–186

Dosanjh N, Hoondal GS (1996) Production of constitutive, thermostable, hyperactive exopectinase from *Bacillus* GK-8. Biotechnol Lett 18:1435–1438

Gadre RV, Driessche GV, Beeumen JV, Bhat MK (2003) Purification, characterisation and mode of action of an endopolygalacturonase from the psychrophilic fungus *Mucor flavus*. Enzyme Microb Technol 32:321–330

Gainvors A, Belarbi A (1995) Detection method for polygalacturonase producing strains of *Saccharomyces cerevisae*. Yeast 11:1493–1499

Gummadi SN, Panda T (2003) Purification and biochemical properties of microbial pectinases—a review. Process Biochem 38:987–996

Hamdy HS (2005) Purification and characterization of the pectin lyase produced by *Rhizopus oryzae* grown on orange peels. Ann Microbiol 55:205–211

Harley JP, Prescott LM (2002) Laboratory exercises in microbiology, 5th edn. McGraw-Hill, New York

Horikoshi K (1972) Production of alkaline enzymes by alkalophilic microorganisms Part III. Alkaline pectinase of *Bacillus* No P-4-N. Agric Biol Chem 36:285–293

Hounsell EF, Davies MJ, Smith KD (1997) Chemical methods of analysis of glycoproteins. In: Walker JM (ed) The protein protocol handbook. Humana, Totowa, NJ, pp 633–634

Karbassi A, Vaughn RH (1980) Purification and properties of poygalacturonic acid trans-eliminase from *Bacillus stearothermophilus*. Can J Microbiol 26:377–384

Kashyap DR, Chandra S, Kaul A, Tewari R (2000) Production, purification and characterization of pectinase from a *Bacillus* sp. DT7. World J Microbiol Biotechnol 16:277–282

Kashyap DR, Vohra PK, Chopra S, Tewari R (2001) Applications of pectinases in the commercial sector: a review. Bioresour Technol 77:215–227

Kim JC, Kim HY, Choi YJ (1998) Production and characterization of acid stable PL from *Bacillus* sp. PN 33. J Microbiol Biotechnol 8:353–360

Laemmli UK (1970) Cleavage of structural proteins during the assembly of the head of bacteriophage. Nature 22:680–685

Martin N, Souza SR, da Silva R, Gomes E (2004) Pectinase production byfungal strains in solid-state fermentation using agro-industrial bioproduct. Braz Arch Biol Biotechnol 47:813–819

Maugeri TL, Gugliandolo C, Caccamo D, Stackebrandt E (2001) A polyphasic taxonomic study of thermophilic bacilli from shallow, marine vents. Syst Appl Microbiol 24:572–587

Nadaroglu H, Taskin E, Adiguzel A, Gulluce M, Demir N (2010) Production of a novel pectin lyase from *Bacillus pumilus* (P9), Purification and characterisation and fruit juice application. Romanian Biotechnol Lett 14:5167–5177

Nakagawa T, Nagaoka T, Taniguchi S, Miyaji T, Tomizuka N (2004) Isolation and characterization of psychrophilic yeasts producing cold-adapted pectinolytic enzymes. Lett Appl Microbiol 38:383–387

Nazina TN, Tourova TP, Poltaraus AB, Novikova EV, Grigoryan AA, Ivanova AE, Lysenko AM, Petrunyaka VV, Osipov GA, Belyaev SS, Ivanov MV (2001) Taxonomic study of aerobic thermophilic bacilli: descriptions of *Geobacillus subterraneus* gen. nov., sp. nov. and *Geobacillus uzenensis* sp. nov. from petroleum reservoirs and transfer of *Bacillus stearothermophilus*, *Bacillus thermocatenulatus*, *Bacillus thermoleovorans*, *Bacillus kaustophilus*, *Bacillus thermodenitrificans* to *Geobacillus* as the new combinations *G. stearothermophilus*, *G. thermocatenulatus*, *G. thermoleovorans*, *G. kaustophilus*, *G. thermoglucosidasius* and *G. thermodenitrificans*. Int J Syst Evol Microbiol 51(2):433–446

Nedjma M, Hoffmann N, Belarbi A (2001) Selective and sensitive detection of pectin lyase activity using a colorimetric test: application to the screening of microorganisms possessing pectin lyase activity. Anal Biochem 291:290–296

Obi SKC, Moneke NA (1985) Pectin lyase and polygalacturonase of *Aspergillus niger* pathogenic for yam tubers (*Diascorea rotundata*). Int J Food Microbiol 1:277–289

Olsson L, Christensen TMIE, Hansen KP, Palmqvist EA (2003) Influence of the carbon source on production of cellulases, hemicellulases and pectinases by *Trichoderma reesei* Rut C-30. Enzyme Microb Technol 33:612–619

Parisot J, Langlois V, Sakanyan V, Rabiller C (2003) Cloning expression and characterisation of a thermostable exopolygalacturonase from *Thermotoga maritima*. Carbohydr Res 338:1333–1337

Perry JJ, Staley JT (1997) Taxonomy of eubacteria and archaea. In: Perry JJ, Staley JT, Fort Worth (eds) Microbiology: dynamics and diversity. Saunders College Publishing, Orlando, pp 388–413

Pilnik W, Voragen AGJ (1993) Pectic enzymes in fruit juice manufacture. In: Nagodawithama T, Reed G (eds) Enzymes in food processing. Academic, New York, pp 363–399

Rahman TJ, Marchant R, Banat IM (2004) Distribution and molecular investigation of highly thermophilic bacteria associated with cool soil environments. Biochem Soc Trans 32:209–213

Rombouts FM, Pilnik W (1986) Pectinases and other cell-wall degrading enzymes of industrial importance. Symbiosis 2:79–89

Sharma HSS (1987) Enzymatic degradation of residual non cellulosic polysaccharides present on dew retted flax fibers. Appl Microbiol Biotechnol 26:358–362

Shevchik VE, Condemine G, Robert-Baudoy J, Hugouvieux-Cotte-Pattat N (1999) The exopolygalacturonase lyase PelW and the oligogalacturonate lyase Ogl, two cytoplasmic enzymes of pectin catabolism in *Erwinia chrysanthemi* 3937. J Bacteriol 181:3912–3919

Soares MMCN, Da Silva R, Carmona EC, Gomes E (2001) Pectinolytic enzyme production by Bacillus species and their potential application on juice extraction. World J Microbiol Biotechnol 17:79–82

Tanabe H, Yoshihara K, Tamura K, Kobayashi Y, Akamatsu I, Niyomwan N, Footrakul P (1987) Pretreatment of pectic waste water from orange canning process by an alkalophilic *Bacillus* sp. J Ferment Technol 65:243–246

Taragano VM, Pilosof AMR (1999) Application of Doehlert designs for water activity, pH, and fermentation time optimization for *Aspergillus niger* pectinolytic activities production in solid-state and submerged fermentation. Enzyme Microb Technol 25:411–419

Whitaker JR (1963) Determination of molecular weight of proteins by gel filtration on sephadex. Anal Chem 35:1950–1953

Zaliha RN, Rahman RA, Leow TC, Salleh AB, Basri M (2007) *Geobacillus zalihae* sp. nov., a thermophilic lipolytic bacterium isolated from palm oil mill effluent in Malaysia. BMC Microbiol 7(77):1–10