VARIATIONS AND SPONTANEOUS MUTATIONS IN THE GENUS LISTERIA IN RESPECT TO FLAGELLATION AND MOTILITY

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In their original publication on *Bacterium monocytogenes* (*Listeria monocytogenes*) Murray *et al.* (1926) described the organism as motile but did not record the nature of the flagellation. Seastone (1935) and Burn (1935) described the organism as having a polar monotrichous flagellum. Subsequent publications (Paterson, 1939; Leifson, 1942) showed conclusively that the organism has peritrichous flagella. Studies by Griffin and Robbins (1944) showed that the number of flagella varies with the incubation temperature, the lower this temperature the more numerous the flagella. At 37 C the organisms were either atrichous or had only one flagellum which was often polar in location.

Through the courtesy of Dr. Griffin of George Washington University the authors received 81 strains of Listeria which had been kept in stock, vacuum dried, for the last decade. A study of these strains is recorded in this paper.

MATERIALS AND METHODS

Cultures. Of the 81 strains of Listeria received from Dr. Griffin 10 were labeled B (bovine), 1 C (fox), 9 F (fowl), 6 G (goat), 39 H (human), 8 R (rodent), and 8 S (sheep). The vacuum dried cultures were transferred to yeast extract broth and incubated at 30 C. Growth resulted in all of the transfers, and the cultures apparently were pure and typical of Listeria: small, gram positive rods which fermented glucose, maltose, and mannose but not lactose, sucrose, xylose, or arabinose. M. R. and V. P. tests were positive, but indole, citrate, urea, and nitrate were negative.

Flagellation. The cultures were transferred to broth composed of peptone (casitone), 0.5 per cent; yeast extract, 0.5 per cent; NaCl, 0.5 per cent; K_2HPO_4, 0.1 per cent; pH 7.0, and incubated at 20 C for 24–48 hr. Formalin (38 per cent formaldehyde) was added to a concentration of 5 per cent (v/v), and the organisms were washed and stained according to the method of Leifson (1951). Two smears were made on each slide. Measurements of flagellar curvature were made with a B & L filar micrometer as described by Leifson, Carhart, and Fulton (1955).

Motility. Moist preparations were made of all the cultures to determine kind and degree of motility. Detection and isolation of motility variants and of motile mutants from the nonmotile variants were made by the following technique: Ten to 15 ml of melted nutrient agar, made by dissolving 1.5 per cent agar in the broth used for flagellation study, were poured into standard 9 cm petri dishes. These plates were streaked with the diluted cultures to give isolated colonies. Over the streaked plates were then poured 7 ml at 50 C of 0.4 per cent melted agar (made by adding 0.4 per cent of agar to the broth used for the flagellation study). These streak-pour plates are more easily prepared than semisolid pour plates. By making the semisolid agar layer thin, the plates may be turned over and handled like solid agar plates. The plates were incubated upright at 20 C and examined daily. The size of the colonies indicates the degree of progressive motility of a culture. Motility variants, if present in sufficient numbers, are readily detected and isolated. These plates also serve for the detection and isolation of mutants of greater motility than the parent stock.

EXPERIMENTAL RESULTS

Normal flagellation and motility. Of the 81 strains of apparently typical Listeria, 70 strains were uniform as to flagellation and motility. These strains may be considered to have the normal flagellation and motility of the genus. The motion is the typical wriggling type exhibited by nonpolar flagellated bacteria. After 2 to 3 days of incubation at 20 C the colonies in the semisolid agar attained a diameter of 10 or more mm. The number of flagella varied considerably from one individual to another and from one strain to another. At 20 C incubation

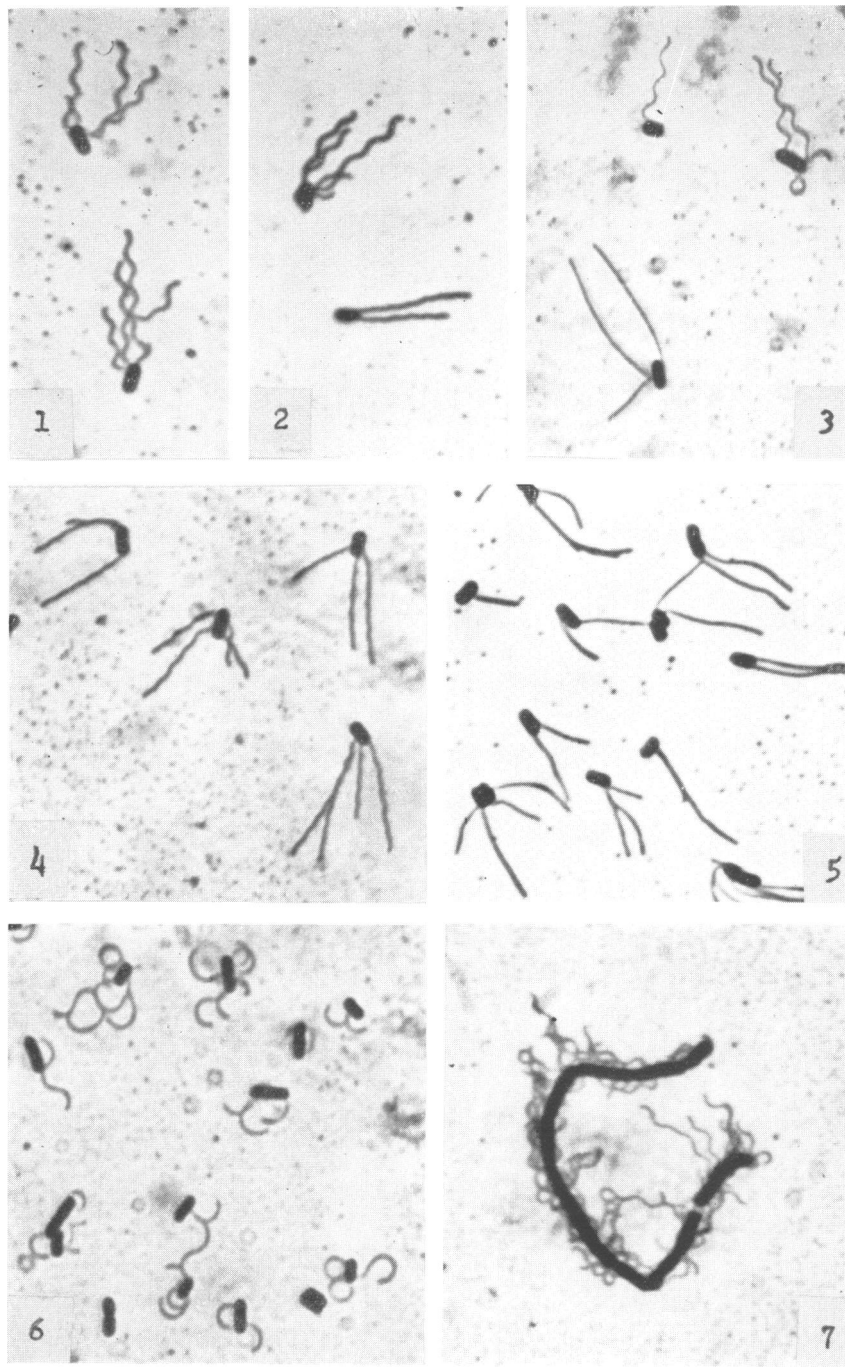

Figure 1. Listeria strain B-2 showing normal flagella.*

Figure 2. Listeria strain H-15 showing one organism with normal flagella and one with small amplitude flagella. The majority of the organisms on the slide had the small amplitude flagella.

Figure 3. Listeria strain B-3 showing 2 organisms with normal flagella and one with straight flagella.

Figure 4. Listeria variant H-15C showing organisms with small amplitude flagella. Isolated from nonspreading colony in semisolid agar prepared from strain H-15.

Figure 5. Listeria variant B-3E showing organisms with straight flagella. Isolated from nonspreading colony in semisolid agar prepared from strain B-3.

Figure 6. Listeria variant B-3D showing coiled flagella. Isolated from moderately spreading colony in semisolid agar prepared from strain B-3.

Figure 7. Listeria strain H-10B showing filamentous organism with normal flagella.

* All stains made by the Leifson method and all photomicrographs × 1,800.

for 24–48 hours the best flagellated strains showed many individuals with 5–6 flagella, but a fewer number was more common (figure 1). At 30 C incubation for 20–24 hours the flagellation was slightly less than at 20 C, and motility was definitely less. At 37 C incubation for 20–24 hours most cultures showed little motility and very few flagella. Somewhat better motility and flagellation were observed after 5 hr incubation at 37 C.

Measurements of wavelength and amplitude were made on 10 flagella of separate organisms in each of 8 selected strains. In table 1 are recorded the mean wavelengths and amplitudes and the standard deviations. Also in this table are recorded the standard deviation of the mean strain wavelengths from the species mean, the standard deviation of the individual wavelengths and amplitudes from the species means, and the standard error of the species mean wavelength. It is obvious from the data presented that the mean wavelengths and amplitudes of the normal flagella of the different strains do not differ significantly. The smallness of the various standard deviations shows how remarkably uniform is the normal curvature of the flagella of Listeria.

Flagellar and motility variants. The term "variant" is applied to an organism or a culture which is typical of the species except for flagellar morphology or motility, and which was obtained by fishing an individual colony on a semisolid plate of the original strain. The term "mutant" is applied to an organism or a culture differing from the parent and obtained by fishing from an outgrowth on an individual colony in semisolid agar. The main distinction we wish to make between the two terms is that the parentage of a variant is not known to us while that of a mutant is. Variants are presumably mutants, but when and where the mutation took place is unknown to us.

Eleven of the 81 original cultures showed the presence of variants of one kind or another. Variants present in small numbers may have been missed, especially if they possessed either normal flagella or no flagella. Nonflagellated individuals in a flagella stain and nonmotile individuals in a moist preparation may have no genetic significance. Only one culture was entirely nonflagellated, and only 2 cultures were entirely nonmotile. The other 9 cultures which yielded variants had a lesser or greater proportion of individuals with normal flagella and normal motion. After 2 days of incubation at 20 C the colonies in the semisolid agar which appeared different were fished to broth and the resulting cultures stained for flagella and examined by moist preparation. Repeated fishings were made wherever the original flagella stain and moist preparation indicated the presence of variants not obtained in pure culture by the first fishings. The 11 strains were classified into 7 groups which may be described as follows:

TABLE 1

Mean wavelengths and amplitudes of selected strains of Listeria and the standard deviations. Ten separate flagella measured in each strain. Units in microns.

Strain	WL	SD	Amp.	SD
B-1AA	2.03	0.09	0.47	0.04
C-1	1.99	0.08	0.49	0.06
F-1A	2.03	0.32	0.49	0.08
G-3	1.99	0.07	0.50	0.05
H-10B	2.03	0.09	0.46	0.07
H-15A	1.99	0.09	0.47	0.05
R-4	2.00	0.12	0.48	0.06
S-2	2.03	0.11	0.47	0.06
Species mean.....	2.01	0.10*	0.48	0.06*

Standard deviations of mean strain wavelengths from species mean = 0.02 μ.

Standard error of species mean wavelengths = 0.01 μ.

* Represents standard deviation of the 80 individual measurements from species mean.

(1) *Strain B-5.* The original culture showed nonflagellated organisms only and was nonmotile. The semisolid agar plate showed only nonspreading colonies. Culture of a single colony was like the original. This strain appeared to be a pure culture of nonflagellated organisms.

(2) *Strain H-6.* The original culture showed normal flagellation only and was nonmotile. The semisolid plate showed only nonspreading colonies. Culture of a single colony was like the original. This strain appeared to be a pure culture of normally flagellated but nonmotile organisms.

(3) *Strains H-4AA, H-5, H-14B, and S-1B.* The original cultures showed only normal flagellation and motility. The semisolid plates showed a variable proportion of normally spreading and nonspreading colonies. Cultures obtained by fishing nonspreading colonies showed only

nonflagellated organisms and were nonmotile. The spreading colonies yielded normally flagellated and motile cultures. These strains were mixtures of normally flagellated, motile organisms and nonflagellated organisms.

(4) *Strains F-4 and S-3.* The original cultures showed normal flagellation and motility. The semisolid plates showed both normal spreading and nonspreading colonies. Cultures obtained from the nonspreading colonies were nonmotile, but the organisms showed normal flagellation. These strains were apparently mixtures of normally flagellated motile organisms and normally flagellated but nonmotile organisms.

(5) *Strain G-3.* The original culture showed about 80 per cent of the flagellated individuals with normal flagella and 20 per cent with straight flagella. Motility appeared normal. The semisolid plate showed a mixture of large spreading colonies and small nonspreading colonies. The spreading colonies yielded cultures of normally flagellated motile organisms. From the nonspreading colonies were obtained two types of cultures, both of which were nonmotile. In the one culture the organisms had straight flagella only and in the other no flagella. This strain was apparently a mixture of normally flagellated, motile organisms; organisms with straight flagella and nonmotile; and organisms without flagella.

(6) *Strain B-3.* The flagella stain of the original culture showed most of the individuals with straight flagella and only a few organisms with flagella of normal curvature. In the moist preparation a majority of the individuals were nonmotile, and only a few showed normal progressive motion. A fair proportion showed a tumbling or wiggling, but not progressive motion. The semisolid plate showed a variety of colonial types: among approximately 100 colonies on the plate were 2 large spreaders, 10 medium spreaders, about 20 small colonies with a fuzzy edge indicating some motion, and 60–70 small nonspreading colonies. Colonies of each type were fished, and pure cultures of 4 variants were obtained:

B-3B. Culture from large spreading colony; organisms with normal motion and normal flagella.

B-3D. Culture from medium spreading colony. The organisms in this culture showed progressive motion but definitely slower and more erratic than the normal. Flagella stain showed organisms with short, coiled flagella (figure 6).

B-3A. Culture from small, fuzzy edged or slightly spreading colony. Moist preparation showed only tumbling or wiggling motion without any marked degree of progression. Flagella stain showed organisms with straight flagella only (figure 5).

B-3E. Culture from a nonspreading colony. Moist preparation showed no motion. Flagella stain showed organisms with straight flagella only, morphologically indistinguishable from those of B-3A.

(7) *Strain H-15.* The original culture showed only an occasional motile individual. Flagella stain showed about 95 per cent of the flagellated individuals with flagella of very small amplitude, and about 5 per cent with normal flagella. The semisolid agar plate showed a few large spreaders but mainly small nonspreaders. By fishing a number of colonies 3 variants were obtained in pure culture:

H-15B. Culture from large spreading colony; normal motion and normal flagella.

H-15A. Culture from a nonspreading colony. Flagella stain showed normal flagella but moist preparation showed the organisms to be nonmotile.

H-15C. Culture from a small nonspreading colony. The organisms were nonmotile with flagella of very small but uniform amplitude (figure 4).

On the bases of flagellation and motility were thus obtained 7 varieties or types from the 81 strains studied:

(1) Nonflagellated; nonmotile (B-5, H-4AAA, H-5A, H-14BA, S-1BA).

(2) Straight flagella; nonmotile (B-3E, G-3A).

(3) Straight flagella; tumbling, spinning, and wiggling motion but little progressive motion (B-3A).

(4) Small amplitude flagella; nonmotile (H-15C).

(5) Coiled flagella; motion progressive but slower and more erratic than normal (B-3D).

(6) Normal flagella; nonmotile (H-6, F-4A, S-3A, H-15A).

(7) Normal flagella; normal progressive motion (70 strains showed this type only, and only B-5 and H-6 showed none of this type).

Another morphological variant may be mentioned although of a different nature from the

above. The original culture of H-14B (figure 7) showed mainly filaments or chains with normal motion in broth and normal flagella. In semisolid agar the colonies at first appeared to be non-spreading, but on prolonged incubation spreading became evident. Cultures obtained from these colonies were like the original. The long filaments apparently move very slowly in semisolid agar although quite well in both.

Measurements were made of wavelength of 20 flagella on separate organisms of variant H-15C with the small amplitude flagella. The mean wavelength was 1.53 μ and standard deviation 0.08 μ. Accurate measurements of amplitude were difficult to get, but the mean of such measurements was 0.25 μ. Comparison of the wavelengths of the normal flagella recorded in table 1 and those of small amplitude show a statistically significant difference. The amplitudes of the two types are obviously different.

The coiled flagella of variant B-3D appear to be less uniform than the flagella of the other types. This may be due to the staining technique in which the flagella become flattened onto the glass surface. A study of these flagella with the organisms in suspension, as in the method of Pijper and Abraham (1954), may show greater uniformity. Measurements of wavelength and amplitude were made of 20 "uncoiled" flagella on as many individuals. The results show the wavelength to be slightly greater than that of normal flagella and the amplitude much greater. The mean wavelength was 2.18μ, standard deviation 0.18μ; mean amplitude 0.76μ, standard deviation 0.13μ. The standard error of the difference of mean wavelength of normal and coiled flagella is:

$$SE_d = \sqrt{\frac{0.18^2}{20} + \frac{0.1^2}{80}} = 0.04 \quad Mc - Mn = 2.18 - 2.01 = 0.17, \quad t = 0.17/0.04 = 4+$$

For amplitude

$$SE_d = \sqrt{\frac{0.13^2}{20} + \frac{0.06^2}{80}} = 0.03 \quad Mc - Mn = 0.76 - 0.48 = 0.28, \quad t = 0.28/0.03 = 9+$$

Spontaneous mutations. The 7 flagellar and motility types of Listeria have been maintained in broth at 20 C, with many transfers, during the past 5 months. Each has been examined several times for motility by moist preparation, by stabbing in semisolid agar, and by plating in semisolid agar; and for type of flagellation by staining. No changes in motility or flagellation have been observed. It may be concluded that the variants have what may be considered normal genetic stability under laboratory conditions.

In our technique for the detection and isolation of motile mutants developing in a nonmotile parent stock, there is one important limitation which must be observed. For several years we have noted, as others must have done, that 2 colonies of motile bacteria developing side by side in semisolid agar will not fuse into a homogenous whole unless they originate close together. Two colonies which originate, say, 0.5 to 2 cm apart, will at first appear spherical; but as they approach each other the adjacent sides become flattened, and a space of about 1 mm remains devoid of visible growth. This phenomenon has been observed with other kinds of motile bacteria we have studied, such as Salmonella and Escherichia, as well as Listeria. The inhibitory effect on the motion of the bacteria in one colony by adjacent colonies was very striking in our mutation studies. If a hundred or more colonies of the nonmotile parent stock develop in the semisolid plate, motile mutants may not become manifest because they cannot migrate into the surrounding medium. In order for motile mutants to develop into visible growth, spreading out from the colony, other colonies must be far removed, at least from one side of the colony.

The technique described for the detection and isolation of variants also serves for the detection and isolation of motile mutants of a nonmotile stock. The diluted nonmotile culture is streaked in series over the two halves of a solid agar plate and the semisolid agar poured over as described. If a nonmotile variant such as B-3E is plated in this manner so that 100 colonies develop on the first half of the plate and only a few or none on the second half of the plate, we may observe: for the first 2–3 days of incubation at 20 C the colonies remain small, compact, and without visible spreading. After about 4 days some of the colonies along the middle of the plate may show spreading growth but only on the side away from the colonies in the first half of the plate. After 5

days a few more colonies may show this unilateral spreading growth, and after 10 days a maximum number may have spread a variable distance into the relatively sterile half of the plate. The other colonies in the first half of the plate do not show spreading growth. Isolated colonies in the second half of the plate may show spreading growth between the 4th and 10th days.

After the 10th day another phenomenon may be observed: From the primary spreading growth, which now has become more or less stationary, a secondary spreading growth may appear. In most instances this secondary spreading growth has had an arboreal texture quite different from the smooth texture of the original colony and the primary spreading growth. In only one instance have we observed a secondary spreader with a smooth texture. With some strains such as B-5 (pure nonflagellated) and to a lesser extent H-6 (pure normally flagellated, nonmotile) the arboreal spreading growth has developed after 5 to 12 days directly from colonies which up to that time had shown no evidence of spreading. The arboreal spreaders were all composed of filamentous forms indicating a nonfilamentous to filamentous mutation. The arboreal spreaders which have been fished have shown the same flagellation and motility as the parent, and those developing directly from the original colony have all been nonmotile. A filamentous form apparently can spread without motility.

Properly diluted semisolid pour plates may also be used. For convenience in handling it is best to use only about 7 ml of semisolid agar and pour it over a layer of solid agar in the plate. Motile mutants may also be detected in semisolid stab culture, but the isolation of the mutant is difficult. This method also seems to be less productive of visible mutants and so far has not shown visible spreading growth with our strains H-6 and B-5. Strain B-3E has invariably shown several areas of spreading growth along the stab after 4 days of incubation.

Types of mutants isolated. Spontaneous mutations to motile types have been observed with each of the 4 types of nonmotile variants (nonflagellated, straight flagella, small amplitude flagella, flagella of normal curvature). By culturing the spreading growth from the colonies in the semisolid agar several types of mutants have been obtained in pure culture. Only a beginning has been made on this problem, but the following will serve to show the nature of these mutants: From variant B-3E (straight flagella, nonmotile) have been isolated as primary mutants B-3E-1 with coiled flagella and fair progressive motility, similar to variant B-3D; B-3E-2 with straight flagella but tumbling motion similar to variant B-3A; and B-3E-13 with small amplitude flagella and tumbling motion. A secondary mutant B-3E-1-1 with coiled flagella, fair progressive motion, and a filamentous soma was isolated from the arboreal outgrowth from the primary spreading growth which had yielded mutant B-3E-1. From variants H-15C (nonmotile, small amplitude flagella), H-15A (nonmotile, normal flagella), H-14BB (nonflagellated) have been isolated mutants with normal flagella and normal motion. From strain B-5 (nonflagellated) have been obtained primary mutant B-5-1 with normal flagella and normal motion; primary mutant (arboreal) B-5-3 with no flagella, no motion, and filamentous soma; secondary mutant B-5-1-1 with normal flagella, normal motion, and filamentous soma; and secondary mutant B-5-2-1 with no flagella, no motion, and filamentous soma. From strain H-6 (normal flagella, nonmotile) have been obtained primary mutant H-6-1 with normal flagella and normal motion; and primary (arboreal) mutant H-6-2 which is filamentous with normal flagella but is nonmotile.

Mutation rates. Our study of this problem is not completed, and the figures given below are only preliminary. With most of our nonmotile variants (exceptions are strains B-5 and H-6) a majority of the well isolated colonies in the semisolid agar develop a spreading outgrowth within about 10 days at 20 C. This outgrowth generally appears to originate from one point on the colony and would appear to be the result of a single mutation. Plate counts showed that the colonies of Listeria on our medium contain an average of 300 million (3×10^8) viable organisms. If one motile mutant only is produced in each colony, the rate of mutation would be approximately 10^{-8} per cell division. With strains B-5 and H-6 the number of colonies which has shown spreading growth between the 4th and 10th days has been only 10 per cent or less of the total, and with these a mutation rate closer to 10^{-9} per cell division seems likely. In contrast to this we found with strain B-5

primary arboreal spreaders, frequently several to a colony, between the 5th and 12th day on practically all of the well isolated colonies, indicating a nonfilamentous to filamentous mutation rate of somewhat greater than 10^{-8} per cell division. With strain B-5 the arboreal spreaders have developed equally readily from well separated colonies on solid media as on semisolid media. More detailed studies of the mutation rate problem are in progress.

DISCUSSION

The flagellar variations observed in the genus *Listeria* would seem to be rather unusual. However, few systematic studies of this nature are recorded in the literature. Nonmotile, and probably usually nonflagellated, variants appear to be common and have been reported in many genera of motile bacteria. Flagellated but nonmotile variants have been reported by several authors (see Friewer and Leifson, 1952). Variants with a flagellar wavelength about one-half the usual (curly flagella) were first reported by Leifson (1951) in *Salmonella wichita*, and later by Leifson and Hugh (1953) in *Salmonella typhimurium*. Curly flagellated variants have also been observed by the senior author in strains of Escherichia and Bacillus. Pijper and Abraham (1954) reported 2 types of flagellar curvature in Sarcina, the wavelength of the one being about one-half that of the other. Leifson, Carhart, and Fulton (1955) reported a variety of flagellar shapes in strains of Proteus. The curious situation with Proteus strains is that curly and normal flagella frequently are found on the same individual, and pure cultures with each of the flagellar types could not be obtained. Furthermore many strains of Proteus showed mainly curly flagella when stained from a slightly acid suspension and mainly normal flagella when stained from a slightly alkaline suspension. With *S. wichita*, *S. typhimurium*, Escherichia, and Bacillus strains the pH of the suspending medium has not influenced the flagellar curvature. Leifson and Hugh (1953) reported a strain of Xanthomonas or Flavobacterium in which many of the organisms had both polar and lateral flagella of different wavelength, as well as some with only polar and some with only lateral flagella. Pure variants could not be isolated. These authors also reported a strain of "Alcaligenes" in which some individuals had lophotrichous flagella, some peritrichous flagella of different wavelength, and some which had both types of flagella. The lophotrichous type apparently mutated to the peritrichous type but not vice versa, or at least at a much lower rate. Pure variants with lophotrichous and with peritrichous flagella were isolated, but not variants with the mixed type of flagella. The latter appeared to be transitory. The senior author has occasionally observed individual organisms of several genera with straight flagella, but attempts were not made to obtain such organisms in pure culture if such was possible. Coiled flagella were reported by Leifson and Hugh (1953) in strains of Aeromonas, by Leifson, Carhart, and Fulton (1955) in strains of Proteus. Flagella with the small amplitude as seen in Listeria have not been observed by us in other bacteria.

Flagellar morphology and flagellar activity are apparently genetically independent. The most usual or normal shape of flagella is a helix with a wavelength about 4 times the amplitude. This shape seems most efficient for locomotion; and with other shapes such as straight, small amplitude and very great amplitude (coiled) the locomotion is inferior. It seems that the normal flagellar shape is usually associated with normal motility in wild strains of bacteria, and the flagellar variants observed by us and others probably occur mainly in laboratory stock cultures. It seems that when the genetic mechanism for motility is permanently (?) lost, the genetic mechanism for flagellation is also lost sooner or later.

Our work seems to indicate that the genetic mechanism for producing normal flagella is present in all strains of Listeria, including those without any demonstrable flagella. Absence of flagella and abnormal flagellar shapes may be the result of "blocks" at various stages in the synthesis of normal flagella. The genetic complexes for motility and for flagellation seem to be closely associated. This arrangement could explain the apparent concomitant change of motility and flagellar shape as a one step mutation.

The genetic complex governing cell separation is independent of both the flagellation and motility complexes. The filamentous mutants which we have isolated always showed the same type of flagellation and motility as the parent.

The difference between a nonflagellated variant which mutates at a finite rate to the flagellated, motile or nonmotile, type and a so-called permanently nonflagellated organism presumably involves, respectively, the presence and absence of a potential genetic mechanism for flagellation and for motility. This mechanism may be present, though usually blocked, in many organisms which are commonly regarded as permanently nonflagellated and nonmotile. Flagellated and motile strains are occasionally encountered in such genera as *Lactobacillus*, *Corynebacterium*, *Streptococcus*, and *Sarcina*, all of which we have personally verified. By making systematic checks for the ability of nonmotile cultures to produce motile mutants, organisms regarded as permanently nonmotile may be found to possess the genetic mechanism for motility and flagellation.

Our data have some bearing on the problem of maintaining stock cultures. Nonmotile variants which mutate to the motile form at a rate of 10^{-8} per cell division would remain nonmotile practically indefinitely if kept in broth or on solid media. In semisolid stab culture, however, the motile mutants may attain appreciable numbers in the first tube provided it is incubated sufficiently long to allow spreading growth. As a general rule motile organisms are best kept motile if maintained in semisolid agar, and nonmotile organisms are best kept nonmotile by culturing in broth or on solid media. Prolonged incubation in semisolid or solid agar may lead to the production of filamentous mutants in great numbers. Broth culture seems best to reduce this type of change to a minimum.

SUMMARY

A study has been made of the flagellar morphology and motility of 81 stock strains of typical Listeria. Best motility and flagellation were found at relatively low incubation temperatures, such as 20 C, and with few flagella and poor motility at 37 C. The flagellar arrangement is peritrichous. Seventy of the 81 strains showed only what we have labeled normal motility and normal flagellation. The normal flagella had a mean wavelength of 2.01 μ +/− 0.01 μ, and a mean amplitude of 0.48 μ +/−0.007 μ. A semisolid agar technique is described for detecting and isolating motility variants and mutants. Flagellar and motility variants were found in 11 strains. From these were isolated 7 different types of variants which are described and illustrated. From the 4 types of nonmotile variants have been isolated several types of motile spontaneous mutants which are described. Preliminary data indicate that flagellar and motility mutations occur at rates in the neighborhood of 10^{-8} to 10^{-9} per cell division. Filamentous spontaneous mutants have been isolated from several of our nonfilamentous strains. These invariably have shown the same flagellation and motility as the parent stock. The problem of maintaining stock cultures is discussed.

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