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NEW EGYPTIAN THERMOPHILIC BACTERIAL STRAINS AS PRODUCER OF THERMOSTABLE PROTEASES

Hassnaa E. EL-Eskafy, A.A. Elzanaty, Marwa S. Abdel-Hamid, R. N. Abbas and H. A. Hamza

Microbial Biotechnology Dept., Genetic Engineering and Biotechnology Institute, Sadat City University, Egypt

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ABSTRACT: *We used Three bacterial isolates RSW-8018, RSS-8028 and HFW-9081 isolated from different Egyptian regions which have the ability to grow up to 90°C and produce thermophilic proteases enzymes. We characterize those isolets Morphologically ,biochemically and molecularly . Morphological tests showed them as Gram positive, aerobes and endospore formers. Biochemical tests showed them to be positive for protease and catalase production. Molecular characterization was done by detection of nucleotide sequencing of 16S rRNA gene obtained data their identity to be Bacillus amyloliquefaciens, Bacillus cereus and Alcaligenes faecalis. We tested characteristics of protease enzyme to its response to heat ,PH ,presence of different metal ions and EDTA.*

Our data showed that this enzyme is isolated over a wide range of PH and temperature. Its activity was slight inhibited by Na+ and Cu+2 and but inhibited by EDTA, Mn+2 , Fe+3 and Ca+2.

This would facilitate its use in different biotechnological applications.

Key words: *Thermostable Protease; Thermophilic Bacteria; 16srRNA; Proteolytic activity.*

INTRODUCTION

Hyperthermophilic bacteria are Gram-positive rods with spore former, usually motile with peritrichious flagella, aerobic, catalase positive and grow above 60ºC Akel et al, (2009). Protease enzymes have been isolated and purified from various sources including, animals, plants, bacteria, fungi and viruses Patel *et al*., 2005. Microorganisms constitute the largest source of proteases including both intracellular and extracellular proteases Tari *et al*., (2006).

Traditional methods for microbial identification require the recognition of differences in growth; enzymatic activity, morphological and biochemical characteristics. Full and partial 16SrRNA gene sequencing methods have emerged as useful tool for identifying aberrant bacteria Petit and Vendeamin, (2005).

Thermostable protease enzymes are useful for numerous industrial processes by increasing the rate of reaction, giving a longer half-life to the enzyme and inhibiting microbial growth which decrease the possibility of microbial contamination Rao *et al*., (1998) .

Proteases can be classified as serine protease (EC3.4.21), cysteine (thiol) protease (EC3.4.22), aspartic protease (EC3.4.23) and metallo protease (EC3.4.24) constitute one of the most important groups of industrial enzymes Adinarayana *et al.,* (2003).

The application of proteases is highly exploited in food, leather, detergent and meat tenderization industries. Proteases are also important tools in studying the structure of protein and peptides. They can hydrolyze proteins to short peptides or free amino acids (Kannan , 2002).

Extracellular protease absorbs and utilizes hydrolytic products from proteinaceous substrates Gupta *et al*., 2002. Besides that they are also used in pharmaceutical; medical diagnostics; waste management, lens cleaning, decomposition of gelatin on X-ray; silver recovery .furthermore ,they are being used textiles as a non-hazardous and bio-alternative; easy control, speed and waste reduction, thus

being eco-friendly Khan 2013; Pawaw *et al.,* 2009 and Sathiya (2013). This investigation aims to isolate thermophilic bacteria from different regions in Egypt; identifing these isolates by studying their biochemical properties 16SrRNA sequencing and studying the stability of the thermostable protease under some environmental stresses.

MATERIALS AND METHODS

Microorganisms

Two samples of water and one sample of soil (Red sea and Hamam Freon regions in Egypt) were collected to isolate thermophilic bacteria which have the ability to produce thermostable protease enzymes. Nutrient agar medium Shiriling and Gottlieb (1966) was used for isolation and enrichment of the thermophilic bacteria.

Identification

Sterilized nutrient agar medium was used to isolate thermosatble isolates RSW-8018, RSS-8028 and HFW-9081 that used in this study, at pH 6.8 and incubated at 80°C and 90°C respectively for three days. Pure isolates were characterized using the criteria of Bergey's Manual of Systematic Bacteriology (Brenner *et al*., 2005). 16S rRNA genes of the new isolates were amplified using universal16S rRNA Primers Bact 27F(5"-AGAGTTTGATC(A/C)TGGCTCAG-3") and Bact1492R (5"-TACGG(C/T)TACCTTGTTACGACTT-3").
PCR amplifications were performed on Thermal Cycler / PCR Model: A100/A200 (Hangzhou Long Gene Scientific Instruments Co., Ltd) in a final volume of 50 µl, reaction mixture containing 0.5 µM of each primer, 10 mM of an equimolar dNTPs mix, 10X PCR buffer supplemented with 20 mM MgCl2, 1.25 U of Taq DNA polymerase and, as template. DNA sequences of 16S rDNA were done by Solgent Co., LTD, South Korea. Oligo (for primer design) was obtained from Gene Bank Database, (www.ncbi.nih.nlm.gov).

The analysis of the sequences and the phylogeny tree were done using Blast sites and phylogeny.fr site. (http://phylogeny.lirmm.fr/phylo_cgi/index.cgi).

Extraction of thermosatble proteases enzymes

The isolates were left to grow in a medium containing: yeast extract 0.5% (w/v), peptone 1.0% (w/v), glucose 0.5 g/l, Na2 HPO4 0.4g/l, Na2 CO3 0.085 g/l, ZnSO4 0.02g/l, CaCl2 0.02g/l, MgSO4 0.02g/l at 50 °C for 50h, centrifuged at 14000xg for 30 min at 4°C. Enzymes were assayed for proteolytic activity in triplicated as described by Huang *et al*., (2006) using casein (Hi media India) as a substrate.

Qualitative determination of proteases activity by casein digestion:

Strains were inoculated on casein agar medium containing casein 2.0%; peptone, 0.5% and agar 1.5% and then incubated at 37°C for 24 hours according to Qureshi *et al*., (2011).

Determination of total protein and specific activity

Bacterial total protein was determined by using diamond total protein kit using Bovine albumin serum was used to calculate specific and total protein activity according to method of Lowry *et al.* (1951).

Effect of purification methods on the stability of protease activity

Purification of the crude enzymes was done by two steps; First by using saturated ammonium sulfate solution (80%) was added the supernatant retained as the source of extracellular enzyme. After 20 min with stirring the precipitated was collected and the pellet suspended in minimum volume of 50 mM Tris-HCl buffered at pH 7.2 and dialyzed in the same buffer for 24h at 4°C. Second the dialyzed enzymes were passed through Sephadex G-100 column (Fluke, Switzerland; 1.5cm x 27cm) equilibrated with 50 mM Tris- HCl buffer ,pH 7.2. The flow rate was 0.5 ml min-1 and 3 ml fractions were collected for further studies Abeer *et al.,* (2014).

Effect of temperature

The thermosatble activity was determined by performing the standard assay procedure after incubating the enzymes at temperatures ranged from 45°C-95°C for 10 min. The residual activity was done at standard assay conditions Akel *et al*., (2009).

Effect of pH

The activity of the protease was measured at different pH values. The pH was adjust the following buffers 50mM sodium acetate (pH 3.8-4.8); 50mM sodium phosphate (pH 5.0-6.8); Tris-HCl (pH 7.2-9.0)and 50mM sodium carbonate (pH 9.2-10.8). The reaction was incubated at 65 °C for 10 min and the activity of the enzyme was measured according to Akel *et al.*, (2009).

Effect of metal ions

The effect of different metals ions on protease activity was determined by the addition of the corresponding ions at a concentration of 5 mM to the reaction mixture and the assay was performed under standard conditions. The tested ions were included NaCl, CaCl2, MgSO4, FeCl3, MnSO4 and CuSO4 according to Akel *et a*l., (2009).

Effect of different concentration of EDTA

Purified enzyme preparation was pre incubated in 50mM Tris-HCl buffer, pH 7.8 containing various EDTA concentrations (ethylene diamine tetra acetic acid) ranged from (0 to 15 mM) in the assay crude and pure enzyme activity was measured at 65°C, Akel *et a*l., (2009).

RESULTS AND DISCUSSION

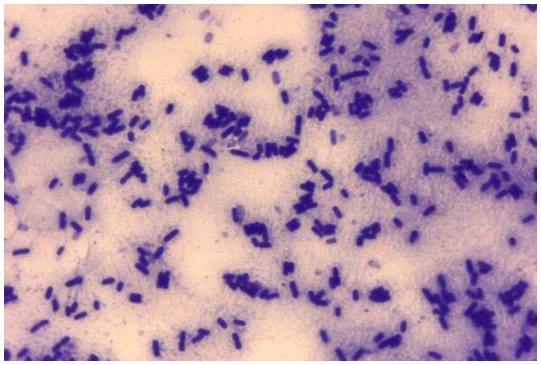
Three isolates RSW-8018, RSS-8028 and HFW-9081 were isolated. Proteolytic activity results in Table (1) presented that isolate RSS-8028 gave higher enzyme activity (445.4U/ml) than both isolates RSW-8018 and HF-9081 which gave 363.4U/ml and 236.4U/ml respectively. The assay showed hydrolysis of the substrates as indicated by no precipitate forming. There was hydrolysis of the casein protease substrates to produce tyrosine.

Morphological and physiological characteristics

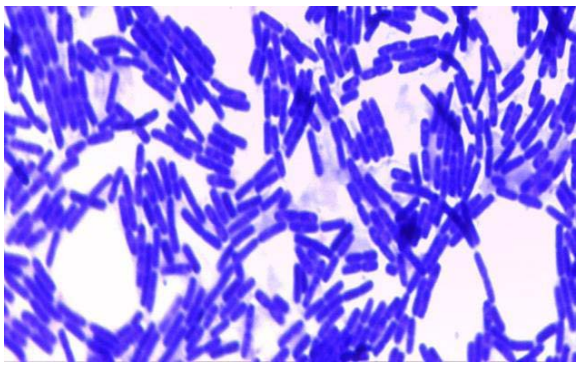
Identification of the three thermophilic isolates RSW -8018, RSS -8028 and HFW -9081promising for thermostable proteases production was carried out based on cell morphology, endospores formation, motility, growth characteristics and several biochemical tests. Cultural characterizations of isolates which showed single white or creamy colony, they are characteristically large and vary in shape from circular to irregular, with entire to undulate, crenate or fimbriate edges; they have mat or granular texture sometimes smooth and moist colonies Fig. (1). Cells showed different shapes rod-shape cells , long chain , endospore-forming chain, ellipsoidal, central and subterminal spores not swelling the sporangia Fig (1a,b) with isolates RRS-8028 and RSW-8018 while isolate HFW-9081 the spores were terminal , subterminal spores swelling sporangia Fig (1c). Morphological and biochemical characteristics were carried out according to Bergey's manual of systematic bacteriology (Brenner *et al*., 2005) and are summarized in Table (2). Bacteria under investigation grow aerobically at 37°C and can tolerate to grow at 80-90°C.

Table (1): Activity of isolated thermophilic bacteria.

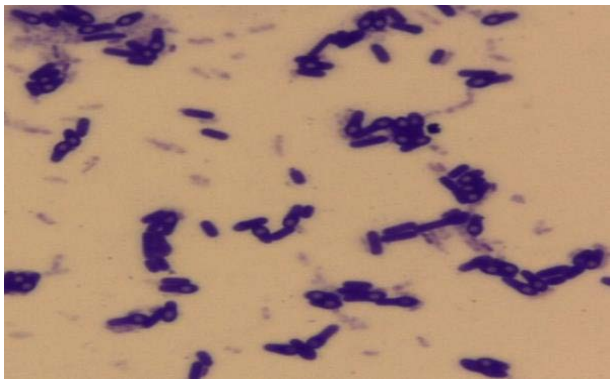
No.	Isolate Code	Source	Activity at 660 (U/ml)
1	RSW -8018	Red sea water	363.4
2	RSS -8028	Red sea soil	445.4
3	HFW -9081	Hamam fareon	236.4



(B) RSW-8018



(A)RSS-8028



(C) HFW-9081

Fig (1): Cultural characterization of the (a, b, c) Isolated thermophilic bacteria staining by Gram stain.

Table (2): Morphological and biochemical characteristics.

Isolate code Characterization	RSW-8018	RSS-8028	HFW-9081
Cell morphology		*Bacilli* rod-shape long or short	
Gram stain	+	+	+
Pigmented colonies		white to creamy	
Motility	+	+	+
Aerobic growth	+	+	+
Anaerobic growth	-	-	-
Catalase test	+	+	+
Acid from D-Glucose	+	+	+
Hydrolysis of casein	+	+	+
Hydrolysis of starch	+	-	-
Salinity tolerance:			
NaCl 5%	+	+	+
NaCl 10%	+	+	+
NaCl 20%	+	-	-
Growth at pH 5	+	+	-
Growth at pH 7	+	+	+
Growth at pH 9	+	+	+
Spore forming	+ not swelling the sporangia	+ not swelling the sporangia	+ with swelling the sporangia

Genetic identification by 16S rRNA Identification of isolates by 16srRNA sequencing

Amplified fragments were sequenced and total of about 1.3 kb (nucleotide) sequences were determined. BLAST program was used for the analysis of the sequence and the homology search showed that the isolates HFW-9081, RSW-8018 and RSS-8028 are relatively close to the bacterial strains *A. faecalis, B amyloliquefaciens* and *B. cereus* respectively as shown in Table (3). The biochemical and molecular identification results of the three Egyptian bacterial isolates showed that they are 98% similarity ratios with the *B. amyloliquefaciens* and *B. cereus* and 99% with *A. faecalis* which was confirmed by the genetic tree in Figure (3).

Determination of protease activity

The new three promising bacterial strains that producing thermostable protease were subjected to a screening program in order to evaluate their proteolytic activities by measuring the hydrolysis of casein. The capability of hydrolysis and digestion of casein agar by forming clear zone were done by spotting with 10 µl culture and incubation for 24 hours at 30◦c. Results in Table (4) showed that the widest zone diameter (3.5, 3.5 and 2.5 cm) were obtained for RSW-2018, RSS- 8028 and HFW- 9081therfor they were able to produce protease enzyme.

Effect of different methods of purification on the stability of protease activity

Purification steps were done by two methods and indicated in Tables (5, 6 and 7). It appears from the obtained data that all the purified enzymes are predominantly endopeptidases as they hydrolyzed larger proteins such as casein and bovine serum albumin. Ammonium sulphate and Sephadex methods were enhanced the yield activity % of strain HFW-9081 (125 and121%) with specific activity 458.9 and 590 in comparison with cell free supernatant (100%) and 328.3. strains RSS-8028 showed that yield activity was partially affected by using Ammonium sulphate and Sephadex which gave 87.6 and 73.2% respectively mean while specific activity was higher (325.3 and 362.6) than crude enzyme (296.9). Yield activity of RSW-8018 was 70.5 and 42.2% while specific activity was 189.9 and 191.7 with Ammonium sulphate and Sephadex respectively.

Effect of temperature on stability of protease activity

Results demonstrated that the optimum temperature for protease activity was 65 ºC where it reached up to 100 % of relative activity with all strains. Exhibited maximum activities at 65°C were gradually decreased up to 95°. The results showed that proteases have good stability between 45 and 65°C. Protease still had about (66 %) activity produced by RSS-8028 at 75°C While 60 and 50 % with strains RSS-8018 and HF-9081. So the protease can be classified as thermophilic. Data showed that the protease activity was affected markedly by the temperature and the enzyme activity decreased and reached 49, 46 and 26% at 95 °C for the three tested strains (Figure 4).

Table (3): Gene sequencing (16S) rRNA of the three Egyptian bacterial isolate.

Samples	Number of bases with primers		Closely related species accessed from Gene Bank			
	27F	1492R	Identification	Strain no.	Accession no.	Similarity
RSW-8018	1243	1228	*Bacillus amyloliquefaciens*	ML361	KC692163	98%
RSS-8028	1250	1177	*Bacillus cereus*	JY9	HQ833026	98%
HFW-9081	1258	1248	*faecalis Alcaligenes*	VIT-RAS	KJ437487	99%

Sample A F

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   1 nnnaanggct aggtgctata atgcagtcga gcggacagat gggagcttgc tccctgatgt
  61 tagcggcgga cgggtgagta acacgtgggt aacctgcctg taagactggg ataactccgg
 121 gaaaccgggg ctaataccgg atggttgttt gaaccgcatg gttcagacat aaaaggtggc
 181 ttcggctacc acttacagat ggacccgcgg cgcattagct agttggtggg gtaacggctc
 241 accaaggcga cgatgcgtag ccgacctgag agggtgatcg gccacactgg gactgagaca
 301 cggcccagac tcctacggga ggcagcagta gggaatcttc cgcaatggac gaaagtctga
 361 cggagcaacg ccgcgtgagt gatgaaggtt ttcggatcgt aaagctctgt tgttagggaa
 421 gaacaagtgc cgttcaaata gggcggcacc ttgacggtac ctaaccagaa agccacggct
 481 aactacgtgc cagcagccgc ggtaatacgt aggtggcaag cgttgtccgg aattattggg
 541 cgtaaagggc tcgcaggcgg tttcttaagt ctgatgtgaa agccccggc tcaaccgggg
 601 agggtcattg gaaactgggg aacttgagtg cagaagagga gagtggaatt ccacgtgtag
 661 cggtgaaatg cgtagagatg tggaggaaca ccagtggcga aggcgactct ctggtctgta
 721 actgacgctg aggagcgaaa gcgtggggag cgaacaggat tagataccct ggtagtccac
 781 gccgtaaacg atgagtgcta agtgttaggg ggtttccgcc ccttagtgct gcagctaacg
 841 cattaagcac tccgcctggg gagtacggtc gcaagactga aactcaaagg aattgacggg
 901 ggcccgcaca agcggtggag catgtggttt aattcgaagc aacgcgaaga accttaccag
 961 gtcttgacat cctctgacaa tcctagagat aggacgtccc cttcgggggc agagtgacag
1021 gtggtgcatg gttgtcgtca gctcgtgtcg tgagatgttg ggttaagtcc ccgcacgagc
1081 gcaccnttga tcttagttgc cagcattcag ttggnacctc taaggtgact gcgnnacaac
1141 cggaganggt ggggatgacg tcancatcat gccccttatg accttgnnnt accacgnnnn
1201 tacantnnnn agaacaatgg nngcnnnaac nngcgcngag ngg
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Sample B F

```
1       gnnnggcgcg cgtgctatac atgcaagtcg agcgaatgga ttaagagctt gctcttatga
 61     agttagcggc ggacgggtga gtaacacgtg ggtaacctgc ccataagact gggataactc
121     cgggaaaccg gggctaatac cggataacat tttgaaccgc atggttcgaa attgaaaggc
181     ggcttcggct gtcacttatg gatggacccg cgtcgcatta gctagttggt gaggtaacgg
241     ctcaccaagg caacgatgcg tagccgacct gagagggtga tcggccacac tgggactgag
301     acacggccca gactcctacg ggaggcagca gtagggaatc ttccgcaatg gacgaaagtc
361     tgacggagca acgccgcgtg agtgatgaag gctttcgggt cgtaaaactc tgttgttagg
421     gaagaacaag tgctagttga ataagctggc accttgacgg tacctaacca gaaagccacg
481     gctaactacg tgccagcagc cgcggtaata cgtaggtggc aagcgttatc cggaattatt
541     gggcgtaaag cgcgcgcagg tggtttctta agtctgatgt gaaagcccac ggctcaaccg
601     tggagggtca ttggaaactg ggagacttga gtgcagaaga ggaaagtgga attccatgtg
661     tagcggtgaa atgcgtagag atatggagga acaccagtgg cgaaggcgac tttctggtct
721     gtaactgaca ctgaggcgcg aaagcgtggg gagcaaacag gattagatac cctggtagtc
781     cacgccgtaa acgatgagtg ctaagtgtta gaggguttcc gcccttagt gctgaagtta
841     acgcattaag cactccgcct ggggagtacg gccgcaaggc tgaaactcaa aggaattgac
901     gggggcccgc acaagcggtg gagcatgtgg tttaattcga agcaacgcga agaaccttac
961     caggtcttga catcctctga caaccctaga gatagggctt ctgcttcggg agcagagtga
1021    caggtggtgc atgggttgtc gtcagctcgt gtcgtgagat gttgggttaa gtcccgcacg
1081    agcgcaaccc ttgatcttag ttgccatcaa tanttggcnn tctagggtgac tgccggtnnn
1141    agcngagagn ggggatgacg gtcagatcat catgccctat gactgngcta acacntgctt
1201    acangnnngg tcaagagctg cagannnnag gnnnnnnann tctcnnannn
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Sample C F

```
  1     gggcnggcgg cagctttaac atgcaagtcg aacggcagcg cgagagagct tgctctcttg
 61     gcggcgagtg gcggacgggt gagtaatata tcggaacgtg cccagtagcg gggataact
121     actcgaaaga gtggctaata ccgcatacgc cctacggggg aaaggggggg atcgcaagac
181     ctctcactat tggagcggcc gatatcggat tagctagttg gtggggtaaa ggctcaccaa
241     ggcaacgatc cgtagctggt ttgagaggac gaccagccac actgggactg agacacggcc
301     cagactccta cgggaggcag cagtggggaa ttttggacaa tgggggaaac cctgatccag
361     ccatcccgcg tgtatgatga aggccttcgg gttgtaaagt acttttggca gagaagaaaa
421     ggtatccgct aatacgggat actgctgacg gtatctgcag aataagcacc ggctaactac
481     gtgccagcag ccgcggtaat acgtagggtg caagcgttaa tcggaattac tgggcgtaaa
541     gcgtgtgtag gcggttcgga aagaaagatg tgaaatcccg gggctcaacc ttggaactgc
601     atttttaact gccgagctag agtatgtcag aggggggtag aattccacgt gtagcagtga
661     aatgcgtaga tatgtggagg aataccgatg gcgaaggcag ccccctgggg taatactgac
721     gctcagacac gaaagcgtgg ggagcaaaca ggattagata ccctggtagt ccacgccgta
781     aacgatgtca actagctgtt ggggccgtta ggccttagta gcgcagctaa cgcgtgaagt
841     tgaccgcctg gggagtacgg tcgcaagatt aaaactcaaa ggaattgacg gggacccgca
901     caagcggtgg atgatgtgga ttaattcgat gcaacgcgaa aaccttacct acccttgaca
961     tgtctggaaa gccgaagaga tttggccgtg ctcgcaagag aaccggaaca cagtgctgca
1021    tggctgtcgt cagctcgtgt cgtgagatgt tgggttaagt cccgcaacga gcgcaaccct
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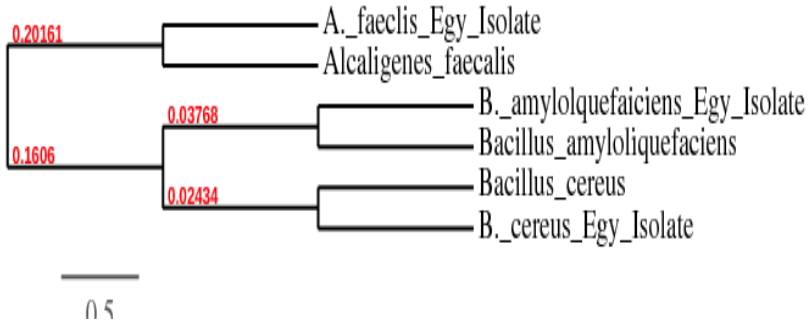

Fig (3): Phylogenetic tree of the three Egyptian bacterial isolates and the most nearest relative standerd strains.

Table (4): Protease activity as reflected by Clear zone diameter.

Isolates code	Crude protease activity U/ml	Clear zone diameter (cm)
RSW- 8018 *Bacillus amyloliquefaciens*	363.4	3.5
RSS - 8028 *Bacillus cereus*	445.4	3.5
HF - 9081 *Alcaligenes faecalis*	236.4	2.5

Table (5): Purification of proteases produced by RSW-8018 *Bacillus cereus.*

Purification Steps	**Total Protein**	**Enzyme activity (U ml-1)**	**Total enzyme activity (U)**	**Specific activity**	**Purification** fold	**Yield (%)**
Cell free supernatant	2.0	1.011	363.4	181.7	1	100
Ammonium sulfate (80%)	1.35	0.904	256.4	189.9	1.04	70.5
Sephadex G-100	0.8	0.801	153.4	191.7	1.1	42.2

Table (6): Purification of proteases produced by *Bacillus amyloliquefaciens* .

Purification Steps	**Total Protein**	**Enzyme activity (U ml-1)**	**Total enzyme activity (U)**	**Specific activity**	**Purification** fold	**Yield (%)**
Cell free supernatant	1.5	1.093	445.4	296.9	1	100
Ammonium sulfate (80%)	1.2	1.038	390.4	325.3	1.09	87.6
Sephadex G-100	0.9	0.975	326.4	362.6	1.2	73.2

Table (7): Purification of proteases produced by *Alcaligenes faecalis.*

Purification Steps	**Total Protein**	**Enzyme activity (U ml-1)**	**Total enzyme activity (U)**	**Specific activity**	**Purification** fold	**Yield (%)**
Cell free supernatant	0.72	0.884	236.4	328.3	1	100
Ammonium sulfate (80%)	0.624	0.934	286.4	458.9	1.3	121
Sephadex G-100	0.504	0.945	297.4	590	1.7	125

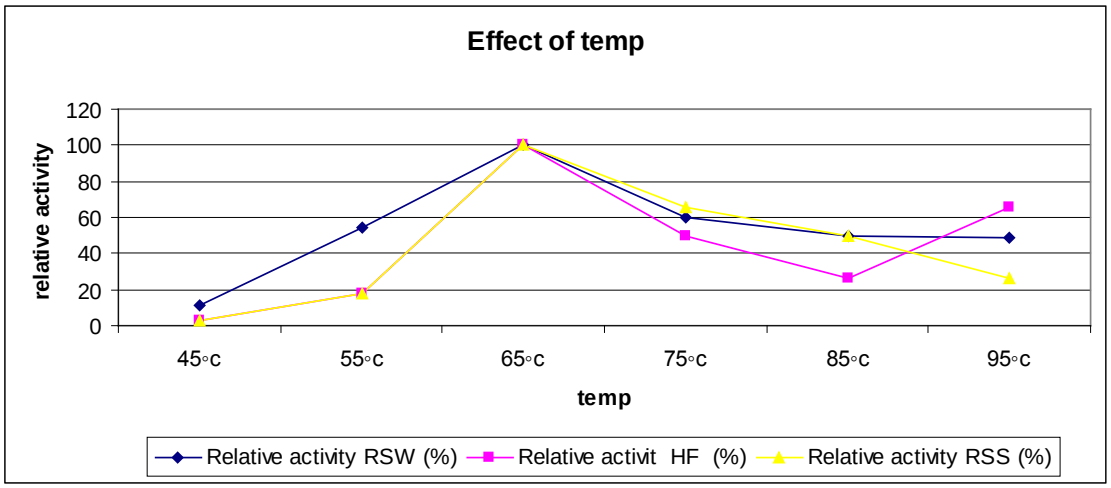

Fig (4): Effect of temperature on activity of protease activity.

Effect of pH-values on activity of protease enzyme

The effect of pH on protease activity and stability for the three studied strains was measured at different pH values 3.5, 5, 6, 7, 8 and 9. Maximum enzyme activity was observed at optimum pH 7 for all strains. The majority of microorganisms producing alkaline proteases show optimum growth and enzyme production under alkaline conditions (Tsujibo *et al*.,1990; Dunaevsky *et al*., 1996). The enzyme activity was greatly affected by the hydrogen ion concentration as mentioned in Figure (5) which showed that the protease remained active between pH 6 and 7 with maximum activity at pH 7 (100%). The activity of the protease began to decrease sharply at pH values between pH 8 (35, 30 and 3 %) and pH 9 then reached to zero level with strain RSS-8028. Similar results were obtained for the optimum pH for proteases enzymatic activity of other *Bacillus* species: pH 7.5 for *Bacillus subtilis* ITBCCB 148 (Akel *et al*.,2009), *Bacillus* sp. HS08 (Huang *et al*., 2006) and *Bacillus* sp. S17110 pH 8.0 for *Bacillus cereus* KCTC 3674 (Kim *et al*., 2001), Thermophilic *Bacillus* SMIA2 (Nascimento andbMartins, 2004) and *Bacillus cereus* BG1 (Ghorbel-Frikha *et al*., 2005). Results presented in this work indicated that extracellular proteases produced by *Bacillus amyloliquefaciens, Bacillus cereus and Alcaligenes faecalis* can be separated into neutral protease.

Effect of various metal ions on activity of protease enzyme

Results in Fig (6) showed that generally all ions tested decreased the activity than control values. The most affected proteases activity was with the strain RSS-8028 in presence of Mn and Fe and RSw-8018 in presence of Na and Cu. Variations of protease activity is very clear between same types of ions and between different ions for the same strain. Nascimento and Martins (2004) reported that protease produced by *Bacillus* spp. was enhanced by metal ions protected the enzyme from thermal denaturation and maintained its active conformation at the high temperature.

Effect of different concentrations of EDTA on activity of protease enzyme

Results presented in Fig (7) show that the chelating agent as EDTA affect the purified protease activity. The relative activity was around to 100% for (0) mM EDTA with all strains and total protease activity was retained up to 98.5% in the presence of 5mM of EDTA with strain HF-9081. Our results indicated that the presence of EDTA had inhibitory effect on protease activity (Fig. 7). But the enzyme activity retained 50% activity in the presence

of 5 mM EDTA, with strain HFW-9081 indicating no requirement for metal cofactor. The stability of the enzyme in presence of EDTA is advantageous for use of enzyme as detergent additive. This may be due to the detergents that contain high amount of chelating agents which function as water softeners and also was useed in stain removal. These agents specifically bind to and chelate metal ions making them unavailable in the detergent solution as (Akel *et al,* 2009) mentioned.

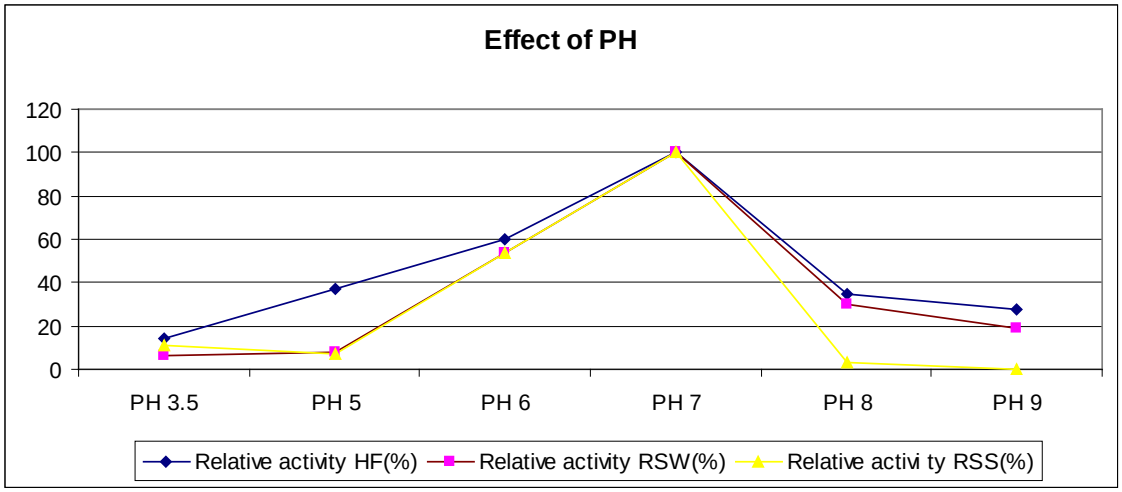

Fig (5) Effect of pH-values on activity of protease.

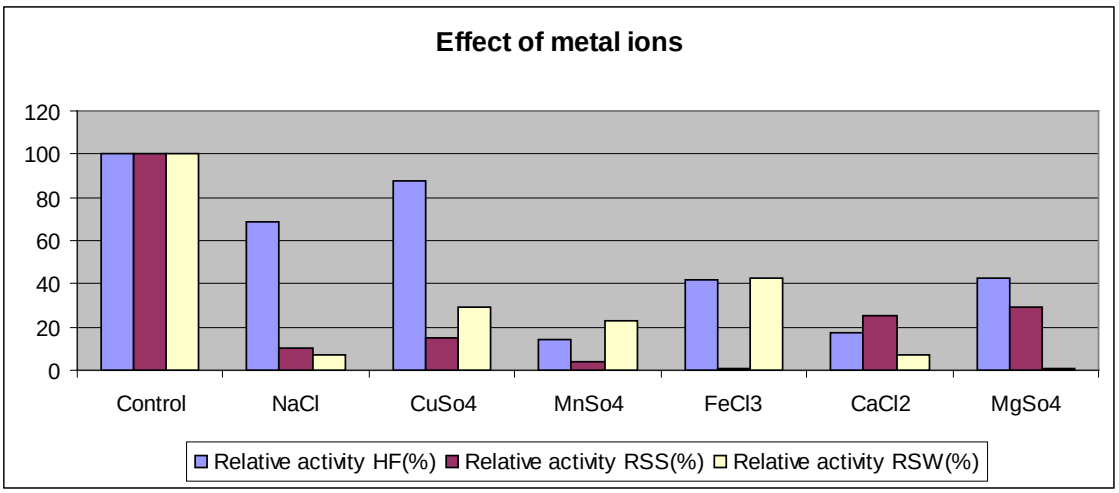

Fig (6): Effect of various metal ions on activity of protease.

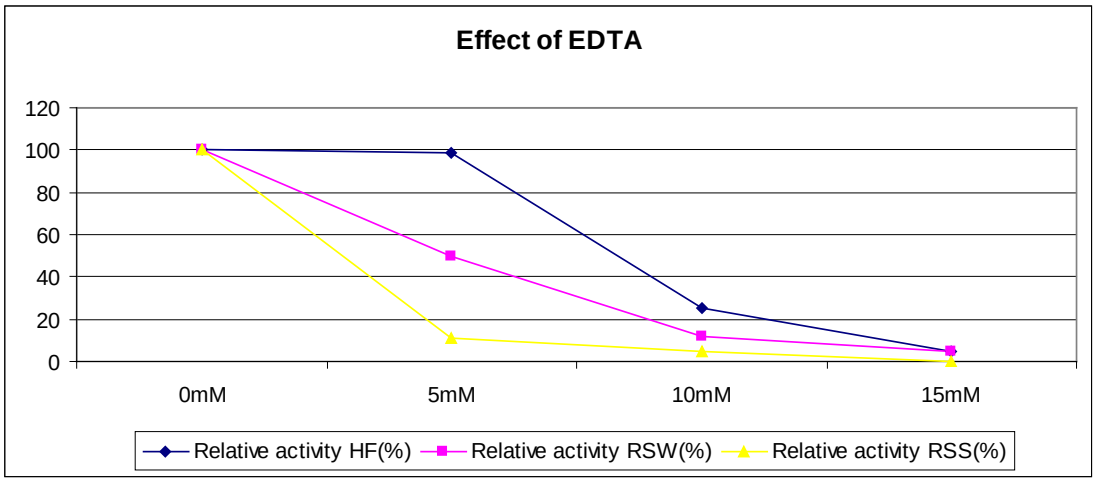

Fig (7): Effect of different concentrations of EDTA on protease activity.

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سلالات بكتيرية مصرية جديدة محبة للحرارة كمنتجة لانزيم البروتياز متحمل الحرارة

حسناء الاسكافى ، عابدين الزناتى ، مروا صلاح ، راتب عباس ، حنفى حمزه

قسم البيوتكنولوجيا الميكروبية بمعهد الهندسة الوراثية والتكنولوجيا الحيوية جامعة مدينة السادات –مصر

الملخص العربى

تم استخدام ثلاث عزلات ميكروبية من مناطق مختلفة فى مصر لها القدرة على النمو فى درجات الحرارة المرتفعة التى تصل الى ٩٠ درجة مئويه ولها القدرة على انتاج البروتيز

تم توصيف السلالات مورفولوجيا وجزيئيا وكيموحيويا وأظهر التوصيف المورفولوجى أن العزلات موجبة لصبغة الجرام–هوائية–لها قدرة على تكوين جراثيم داخلية وموجبة لإنزيمى البروتيز واللاكتيز .تم توصيف الجزئ بتحديد تواليات القواعد الازوديه لجين 16S حمض الريبوسوم النووى rRNA هذه المواصفات أثبتت أن العزلات تنتمى إلى جنس الباسلس .

تم دراسه عده عوامل مؤثرة على نشاط إنزيم البروتيز الناتج من هذه العزلات مثل درجة الحرارة ، درجة الحموضة، أيونات المعادن الكالسيوم – صوديوم– نحاس – منجنيز حديد وكذلك الايديتا . تم التوصل إلى أن الإنزيم الخارجى يصل إلى أعلى قيمة بعد ٤٨ ساعة من نمو العزلات على درجة حرارة ٦٥ درجة مئوية ودرجة حموضة ٧ بينما ثبط نشاط الإنزيم إلى حد ما فى وجود أيونات الصوديوم والنحاس والمنجنيز والايديتا.

وعليه يمكن استخدام الإنزيم فى التطبيقات الحيوية.