SUMMARY

1- The present thesis deals with the study of thermo-alkalophilic bacteria of soil samples representing various localities of Egypt.

2- Thirty five soil samples were collected from five regions of Egypt i.e. Wady El-Natroon (WN) depression, Port Said salt marshes (PS), Al-Ameria salt marshes (AS), Mariut Lake (ML) and Qalubyia Governorate (QG). The samples were collected as possible, from alkaline and desert regions.

3- The counts of thermoalkalophilic bacteria in the collected soil samples, showed that highest counts of thermo-alkalophilic bacteria were recorded for wadey El-Natroon (WN) region, Port Said (PS) salt marshes and Al Ameria salt marshes (AS). Moderate counts of thermo-alkalophilic bacteria were recorded for Mariut lake (ML) and Qalubyia Governorate (QG).

4- 170 Thermo-alkalophilic bacterial isolates were selected from bacterial flora of the thirty five soil samples.

5- These thermo-alkalophilic bacterial strains were allowed to grow on growth medium, viz Dox's - yeast extract -gelatin agar medium containing 1-2% $Na_3 PO_4$ for the isolation of thermophilic alkalophilic proteolytic strains at PH12 and incubation temperature 65°C.

6- The incidence of the isolated 170 bacterial strains in relation to their originating soils was studied. The highest number of thermo-alkalophilic bacterial isolates was recorded in Wady El-Natroon depresion (34.7%), followed by Mariut Lakes (19.4%), Al-Ameria salt marshes (15.9%), Port said salt marshes (15.3%) and Qalubyia Governorate (14.7%) respectively.

7- A screening program for the proteolytic activities of the 170 thermo-alkalophilic bacterial isolates was carried out. This screening exhibited that 16 isolates were characterized by proteolytic activities at pH12 and 65°C while the remaining isolates were non-proteolytic. The most potent isolate which was capable of producing the highest yield of proteases at 65°C and PH12 was isolate No. WN1616 B.

8- Identification procedures of the 16 thermo-alkalophilic proteolytic bacterial isolates were carried out using the international keys. Cells of these isolates are rod - shaped occur singly, in pairs or in chains. Gram positive, strict aerobic. Isolates do not produce acetyl methyl carbinol except isolates Nos 1201, 2305 and 611 produce acetyle methyl carbinol; isolates Nos 1616B, 1515A, 1201, 2305, 611 and 3403B produce acid from D-glucose and D-mannitol but not from D-xylose or L-arabinose, the remaining isolates do not produce acid from D-glycose, D-xylose, L-arabinose or D-mannitol; they hydrolyse gelatin, casein and starch except isolates Nos. 1515A, 1201, 2716 A and 611 do not hydrolyse casein and isolates Nos. 1616B, 2715 A and 2101 do not hydrolyse starch. They degrade tyrosine except isolates Nos. 3403 K, 1616B, 1515A, 305 B, 2305, 2716 A and 611 do not degrade tyrosine. All isolates reduce nitrate to nitrite; they produce indole except isolates Nos. 1616B, 1515A, 2305, 2716 A, and 611 do not produce indole; they do not produce gas from D-glucose and nitrate except isolates Nos 3403K, 2305, 2716A and 3403B produce gas from nitrate. They produce catalase. They are thermophilic, the growth is produced from 45°C to 80°C and no growth is produced at 40°C or below and exhibit optimum growth from 55 °C to 65°C. They are alkalophilic where the growth is produced at pH values from pH7.5 up to pH13.3 and no growth is produced at pH values higher than

pH13.3 or lower than pH7.5, while isolates Nos 305B and 3503B exhibited growth from pH8 to pH 13.3 and no growth is produced at pH lower than pH8 or higher than pH13.3. Isolates give growth with NaCl concentrations from 2 to 6%.

9- According to Bergey's Mannual of Systematic Bacteriology (1986) and other related Keys, All the 16 bacterial isolates belonging to the genus *Bacillus* i.e. *Bacillus stearothermophilus* ***(Donk, 1920).***

10- A special study has been under taken concerning the productivity of thermo-alkaline protease(s) by the proteolytic thermoalkalophilic *Bacillus stearothermophilus* S- WN1616B isolated from Wady El-Natroon since this strain was found to be the most potent protease(s) producer.

11-Factors affecting protease(s) productivity by *Bacillus stearothermophilus* S-WN1616B were investigated. The following data were found to be optimal for a maximal yield of protease(s).

a) An inoculum size of 0.5 ml of the bacterial stock suspension (containing 229.3 x 10^6 cells) was found to be the optimum inoculum for maximum enzyme yield.

b) The maximal enzyme yield was attained within 30 days incubation period at 55°C.

c) The optimum incubation temperature at which *B. stearothermophilus* S-WN 1616B produced its maximum yield of the extracellular thermo-alkaline protease was at 55°C.

d) The optimum pH value for protease(s) production was found to be at pH10 with 1% Na_3PO_4.

e) It was found that the most suitable buffer is Borax NaOH at pH 10.2) (316.9 u/ml) This is followed by carbonate bicarbonate at pH10.7

ug/ml
or umol/m

(282.5 u/ml) then, glycine NaOH at pH10.4 (178.2 u/ml), tris-buffer at pH9 (126.2 U/ml) and Boric Borax at pH 9.2 (95.7 u/ml) respectively.

f) The maximum amount of protease production was obtained with 3% NaCl.

g) Introducing different 15 amino acids into the production medium as organic nitrogen sources instead of $NaNO_3$ (as the inorganic basic source) resulted in the following main data.

I) L-threonine exerted the highest effect of protease(s) production (893.4 units / ml) in comparison with $NaNO_3$ (126.2 units / ml). This is followed by DL alanine, L-Cysteine, B-alanine and L-aspartic acid.

II) L-tyrosine, glycine, DL-leucine and L-histidine had not efect on protease production comparable to $NaNO_3$ which exerted the same yield.

III) L-cystine, DL-phenylalanine, L-asparagine, and DL-Serine exerted 40% loss of protease productivity while L-glutamic acid and L-tryptophane exerted 74% loss of enzyme production comprable to $NaNO_3$.

h) Introducing different nitrogen sources (ammonium molybdate, ammonium chloride, ammonium nitrate, ferrous ammonium sulphate, potassium nitrate, ammonium dihydrogen phosphate, ammonium sulphate, ammonium oxalate, urea and calcium nitrate). Some of them resulted in increasing yield of protease (s) and reached up its maximum in presence of calcium nitrate.

I) The effect of elimination of one or more of ingredients of mineral salts of Dox's medium revealed that production medium containing only tap water, gelatin and Na_3PO_4 resulted in increasing the yield of protease(s) than in the presence of any ingredients.

J) Supplying different protein sources (casein, peptone, protease peptone, tryptone, egg albumine and gelatin) resulted in increased protease production and reach its maximum with peptone. In absence of protein source no yield of protease was recorded.

k) The best vitamin, which induced protease production could be arranged according to the following pantothenic acid (500 ppm), thiamine (250 ppm), L-ascorbic acid (500 ppm), folic acid (250 ppm). Nicotinic acid (250 ppm) and Riboflavin (250 ppm).

L) Introducing different concentrations of available heavy elements (such as cupper sulphate cobalt sulphate, zinc sulphate and lead acetate) resulted in increasing the yield of protease(s) and reached up its maximum in cases of zin sulphate at 50 p.p.m. but in presence of cupper sulphate, cobalt sulphate and lead acetate at all applied concentrations protease productivity by *B. stearothermophilus* S-WN1616B were inhibited in comparison with the control (tap water).

12- The produced thermo-alkaline protease under all the previously mentioned optimal conditions was subjected to a purification procedure and the purified enzyme preparation was investigated for same factors affecting its activity while in the purified form.

13- Purification steps included preparation of cell free filterate, ammonium sulfate fractionation at 60% saturation, dialysis against H2O then against pure sucrose crystals, and gel - filtration using sephadex G200 and G100 column chromatographic techniques which increased the purity of the enzyme up to fourty eight folds with a specific activity of 1267.6 (u/mg. pr//ml).

14- Factors affecting the activity of the purified extracellular enzyme of *B. stearothermophilus* S-WN 1616B were investigated. The resulting data is given in the following :

a) The activity of the purified enzyme increased gradually by increasing temperature and reached its maximum at 60°C.

b) The optimum pH for a maximum activity of the protease enzyme was found to be pH11.

c) The purified enzyme recorded its maximum activity at incubation period of 62 h.

d) The optimum concentration of substrate (gelatin) was found to be (0.5%).

e) Calcium chloride and EDTA exerted the best stimulatory effect on protease activity at 1.0mM concentration. This was followed by the stimulation of magnesium sulfate within the range of 1-5 mM and Na-dedocyle benzene sulphonate at 1.0 mM concentration but mercuric chloride inhibited completely the enzyme activity at all concentrations, i.e. within the range of 1.0 - 10 mM.

f) The purified enzyme was stable at 60°C and 70°C for 18h and at 80°C it retained about 50% of its original activity after ten minutes.

15) The amino acid detected in the purified protease enzyme were as the following : Isoleucine, leucine, tyrosine, phenylalanine, Histidine, Lysine, Arginine and glysine.