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Original Article

Purification and characterization of an alkaline protease from *Bacillus licheniformis* UV-9 for detergent formulations

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Abstract

Alkaline protease produced by mutant strain *B. licheniformis* UV-9 was purified and characterized for its exploitation in detergent formulation. The enzyme was purified to homogeneity by employing ammonium sulphate precipitation and sephadex G-100 gel filtration chromatography with a 36.83 fold increase in specific activity and 11% recovery. The molecular weight of the protease was found to be 36.12 kDa by SDS-PAGE. The *K*m and *V*max values exhibited by purified protease were 5 mg/ml and 61.58ìM/ml/min, respectively, using casein as substrate. The enzyme exhibited highest activity at pH 11 and temperature 60°C. Stability studies showed that the enzyme retained higher than 80% residual activity in the pH and temperature ranges of 8 to 11 and 30 to 50°C, respectively. However, in the presence of 10 mM Ca^{2+} ions the enzyme tained more than 90% of its residual activity at pH 11 and temperature 60°C. Phenyl methyl sulphonyl fluoride (PMSF) completely inhibited the enzyme activity suggesting that it was serine protease. Among metal ions, the Mg^{2+} and Ca^{2+} ions enhanced activity up to 128% and 145%, respectively. The purified enzyme showed extreme stability towards various surfactants such as Tween-20, Tween- 45, Tween-65 and Triton X-45. In addition, the enzyme also exhibited more than 100% residual activity in the presence of oxidizing agents, H_2O_2 and sodium perborate. These biochemical properties indicate the potential use of *B. licheniformis* UV-9 enzyme in laundry detergents.

Keywords: alkaline protease, *B. licheniformis,* purification, characterization, oxidant stability

1. Introduction

Proteases represent one of the three largest groups of industrial enzymes and account for about 60% of the total worldwide sale of the enzymes (Rao *et al.,* 1998). They have diverse applications in various industries such as food, pharmaceuticals, silk and diagnostic with predominant use in detergent and leather industries (Kumar and Takagi, 1999; Oberoi *et al.,* 2001). Proteases in detergent formulations facilitate washing efficiency of detergents by releasing proteinaceous stains. The applications in detergent industries require their improved stability at elevated temperatures and pH and compatibility with various oxidants and metal ions (Jaswal and Kocher, 2007; Haddar *et al.,* 2009).

Several alkaline proteases have been purified and characterized from many *Bacillus* strains (Bhaskar *et al*., 2007; Doddapaneni *et al.,* 2007, Padmapriya *et al*., 2012). Subtilisin Carlsberg produced by *Bacillus licheniformis* (Jacob *et al.,* 1985) and Subtilisin Novo produced by *Bacillus amyloliquefaciems* (Wells *et al.,* 1983) have been the enzyme of choice for detergent industries. Both enzymes have similar molecular mass of 27.5 kDA but differ from each other in the constitution of amino acids. These enzymes exhibit maximum activity at alkaline pH values ranging from 8-10 (Horikoshi, 1999). Generally the alkaline proteases for detergent applications should be active at temperature higher than 40-50°C and pH in the range of 9-12 (Sellami-Kamoun *et al.,* 2008; Haddar *et al.,* 2009). Appropriate specificity and compatibi-

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lity with various detergent constituents continuously stimulate the thrust of new enzymes in the market. Purification and characterization is the basic need to elucidate such precise properties of a newly isolated enzyme for its applications in the industry. The purification process also increases the specific activities of enzymes, making them more specific for industrial applications (Kumar, 2002; Adinarayana *et al.,* 2003). In the present study, we report purification and characterization of alkaline protease produced by mutant strain *Bacillus licheniformis* UV-9. Further studies regarding stability and compatibility of the enzyme with various detergent constituents were also conducted to examine its potential use as detergent additive.

2. Material and Methods

2.1 Enzyme production

Production of alkaline protease from mutant *B. licheniformis* UV-9 was carried in a 2 L lab scale bioreactor (Eyela, Japan) having 1.5 L growth medium comprising of glucose, 1% (w/v); soybean meal, 1% (w/v); K_2HPO_4, 0.5% (w/v); $MgSO_4.7H_2O$, 0.05% (w/v); NaCl, 0.05% (w/v) and $CaCl_2.2H_2O$, 0.05% (w/v) at optimum conditions described elsewhere (Nadeem *et al.,* 2009). Cell-free supernatant was used for subsequent studies.

2.2 Enzyme assay

Protease activity was determined by the method of Yang and Haung (1994) with slight modifications in pH of substrate and incubation temperature. The reaction mixture containing 2 ml of 1% (w/v) casein solution in 0.05 M glycine-NaOH buffer (pH 11) and 1 ml of enzyme solution was incubated at 60°C for 15 min. The reaction was then stopped by adding 3 ml of 10% (w/v) trichloroacetic acid. After that the entire mixture was centrifuged at 9000 x *g* for 10 min at 4°C and absorbance of the liberated tyrosine was measured at 280 nm against blank. One proteolytic unit (IU) was defined as the amount of the enzyme that released 1 µg of tyrosine per minute, under the assay conditions.

2.3 Protein assay

Total protein contents were determined according to the method of Lowry *et al.* (1951), using bovine serum albumin as a standard.

2.4 Protease purification

2.4.1 Ammonium sulphate precipitation

Cell free supernatant was precipitated by adding ammonium sulphate at different saturation levels (40-80%). After each addition, the enzyme solution was stirred for 1 h at 4°C. The protein precipitated was collected by centrifugation at 12,000 x *g* for 20 min at 4°C and resuspended in minimum volume of 0.05M Tris-HCl buffer, pH 8.0 to get the concentrated enzyme suspension. After that the enzyme suspension was dialyzed against the same buffer with 4-6 changes.

2.4.2 Sephadex G-100 gel filtration chromatography

The concentrated enzyme sample was purified on sephadex G-100 (Pharmacia) column (1.5 cm x 30 cm) by suing FPLC system (Biologic LP, Bio-Rad, USA). The Sephadex column was equilibrated with 0.05 M Tris-HCl buffer of pH 8.0. The dialyzed enzyme sample was loaded onto a Sephadex G-100 column and then eluted with the same buffer. Fractions each of 4 ml were collected at a flow rate of 30 ml/h by fraction collector (Model 2110, Bio-Rad). The fractions showing absorbance at 280 nm were analyzed for protease activities and the active fractions were pooled, dialyzed and then freeze dried by freeze dryer (Eyela, Japan). The freeze dried preparation was stored at -20°C for further studies.

2.4.3 SDS-polyacrylamide gel electrophoresis

SDS-PAGE (12%) was performed according to the method described by Laemmli (1970) using a mini slab gel apparatus (8x8 cm glass plate). The molecular weight was determined by interpolation from linear semi-logarithmic plot of relative molecular weight versus the R_f value (relative mobility) using standard molecular weight markers (Fermentas).

2.5 Characterization of purified protease

2.5.1 Effect of pH on enzyme activity and stability

The activity of purified protease was measured at different pH values (6-12) by using 1% (w/v) solution of casein as a substrate dissolved in different buffers (0.05 M): phosphate (pH 6-7) tris–HCl (pH 8-9) and glycine-NaOH (pH 10-12). To determine pH stability, the enzyme was incubated in different buffers of pH values ranging from 6 to 12 for 12 h at 40°C in the absence and presence of 5 and 10 mM $CaCl_2.2H_2O$. The residual activities were then measured according to the standard assay procedure.

2.5.2 Effect of temperature on protease activity and stability

Influence of temperature on purified protease activity was studied by incubating reaction mixtures at different temperatures ranging from 30 to 80°C by using 1% casein solution. Thermal inactivation was examined by incubating purified enzyme at different temperatures (30-80°C) for 1 h in a water bath (Eyela, Japan) in the absence and presence of Ca^{2+} ions at 5 mM and 10 mM of $CaCl_2.2H_2O$. The residual activities were then measured as described above while

taking the activity of non-heated enzyme as 100%.

2.5.3 Effect of inhibitors and metal ions on protease activity

Effects on protease activity of various inhibitors (each at 5mM) such as phenyl methyl sulphonyl fluoride (PMSF), 1,10-phenanthroline, pepstatin, di-isopropyl fluorophosphate (DFP), cysteine inhibitors *p*-chloromercuric benzoate (*p*CMB), ethylene diamine tetra acetic acid (EDTA) and metal ions (Ca^{+2}, Zn^{+2}, Mg^{+2}, Na^{+2}, Hg^{+2}, Cu^{+2}, Al^{+3}, Ni^{+2}, Cd^{+2} and Co^{+2}), each at 5mM concentration on protease activity were studied. The purified enzyme was pre-incubated with the above inhibitors and metal ions for 30 min at 40°C. Then the remaining activity was measured routinely, taking activity in the absence of inhibitors and metal ions as 100%.

2.5.4 Effects of surfactants and oxidants on stability of alkaline protease

Effects of different surfactants like Tween-20 (0.5%, 1.0%); Tween-45 (0.5%, 1.0%); Tween-65 (0.5 %, 1.0 %); Triton-X-405 (0.5 %, 1.0 %); SDS (0.5 %, 1.0 %, 5.0 %) and oxidizing agents like H_2O_2 (0.5%, 1.0%, 5.0%) and sodium per borate (0.5%, 1.0%, 5.0%) on the alkaline protease stability were studied by incubating the mixtures for 1 h at 40°C. The residual activities were then measured and the activity of enzyme without any additive was taken as 100%.

2.5.5 Substrate specificity

Effects of various substrates such as casein, bovine serum albumin, gelatin, hemoglobin and keratin on purified alkaline protease activity were determined according to the method of Yang and Huang (1994) as described earlier. The protease activity towards casein was taken as control.

2.5.6 Enzyme kinetics

The K_m and V_{max} values for alkaline protease were calculated by linear regression analysis by Lineweaver-Burk plot (double reciprocal plot) using various concentration of casein (5, 10, 15, 20, 25 and 30 mg/ml). The experiments were carried out in triplicate and then the activity measured according to the standard assay conditions.

3. Results and Discussion

3.1 Purification of alkaline protease

Purification of alkaline protease of mutant *B. licheniformis* UV-9 is summarized in Table 1. Initially the enzyme solution was precipitated with ammonium sulphate and 60-70% saturation level increased the protease activity 6.41 fold with 62% recovery, showing specific activity of 2106.02 U/mg. The dialyzed enzyme suspension of 60-70% saturation level was then subjected to gel filtration chromatography on a Sephadex G-100 column for further purification. The elution profile yielded a well-resolved single peak showing protease enzyme after activity measurement (Figure 1). The active fraction of this peak gave a 36.83 fold increase in protease activity with a recovery of 11% and specific activity of 12102.96 U/mg of protein. The results indicate that gel filtration chromatography yielded a pure enzyme. Adinarayana *et al.* (2003) and Alam *et al.* (2005) have reported purification of protease enzymes by gel filtration chromatography with Sephadex G-200 and Sephadex G-100 columns, respectively. Wide-ranging results of protease purification (4.25-200-folds) with various specific activities (13.33-159381 U/mg of protein) and % recovery (2-21%) have been described for different microbial species (Tunga *et al.,* 2003; Tang *et al.,* 2004; Kim and Kim, 2005; Guangrong *et al.,* 2006; Yossan *et al.,* 2006).

The enzyme purity was confirmed by SDS-PAGE which demonstrated a single band, indicating homogeneous preparation (Figure 2a). Molecular weight of the protease was determined by interpolation from a linear logarithmic plot of relative molecular mass versus the R_f value. The molecular weight of the protease band was accordingly calculated and found to be as 36.12 kDa (Figure 2b). Beg and Gupta (2003) reported a 30 kDa molecular weight serine alkaline protease produced by *B. mojavensis*. Generally, the molecular masses of alkaline proteases from various *Bacillus* species range between 17-40 kDa (Tang *et al.,* 2004; Kim and Kim, 2005; Guangrong *et al.,* 2006; Yossan *et al.,* 2006, Haddar *et al.,* 2009; Padmapriya *et al.,* 2012), with few exception of high molecular mass, such as up to 90 kDa from *Bacillus* subtilis (Kato *et al.,* 1992). The molecular masses of most popular Subtilisin Carlsberg and Subtilisin BPN are 27.3 and 27.5 kDa, respectively (Wells *et al.,* 1983; Jacobs *et al.,* 1985).

Table 1. Summary of purification of alkaline protease produced by *B. licheniformis* UV-9 in submerged fermentation process

Purification Steps	Total activity (U)	Total protein (mg)	Specific activity (U/mg)	Purification fold	Yield (%)
Crude enzyme	2530620	7700	328.65	1	100
Ammonium sulphate precipitation (60-70%)	1568984	745	2106.02	6.41	62
Sephadex G-100	278368	23	12102.96	36.83	11

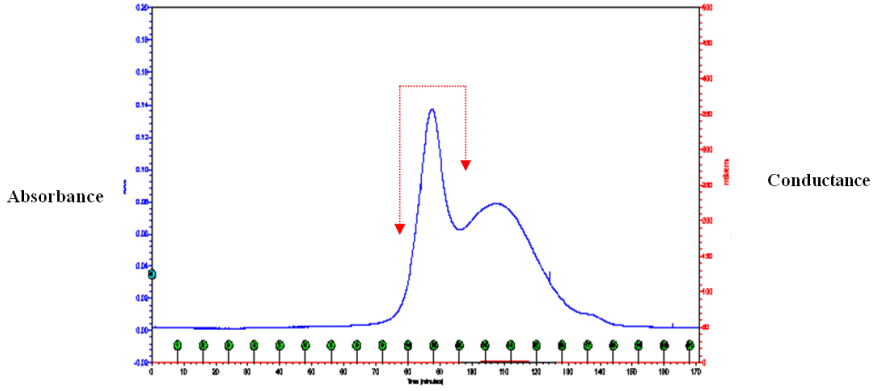

Figure 1. Elution profile of alkaline protease of *B. licheniformis* UV-9 from Sephadex G-100 column by FPLC

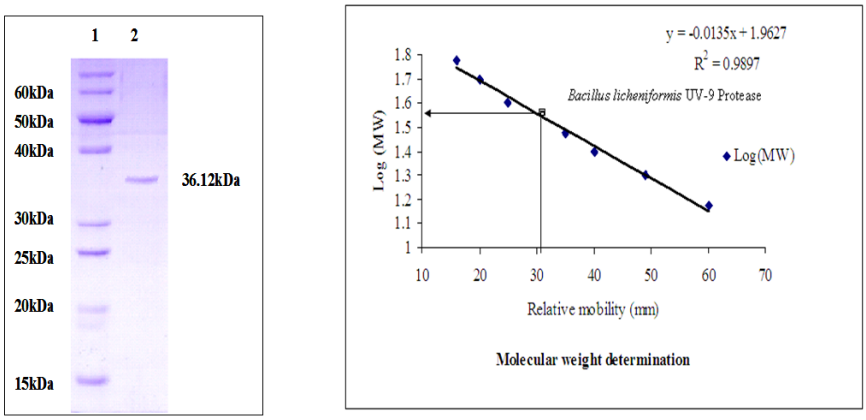

Figure 2. SDS-PAGE of the purified protease. Molecular mass markers and the purified protease were applied to lanes 1 and 2, respectively. a: SDS-PAGE; b: Molecular weight determination

3.2 Characterization of purified protease

3.2.1 Effect of pH and temperature on enzyme activity and stability

The present protease was found to be active over a broad range of pH 8-12 with optimal activity at pH 11 indicating alkaline nature of the enzyme (Figure 3a). Generally, commercial proteases from microorganisms have maximum activity in the alkaline pH range of 8-12 (Rao *et al.,* 1998; Kumar *et al.,* 1999; Gupta *et al.,* 2002). Optimum pH of 10 of alkaline proteases from various *Bacillus* species has been described by some workers (Adinarayana *et al.,* 2003; Uchida *et al.,* 2004; Gupta *et al.,* 2005).

pH of the laundry detergent is usually in the range of 9-12. The results of pH stability study indicated that the enzyme was stable over a wide range of pH (8-11) and retained its 80% activity at pH 11 (Figure 3b). However, 40% remaining activity was observed at pH 12 in the absence of Ca^{2+} ions. The presence of 5 and 10 mM Ca^{2+} ions increased the stability against various pH. About 90 and 93% activities are noted at pH 11 in the presence of 5 and 10 mM Ca^{2+} ions, respectively. However, the enzyme retained its 60 and 72% activity in the presence of Ca^{2+} ions at pH 12. Proteases produced from *Bacillus subtilis* PE-11 and *Bacillus subtilis* CN2 have been described as remaining stable in the ranges of 8-11 and 7-11, with relative activities of more than 90% and 70%, respectively (Adinarayana *et al.,* 2003; Uchida *et al.,* 2004). Comparable results have been reported by Sookkheo *et al.* (2000). These workers found 60% proteolytic retention at pH 10 in the presence of 5 mM Ca^{2+} ions. All these investigations indicate that pH stabilities of enzymes depend on the available concentration of Ca^{2+} ions in the enzyme solution.

Optimum temperature for protease of *B. licheniformis* UV-9 was found to be 60°C when tested at pH 11 using casein as substrate (Figure 4a). Maximum proteolytic activity of *Bacillus* strains HR-08 and KR-8102 isolated from soil of western and northern parts of Iran have been recorded at 65°C and 50°C, respectively (Moradian *et al.,* 2006). Proteases from *P. aeruginosa* MN1 and some other *Bacillus* species have been described with optimum temperature of 60°C (Banerjee *et al.,* 1999; Beg and Gupta, 2003; Nascimento and Martins, 2004: Khosravi-Darani *et al.,* 2008; Olajuyigbe and Ajele, 2008). These findings indicate that variation in the characteristics of the extracellular proteolytic enzymes could

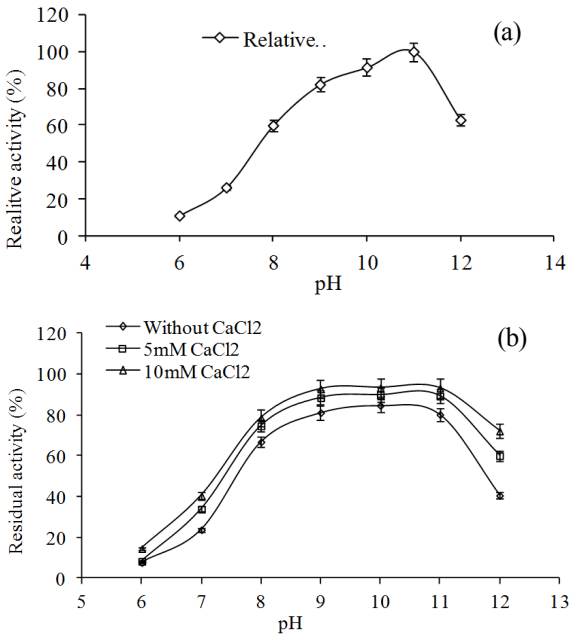

Figure 3. (a) Effect of different pH levels on alkaline protease activity produced by *B. licheniformis* UV-9. Bars represent ± S.D. (b) Effect of various pH on the stability of protease produced by *B. licheniformis* UV-9 in the presence and absence Ca2+ ions. The stability is expressed in percentage of residual activity. Bars represent SD.

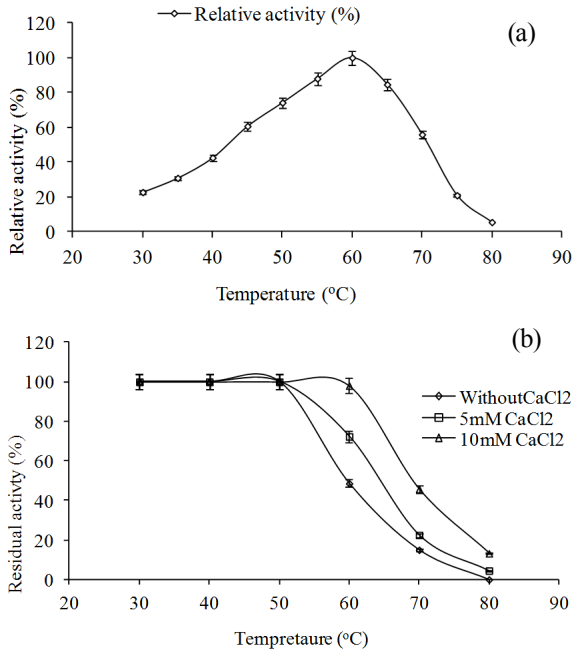

Figure 4. (a) Effect of different temperatures on protease activity produced by B. *licheniformis* UV-9. Bars represent ± S.D. (b) Effect of various temperatures on stability of protease produced by *B. licheniformis* UV-9. The stability is expressed in percentage of residual activity. Bars represent SD.

exist among various *Bacillus* species.

The investigations regarding thermostability of alkaline protease showed that the enzyme was stable up to 50°C and above this temperature its activity decreased. However, presence of Ca^{2+} ions improved thermostability of the alkaline protease produced by *B. licheniformis* UV-9. The enzyme retained 72 and 98% residual activities at 60°C in the presence of 5mM and 10 mM Ca^{2+} ions, respectively (Figure 4b). Ca^{2+} ions have been described to keep 78% of residual activity of thermostable bacterial enzymes after incubation at 80 °C for 1 h in the presence of 10 mM Ca^{2+} ions (Johnvesly and Naik, 2001). Most alkaline proteases have been reported to remain stable at high temperature in the presence Ca^{2+} ions (Rahman *et al.,* 1994; Kumar *et al.,* 1999). The Ca^{2+} ions may stabilize the structure of the present enzyme which consequently increased its thermal stability at elevated temperatures.

3.2.2 Effects of inhibitors and metal ions on enzyme activity

Effects of different inhibitors and metal ions on alkaline protease of *B. licheniformis* UV-9 are given in Table 2. The results showed that the enzyme was completely inhibited by serine protease inhibitor phenylmethyl sulphonyl fluoride (PMSF), suggesting its serine nature. PMSF is a well known serine protease inhibitor which results in complete loss of the enzyme activity after inhibition (Tsuchida *et al.,* 1995; Jeong

Table 2. Effect of inhibitors and activators on the relative activity of alkaline protease produced by *B. licheniformis* UV-9

Inhibitors/ Activator	Relative Activity (%)
Control	100
PMSF	0
1,10-phenanthroline	97
Pepstatin	103
DFP	03
$_{P}$CMB	88
EDTA	90
Ca^{2+} ($CaCl_2$)	145
Zn^{2+} ($ZnCl_2$)	80
Mg^{+2} ($MgCl_2$)	128
Na^{2+} ($NaCl_2$)	102
Hg^{2+} ($HgCl_2$)	70
Cu^{2+} ($CuCl_2$)	106
Al^{3+} ($AlCl_3$)	65
Ni^{2+} ($NiCl_2$)	98
Cd^{2+} ($CdCl_2$)	90
Co^{2+} ($CoCl_2$)	93

PMSF = Phenylmethyl sulphonyl fluoride; DFP = di-Isopropyl fluorophosphate; pCMB = p-Chloromercuric benzoate; EDTA = Ethylene diamine tetra acetic acid. The concentration of all inhibitors and metal ions was adjusted at 5 mM.

et al., 2000; Adinarayana *et al.,* 2003). The inhibitor di-isopropyl fluorophospahte (DFP) reduced the protease activity up to 97%. Similar results of inhibition by DFP have been recorded for serine protease produced by *B. licheniformis*, *B. pumilus* and *B. inetermedius* 3-19 (Aoyama *et al.,* 2000; Huang *et al.,* 2003; Tang *et al.,* 2004). The presence of suitable metal ions plays an important role in maintaining active conformation of enzyme against thermal denaturation (Donaghy and Mckay, 1993). Therefore, identification of proper metal ions has significant impact on enzyme applications at commercial level. Results of some metal ions on the protease activity indicated that Ca^{+2} and Mg^{+2} ions increased relative enzyme activity up to 145 and 128%, respectively. More than 100% relative activities also appeared in the presence of Cu^{+2} and Na^{+1} ions. Siriporn *et al.* (2006) described that Mn^{+2}, Ca^{+2} and Mg^{+2} ions increased relative activity of protease produced by *Bacillus megatarium*. These cations have also been reported to increase activity and thermostability of *Bacillus* alkaline proteases (Johnvesly and Naik, 2001).

3.2.3 Effect of surfactants and oxidants on stability of alkaline proteases

In addition to activity and stability at high temperature and pH ranges, a good detergent protease must be compatible and stable with all commonly used detergent compounds such as surfactants, bleaches, oxidizing agents and other additives which might be present in the formulation (Gupta *et al.,* 1999; Kumar and Takagi, 1999; Oberoi *et al*., 2001). Therefore, the effects of various surfactants and oxidizing agents at different concentrations on activity of the purified protease of *B. lichenifomis* UV-9 was studied after pre-incubation at 40°C for 1 h (Table 3). The residual activity of *B. licheniformis* UV-9 protease were found 90.45%, 90.15% and 97.56% at 1% each of Tween-20, Tween-45 and Triton-X-405, respectively. While 1% of Tween-65 showed 121.06% residual activity. However, a minimum residual activity of 45.83% was measured at 1% of SDS. These findings indicated that Tween-65 might improve the interaction of enzyme with substrate and this could result in increased residual activity. Similar effects of SDS on alkaline proteases of various *Bacillus* species have been reported in earlier investigations (Matta and Punj, 1998; Banerjee *et al.,* 1999). Oxidizing agents such as peroxide and perborates are common ingredients of modern bleach-based detergent formulations. The enzymes have significant importance for detergent industries and show extreme stability towards oxidizing agents. Interestingly, 1% of H_2O_2 and sodium perborate stimulated the activity and enzyme expressed 108 and 115% residual activities, respectively. Joo *et al.* (2003) reported that *Bacillus clausii* 1-52 protease exhibited residual activity of up to 114% after treatment with 1% H_2O_2. Haddar *et al.* (2009) reported alkaline protease from *B. mojavensis* A21 showing residual activity of up to 79.40% and 35% after incubation with 1% of H_2O_2 and sodium perborate. However, the present enzyme showed >100% residual activities at 5% of H_2O_2 and sodium perborate indicating its eventual use in detergent formulations.

Table 3. Effect of different oxidizing agents and surfactants on alkaline protease activity produced *by B. licheniformis* UV-9

Surfactants/ Oxidizing agents	Concentration (%)	Residual activity (%)
Control	-	100
Tween 20	0.5	105.80
	1.0	90.45
Tween 45	0.5	98.46
	1.0	90.15
Tween 65	0.5	128.40
	1.0	121.06
Triton X 405	0.5	102.72
	1.0	97.56
	5.0	15.32
SDS	0.5	54.64
	1.0	45.83
	5.0	25.71
H_2O_2	0.5	110.96
	1.0	108.56
	5.0	100.47
Sodium perborate	0.5	118.78
	1.0	115.56
	5.0	101.92

Residual activity was expressed as a percentage of the activity level in the absence of surfactants and oxidizing agents. Separate blank was processed for each agent

Table 4. Effect of various substrates on alkaline protease activity of *B. licheniformis* UV-9

Substrate	Relative activity(%)
Casein	100
Albumin (bovine)	52
Hemoglobin	72
Gelatin	11
Albumin (egg)	42
Keratin	8
Collagen	2

3.2.4 Substrate specificity

Activity of the purified alkaline protease against various proteinaceous substrates was examined (Table 4). The enzyme showed a high level of catalytic activity against casein, hemoglobin and albumin (bovine) indicating its ability to hydrolyze several proteins. Substrate diversity is an important criterion to analyze the potency of a protease. In

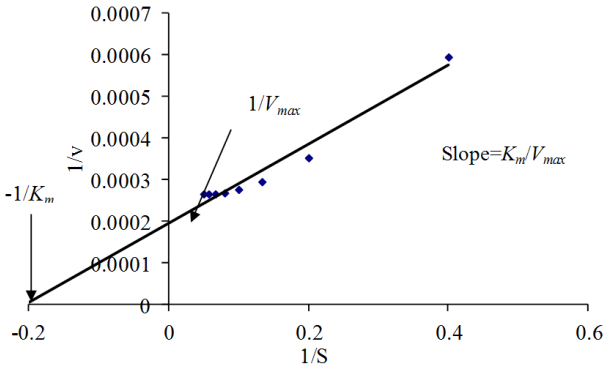

Figure 5. Linewaever-Burk Plot for alkaline protease for varying substrate (casein) concentrations (2.5-20 mg/ml) indicating K_m and *Vmax* values

contrast, the enzyme showed very poor hydrolysis against collagen, keratin and gelatin corresponding 2, 8 and 11% relative activities, respectively. Adinarayana *et al.* (2003) reported similar finding that casein was a good substrate for serine protease enzyme produced by *B. subtilis*.

3.2.5 Kinetic studies

The kinetic parameters K_m and V_{max} of the alkaline protease produced by *B. licheniformis* UV-9 were estimated by Lineweaver-Burk Plot employing various concentrations (2.5-20 mg/ml) of casein as substrate (Figure 5). The Lineweaver-Burk Plot for the proteolytic reaction of the casein revealed that the K_m and V_{max} values of the reaction were 5 mg/ml and 61.58 uM/ml/min, respectively. Kaur *et al.* (1998) reported K_m of 3.7 mg/ml for *B. polymyxa* protease, while Thangam and Rajkumar (2002) reported a K_m and V_{max} of 1.66 mg/ml and 526 U/min/mg, respectively, for alkaline protease of *Alcaligenes faecalis* using casein as a substrate. Jaswal and Kocher (2007) plotted a double reciprocal plot (Lineweaver-Burk Plot) and estimated an apparent K_m of 5 mg/ml and V_{max} of 1000 mol tyrosine/min/ml for protease of *B. circulans*. The K_m value represents the dissociation constant (affinity for substrate) of the enzyme-substrate (ES) complex. Low values of K_m indicate that the ES complex is held together tightly and dissociates rarely before the substrate is converted to product. The value of K_m further proved that the enzyme may preferably be used for protein hydrolysis due to its catalytic efficiency.

4. Conclusion

The alkaline protease enzyme of mutant *Bacillus licheniformis* UV-9 was purified up to homogeneity level by employing ammonium sulphate precipitation (60-70%) and gel filtration through Sephadex G-100 chromatography. After final purification step, the enzyme was purified 36.83 fold with a specific activity of 12102.96 U/mg and 11% recovery. The molecular weight of the enzyme was estimated to be 36.12 kDa by SDS-PAGE. PMSF completely inhibited the enzyme activity, suggesting its serine nature. The purified enzyme showed desirable properties such as high activity and stability at broad ranges of pH and temperature. The enzyme was also found compatible and stable with most of surfactants and oxidizing agents tested and retained its more than 100% residual activity. These properties indicate the possibilities of commercial exploitation of the alkaline protease in detergent formulations.

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