THE EFFECT OF pH AND TEMPERATURE ON MOTILITY OF LISTERIA SPECIES

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SUMMARY: The effect of pH of the medium and temperature of incubation was studied on motility of four strains of 2 Listeria species. All the strains were found to be non-motile at pH 4. The motility was observed at a pH range of 5.5 to 9.0. However, the optimum pH and temperature for motility was 7.0 and 22°C respectively. The strains NCTC 7973 and C-274 were, however, motile at pH 5.0. At pH 10, NCTC 5214 m, C-274 and NCTC 7973 were motile at 22°C with the exception of C-274 which was motile also at 35°C. NCTC 5214 m was motile at pH 11 only at 22°C whereas C-274 was motile at both 22 and 35°C.

Key Words: Listeria, pH.

INTRODUCTION

The members of the genus *Listeria* are pathogenic to a very wide range of animals (6, 7) causing multiple clinical manifestations including abortion or still birth in pregnant farm animals (1, 8, 9, 12-17).

Listeria that has previously been placed as a member of the family Corynebacteriaceae (2) has now been classified with "Erysipelothrix" and "Caryophanon", a group entitled as Genera of uncertain affiliation (3,11,19, 20). The cells are small, gram positive, non-sporing, usually non- capsulated (5, 21), non-acid-fast, diptheroid rods (12). All members when grown at 20-22°C in liquid media show characteristic tumbling motility but appear sluggishly motile when grown at 37°C for 24 hours (12). The motility of this group is attributed to by the presence of peritrichous flagella (18, 22-24).

To elucidate whether the motility is the function of temperature alone, we designed this experiment to study the motility at two different temperatures in the medium having different pH values. We also wanted to determine whether the organisms only loose motility/growth or both at different temperatures when exposed to different pH values.

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MATERIALS AND METHODS

Organisms and media

Three strains of *L. Monocytogenes* (NCTC 5214 m, NCTC 7973 and C-274), serotype 4a, 1/2a and 4b respectively, and one strain of *L. seeligeri* (SLCC 3954) were used. The organisms were maintained at 5°C in tryptose phosphate agar (TPA pH 7.3 oxoid). From these stock cultures maintained at 5°C, inoculum was taken into TPB and incubated at 30°C for subsequent experiment.

Effect of temperature and pH

Motility medium containing gelatin (Oxoid) 30 gm; brain heart infusion broth (Merck), 25 g; K_2HPO_4, 02 g; KNO_3, 02 g; agar, 1 g and distilled water 1 liter (quantity of agar varied when pH was above 9 and below 6), was prepared and adjusted to appropriate pH values (4, 5, 5.5, 6, 7, 9, 10 and 11). pH was adjusted using 2.5 M Lactic acid (Merck) and Sodium hydroxide 1 M. The medium was dispensed in U-tubes and autoclaved at 121°C for 15 minutes.

Cultures of each strain was grown for 24 hours in TPB at 30°C. From this growth 0.1 ml of inoculum was added to one of the arms of U-tube which was then incubated at 35°C and 22°C, for 24 and 72 hours respectively. After incubation distance traveled by the organisms in the inoculated arms of U-tube was noted.

RESULTS

Tables 1 and 2 show the results of motility of *Listeria* species at 22°C and 35°C in media having varying pH values. No growth and consequently no motility was

Table 1: Motility of *Listeria species* incubated at 22°C on various pH values.

Listeria species	Time of incubation	Swarming index at various pH values							
		4.0	5.0	5.5	6.0	7.0	9.0	10.0	11.0
NCTC 5214 m	24 hours	-	-	++	++	++	+	+	+
	72 hours	-	-	+++	++++	++++	++++	+++	++
C-274	24 hours	-	+	++	+++	+++	+	+	+
	72 hours	-	++	++	+++	+++	+++	++	+
NCTC 7973	24 hours	-	++	++	++	+++	++++	+	-
	72 hours	-	++	+++	++++	++++	++++	+++	-
SLCC 3954	24 hours	-	-	++	++	+++	+	-	-
	72 hours	-	-	+++	++++	++++	+	-	-

- = No growth,
+ = 0.1-1.5 cm,
++ = 1.6-3.0 cm,
+++ = 3.1-4.5 cm,
++++ = 4.6 cm and above.

Table 2: Motility of *Listeria species* incubated at 35°C on various pH values.

Listeria species	Time of incubation	Swarming index at various pH values							
		4.0	5.0	5.5	6.0	7.0	9.0	10.0	11.0
NCTC 5214 m	24 hours	-	-	+	++	+++	+	-	-
	72 hours	-	-	++	++++	++++	++	-	-
C-274	24 hours	-	+	+	+	++	+	+	+
	72 hours	-	+	++	++	+++	+	+	+
NCTC 7973	24 hours	-	+	++	++	++++	++++	-	-
	72 hours	-	+	++++	++++	++++	++++	-	-
SLCC 3954	24 hours	-	-	+	++	++	+	-	-
	72 hours	-	-	+	++++	+++	++	-	-

- = No growth,
+ = 0.1-1.5 cm,
++ = 1.6-3.0 cm,
+++ = 3.1-4.5 cm,
++++ = 4.6 cm and above.

exhibited at pH 4 by any of the *Listeria* strains used in this study. However all the four strains showed a positive viable test on TPA even after one month incubation.

At pH 5 similar results of motility was observed in tubes inoculated with strains NCTC 5214 m and SLCC 3954. C-274 traveled to the same distance at 22°C and 35°C after 24 hours of incubation. Motility was, however, considerably increased in the tubes incubated at 22°C after 72 hours. NCTC 7973 behaved differently and distance traveled by this strain at 22°C after 24 hours was comparatively greater and no further increase in distance was observed even after 72 hours incubation. In contrast to 22°C, at 35°C the movement continued up to 72 hours. Nonetheless the overall distance traveled by the bacterium was less what was covered at 22°C by the same strain.

All four strains were motile at pH 5.5 and approximately same distance was observed in tubes incubated at 22°C after hours. There was an increase in distance covered, with increasing time of incubation up to 72 hours, the only exception was C-274. The overall distance covered was less in the tubes incubated at 35°C except NCTC 7973 which traveled approximately the same distance after 24 hours as at 22°C, but the overall distance after 72 hours was much greater. A slight increase in the distance covered was evident in other three strains until 72 hours.

At pH 6, 7 and 9 a characteristically similar pattern of motility was observed. The organisms showed motility after 24 hours of incubation which continued to improve, with time up to 72 hours at both the temperatures i.e. 22°C and 35°C. The distance covered after 24 hours being lesser and greater after 72 hours. Only NCTC 7973 contradicted these findings as they cove hours of incubation with no further improvements in tubes at pH 9.0 at 22°C and pH 7.0 and 9.0 at 35°C.

At pH 10 except SLCC 3954 all the three strains showed their characteristic pattern of motility at 22°C. There was hardly any movement observed except for growth on the top after 24 hours. However, there was a marked increase in the distance traveled by NCTC 5214 m, C-274 and NCTC 7973 after 72 hours. At 35°C only C-724 has shown the evidence of movement and growth, the pattern was similar to that at 22°C, but the overall distance covered was lesser.

At 22°C, the medium having pH 11, only two strains NCTC 5214 m and C-274 revealed motility as merely the growth on surface after 24 hours with slight increase in distance after 72 hours. Only C-724 was motile at 35°C with similar result as for 22°C incubation.

DISCUSSION

Results of the present investigation revealed that all the three different strains of *Listeria monocytogenes* and one strain of *L. seeligeri* remain motile over a wide range of pH from 5-9 with a characteristic slightly sluggish motility at 35°C than at 22°C. It also supports the evidence provided by previous workers that a pH value 7 and temperature of incubation 22°C is optimum for the motility (12).

The significant observation made in this investigation is that the strains of *Listeria monocytogenes* can tolerate extreme pH values, with intact motility, at as low pH as 5 by NCTC 7973 and C-274 both at 22°C and 35°C, and as high as pH 11 by *Listeria monocytogenes* NCTC 5214 m at 22°C and C-274 both at 22°C and 35°C. None of the strains was, however, able to provide any evidence of motility at pH 4.

The literature reveals a little information concerning the motility of *Listeria* species at extreme pH values. The results of the previous experiments were reported that *Listeria monocytogenes* can demonstrate growth and tolerance to environmental stresses, such as moderate concentrations of sodium chloride and low pH values (4). No report is available that would indicate the tolerance of different strains of *Listeria* species to alkaline pH, as high as 11. Further research is needed to identify the factors responsible for the motility, the chemical nature of flagella at extreme conditions, the influence of these environmental stresses on cellular and flagellar morphology, as well as the influence of these factors on the Pathogenicity of *Listeria* species.

The rapid detection of *Listeria* species in environmental samples is difficult and in most cases troublesome. The low level of *Listeria* cells in samples often goes undetected. Based on the present study it may be the possibility that to characterize the complete profile of flagellar proteins of *Listeria* by polyacrylamide gel electrophoresis produced at different pH values. This technique may provide a clue to develop a method to detect the presence of *Listeria* species in environmental samples by detecting proteins, commonly found in flagella produced by *Listeria* species under extreme environments (pH values).

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