OBSERVATIONS ON THE MORPHOGENESIS OF ARTHROBACTER CITREUS, SPEC NOV

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The genus *Arthrobacter* was proposed by Conn and Dimmick (1947) to include the strictly aerobic, gram variable members of the *Mycobacteriaceae* (into which were also placed the genera *Corynebacterium* and *Mycobacterium*) existing as rods in young culture and as cocci in older cultures. Taxonomic studies of an unidentified bacterium used in this laboratory for the assay of subtilin indicated that it belongs to this genus and moreover constitutes a new species of this genus.

The organism is described in the present communication and the name *Arthrobacter citreus*, spec nov, is proposed for it.[2] In addition, the morphological development of the organism was studied in shake cultures, which permitted a correlation of the morphological types with the growth curve as determined by colony counts and cell nitrogen.

METHODS

The shake culture experiment was carried out in Erlenmeyer flasks, incubated at 25 C on a rotary shaking machine. The medium had the following composition, in grams per liter: K_2HPO_4, 2.18; KH_2PO_4, 1.68; NaCl, 1.67; glucose, 1.0; yeast extract, 5; NZ amine, 20. Cell counts were made by plating on nutrient agar (Difco) after agitating the dilution bottles vigorously on a mechanical shaker to break up clumps. Cell nitrogen was determined by micro-Kjeldahl analysis on cells washed twice in physiological saline.

Photomicrographs of the live cells were made with the phase microscope using a medium dark contrast objective. The cells were centrifuged first in order to concentrate them and then were resuspended in water to which 0.25 per cent of carboxymethyl cellulose had been added to eliminate Brownian motion. Also, safranin stained smears were photographed in bright field with a green filter.

The optimum pH level for growth was determined by cultivating the cells in shallow layers of a liquid medium containing yeast extract, tryptone, $MgSO_4$, and K_2HPO_4, adjusted to pH levels ranging from 6.0 to 9.0.

Standard methods of identification were employed to determine the taxonomic position of the organism.

DESCRIPTION OF ARTHROBACTER CITREUS, SPEC NOV

Morphology. Gram variable rods, 0.8 by 5 μ, frequently in V-shaped pairs, becoming coccoid, 0.7 μ diameter, usually within 24 hours or less. Feebly motile prior to fragmentation.

Colonial characteristics. Agar streak: filiform; agar colonies: entire, low convex. Pigment: lemon yellow, becoming intense as the culture ages; does not diffuse into the medium; insoluble in ether or acetone.

Physiology. Strict aerobe; catalase positive. No appreciable acid production on sugar media; glucose is oxidized by resting cells grown in its presence (manometric test). Optimum temperature, 25 to 32 C. Growth at 35 C is reduced very markedly. The organism will grow at temperatures as low as 10 C.[3] Slow, crateriform gelatin liquefaction. Nitrates reduced to nitrites. Indole is not formed. Starch is not hydrolyzed. No change in litmus milk. No cellulolytic activity.[4] Optimum pH range is between 7.3 and 8.5 or 9.0. Scant growth at pH 6.5 or below. No growth on inorganic nitrogen media in the absence of ac-

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[2] A culture has been deposited with the American Type Culture Collection.

[3] First observed at the Northern Regional Research Laboratory.

[4] The author is indebted to Dr. F. E. Clark for this observation.

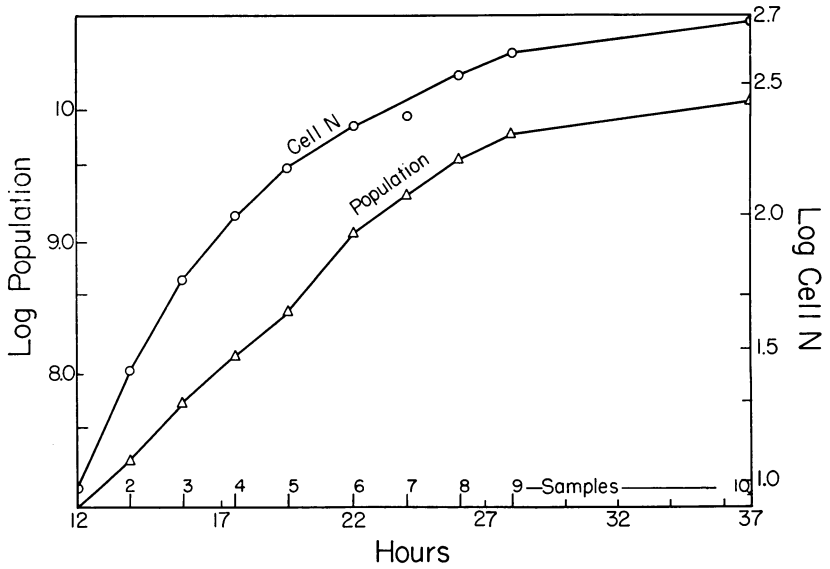

Figure 1. Growth curves of *Arthrobacter citreus*, as determined by colony counts (per ml) and cell nitrogen analyses (Ng cell N per ml). The numbers shown on the abscissa, above the time units, represent the sample designations for the samples taken at that particular time.

cessory growth factors.[5] This bacterium, heretofore designated as C7, was isolated by D. M. Reynolds at this laboratory in 1948 while searching for suitable subtilin assay organisms. The organism was isolated from chicken feces, but its temperature optimum makes it seem almost certain that it was a contaminant from dust or soil.

GROWTH IN SHAKE CULTURES

The growth and morphology of *A. citreus* were followed in shake culture. At two hour intervals, total viable counts and total cell nitrogen were determined; simultaneously photomicrographs were made to demonstrate the organism's morphology. In constructing a growth curve for this organism, conventional viable counts by themselves would be inadequate. In shake culture, *A. citreus* tends to grow in clusters when young, giving deceptively low counts; moreover, the fragmentation which breaks the rod forms into coccoid forms as the culture ages is not the sort of bacterial multiplication commonly encountered. Counts tend to show a sharp upswing at this point. Therefore, "growth curves" obtained from both viable counts and total cell nitrogen are shown in figure 1. Sample 6 (22 hours) shows a count sufficiently high to make the slope of the curve increase markedly; fragmentation has occurred here. Fragmentation is shown even more clearly if the *rate* of viable

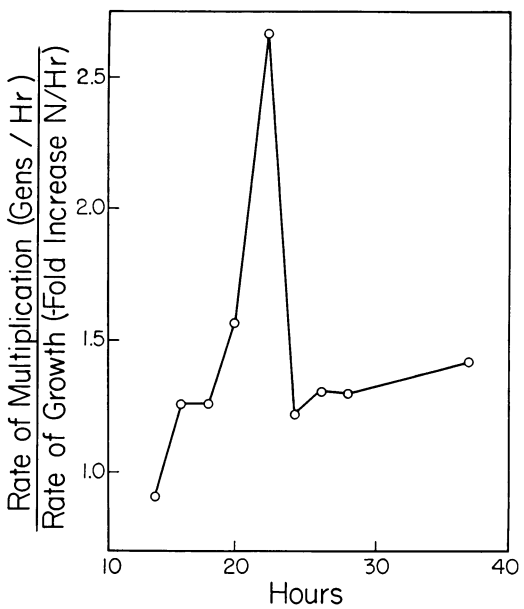

Figure 2. Ratio of multiplication rate to growth rate. Sharp increase at 22 hours (sample 6) indicates that fragmentation has occurred.

[5] The organism has been grown successfully on a medium having the following composition, in grams per liter: 1(+)-sodium glutamate, 20; KH_2PO_4, 0.5; K_2HPO_4, 0.5; $MgSO_4$, 0.2; $CaCl_2$, 0.1; yeast extract, 0.5; and $FeCl_3$, 0.006 (added separately after sterilization).

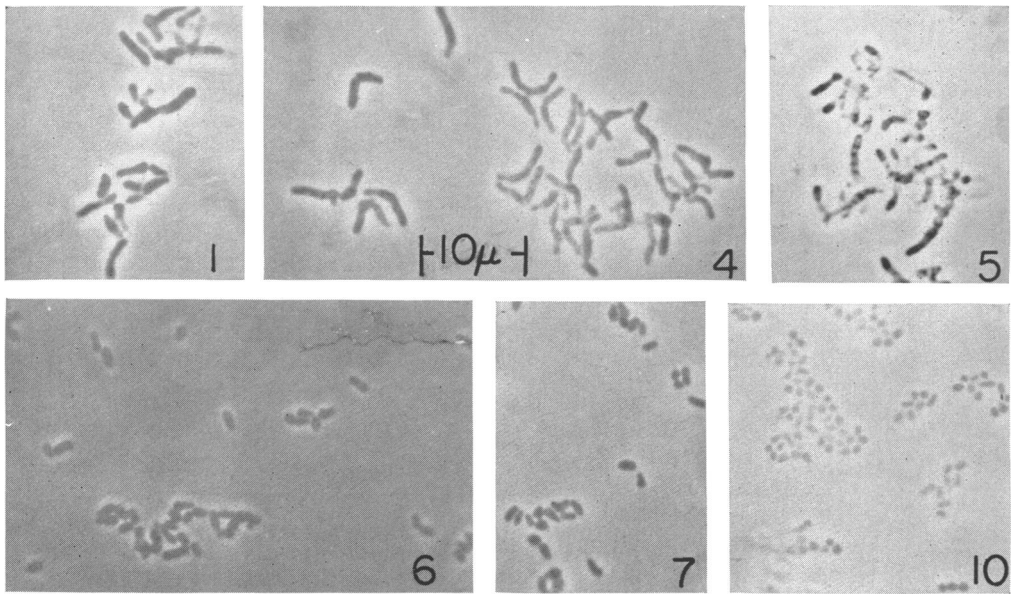

Figure 3. Some stages in the morphogenesis of *Arthrobacter citreus*. Wet mounts, dark phase contrast. Sample designations in lower part of photos indicate location on growth curve (figure 1). 1,455 ×.

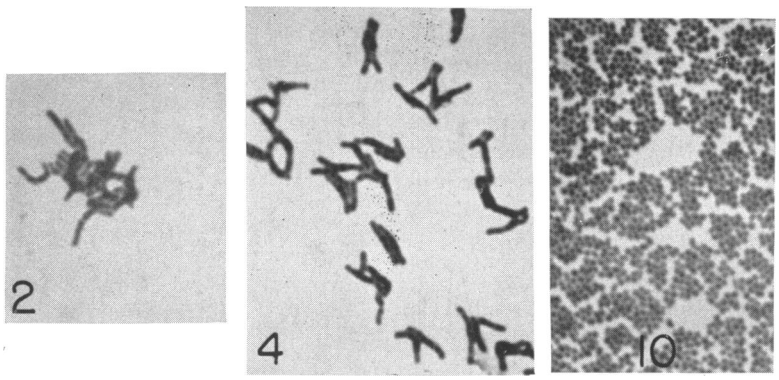

Figure 4. Safranin stained smears of *Arthrobacter citreus*. Sample designations in lower part of photos indicate location on growth curve (figure 1). 1,425 ×. Green filter.

cell increase per hour (commonly called the rate of multiplication expressed as generations per hour) is compared with the rate of cell N increase per hour (sometimes called the rate of growth). Figure 2 shows the ratio of multiplication rate (expressed as generations per hour) to the growth rate (expressed as "fold" increase of N per hour). The sharp peak at 22 hours corresponding to sample 6 indicates that many new viable cells have been formed without an equivalent amount of protoplasmic synthesis; that is, fragmentation has occurred. From the figure it appears that the rod forms probably produce 2, 3, or even 4 fragments per cell. The photographic evidence (figure 3) is in harmony with this hypothesis. Sample 5, taken at 19.5 hours just prior to fragmentation, shows transverse bands or septa forming in the rods. Sample 6, taken at 22 hours, shows much shorter rods and coccoid forms. During the next few hours, the short rods become progressively shorter until they are indistinguishable from cocci.

The resemblance to true cocci is shown even more clearly in the safranin stained preparations (figure 4, sample 10). The stained rod preparations (figure 4, samples 2 and 4) show several densely staining areas within a single rod. Dark field examination of these rods, however, clearly shows only one cell membrane encompassing the entire rod.

The rod forms show a slight tendency to branch, and occasionally buds can be seen. The V-shaped pairs observable in sample 4 are very typical of the later stage of rod development and are probably an indication of snapping division, prevalent among the related *Corynebacteria*. Topping (1937) also remarked on this point.

As already indicated, the rods are only feebly motile, and in shake cultures motility occurs for only a short period prior to the appearance of cocci. The latter are not motile.

DISCUSSION

The *Arthrobacter* strain described here does not appear to fit the description of any of the three species listed by Conn and Dimmick (1947). It resembles *Arthrobacter helvolum* most closely but differs in the following respects: (a) it will not grow on a chemically defined medium without added growth factors, (b) it does not hydrolyze starch, and (c) agar growth, examined microscopically, often resembles a pure *Micrococcus* culture. Recently, Lochhead and Burton (1953) described a strain of *Arthrobacter* (*A. terregens*) which requires accessory growth factors but differs from our organism in its inability to liquefy gelatin, its yellow-brown pigment, nonmotility, and the need for growth factors not present in yeast extract.

Although Conn and Dimmick (1947) included in their definition ability to grow on inorganic nitrogen without added accessory growth substances, Lochhead and Burton (1953) have now extended the genus to include more fastidious forms. Jensen (1952) contended that placing the nonexacting species in a separate genus *Arthrobacter* seemed somewhat arbitrary; however, Lochhead (1948) and Clark (1952) pointed out the desirability of creating a separate genus for the soil globiform organisms, in order to prevent the genus *Corynebacterium* from becoming too inclusive and unwieldy. Clark (1952) also mentions that *Corynebacterium* cultures could, almost without exception, be differentiated culturally from cultures of *Arthrobacter*. The fact that the *Arthrobacter terregens* (Lochhead and Burton, 1953) does not liquefy gelatin recalls a sentence of Clark (1952): "The finding of occasional intermediate strains that are difficult to classify is by no means limited to the group of bacteria under discussion, nor does it invalidate the use of *Arthrobacter* for the soil *Corynebacteria*."

The fragmentation type of cell multiplication described in the past for several coryneform bacteria is amply confirmed with our strain of *Arthrobacter* by the cell count per cell N ratios, as well as by the photographic evidence, especially the evidence for septa formation. The multiplication processes appear to resemble those described diagrammatically by Krassilnikow (1934) and Topping (1937).

ACKNOWLEDGMENT

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SUMMARY

A bacterium, useful in the assay of subtilin by the cup plate technique, has been identified as a member of the genus *Arthrobacter*. A description of the organism, which apparently belongs to a hitherto undescribed species, is given. The name *Arthrobacter citreus*, spec nov, is proposed for it.

The growth and morphogenesis of the organism were followed by viable cell counts, cell nitrogen determinations, and photomicrography on both living and dried, stained cell preparations. The fragmentation type of multiplication, which resulted in the transformation of the rods into coccoid forms, was clearly shown, and the morphological types were correlated with the growth curve.

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