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Extracellular proteolytic activity of *Deinococcus geothermalis*

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Production of extracellular protease by extremophilic bacteria *Deinococcus geothermalis* cultivated in liquid media containing 0.1% (w/v) of peptone K, 0.1% yeast extract and 0.2% marine salt reached a maximum in 14 h of the cell growth at 45°C and pH 8.0. The enzyme was purified by a two-step procedure using fractionation by a graded ammonium sulphate precipitation technique and gel filtration on Sephadex G-100 column. Protease from *D. geothermalis* with a molecular mass of 24 kDa was active at 60°C and pH 9.0. The enzyme solution was stable for 1 h at 60°C and displayed about 60% of the initial activity after 1 h incubation at pH values 5.0 and 11. The phenylmethanesulfonyl fluoride (PMSF) at 1 mM concentration decreased proteolytic activity up to 27.4% of the initial value and it suggests that the enzyme is a serine protease. The activity was stimulated by Ca^{2+}, Na^{+}, Mg^{2+} and strongly inhibited by Hg^{2+}, Cu^{2+}, Zn^{2+} and Fe^{3+}. Nonionic detergents like Triton X-100 and Tween 80 did not affect catalytic properties. It suggested that the enzyme produced by *D. geothermalis* could be used as a component of detergents.

Key words: *Deinococcus geothermalis*, alkaline protease, detergents, thermostability.

INTRODUCTION

During the past decades, many studies on microbial proteases were reported and some of these enzymes find numerous applications (Gupta et al., 2002). Depending on enzyme source, proteases show different activity and substrate specificity. Usefulness of proteases is restricted by its selectivity, activity at high or low temperatures and the resistance to some chemicals sourced from the substrate or added to reaction media. Thus, the continuous search of microorganisms producing novel proteases is necessary.

Proteases from moderate thermophiles are of particular interest because they are stable and active at temperatures around 60 to 70°C. They have high affinity towards proteinaceous substrates since unfolded form of proteins created at elevated temperatures is more accessible to enzyme action (Suzuki et al., 2006). Performing an enzymatic reaction at enhanced temperatures allows for higher substrate concentration, lower viscosity, reduction of microbial contamination and high reaction rates (Synowiecki, 2010). Furthermore, the resistance against organic solvents makes some proteases useful for synthesis of certain oligopeptides, carried-out in reaction media with low water content (Isono and Nakajima, 2000; Nagashama et al., 1992). Enzymes having high activity in the pH range above 8.0 are important group of enzymes widely used as

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Abbreviations: PMSF, Phenylmethanesulfonyl fluoride; **TCA,** trichloroacetic; **SDS/PAGE,** sodium dodecylsulphate polyacrylamide gel electrophoresis; **BSA,** bovine serum albumin; **EDTA,** ethylene diamine tricholoroacetic acid.

ingredient for laundry detergents. Biocatalysts useful for this purpose must be stable and active in the presence of surfactans, builders bleaching agents, softeners and other typical detergent components (Banerjee et al., 1999). Moreover, proteases active at alkaline environments are useful for other purposes, such as isolation of nucleic acids, degumming of silk, selective delignification of hemp and recovery of silver from used photographic films (Kyon et al., 1994; Kanehisa, 2000; Fujiwara et al., 1991; Dorado et al., 2001; Klibanov et al., 2001).

We report the proteolytic activity of post-cultivation broth of *Deinococcus geothermalis* and this study allows broadening our knowledge about biodiversity of microbial sources of proteases. *D. geothermalis* is red-pigmented, Gram-positive moderate thermophile, extremely resistant to ionizing radiation, ultraviolet light and desiccation. This bacterium can be cultivated on inexpensive substrates in aerobic condition (Ferreira et al., 1997).

MATERIALS AND METHODS

Organism and culture conditions

D. geothermalis (DSM 11300) was routinely cultivated under aerobic conditions at pH 7.2 in a media containing 0.1% of peptone K (BTL, Poland), 0.1% of yeast extract (BTL, Poland) and 0.2% marine salt as a source of microelements (Dako-Art, Poland). The pH was adjusted with 4 M NaOH solution and the growth media were sterilized for 30 min at 121°C. The cultures were grown in 1 L Erlenmeyer flasks containing 200 mL of liquid medium. The flasks were inoculated with 5 mL of *D. geothermalis* cell suspension prepared according to DSM recommendation and incubated at 45°C with the agitation rate of 160 rpm on a rotary shaker (Forma Orbital, Thermo Scientific, Marietta, OH, US) for 24 h. After desired time of growth without additional aeration, the culture was centrifuged at 6,000 x *g* for 15 min (15°C). Then, the supernatant fluid was lyophilized.

Effect of cultivation condition

The influence of pH and temperature on cultivation of *D. geothermalis* was investigated in the range 4 to 11 and 30 to 60°C, respectively. Effect of different carbon and nitrogen sources in a growth media was also assayed. Cultivation was carried out for 24 h using inorganic nitrogen sources (1% w/v) such as urea, NH_4NO_3 and $(NH_4)_2SO_4$. As carbon source, glucose, maltose, trehalose, saccharose and starch at 1% (w/v) were used. In all cases, enzyme activity and biomass yield were determined.

Enzyme purification

A known part of lyophilized post-cultivation broth was dissolved in 20 mL 20 mM phosphate citrate buffer (pH 7.2) and was fractionated with $(NH_4)_2SO_4$ at 30, 45 and 90% of saturation. Proteins precipitated at 90% saturation were concentrated and partially purified on a Centrifugal Filter Device (Amicon® Ultra-15) 10,000 MWCO, Carrightwohill, Cork, Ireland) and then polished with gel filtration. Gel filtration was carried out on Sephadex G-100 column (2.3 x 45 cm). Proteins were eluted with 50 mM phosphate citrate buffer (pH 7.0) at flow rate 1 mL/min. At each fraction of 4 mL volume, enzymatic activity and protein concentration were measured. Protein concentration was determined at 280 nm. Fractions with proteolytic activity were collected and concentrated.

Enzyme assay

The enzyme activity was assayed using azocasein (Serva Feinbiochemica) as the substrate according to modified method of Sarath et al. (2001). The reaction mixture consisted of 0.25 mL 20 mM phosphate citrate buffer (pH 7.2), containing 0.2% (w/v) azocasein and 0.15 mL of crude enzyme. After incubation at 60°C for 15 min, the reaction was stopped by addition of 1.2 mL of 10% (w/v) trichloroacetic acid (TCA) solution. The samples were left at 4°C for 15 min and then centrifuged 12,000 x *g* for 10 min to remove the precipitate. The supernatant was added to 2 mL of 1 M NaOH solution and absorbance was measured at 440 nm. Control samples were prepared in a similar manner except that the enzyme was replaced by appropriate buffer. Enzyme blank samples were prepared by mixing enzyme, TCA solution and substrate in that order. One unit of activity was defined as the amount of enzyme required to produce an absorbance change of 0.01 under described reaction conditions. All the experiments were carried out in triplicate and the mean values with standard deviation are presented. The relative activity (%) was defined as the percentage of proteolytic activity in the control.

Effect of reaction conditions on enzyme activity and stability

The effect of temperature on the enzyme activity was determined by performing the standard assay procedure at pH 7.2 in the range of 30 to 80°C. To determine the stability against thermal denaturation, the enzyme samples were incubated at various temperatures for 1 h and then the residual activity was assayed. The optimum pH was determined with azocasein as substrate dissolved in 100 mM Britton Robinson buffers of different pH values in the range of pH 4.0 to 12.5. The pH-stability were determined by measuring the residual activities of the sample after 1 h incubation (15°C) in 50 mM Britton-Robinson buffers at various pH values.

Effect of metal ions on enzyme activity

The effects of various cations on enzyme activity were assayed by adding to the crude enzyme the chloride salts solution, specified in Table 4, containing the corresponding cation up to a final concentration of 1, 5 or 10 mM. After pre-incubation at 4°C for 1 h, the activity was determined in triplicate under standard conditions. Separate blanks with individual metal ions were prepared for each sample. The activity of the enzyme without metal ions addition was considered as 100%.

Effect of inhibitors and some other chemicals on enzyme activity

The effect of chemicals, specified in Table 3, on enzyme activity was determined by addition to the crude enzyme inhibitors up to a final concentration of 1 mM and at final concentrations of 5 mM and 10 mM in case of other chemicals. After pre-incubation at 4°C for 1 h, the activity was assayed in triplicate under standard conditions. Separate blanks with individual chemicals were prepared for each sample. The activity of enzyme measured in absence of chemicals was taken as control.

Organic solvent stability assay

The aqueous solution of the crude enzyme was incubated for 1 h

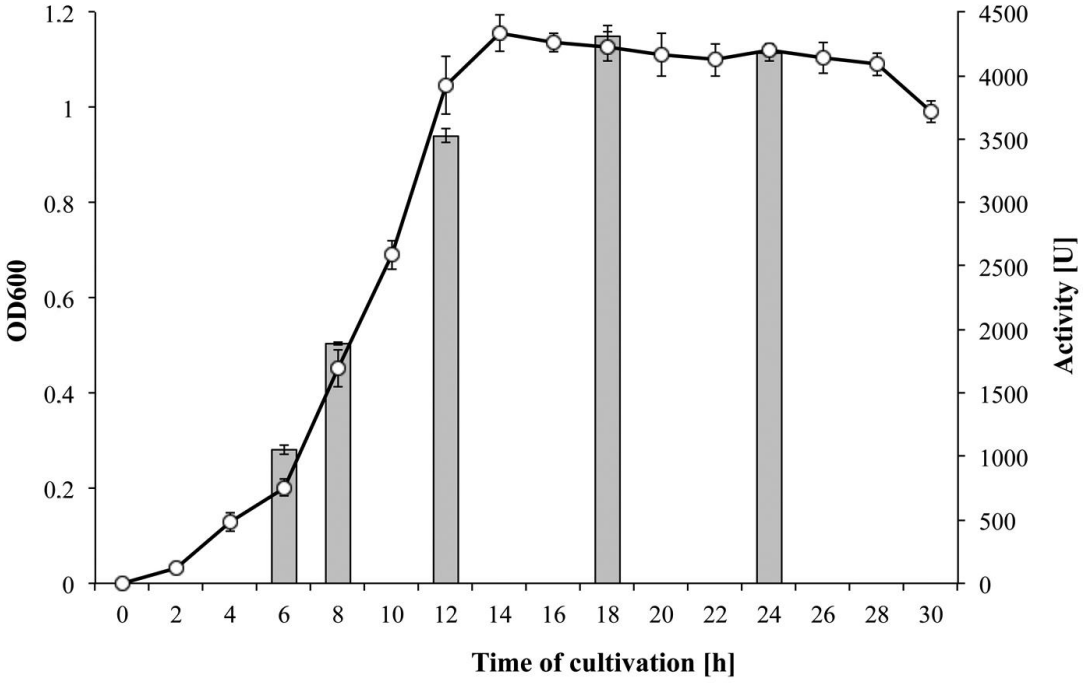

Figure 1. The effect of cultivation time on *Deinococcus geothermalis* growth (○-○) and activity (□) of culture broth (U/L). The results are mean values of data from three replicates ± standard deviations.

at 25% (v/v) concentration of organic solvents: Acetone, dimethyl sulfoxide (DMSO), methanol, ethanol and isopropanol. The remaining enzyme activity was determined in triplicate under standard conditions. The activity of the enzyme, determined without organic solvents, was taken as control.

Molecular mass determination

The purified protease from *D. geothermalis* was applied on a Sephadex G-100 column (2.3 x 45 cm) equilibrated with 50 mM phosphate citrate buffer (pH 7.0) and then was eluted with the same buffer. Absorbance at 280 nm was measured to monitor the elution profile. The elution patterns of protease were compared with those of standard proteins: Lysozyme (14.6 kDa), ovoalbumin (43 kDa), monomer of bovine serum albumin (66 kDa), and α-amylase (51 kDa). The molecular mass of the enzyme subunit was determined by sodium dodecylsulphate polyacrylamide gel electrophoresis (SDS/PAGE) using 12% (w/v) polyacrylamide gel in a Tris-glycine buffer (pH 8.3) (Laemmli, 1970). The samples (20 µL) denatured by β-mercaptoethanol as a reducing agent and SDS were layered on the gel and separated using a voltage gradient of 15 V/cm. Protein bands were located by staining with silver according to the method of Shevchenko et al. (1996). The molecular mass was determined using a molecular mass marker kit (Fermentas). Purity and molecular mass were determined from SDS/PAGE gels with Quantity One Software (BioRad, CA, USA).

Substrate specificity

The ability to hydrolyze different protein substrates was assayed using 2% (w/v) solutions of casein, hemoglobin, lyzozyme, ovoalbumin, bovine serum albumin (BSA), bovine β-globulin, gelatin and keratin hydrolyzate. 2.5 mL solution of given protein substrate was incubated for 2 min at 60°C. Then, 0.5 mL of enzyme solution was added and reaction was carried out for 15 min. Reaction was terminated by addition of 2.5 mL of 10% trichloroacetic acid, the samples were incubated for 15 min at 4°C and centrifuged (10 min, 12000 x *g*). 2 mL of supernatant was transferred to 5 mL 0.5 M Na_2CO_3 solution, 1 mL of Folin reagent was added and absorbance was measured at 660 nm. Control samples were prepared in a similar manner except that the enzyme was replaced by appropriate buffer. Enzyme blank samples were prepared by mixing enzyme, TCA solution and substrate in that order. One unit of activity was defined as the amount of enzyme required to produce 1 nmol of tyrosine under described reaction conditions. All the experiments were carried out in triplicate and the mean values with standard deviation were given.

Kinetic calculation

The K_m and V_{max} value of protease from *D. geothermalis* were determined by measuring the enzyme activity with various concentrations of azocasein solution as substrate (0.625 to 40 mg/mL). Kinetic constants were calculated from Lineweaver and Burk (1934).

RESULTS AND DISCUSSION

Cultivation condition

Figure 1 shows that the total proteolytic activity in the cultures cultivated on standard growth medium reached a maximum (4412 U/L) in 14 h of growth and was highly correlated (r = 0.981) with the cell density. This suggests

Table 1. The effect of different media on proteolytic activity of extracellular proteases from *Deinococcus geothermalis*.

S/N	Media	Amount of cells from 1 L culture (g)[1]	Activity of 1 L of post cultivation liquid (U)[1]
1	Standard	3.50 ± 0.10^{f}	3661.1 ± 38.5^{h}
2	Beef extract	3.51 ± 0.20^{f}	2800.0 ± 76.4^{g}
3	Peptone K	2.74 ± 0.09^{d}	2355.6 ± 50.9^{f}
4	Yeast extract	3.12 ± 0.04^{e}	4077.8 ± 113.4^{i}
5	Urea	0.10 ± 0.01^{a}	55.6 ± 9.6^{a}
6	NH_4NO_3	0.18 ± 0.01^{a}	88.9 ± 9.6^{a}
7	$(NH_4)_2SO_4$	0.26 ± 0.11^{a}	50.0 ± 0.0^{a}
8	Glucose	0.48 ± 0.03^{b}	438.9 ± 25.5^{b}
9	Maltose	0.56 ± 0.04^{b}	550.0 ± 16.7^{c}
10	Trehalose	0.60 ± 0.06^{b}	527.8 ± 25.5^{bc}
11	Saccharose	0.43 ± 0.37^{b}	905.6 ± 9.6^{d}
12	Starch	0.88 ± 0.11^{c}	1138.9 ± 9.6^{e}

[1]The values for a particular columns followed by different letters (a–g), differ significantly (P<0.05).

Table 2. Purification of protease from *Deinococcus geothermalis*.

Purification step	Total protein (mg)	Total activity (U)	Specific activity (U/mg)	Recovery (%)	Purification fold
Culture supernatant[a]	115.4	4513.8	39.11	100	1
Ammonium sulphate precipitation at 90% (w/v) saturation	40.89	1975.0	48.3	43.75	1.23
Sephadex G-100	0.96	289	301.1	6.40	7.7

[a]Culture supernatant from 1 L of broth.

that production of protease by *D. geothermalis* is dependent on cell growth. The effect of carbon and nitrogen sources on enzyme production was studied (Table 1). The maximum protease production was obtained with yeast extract (4077.8 U/L), beef extract (2800 U/L) and peptone K (2355.6 U/L). They are balanced source of carbon, nitrogen and mineral substances and may constitute the sole component of the medium suitable for production of biomass and biocatalyst production. *D. geothermalis* produced markedly less protease activity in the presence of sugars. Almost complete inhibition of growth and therefore loss of activity was observed in the case of inorganic nitrogen compounds. The similar situation was observed in the case of *Pseudomonas aureginosa* MCM B-327 that exhibit highest activity with beef extract, peptone and soybean meal as a growth media (Zambare et al., 2011).

The effect of initial pH of the culture medium on the cell production was studied in pH range 4 to11. The highest cells yield was observed during growth at pH 8.0 and it decreased by about 11.2 and 8.9% at pH values 4.0 and 9.0, respectively. No growth was found at 11.0. The bacterial growth and the enzyme secretion were also measured at temperature range from 30 to 60°C. The highest activity and wet cell yield (3.45 g/L of medium) were achieved after cultivation in a standard medium (pH 7.2) at 45°C. The cell growth at 35 and 55°C decreased total enzyme activity up to 75.8 and 16.7% of the maximal value, respectively.

Enzyme purification

Protease from *D. geothermalis* was purified by the two-step procedure (Table 2). In the first step, the post-cultivation broth was fractionated with ammonium sulphate and then polished with gel filtration on Sephadex G-100 column. The results show that fraction precipitated at 90% (w/v) $(NH_4)_2SO_4$ saturation had the highest proteolytic activity. A significant purification degree of the enzyme was confirmed by electrophoresis (SDS-PAGE) (Figure 2). The protein was purified 1.23-fold and its specific activity was 48.3 U/mg.

Due to this fact, it was decided to use fraction of protein precipitated at 90% $(NH_4)_2SO_4$ saturation in the next purification step. Consequently, after gel filtration, the protease was purified 7.7-fold and 6.40% of the total activity units were recovered (Table 2). The specific activity of purified enzyme was 301.1 U/mg. Similar situation was noticed in the case of protease from

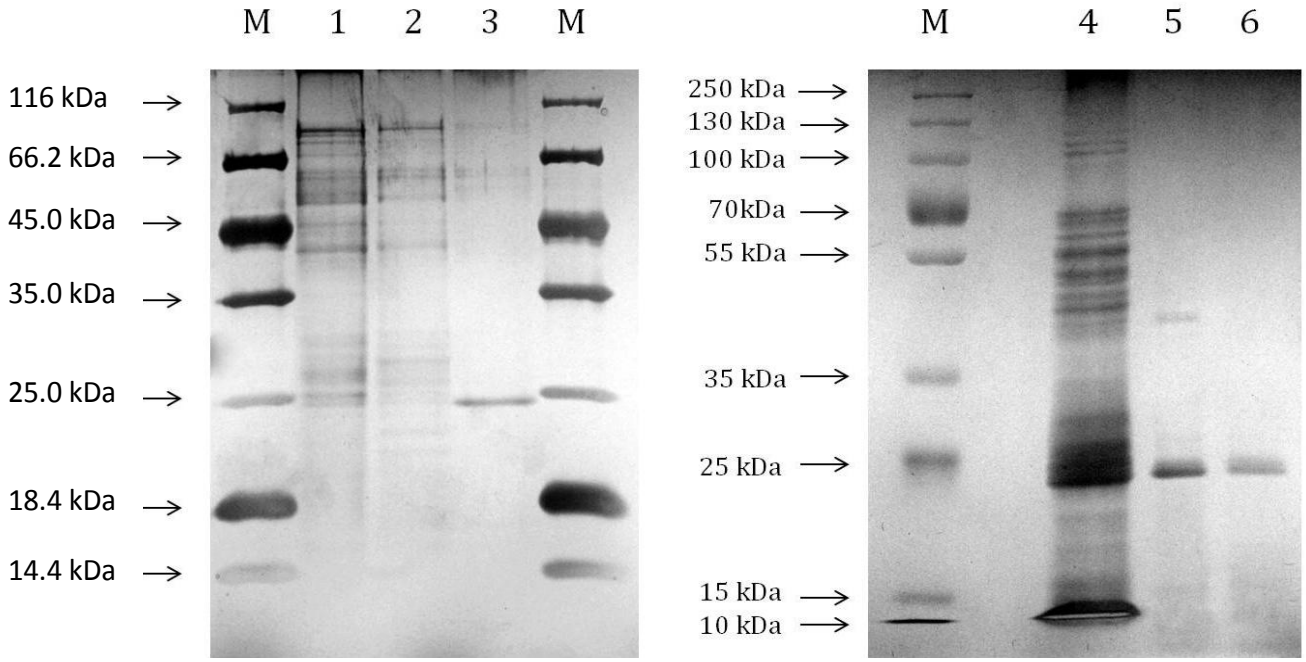

Figure 2. SDS-PAGE of post-cultivation broth proteins from *D. gethermalis* in the fraction during purification steps. M, Molecular weight marker; 1, fraction precipitated at 30% $(NH_4)_2SO_4$ saturation; 2,fraction precipitated at 45% $(NH_4)_2SO_4$ saturation; 3, fraction precipitated at 90% $(NH_4)_2SO_4$ saturation; 4, post-cultivation broth; 5, fraction precipitated at 90% $(NH_4)_2SO_4$ saturation; 6, fraction after gel filtration.

Table 3. The effect of chemicals on activity of extracellular protease from *D. geothermalis*.

Chemical compound	Concentration	Relative activity (%)
Control	-	100
SDS	5 mM	66.56 ± 2.4
	10 mM	60.25 ± 4.3
2-mercaptoethnol	5 mM	96.61 ± 0.9
	10 mM	91.22 ± 1.4
DTT	5 mM	78.43 ± 1.6
	10 mM	64.71 ± 0.8
Tween 80	5 mM	96.80 ± 0.7
	10 mM	94.31 ± 1.2
Triton X-100	5 mM	90.29 ± 1.7
	10 mM	87.83 ± 0.9
Tris	5 mM	99.78 ± 0.7
	10 mM	101.08 ± 1.1
	20 mM	100.86 ± 1.3
Urea	1 M	98.92 ± 1.5
	4 M	93.30 ± 0.6
Pepstatin	1 mM	95.66 ± 1.8
pCMB	1 mM	96.46 ± 1.4
Iodoacetamide	1 mM	100.32 ± 2.4
PMSF	1 mM	28.46 ± 0.5
EDTA	1 mM	99.7 ± 1.3
	10 mM	65.6 ± 0.9

Mean value of three replicates ± standard deviation.

Bacillus circulans. Two-step purification of post-cultivation broth, consisting of precipitation at 60% $(NH_4)_2SO_4$ saturation and gel filtration chromatography on Sephadex G-100, was sufficient to obtain a homogenous enzyme. According to Subba Rao et al. (2009), this procedure led to 12-fold purification of enzyme with specific activity of 9000 U/mg.

The purified enzyme was a monomer and its molecular mass was estimated to be 24 kDa. It was confirmed by SDS-PAGE (Figure 2.) and gel filtration. According to literature reports, extracellular proteases in most cases have monomeric structures. For example, extracellular alkaline serine proteases from *Bacillus pumilus* A1 (34 kDa), *Geobacillus* sp. YMTC 1049 (59.2 kDa), *B. circulans* (39.5 kDa), *B. pumilus* strain MS-1 (33 kDa) (Fakhafakh-Zouari et al., 2010; Zhu et al., 2007; Subba Rao et al., 2009; Miyaji et al., 2006).

Enzyme properties

The investigated enzyme was not inhibited by iodoacetamide which acts as cysteine protease inhibitor (Table 3). On the other hand, strong inactivation by 1 mM PMSF suggests that the enzyme is a serine protease. However, EDTA also inhibited the enzyme which retained only 65.6% of its activity when pre-incubated with 10 mM EDTA. This finding is in agreement with several earlier reports showing that active structure of serine proteases

Table 4. The effect of metal ions on activity of extracellular protease from *D. geothermalis*.

Parameter	Relative activity (%) at concentration		
Cation	1 mM	5 mM	10 mM
Control	100	100	100
Cu^{2+}	75.40 ± 2.7	59.18 ± 1.9	45.45 ± 1.2
Al^{3+}	94.92 ± 2.4	88.97 ± 3.0	69.02 ± 1.3
Ni^{2+}	92.3 ± 2.2	84.17 ± 0.4	71.52 ± 1.6
Ca^{2+}	101.12 ± 2.7	100.1 ± 1.7	97.58 ± 1.6
Co^{2+}	92.99 ± 1.7	63.12 ± 1.2	51.68 ± 1.0
Zn^{2+}	91.44 ± 2.1	72.13 ± 2.1	61.81 ± 0.6
Mg^{2+}	101.40 ± 1.7	102 ± 2.0	107.27 ± 3.1
Ba^{2+}	100 ± 1.6	81.18 ± 1.2	70.98 ± 1.5
Hg^{2+}	69.3 ± 2.0	42.50 ± 0.9	29.29 ± 3.0
Mn^{2+}	99.19 ± 2.0	79.04 ± 1.4	76.76 ± 1.6
Fe^{3+}	97.86 ± 4.4	84.11 ±2.1	62.20 ± 2.0
Na^{+}	101.94 ± 1.3	101.91 ± 0.8	101.73 ± 1.9

Mean value of three replicates ± standard deviation.

contains contains Ca^{2+} binding site and the removal of Ca^{2+} is associated to a significant reduction of enzyme activity. 5 mM DTT decreased the proteolytic activity up to 78.4% of the initial value, which suggests that the reduction of disulfide bridges disturbed the active structure of the protein.

The protease produced by *D. geothermalis* was stable in the presence of non-ionic surfactants like Triton X-100 and Tween-80 and showed moderate stability to SDS which is strong anionic detergent. The enzyme retained 60.2% of initial activity after 1 h pre-incubation with 10 mM SDS. Table 3 shows that protease from *D. geothermalis* was resistant against denaturation by 4 M urea. It suggests, that this protein has well-packed structure and the native conformation is very rigid. The stability in organic media is an important attribute of some proteases which can be used for peptide and ester synthesis under non-aqueous conditions. The enzyme maintained about 96.8% of initial activity after incubation with dimethyl sulfoxide (DMSO). Methanol caused slight decrease of activity of about 13%. Isopropanol, ethanol and acetone caused decrease of activity up to 62.91, 66.80 and 70.78%, respectively.

The investigated enzyme was salt tolerant and in the presence of 10 mM NaCl retained about 100% of activity. The examined bivalent metal cations at a concentration up to 10 mM affected protease activity at various degrees and further details are shown in Table 4. The calcium, sodium and magnesium stimulated the enzyme activity indicating that these ions had a functional role in the molecular structure of the protein whereas, the highest inhibitory effect exerted ions of transition metal Hg^{2+}, Cu^{2+}, Zn^{2+} and Fe^{3+}. It is known that the ions mercury, cadmium and lead react with protein thiol groups (converting them to mercaptides), as well as with histidine and tryptophan residues. Moreover, by action of Hg^{2+}, the disulphide bonds are degraded (Vieille and Zeikus, 2001). Other ions influenced negatively depending on their concentration which was presumably a consequence of the ionic strength increase in reaction fluid. The same effect of metal ions was observed for serine proteases from *B. circulans* and *Bacillus* sp. JB-99 (Subba Rao et al., 2009; Johnvesly and Naik, 2001). In both cases, presence of calcium, sodium and magnesium ions had positive effect while zinc and mercury caused inactivation of biocatalyst.

Extracellular protease from *D. geothermalis* was optimally active at pH 9.0 and the retention of about 80% of maximal activity was observed between pH 6.5 and 10.5 (Figure 3). A broad stability range of this protease confers it a potential to be used in many industrial applications. In comparison, the important detergent enzyme, subtilisin Carlsberg, showed maximum activity at pH values of 8.0-10.0. Furthermore, the enzyme from *D. geothermalis* retained 60% of its initial activity after 1 h of incubation at the pH values 5.0 and 11. The optimum temperature for activity towards azocasein was observed at 60°C. One of the most important characteristic is that the protease displayed about 80% of relative activity on the broad range of temperatures comprised between 45 and 70°C (Figure 4). The enzyme was stable after 1 h of pre-incubation at 60°C.

The protease from *D. geothermalis* exhibited wide substrate specificity and caused the degradation of structural proteins. The activities towards casein, hemoglobin and bovine β-globulin were 944.7 ± 34.0, 1050.1 ± 29.9 and 1238.0 ± 31.8 U, respectively. Markedly lower proteolytic activity was reported for keratin (170 ± 8.2 U), gelatin (585.3 ± 14.2 U), ovalbumin (63.6 ± 4.7 U) and lyzozyme (103.9 ± 10 U). The similar substrate preferences were noticed for extracellular protease from *P. aeruginosa* MCM B-327

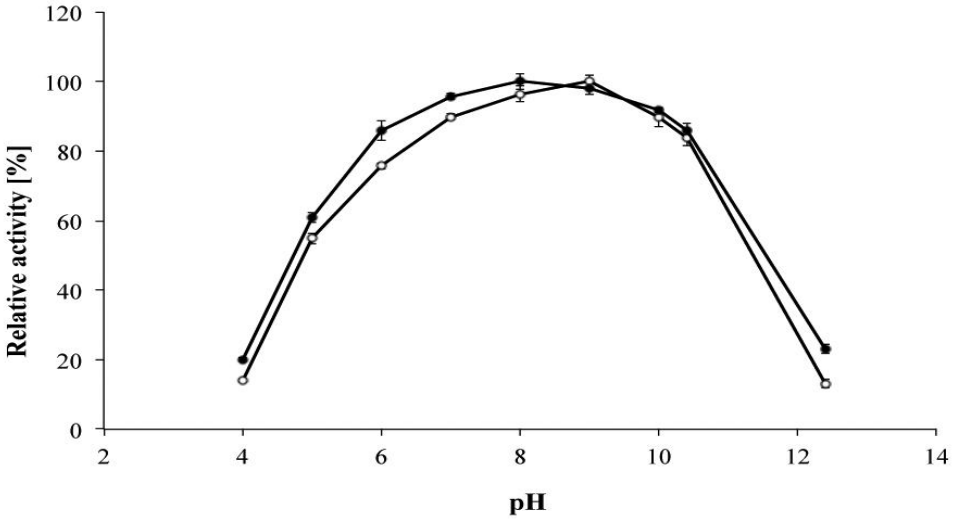

Figure 3. The effect of pH on activity (○) and stability (●) of extracellular protease from *D. geothermalis*. To examine the thermal stability, the samples were pre-incubated at various temperatures (30-80°C) for 1 h. The results are mean values of three determinations ± standard deviations.

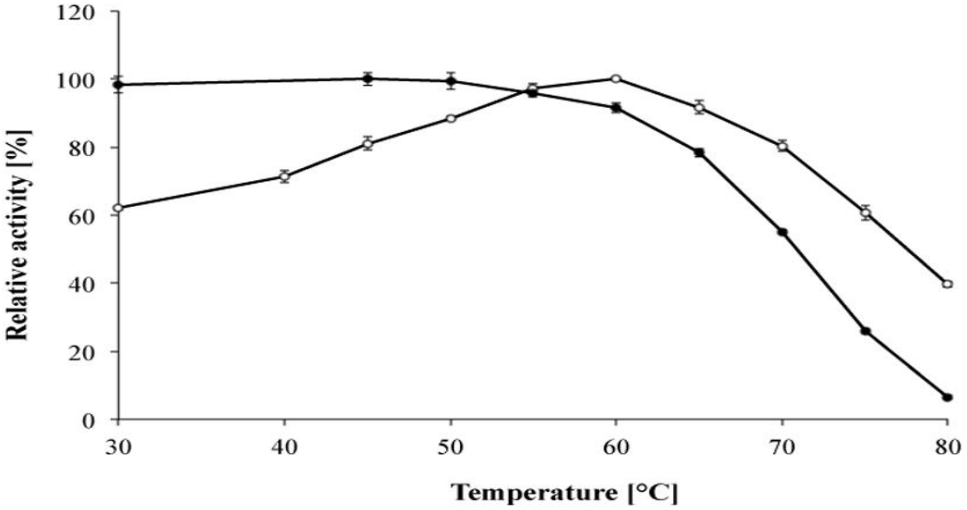

Figure 4. The effect of temperature on activity (○) and stability (●) of extracellular protease from *D. geothermalisr*. To examine the pH stability, the samples were incubated in Britton Robinson buffers (pH range 4-12.5) for 1 h. The results are mean values of data from three determinations ± standard deviations.

(Zambare et al., 2011). The kinetic study was carried out with azocasien as a substrate. Maximum velocity (V_{max}) and Michaelis-Menten's constant (K_m) were calculated from Linewaver-Burk's plot and were 2.18 U/min and 1.64 mg/mL, respectively. Approximate values of kinetic constants were reported for proteases from *Streptomyces* sp. XZNUM 00004 (K_m = 0.96 mg/mL), *Streptomyces* sp.CS684 (K_m=4.2 mg/mL) or *Enterococcus faecalis* TN-9 (K_m=0.98 mg/mL) (Ju et al., 2012; Simkhada et al., 2010; Yuan et al., 2009).

In conclusion, the properties reported in this article indicate that thermostable protease secreted by *D. geothermalis* showed activity at a broad range of temperature and pH that might have potential application in detergent, medical and food industries, such as meat tenderization or production of protein hydrolysates. It also allowed expanding knowledge about biodiversity microbial sources of the thermostable proteases.

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