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Isolation and Characterization of Thermophilic *Bacillus* Species from Thermal Ponds in Jordan

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Abstract: In the last tow decades Thermophilic *Bacilli* were classified as either *B. coagulans or B. sterothermophilus*, but it is becoming increasingly evident that they comprise a diverse group of organisms. In present study thermophilic *Bacillus* (gram positive, motile, spore forming) was isolated, characterized and identified from recreational thermal spring located along the Jordan Rift valley. The isolated strain was distinguished by the formation of terminal cylindrical spore, it was named *Bacillus brevis*. The optimum growth temperature for this strains was 57°C. Studying thermostable proteases (from thermophilic *Bacilli*) as biocatalysts for industrial applications is going to be considered for future work.

Key words: Thermal springs, thermophilic bacteria, *Bacillus brives*, gram positive, spore forming

Introduction

Fifteen species of the thermophilic *Bacilli* have been described in the first edition of Bergeys Manual (Bergeys *et al.*, 1923). In the sixth edition some eighteen thermophilic species able to grow above 60°C were described (Breed *et al.*, 1948). The first systematic study of aerobic thermophilic sporeformers was made by Ruth Gordan and Nathan Smith in which they examined 216 cultures from variety of sources (Gordon and Smith, 1949; Smith *et al.* (1952). Their extensive investigation resulted in the seventh edition of Bergeys Manual (Breed et al., 1957) listing only tow species with optimum growth at 55°C or above, These tow species were *B. sterothermophilus* (Donk *et al.*, 1920) and B. coagulans (Hammer, 1915).

The description of *Bacillus stearothermophilus* in the eighth edition of Bergey's Manual (Gibson and Gordon, 1974) described the distinguishing features as; maximum growth temperature 65-75°C, a minimum growth temperature of 30-45°C, inability to grow on sabouraud dextrose agar and in 0.02% (w/v) sodium azide, starch hydrolysis was considered to be variable. Schenk and Aragno (1979) described an obligate thermophile which was strictly aerobic, oxidizing hydrogen in the presence of O_2 and CO_2 and producing spherical spores within a swollen sporangium.

Isolation of six obligate thermophilic aerobic sporeformers from soil in Japan, which they named *Bacillus thermoglucosidasius* were also reported (Suzuki *et al.*, 1983). Nine thermophilic *Bacillus* strains were isolated from thermally treated waste water. The group of strains named *B. pallidus* had a growth temperature range 30-70°C with optimum of 60-65°C (Scholz *et al.*, 1987). Other thermophilic *Bacilli* were also isolated from deep-sea hydrothermal vents (Marteinsso *et al.*, 1996).

Ten obligatory thermophilic *Bacilli* able to utilize n-alkanse as growth substrate were isolated. The organisms were isolated following the enrichment of mud and water samples on a medium with n-heptadecane added as substrate. Growth occurred between 45-70°C. The strains were named *B. thermoleovorans* (Zarilla and Perry, 1987).

Thermotolerant and thermophilic bacteria were isolated and identified from recreational thermal spring located along the Jordan Rift valley. Thermophilic *Bacillus* spp. was dominant over other gram-positive and gram-negative bacteria (Khalil *et al.*, 1998). In this study, aerobic, rod-shaped, gram-positive, sporeforming thermophilic *Bacillus* isolated from thermal ponds in Jordan was described and discussed.

Materials and Methods

Sampling sites: Water samples were collected from several thermal hot springs located along the Jordan Rift Valley during spring season, using 500 ml sterile thermal glass containers which keep the temperature of the water samples constant (Khalil *et al.*, 1998).

Strain growth and culture: Thermophilic *Bacillus brevis* was used. Cells were grown on 57°C in shake flask and agar plate cultures in thermus media (ATCC medium 697).

Identification of bacteria and biochemical studies: Different morphological colonies were subcultured on thermus agar media and the isolated pure cultures were identified according to Holt (1989) and Cowan and Steel (1985). Additionally, biochemical tests were done to characterize the isolated strain (MacFaddin, 1980).

Morphological studies: To determine the presence or absence of morphological features of the isolated strain, it was inoculated and grown in thermus media plates or liquid broth at 57°C in shaking incubator at an aeration rate of 300 RPM. Sample taken from culture was prepared for taking photographs under the light microscope (Olympus BX50), gram stain and spore stain microscopic slides were prepared for this purpose.

Determination of optimal growth temperature: Isolated pure culture was incubated at different temperatures i.e., 20 to 65 with 5°C difference then 1°C difference, to find the maximum and minimum temperature for its growth. The optimal growth temperature was taken for the isolated pure culture by comparing the total number of bacteria grown at different temperatures.

Results and Discussion

Twenty three strains of aerobic thermophilic or thermotolerant bacteria were isolated from several hot springs located along the Jordan Rift valley, whose temperature ranges were between 24 and 64°C. *Bacillus* spp. was dominated over other gram-positive and gram-negative bacteria, (Khalil *et al.*, 1998). *Bacillus brevis* has a minimum temperature requirement of 40°C. The maximum growth temperature for such isolate reached 60°C, with the optimal growth temperature 57°C (Table 1).

Biochemical studies: The fact that *Bacillus brevis* is strictly aerobic microorganism was further supported by the presence of catalase and oxidase activity. Additionally, degradation of tryptophan and the formation of indole that would react with p-dimethyl amino benzyldehyde to give red complex, was not observed. However, isolated strain did not produce acetone from glucose, and was unable to release strong acids like acetic, formic or lactic acid and could not use citrate as the sole source of carbon. *Bacillus brives* gave turbid regions around the colonies when incubated in the presence of Tween-80 but not Tween-20. and showed negative results for urease test. It was capable of starch, casein and gelatin hydrolysis (Table 2).

Morphological studies: *Bacillus brevis* was gram-positive, motile. Sporulation is clearly detected under the light microscope, it is important distinguishing characteristic when grown on thermus

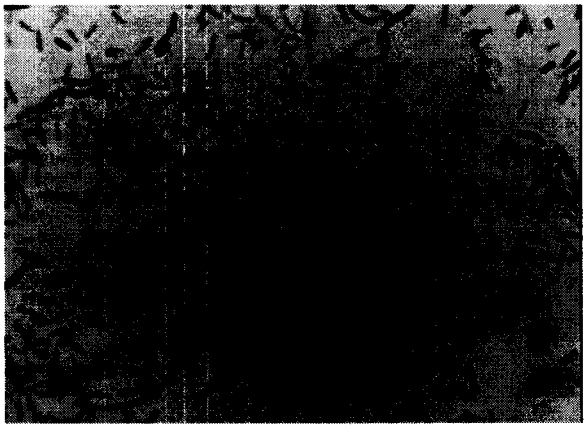

Fig. 1: Staining and morphological structure of *Bacillus brevis* as appeared under the light microscope, with magnification power of 10X100

Table 1: Growth temperatures of *Bacillus brevis*

	Growth temperature (°C)		
Species	Minimum	Optimum	Maximum
Bacillus brevis	40	57	60

Table 2: Biochemical tests of *Bacillus brevis*

Biochemical test	*Bacillus brevis*
Nitrate reduction	+
Indole formation	-
Citrate utilization	-
Methyl red	-
Voges-Proskouer	-
Starch hydrolysis	+
Gelatin hydrolysis	+
Casein hydrolysis	+
Tween-80 hydrolysis	+
Tween-20 hydrolysis	-
Deamination (phenyl ala)	-
H_2S production	-
Gas from nitrate	-
Oxidase	+
Catalase	+
Urease	-
Anaerobic growth	-
Motility	+
Sporulation	+
Gram's reaction	+
Morphology	Rods

media plates or broth (Fig. 1). The elongated structure of this strain was observed under the light microscope when cultivated in broth media. However, these elongated structures are maintained and could be detected even after 48 h of incubation.

Cultivation of *Bacillus brevis* in broth media, followed by their examination using the light microscope, indicated that it is normally available in the form of short rod single or in a chain of two, some show long curve *Bacilli*, it also showed terminal and sub terminal spores (Fig.1).

The aerobic endospore-forming bacteria are a rich source of extracellular depolymerases active on carbohydrates, proteins, nucleic acids and various other compounds (Priest, 1977). The extracellular enzymes produced by thermophilic *Bacilli* could be described as the scavenger enzymes, involved in degrading polymeric molecules in the environment, that are too large into the cell (Priest, 1989).

A wide range of industrial and environmental applications involve the utilization of enzymes being operating under extreme physical parameters of temperature, pH and pressure. Enzymes suited to operate under extreme conditions could be obtained only from the microbial sources that are naturally occurring in a range of environmental conditions considered as being extreme for some, while for others are essential for their normal growth and development (Antranikian *et al.*, 1987).

Thermophilic *Bacilli* are of interest for more than just basic biological reasons. They offer some major advantages for biotechnological process, many of which run more rapidly and efficiently at high temperatures.

Future work will focus on the bioactive substances produced by aerobic sporulating bacteria of *Bacillus* genus, such as, antibiotics synthesis by *Bacilli* and thermostable proteins and enzymes, which are capable of catalyzing biochemical reactions at high temperature and are generally more stable than enzymes from mesophiles.

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