

# Seasonal Incidence of Bacterial Temperature Types in Louisiana Soil and Water

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Received for publication 23 March 1970

Psychrophilic bacteria were not detected in soil, mud, and water in the summer. In winter, they were present in water and mud and constituted a significant portion of the bacterial flora in lake water.

In recent years, investigations of psychrophilic bacteria (those capable of growing well at 0 C) have revealed that they exist in nature in larger numbers than previously suspected and that many bacterial genera contain psychrophilic members. For example, from the Antarctic region, psychrophiles were found in 8 of 11 samples (6), 18 of 41 samples (4), and 52 of 126 samples (1). Psychrophilic *Bacillus* species were isolated from three of four samples obtained at Macquarie Island (3) and from 60 of 75 samples obtained in Washington and Idaho (2).

The frequency and ease with which psychrophiles have been isolated prompted Stokes and Redmond (5) to determine the numbers of psychrophilic bacteria and molds from various sources to see if they existed in nature in sufficient numbers to be a significant portion of the microbial population. Psychrophiles accounted for 0.5 to 86%, 16 to 76%, and 11 to 33% of the bacterial populations in soils, various waters, and lake muds, respectively. Thermophiles usually accounted for less than 1% of the total population. These results indicate that psychrophiles are both widespread and numerous in nature and, as such, are probably important in the various cycles of matter.

Previous studies on psychrophilic bacteria have concentrated on materials and samples obtained from relatively cold regions of the world. Limited data are available on the incidence of psychrophilic bacteria in the more temperate climates. This study was undertaken to determine if psychrophilic bacteria make up a significant portion of the bacterial population in a warm climate area, namely, Baton Rouge, La., and to determine if the level of the three temperature groups of bacteria changes with the seasons.

Samples of soil, mud, and water were collected in sterile jars and were stored in an ice chest until used. Samples were always used within 5 hr of

collection. Water and mud samples were obtained from within a meter of the shore of lakes, rivers, and bayous. Soil samples were collected from the top few centimeters.

Samples were serially diluted in 0.1% peptone water and plated in triplicate with Trypticase Soy Agar. Counts of psychrophilic and mesophilic bacteria were obtained by the spread plate technique, and the pour plate technique was used, to reduce colony spreading, for counts of the thermophilic bacteria. Plates used for thermophilic counts were incubated 2 to 3 days at 55 C, whereas counts of psychrophiles were obtained by incubation at 0 C for 2 weeks. Occasionally, a plate would freeze and be unusable. A third set of plates was incubated at 20 C for 4 to 5 days, thus allowing both mesophiles and psychrophiles to grow. The mesophilic population was determined by subtracting the 0 C count from the 20 C count.

Representative results showing the bacterial population of samples collected in the summer are shown in Table 1. Mesophilic bacteria accounted for most of the total population, with thermophiles comprising 4% or less in all but one sample. No psychrophiles were found. Mud and water temperatures were  $31 \pm 2$  C when collected. Soil temperature was not measured but, presumably, was near air temperature of 33 to 34 C.

The same sites were sampled again the following winter and the results are shown in Table 2. In the water samples, the bacteria in each temperature group generally increased in numbers, although the thermophilic population still made up only 0 to 3% of the population. Psychrophiles, which were absent during the summer, were present in four of five samples and comprised 0.1 to 31% of the total number. In the mud samples, psychrophilic bacteria were found in about the same percentage as thermophiles,

TABLE 1. *Bacterial population of water, mud, and soil in the summer*

Source	Psychrophiles		Mesophiles		Thermophiles	
	No./ml or g	Per cent	No./ml or g	Per cent	No./ml or g	Per cent
<b>Water</b>						
City park lake.....	<10	0.0	1,540	96.3	60	3.7
University lake.....	<10	0.0	2,500	99.7	8	0.3
Campus lake.....	<10	0.0	376,000	100.0	42	0.0
Mississippi River.....	0	0.0	90,000	99.9	68	0.1
Elbow Bayou.....	0	0.0	550,000	100.0	162	0.0
<b>Mud</b>						
City park lake.....	<10	0.0	1,500,000	91.0	148,000	9.0
University lake.....	<10	0.0	480,000	99.1	5,000	0.9
Campus lake.....	<10	0.0	960,000	99.6	4,200	0.4
Mississippi River.....	<10	0.0	690,000	99.3	5,000	0.7
Elbow Bayou.....	<10	0.0	1,060,000	99.5	4,800	0.5
<b>Soil</b>						
Garden.....	<10	0.0	5,100,000	97.9	106,000	2.1
Garden.....	<10	0.0	4,000,000			
Foot path.....	<10	0.0	760,000			
Lawn.....	<10	0.0	7,900,000	99.8	18,300	0.2

TABLE 2. *Bacterial population of water, mud, and soil in the winter*

Source	Psychrophiles		Mesophiles		Thermophiles	
	No./ml or g	Per cent	No./ml or g	Per cent	No./ml or g	Per cent
<b>Water</b>						
City park lake.....	3,300	30.8	7,100	68.9	320	0.3
University lake.....	1,630	16.8	7,970	83.1	110	0.1
Campus lake.....	3,100	15.4	17,000	84.6	14	0.0
Mississippi River.....	1,310	0.1	1,500,000	99.4	8,400	0.5
Elbow Bayou.....	<10	0.0	530,000	97.9	11,000	2.1
<b>Mud</b>						
City park lake.....	1,000	0.5	186,000	98.2	2,500	1.3
University lake.....	9,000	0.8	1,140,000	96.2	36,000	3.0
Campus lake.....	6,900	2.9	233,000	97.1	14	0.0
Mississippi River.....	<10	0.0	1,160,000	97.5	30,000	2.5
Elbow Bayou.....	20,800	0.1	28,000,000	98.2	470,000	1.7
<b>Soil</b>						
Garden.....	<100	0.0	3,200,000	99.2	24,000	0.8
Garden.....	<100	0.0	14,300,000	100.0	520	0.0
Foot path.....	<10	0.0	6,600,000	99.6	26,000	0.4
Lawn.....	<100	0.0	36,000,000	100.0	600	0.0

i.e., as much as 3%. In soil samples, no psychrophiles were found, and the ratio of thermophiles to mesophiles remained the same as in the summer. Mud and water temperatures were  $6 \pm 1$  C, whereas soil temperatures were probably near 0 C.

These data showed that there was a seasonal fluctuation in the numbers of psychrophilic bacteria found in water and the underlying mud. During the hot summer they were either absent, or present in such low numbers that they went

undetected. In the winter, however, they were present, and in lake water they accounted for a significant percentage of the total population. Stokes and Redmond (5) also showed a high percentage of psychrophiles in lake water. No psychrophiles were found in the soil, even though the total bacterial counts were high, but this may indicate that those psychrophiles found in the lakes were indigenous to those waters and were not merely washed into the lakes during the winter rains. Gram stains showed that the psy-

chrophilic population was exclusively gram-negative rods. None of the psychrophiles was pigmented even though many mesophiles were.

Although a considerable amount of work must still be done to establish the relative importance of psychrophilic bacteria in nature, the available evidence indicates that, at least in some environments, psychrophiles, because of their presence in large numbers, must be playing some role in the cyclization of matter.

This research was supported in part by a grant from the Graduate Research Council of Louisiana State University.

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