

ON THE OCCURRENCE OF PSYCHROPHILIC  
BACTERIA IN ASIR SOILS

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ABSTRACT

*An attempt was made to shed some light upon the occurrence of psychrophilic bacteria in soil samples collected from various localities of Asir Region, in south-western Saudi Arabia. Results indicated that about 95% of the investigated samples harboured psychrophilic bacteria. Counts of these bacteria ranged from 3200 to 42000 colonies per gram oven dry soil.*

*Isolates of psychrophilic bacteria in the investigated samples belong to the genera Pseudomonas, Flavobacterium, Bacillus, Streptococcus and Micrococcus. Results indicated the absence of obligate psychrophiles and revealed a distinct ecological group of bacteria with respect to temperature distributed in this region.*

INTRODUCTION

Although many investigations have been carried out on the distribution of psychrophilic bacteria in various countries since they were first described by Forster in (1887), yet little information is available as for the occurrence of this important group of bacteria in Saudi Arabia.

The significance of psychrophiles in nature is not fully clear since data in natural habitats are limited. Most of the quantitative data in literature are for dairy products (e.g. Thomas; Griffiths and Fouldes (1960) ;

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Baumann and Reinbold (1963) and Elliott and Michener (1965).

The present investigation was designed to throw light upon the occurrence and the quantitative distribution of psychrophilic bacteria in 56 soil samples collected from various localities of Asir Region (south-west of Saudi Arabia) as determined by counts at 0 °C. Psychrophiles as they are described in this investigation fit Stokes (1963) definition.

### MATERIALS AND METHODS

Fifty-six soil samples (uncultivated and cultivated) were collected under aseptic condition in polyethylene bags from surface layer (30 cm depth) from different localities of Asir Region. Samples (in all 56) were from Abha and its districts 10, South Abha 10; North Abha 8; East Abha 8 and Tohamet Asir 12.

The dry weights of the soil samples were determined by drying portions to constant weight at 105 °C.

Counts were made on Trypticase Soy Agar using the standardized spatula spray technique (Elwan and El-Sayed 1964). Plates were incubated for 14 days at 0 °C.

Optimum and maximum temperatures for growth were determined by growing the pure isolates in Trypticase Soy Broth. Incubation was made for 7 days at different temperatures investigated. The dry weights were estimated.

Media and techniques used for identifying bacteria were similar to those recommended in the Microbiological Methods (1957).

The Keys of Bergey (1974) were used for identifying the pure isolates of bacteria.

On the occurrency of psychrophilic....

## RESULTS

Results of investigating cultivated and uncultivated soil samples are presented in Table 1. Results show that about 95% of the investigated samples harboured psychrophilic bacteria. Counts ranged from 3200 to 42000 colonies per gram oven dry soil. The cultivated soils had fewer psychrophilic bacteria than did the uncultivated soils.

Chromogenic (Pale yellow to deep orange) and nonchromogenic bacterial colonies appearing after 14 days incubation at 0 C° were isolated, purified and partially identified to the genus level only due to some technical difficulties. Table 2 shows the distribution of psychrophilic bacteria in the investigated soil samples. Five psychrophilic bacterial genera viz: *Pseudomonas*; *Flavobacterium*; *Bacillus*; *Streptococcus* and *Micrococcus* were identified.

*Pseudomonas* and *Flavobacterium* were recorded in all the investigated regions. *Bacillus* was recorded in the uncultivated soil samples from south, north and west of Abha. *Streptococcus* was recorded in Tohamet Asir whereas *Micrococcus* was found in uncultivated soils of south and west of Abha.

Table 3 represents the distribution of optimum and maximum temperatures for growth of 24 morphologically distinct isolates. The optimum temperatures for growth of the isolates ranged from 25 - 33 C° whereas the maximum temperatures ranged from 30 - 40 C°.

## DISCUSSION

Results gave evidence of the occurrence and distribution of psychrophilic bacteria in soils of Asir Region. Most of the investigated samples (about 95%) harboured psychrophilic bacteria in appreciable numbers. In analogy

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TABLE 1 : The occurrence of psychrophilic bacteria in soil samples collected from various localities of Asir Region

Regions	No. of investigated samples	No. of samples containing psychrophilic bacteria	Range of counts of psychrophilic bacteria/g oven dry soil
<b>Abha and Districts</b>			
Uncultivated	5	5	5200 - 14400
Cultivated	5	5	4600 - 6200
<b>South Abha</b>			
Uncultivated	5	5	4600 - 9600
Cultivated	5	4	3400 - 9400
<b>North Abha</b>			
Uncultivated	4	4	5200 - 12600
Cultivated	4	4	4800 - 9400
<b>East Abha</b>			
Uncultivated	4	4	7600 - 9200
Cultivated	4	4	5600 - 7800
<b>West Abha</b>			
Uncultivated	4	4	12800 - 42000
Cultivated	4	4	7600 - 16400
<b>Tohamet Asir</b>			
Uncultivated	6	6	3800 - 5200
Cultivated	6	4	3200 - 4200

On the occurrency of psychrophilic....

TABLE 2 : Distribution of psychrophilic bacterial genera isolated from the investigated samples.

Regions	No. of samples containing the bacterial genera:				
	<u>Pseudo-</u> <u>monas</u>	<u>flavobac-</u> <u>terium</u>	<u>Bacillus</u>	<u>Strepto-</u> <u>coccus</u>	<u>Micro-</u> <u>coccus</u>
<b>Abha and Districts</b>					
Uncultivated	5	5	0	0	0
Cultivated	5	5	0	0	0
<b>South Abha</b>					
Uncultivated	5	5	2	0	1
Cultivated	4	4	0	0	0
<b>North Abha</b>					
Uncultivated	4	4	1	0	0
Cultivated	4	4	0	0	0
<b>East Abha</b>					
Uncultivated	4	4	0	0	0
Cultivated	4	4	0	0	0
<b>West Abha</b>					
Uncultivated	4	4	3	0	0
Cultivated	4	4	0	0	0
<b>Tohamet Asir</b>					
Uncultivated	6	6	0	2	0
Cultivated	4	4	0	1	0

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TABLE 3 : Distribution of optimum and maximum temperature for growth of 24 isolates of psychrophilic bacteria

Bacterial genera	No. of isolates	OPTIMUM °C					MAXIMUM °C				
		20	25	30	35	40	25	30	35	40	45
<u>Pseudo-</u> <u>monas</u>	8	0	2	6	0	0	0	2	4	2	0
<u>Flavoba-</u> <u>cterium</u>	9	0	4	5	0	0	0	3	6	0	0
<u>Bacillus</u>	3	0	0	2	1	0	0	0	2	1	0
<u>Strepto-</u> <u>coccus</u>	2	0	0	2	0	0	0	0	2	0	0
<u>Micro-</u> <u>coccus</u>	2	0	2	0	0	0	0	2	0	0	0

## On the occurrence of psychrophilic....

with the finding of Stokes and Redmond 1966, counts of psychrophilic bacteria were higher in uncultivated than in cultivated soils. As it has been shown by previous investigators (e.g., Forster (1887), Lochhead (1926) and Stokes and Redmond (1966) that mesophilic bacteria in garden and cultivated soils predominated those in uncultivated soils. It is possible that cultivation favours mesophilic bacteria and this reduces the percentage of psychrophiles. The counts in this investigation, however, are much lower than those recorded by Stokes and Redmond (1966). It should be remembered when comparing psychrophilic bacteria counts that Stokes and Redmond (1966) used the spread plate technique for estimating the numbers of viable psychrophilic bacteria.

Identification studies to the genus level revealed the occurrence of *Pseudomonas*, *Flavobacterium*, *Bacillus*, *Streptococcus* and *Micrococcus*. Isolates of the first two genera are common psychrophiles and are found in all the investigated regions. However, it is now accepted that psychrophilic bacteria belonging to a large number of genera exist. Larkin and Stokes (1966) isolated ninety psychrophilic isolates of *Bacillus* from soil, mud and water. *Bacillus* in this investigation was isolated from three regions, *Micrococcus* from two regions and *Streptococcus* was recorded only in Tohmet Asir.

Psychrophiles as defined by Stokes (1963) are organisms that grow at 0 C° so that they can be detected visually within a week; facultative psychrophiles have an optimum growth temperature of 20 C° or higher whereas the optimum growth temperature of obligate psychrophiles is 20 C° or lower.

Temperature optima of the isolates were in the range

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of 25 - 35 C° even though all of the precautions in technique described by Morita (1966) were taken. According to Stokes (1963) definition all the isolated psychrophilic bacteria are facultative. Since soils become warmed to temperatures above 30 C° in summer, it seems unlikely that obligate psychrophilic bacteria could survive. Facultatively psychrophilic bacteria are wide spread in terrestrial and fresh water habitats (Larkin and Stokes, (1966); Stokes and Redmond, (1966) and Druce and Thomas (1976). The occurrence of psychrophiles in appreciable numbers in many habitats might reveal a bacterial group of considerable importance in various cycles of matter.

On the occurrence of psychrophilic....

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