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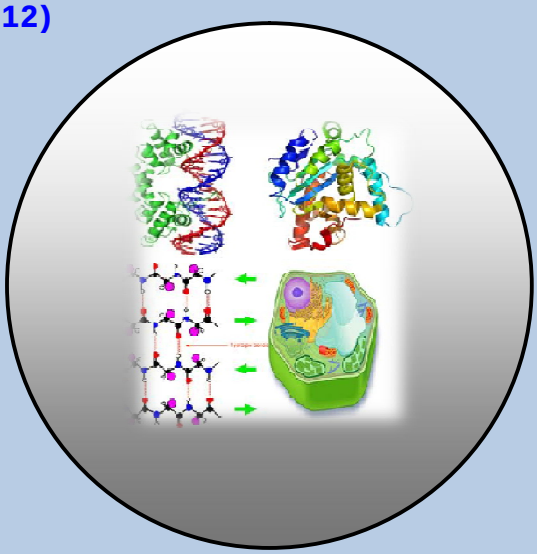

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Biochemical and Molecular Characterization of Alkalo-Thermophilic Proteases Purified from *Aspergillus fumigatus*

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ABSTRACT

Among the fungal species isolated from wastes water of detergent factory (pH 10.5), Aspergillus fumigatus was found to be the most potential proteolytic species. The phylogenetic analysis using 16 S rRNA amplified sequence indicated that the tested fungal species is A. fumigates which separated from other groups belonged to Asperigillus species. Purification of alkaline protease was made in a four-step procedure involving ammonium sulphate precipitation, DEAD Cellulose, Sephadex G-200 and Sephadex G-150 column chromatographies. The apparent molecular weight of the enzyme was determined by SDS-PAGE to be 24 KDa. A. fumigatus protease was found to be thermo-alkalophilic enzyme acting optimally at pH 11 and 50°C and inhibited by Na^{+}, Cu^{+2}, Urea, Zn^{+2}, Mg^{+2}, EDTA and Fe^{+3}.The phylogenetic analysis of partially sequenced alkaline protease gene(ALP) was carried out by the neighbor joining method using MEGA version 5, it showed 100% identity to the portion of the PCR fragment containing the alkaline protease (ALP)region sequence and 99% of A. fumigatus (GenBank accession number BAN04643). Application of the enzyme by adding it to some commercial detergents in Egyptian market indicated that the enzyme retained activity ranged between 32.05% to 91.10% after 60 minutes incubation at 45°C in the presence of all tested detergent Xtra, Persil, Oxi, Ariel and leader, proving its suitability as bio detergent. The purified enzyme despotted dried blood spots from cloth pieces (silk, cotton and polyster). Furthermore, it dehaired skin when incubated with a piece of goat leather indicating its suitability for application in leather industry.

Keywords: Aspergillus fumigates, Alkaline protease, Purification, Bio detergent, Dehairing and Molecular studies.

INTRODUCTION

Protease represent one of the most important groups of industrial enzymes accounting for about 60% of the total worldwide enzymes market because of their many various application in many industries such as the detergent , food ,leather , silk , photographics, dairy and pharmaceutical industries(Tunga et al .2003, Merheb et al. 2007).Many proteases are a major focus of attention for the pharmaceutical industry as potential drug targets or as diagnostic and prognostic biomarkers (Otín and Bond, 1999).Based on the catalytic site on the substrate, proteases are mainly classified into endoproteases and exoproteases (Rao et al. 1998). Endoproteases preferably act at the inner region of the polypeptide chain. By contrast, exoproteases preferentially act at the end of the polypeptide chain. Exoproteases are further classified into amino peptidases (which act at the free N-terminus of the polypeptide substrate), and carboxy peptidases (which act at the free C-terminal of the polypeptide chain) (Rao et al. 1998).Proteases have been isolated and purified from microorganisms, animals and plants. Microorganisms represent the most common source of enzymes because of their broad biochemical diversity, feasibility of mass culture and ease of genetic manipulation (Zhang and Kim. 2010). Two third of the industrial produced proteases are from microbial sources (Adinarayana and Ellaiah. 2002).The various microorganisms such as bacteria, fungi, yeast and Actinomycetes are known to produce these enzymes (Madan et al. 2002; Devi et al. 2008). In 1960, Dane first isolated alkaline protease from *Bacillus licheniformis*. Molds of the genera *Aspergillus*, *Penicillium* and *Rhizopus* are especially useful for producing proteases, as several species of these genera are generally regarded as safe (Devi et al. 2008). *Aspergillus clavatus ES1* has been recently identified as a producer of an extracellular bleaching stable alkaline protease (Hajji et al. 2008).As detergent additives, alkaline proteases dominate commercial applications market. Alkaline proteases added to laundry detergents enable the release of proteinaceous material from stains. Increased usage of these proteases as detergent additives is mainly due to the cleaning capabilities of these enzymes was environmentally acceptable (Kumar and Takagi. 1999). Dehairing is an important operation in tanneries conventionally practiced using lime and sodium sulphide (Thanikaivelan et al. 2004). In this process, lime contributes to the dehairing process by opening up the collagen fiber structure. The use of alkaline protease has proven superior and efficient for selective removal of the non-collagen part of hide/skin (Kamini et al. 1999).

The objectives of the present study were focused onisolation and molecular identification of *A. fumigatus* from air and soil around detergent factory with pH 9.5, purification and characterization of alkaline protease produced by *Aspergillus fumigatus*, application of alkalophilic protease as despotting agent in bio detergent and as dehairing agent of leather.

MATERIAL AND METHODS

Test Microorganisms

Aspergillus fumigatus was isolated from the waste water, air and soil around detergent factory in Egypt having pH 9.5 – 10

It was grown on production medium containing (g/l): Malt extract 20g, Fructose 5g, Thio urea 5 mg, KCl 1.0 g, FeSO4.7 H_2O 0.02 g, MgSO4. 7 H_2O0.5g, K2HPO4, 1.0g; pH 9.0 (Saravana Kumar et al.2010). The plates were incubated at 45°C for at least 7 days.

Assay of proteolytic activity

Aspergillus fumigatus assayed for its activity using cup plate method according to Dorey et al. (1965).

Protein assay

Protein was quantified by the method of Lowry et al., (1951), using bovine serum albumin as Standard.

Purification of alkaline protese

Ammonium sulfate fractionation: Solid ammonium sulfate was added to the crude extract to 40-80% saturation. The precipitate was collected by centrifugation, dissolved in minimal volume of 0.1%Tris-Hcl buffer (pH8) and dialyzed against same buffer at 4°C over night

DEAE-cellulose chromatography

The daily sedenzyme solution was applied to DEAE-cellulose column (2.0 X 25cm) pre equilibrated with 0.1 M Tris-HCl buffer (pH 8). The enzyme was eluted with the same buffer at a flow rate of 150 ml/h.

Sephadex G-150 column chromatography

The active fractions collected from DEAE-cellulose were then applied to the Sephadex G-150column previously equilibrated with 0.1 M Tris-HCI buffer pH 8.0. The enzyme was eluted with the same buffer at a flow rate of 150 ml/h and the fraction volume of 5.0 ml/tube.

Sephadex G-100 column chromatography

The active fractions from sephadex G-150 column were then applied to the Sephadex G- 100 column previously equilibrated with 0.1 M Tris-HCI buffer pH 8.0. The enzyme was eluted with the same buffer at a flow rate of 150 ml/hour and the fraction volume of 5.0 ml/tube.

Characterization of thermo stable alkalophilic protease

Effect of pH on the enzyme

The reaction mixture was prepared containing a constant volume of 0.5 ml of 1% casein, 3 ml of 0.2 MTris HCl buffer, of pH ranged from pH 7 to pH 11, and 1.0 ml of enzyme .The mixture was incubated at 45 °C. After 10 min, the reaction was stopped by adding 3 ml of cold 10% TCA then centrifuged at 8000 rpm for 5 min to remove the precipitate. Five ml of Na_2CO_3 was added and incubated at 37 °C for 10 min then 0.5 ml of folin reagent was added and kept for 30 min for 37 °C. Absorbance was read spectro photometrically at 630 nm. Enzyme activity was calculated by measuring µg of tyrosine equivalent released and compared with the standard. One unit (U) of enzyme activity represents the amount of enzyme required to liberate 1 µg of tyrosine under standard assay condition (Upadhyay et al. 2010).

Effect of Temperature on Protease activity

The reaction mixture conataining0.5 mL of 1% casein and 3 mL of 0.2 M Tris HCl buffer (pH 11) and 1.0 mL of enzyme was treated at different temperature ranging from 10 °C to 70 °C (Chanda et al., 2011). After 10 min. Protease activity was assayed by the method mentioned above.

Effect of various metal ions and protease inhibitors

The effects of various metal ions and inhibitors on the activity of the purifiedalkalophilic protease were assessed by adding the following metal ions and inhibitors ($FeCl_3$, $MgSO_4$, $ZnSO_4$, $CuSO_4$, $MnSO_4$, $CaCl_2$, NaCl , EDTA , Urea) with concentration 5 mMto the reaction mixture , after 10 min. Protease activity was assayed.

Substrate specificity of protease

The effects of various type of substrate on the activity of the purified protease were assessed by adding different substrate as (casein, albumin, and gelatin) in the reaction mixture. After 10 min Protease activity was assayed.

Molecular mass determination

SDS-PAGE: SDS-polyacrylamide gel electrophoresis (SDS-PAGE) was performed on12.5% (w/v) acrylamide slab gel with 25 mM Tris / 192 mM glycerin buffer (pH 8.3) that contained 0.1% (w/v) SDS as the running buffer, as described by Laemmli (1970).

Durability of alkaline protease with commercial detergents

Detergent solutions (Tide, Persil Oxi, Leader, Xtra, Ariel) purchased from local market were used at a concentration of 0.07 mg/mL in distilled water. These solutions were boiled for 10 min to destroy any protease already present and cooled. A reaction mixture was prepared by adding 0.5 mL of 1.5% casein as a substrate, 10 ml of enzyme solution and 0.5 mL of detergent solution. After 10 min incubation, Protease activity was assayed by the method of Adinarayana et al.(2003).

Effect of purified alkaline protease on blood stained cloth

Purified enzyme as a detergent additive was studied on white cotton, silk and polyster cloth pieces stained with blood. The stained cloth pieces were taken in separate flasks. The following sets were prepared and studied according to the method of (Kunamneni Adinarayana et al. 2003).

- Flask with distilled water (25 ml) + cloth stained with blood (control)
- Flask with distilled water (25 ml) +stained cloth + 1 ml purified protease
- Flask with distilled water (25 ml) + stained cloth + 0.25 ml Xtra detergent (0.07mg/ ml)
- Flask with distilled water (25 ml) + stained cloth + 0.25 ml Xtra detergent (0.07mg/ ml)+ 1 ml purified enzyme

The above flasks were incubated at 50^{o}C for 15 min. with shaking. After incubation, cloth pieces were taken out, rinsed with water, and dried. Visual examination of various pieces exhibited the efficiency of enzyme in removal of stains.

Dehairing capability of purified alkaline protease

Skin of goat was cut and incubated with 5 ml of purified protease in 10 ml of 50 mM Tris-HCl (pH 9) at 50^{o}C .The skin was checked for removal of hair at different incubation periods (5,12,18,24 hours). After incubation times the hair was removed from the skin using blunt knife (Mohsen Fathi Najafi et al. 2005).

Molecular identification of the producing fungus and expression of alkaline protease gene (ALP_1)

DNA extraction

Fungal mats were harvested from the cultured Potato Dextrose Agar (PDA, Difco Lab., USA) and suspended into Hanks Balanced Salt Solution (HBSS, Sigma Chemical Co, St. Louis, MO, USA) in a ratio of 1 whole fungal mats mixture: 2 HBSS (v/v). The fungal suspension was vortexed with glass beads. Then, DNA was extracted using the Qiagen plant cell mini kit (Qiagen, Germantown, MD, USA) according to the manufacturer's protocol. DNA purity was measured spectro photometrically at 260 / 280 Φ wave length.

Detection of ALP gene by PCR

The 747 bp of the Alkaline protease (ALP) gene was amplified by PCR using two ALP gene primers: 5'- AGCACCGACTACATCTAC-3' (ALP1) and 5'- GAGATGGTGTTGGTGGC-3' (ALP2). Amplification was done after 42 cycles of de naturation at 94°C for 0.5 min, annealing at 63°C for 45 sec, and extension at 72°C for 2 min, using a PCR gradient thermal cycler (TC-3000G, Bibby Scientific Ltd, Staffordshire, United Kingdom) (Urata et al. 1997). As a precaution to prevent DNA template contamination, preparation of DNA samples, preparation of PCR master mix solutions, and PCR amplification were performed in different rooms using a safety cabinet or clean bench and aerosol resistant pipette tips. The PCR products were electro phoresed in 2% agarose gel (Invitrogen), stained with ethidium bromide, visualized with ultraviolet light, and photographed using Kodak EDAS System (Eastman Kodak, Rochester, New York, USA).

Cloning and sequencing of ALP gene

The purified amplified fragments were cloned into pGEM-T Easy vector® plasmid by T4 ligase (Promega, Madison, WI, USA), and then the cloned plasmid transformed into *Escherichia coli DH5α* by the heat shock method. The transformants clones were screened by colony PCR with the oligonucleotide primers SP6 (5'-ATTTAGGTGACACTATAGAA-3') and T7 (5'-TAATACGACTCACTATAGGG-3'). The plasmid DNA of clones containing the correct insert segments were then purified using the QIA prep Spin Mini prep kit (Qiagen, Germantown, MD, USA) to be sequenced (Abdelsalam et al. 2010). Sequence reactions were then performed using the ABI Prism Big Dye Terminator Cycle Sequencing Ready Reaction Kit (Perkin Elmer Applied Bio systems, CA, USA) with the oligonucleotide primers SP6 and T7. The samples were then loaded into the ABI Prism 310 Genetic Analyzer (Perkin Elmer Applied Bio systems, CA, USA), and the nucleotide sequences were determined. The nucleotide sequences were analyzed using Bio Edit version 7.0 (Hall, 1992). The phylogenetic analysis of partially sequenced (ALP) gene was carried out by the neighbor joining method using MEGA version 5 (Tamura et al. 2011)

Nucleotide sequence accession number

The nucleotide sequences determined in this study were submitted to GenBank nucleotide sequence database, and the accession numbers were given (AB807664.1).

RESULTS

Assay of proteolytic activity

Aspergillus fumigatus showed an effective zone of casein hydrolysis of diameter 1.0 Cm.

Figure 1.Proteolytic activity in *Aspergillus fumigats* using cup plate method.

Purification of *A. fumigates* alkalophilic protease

The purification steps and yields of a typical experiment are summarized in table (1).

A single peak of proteolytic activity was obtained after G-150 gel chromatography with 293.8 U/mg specific activity, 50.6 purification fold and 35.9 recovery of the crude enzyme.

Table 1 . Full purification protocol of *A. fumigates* protease.

Purification steps	Total protein (mg/ml)	Total activity (units/ml)	Specific activity (U/mg)	Purification fold	Recovery
Crude	151.28	882.0	5.8	-	100
Ammonium sulphate & Dialysis	54.4	790.2	14.5	2.5	89.5
DEAE-Cellulose	3.21	560.6	174.6	30.1	63.5
Sephadex G100	1.989	398.0	200.1	34.5	45.1
Sephadex G150	1.2	352.6	293.8	50.6	35.9

The purified enzyme migrated as a major protein band in SDS-PAGE (figure.2).The SDS-Page indicated that the apparent molecular weight of *A. fumigatus* was found to be approximately 24 KDa.

Fig. 2. SDS - PAGE showing the molecular weight of alkaline protease produced by *Aspergillus fumigatus* (A) Enzyme after purification using G100. (B) Enzyme after purification using G150

Characterization of thermo stable alkalophilic protease

Effect of different pH

The effect of pH on the activity of alkaline protease in A. fumigatus showed that the optimum pH was 11. This proves the alkalophilic activity of the enzyme (Figure. 3)

Effect of temperatures on protease activity

The effect of temperatures on protease activity was shown in figure (4) indicates that the optimum temperature was found to be 50 °C. An initial increase in temperature increased the rate of the enzyme catalyzed reaction but the enzyme begins to denature on exposure to higher temperature than its optimum temperature.

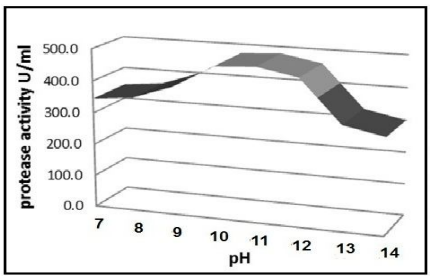

Fig 3. Effect of PH on enzyme activity

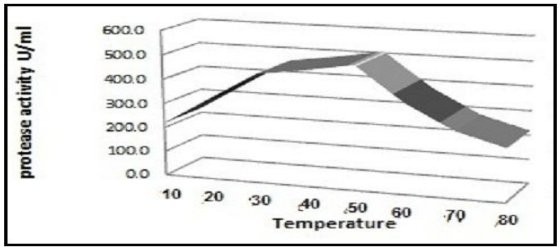

Fig 4. Effect of temperature on enzyme activity

Substrate specificity of protease

The purified protease in *A. fumigatus* was non specific protease. It could hydrolyse bovine albumine, gelatine in addition to casein . It showed higher specificity to gelatin hydrolysis than the other two substrates (Figure. 5)

Effect of different metal ions and inhibitors on protease activity:

The influence of various metal ions and inhibitors on enzyme activity was studied. Addition of 5 mM $CaCl_2$ enhanced the activity by 117% of control. $ZnSO_4$ and $CuSO_4$ inhibited alkalophilic protease activity to the level of 69 and 80% of control. Maximum inhibition was obtained by EDTA (Figure 6).

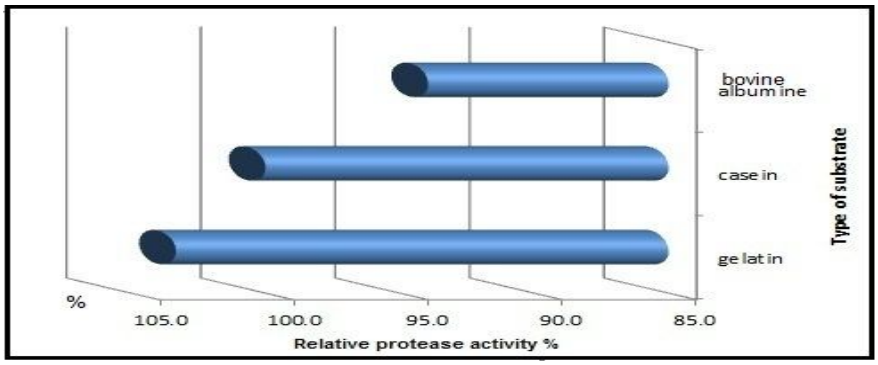

Fig 5. Effect of substrate type on Enzyme activity

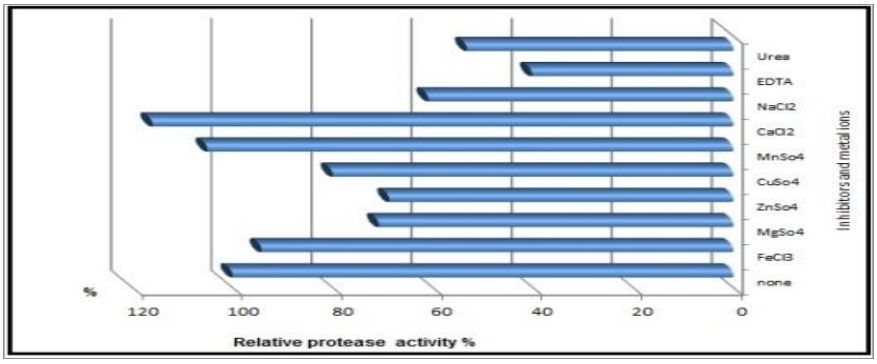

Fig 6. Effect of metal ions and protease inhibitors on enzyme activity.

Table 2. Characterization of the alkaline protease in A. *fumigatus*.

Properties	*Aspergillus fumigatus*
Optimum pH	11
Optimum temperature (°C)	50
Most specific substrate	Gelatin
Molecular weight (kDa)	24
Activator	Ca^{+2}
Inhibitors	Zn^{+2} ,Ca^{+2} , EDTA

Durability of Alkaline Protease with Commercial Detergents

Compatibility of alkaline protease was varied depending on the detergent type. Alkaline protease of *A. fumigatus* retained 32% of its activity in presence of Tide while it retained 91.1% of the activity in presence of Xtra detergents (figure 7).

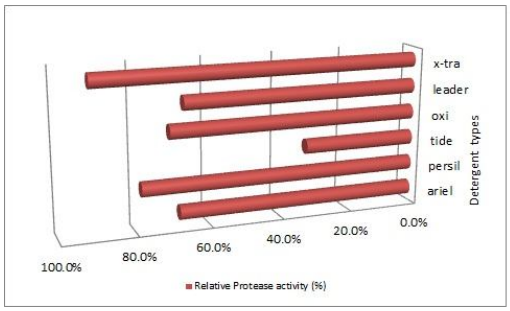

Figure 7. Durability of purified alkaline protease with various types of detergents.

Application of purified alkaline protease enzyme

(A) Despotting action

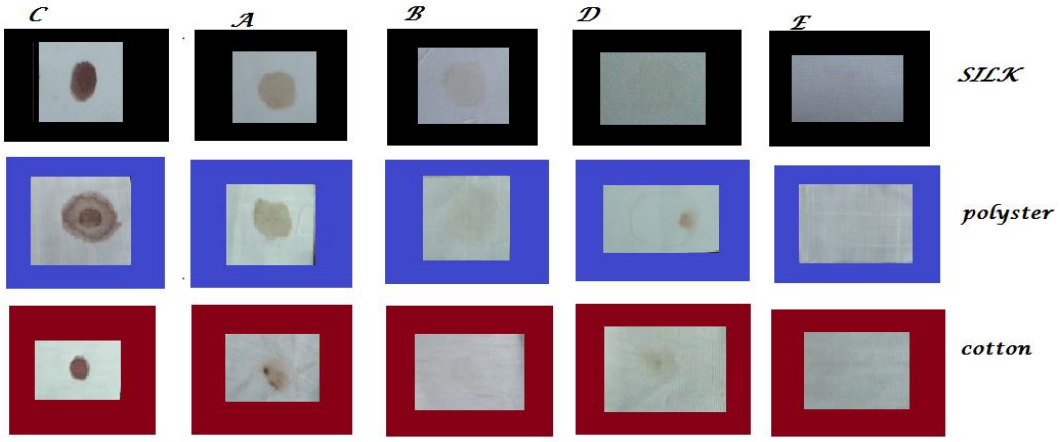

Fig 8.Column c: Control (without detergent and enzyme); A: Treated with 25 ml distilled water only ; B: treated with 1 ml purified enzyme solution+ 25 ml distilled water ; D: Treated with 0.25 ml detergent solution (7µg/mL)+25 ml distilled water; E : Treated with 0.25 ml detergent solution (7 µg/mL) + 1 mL of purified enzyme sample.

The qualitative observation indicated the higher efficiency of *A. fumigatus* protease(when added singly B) as despotting agent than the single addition of detergent (D) combination of enzyme and detergent resulted in promising cleaning of the clath piece from blood spot (E).

(B) Dehairing

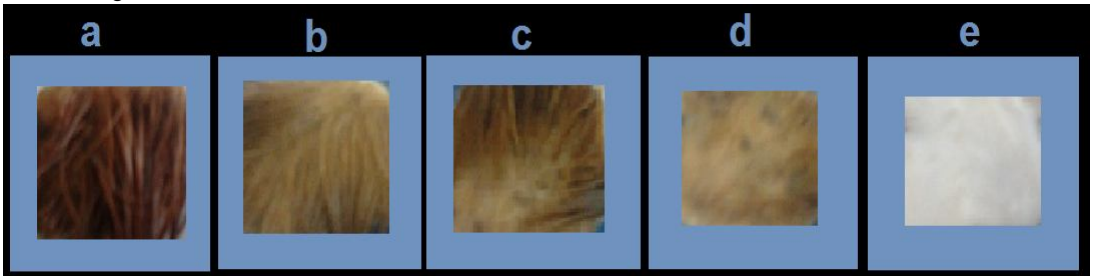

Fig 9. A; goat skin without treatment , B; goat skin after treatment with alkaline protease for 5 hours , C; treated skin for 12 hours, D; treated skin for 18 hours, E; Treated skin for 24 hours.

The data showed clearly the dehairing action of protease which become more evident with time (Figure 9, a-e)

PCR and phylogenetic analysis

A 747 bp fragment was amplified from high-quality DNA extracted from 1 replicate of *A. fumigatus* isolates using primers targeting the ALP gene (Fig.11). The ALP region' sequence achieved from the highest-scoring segment pairs from the BLAST search using the ALP region sequence were from A. fumigatus. The ALP region sequence from *A. fumigatus* (figure 10- GenBank accession number AB807664.1) showed 100% identity to the portion of the PCR fragment containing the ALP region sequence and 99% from *A. fumigates* (GenBank accession number BAN04643). The phylogenetic analysis using neighbor joining method showed that the amplified sequence were grouped with known sequences of *A. fumigatus* and separated from other groups belonged to *Asperigillus* species (Figure. 11)

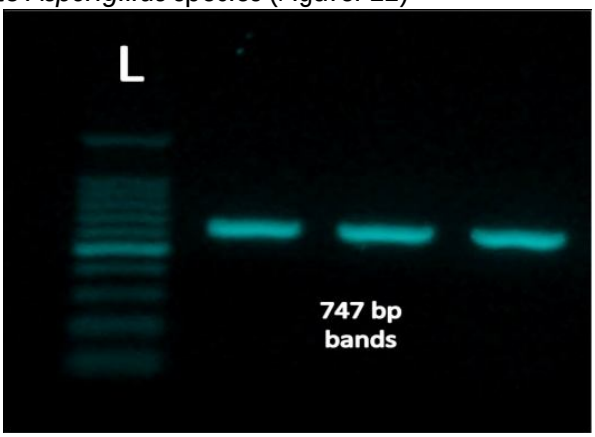

Fig. 10. A gel electrophoresis picture showing A 747 bp fragment (band) that was amplified from high-quality DNA extracted from 1 replicate of *A. fumigatus* isolates using primers targeting the ALP gene.

L: 100 bp increment ladder (DNA marker)
<egyptst1 *Aspergillus fumigatus*
gacaccagcgctggcgcaggcacctatgcctacgttgtcgacagtggcatcaatgtcaac
cacgtcgagttcgagagccgcgcatcgctggcatacaacgccgctggtggcagccatgtt
gacagcatcggccacggaacgcacgttgctggtaccattggcggcaagacctacggagtg
gccaagaagaccaaccttctgtccgtcaaggtcttccagggcgagtcctctagcacctcc
atcatccttgacggcttcaactgggctgtcaatgacattgtgagcaagggtcgtactaag
aaggctgcgatcaacatgagccttggtaagatagacccctcttgctcgttcaactggact
gaaagtaacggcggctgatggataatgacaggtggtggttactcttatgccttcaacaac
gctgttgagaacgctttcgatgaaggtgtcctttctgtcgtcgctgctggaaacgagaac
gtatgtatcccccgttttagaaaagttacaaatgaacattgcaggtttggtcggactaac
atgttgtgccagagtgatgcctcaaataccagccctcgttccgctcctaacgctttgacg
gttgctgcgatcaacaagagcaacgcccgcgcctccttctccaactatggttccgttgtc
gacatcttcgctcccggtcaggatatcctttcggcctggattggctccaccact
Primers
ALP1:AGCACCGACTACATCTAC
ALP2:GAGATGGTGTTGGTGGC
gene: Alkaline protease (AIP)
Product: Alkaline protease enzyme
Partial sequence

Fig 11. Nucleotide Sequence of the Alkaline Protease Gene of *A. fumigates*.

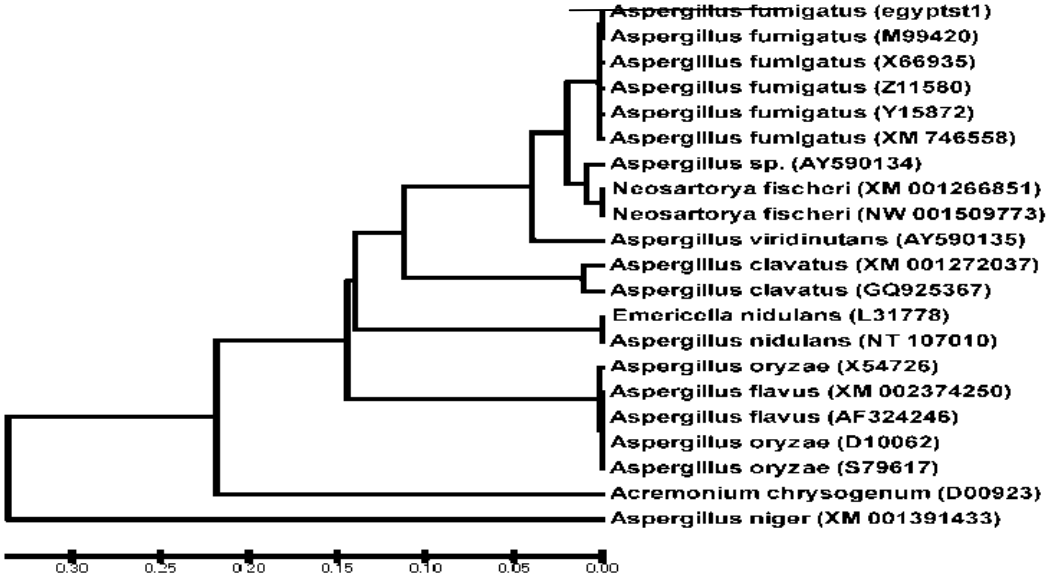

Figure 12 . Dendogram showing phylogenetic analysis using neighbor joining method.

DISCUSSION

Alkaline protease was isolated from *A. fumigates* and then purified by different procedure and characterized. The protein content of *A. fumigates* purified alkaline protease in a solution was determined to be 1.2 mg/ml with 352.6 U/ml total activity, 293.8U/mg specific activity ,50.6 purification fold and 35.9 recovery .

Maximum protease production was found at pH 11. The results clearly indicated the alkalinophilic nature of the fungus and the protease. It was reported by Negi and Banerjee (2010) that pH 9 was the optimum pH for alkaline protease enzyme from *Aspergillus fumigatus*. Hussain et al. (2010) has found that the optimum pH was between 7-11 for production of serine protease by *Aspergillus terreus*. Alkaline protease from *Microbacterium luteolum* showed maximum activity at pH 7.5 with 71% residual activity atpH 10 and pH 10.5 (Malathu et al., 2008).The optimum activity of protease from *Aspergillus oryzae* was found to be at pH 8-9 (Samarntarn et al., 1999) Optimum alkaline protease activity from *Aspergillus versicolor* was observed at pH 9 and found to retain residual activity at pH 8 (Vaishali, 2012). *Pseudomonas aeruginosa* showed maximum protease activity at pH 9 (Sathees Kumar et al. 2013).

Maximum protease activity was observed at 50°C and decreased at higher temperature (60 °C).Similar observation was shown by Morimura et al. (1994) for *Aspergillus usami*. Hashimoto et al. (1972) found optimum temperature for acid protease production by *Penicillium duponti* to range between 45°C to 50°C.Optimum alkaline protease activity from *Aspergillus versicolor* was observed at 40°C (Vaishali, 2012).*Pseudomonas aeruginosa* showed maximum protease activity at 50 °C (Sathees kumar et al. 2013).

The *A. fumigatus* alkaline protease proved to have broad substrate specificity, could hydrolyse gelatin> casein > bovine albumine. Similar result was reported by Srividya Shiva Kumar (2012) asit was found that the protease enzyme from *Aspergillus sp*. showed high level of hydrolytic activity with various protein substrates including BSA, casein, egg albumin and gelatin with highest specificity for gelatin (130 %), these results are in agreement with the reports on fungi like *Rhizopus oligosporus* NRRL 2710, *Mucordisperses* NRRL 3103 and *A. elegans* NRRL 3104(Wang HL et al.1974)..The highest protease activity was observed towards casein as substrate *for Bacillus stearo thermophillus F1*by (Rahman et al., 1994), similar results were reported by (Olajuyigbe and Ehiosun 2013) for Bacillus *coagulans PSB- 07*.

The effect of different inhibitors and metals on the purified alkalo-thermophilic protease was studied and showed that the enzyme retained 94.2% activity when incubated in FeCl3(5mM) for 1 h, the enzyme was inhibited by metal chelator EDTA indicated that it is a metaloprotease . Zn^{+2}, Ca^{+2} inhibited the enzyme activity. Close results were observed by Madan et al. (2002) as A*. fumigates* activity enhanced when incubated $CaCl_2$, and was inhibited 60% by EDTA. Addition of 5mM $CaCl_2$ enhanced the activity 105.3% of alkaline protease enzyme produced by *Aspergillus niger* (Kalpana Devi et al.2008). Mg inhibite our enzyme activity with 29.2% but it was reported by Nehra et al. (2004) that Mg^{+2} was found to be an activator of the alkaline protease enzyme produced by *Aspergillus sp*.

The molecular weight of purified *A. fumigatus* was determined by SDS-PAGE to be 24KDa. Reichard et al. (1990) reported that the purified proteases from *A. fumigatus* were 32 KDa serine protease.124KDa serine protease was reported from *Aspergillus fumigatus* TKU0039 Lercher et al. 1992).

The molecular weight of purified enzyme produced by *Aspergillus niger* as determined by SDS-PAGE was found to be 38 kDa. (Kalpana Devi et al.2008), the same observation was reported by Ogundero and Osunlaja (1986) for *Aspergillus clavatus* .33 KDa serine protease from *Aspergillus fumigatus* CBS113.268 was reported by (Wang et al. 2005). Aspartic protease with 34KDa molecular weight from *Rhizopus oryzae* was observed by (Kumar et al. 2005). *Thermoascusaurantiacus* was found to produce protease with 24.5 KDa (Dini et al. 2009). *Aspergillus parasiticus* also produces alkaline protease with 23 KDa (Tunga et al. 2003).The purified protease enzyme produced by *Pseudomonas aeruginos* was recorded at a molecular weight of 60 kDa on SDS-PAGE (Sathees kumar et al. 2013).

Enzyme activity and stability in presence of some available commercial detergents was studied with a view to exploit the enzyme in detergent industry. Alkaline protease of *A. fumigatus* retained32.05% to 91.10% activity in presence of different detergents. The highest activity was retained in presence of Xtra and the least activity was observed in case of Tide. Madan et al. (2002) have founded that the compatibility of alkaline protease from *Bacillus polymyxa* retained 20-84.5% of its activity in various detergents. Adinarayana (2003) also reported 16, 11.4 and 6.6% activity in Revel, Ariel and Wheel respectively. Stability of protease for laundry detergents up to 80 % was also reported by Venugopal and Saramma (2006). Purified alkaline protease from *Pseudomonas aeruginosa* was retained 95% of activity in Tide and only 74% activity in Surf-Excel, in Ariel and Power only 15% of activity was reduced from the optimum level (Sathees Kumar et al. 2013)

In this study when purified protease was incubated with a piece of different types of clothes stained with blood it results in destaining for the blood within 30 minutes and it also give more preferable result when incorporated with the most compatible detergent (Xtra). Alkaline proteases have been incorporated in the detergents to hydrolyze and remove proteinaceous stains on clothes(Kobayashi et al., 1995; Ito et al., 1998).The efficiency of alkaline proteases in removing stains has been proved with different washing conditions and detergent compositions (Greene et al., 1996; Kalisz, 1998; Banerjee et al., 1999).The partially purified protease from *Nomuraearileyi* had high capability of removing the staining dye methylene blue and blood stain (Ramachandran and Arutselvi, IJPSR, 2013).Based on the results reported here, it is proposed that the alkaline protease produced by *Aspergillus fumigatus* isolated in the present study may have great commercial value in bio detergents technology.

In this study the alkaline protease enzyme produced by *A. fumigatus* show complete removal of hairs from goat skin without any additives after 24 hours. Complete removal of hairs has been achieved through protease without chemical assistance (Thangam et al., 2001; Dayanandan et al., 2003; Macedo et al., 2005). The alkaline protease produced by a Mutant of *Bacillus licheniformis* show a complete dehairing of skin with clean hair pore and clean grain structure after 12 h (Nadeem, et al. 2010). *Bacillus pumilus* strain can produce high levels of alkaline proteases that are able to efficiently remove hair from skin (H.Y. Wang et al. 2006).Alkaline protease isolated from a new strain *Alcaligenes faecalis* is suitable for unhairing (Berla Thangam et al., 2001). Enzyme preparation produced by a new strain of *Bacillus subtilis* show hide unhairing (Aline Dettmer et al. 2013). The leather processed by the use of protease from *Bacillus subtilis* is found to have maximum softness (Sathiya G 2013).

The enzymatic dehairing on industrial scale is generally accompanied by the use of small amount of lime to improve the dehairing efficacy and to reduce the cost of the dehairing process (Thanikaivelan et al., 2004).The appreciably high enzyme activity makes this isolate an industrially promising and of special interests for basic and applied research.

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