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MICROBIOLOGY

Characterization of alkaline metalloprotease isolated from halophilic bacterium *Bacillus cereus* and its applications in various industrial processes

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Abstract: Microbial proteases are one of the most demanding enzymes for various industries with diverse applications in food, pharmaceutics, and textile industries to name the few. An extracellular alkaline metalloprotease was produced and purified from moderate halophilic bacterial strain, *Bacillus cereus* TS2, with some unique characteristics required for various industrial applications. The protease was produced in basal medium supplemented with casein and was partially purified by ion exchange chromatography followed by ammonium sulphate precipitation. The alkaline metalloprotease has molecular weight of 35 kDa with specific activity of 535.4 µM/min/mg. It can work at wide range of pH from 3 to 12, while showing optimum activity at pH 10. Similarly, the alkaline metalloprotease is stable till the temperature of 80 °C and works at wide range of temperature from 20 to 90 °C with optimum activity at 60 °C. The turnover rate increases in the presence of NaCl and Co^{+2} with k_{cat}/K_M of 1.42×10^3 and 1.27×10^3 $s^{-1}.M^{-1}$ respectively, while without NaCl and Co^{+2} it has a value of 7.58×10^2. The alkaline metalloprotease was relatively resistant to thermal and solvent mediated denaturation. Applications revealed that the metalloprotease was efficient to remove hair from goat skin, remove blood stains and degrade milk, thus can be a potential candidate for leather, detergent, and food industry.

Key words: Bacterial proteases, halophiles, metalloprotease, purification, thermostable.

INTRODUCTION

Microbial enzymes have enormous potential and were used from centuries to produce various products. These enzymes are the key players in various industrial processes due to their ability to work under mild conditions, their selectivity and their least toxicity towards the environment (Choi et al. 2015). Although the enzymes isolated from various microbes depending on their habitat are equally important but, in some circumstances, the extremozymes are the only option for certain industries due to their unique features. Most of the mesophilic enzymes that were isolated from soil, water and food etc. are not operational under harsh conditions of industries, thus it is essential to get enzymes with robust nature that can tolerate high temperature, pH, organic solvents etc. (Eichler 2001, Irwin & Baird 2004, de Champdoré et al. 2007). Extremophiles are the only option for such type of enzymes as these organisms live under extreme conditions such as very high temperature (55 to 120 °C) and low temperature (-2 °C to 20 °C), pH (pH < 4 , pH > 8), salinity (2-5

M KCl / NaCl) etc. (Seitz et al. 1997). Therefore, the enzymes from such organisms are unique in nature and stable under extreme conditions, which are more demanding for industrial applications (Chapman et al. 2018).

Proteases are very important enzyme as far as the commercial aspects are concern with enormous applications. It has been reported that out of 60 % of whole enzymes marketed worldwide, proteases account for 20 % (Subba Rao et al. 2009, Razzaq et al. 2019). Proteases are hydrolytic enzymes that hydrolyze the peptide bonds present in polypeptide chain of amino acids (Razzaq et al. 2019). Majorly the proteases can be divided into two main groups i.e. endoproteases and exoproteases, depending on their site of action. Proteases can be further classified into four major groups depending upon the functional groups possessed by them, these are serine proteases, aspartic proteases, cysteine proteases and metalloproteases (Rao et al. 1998). Microbial proteases are preferred over plants and animals because of their high yield, less time consumption, less space requirement, lofty genetic manipulation and cost effective (Ali et al. 2016). Alkaline proteases produced by bacteria are majorly involved in various industrial processes and are used in the production of detergents, food processing, medical formulations, tannery industry, bioremediation, etc. (Genckal & Tari 2006, Geng et al. 2016). The bacteria belong to genus *Bacillus* are the major producer of commercially important alkaline proteases that works in wide range of pH (Saggu & Mishra 2017, Zhao et al. 2011). The alkaline proteases are mostly used in detergent industry from which the serine protease works well in wide pH range (Singh et al. 2001). Metalloproteases is one of the diverse group of proteases depending on divalent ions for their function (Rawlings & Barrett 1995). Alkaline metalloproteases isolated from the *Pseudomonas aeruginosa* and *Serratia spp*. can be able to catalyze in pH range of 7 to 9. The matrix metalloprotease play distinct role in the degradation of extracellular matrix during tissue morphogenesis, differentiation and wound healing and may be the potential clinical enzyme for the treatment of cancer and arthritis (Bramono et al. 2004). Although number of alkaline proteases are reported earlier but still there is need to explore some novel protease with unique properties for the industrial and clinical applications. Thus, in the present study alkaline metalloprotease was isolated from the halophilic bacterial strain and detailed kinetics study was performed along with some important applications required for industrial processes.

MATERIALS AND METHODS

Isolation

Samples were collected from the Karak salt range, Kohat, KPK. The soil was collected and preserved in the sterilized bags at 4 °C till further analyses. Serial dilution was performed for the isolation of bacterial strains, where the nutrient agar plates amended with 5 %, 10 %, 20 % and 30 % sodium chloride were spread with 100 µl of diluted samples. The plates were incubated at 37 °C for about 72 hours to get salt-tolerant isolates. The isolated strains were further purified by sub-culturing in the same media from where they have been isolated (Moshfegh et al. 2013).

Screening test for proteases positive strains

Skim milk agar assay was performed for the initial screening of protease active strain. It contains 2 g agar and 10 g skim milk in 100 ml distilled water, both were sterilized separately by autoclaving at 121 °C for 5 minutes (Jones et al. 2007). Two bacterial strains were identified as protease positive as they show zone of

hydrolysis in skim milk plates. These were further confirmed by sub-culturing on skim milk plates as well as by protease assay using casein as a substrate.

Strain identification and phylogenetic analysis

Identification of bacterial strains was carried out by sequencing 16S rDNA gene. Genomic DNA was extracted by using EZ-10 spin column genomic DNA extraction kit (Biobasic Inc., Canada). The amplified DNA was sequenced and compared with already existing sequences available in NCBI database. The phylogenetic tree was constructed based on available blast results by using the Mega X software.

Protease production from *Bacillus cereus*

Seed culture

To produce protease from strain TS2, a freshly prepared seed culture of about 16 to 18 hours of growth was always used. A single isolated colony of the bacterium was inoculated in sterilized LB media while incubating it in a shake flask incubator at 37°C and 200 rpm (Fareed et al. 2017).

Protease production

The pellet of the bacterium was obtained from freshly prepared seed culture by centrifugation at 8000 rpm for 15 minutes while keeping it at 4 °C and was washed three times with sterilized distilled water. An inoculum of 100 µl was inoculated in production medium that contains (w/v); (yeast extract 0.55 %, peptone 0.5 %, $MgSO_4$ 0.2 %, Na_2CO_3 1 %, glucose 1 %, casein 0.5 %, KH_2PO_4 0.2 %, pH 7). The culture was incubated at 37 °C with continuous shaking at 220 rpm for 24 hours. The cells were pellet down by centrifugation at 10,000 rpm for 15 minutes at 4 °C and the cell-free extract (CFE) obtained was used as a protease source (Sharma & Bisht 2017).

Protease assay

A caseinolytic assay was performed to measure the protease activity. A total volume of reaction mixture i.e. 1 ml contains 0.65 % casein and 50 mM phosphate buffer (pH 7.5) along with 50 µl CFE in treatments while the control has no CFE. The reaction mixture was incubated at 37 °C for 10 minutes and was terminated by adding trichloroacetic acid (312 µl) and again incubated for 30 minutes at 37 °C. The reaction mixture was filtered to remove any insoluble from the mixture and 50 µl folin reagent was added to it that will bind with the free tyrosine produced after the catalysis of casein. The pH of the mixture was dropped due to the addition of folin which was adjusted by adding 312 µl of sodium carbonate. The reaction mixture was again incubated for further 30 minutes at 37 °C. Folin reacted with tyrosine, a catalytic product of casein, and formed a bluish adduct with an absorbance maximum at 660 nm. One unit of protease activity was defined as the quantity of protease required to produce one micromole of tyrosine per unit time at its optimum conditions (Folin & Ciocalteu 1927).

Protein estimation

Protein concentration was determined according to the Bradford method (Bradford 1976), using bovine serum albumin (BSA) as standard.

Purification of protease

Ammonium sulphate precipitation

Ammonium sulphate (100 %) was added in the CFE (60 ml) to reach 60 % saturation and incubate overnight at 4 °C. The precipitates were obtained after centrifugation at 10,000 rpm at 4 °C for 20 minutes, the precipitates were dissolved in 100

mM phosphate buffer (pH 7) and were dialyzed with the same buffer thoroughly for 24 hr with constant stirring.

DEAE-cellulose separation

The dialyzed sample was applied to the DEAE-cellulose column, which was pre-equilibrated with 50 mM phosphate buffer (pH 7). The sample was eluted with the same buffer and fractions of 0.5 ml were collected. Each fraction was assayed for protease activity and fractions showing protease activity was monitored by using 10 % acrylamide SDS-PAGE for their purity (Rafiei et al. 2016). SDS-PAGE was performed at each step to confirm the purification of protein and was used to determine the molecular weight of the purified protein.

Kinetic studies

Time courses for kinetic analysis of protease were carried out by using the above-mentioned assay (2.4.3). Kinetic parameters were determined by using various concentrations of casein and a constant amount of protease enzyme. Reaction mixtures were incubated at 37 °C for 60 minutes and absorbance was recorded for every 5 min interval following the same protocol as described in the assay. Similarly, kinetic parameters were determined in the presence of NaCl (2M) as well as in the presence of cobalt (10 mM) while keeping all other conditions same as described above.

Characterization of protease at different parameters

Temperature, as well as pH optimization, was performed at various temperatures (5 °C to 120 °C) and pH (2 to 12). The effect of temperature on the stability of protease was also determined by incubating the protease at various temperatures (5 to 120 °C) for 5 min and then performed the assay at 37 °C as described above. The effect of NaCl on the activity of protease was determined by investigating the activity of protease in the presence of various concentrations of NaCl (0 to 4 M) in the reaction mixture. The effect of solvents exposure on protease activity was investigated by varying the buffer concentrations in the assay to include 0 to 20 % (v/v) ethanol, methanol, ethyl acetate, acetone, or hexane in reaction mixture.

Effect of metal ions on enzyme activity

The effect of various metal ions on the activity of proteases was evaluated by incubating the proteases along with 10 mM concentrations of various metals ions (Mg^{+2}, Zn^{+2}, Cu^{+2}, Ni^{+2}, Co^{+2}) for 10 min followed by protease assay in same conditions as defined above.

Effect of inhibitors on enzyme activity

To investigate the effect of inhibitors on protease activity EDTA and PMSF were added in a final concentration of 10 mM in the reaction mixture. The protease activity was monitored after 30 min of incubation in the same manner as described in the section Protease Assay.

Application of metalloprotease

Destaining test

For investigating the destaining activity of metalloprotease a white cloth was cut into pieces (10 cm by 10 cm) and stained with fresh blood in center of the cloth. The cloth was treated with simple water, commercial detergent and with 3 % metalloprotease and an untreated cloth with blood stain was used as a control. Each experiment was performed in separate flask and incubated at 60 °C for 30 minutes. After incubation visual examination of each piece exhibited the effect of enzyme on removal of blood stain (Annamalai et al. 2014).

Dehairing of goat hide

The dehairing ability of metalloprotease was determined by using the goat skin obtained from the slaughterhouse. The skin was cut into pieces of about 5 cm^2 and soaked in water to eliminate dirt and other contaminants. Each piece is then treated with various percentages (1%, 2% and 3%) of metalloprotease while using only water as negative control and lime (10 %) with 3 % sodium sulfide was used as a positive control (Anandharaj et al. 2016a).

Milk clotting assay

Skimmed milk agar plates were used for determining the milk clotting ability of metalloprotease as it has been explained earlier. The CFE was used to determine the milk clotting activity, while adding protease in wells preformed in skim milk agar plates and incubate for 24 hrs and a clear zone around the wells was visually examined.

Feather degradation and keratinase activity

The feather degradation and keratinase activity of the metalloprotease was determined following the method described earlier (Ramakrishna Reddy et al. 2017). Feather degradation was determined by weight loss approach, where the feathers were washed, dried and placed in a medium containing various concentrations of metalloprotease, while the same amount of feather were incubated in buffer without any enzyme.

RESULTS

Isolation, screening, and identification of bacterial strain

Bacterial strains were isolated from the salt range, Karak, Pakistan. A total of 33 strains, 2 were found to be protease positive with the ability to grow in the presence of 10 % salt. The strain TS2 is classified as moderate halophile and has high protease activity as compared to the other strain. The strain TS2 was identified by 16S rRNA sequence, which exhibited more than 99 % similarity with *Bacillus cereus*, and the sequence is already submitted in NCBI database with accession number KX710326.1. A phylogenetic tree was constructed based on blast results which is shown in Fig. 1.

Purification of protease from strain TS2

The summary of purification of metalloprotease from strain TS2 is presented in Table I. It has been demonstrated that with the step of the purification fold, protein contents decreased but the specific activity increased from CFE to the purified enzyme. Finally, the specific activity of purified enzyme increases from 50.362 (μM/min/mg) to 535.476 (μM/min/mg) with purification fold 10.632 and yield 37.391 %. The molecular weight of the protease was 35 kDa which is shown in Fig. 2.

Kinetic properties of protease

The kinetic parameters were measure in absence and in the presence of NaCl and Co. A significant increase in rate of catalysis was observed when NaCl and Co^{2+} were added separately in the reaction mixture as shown in Table II. The k_{cat}/K_M of the protease in the presence of NaCl was $1.42 \times 10^3\ s^{-1}$. M^{-1} which was significantly high as compared to the k_{cat}/K_M obtained when protease

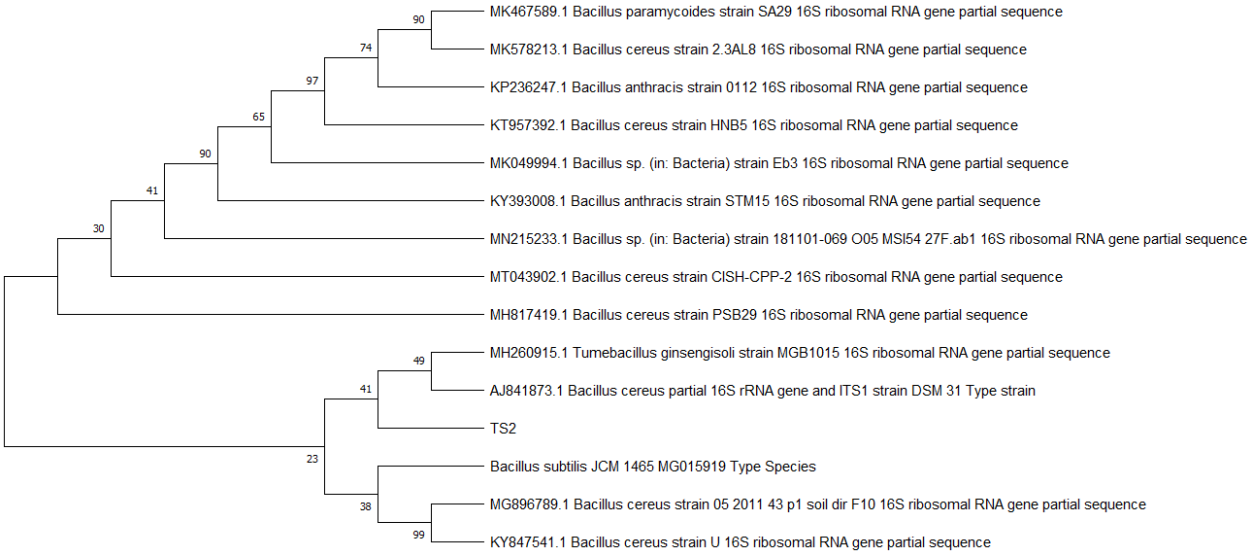

Figure 1. Neighbor-joining phylogenetic tree of the representative bacterial strain TS2 and its related species based on the 16S rRNA gene sequences.

Table I. Summary of the purification of extracellular protease from *Bacillus cereus* TS2.

	Total Protein content (mg)	Unit activity (μM/min)	Specific activity (μM/min/mg)	Purification fold	Yield (%)
Cell free extract	0.22	294.512	50.362	1	100
Saturated Dialyzed	0.041	201.976	492.624	9.781	68.579
DEAE cellulose	0.055	111.302	535.476	10.632	37.391

was used alone without addition of NaCl i.e. 7.58 × 10^2 s^{-1}. M^{-1}. Similarly, the K_{cat}/K_M was improved with value of 1.27 × 10^3 s^{-1}. M^{-1} when cobalt was added in the reaction mixture as shown in Table II.

Characterization of protease

Effect of salt, pH, and temperature on protease activity

The alkaline metalloprotease exhibits clear evidence of salt tolerance as it was quite stable under high concentrations of salt and acquired an optimum activity in the presence of 2 M NaCl, it was finely stable with a 50 % residual activity when 6 M NaCl was added in reaction mixture as shown in Fig. 3a. The protease was capable to work in a wide range of pH, and the maximum activity was observed at value of 10. The enzyme retains its activity till pH of 12 but found to be very less active at lower values of pH as shown in Fig. 3b. The protease was quite active at various temperatures while the maximum activity was observed at a temperature of 70 °C (Fig. 3c), similarly the thermostability assay indicates that the enzyme was quite stable in a wide range of temperatures and has shown activity still after incubation at 90 °C as shown in Fig. 3d.

Effect of metal ions and inhibitors on protease activity

To determine the effect of metal ions on the activity of enzyme the reaction mixture was amended with 10mM of each divalent ion separately, and it was observed that the addition of Mg^{+2} and Co^{+2} significantly increased the

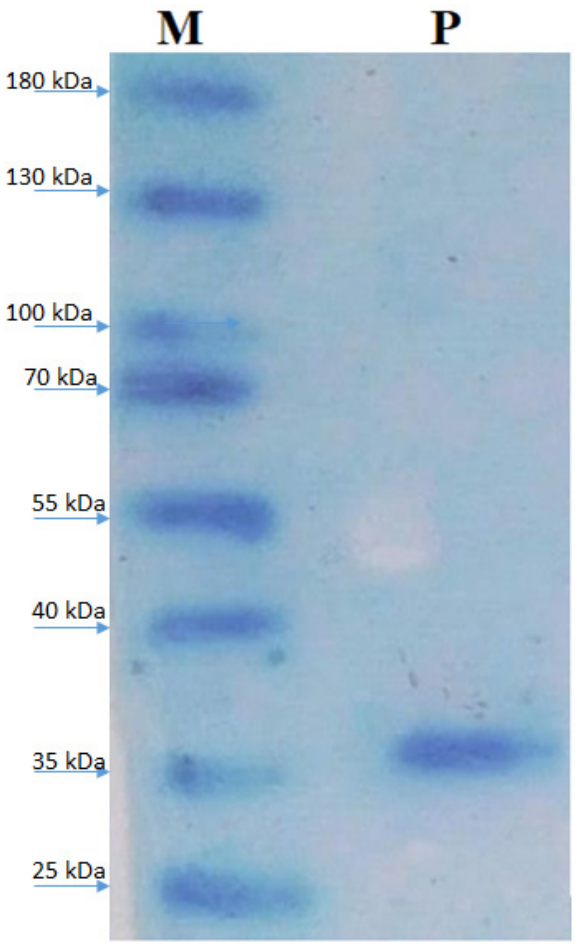

Figure 2. SDS-PAGE of purified metalloprotease isolated from strain TS2. The well "M" loaded with protein ladder and the "S" loaded with protease sample.

activity of protease while the other three ions i.e. Zn^{+2}, Cu^{+2}, Ni^{+2} has decreased the activity as compared to control shown in Fig. 4a. The effect of two inhibitors was studied on the activity of protease i.e. EDTA (10 mM) and PMSF. It was found that the protease activity was inhibited in the presence of 10 mM EDTA, but PMSF has no effect on the protease activity as shown in Fig. 4b. These findings suggest that the protease is metalloproteases, as their proteolytic activity was inhibited by the metal chelating agent EDTA.

Effect of organic solvents on protease activity

Protease isolated from the strain TS2 is quite stable to the organic solvents, retaining its activity in the presence of 20 % of ethanol, methanol, ethyl acetate, acetone and hexane as shown in Fig. 5. Surprisingly, protease shows a distinct characteristic, and the activity was increased in the presence of ethanol, methanol, and hexane till 9 % and above that, the relative activity starts decreasing but still it was active till 20 % of these solvents. For ethyl acetate and acetone, the activity of protease was increased to 12 % and then it gradually decreases as their concentrations increase but still active till the concentration of 20 % of these solvents (Fig. 5).

APPLICATIONS OF METALLOPROTEASE

Destaining test

The metalloprotease from TS2 was examined to determine its ability for removing the blood stain. The cloth with blood stain was treated with 3 % crude enzyme and it was found that the stain was removed within given period as shown in Fig. 6a.

Dehairing test

The metalloprotease has no keratinase activity as it has been confirmed by performing the assay with feathers. The metalloprotease from TS2 selectively remove the hairs from goat skin without damaging the leather strength and facilitate the removal of intact hairs from the skin. The goat skin treated with chemical has short hairs on the surface while the skin treated with enzyme clean, white, smooth, and silky as shown in Fig. 6b.

Table II. Kinetic parameter of purified extracellular protease from Bacillus cereus TS2 at various conditions.

	k_{cat} (s^{-1})	K_M (μM)	k_{cat}/K_M (s^{-1} M^{-1})
Protease	746.66	984.6	7.58×10^2
Protease with NaCl	1297.77	916.3	1.42×10^3
Protease with Co^{+2}	1550.55	1220	1.27×10^3

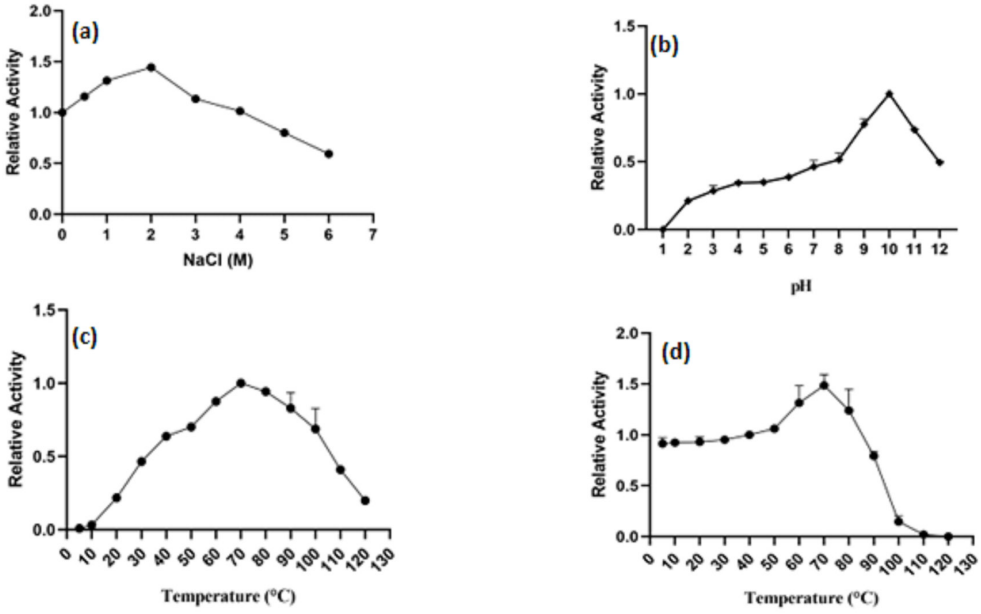

Figure 3. Relative activities of metalloprotease under different conditions: (a) Effect of various concentrations of NaCl (0-6 M); (b) Effect of pH on activity of metalloprotease; (c) Effect of temperature (5°C to 120°C); (d) Thermostability (5°C to 120°C).

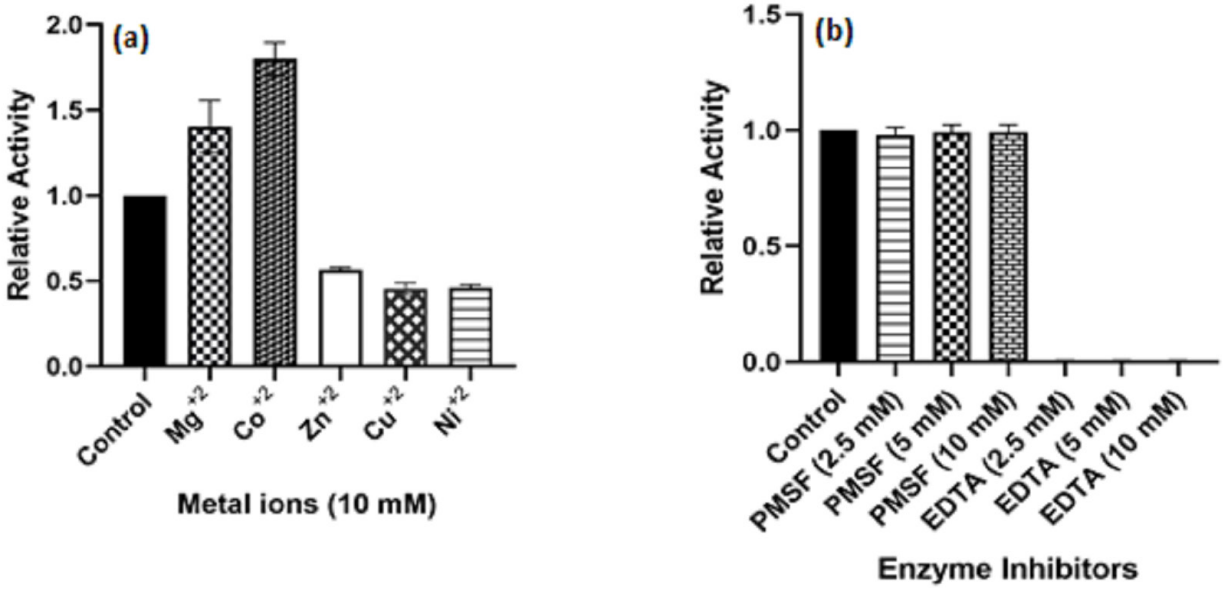

Figure 4. (a) Effect of metal ions on the activity of metalloprotease; (b) Effect of inhibitors on the activity of metalloprotease.

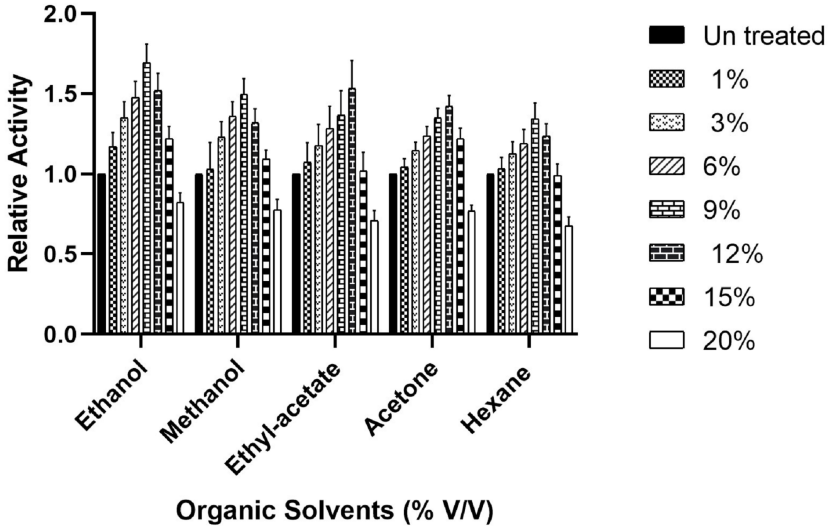

Figure 5. Effect of organic solvents on the activity of metalloprotease.

Figure 6. (a) Dehairing activity of metalloprotease of TS2;"A" control treated with water only; "B" treated with 10 % lime and 3 % sodium sulfide; "C" treated with 1 % metalloprotease; "D" treated with 2 % metalloprotease and E treated with 3 % metalloprotease; (b) Destaining activity of metalloprotease; "A" control treated with distilled water; "B" treated with commercial detergent; "C" treated with 3 % metalloprotease and "D" treated with detergent plus 3 % protease.

Milk clotting ability

The metalloprotease from strain TS1 has ability to degrade milk protein as shown in Fig. 7. The well diffusion assay as well as the skimmed milk agar plates shown a clear zone that exhibit the ability of metalloprotease to clot milk as shown in Fig. 7.

Feather degradation

Metalloprotease from TS2 has no activity when used against the feather and no reduction in weight was observed with various concentration of enzymes while incubating at various time intervals. This also confirmed that the protease has no keratinase activity.

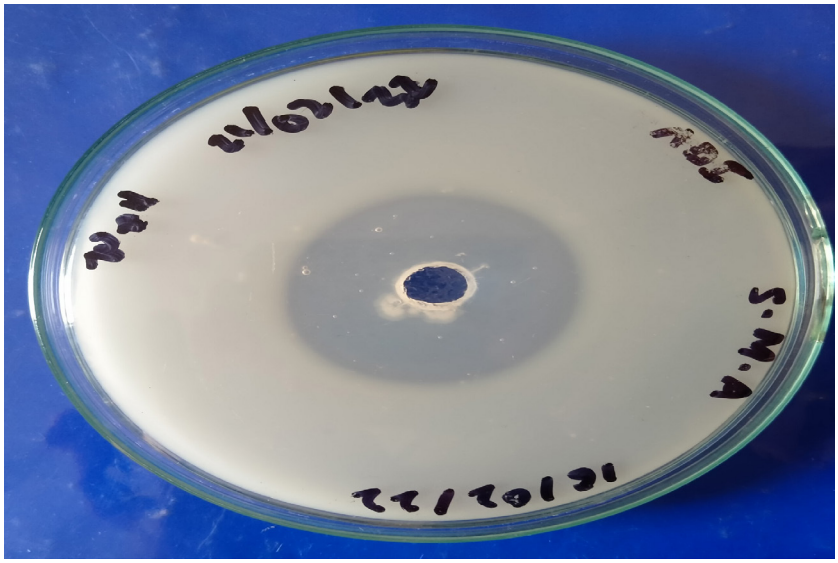

Figure 7. Skimmed milk agar assay showing milk protein degrading ability of metalloprotease of TS2.

DISCUSSION

Extremozymes are the enzymes derived from microorganism that can survive under extreme conditions such as extreme temperatures, pressure, salt concentrations etc., therefore their enzymes are adapted to withstand harsh industrial conditions (Elleuche et al. 2014). Halophiles are such organisms that can survive under high salt concentrations; therefore, it is the hypothesis that enzymes required for the processes where involve high salt concentration can be isolated from such type of microorganisms. A microorganism identified as *Bacillus cereus* was isolated from the salt range found to be protease activity and able to grow in the presence of 10 % salt. The *Bacillus cereus* strain TS2 is classified as moderate halophile as it can grow in the presence of 10 % NaCl as classified earlier (Ventosa et al. 1998). *Bacillus* is one of the known genera as a producer of alkaline proteases (Rifaat 2007) and number of species belong to *Bacillus* has been reported as excellent producer of proteases which has been used in various industrial processes (Ali et al. 2016, Kalwasińska et al. 2018, Alnahdi 2012). Similarly, an alkaline protease was isolated from *Bacillus infantis* with unique properties having ability to work at high temperature and in presence of acetyl acetone (Saggu & Mishra 2017). The protease from *Bacillus cereus* strain TS2 has profound activity when NaCl was added in concentration of 2 M, although it also works without NaCl but significant increase in catalytic activity was observed while adding NaCl. Halophilic proteins have adapted to work under high salt concentration and therefore, their amino acid residues have different composition as compared to their mesophilic counter parts. Thus, these enzymes have higher composition of acidic residues with short polar side chains while the lysine and bulky hydrophobic amino acids were depleted (Ortega et al. 2015). This unique composition of amino acids helps them to be active in high salt concentration, therefore the high concentration of salt for halophilic proteins helps them to get proper conformation and that is the reason the halophilic enzymes become more active in the presence of salt and some of the halophilic proteins even lost their activity in absence of particular salt concentration (Ortega et al. 2011, Madern et al. 2000, Siglioccolo et al. 2011). There is clear evidence that salt is a prerequisite for the functioning of halophilic proteins and help to adapt the proper conformation of halophilic

proteins (Sinha & Khare 2014, Madern et al. 2000, Mevarech et al. 2000). Therefore, the addition of salt exhibited a positive impact on the activity of the enzyme and thus the protease which was isolated from the halophilic bacterium works more efficiently in the presence of 2 M salt following the same findings as described in previous studies (Karan & Khare 2011, Chuprom et al. 2016). Enzymes isolated from various halophilic species has a remarkable ability to work under high salt concentrations (Gupta et al. 2016). The distinct ability of the protease to work under high saline conditions was like the previously reported enzymes isolated from the halophilic species, as an alkaline protease isolated from *Bacillus luteus* H11 have optimal activity at 3 M of NaCl (Kalwasińska et al. 2018). Similarly, the alkaline protease from *Natronolimnobius innermongolicus* WN18 and halophile Chromohalobacter TVSP101 has optimal activity at 2.5 and 4.5 M of NaCl respectively (Vidyasagar et al. 2009, Selim et al. 2014).

The activity of protease isolated from strain TS2 has been stimulated by the addition of Mg^{+2} and Co^{+2} ions but the Zn^{+2}, Cu^{+2}, and Ni^{+2} has significantly inhibited the activity of protease. The results also confirmed that the protease isolated from strain TS2 is metalloprotease as its activity was diminished while using the EDTA that removes the metal ions from the protease, and it stops working. Metalloproteases are the proteases that contains one or two metal ions at their center, these metal ions activate water molecule and serve as nucleophile during catalysis (Rawlings & Barrett 2013). Similarly, an alkaline metalloprotease was isolated from the *Bacillus alkaliterlluris* TWI3, where the Ca^{+2}, Mn^{+2} and Mg^{+2} increased the activity of metalloprotease while Hg^{+2}, Cu^{+2}, Zn^{+2} and Fe^{+2} inhibited enzyme activity (Anandharaj et al. 2016b, Yilmaz et al. 2016). The metalloprotease from the TS2 has optimum activity at pH of 10 and at 80 °C, which was also quite similar with previous findings where the alkaline protease showed maximum activity at high temperature and pH (Abu-Khudir et al. 2019, Yang et al. 2020).

The metalloprotease from strain TS2 was quite promising in the presence of organic solvents and it can tolerate ethanol, methanol, ethyl acetate, acetone, and hexane, mostly the relative activity of the enzyme increases in the presence of observed organic solvents. Microbial enzymes that can tolerate organic solvents have numerous industrial applications, recently numerous studies have been conducted to isolate such enzymes from various extremophiles such as thermophiles, halophiles etc. (Doukyu & Ogino 2010). Similarly, a protease from halophilic *Bacillus* sp. APCMST-RS7 was isolated that can tolerate hexane, butanol, ethyl acetate, methanol and benzene (Maruthiah et al. 2017).

The protease isolated from various microbes have several applications in various industries, therefore the potential of metalloprotease isolated from TS2 was determined by performing various activities (Kalwasińska et al. 2018). The dehairing ability of metalloprotease was proved when it was used against the goat skin as shown in Fig. 6a, which was also be supported by previous findings where the alkaline protease has ability to dehair goat skin when used in various concentrations (Anandharaj et al. 2016a). Proteases are one of the potential candidates for detergent industry as it helps to de-stain various stains having protein such as blood stains etc. Several proteases have been reported having role in destaining of cloths (Razzaq et al. 2019). The metalloprotease has efficient to remove blood stains which was also in accordance with previous studies where proteases were applied to remove various stains like blood (Anandharaj et al. 2016a, Jayashree et al. 2014).

CONCLUSION

Metalloprotease from strain TS2 has remarkable features that make it a future candidate for industrial applications. It can tolerate various organic solvents along with the ability to catalyze under extreme conditions of pH as well as temperature. The metalloprotease has ability to dehair goat skin with no keratin activity and able to destain when used against blood stain that make it potential candidate for leather as well detergent industry. The ability of metalloprotease to degrade milk protein make it applicable for food industry as well.

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