A Microcultural Study of the Growth of Cystites, Cocci and Rods of *Arthrobacter globiformis*

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During the transition from cocci to rods of *Arthrobacter globiformis* grown under constant conditions in perfusion microculture, the shape of the original coccus was maintained for many hours during a sequence of multiple bud formation. Buds matured into rods in which the tips were phase-dark. Cross-wall formation, which cut off the buds from the parent cells, occurred by centripetal growth. V-forms were produced by angular growth, germination of adjacent cocci and slow bending division. Rods could be transformed into cystites by omitting fixed nitrogen from the medium. Cystites were capable of budding on restoration to complete media. Budding, originally occurring at the poles of the cystite, also occurred at the middle of the cystite, following the growth of a cross-wall at this point.

INTRODUCTION

Cystites are common in many strains of *Arthrobacter* but their origin and ability to regenerate rods and cocci are not clear. Germination has been demonstrated by several workers, including Schippers-Lammertse, Muijsers & Klatser-Oederkerk (1963), but most of these studies did not involve continued observations on the formation and germination of individual cystites under controlled conditions. This paper reports the use of perfusion microculture to achieve this, allowing a comparison to be made with the growth of cocci and rods under similar conditions. Because organisms can be grown on a cellophane membrane and supplied with nutrients by diffusion through the membrane from a flowing medium, the effect on the organism of changing the medium could be observed without disturbing the individuals being studied.

METHODS

Preparation of inocula. Cultures of *Arthrobacter globiformis* NCIB10683 were grown in Oxoid nutrient broth, pH 7.2, at 25 °C on a rotary shaker and harvested after 18 h (rods) or 6 days (cocci). To produce cystites, 1 ml of a 24 h nutrient broth culture was inoculated into 50 ml of PYEBG medium [bacteriological peptone, 5 g; yeast extract, 4 g; glucose, 1 g; basal salts medium (Lochhead & Burton, 1954), 750 ml; water, 250 ml] and incubated at 25 °C on a rotary shaker for 40 h. One ml of this culture was transferred to 50 ml of BG medium [basal medium of Lochhead & Burton (1954) containing 1 % (w/v) glucose] and incubated at 25 °C on a rotary shaker. Cystites were harvested after 4 days incubation.

Perfusion microculture. Organisms were grown on PT300 cellophane (British Cellophane Co.) membranes held in a microculture chamber (Duxbury, 1977) and perfused with nutrient broth at a flow rate of 10 ml h⁻¹. The chamber was kept at 25 °C and observations made with a Zeiss Photomicroscope I, fitted with Neofluar phase contrast objectives and a condenser with an oil immersion front lens. Photographs were made on Ilford Pan F film, and a selection of these from several sequences are shown below.

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Figs 1-11. The development of a coccus supplied with nutrient broth (25 °C). Time sequence: Fig. 1, 0 h; Fig. 2, 4 h 30 min; Fig. 3, 5 h 50 min; Fig. 4, 6 h 20 min; Fig. 5, 6 h 50 min; Fig. 6, 7 h 20 min; Fig. 7, 7 h 50 min; Fig. 8, 8 h 05 min; Fig. 9, 8 h 25 min; Fig. 10, 9 h 05 min; Fig. 11, 10 h 45 min. Note the formation of a cross-wall by centripetal growth at the point arrowed in Figs 7-9, the retention of the original coccus shape, even after 9 h 05 min in Fig. 10, and the phase-dark appearance of cell tips and some new cells in Figs 2, 6 and 11. Bar marker represents 2 μ m.

RESULTS

Transition of cocci into rods

Cocci inoculated on the cellophane membrane (Fig. 1) began to elongate to form slightly club-shaped rods in which the new outgrowth had a smaller diameter than the original coccus (Fig. 2). A cross-wall was then formed at the site of the original coccus wall (Fig. 3) to produce a two-part structure consisting of a coccus and a rod. The coccus then began to elongate a second time to produce another bud, again of smaller diameter than the parent cell, which was eventually separated from the coccus by formation of another cross-wall (Figs 3-7). This budding and cross-wall formation occurred several times but the outline of the original coccus was still discernible after 9 h (Fig. 10). Mulder & Antheunisse (1963) noted two or three growth points on cocci but multiple growth points, and the essentially sequential nature of bud formation, have not been recorded before.

Following the formation of cross-walls, the rods increased in diameter and then began to branch by producing buds (Figs 4-10) close to the sites of previous cross-wall formation, a feature also noted by Sundman (1958). Very frequently, the tips and younger rods in the microcolonies were more phase-dark than the rest of the colony (Figs 2, 6, 11) which may be due to the concentration of ribosomal material previously noted in electron micrographs of dividing arthrobacters (Duxbury, Gray & Sharples, 1977).

Cross-wall formation occurred by centripetal growth (see Figs 7-9) which sealed off the new bud from the parent cell. The concentration of budding activity and cross-wall formation at the same sites suggests that the metabolic activities associated with growth and division are both highly controlled and highly localized in this organism. This was confirmed by the development observed in a second sequence where not only were the shapes of the original cocci (Fig. 12) maintained for at least 10 h but they could be identified as phase-dark regions (Figs 13-17) quite distinct from the phase-dark tips of young buds. After 11 h, some of this original phase-dark material was less clear due to renewed budding from the parent cell (Fig. 18).

Figs 12-18. The development of cocci under identical conditions to the sequence in Figs 1-11. Time sequence: Fig. 12, 0 h; Fig. 13, 4 h 30 min; Fig. 14, 6 h 20 min; Fig. 15, 7 h 35 min; Fig. 16, 9 h 05 min; Fig. 17, 10 h 05 min; Fig. 18, 11 h 20 min. Note the retention of the original coccus shape in Figs 13-17. Bar marker represents 2 μ m.

Several processes leading to the formation of V-forms (Starr & Kuhn, 1962) can be seen in Figs 13-18. These are angular growth of two buds (Figs 13-16), germination of adjacent cocci (Figs 13-17, lower pair of cocci) and slow bending of dividing rods (upper left, Figs 17, 18).

Transition of rods into cystites

Growth from rods inoculated on cellophane and perfused with PYEBG medium followed much the same pattern as the development of colonies from cocci although it was not possible to determine if part of the original rods were conserved during growth (Figs 19-22). Division by binary fission and budding were both observed.

After the colony had been growing for 8 h, the effect of decreasing the concentration of the carbon source was studied by replacing the complete medium with Lochhead & Burton's basal medium without glucose. As the glucose became progressively more dilute, changes in the colony occurred and the average length of cells became shorter. Thus at 14 h (Fig. 23) the length to width ratio was 3.5 but this had decreased to 2.4 at 20 h (Fig. 25). This change did not occur uniformly throughout the colony for cells at the edge had a length to width ratio of 3 while those in the centre had a ratio of 2.

Just prior to this (at 15 h 30 min), the medium was changed again by including 1% glucose but omitting nitrogen, a change stated to induce cystite formation (Mulder & Antheunisse, 1963). The rods became shorter but larger in diameter (Fig. 26) and after 28 h (Fig. 27) were mostly cocci or enlarged cocci. After 64 h, the colony was disturbed and some of the cells formed are shown in Fig. 28. They were like cystites but remained phase-dark.

Growth of cystites

The growth of cystites was characterized by very early cross-wall formation (Figs 29-32). Indeed Duxbury *et al.* (1977) have observed that cross-walls are already present in cystites immediately after their formation. Buds arose simultaneously from the two poles of the cell and had about the same diameter as mature rods (Fig. 31). Cross-wall formation at the base of the buds (Figs 33, 34) maintained the original shape of the cystite but this was sometimes obscured by wall synthesis near the median cross-wall of the cystite and further

Figs 19–28. The development of cystites from rods. The medium was changed from the original PYEBG medium after 8 h to Lochhead & Burton's basal medium without glucose, and after 15 h 30 min to a medium containing 1 % glucose but no nitrogen source. Time sequence: Fig. 19, 3 h 30 min; Fig. 20, 5 h 30 min; Fig. 21, 8 h 10 min; Fig. 22, 10 h; Fig. 23, 14 h; Fig. 24, 15 h 30 min; Fig. 25, 20 h; Fig. 26, 22 h; Fig. 27, 28 h; Fig. 28, 64 h 20 min. Note the increasingly cystite-like organisms developing after 15 h 35 min. Arrows show the same cell on Figs 23–27. Bar marker represents 2 μ m.

Figs 29–37. The 'germination' of a cystite in nutrient broth at 25 °C. Time sequence: Fig. 29, 0 h; Fig. 30, 3 h 30 min; Fig. 31, 5 h 15 min; Fig. 32, 6 h 30 min; Fig. 33, 7 h 15 min; Fig. 34, 7 h 30 min; Fig. 35, 8 h 30 min; Fig. 36, 9 h 30 min; Fig. 37, 10 h 30 min. Note the formation of cross-walls in the cystites (Fig. 31), the occurrence of bending division (arrowed in Figs 33–37) and multiple budding (Figs 35–37). Bar marker represents 2 μ m.

bud formation at this point. These buds began as small protruberances broadening as they elongated. Multiple budding was observed (Figs 35–37) and it was restricted to the central cross-wall region. The formation of V-forms was seen and occurred by bending movements (Figs 33–37). In Fig. 37, the rod produced after budding seems to be still held by the parent cell, possibly by a strip of wall material.

DISCUSSION

Arthrobacter globiformis NCIB10683 grows by a sequential, multiple budding process which ensures that each bud is sealed off from the parent cell before a second bud is initiated. This process ensures the integrity of the original parent cell for several hours, even while the daughter buds are themselves dividing. Presumably, there is an upper limit to the number of buds that a parent cell can produce as electron micrographs of dividing cells suggest that parent cells undergo ageing as shown by a decrease in ribosome concentration (Duxbury *et al.*, 1977). This number of buds is certainly no less than four.

Budding accounts for the appearance of club-shaped cells in arthrobacter cultures which can be interpreted as cocci forming rod-shaped buds, and also for the frequent occurrence

of lemon-shaped cystites where buds are produced terminally, often prior to their production near the median cross-wall.

Budding may also be responsible for the occurrence of V-forms in cultures of arthrobacters. The presence of wall scars, especially at the base of detached buds (Duxbury *et al.*, 1977), suggests that the outer part of the wall may be different from the inner part. If the outermost wall layer ruptures locally due to internal pressure at the point of bud formation then, as the bud grows, so it will push against the mother cell, causing the pair to hinge on the unbroken wall layer. Snapping post-fission movement (Krulwich & Pate, 1971) may be interpreted as a sudden rupture of an outer wall layer, while bending movement (Komagata, Yamada & Ogawa, 1969), as seen in the organism studied here, would be caused by slow tearing. The difference between snapping and tearing may be correlated with a difference in either chemical composition or elasticity of the outer wall region or the presence or activity of lytic enzymes. The sequence of events following slow tearing of the outer wall can be followed in Figs 33–37.

This study has also confirmed that cystites may be produced by growing arthrobacters in nutritionally unbalanced conditions, that they may form from rods as well as cocci and that they are capable of reverting to normal cells upon restoration to normal nutrient conditions. The occurrence of cross-walls in some cystites also suggests that they may be capable of division to form new cystites and this has recently been confirmed by Chapman (1976) who has been able to culture enlarged cystite-like cells in a chemostat under conditions of nitrogen-limitation and at low growth rates.

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