MORPHOGENESIS IN ARTHROBACTER SPECIES

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ABSTRACT

This paper presents data to show that arthrobacterial morphology depends upon the nature of nutrients in the culture media and the age of the cultures. Biotin has been shown to be a key nutrient affecting morphogenesis. Angular growth due to subpolar and bipolar germination and germination of two adjacent cocci have been indicated as the cause for the appearance of ‘V’ forms in Arthrobacter. Life-cycle of A. ruber n. sp. has been photomicrographically illustrated.

INTRODUCTION

In the general introduction to the genus Arthrobacter Bergey’s Manual (1957) describes the member species as follows:

“In young cultures the cells appear as rods which may vary in size and shape from straight to bent, curved, swollen or club-shaped forms; snapping division may show angular cell arrangement. Short filament formation with rudimentary budding may occur, especially in richer liquid media. Gram negative or Gram variable coccoid cells are characteristically observed in cultures after one or more days.”

Skerman (1959) held the view that true persisting branching in Arthrobacter has never been observed and suggested that this should be the guideline between Nocardia and Arthrobacter, provided in the latter organism rod forms get completely transformed into cocci or coccoids.

The morphological features and life-cycle of A. globiformis, the type species, has been studied by several workers (Conn and Dimmick, 1947; Stevenson, 1961; Veldkamp et al., 1963). Mulder (1963) examined in detail the morphological pattern in A. globiformis and made a comparison thereof with that of Mycobacterium phlei, Brevibacterium linens and Cellulomonas species.

Sacks (1954) observed a correlation between morphogenesis and growth of A. citreus. Sguros (1955; 1957) reported on the life-cycle of A. oxydans and the origin of ‘V’-shaped cell arrangement. The origin of ‘V’ forms
was also traced in *A. astrocyaneus* by Starr and Kuhn (1962). Chaplin (1957) described the life-cycle of two species, *viz.*, *A. pascens* and *A. terregenes* and Sundman (1958) made a comparative morphological study of six other species.

The abnormal morphological patterns due to nutrient deficiency conditions have also been described in this genus. Chaplin and Lochhead (1956), for example, observed on the abnormal morphology caused by vitamin B$_{12}$ deficiency and Chan (1964) that due to biotin deficiency. Likewise, the development and significance of the enlarged ‘cystites’ in *A. globiformis* and *A. pascens* species was explained by Stevenson (1963) as the result of passage of cells grown in a rich to a minimal medium. The purpose of this paper is to present the general observations made by the authors on the morphology of several *Arthrobacter* strains and to illustrate the life-cycle of *Arthrobacter ruber* n. sp. in particular.

**MATERIALS AND METHODS**

**Arthrobacter cultures.**—One strain each from 3 groups of *Arthrobacter*, *viz.*, *Arthrobacter ruber* group I (Strain 1); *Arthrobacter* group X (Strain 75) and *Arthrobacter* group XI (Strain 86) was chosen for this investigation. Characteristics of all the groups have been dealt with elsewhere (1966).

**Media.**—The cultures were cultivated both in liquid and solid media. For studying the life-cycle, nutrient agar medium was used throughout. Effect of biotin was recorded by growing them in a chemically defined medium containing glucose as the carbon source.

**Methods.**—For observing continuously the morphology, agar plates were inoculated by spreading a small volume of a suspension of the stock culture over the surface. Excess of the suspension was poured off before the plates were incubated at room temperature (20–28° C.). At various time intervals, agar blocks were cut out of the plates and contact prints were made for microscopical examination. In old cultures ordinary heat-fixed smears were prepared.

**Staining procedures.**—Gram staining was performed as described in the *Manual of Microbiological Methods* (1957). Metachromatic granules were stained by Loffler's methylene blue. Cell-wall stained preparations were made by the method of Webb (1954).

**Photomicrography.**—The microscope employed for observation and photomicrography was the Bausch and Lomb Research Microscope; a X 90 oil immersion objective and a X 10 eyepiece were used and the selected
fields were photographed at full or half their magnification on Kodak Microfile 35 mm. panchromatic film using Leica camera attachment. Final magnifications reached are indicated in the text.

RESULTS AND DISCUSSION

Morphological Patterns in Arthrobacter—A General Consideration

The arthrobacterial cell morphology was found to depend on the nutrient supply and the age of the culture. During the first six hours of incubation on nutrient agar, Arthrobacter formed long, filamentous, irregular, sometimes branched, rods arising from single oval-shaped coccus which acted as resting cell or spore. During first 24 hours of incubation the long filamentous rods gave rise to short pleomorphic rods and it is at this stage that the ‘Chinese letter’ cell morphology was observable. During the next 24 hours of growth all the rod forms were converted into either uniform small cocci in irregular bunches (appeared as micrococi) or as a mixture of very short coccobacillary rods and spheres. Large cocci, known as cystites, appeared in older cultures (48 hours old) especially in the liquid medium.

Germination of Coccoid Cells

On transference to a fresh nutrient medium the germination of cocci and cystites presented a characteristic, typical of Arthrobacter. The coccoids first grew in size and appeared swollen. After 2–4 hours they formed germination tubes, one, two, or even three in number from a single coccoidal element. These then developed into long rod-shaped cells. Large cocci, known as cystites, showed evidence of formation of two germ tubes from two ends which then developed into a long, curved or 'V'-shaped cell. Depending upon the number of germ tubes, the cells assumed a short branching or rudimentary budding appearance. These branching forms were, however, not persistent as in Nocardia, for soon after their formation, they fragmented into long pleomorphic rods which slowly got shortened to become coccoids all over again.

Formation of Coccoid Cells

Coccoidal cells usually were seen to arise either from the long filamentous rods, rudimentary buds or branches, or from short rods themselves. They rounded off either by gradual shortening of the dividing rods or by simultaneous formation of a number of septa (transverse septum in each individual cell) in the filamentous cells. The septated cells subsequently
fragmented to give rise to short rods and coccoids. The coccoids either remained thereafter as resting cells, *i.e.*, without further multiplication, or underwent a slow division to gain in their cell mass. The cocci of old cultures in stained preparations appeared as bunches of grapes.

*Life-cycle of Arthrobacter ruber* *n. sp.*

From the photomicrographic illustrations (Plate XV) it may be seen that the uniform oval cells from a old 48-hr. agar slant (Fig. 1) on transference to a fresh medium, got germinated to long curved, filamentous, occasionally branched, rods within 4 hr. (Fig. 2). Cell wall stained preparations at this stage revealed the formation in a coccus of one, two or sometimes even three germ tubes. Every germ tube, during the course of development, lengthened considerably and revealed within, the presence of two or three transverse septa (Figs. 3, 4, 5, 6). The septate cells were also observable during 8 hr. growth period (Figs. 7 and 8) but during this period the cells got shortened and finally started fragmenting giving rise to three to four segments in the form of cocci and coccobacillary rods (Fig. 9). The cocci remained attached to each other and appeared beaded and their metachromatic granules were stainable after 24 hr. by Löffler's alkaline methylene blue. Each individual cell revealed two or three granules. After 48 hr. of incubation period the short rods also got converted into cocci once again (Fig. 1). This cycle was repeated every time a transfer of these cocci to a fresh nutrient medium was made.

*Origin of 'V' Forms in Arthrobacter*

The classical 'V' forms in arthrobacterial morphology is a characteristic typical of the genus. The two probable modes of their formation is as follows:

(a) *Due to germination of adjacent cocci.*—Old cultures of *Arthrobacter ruber* revealed the presence of both small and large cocci and coccobacillary rods. When seeded on a fresh medium these cells began to swell and gave outgrowths. From Figs. 5, 6, 7 and 8 it can be seen that two adjacent cocci on germination gave a 'V'-shaped cell. Such 'V' forms were seen mostly in very young cultures of *Arthrobacter*.

(b) *Due to angular growth.*—'V' forms originate in *Arthrobacter* from the angular growth due to subpolar or bipolar germination of cocci. As can be seen from Figs. 5 and 6 the two outgrowths from a single coccus sometimes assumed the shape of a curved rod; but occasionally when the angles were not acute other forms appeared. According to Starr and Kuhn
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(1962) these ‘V’ forms can arise in several different ways, viz., due to snapping post-fission movement or germination of adjacent cocci or the angular growth or the germination of cocccoidal element.

Branching Forms in Arthrobacter

True branching in Arthrobacter is a rare phenomenon. Four strains were encountered in group XI which exhibited branching in young cultures; Figures 10 and 11 typify true branching observed in a 4-hr. old culture of strain 86. These branching did not persist in older cultures as after 24 hr. the cells reverted to their uniformly shaped rods exhibiting true arthrobacterial morphology. In older cultures they were, however, converted into spherical forms. These strains represented morphological features midway between Arthrobacter and Nocardia though were more akin to Nocardia than Arthrobacter. As their physiological and growth characteristics in both liquid and solid media resembled closely with those of Arthrobacter, they were included in this genus.

Effect of Nutrient Conditions on the Morphogenesis of Arthrobacter Species

When the Arthrobacter cultures were cultivated on a nutritionally poor medium, they exhibited less pronounced pleomorphism. The cells remained as short rods in young and spherical in older cultures. The morphological patterns of Arthrobacter may therefore be considered as those dependent on the nutritional status of the medium and/or age of the cultures; changes in the composition of the medium, however, induced abnormal morphology besides pleomorphism. For example, the group X Arthrobacter strains, represented by Strain 75, exhibited clear-cut difference in their morphogenesis in the presence and absence of biotin, in the synthetic medium made with glucose and salts. In the biotin-deficient medium cells remained unfragmented even after 4 days (Mullakhanbhai and Bhat, 1966) and revealed a highly granulated structure; and, though were only weakly Gram-positive, contained strongly Gram-positive granules. The cells in biotin-sufficient condition, on the other hand, exhibited the true arthrobacterial morphology, viz., pleomorphism characterized by formation of both short rods and cocci. Shake flask experiments in medium, without biotin, showed a suppression of growth accompanied by the accumulation of morphologically ‘abnormal’ cells, which failed to fragment further. In other words, biotin deficiency brings about a nutritional stress and cessation of the normal lifecycle witnessed in a biotin sufficient medium,
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REFERENCES


EXPLANATION OF PLATE XV


**Fig. 1.** Cells from a 48-hr. old nutrient agar culture; crystal violet stain (× ca. 2,625).

**Fig. 2.** Cells from a 4-hr. old nutrient agar culture; crystal violet strain (× ca. 1,312) illustrating the long pleomorphic rods.
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Figs. 3 and 4. Cells from a 4-hr. old nutrient agar culture; cell wall strain (× ca. 2,625), the enlarged portion showing the cells with one or two transverse septa.

Figs. 5 and 6. Cells from a 4-hr. old nutrient agar culture; cell wall strain (× ca. 2,625), showing the angular growth. 'V' forms and germinating cocci; cells with one or two septa.

Fig. 7. Cells from a 8-hr. old nutrient agar culture; crystal violet strain (× ca. 2,625).

Fig. 8. Cells from a 8-hr. old nutrient agar culture; cell wall strain (× ca. 2,625), showing the 'V' forms and septate cells.

Fig. 9. Cells from a 24-hr. old nutrient agar culture; cell wall strain (× ca. 2,625).

Figs. 10 and 11. Morphogenesis of Arthrobacter Group XI, Strain 86.

Fig. 10. Cells from a 4-hr. old nutrient agar culture; crystal violet strain (× ca. 1,312), showing the branching forms.

Fig. 11. Cells from a 4-hr. old nutrient agar culture; cell wall strain (× ca. 2,625).