A NEW SPECIES OF THE GENUS BACILLUS EXHIBITING MOBILE COLONIES ON THE SURFACE OF NUTRIENT AGAR

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Received for publication, July 23, 1934

During an investigation of the intestinal flora of several termites from central Texas, a characteristic bacterial species was frequently observed. The uniformity of its occurrence seems to indicate that this organism is a normal inhabitant of the termite intestine. Isolation was accomplished by dissecting the intestine, mashing its contents into a drop of sterile water, and streaking the diluted excreta over the surface of nutrient agar. Subsequent studies on pure cultures have shown that the organism is not identical with any which has been found previously reported in the literature. It appears to be an undescribed species belonging to the genus *Bacillus*. The following are the principal diagnostic characteristics of the organism:

CULTURAL CHARACTERISTICS

Nutrient agar plate. The growth of the organism on the surface of nutrient agar is only moderate, even under the best conditions for cultivation. Young colonies are translucent and watery. Due to certain characteristic behavior of young colonies, a more detailed description of their sculpture is rendered impossible. Older colonies (three to five days) are slightly opaque and of butyrous consistency.

The outstanding characteristic of the organism is the mobility of young colonies on the surface of nutrient agar entirely free from excess moisture. This mobility expresses itself in two characteristic forms. In certain colonies a clearly visible rotary motion occurs, whereas in other cases the entire colony, consisting

of thousands of cells, may exhibit a migratory mobility, moving over the agar at the rate of 0.01 mm. per second. Although the movement of such colonies is not visible to the unaided eye, in later stages the colonies and the paths over which they have traversed may be seen without the aid of a lens (fig. 1).

In order to determine the nature and cause of mobility, the series of morphological changes undergone by the colony during its development was studied in detail. An agar plate was inoculated from a glucose broth culture of the organism in the maximum growth phase. The colonial development was observed with the low power objective (× 150) at ten-minute intervals for the following thirty hours.

After five hours of incubation the colonies were microscopic, each consisting of a single-layered, somewhat loosely arranged plate of cells. After about seven hours the first indications of motility appeared. The initiation of movement in different colonies was almost simultaneous, practically all the colonies being somewhat mobile after seven and one-half hours. The cells of a non-mobile colony could not be made to move by smearing the edge of the colony with a probing needle. Movement within the colony usually began at a short distance inward from the periphery. With few exceptions the cells at the outer boundaries of the colony were non-motile. In some cases this periphery was never broken, movement being limited to the interior. In this event motility expressed itself first through unorganized slowly milling masses of cells which finally synchronized their motion and produced either levo- or dextro-rotary plates, the cells of which changed their relative positions but little or not at all (fig. 4). The rotation caused the colonies to become somewhat rounded.

In the majority of cases the periphery was broken by the moving cells before a round colony was formed. The release of the cells from the inner portion allowed the colony to move over the agar with a rapid migratory mobility. Such colonies assumed the shape of a bullet with a round but somewhat pointed anterior portion and a deeply concave posterior portion. From the edges of these colonies cells were constantly being lost, thus forming a

"colony track" composed of two parallel streaks seeded by the moving colony (fig. 2). The cells which were detached from the mother colony immediately became non-motile; a mass of cells seemed necessary for motility. Subsequent growth of the detached organisms made the tracks clearly visible during later stages of growth (fig. 1). Migratory colonies always described arcs, or spiral paths until the cell mass finally stopped and rotated in the center of the described spirals (fig. 2). Smaller sub-colonies sometimes broke from the mother colony and moved over independent courses.

After eighteen hours the rotating colonies were composed of numerous concentric plates of cells stacked upon each other in pyramidal fashion (fig. 3). After thirty hours the motility had ceased. After forty hours the surface of the colonies became smooth or slightly contoured, rising above the agar in helicoid fashion.

Sub-surface colonies show no tendency to be mobile and are ellipsoid, often with a fuzzy growth at one end. Various consistencies of agar have been tested and the organism has been found motile in every case. The agar is never liquefied.

No entirely satisfactory explanation for the mobility of the colony is offered. Observation of the morphological changes undergone by the colony has yielded insufficient evidence to warrant definite conclusions. The nature of the movement would indicate that it is due to the action of the flagella within a watery secretion produced by the cells themselves. The unique character, or combination of characters, which enables this organism to exhibit mobility on dry solid media, exclusive of other motile, mucous-forming species, has not been determined. It is possible that the cells become entangled by their flagella to such an extent as to form a more or less connected cellular mass similar to such colonial forms as Volvox or Pandorina. Mere entangling of the flagella would serve to explain the tenacity with which the colony is held together during migration but would not in itself explain the unified synchronism of motility. We offer no explanation for the harmony which exists between the moving cells but merely call attention to the fact that when the organisms are held in

close proximity as they are on the surface of nutrient agar, maximum inter-cellular influences are exerted.

The rotary movement and the spiral migratory mobility of the colony as a whole are in harmony with an unexplained tendency toward spiral growth and motion which occurs throughout nature. The common occurrence of spirality in animals and plants tends to support the view that it is due to something fundamental in organized matter.

Plain broth. Growth in nutrient broth after twenty-four hours of incubation is moderate with an even clouding. Older cultures have a delicate pellicle and a slightly viscid sediment.

Agar slope. The growth is moderate, spreading and translucent.

Gelatin. Gelatin is completely liquefied after thirty days incubation.

Litmus milk. There is a slight reduction of the litmus in the bottom of the culture tube after ten days of incubation. Otherwise no change is noticeable.

PHYSIOLOGICAL CHARACTERISTICS

Fermentation. Peptone water was adjusted to pH 7.2, sterilized, and various carbohydrates in distilled water solutions were added in 0.5 per cent amounts under aseptic conditions. A stock strain of the organism, which had been in culture for three months was used in the tests. An alkaline reaction was produced in all the carbohydrate media tested. These reactions have been consistent on repeated tests. Quantitative tests for the presence of glucose indicate that this carbohydrate is utilized without acid production. Starch-agar plate cultures of the organism, flooded with iodine after four days of incubation, gave no indication that starch is hydrolyzed. Cellulose is not decomposed. No acid is produced from mannitol, sorbitol, adonitol or dulcitol.

Indol production. No indol was present in seventy-two-hour tryptophane broth cultures tested by the method of Goré.

Production of hydrogen sulfide. Hydrogen sulfide is not produced in lead acetate agar stabs as indicated by the failure of the medium to blacken within ten days.

Reduction of nitrates. Cultures prepared on nitrate agar slopes and subsequently tested for the presence of nitrites by the sulphanilic acid-naphthylamine method showed that nitrates are not reduced to nitrites.

Temperature relations. The organism grows best at 22°C.; growth and sporulation are markedly inhibited at 37°C. There is no motility at 37°C.

Oxygen relations. Best growth occurs under aerobic conditions. Some growth was obtained, however, under anaerobic conditions produced by oxidising phosphorus in a sealed jar.

MORPHOLOGY

Vegetative cells cultured on nutrient agar at 22°C. for twenty-four hours are from 3 to 5μ in length by 0.5μ in diameter. The cells occur singly and in short chains. The ends of the cells are rounded. Terminal endospores are formed after forty-eight hours growth on nutrient agar; swellings on the vegetative cells at the site of spore formation indicate the initial steps in sporulation (fig. 5). The mature spore is quite large, almost entirely filling the swollen sporangium, and is spherical to ovoid in shape.

In liquid media very active motility is accomplished by means of numerous peritrichic flagella.

The Gram reaction is negative in cultures varying in age from 9 to 36 hours when stained by that variation of the Gram stain suggested by Kopeloff and Cohen (1928). The bacteriostatic action on the organism, however, is typical of that on Gram-positive forms, growth being inhibited in crystal violet diluted 1:200,000. We realize that Gram-negative, spore-forming aerobes are unusual if they occur at all. Since Gram-variable forms have been shown to exist, and in view of the atypical bacteriostatic action, we hesitate definitely to characterize this organism as Gram-negative in spite of its consistency with regard to the technique employed.

DISCUSSION

This organism does not seem to agree in all particulars with any species heretofore described. According to Bergey's Manual

(1934) there are only eight aerobic, facultative, mesophilic, motile bacilli with terminal spores which cause a swelling of the vegetative cell at sporulation, and which do not produce a yellow pigment. The organism is definitely different from these eight. It differs from all the described species within this group with regard to optimum temperature and the mobility of colonies on the surface of nutrient agar. The characteristics of the organism are not in agreement with those of any of the eight, even with regard to the more salient characteristics. In view of these facts introduction of this organism as a new species is believed justified and the descriptive name *Bacillus rotans* is suggested. Cultures of this organism have been placed in the American Type Culture Collection.

While many of its characteristics, particularly the mobility of colonies, are strongly suggestive of the *Myxobacteriales*, we believe that the organism is a true bacterium. Failure to produce fructifications after long incubation and the ability to form flagella and endospores are evidence for placing the organism in the genus *Bacillus*.

RÉSUMÉ

Bacillus rotans, n.sp.

A medium sized rod with rounded ends, occurring singly and in short chains; actively motile by means of numerous peritrichic flagella; characteristic mobility of colonies on the surface of nutrient agar; terminal spores which cause a marked swelling of the vegetative cell at sporulation; no pigment produced; gelatin liquefied after thirty days; no coagulation or digestion of milk, but reduction of litmus; no hydrogen sulfide or indol formed; nitrates not reduced; alkaline in all carbohydrate media; optimum temperature 22°C.; oxygen relation, aerobic preference; Gram-negative but sensitive to dilute crystal violet.

REFERENCES

BERGEY, D. H. 1934 Manual of Determinative Bacteriology. Williams & Wilkins Co., Baltimore.

KOPELOFF, N., AND COHEN, P. 1928 Stain Tech., **3**, 64.

PLATE

PLATE 1

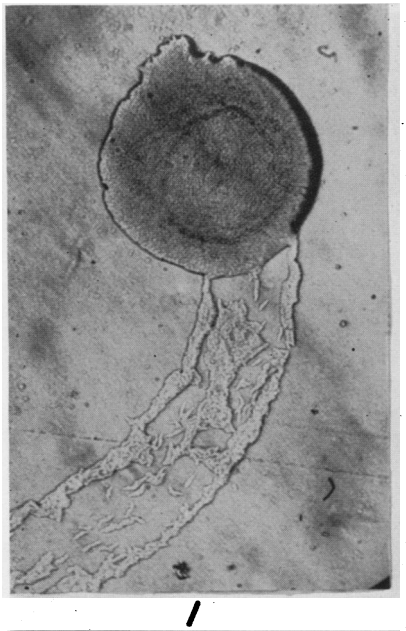
FIG. 1. Old migratory colony after the period of active mobility, showing growth of detached cells in the path of the colony. × 150.

FIG. 2. Migratory colony in active mobility, showing the spiral path of the colony. × 150.

FIG. 3. Old rotating colonies trapped in a mass of cells, showing concentric plates of cells stacked in pyramidal fashion. × 150.

FIG. 4. Typical rotating colony after twelve hours growth on nutrient agar. × 150.

FIG. 5. The individual cells after forty-eight hours growth on nutrient agar, showing the swollen sporangium. × 1500.



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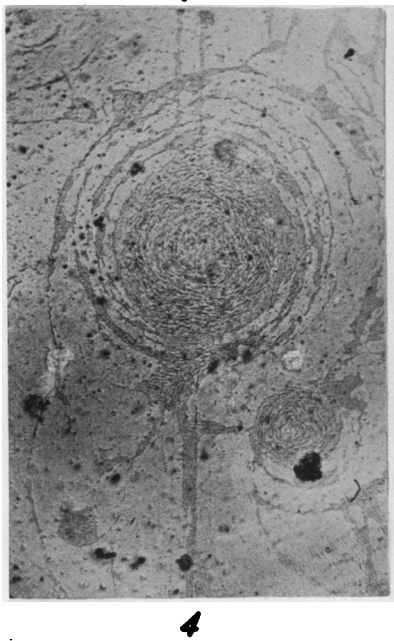

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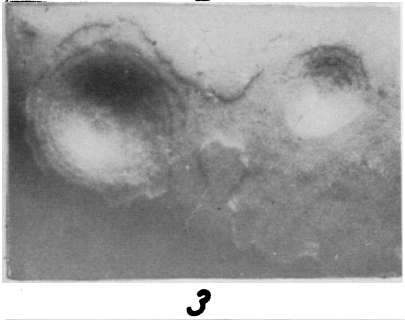

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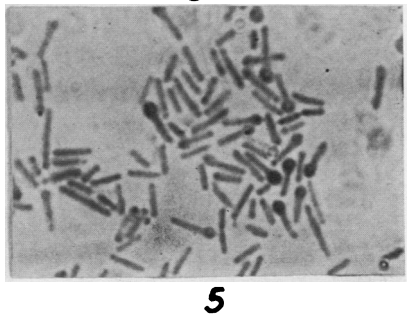

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(J. Lemoyne Roberts: New Species of Genus Bacillus)