SYSTEMATIC BACTERIOLOGICAL STUDY OF MANGHOPIR HOT SPRING WATER

Pages with reference to book, From 34 To 40

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Abstract

The thermophilic islolates of Mangho-pir spring water were of bacillary forms. All were gram positive, sporing rods, varying in sugar reactions; one being non saccharolytic, two monosaccharolytic, one disaccharolytic and nine fermented four or more sugars. All belonged to genus Bacillus of family Bacillaceae. All were found to be thermophilic varieties of ter-ristrial mesophilic species of genus Bacillus viz. B. circulans, B. pumilus, B. badius and B. pulvifacience (JPMA. 30:34, 1980).

Introduction

A number of hot spring have been known since long though little was known about their microflora. Many workers have isolated some thermophilic bacterial species and strains from such waters (Visintin and Zavattiero, 1961; Bairiev and Mamedov, 1962; Egorova and Deryungina, 1963).
Majority of the thermophiles reported so far belong to the family Bacillaceae. Certain important thermophilic species of family Bacillaceae including aerobes and anaerobes; B. thermocellum, B. thermocllesum, Bstearo-thermophiles and CI nigrificans (Bergey's Manual of Determinative Bacteriology, 1957).
Visintin and Zavattiero (1961) isolated a thermophilic bacterium from a hot spring. It had an optimum temperature of about 55°C. Its resistance to temperature as reported was comparatively higher than other; 71.5°C; with a little difference in sugar fermentations, it was included as a strain Bacillus stearothermo-philes. Bairiev and Mamedov (1962) reported a new species named as Ozokerite bacillus, with a wide range of optimum temperature, from 37°C to 60°C. A thermophilic thiobacterium, Thiobacillus thermophilica, was reported by Egorova and Deryungina (1963). It was reported that the culture grew only in mineral nutrient medium upon addition of various sulphides.
At Manghopir, Karachi, number of hot springs are present. In the present communication, attempts were made to isolate and identify the thermophilic bacterial flora of these waters. Qualitative as well as quantitative analysis of the water was carried out.

Material and Methods

Simple nutrient medium was used for the quantitative and qualitative analysis. Certain other media; Nutrient broth, Glucose Agar; Simon's citrate Agar, Lactose Egg-Yolk Agar; Sugars, M.R. & V.P. medium (Difco), tryptone water, Nitrate broth, Gelatin stabs were also used for the characterization of isolates. All the media were prepared in spring water instead of distilled water.

ENUMERATION OF THERMOPHILIC BACTERIA IN HOT SPRING WATER:

Pour plate method was employed for enumeration of most probable number of viable bacteria in water samples. Dilution of samples were made upto 1/100,000 in sterile spring water.

ISOLATION AND CHARACTERIZATION OF BACTERIA:

After getting the pure cultures of isolates; their morphological characters (such as shape, arrangement and Gram's reaction), physiological characters, growth on different media, biochemical reactions and ability to ferment sugars were determined.

Results and Discussion

SEASONAL VARIATIONS:

The temperature and pH values were obtained at the time of collection of each sample. The observations were made at weekly intervals during the month of January through November. No observations could be made during the month of July, August and September.

Table I: Seasonal Variations of Thermophillic Bacteria from Manghopir Hot Water Spring.

Month	*Temperature*	*pH values*	*Mean Total Bacterial counts/ml. (Undilured Samples)*
January	44°C	7.3 - 7.4	20 - 38
February	44° - 45°C	7.4	17 - 26
March	45 - 46°C	7.4 - 7.5	19 - 44
April	46°C	7.5 - 7.6	31 - 47
May	47 - 48°C	7.5 - 7.6	58 - 78
June	48°C	7.6	72 - 86
October	46 - 47°C	7.3 - 7.4	62 - 57
November	46 - 47°C	7.3 - 7.4	48 - 72

Table I shows the seasonal variations in temperature and pH value during the course of observations and also the count of thermophilic bacteria of water.

The observations showed that the temperature fluctuated between 44 °C The highest temperature of 48 °C was recorded during the month of May and June while the lowest temperature of 44 °C was observed during the month of January.

The pH values showed a little fluctuations throughout the period of observations; the highest pH value of 7.6 was recorded during the month of June, whereas the lowest pH values being 7.3 was observed

during the month of October.

QUANTITATIVE ANALYSIS:

The bacterial counts during the course of observations showed a maximum count of 86 bacterial cell/ml in the month of June, and a minimum count of 17 bacterial cell/ml in the month of February. Though there were fluctuations in counts within the same month, however the highest count was observed in the month of May and June. All the isolates were bacillary forms, thermophilic, sporing and motile.

QUALITATIVE ANALYSIS:

Qualitative analysis of spring water was carried out. Over all 13 aerobic thermophiles could be isolated in pure culture. Attempts were made for isolation of anaerobic thermophiles as well, but no anaerobic thermo-phile was detected.

Qualitative and quantitative analysis of Manghopir spring revealed as follows: The temperature fluctuated between 44°C-48°C during the month of January-November excluding July, August and September. Shah al (1964) reported the temperature of Water was 48°C in August. The temperature variations were supposed to be due to differences in external temperature. As soon as the water was collected from the spring pH fluctuated from 7.6. Earlier report recorded 7.6 pH in the month of June and August (Shah et al., 1964). The variations in the distribution and occurrence of thermophilic bacteria (Table-I) describes the highest number of 86 bacterial cells/ml during the month of June as compared to the minimum of 17 bacterial cells/ml during the month of February. There was no direct correlation of abundance of bacteria with the temperature and pH values-Such little increase in the number of bacteria as noted with the increase of temperature may be accounted for storage of the spring water in transit, as such a rise in the number of marine bacteria from initial 160 to a maximum 333 bacteria during storage in flasks for 24 hours was reported (Kriss 1963).

Thirteen aerobic thermophilic strains could be isolated in pure cultures (Table IV). In spite of repeated attempts, no anaerobic thermophile could be isolated. However, it did not rule out the presence of anaerobes in Manghopir waters.

Characterization of the isolate has been made following the Bergey's Manual (1957). Colonial and morphological characters, biochemical reactions and physiological characters were observed and on the basis of these attempts were made to bring the isolates to nearest possible species according to Bergey's Manual (1957). Majority of thermophiles have been reported in the family Bacillaceae however species related to other families have also been reported (Egorova and Deryungina, 1963). All the isolates in the present workwere found to be gram positive and sporing rods, suggesting that they belong to the family Bacillaceae. The isolates were found to be microaerophilic and were catalase positive thus belonging to genus Bacillus. Majority of the species in the genus Bacillus are motile with peritrichous flagella (Table-II)

Table II : Morphological Characters

Isolate No.	Gram's staining	Morphology and arrangement	Spores	Motility	Capsule
SB1	+	Rods, 0.6×3 u, little pointed ends, small chains.	Ellipsoidal 0.9×1.2 u, sub-terminal sporangium swollen	Motile with peritrichors flagella	—
SB2	+	Rods, 0.7×3.2 u rounded ends, no chain.	Ellipsoidal 0.8×1.3 u sub-terminal sporangium spindle shape.	"	—
SB3	+	Small rods, 0.5×1.8 u no chains.	Ellipsoidal, 0.7×1.3 u sub-terminal.	"	—
SB4	+	Rods, 0.8×2.5 u, no chains.	Ellipsoidal 0.6×1.0 u, sub-terminal, not swollen sporangium.	"	—
SB5	+	Rods 0.8×2.2 u, few small chains.	0.5×1.0 u, not swollen sporangium.	"	—
SB6	+	Rods 0.7×2.5 u, not in chains.	Ellipsoidal 0.9×1.3 u sporangium swollen.	"	—
SB7	+	Rods 0.6×2.8 u, pointed ends, no chains.	0.8×1.2 u, sub-terminal swollen sporangium.	"	—
SB8	+	Rods 0.6×2.5 u no chains.	1.0×1.5 u swollen sporangium.	"	—
SB9	+	Rods 0.9×2.5 u few short chains.	Ellipsoidal 0.8×1.2 u sub-terminal sporangium not swollen.	"	—
				"	—
SB10	+	Small rods, 0.6×2.2 u no chains.	0.8×1.2 u sub-terminal sporangium swollen.	"	—
SB11	+	Rods, 0.6×2.6 u rounded ends, few chains.	Ellipsoidal 0.8×1.3 u sub-terminal.	"	—
SB12	+	0.6×2.5 u, no chains.	0.8×1.2 u, sub-terminal sporangium swollen.	"	—
SB13	+	Rods, 0.5×2.5 u no chains.	0.8×1.2 u, sub-terminal swollen sporangium.	"	—

(Bergey's Manual, 1957). The 13 isolates in the present work were motile with peritrichous flagella. Variable reactions of the strains and species have been recorded (Table-III).

Table III : Growth Characters of Isolates on Different Media

Isolate No.	Nutrient Agar	Nutrient broth	Glucose Agar	Simon's Citrate Agar	Lactose Egg-yolk Agar			
					Opalescence	Pearly layer	Proteolysis	Lact.
SB1	Thin, spreading whitish	Even turbidity little sediment	Heavier than on N. Agar	Small, round bluish	—	—	Partial	—
SB2	Small, round, flattened, opaque	Even turbidity thin pellicle	Same as on N. Agar	Scant, Smooth, round, blue	—	—	Partial	—
SB3	Small, round, flattened, adherent	Even turbidity	Same as on N. Agar	Small, round, Smooth, bluish	—	—	Partial	—
SB4	Small, thin smooth, flattened, translucent	Even turbidity thin pellicle	Small, thin, smooth, translucent	Scant, small, round, smooth bluish	—	—	Partial	—
SB5	Small, round, smooth, flattend	Even turbidity thin white pellicle	Heavier than on N. agar flattened opaque	Scant, round smooth, bluish	—	—	—	—
SB6	Spreading flattened, opaque	Granular turbidity	Heavier than on N. Agar.	Scant, small, round, smooth bluish	—	—	Partial	—
SB7	Spreading, Rough white hairy outgrowth	Even turbidity thin pellicle	Heavier than on N. Agar.	Small, at the streak line, round, smooth	—	—	Partial	—
SB8	Medium size, round, flattened opaque	Granular turbidity pellicle	Same as on N. Agar	Small, round blue	—	—	Partial	—
SB9	Large, rough, hairy outgrowth, whitish	Even turbidity thin white pellicle	Large rough spreading, opaque whitish	—	—	—	Strong	—
SB10	Large, thick white, spreading	Even, dense, turbidity few granules	Same as on N. Agar	Small, round raised, smooth, blue	—	—	Strong	—
SB11	Small, round, flattened, rough	Even turbidity few granules	Same as on N. Agar	Scant, small at the streakline smooth, blue	—	—	Partial	—
SB12	Large size, spreading, rough flattened	Even turbidity little sediment	Heavier than on N. Agar	Small, round, bluish	—	—	Strong	—
SB13	Small, round rough, flattened	Granular turbidity thin pellicle	Same as on N. Agar	Scant on point of streak, round blue	—	—	Partial	—

Table IV : Temperature Tolerance of Thermophilic Isolates.

Isolate No.	*Temperature Relations*	
SB1	Opt. 47° - 32°C	No growth at 63°C
SB2	Opt. 48° - 53°C	No growth at 64°C
SB3	Opt. 45° - 53°C	No growth at 64°C
SB4	Opt. 46° - 54°C No growth at 64°C	Lower growth at 41°C
SB5	Opt. 45° - 53°C	No growth at 64°C
SB6	Opt. 42 - 54°C 40°C, No growth at 44°C	Lower growth below 64°C
SB7	Opt. 47° - 53°C	No growth at 63°C
SB8	Opt. 47°C-53°C	No growth at 64°C
SB9	Opt. 48° - 51°C	No growth at 61°C
SB10	Opt. 47° - 53°C	No growth at 62°C
SB11	Opt. 46° - 53°C	
SB12	Opt. 48° - 53°C	No growth at 63°C
SB13	Opt. 44 - 53°C	No growth at 64°C

All the isolates except SB9 fermented glucose. Of the fifteen sugars used (Table V),

Table VI : Classification of the Isolates from Manghopir Water Based on Biochemical Reactions Following Bergey's Manual (1957).

Isolate No.	*Reactions common to isolates and proposed species and strains Bergey's Manual (1957)*		*Related species Bergey's Manual (1957)*	*Remarks*
	Positive reactions	*Negative reactions*		
SB 1, 7 10 & 12	Sta., Gel. Gluc., Mannit., citrate	Lact., V.P., Indol., Nitrate	B. circulans Xyl.+ ve gram-ve Mesophillic	B. circulans Var. Xyl gram+ve thermophillic
SB 4	Gluc., Suc., Gel., citrate	Starch, Lact., Nitrate	B. pumilus. v.p+ ve Mannitol+ve, Mesophillic	B. pumilus var. —ve, thermophillic Mannitol—ve
SB 5	Gluc., citrate	Starch, Lact., Nitrate	B. pumilus v.p.+ ve Mannit.+ve, Gel.+ve. Mesophillic	B. pumilus var. V.P.—ve Mannit. —ve Gel.—ve Thermophillic
SB 2, 3. 8, 11 & 13	Gluc., Mannit., Mannose, Gel.	Starch, Indol, V.P.	B. pulvifacience Nitrate+ve, Mesophillic	B. pulvifacience Var. Mannitol. -- ve thermophillic
SB 6	Glu., Gel., Nitrate	Starch. V.P. Indol	B. pulvifacience Mannit.+ve	B. pulvifacience Var. Mannit. — ve thermophillic
SB 9	Gelatin	All Sugars, V.P. Indol, Starch, Nitrate	B. badius Mesophillic	B. badius var. thermophillic

Key : Glu. = Glucose, Suc. = Sucrose, Mannit = Mannitol, Lact = Lactose, xyl = Xylose.

six were not fermented by any of the isolates, viz; Lactose, xylose, Rhamnose, Inulin, Arabinose and Dulcitol. None of the isolates produced gas from any of the sugars when fermented, 33% isolates metabolized starch, Mannitol, a key factor in characterization of species of genus Bacillus, was fermented by about 69% of the isolates. Gelatin liquifaction was demonstrated by all the isolates except SB 3, SB 5 and SB 13. About 9.2% of isolates showed variable proteolytic action on lactose Egg Yolk Milk Agar (Willis 1960). They were lipase and lecithinase negative. Sodium citrate was utilized as sole source of carbon by all the did not ferment xylose whereas B. circulans was positive. Therefore these species were suggested as gram positive thermophilic varieties of B. circulans, SB 1 and SB 10 were isolates except SB 9. On the basis of morphological and biochemical differential characters the isolates have been identified (Table-VI).

Table V : Saccharolytic and Other Characters

Isolate No.	SB1	SB2	SB3	SB4	SB5	SB6	SB7	SB8	SB9	SB10	SB11	SB12	SB13
Glucose	+	+	+	+	+	+	+	+	|	+	+	+	+
Sucrose	+	+	+	+	|	|	+	+	|	+	+	+	+
Maltose	+	|	+	|	|	|	+	|	|	+	+	+	|
Mannital	+	+	+	+	|	+	+	+	|	+	+	+	+
Mannose	+	+	+	+	|	+	+	+	|	+	+	+	+
Dextrin	+	|	+	|	|	|	+	+	|	+	+	+	+
Sorbitol	+	|	|	+	|	|	+	|	|	+	|	+	|
Inosital	|	|	|	|	|	|	|	|	|	+	|	|	|
Starch	+	|	|	|	|	|	+	|	|	+	|	+	|
Dulcitol	|	|	|	|	|	|	|	|	|	|	|	|	|
Xylose	|	|	|	|	|	|	|	|	|	|	|	|	|
Rhamnose	|	|	|	|	|	|	|	|	|	|	|	|	|
Inulin	|	|	|	|	|	|	|	|	|	|	|	|	|
Arabinose	|	|	|	|	|	|	|	|	|	|	|	|	|
Lactose	|	|	|	|	|	|	|	|	|	|	|	|	|
Indol	|	|	|	|	|	|	|	|	|	|	|	|	|
V.P.	|	|	|	|	|	|	|	|	|	|	|	|	|
M.R.	+	|	+	|	|	+	+	|	|	+	|	+	|
Nitrate Reduction	|	|	|	|	|	+	|	|	|	|	|	|	|
Gelatin	+	+	+	|	+	+	+	+	+	+	+	+	+	+
Citrate	+	+	+	+	+	+	+	+	|	+	+	+	+	+
Catalase	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Urea	|	|	|	|	|	|	|	|	|	|	|	|	|	|

Key : + = Acid, — = No Fermentation, S = Slight fermentation
Gas was not produced in any sugar.

Isolates SB 1, 7, 10 and 12 correspond to B. circulans differing in that the isolates found to ferment inositol while SB 7 and SB 12 did not. Morphologically SB 1 different from SB 10 as the former was found in small chains and later occurred as single short rods SB 7 differed from SB 12 in

morphological characters and proteolysis SB 7 was 2.8 u inlength and showed partial proteolysis of Lact. Egg Yolk Milk Agar while SB 12 had a size of 2.5 u and was strongly proteolytic.
Isolates SB 4 and SB 5 were found to correspond to B. pumilus. SB 4 did not produce acetylmethyl carbionol and did not ferment Mannitol while B. pumilus did. SB 5 showed no proteolysis on Lact. Egg Yolk Milk Agar, Gelatin was not liquified as well, mannitol and V.P. were negative. SB 4 and SB 5 may be suggested as thermophilic varieties of B. pumilus.
SB 9 can be suggested as a thermophilic variety of B. badius, all the reactions being identical to the species.
SB 2, 3, 6, 8, 11 and 13 were found corresponding to B. pulvifacience. However the six isolates differed from B. pulvifacience as they did not reduce nitrates. SB 6 being sucrose negative, differed from B. pulvifacience as well as from other five isolates. SB 2 and SB 11 differed from SB 6 and SB 13 as they did not ferment Dextrin. SB 3 differed from SB 2 and SB 11 as it was Dextrin positive and M.R. positive. SB 2 and SB 11 being identical in almost all the reactions differed from each other only in morphological characters, SB 2 being long rods of 0.7 x 3.2 u occuring singly and SB 11, a short rod of 0.6x2.6 u occuring singly or in short chains. SB 8 and SB 13 differed only in colonial characters and in spores size. SB 8 formed round opaque and adherent colonies on N. Agar and had spores measuring about 1.0x 1.5 u while SB 13 formed small rough non adherent colonies and had spores measuring about 0.8x1.2 u.

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