microbiological study on the rmo-alkolophilhc bacteria

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1- The present thesis deals with the study of thenno-alkalophilic bacteriaof 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 QalubyiaGovernorate (QG). The samples were collected as possible, fromalkaline and desert regions.3- The counts ofthermoalkalophilic bacteria in the collected soil samples,showed that highest counts of thermo-alkalophilic bacteria wererecorded for wadey El-Natroon (WN) region, Port Said (PS) saltmarshes and Al Ameria salt marshes (AS). Moderate counts of thermoalkalophilicbacteria were recorded for Mariut lake (ML) and QalubyiaGovernorate (QG).4- 170 Thermo-alkalophilic bacterial isolates were selected from bacterialflora of the thirty five soil samples.5- These thermo-alkalophilic bacterial strains were allowed to grow ongrowth medium, viz Dox's - yeast extract -gelatin agar mediumcontaining 1-2% Na3 P04 for the isolation of thermophilic alkalophilicproteolytic strains at PH12 and incubation temperature 650C.6- The incidence of the isolated 170 bacterial strains in relation to theiroriginating soils was studied. The highest number of thermoalkalophilicbacterial isolates was recorded in Wady EI-Natroondepresion (34.~%), followed by Mariut Lakes (19.4%), Al-Ameria saltmarshes (15.9%), Port said salt marshes (15.3%) and QalubyiaGovernorate (14.7%) respectively.7- A screening program for the proteolytic activities of the 170thermoalkalophilicbacterial isolates was carried out. This screeningexhibited that 16 isolates were characterized by proteolytic activities atpH12 and 65°C while the remaining isolates were non-proteolytic. Themost potent isolate which was capable of producing the highest yieldof proteases at 65°C and PHI2 was isolate No. WNI616 B.8- Identification procedures of the 16 thermo-alkalophilic proteolyticbacterial isolates were carried out using the international keys. Cells ofthese isolates arerod - shaped occur singly, in pairs or in chains. Grampositive, strict aerobic. Isolates do not produce acetyl methyl carbinolexcept isolates Nos 1201, 2305 and 61I produce acetyle methylcarbinol; isolates Nos 1616B, 1515A, 1201,2305,611 and 3403Bproduce acid from D-glucose and D-mannitol but not from D-xylose orL-arabinose, the remaining isolates do not produce acid. fromD-glycose, D-xylose, L-arabinose or D-mannitol; they hydrolysegelatin, casein and starch except isolates Nos. 1515A, 1201,2716 Aand 61I do not hydrolyse casein and isolates Nos. 1616B, 2715A and2101 do not hydrolyse starch. They degrade

tyrosine except isolatesNos. 3403 K, 1616B, 1515A, 305 B, 2305, 2716 A and 6II do notdegrade tyrosine. All isolates reduce nitrate to nitrite; they produceindole except isolates Nos. 1616B, 1515A, 2305, 2716 A, and 6II donot produce indole; they do not produce gas from D-glucose andnitrate except isolates Nos 3403K, 2305, 2716A and 3403B producegas from nitrate. They produce catalase. They are thermophilic, thegrowth is' produced from 45°C to 80°C and no growth is produced at40°C or below and exhibit optimum growth from 55°C to 65°C. Theyare alkalophilic where the growth is produced at pH values from pH7.5up to pH13.3 and no growth is produced at pH values higher thanpHI3.3 or lower than pH7.5, while isolates Nos 305B and 3503Bexhibited growth from pH8 to pH 13.3 and no growth is produced atpH lower than pH8 or higher than pH13.3. Isolates give growth withNaCI concentrations from 2 to 6%.9- According to Bergey's Mannual of Systematic Bacteriology (1986) andother related Keys, All the 16 bacterial isolates belonging to the genusBacillus i.e. Bacillus stearothermophi/us (Donk, 1920).10- A special study has been under taken concerning the productivity ofthermo-alkaline protease(s) by the proteolytic thennoalkalophilicBacillus stearothermophilus S- WNI616B isolated from Wady EINatroonsince this strain was found to be the most potent protease(s)producer.l l-Factors affecting protease(s) productivity by Bacillusstearothermophilus S-WN1616B were investigated. The followingdata were found to be optimal for a maximal yield ofprotease(s).a) An inoculum size of 0.5 ml of the bacterial stock suspension (containing229.3 x 106 cells) was found to be the optimum inoculum for maximumenzyme yield.b) The maximal enzyme yield was attained within 30 days incubationperiod at 55°C.c) The optimum incubation temperature at which B. stearothermophilus SWN161(;8 produced its maximum yield of the extracellular thermoalkalineprotease was at 55°C.d) The optimum pH value for protease(s) production was found to be atpHlO with 1% Na3P04.e) It was found that the most suitable buffer is Borax NaOH at pH 10.2) ([16.9 ~~ This is followed by carbonate bicarbonate at pHlO.7'-9 »: ~.,lA., I,p. p-~(--(282.5 u/ml) then, glycine Na