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Microbial Production of Thermoalkaliphilic Enzymes for Application in Biodetergent Technology

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Abstract: Detergents are an undetectable source of pollution, which hidden in most of our daily activities. Detergents could cause harmful effects before they are completely degraded. It is wise to reduce the amount of detergents that usually used by invention new bio-friendly formula contains efficient enzymes such as protease. Screening studies were carried out for one hundred and fifty bacterial isolates with respect to their ability to produce protease(s), after growing on slaughter house wastes (SHW) isolated from El-Khorma governorate, Taif, King Saudia Arabia (KSA) at 55°C and pH 9. The most potent thermophilic bacterial isolate concerning of alkaline thermostable protease(s) production was identified as *Bacillus licheniformis* EGT50. Alkaline thermostable proteases productivity by the most potent bacterial isolate was affected by substrate concentrations (solid substrate), carbon source, nitrogen source, amino acid supplements, incubation temperature, incubation period and inoculum size. Maximum both enzymes production by *B. licheniformis* EGT50 was obtained on SHW concentrations, 7.5 %;galactose; diammonium hydrogen phosphate; arginine at 55°C for 72 h. when inoculated by 0.5 ml. The protease production under all optimal conditions was increased many folds from 563.68 to 17825 U/ml (31 fold). The purification fold of *B. licheniformis* EGT50 alkaline thermostable protease increased to 394.7 after applying Sephadex G200 column chromatography techniques. The enzyme productivity of protease has been determined and the result proved the possibility to use the crude and purified enzymes in biodetergent technology.

Key words: Thermostable · Alkaliphilic · Protease · Biodetergent · *Bacillus licheniformis*

INTRODUCTION

Enzymes play a significant role in our life. Their existence had been associated with the history of ancient civilizations. Enzymes from plant and microorganisms had been used in brewing, baking, alcohol production, cheese, vinegar making….. etc. The uses of enzymes were variable ranging from just making wine or bread to producing complicated fermentations processes. Technical enzymes represent 1 billion USD in 1999 [1-3]. Part of these enzymes is the thermostable enzymes, which are better, suited for harsh industrial processes and constitute more than 65% of the global market [4-11, 3].

Protease constitutes one of the most important groups of industrial enzymes, accounting for about 60% of the total enzyme market [12]. Protease are of commercial value and find multiple applications in various industrial sectors. Proteases are widely used in detergen, food and leather tanning industries [13]. Alkaliphilic *Bacillus* sp. are considerable as prolific producers of alkaline proteases, which exhibit significant activity and stability at high pH and temperatures [14-17, 2]. Enzymes have many applications especially in paper industry, detergents, drugs, degradation of different wastes, textile, food, pharmaceutical, leather, degumming of silk goods, manufacture of liquid glue, cosmetics, meat tenderization, cheese production, growth promoters …etc [1, 18, 3]. Meanwhile one of the most important and profitable applications for enzymes is in detergents, where the (Novozymes data) [19]. The first use of enzymes in detergents occurred in 1913 when Röhm and Haas introduced crude trypsin into their detergent. Burnus based on a German patent issued to Otto Röhm (1913) [1]. To provide desirable benefits, enzymes must

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be stable and function well in the presence of a variety of potentially unfriendly detergent ingredients (e.g., anionic/ nonionic/cationic surfactants, chelants, builders, polymers, bleaches) and in various forms of detergent products (i.e., liquids and powders) [1, 8, 9, 10].

In present study, isolation, purification and identification of thermophilic bacterial isolates from El-khorma governorate, king Saudi Arabia; production of an alkaline thermostable protease from *B. licheniformis* EGT50 and optimization of the alkaline thermostable protease production parameters for potential use as a detergent industry.

MATERIALS AND METHODS

Microorganisms: An alkaliphilic *B. licheniformis* EGT50 strain, which produce were originally isolated from different desert soil samples collected from different localities of El-khorma governorate,Taif, king Saudi Arabia. Both strains found to be capable of producing extracellular alkaline protease were identified and the criteria laid down in Bergey's Manual of Systematic Bacteriology [20] and Schallmey *et al.* [21].

Construction of Standard Enzyme and Protein Curves: A stock solution of (50,000 μg/ml) purified protease enzyme supplied by Sigma chemicals Co. was prepared in Tris-HCl buffer (0.2M) at pH 9, where descending dilutions were prepared. After preparing the required dilutions for protease, only 0.1 ml of each dilution was transferred to each well in the gelatin-substrate medium using gelatin clearing zone (GCZ) technique. The obtained standard curve was used for estimating the enzymes activities in terms of μg/ml and then translated into units (U). One unit is defined as the amount of enzyme protein (mg) required to exert one unit of clearing zone (mm) in one unit time under all the specified conditions of enzyme assay (clearing zone technique). The total protein determination was made according to the method of Lowry *et al.* [22] using bovine serum albumin as a standard protein.

Production Medium: Alkaline thermostable protease production was determined by applying a modified basal medium given by Vincent [23], containing of the following ingredients (g/l): SHW, 10; NaCl, 6; $(NH_4)_2SO_4$, 1; yeast extract, 1; KH_2PO_4, 0.5; $MgSO_47H_2O$, 0.1; $CaCl_2$ $6H_2O$, 0.1; $FeSO_47H_2O$, all ingredients were dissolved in distilled water and completed up to one liter. The initial pH of the culture medium was adjusted at 9.

Protease(s)Assay Medium for (Gelatin Clearing Zone (GCZ) Technique: This medium was devoted to gelatin clearing zone (GCZ) technique according to Ammar *et al.* [24]. The assay plates contained 1% gelatin and 1.5% agar for solidification, to be dissolved in 100 ml of Tris-HCl buffer (pH 9). At the end of incubation period, protease (s) activity was detected by flooding each plate with 10 ml freshly prepared acid mercuric chloride solution [25]. Mean diameters of clearing zones were measured and taken as indication for proteolytic activities. The standard errors of mean values were less than 3 %.

Solid State Fermentation (SSF): Five grams of SHW were introduced moistened with 5 ml of production medium in 250 ml Erlenmeyer flask and autoclaved at 15 psi for 20 min, was taken as the basal medium for SSF studies was adjusted at pH of 9.0. Medium was left to cooled, inoculated with 0.5 ml of the inoculum (A 600;0.8), having 1.5 x 10^6 cells /ml in case of *B. licheniformis* EGT50 from 12 h. old shake culture and incubated at 65°C. Visual observations regarding growth were made each day and the SHW was mixed with 10 ml of tap water and filtered through a metallic sieve. The extracted filtrate was centrifuged (10.000 xg; 4°C) for 15 min and cell free filtrate was used as the source of crude alkaline thermostable protease.

Optimization of Fermentation Conditions for Alkaline Thermostable Protease Production: Protease production was optimized under SSF on SHW, unless otherwise stated, by altering various physicochemical conditions. Effect of different temperatures, pH values, different substrate concentrations, various supplements on the alkaline thermostable protease production by adding different carbon sources (1% w/w), nitrogen sources, inoculum sizes and incubation periods were carried out by allowing the *B. licheniformis* EGT 50 strain to grow on SHW and incubated at different incubation periods at 55°C at pH 9.

Protease Production by *B. licheniformis* EGT50: *B. licheniformis* EGT50 was allowed to grow under the optimal static natural substrate under solid state fermentation conditions on slaughter house wastes for protease(s) production. The optimum protease(s) production medium contained (g/l,w/v), 10g of slaughter houses wastes per flask of 1000 ml capacity were used and supplemented by 20 ml of production medium, which contained (g/l, w/v): NaCl, 6; KH_2PO_4, 0.5; $MgSO_47H_2O$, 0.1; $CaCl_26H_2O$, 0.1; yeast extract,

1 in addition to galactose, ammonium dihydrogen phosphate and arginine, pH was adjusted at 9 and inoculated with bacterial suspension and incubated at 55°C for 72 h.

Enzyme Purification: To the cell free supernatant, ammonium sulphate was added up to 80 % saturation and centrifuged (15.000 rpm for 15 min) after 2 h of incubation at 4°C. The precipitate was dissolved in a minimum amount of Tris-HCl buffer (0.2M) at pH 9, dialyzed overnight against the same buffer and retained for further purification steps by sephadex G200. After dialysis, the supernatant, containing enzyme protein, was applied to a sephadex G200 (particle size 200M) column (50 cm x 2.5 cm) pre-equilibrated with Tris-HCl buffer (0.2M) at pH 9. Fractions (5 ml each) were collected at the flow rate of 20 ml /h and assessed for enzyme activity.

Data Analysis: All data used for this experimentation is obtained from triplicate experiments.

RESULTS AND DISCUSSION

Enzymes have long been of interest to the detergent industry for their ability to aid in the removal of proteinaceous stains and to deliver unique benefits that cannot otherwise be obtained with conventional detergent technologies. Due to potential usefulness of alkaline thermostable protease in bio-detergent industry, the development of methods for cheaper production of enzyme is very important. SSF holds tremendous potential for the production of enzymes and it can be used of special interest in these processes where the crude fermented product may be used directly as enzyme source [26-28, 3, 11].

Out of one hundred and fifty thermophilic bacterial isolates was found that only 20 isolates gave higher protease productivity. Data recorded in Table (1) showed the ability of twenty bacterial isolates selected from the qualitative screening to attack SHW for protease(s) production. It was found that bacterial isolates number EGT50, EGT95, EGT106 and EGT147 gave the highest proteolytic productivities where it reached up to 28,23.16,22.5 and 22.8 mm respectively. From the previous results, bacterial isolate viz. EGT50 was selected as the most potent bacterial isolates for their potentiality to highest produce alkaline thermostable protease.

Optimization of Solid State Fermentation (SSF): There are several factors, affecting SSF processes for protease production. Among these, selection of a suitable strain and selection of process parameters are crucial [29]. While efforts largely continued to exploit filamentous fungi and yeast for the production of various products, attempts have also been made to explore the possibilities of using bacterial strains in SSF systems [30]. The two most potent producers bacterial strain viz. *B. licheniformis* EGT50 for concerning alkaline thermostable protease, used in present manuscript, were capable of growing at 55°C and pH 9.

From industrial point of view, in order to production of low cost of enzymes, these bacterial isolates under study were allowed to grown on natural substances such as SHW under SSF conditions, However, the selection of the previously mentioned substrate for the process of enzymes biosynthesis was based on the following factors viz (i) they represent the most cheapest agro-industrial wastes in Egypt; (ii) they are available at any time of the year; (iii) Their storage represents no problem in comparison with other substrates and (iv) they resist any drastic effect due to the exposure to other

Table 1: Screening program of protease production by the most potent thermophilic bacterial isolates by growing on SHW at 55°C for 48 h. using GCZ technique

No.	Code number	Proteases(s) production GCZ technique (mm)	No.	Code number	Protease(s) production GCZ technique (mm).
1	EGT15	20.0 ± 0.13	11	EGT 78	21.8 ± 0.0
2	EGT 38	18.5 ± 0.0	12	EGT 80	18.0 ± 0.47
3	EGT 42	19.5 ± 0.0	13	EGT 89	20.6 ± 0.0
4	EGT 47	22.6 ± 0.0	14	EGT 90	18.16 ± 0.0
5	EGT 50	28.0 ± 0.0	15	EGT 95	22.16 ± 0.52
6	EGT 60	20.0 ± 0.1	16	EGT 106	20.5 ± 0.49
7	EGT 64	20.0 ± 0.0	17	EGT 108	17.5 ± 0.0
8	EGT 70	19.16 ± 0.0	18	EGT 110	19.0 ± 0.0
9	EGT 71	20.0 ± 0.0	19	EGT 141	20.3 ± 0.58
10	EGT 72	18.6 ± 0.0	20	EGT 147	20.8 ± 0.0

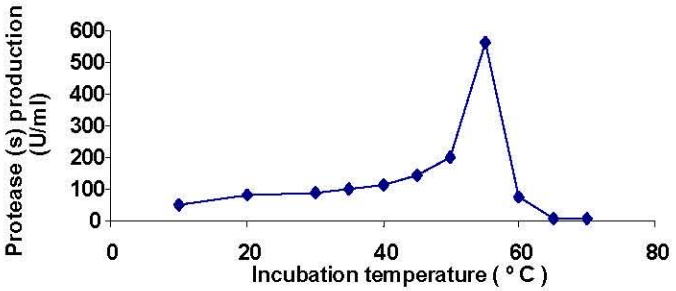

Fig. 1: Effect of different incubation temperatures on protease(s) productivity by *B. licheniformis* EGT50.

environmental conditions e.g temperature, variation in the weather from season to season and or from day to night. Interestingly slaughter house wastes are an important pollutant factor for the environment, many pathogenic microorganisms can grow on it, this may cause many diseases for man and animals, thus its use for enzymes production help in prevention disease distribution. Therefore, the purpose of the present work is to determine the best factors controlling the enzyme(s) productivities by *B. licheniformis* EGT50. On the other hand, the economic feasibility of the microbial enzymes production for its application generally depends on the cost of its production processes. In order to obtain high and commercially viable yields of alkaline-thermostable enzymes, it was essential to optimize the fermentation medium used for bacterial growth and enzymes production from both thermophilic *Bacillus* strains. Optimal parameters of the alkaline-thermostable enzymes biosynthesis from microbial origin, varied greatly, with the variation of the producing strain, environmental and nutritional conditions.

The maximum protease(s) productivity was attained at 55°C in the presence of SHW viz. 563.68 U/ml for both *B. licheniformis* EGT50 bacterial strain (Fig. 1).

pH is among the other most important factors for any fermentation process and dependent on the type of the moistening agent used in the medium. Each microorganism possesses a pH range for its growth and activity with a optimal value in between the range. The pH of the culture medium plays a critical role for the optimal physiological performance of the microbial cells and the transport of various nutrient components a cross the cell membrane aiming at maximizing the alkaline enzymes yields. Thus, the pH of the fermentation medium has a marked effect on the cell growth and enzyme production [31, 32, 3, 9]. Furthermore the optimal pH values may be affected by the incubation temperature in many thermophiles. However, it was not surprising to find that, not only the incubation temperature was affecting the optimal pH value, but also many factors in the environment may change it i.e secretion of alkaline solutions like ammonia or acids like oxalic acid in the medium [33], incubation period, growth changes in medium, growth factors supply, the different minerals and nitrogen source. The variation in the pH value for protease production was affected by strain or species difference. Moreover, several workers indicated that, the variations in the pH value for proteases production even within the same bacterial species owing to the difference in method applied and different environmental conditions. The optimum initial pH value capable of promoting protease(s) production by *B. licheniformis* EGT50 was found to be at the value of 9.0 since the enzyme(s) yields reached up to 447.74 U/ml (Table 2). The maximum protease(s) productivity was reached up to 316.98 u/ml with SHW concentration of 1.5 g/ flask 100ml produced by *B. licheniformis* EGT50 by incubation at 55°C for 48 h. These results means that, protease(s) biosynthesis depends not only on the substrate concentration but also on the kind of the producing strain. *B. licheniformis* B42 was able to utilize D (+) galactose which increased protease(s) production on SHW basal medium (Table 3).

Ammonium dihydrogen phosphate was considered to be the best induced for the highest protease productivity by *B. licheniformis* EGT50 where the enzymes productivity reached up to 2377 U/ml with SHW (Table 4). The maximum protease(s) productivity was reached up to 1888.12 U/ml at inoculum size of 0.5 ml/flask by *B. licheniformis* EGT50. The most potent bacterial strain viz. *B. licheniformis* B50 was exhibited its maximum ability to biosynthesis protease(s) with 72h incubation period, since, the productivity reached up to 3556.5 U/ml (Table 5).

Concentration of 500 ppm EDTA supplementation induces the highest protease(s) productivity where it reached up to 13366.8 U/ml. Also a concentrations from 125-1000 ppm EDTA and 1000 ppm $ZnCl_2$ exhibited a higher inductive effect on protease(s) produced by *B. licheniformis* EGT50.

Table 2: Relation of different incubation temperatures, pH and substrate concetration to protease(s) production by *B.licheniformis* B-50 allowed to grow on SHBM under Submerged fermentation (SmF) condition

Incubation temperature (°C)	Protease(s) production (unit/ml).	Initialc pH value	Protease(s) production (unit/ml).	Substrate concentration (g/flask)	Protease(s) production (unit/ml).
10	50.23 ± 2.08	3.0	0.0	0.05	2.82 ± 0.90
20	79.62 ± 1.5	5.0	10.02 ± 0.00	0.10	13.36 ± 0.52
30	89.33 ± .38	6.0	17.82 ± 0.00	0.20	44.77 ± 0.49
35	100.2 ± 0.0	7.0	100.23 ± 0.00	0.50	89.33 ± 0.70
40	112.47 ± 3.09	7.5	178.25 ± 0.00	1.00	188.8 ± 0.380
45	141.59 ± 0.98	8.0	316.98 ± 0.00	1.50	316.98 ± 0.60
50	200.0 ± 2.3	8.5	422.69 ± 0.00		
55	563.68 ± 2.0	9.0	447.74 ± 1.04		
60	75.16 ± 1.4	9.5	10.02 ± 0.00		
65	4.23 ± .52	10.0	5.64 ± 0.00		
70	3.16 ± 0.0				

Table 3: Relation of application of different carbon sources to protease(s) productivity by *B.licheniformis B*-50 allowed to grow under SmF conditions at 55°C.

Carbon source	Protease(s) production (U/ml)	Carbon source	Protease(s) production (U/ml)
Control	316.98 ± 0.6	Disaccharides	563.68 ± 0.0
Monosaccharides		Maltose	1002.37 ± 0.0
Ribose	1002.37 ± 0.0	Sucrose	563.68 ± 0.0
D(+) Xylose	316.98 ± 0.6	Cellobiose	1782.5 ± 0.0
D(-) Arabinose	316.98 ± 0.0	Trisaccharides	
D(-) Glucose	1782.5 ± 0.0	Raffinose	56.36 ± 0.16
D(+) Galactose	1957.6 ± 0.38	Polysaccharides	
D(+) Mannose	563.68 ± 0.3	Starch	178.25 ± 0.0
D(-) Fructose	1002.37 ± 0.0	Cellulose	1002.37 ± 0.0
Rhamnose	1002.37 ± 0.0	Dextrin	1869.7 ± 0.11
Trehalose	1002.37 ± 0.0	Inuline	1782.5 ± 0.0
Disaccharides		Polyhydric alcohol	
Lactose	316.98 ± 0.28	Mannitol	563.68 ± 0.0

Table 4: Relation of application of different nitrogen sources to protease(s) productivity by *B. licheniformis* EGT50 allowed to grow on SHW under SSF conditions at 55°C

Nitrogen source	Protease(s) production (U/ml).
Control	1957.6 ± 0.0
Ammonium acetate	1002.37 ± 0.0
Ammonium molybdate	316.98 ± 1.04
Ammonium nitrate	1336.89 ± 0.52
Ammonium dihydrogen phosphate	2377 ± 0.52
Ammonium monohydrogen phosphate	1782.5 ± 0.0
Ammonium chloride	1336.69 ± 0.52
Ammonium sulphate	1336.69 ± 0.52
Sodium nitrate	422.69 ± 0.52
Potassium nitrate	422.69 ± 0.52
Magnesium nitrate	1002.37 ± 0.0
Peptone	1002.37 ± 0.0
Urea	1782.0 ± 1.04

The maximum protease(s) production reached up to 25178.5 U/ml by introducing thiamin into the production medium, this followed by L-Ascorbic acid and nicotinic acid which reached up to 23770.0 U/ml by *B. licheniformis* EGT50. The alkaline thermostable protease(s) productivity reached its maximal value 2377.0 U/ml by the addition of L-arginine to SHW production medium by *B. licheniformis* EGT50. Also, DL-serine and L-glutamine exerted high stimulatory effect on protease production by the same bacterial strain (Table 6). The best production vessel for maximum protease(s) production was 1000 ml capacity for the two most potent bacterial strains where, the yield of constitutive protease(s) production was 1785.0 and 7096.25 U/ml by *B. licheniformis* EGT50.

Data recorded in Table (7) showed a summary of the optimal nutritional and physicho-chemical conditions for alkaline thermostable protease production by *B. licheniformis* EGT50 grown on SHW as preferable substrates.

Table 5: Relation of different inocula sizes, different incubation periods and vitamins to protease(s) productivity by *B. licheniformis* B-42 allowed to grow under SmF conditions at 55°C for 48h

Inoculum size (ml).	Protease(s) production (U/ml).	Incubation period (hours)	Protease(s) production (U/ml)	Vitamin	Protease(s) production (U/ml)
0.1	100.23 ± 1.04	6	751.67 ± 0.79	Control	17825.01 ± 0.0
0.2	447.74 ± 1.04	12	1336.69 ± 0.52	Ascorbic acid	23770.0 ± 0.79
0.4	597.07 ± 0.9	24	2377 ± 0.52	Nicotinic acid	23770.0 ± 1.3
0.5	1888.12 ± 1.04	48	2825.07 ± 0.38	Thiamine (B_1)	25178.5 ± 0.93
1	1002.37 ± 1.04	72	3556.5 ± 0.42	Pyridoxin	10023.7 ± 0.98
1.5	1002.37 ± 0.0	96	2825.07 ± 0.75	Riboflavin	13366.87 ± 0.52
2	316.98 ± 1.04	120	1336.69 ± 0.71	Folic acid	7096.26 ± 0.0
2.5	178.25 ± 0.0	144	316.98 ± 1.04		
5	1336.69 ± 0.52				
10	56.36 ± 0.0				

Table 6: Relation of different amino acids application to protease(s) productivity by *B. licheniformis* B-50 allowed to grow on SHW under semi solid fermentation conditions at 55°C

Side chain (SC).	Amino acid.	Protease(s) production (U/ml).
Control		1336.7 ± 0.520
Aliphatic SC	Glycine	316.98 ± 0.24
	DL-Alanine	316.98 ± 0.00
	DL-Valin	1336.69 ± 0.52
	L-Leucine	237.7 ± 0.520
	DL-Isoleucine	422.69 ± 0.52
Hydroxylic (OH) Group- containing SC	DL-Serine	2118.5 ± 0.490
	DL-Threonine	751.67 ± 0.52
	DL-Tyrosine	751.67 ± 0.52
Sulphur atom- containing SC	L-Cystein	316.98 ± 0.00
	L- Methionine	1002.37 ± 1.04
Acidic groupes or their amides	DL-Aspartic acid	1002.37 ± 0.52
	L-Glutamine	1888.12 ± 1.20
Basic group	L-Arginine	2377.0 ± 0.520
	L-Lysine	563.68 ± 0.00
	L- Histidine	1002.37 ± 1.04
Aromatic group	L -Phenylalanine	893.36 ± 0.38
	Tryptophan	1336.69 ± 0.52
Imino group	L- Proline	316.98 ± 0.00

Table 7: A summary of the optimal nutritional and environmental parameters controlling of protease productivities by *B. licheniformis* EGT50 under solid state fermentation conditions

Parameter	*B. licheniformis* B42	Parameter	*B. licheniformis* B42
Temperature (°C)	55	Inoculum size (ml)	0.5
pH value	9	Incubation period (hour)	72
Substrate concentration	1.5	Amino acid	Argenine
Carbon source	Galactose	Bottle capacity (ml)	1000
Nitrogen source	$(NH_4)_2H2PO_4$		

Partial Purification of the Alkaline Thermostable Enzyme and its Kinetic Characterization: The application in the bio-detergent industry does not require highly-pure alkaline thermostable protease and generally makes use of crude or partially purified preparations is valid. However, it is significant to obtain enzymes with higher specific activity for their kinetic characterization. Traditionally, the purification of alkaline thermostable proteases from fermentation media has been done in several steps which include centrifugation of culture filtrate, selective precipitation of the enzyme by ammonium sulphate, followed by gel filtration [34, 9, 3].

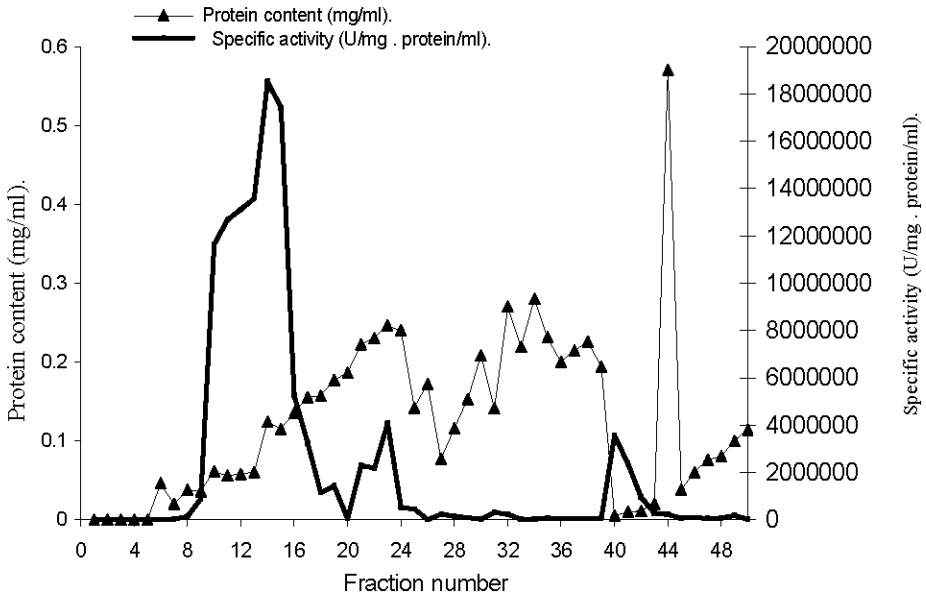

Fig. 2: Fractionation pattern of protease(s) produced by *B. licheniformis* B-42 at 55°C using sephadex G-200 column chromatography technique.

Table 8: A summary of the purification steps of protease produced by *B. licheniformis* EGT50 allowed to grow on SHW substrate at 55°C under SmF

Purification step	Volume (ml)	Protein concentration (mg/ml)	Total protein (mg/ml)	Protease activity (U/ml)	Total activity	Specific activity (U/mg, protein)	Purification fold	Yield (%)
CFF	1140.0	0.380	433.20	17825.01	20320510.94	46907.92	1.00	100
Ammonium sulphate	100.0	0.965	96.50	2000000.00	200000000.00	2072538.86	44.00	980
Dialysis against sucrose	4.5	1.100	4.95	5636765.86	25365446.37	5124332.60	109.24	124
Sephdex G-200	5.0	0.124	0.62	2296307.24	11481536.20	18518606.70	394.70	56

An attempt to purify alkaline thermostable protease from *B. licheniformis* EGT50 partially, the cell free filtrate supernatant was subjected to ammonium sulphate precipitation, gel filtration on Sephadex G200 columns chromatography. *B. licheniformis* EGT50 was allowed to grow on the production medium under all optimal semisolid fermentation conditions as shown in Table (7) for the production of alkaline thermostable protease. At the end of incubation period, 1000 ml of protease(s) was extracted. Results presented in Figure (2) showed that three active peaks were obtained after purification of protease(s) by applying sephadex G200 column chromatography in fractions (9-18), fractions (21-26), fractions (39-42) and the fraction 14 reached the highest specific activity up to 18518606.7 U/mg protein.

A summary of the purification steps of protease produced by *B. licheniformis* EGT50 was presented in Table (8). The alkaline thermostable enzyme was partially purified 394.7 folds with sephadex G200.

CONCLUSION

Bacillus licheniformis was isolated from soil samples collected from different localities of El-khorma governorate, Taif, king Saudi Arabia. and the organism was studied for the production of thermoalkaliphilic protease under different environmental and nutritional requirements on cheap raw material viz. slaughter house wastes (SHW). This enzyme was found to have ability to stability at high temperature and alkaline pH be useful for many industrial processes especially in biodetergent industry.

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