NONMOTILE VARIANTS OF BACILLUS ALVEI[1]

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The ability of *Bacillus alvei* to form migrating colonies upon the surfaces of solid media free from excessive moisture was reported previously by Smith and Clark (1938).[2] A similar property has been noted by Roberts (1935) for *Bacillus rotans*. Recently Shinn (1938), in a cinematographic study of colony motility of *B. alvei*, remarked that it was surprising that such motility had been so long overlooked, and questioned whether the phenomenon applied to *B. alvei* or to a bacterium described as "*Bacillus helixoides*" by Muto (1904).

Following our original demonstration of colony motility in *B. alvei*, additional cultures, isolated from foulbrood of bees and labelled *B. alvei* or *Bacillus para-alvei* have been received which likewise show colony migrations. All of our own isolates from soil, considered identical with named cultures of *B. alvei*, have shown motile colonies. Reports of earlier workers (Cheshire and Cheyne, 1885; Harrison, 1900; White, 1906; Tarr, 1935) upon *B. alvei* contain descriptions of its growth upon solid media which suggest that the phenomenon of colony motility had occurred, even though the actual migration had not been observed. Such references, together with the motility of authentic cultures, have led to the present study to determine whether colonies of *B. alvei* always show motility under suitable conditions.

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ISOLATION OF COLONY VARIANTS

The culture of *B. alvei* employed in these studies was obtained from J. I. Hambleton, Bureau of Entomology and Plant Quarantine, Beltsville, Md. This culture, found morphologically and culturally identical with a second culture from Hambleton and with a culture of *B. alvei* obtained from A. G. Lochhead, Central Experiment Farm, Ottawa, has been under observation for a period of 20 months for variations in its colony motility.

Isolation of variants from this culture was accomplished by streaking single lines of inoculation across Petri dish surfaces of sterile nutrient agar. After incubations of from 1 to 4 days at room temperatures, such surfaces were covered by migrating colonies. Occasional colonies could be observed which exhibited more elevated or more mucoid appearances. Upon re-streaking these to subsequent plates, it was possible to obtain, after a few subcultures, plates showing colony formation limited to the line of inoculation. In further subculturing, such daughter strains quite frequently showed motile out-growths at one or more points along the line of inoculation, and it was only after continued selection of nonmotile colonies that strains were established which could be carried for successive subcultures on solid agar without showing motile habit of growth.

PROPERTIES OF NONMOTILE VARIANTS

The nonmotile variants showed the cultural, tinctorial and physiological responses of the motile types except in case of the following characteristics:

Appearance of growth on solid agar

Nonmotile strains produced round, raised, convex colonies on solid agar, with growth limited to the line or point of inoculation. On the other hand, motile strains produced many irregular colonies, frequently vermiform or helicoid, and growth was not limited to the line or point of inoculation.

Motility

Hanging drop mounts prepared from nonmotile colonies did not show the presence of motile organisms; in contrast, active motility was exhibited by cells from motile colonies.

Flagella

From motile colonies, peritrichic flagellation of cells could be demonstrated readily; on the contrary, efforts to stain flagella on cells obtained from nonmotile colonies failed repeatedly, although areas of precipitation or of light staining were obtained about the individual cells.

Capsules

Using the India ink method of demonstration, cells from nonmotile colonies showed the presence of large capsular areas, in contrast to the lack of such distinct encapsulation in the motile strains.

Glucose broth

Nonmotile variants produced a granular type of growth, with sediment, whereas motile variants produced an early uniform turbidity following inoculation.

In all physiological responses the two types were found similar. Differences were not observed in sizes of cells or spores, or in numbers of spore-bearing rods or free spores in cultures of comparable ages. In stained smears prepared from suitably aged nutrient agar cultures of either the motile or nonmotile type, long rows of cylindrical spores, with the long axes of the spores lying parallel, were observed. The formation of such rows of spores by *B. alvei* has been noted frequently by earlier workers (McCray, 1917; Lochhead, 1928; Tarr, 1935), and is considered almost diagnostic of this species.

STABILITY OF NONMOTILE VARIANTS

The nonmotile habit of growth was difficult to establish, but once obtained, nonmotile variants were relatively stable on solid

media. Such variants could also be passed through broth for limited periods without loss of their characteristic nonmotile form of growth. Suspensions of their spores boiled for from 5 to 10 minutes and plated again gave rise to nonmotile colonies. On the other hand, after several transfers through broth media, after aging of a single culture in broth for several days, or occasionally spontaneously, nonmotile variants would again show the motile habit of growth. In contrast, motile strains were never observed to become spontaneously nonmotile, either after boiling of spores, after subculturing on various common laboratory media, or after incubations of inoculated plates under different conditions of light, temperature or position.

DISCUSSION

These studies upon *B. alvei* have shown that, even when conditions suitable for colony migration exist, nonmotility of its colonies may occur. The tinctorial and cultural evidence indicates that lack of motility in selected cultures is associated with an excessive amount of capsular or extracellular material. The production of variants unusually well endowed with capsular material raises again the question of the importance of extracellular material in the phenomenon of colony migration generally. Roberts (1935) has suggested that the migration of colonies of *B. rotans* may result from the action of flagella within a watery secretion produced by the cells themselves. A limited amount of cohesive extracellular material could undoubtedly contribute to the unity exhibited by a number of cells grouped into a mobile unit.

The occurrence of long rows of spores in stained preparations from nonmotile cultures, identical to rows of spores produced by motile cultures, is of especial interest. In direct microscopic observation of motile colonies, the individual bacilli are observed to lie parallel to one another, an arrangement significant in the motility of a group of cells as a unit, and it is not surprising that in stained preparations spores are observed lying in lateral rows. The appearance of similar rows of spores in stains from nonmotile colonies indicates an orientation of cells, even though actual migration does not occur.

The greater stability of the motile over the nonmotile type of growth makes it plausible to assume that earlier descriptions of plate growths of *B. alvei* were based on cultures showing motility of colonies, even though the actual phenomenon of migration was not observed. Cheshire and Cheyne (1885) noted that on gelatin plates outgrowths from the line of inoculation occurred, that such outgrowths might grow round so as to form a circle, and that from such circles other fresh circles might be formed. Harrison (1900) observed spreading and repeated branching of growth on agar plates, and believed the seaweed appearance "distinctively characteristic, and as the growth is very rapid, this method commends itself for making a quick diagnosis. . ." That White (1906) possibly encountered colony motility is indicated by his observation that colonies were often "surrounded by numerous smaller but similar growths." More recently, Tarr (1935) has noted that on agar (dried), "there is marked spreading, the growth appearing as a mass of colonies over the surface of the medium."

These references suggest that the spreading habit of growth has long been considered characteristic of *B. alvei.* Some observations on variability in this species have also been noted. Lochhead (1928) reported an asporogenic habit of growth by *B. alvei* on certain sugar-containing media, and that the further aging of the non-sporulating rods on suitable media produced coccoid types, relatively stable in further culture. Burnside (1934) produced asporogenic cultures of *B. alvei* by repeated passages through broth, and noted that such cultures were nonmotile. Whether the sporogenic nonmotile variants which we have secured on ordinary nutrient agars are related in any manner to, or are intermediate between, the streptococcus-like types noted by Lochhead or the asporogenic nonmotile types noted by Burnside is not known.

Shinn (1938) has questioned whether *B. alvei* and *Bacillus helicoides* (the latter described by Muto in 1904 as *B. helixoides,* and amended to *B. helicoides* by Kitasato (Lehmann and Neumann, 1931)) are not "one and the same," noting that his strain (*B. alvei* A. T. C. C.) "would, on cursory examination, answer

best to Muto's description." Apparently Shinn did not fully consider the original description, because *B. helicoides* was described as a non-sporing gram-negative rod, failing to withstand heating to 60°C. for 10 minutes, and failing to attack gelatin or milk. Lehmann and Neumann (1931) have considered *B. helicoides* to be related to *Proteus vulgaris*; it should not be confused therefore with *B. alvei*. It has been noted previously by Smith and Clark (1938) that colony motility is not limited to the genus *Bacillus*; the colonies of a *Bacterium* isolated from the intestinal tract of an angle-worm showed motility.

Russ-Munzer (1938) has also reported aerobic gram-positive spore-bearing rods showing colony migrations. Her cultures have been identified by N. R. Smith (personal communication) as *B. alvei* and as *Bacillus circulans*.

SUMMARY

From a culture of *Bacillus alvei* showing motile colonies on dried agar surfaces, variant daughter strains which fail to show colony motility were obtained by selective picking. Such nonmotile variants showed reversion to the motile type when aged in glucose broth.

Lack of colony motility is associated with lack of demonstrable flagella, the presence of a large amount of extracellular or capsular material, and with a granular, rather than a turbid, type of growth in broth. Otherwise, the nonmotile variants were identical with the motile parent culture, even insofar as showing orientation of spores in long lateral rows.

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