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Characterization of Crude and Partially Purified Thermo Active and Thermo Stable Alkaline Protease Produced by *Bacillus cereus* FT1

Asha B [1, 2] and Palaniswamy M*[1]

[1]*Department of Microbiology, Karpagam University, Karpagam Academy of Higher Education, Coimbatore- 641021, Tamil Nadu, India*
[2]*SIAS Centre for Scientific Research, Vazhayoor, Malappuram- 673633, Kerala, India*

Abstract

The *Bacillus cereus* FT1 isolated from soil is found to be a producer of alkaline protease enzyme. The crude and partially purified enzymes are characterised by studying the effect of substrate concentration, pH, temperature, metal ions, detergents, surfactants and oxidising agents on enzyme action. The maximum enzyme activities of crude and partially purified enzyme are observed at substrate concentrations of 2 and 3% respectively. The maximum activity of crude enzyme is at pH 9.5 and partially purified enzyme is at pH 10. Maximum stability for crude and partially purified enzyme are at pH 9.5 when incubate for 1h with residual activities of 98 and 100% respectively. The optimum temperature for action is 60°C for crude enzyme and 65°C for partially purified enzyme. The crude enzyme retains more than 95% of residual activity at temperature ranges of 5°C to 55°C and partially purified enzyme retains more than 95% of residual activity at temperature ranges of 5°C to 60°C after 1h of pre incubation. The metal ions Ba^{2+} and Mn^{2+} enhance the activity of both. They maintain 90% and above of residual activity when incubate with surfactants and detergents for up to 1h and the detergent Sunlight even increases the activity to above 100% for both. Different concentrations of H_2O_2 also retain 84% and more of residual activity for both enzymes. The partially purified enzyme retains its optimum activity for almost eight weeks at room temperature and there after only an insignificant decrease is noted.

Key words: Thermo stable alkaline protease, characterisation, *Bacillus cereus*

INTRODUCTION

Proteases are the enzymes that breakdown proteins in to its component amino acid. They are studied intensively not only due to their importance in cellular metabolism, but also because they have gained importance in the industrial community [1, 2]. The demand for the proteolytic enzymes which are specific and stable towards a particular pH, temperature, surfactants, metal ions, and organic solvent are now rising and stimulating the searches for more new enzyme sources [3]. Proteases account for about 60% of total worldwide enzyme sale and find many applications in the detergents, food processing, pharmaceuticals, bioremediations, production of digestives and treatments of inflammation and virulent wounds [4, 5, 6, 7, 8].

Alkaline proteases are generally active and stable at pH ranges of 9-11 and find applications mainly in detergent industry as the pH at which the laundry detergents are active lie between 9 and 12 [3, 9, 10]. The proteases must exhibit important properties to be used as a suitable detergent additive, like optimum alkaline pH activity, effectiveness at low temperatures, stability at high temperatures, stability in presence of detergent ingredients and ability to degrade variety of proteins [11]. Based on all these properties, alkaline proteases are industrially applicable due to their high stability and high activity under harsh conditions [12, 13, 14, 15].

Among the bacterial strains reported to be producing alkaline proteases, *Bacillus* genus gained a notable importance as a significant source of neutral and alkaline proteases. They are found to be highly stable at extreme pH and temperature ranges [16, 17, 18, 19]. Alkaline proteases derived from *Bacillus* have been extensively used due to their significant proteolytic activity and stability, broad substrate specificity, low cost production and purification [9, 20].

The detergent industry now demands an alkaline protease enzyme which is specific and stable towards pH, temperature, organic solvents, metal ions and surfactants, stimulating the searches for new enzyme sources [3]. In the present study, an attempt is made to characterise the protease enzyme produced by soil isolated *Bacillus cereus* FT1 strain. The characterization and stability of the enzyme are aimed to study and determine a possible eco-friendly application of enzyme in the detergent industry.

MATERIALS AND METHODS

Microorganism

The microorganism that is used in this study is *Bacillus cereus* FT1, which was previously isolated and maintained in our laboratory, SIAS Centre for Scientific Research, Vazhayoor, Malappuram, Kerala. The isolate was identified based on the methods recommended in the Bergey's manual of determinative bacteriology and diagnostic microbiology depending upon it's morphological, cultural and biochemical characteristics [21] and further confirmed based on the 16S rRNA sequence analysis and BLAST identification.

Enzyme production

Protease enzyme production was carried out using the previously standardised media (Composition [%]: lactose 2%; casein 4%; KH_2PO_4 0.2%; K_2HPO_4 0.2%; $MgSO_4.7H_2O$ 0.1%; $MnSO_4$ 0.1%) with pH 9.5. Inoculated media was incubated at 35°C for 48 h at 200 rpm on a rotary shaker. Cell free supernatant was collected by centrifugation at 10,000 rpm in a cooling centrifuge for 20 min to obtain crude extract, which can be used as the enzyme source for characterization. The crude extract was also partially purified and characterised.

Assay of alkaline protease enzyme activity

The crude or partially purified enzyme (0.1ml) was mixed separately with 1 ml of casein solution (2% w/v in 0.05M Tris-HCl buffer having pH 9) and incubated for 10 min at 37°C in the water bath. The reaction was terminated by adding 3 ml of 20% ice cold TCA and the final volume was made 5ml by adding 0.9 ml of the distilled water. The mixture was then centrifuged at 5000 rpm for 15 min and the supernatant (0.5ml) was mixed with 2.5 ml of 0.5M Na_2CO_3 and kept for 20 min at room temperature. Finally, added appropriately diluted Folin's phenol reagent, kept for 10 min and absorbance was measured (660 nm). The amount of tyrosine liberated was calculated from a standard graph generated using standard tyrosine of 10-100 µg/ml and protease activity was calculated from tyrosine concentration. One unit of protease activity is equal to the amount of enzyme required to liberate 1 µg tyrosine per ml per minute under standard assay parameters. [22].

Partial purification of enzyme by ammonium sulphate precipitation

The crude enzyme extract obtained in the previous step was subjected to various concentrations of ammonium sulphate (40 to 90%) to precipitate the proteins. The precipitates obtained from each saturation were collected by centrifugation at 5000 rpm for 20 min at 4°C and dissolved in 0.05M Tris HCl buffer, pH 9 and dialysed against the same buffer at 4°C. Protease activities of the dialysed samples were determined and the concentration which gave the desired precipitate was used to precipitate the enzyme from larger volumes of production medium. [23, 24]. The partially purified enzyme obtained after the precipitation and dialysis was taken for the characterisation studies.

Protein estimation

Protein estimation of both the crude and the partially purified enzyme was done according to the Lowry's method using bovine serum albumin as standard. [25] Specific activity of the enzyme was also calculated [24].

Characterisation of crude and partially purified protease enzyme

The effect of substrate concentration, pH, temperature, surfactants, detergents, metal ions, metal chelators and oxidising agents on the activity and stability of crude and partially purified enzyme was studied.

Effect of substrate concentration on enzyme activity

Casein was the substrate used for the enzyme assay. Different concentrations of casein (0.5% to 4%) in Tris-HCl buffer having pH 9 was used as enzyme substrate for protease assay to determine the optimum concentration of substrate for maximum enzyme activity [26].

Effect of pH on enzyme activity and stability

Optimum pH for protease activity was determined by conducting protease assay at different pH ranges (6 to 10.5). The assay substrate casein was dissolved in the different buffers with varying pH. Stability of the enzyme were determined by pre-incubating equal volume enzyme and buffers (pH 3.5 to 10.5) at 37°C for 30 min and 1 h. Assay procedure was followed after incubation and residual activity of the enzyme was calculated [24, 27]. Buffer systems used for this study included: acetate buffer (pH 3.5 to 5.5); phosphate buffer (pH 6 to 7); tris-HCl buffer (pH 7.5 to 9); sodium hydroxide/di-sodium hydrogen phosphate buffer (pH 9.5 to 10.5).

Effect of temperature on enzyme activity and stability

The optimum temperature for enzyme action was determined by measuring protease activity at different temperatures (20 to 65°C). The assay mixture was incubated at respective temperatures and enzyme activity was determined. Thermal stability was determined by pre-incubating the enzyme for 30 min and 1h at temperatures ranging from 20 to 65°C and measuring the residual protease activity [28].

Effect of metal ions on enzyme activity and stability

The metal ions tested included Ba^{2+}, Ca^{2+}, Cd^{2+}, Mg^{2+}, Co^{2+}, Fe^{2+}, Mn^{2+}, Zn^{2+}, Hg^{2+}, K^{+}, Na^{+} and Cu^{2+}. The effect of metal ions on the enzyme activity was checked by mixing 5mM of the metallic chloride with the assay mixture and protease activity was determined. The enzyme was pre-incubated in the presence of 5mM of the metallic chloride for 30 min and 1h at 37°C to check the stability of enzyme. Then the residual protease activity was measured [24, 28, 29].

Effect of surfactants, commercial detergents and oxidising agents on enzyme activity and stability

The effect of surfactants (SDS, tween 80, tween 20, triton X 100) and commercial detergents (Sunlight, Ariel, Henko, Surf Excel, Ujala, Surya, Tide) on enzyme activity was investigated by conducting protease assay in the presence of 1% surfactant and detergent solutions. Stability of the enzyme with surfactants and detergents were studied by pre-incubating the enzyme with 1% surfactant and detergent solutions for 30 min and 1h at 37°C and the residual activity of the enzyme was calculated [24, 28, 30]. To check the effect of oxidising agent on the activity and stability of the enzyme, 0.5% to 2% H_2O_2 was used [31].

Enzyme stability at the room temperature

The partially purified enzyme was stored at room temperature and enzyme activity was determined at weekly intervals for a period of three months. Alkaline proteases can improve the cleansing efficiency of detergents and hence are important in detergent industry. The detergents are usually stored at room temperature, hence the stability of the enzyme at room temperature should be determined.

RESULTS AND DISCUSSION

Partial purification of protease enzyme

Purification of alkaline protease by *Bacillus cereus* FT1 is summarised in the table 1.

The crude enzyme showed an enzyme activity of 270 U/ml and a specific activity of 82 U/mg. The enzyme precipitated with 60-80% saturation of ammonium sulphate increased the protease activity 4.88 fold, showing the specific activity of 400 U/mg. Different microbial species showed varying specific activities (13.33- 159381 U/mg of protein) with wide ranges of protein purification (4.25-200-folds) [32, 33, 34, 35, 36]. The alkaline protease K3 from *Bacillus aryabhattai* K3 showed approximately 6.65-fold purification during ammonium sulphate precipitation (50%-70%) with a specific activity of 3357.97 U/mg for the final partially purified enzyme [29]. A 4.62-fold purification for the protease enzyme by ammonium sulphate precipitation (80%) was reported for *Virgibacillus pantothenticus* MTCC 6729 [37].

Characterisation of crude and partially purified enzyme

Effect of substrate concentration on enzyme activity

The requirement of the substrate concentration for maximum enzyme activity for crude enzyme and partially purified enzyme is observed as 2, and 3% w/v respectively. (Figure 1).

The enzyme activity gradually increased as the substrate concentration increased for both the crude and the partially purified enzymes and at higher concentrations, there was a gradual decrease in the activity which may be due to substrate level inhibition of the enzymes. However in the present work, the enzyme could tolerate up to 4 % w/v casein concentration. Earlier studies have reported *Bacillus lichenifiormis* U1 showing substrate tolerance up to 2% w/v of casein suggesting its applicability in the substrate rich system [38].

Effect of pH on enzyme activity and stability

Proteolytic *Bacilli* have been reported to produce two different types of extracellular proteases. Neutral/ metalloprotease with optimal activity at pH 7.0 and alkaline protease with optimal pH range between 9 and 11 [39, 40]. The protease enzyme produced by *Bacillus cereus* FT1 showed highest protease activity at a pH range of 9 to 9.5. The crude enzyme showed a maximum protease activity of 260 U/mL at a pH of 9.5 and partially purified enzyme showed a maximum protease activity of 330 U/mL at a pH of 10 (Figure 2). The optimum activity at alkaline pH ranges suggests that the enzyme is an alkaline protease. A drastic decline in the activity was observed in acidic range i.e. activity decreased below 60% at these ranges. The crude enzyme showed a residual activity of 98% upon incubation up to 1 h at 9.5 pH and more than 75% of residual activity at pH ranges of 7.5 to 9 on incubating for 30 min to 1 h. The partially purified enzyme maintained 100% enzyme activity upon incubation at pH 9.5 up to 1 h and more than 90% of residual activity upon incubation for 30 min to 1 h at pH ranges of 7.5 to 10.5. Figure 3 and Figure 4 show the residual activities of crude and partially purified enzymes on 30 min and 1 h pre incubation respectively.

Table 1: Purification of *Bacillus cereus* alkaline protease

Purification level	Enzyme activity (U/ml)	Total protein content (mg/ml)	Specific activity (U/mg)	Purification fold
Crude enzyme	270	3.3	82	1
Partially purified enzyme	320	0.8	400	4.88

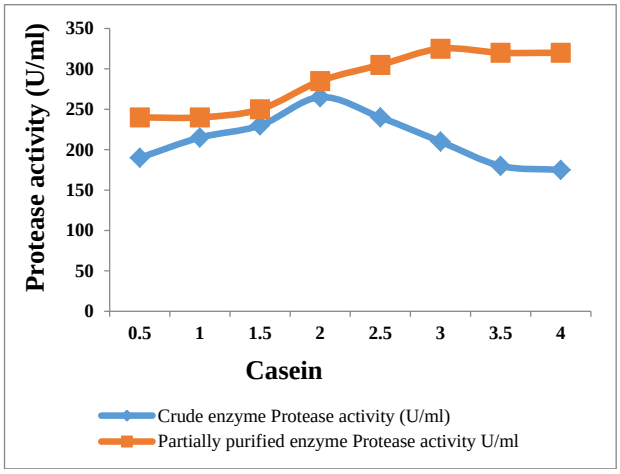

Figure 1: Effect of substrate concentration on activity of crude and partially purified enzymes

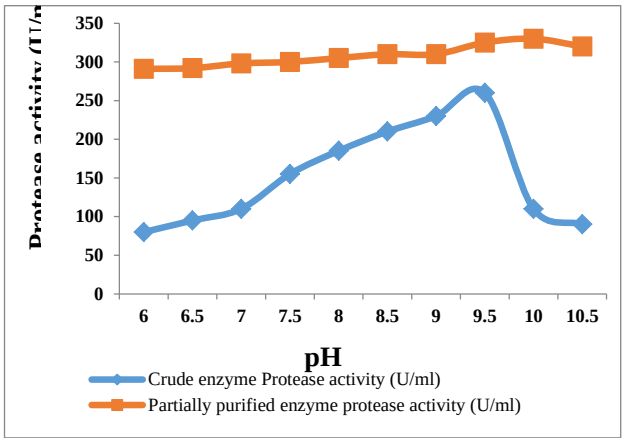

Figure 2: Effect of pH on activity of crude and partially purified enzymes

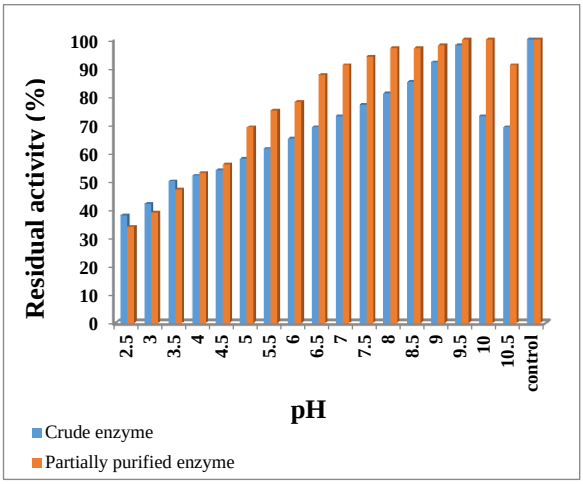

Figure 3: Residual activities of crude and partially purified enzyme on 30 min pre-incubation

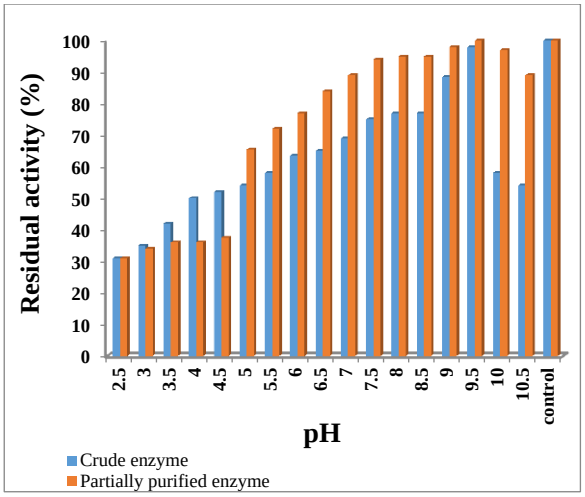

Figure 4: Residual activities of crude and partially purified enzyme on 1 h pre-incubation

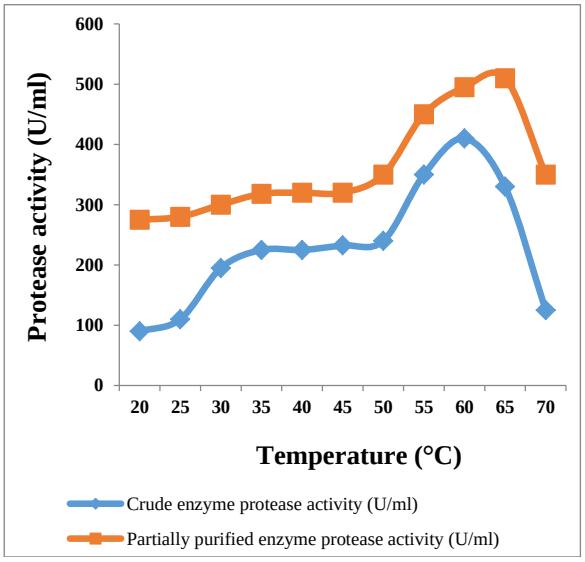

Figure 5: Effect of temperature on activity of crude and partially purified enzymes

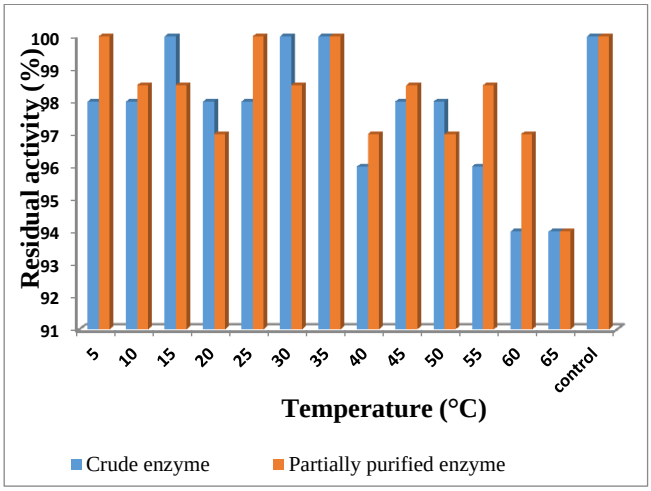

Figure 6: Residual activity by crude and partially purified enzyme after 30 min pre incubation

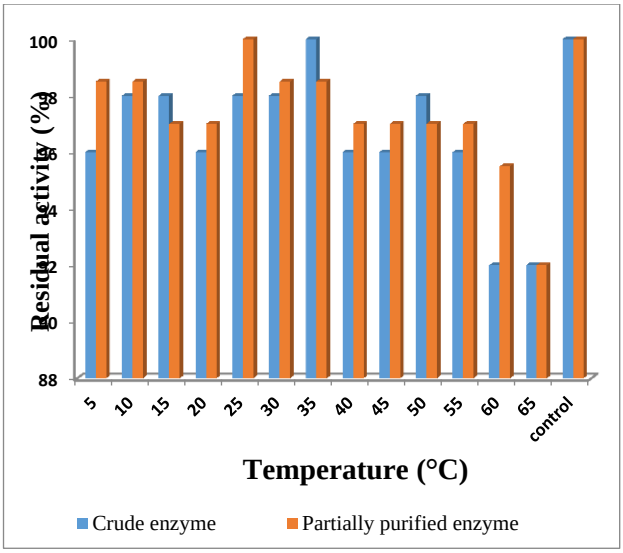

Figure 7: Residual activity by crude and partially purified enzyme after 1 h pre incubation

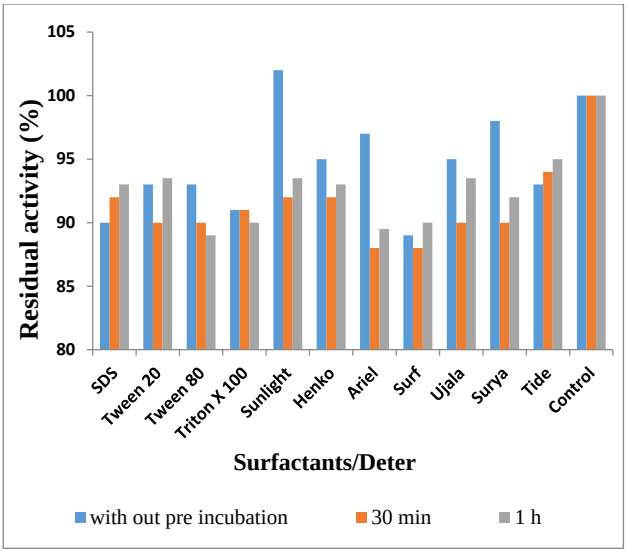

Figure 10: Residual activities of crude enzyme on incubation with detergents and surfactants at different time periods

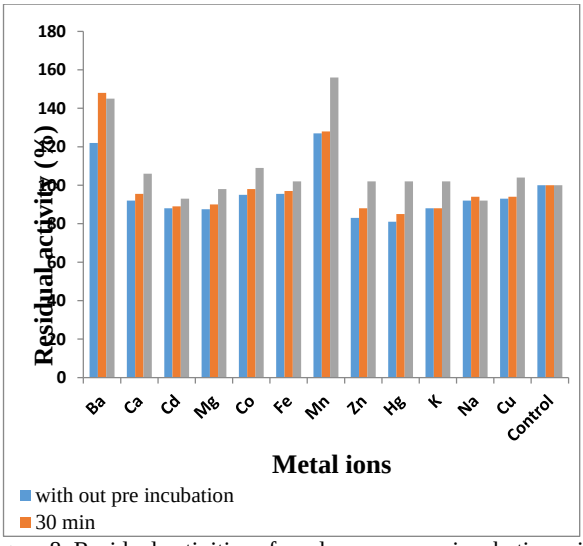

Figure 8: Residual activities of crude enzyme on incubation with metal ions at different time periods

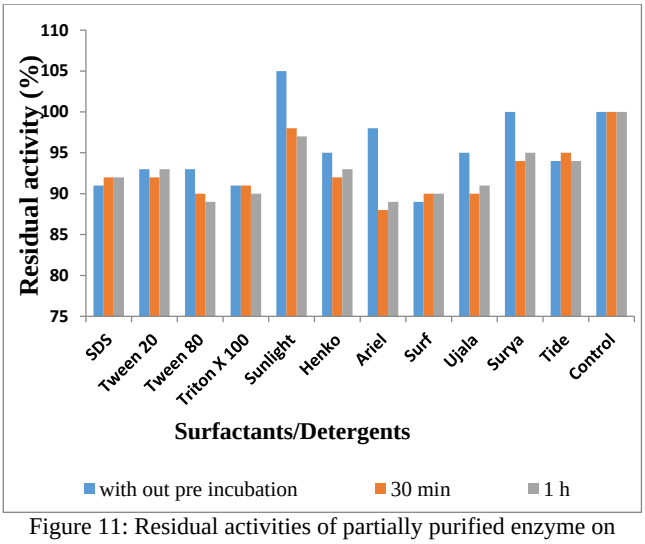

Figure 11: Residual activities of partially purified enzyme on incubation with detergents and surfactants at different time periods

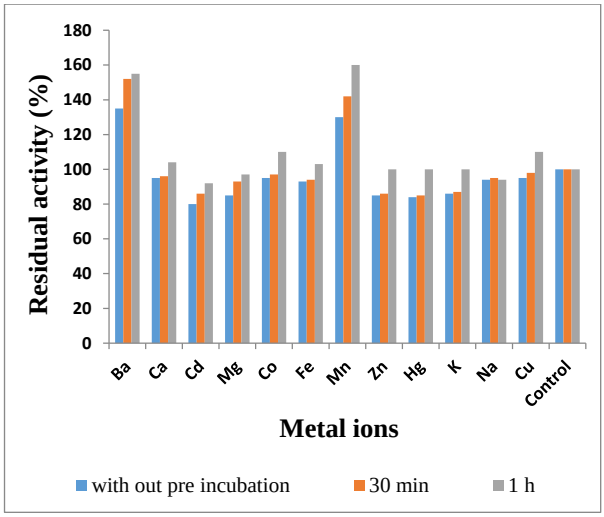

Figure 9: Residual activities of partially purified enzyme on incubation with metal ions at different time periods

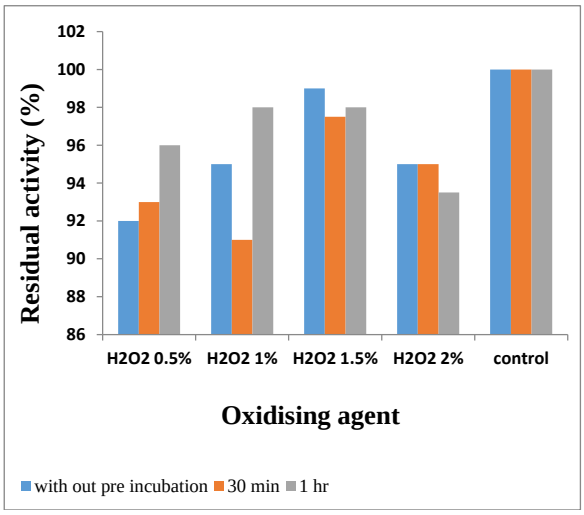

Figure 12: Residual activities of crude enzyme on incubation with different concentrations of H_2O_2 at different time periods

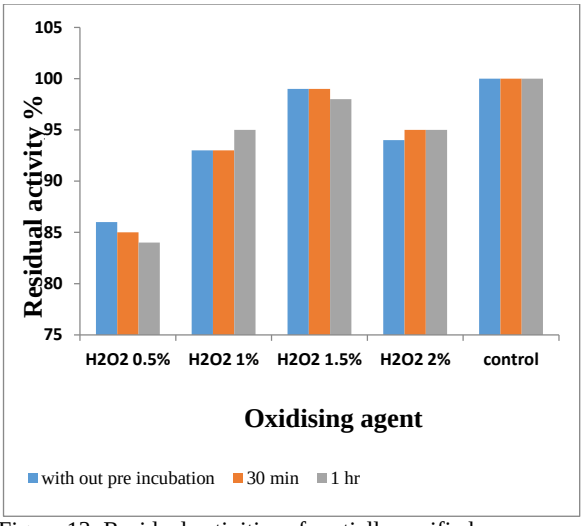

Figure 13: Residual activities of partially purified enzyme on incubation with different concentrations of H_2O_2 at different time periods.

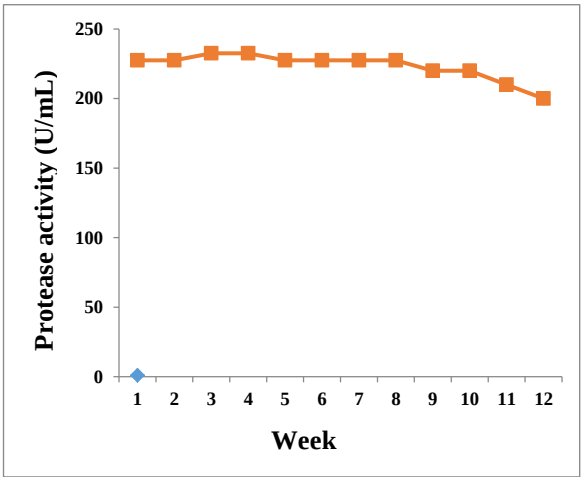

Figure 14: Stability of partially purified enzyme in room temperature.

Generally, commercial proteases derived from microorganisms have optimum activity in the alkaline pH range of 8-12 [5, 41, 42]. The pH of laundry detergent falls in the range of 9-12 [31]. Different *Bacillus* species are reported to produce alkaline proteases with optimum activity within this range. *Bacillus subtilis* VSG-4 produced an alkaline protease with maximum activity at pH 9. The enzyme activity declined at acidic pH ranges [43]. The other *Bacillus* species with optimum pH 9 include *Bacillus* sp. strain CR-179 isolated from soil sample collected from Lavizan junglepark [44], *Bacillus subtilis* SHS-04 grown on groundnut meal [45], *Bacillus horikoshii* isolated from the body fluid of a Korean polychaeta (Yellow Sea) [46], and *Bacillus licheniformis* LHSB-05 isolated from Nigerian hot spring [47]. *Bacillus* species with optimum pH 10 include *Bacillus subtilis* strain VV [48], and *Bacillus licheniformis* Bl8 [49]. The protease derived from *Bacillus subtilis* NCIM 2713 had an optimum activity at pH 8 and stability at pH 6.5 to 9 [50]. The novel species *Bacillus caseinilyticus* exhibited maximum enzyme activity at pH 8[51].

At non suitable pH conditions, modification of three dimensional structures of proteins occurs together with the ionization state alteration of amino acids in the enzyme active site resulting in the loss of enzyme activity [52]. The enzyme with high stability and activity at alkaline pH ranges are most suitable for detergent industry and for cleaning the ultra filtration membranes [24].

Effect of temperature on enzyme activity and stability

Study on the effect of different temperatures showed that the enzyme was active at different temperatures, with an optimum temperature of 60°C for the crude enzyme and 65°C for the partially purified enzyme, which supports the thermo active nature of the enzyme (Figure 5). In the temperature stability studies, the enzyme was pre incubated for the time period of 30 min and 1h at different temperatures. The crude enzyme showed more than 95% residual activity at the temperature ranges of 5°C to 55°C. At 60°C and 65°C, there was a slight decrease in the enzyme activity with 94% and 92% of residual activity for 30 min and 1 h pre incubation respectively for both the temperatures. The partially purified enzyme showed more than 95% of residual activity at 5°C to 60°C after 30 min and 1 h pre incubation. At 65°C, the 30 min pre incubated enzyme showed 94% residual activity and on 1 hr pre incubation, the residual activity was 92%. According to the studies, it is clear that the protease enzyme produced by *Bacillus cereus* FT1 is a thermo stable enzyme. Figure 6 and Figure 7 show the residual activities of crude and partially purified enzymes 30 min and 1 h pre incubation respectively.

The optimum temperature range of alkaline proteases is generally from 50°C to 70°C [53]. A strain, *Bacillus licheniformis* KBDL4 isolated from lonar soda lake with maximum enzyme activity at 60°C showed 40% residual activity after heating for 1h at 60°C [54]. The *Bacillus brevis* strain isolated from hot springs also had an optimum activity at 60°C [12]. A thermo stable serine protease produced by *Bacillus licheniformis* U1 was reported to be showing a maximum enzyme activity at 50°C [38]. The enzyme remained stable at 40°C to 50°C for 30 min and nearly 50% residual activity was shown at 60°C. A *Bacillus* sp. isolated from abattoir soil in the north region of Cameroon showed maximum enzyme activity and stability at 80°C [55]. A similar result was reported in which the bacterium *Bacillus stearothermophilus* F1 showed the optimum temperature of action as 85°C [56]. The *Bacillus* species with activity and stability at higher temperature ranges also include *Bacillus horikoshii* with an optimum temperature of 45ºC [46], *Bacillus* sp. SSR1 with an optimum temperature of 40ºC [57] and *Bacillus subtilis* WIFD5 with optimum temperature of 55ºC [58].

Effect of metal ions on enzyme activity and stability

Among the different metal ions tested, Ba^{2+} and Mn^{2+} were found to be enhancing the protease activity resulting in the residual activity of 122% and 127% respectively for the crude enzyme. On pre incubation for 30min and 1 h with these ions, residual activity again increased for crude enzyme. Among the other metal ions tested, $Ca^{2+,}$ Co^{2+}, Fe^{2+}, Zn^{2+}, Hg^{2+}, K^{+} and Cu^{2+} were also resulted in the enhanced protease activity for crude enzyme upon pre incubation up to 1 h. Cd^{2+}, Mg^{2+} and Na^{+} also retained more than 90% of the residual activity for crude enzyme upon I h pre incubation (Figure 8).

Ba^{2+} and Mn^{2+} enhanced the activity of partially purified enzyme with a residual activity of 135% and 130% respectively and upon further incubation for 30 min and 1 h, the residual activity increased to 152% and 155% for Ba^{2+} and 142% and 160% for Mn^{2+}. $Ca^{2+,}$ Co^{2+}, Fe^{2+}, and Cu^{2+} slightly increased the enzyme activity on incubating up to 1h. All the other metal ions decreased the residual activity for partially purified enzyme, but not less than 85% (Figure 9).

The presence of suitable metal ions plays a major role in maintaining the active conformation of enzyme against heat denaturation [59]. Therefore, identification of proper metal ions has significant impact in the commercial application of enzymes. Increase in the relative activity of protease produced by *Bacillus megatarium* in presence of Mn^{2+}, Ca^{2+} and Mg^{2+} was reported

[36]. These cations have also been reported to increase the stability and activity of alkaline proteases produced by a thermophilic and alkalophilic *Bacillus* sp. JB-99 [60]. The Ca^{2+} and Mg^{2+} ions enhanced the activity of alkaline protease produced by *Bacillus licheniformis* UV-9 [31]. The activity of serine alkaline metalloprotease produced by *Bacillus brevis* MWB-01 was enhanced by Ca^{2+} and Mn^{2+} ions with 110% and 103.5% residual activity respectively [28]. The study revealed the role of these ions in maintaining the conformation of active site of the enzyme [56]. The activity of alkaline protease produced by an alkaliphilic *Bacillus licheniformis* KBDL4, was enhanced by Ca^{2+}, while Mg^{2+}, Ba^{2+}, Cu^{2+}, Mn^{2+} and inhibited by Hg^{2+} [54]. The protease activity of *Bacillus tequilensis* was enhanced with addition of Ca^{2+} and Mg^{2+} ions resulting in the residual activity of 105% and 107% respectively. Other metal ions Zn^{2+}, Cd^{2+}, Na^{+} and K^{+} resulted in more than 60% residual activity whereas Hg^{2+} exhibited only 12.45% residual activity [23].

Effect of detergents and surfactants on enzyme activity and stability

A good detergent additive protease enzyme must be stable and compatible with the compounds present in the commonly used detergent such as surfactants, bleaches and oxidizing agents [53, 61]. Therefore, the effects of various oxidizing agents and surfactants on the activity of protease enzyme were studied. Stability of the enzyme towards these factors was also checked after pre-incubation at 37°C for 30 min and 1h. All the surfactants (SDS, triton X-100, tween-20 and tween-80) and commercial detergents (sunlight, henko, ariel, surf, ujala, surya, and tide) tested maintained the residual enzyme activity of crude and partially purified enzymes above 90% and the detergent Sunlight even increased the activity showing a residual activity of 102% and 105% for both enzymes. Studies on the effects of surfactants and commercial detergents on protease stability revealed that upon the incubation up to 1hr with surfactant and commercially available detergents tested, both the crude and the partially purified enzymes retained the residual activities above 90% (Figure 10 and Figure 11).

Protease stability in presence of commercially available detergents has been reported in many works with varying stabilities and activities [62, 63, 64]. Stability and activity in presence of detergents shows the potential of an enzyme as a detergent additive for effective stain removal [24]. An alkaline protease produced by *Bacillus brevis* MWB-01 showing highest compatibility with a commercial detergent sunlight, with 76% residual activity is reported in an earlier study [28]. The alkaline protease produced by *Bacillus aryabhattai* K3 was stable in presence of the detergents, with more than 90% activity in presence of Ariel, Surf Exel and Nip; more than 80% activity in presence of Tide and Vim and more than 70% activity in presence of Ghadi, after 1 h of incubation [29]. The purified alkaline protease from *Bacillus licheniformis* UV-9, was extremely stable towards various surfactants such as tween-20, tween-65, tween-45, and triton X-45 [31]. Studies on alkaline protease from the strain B*acillus tequilensis* CSGAB0139 revealed that upon the incubation with triton X-100 and tween-20, the enzyme showed enhanced residual activities 102% and 108% respectively while other surfactants including commercially available detergents (Surf Excel, Ariel and Surf Blue) exhibited more than 90% residual activity except SDS which showed 60% residual activity [23].

For protease based detergent formulations, the enzyme should have the ability to tolerate oxidizing agents. The residual activity of crude enzyme from *Bacillus cereus* FT1 was 98% in presence of 1% and 1.5% H_2O_2 after 1 h of incubation. A residual activity of 96% is maintained in presence of 0.5% of H_2O_2 and 2% H_2O_2 maintained 93.5% of residual activity of crude enzyme after 1 h of incubation. For partially purified enzyme, residual activity after 1 h of incubation is, 98% for 1.5% H_2O_2, 95% for 1% and 2% H_2O_2 and only 84% for 0.5 % of H_2O_2 (Figure 12 and Figure 13). The enzyme with extreme stability towards the oxidizing agents is commercially significant as the peroxides are common ingredients of bleach-based detergent. The enzyme which is compatible and stable with the detergent and its formulation compounds like bleaching agents, anionic surfactants, and water-softening agents is the most effective one while washing [65].

Oxidizing agents are major ingredients of modern bleach-based detergents. Alkaline protease from *Bacillus licheniformis* UV-9 show extreme stability towards oxidizing agents. The enzyme showed 108% residual activity in presence of 1% of H_2O_2 [31]. An earlier report stated that the protease enzyme produced by *Bacillus clausii* 1-52 showed a residual activity of maximum 114% after treatment with 1% H_2O_2 [66]. The *Bacillus mojavensis* A21 showed 79.40% and 35% residual activities after incubation with 1% of H_2O_2 and sodium perborate respectively and more than 100% residual activities at 5% of H_2O_2 and sodium perborate. Protease residual activity of *Bacillus tequilensis* was 127% in presence of 2% H_2O_2, indicating its immense commercial significance for detergent industry [23].

Enzyme stability at room temperature

The enzyme preparations are conventionally stored under low temperatures which will prevent its denaturation. If the enzyme can withstand room temperature, it could be advantageous for commercial applications. By considering the practical problems during the storage and transport, the ability of partially purified enzyme to withstand ambient temperature is studied by storing it at the room temperature (30 ± 2°C) for a time period of twelve weeks and assaying at regular weekly intervals. The results indicate that the enzyme from *Bacillus cereus* FT1 stored at room temperature retained its activity without any decrease in it for the first eight weeks and then there is an inconsiderable decrease in it for the next weeks (Figure 14). This property reveals its use as a good detergent additive with a longer shelf life.

CONCLUSION

The crude and partially purified protease enzymes isolated from *Bacillus cereus* FT1 shows optimum activities at high temperatures, alkaline pH ranges and high substrate concentrations. Both the enzymes are stable in the presence of tested metal ions, surfactants, commercial detergents and oxidising agents. The partially purified enzyme also shows long term stability in the room temperature also. All these desirable properties are required for being applicable as a good detergent additive. All the characterisation studies conducted for the enzyme reveals its promising nature in commercial applications.

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