The Life Cycle of *Sporocytophaga*

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SUMMARY: The life cycle in *Sporocytophaga* closely resembles that of the non-sporing eubacteria.

The haploid microcyst germinates, the resulting cell containing two paired chromosomes reproducing vegetatively. An autogamous fusion and subsequent reduction division precede the maturation of the microcyst.

Sporocytophaga differs from *Myxococcus* by the absence of fruiting body formation, and in the details of its nuclear cycle. These bacteria may possibly be regarded as the ancestors of the higher myxobacteria.

The occurrence of a complex life cycle in the cellulose-decomposing cytophagas, which form large microcysts (*Sporocytophaga*, Stanier, 1942), is well known. It was first recognized by Hutchinson & Clayton (1919), who described the nuclear changes that occurred during the transformation of the rod-shaped bacteria into the large 'cocci' observed by earlier workers on decomposing cellulose fibres (van Iterson, 1904; Merker, 1911). Hutchinson & Clayton recognized that the 'cocci' were the resting stage and termed them sporoids, but did not observe their germination. Their preparations were dried and fixed by heat. This method distorts the vegetative cells, so that they appear as twisted, spiral rods, an appearance that misled these authors into believing that they were dealing with a spirochaete.

Bokor (1930) described the vegetative cells as long filaments which broke up into short rods, and finally divided into spores by fragmentation. This process is characteristic of many actinomycetes and, from his descriptions, it appears that he was dealing with mixed cultures containing one of these micro-organisms. Both Bokor and Winogradsky (1929) considered that the 'sporoids' were contaminants.

Using more suitable cultural and staining techniques, Krzemieniewska (1930) confirmed most of the observations of Hutchinson & Clayton. She recognized the resemblance to the life cycle in myxobacteria, and also realized that the sporoids were microcysts. She described the nuclear changes during the germination of the microcysts, and observed an autogamous fusion preceding their maturation. Winogradsky (1935) later acknowledged the correctness of this interpretation of the life cycle.

MATERIAL AND METHODS

Fourteen strains of *S. myxococcoides* freshly isolated from soils and composts were examined. They were cultivated at 28° on filter-paper or cotton-wool moistened with a mineral salt solution containing 0.5 % NaCl; 0.1 % $MgSO_4 \cdot 7H_2O$, 0.1 % KNO_3 and 0.1 % K_2HPO_4 dissolved in tap water and the pH adjusted to 7.2.

Preparations were mounted in water and were not fixed, because it was observed that fixation by sublimate alcohol or osmic acid caused the vegetative cells to shrink considerably. The chromatin arrangement was identical in fixed and in unfixed preparations.

The chromatin material of eleven strains was demonstrable at all stages by staining with dilute aqueous Giemsa, provided that the dilution and time of application of the stain were adjusted to the individual strain. The vegetative cells of the remaining three strains could not be stained suitably by this technique; for these the acid-Giemsa technique (Robinow, 1942) was used.

Confirmation of the life cycle was obtained by the observations of living cells growing on agar containing finely divided cellulose. The cellulose was prepared by dissolving filter-paper in the minimum amount of concentrated sulphuric acid, and then precipitating it from this solution by rapid dilution with water; after washing to remove excess acid, it was added to a 2% agar containing the mineral salts.

OBSERVATIONS

The mature microcyst. The mature microcyst is found in culture about 2 weeks' old. It is a spherical structure, *c.* 2μ in diameter, containing a small, central mass of chromatin. The outer layer of the cell stains deeply (Figs. 1 *a*, 2 *a*).

Fig. 1. Diagrammatic representation of the life cycle of *Sporocytophaga*. *a*, mature microcyst; *b-d*, germination of the microcyst; *e-g*, vegetative reproduction; *h-q*, microcyst formation.

Germination of the microcyst. When transplanted on to fresh medium, germination usually begins after a lag period of about 8 hr. The central mass of

chromatin enlarges and is transformed into a thick, central bar. The cell shrinks slightly and begins to elongate, and the chromatin bar divides. At the same time, the envelope of the cell splits and is discarded (Figs. 1*a-d*, 2*b*, *c*). As the cell continues to elongate, the chromatin bars migrate towards the poles

Fig. 2

Fig. 3

Fig. 4

Figs. 2–4. Drawings from Giemsa-stained preparations of cells of *Sporocytophaga*. Fig. 2. Germinating microcysts of *Sporocytophaga*. *a*, mature microcyst; *b*, first stage in the germination of the microcyst; *c*, liberation of the vegetative cell from the microcyst envelope. Fig. 3. Vegetative cells of *Sporocytophaga*. *a*, vegetative cell; *b*, stages in normal vegetative reproduction; *c*, trinucleate cell; *d*, filamentous cells; *e*, first stage in microcyst formation. Fig. 4. Stages in microcyst formation of *Sporocytophaga*. *a*, *b*, behaviour of chromatin material preceding fusion; *c*, nuclear fusion; *d*, reduction division; *e*, vesicular nucleus with tadpole-shaped chromatinic body; *f*, mature microcyst.

of the cell and are transformed into the paired chromatinic bodies, characteristic of the vegetative cell (Figs. 1*d*, *e*, 3*a*).

Vegetative reproduction. Normal living vegetative cells are weakly refractile rods, approximately $0.3 \times 5 \mu$. or more in size, with blunt rounded ends.

Spindle-shaped or spiral cells are only seen in stained preparations, especially those which have been fixed by heat or allowed to dry (Fig. 3*e*).

Ordinary vegetative reproduction is by fission. Each of the four chromosomes divides longitudinally, the cell begins to elongate and to constrict at the centre until two daughter cells are formed, each containing two pairs of chromosomes (Figs. 1*e-g*, 3*b*).

Filamentous cells were often observed in young cultures, but in most preparations it was impossible to observe the exact chromosomal arrangement. Preparations made from young cultures of all strains, however, showed cells containing three pairs of chromosomes, and in a few preparations, cells containing six and twelve pairs were observed (Fig. 3*c, d*). It seems probable, therefore, that the secondary method of vegetative reproduction, described in the non-sporing eubacteria by Bisset (1948), also occurs in *Sporocytophaga*. In this process the three chromosome pairs fuse at the centre of an enlarged cell. After two nuclear divisions, the chromatinic material is distributed throughout the growing filament, which finally fragments into six typical vegetative cells.

Microcyst formation. Microcyst formation may begin after 2-8 days, the exact time depending on the individual strain. The cell becomes shorter and the chromosomes are transformed into a central chromatin mass (Figs. 1*g-j*, 3*e*). The whole cell then enlarges slightly and the chromatinic material elongates and divides. This process is accompanied by a central constriction of the cell, which sometimes progresses to such an extent that two daughter cells are formed (Figs. 1*k, l*, 4*a, b*). The chromatin bodies then fuse again at the centre of the constricted cell, which becomes spherical (Figs. 1*m, n*, 4*c*). The fusion nucleus usually divides into two, but occasionally four bodies of equal size are formed. One (or, in the latter case, three) of these bodies gradually disappears. The remaining one is transformed into a large vesicular nucleus. The chromatinic portion of the latter takes the form of a body which appears to be shaped like a tadpole, but this appearance may be an optical illusion (Figs. 1*o-q*, 4*d, e*). Later this chromatinic material contracts into the small central body characteristic of the mature microcyst. The outer layer of the cell thickens during the latter stages of microcyst formation and begins to stain deeply.

DISCUSSION

The life cycle of *Sporocytophaga* appears to be as follows. The envelope of the microcyst is shed on germination. This process can be observed both in living cells, and in stained preparations as shown also by Krzemieniewska (1930), and Stanier (1942).

The vegetative cell contains two paired chromosomes, similar both in appearance and behaviour to those found in most Gram-negative bacteria (Bisset, 1950*a*). The diffuse distribution of the chromatinic material, described by Hutchinson & Clayton (1919), and by Krzemieniewska (1930), is only apparent in unhydrolysed preparations of those strains which cannot be stained directly with diluted Giemsa.

The maturation of the microcyst is preceded by a process suggestive of

nuclear fusion. Hutchinson & Clayton depicted the fusion of the nuclear material at the centre of the constricted cell, but they claimed that a complete cell division took place with the formation of two sporoids. They apparently mistook the nuclear fusion for a cell division.

Krzemieniewska did not observe a reduction division, although she claimed that some of the chromatin material was rejected during the formation of the vegetative cells. From my observations, it appears that the reduction division immediately follows the nuclear fusion. The mature microcyst, therefore, contains a simple, central, haploid nucleus. This condition appears to be general in bacterial resting cells (Bisset, 1950*a*).

Sporocytophaga has been classified in the Myxococcaceae (Stanier, 1942), which it resembles in its creeping motility, but it differs from the other members of this family (*Myxococcus*, *Chondrococcus*) by the absence of fruiting-body formation. The formation of microcysts, which Stanier considers to be the main point of resemblance, is shared by many apparently unrelated groups of bacteria (Bisset, 1949, 1950*a*). In the details of the nuclear cycle, moreover, *Sporocytophaga* more closely resembles the non-sporing eubacteria than the myxococci (Klieneberger-Nobel, 1947).

The tendency to develop from an aquatic to a terrestrial mode of existence can be observed in bacteria (Bisset, 1950*b*), and an interesting evolutionary development can be seen in the Myxobacterales. These bacteria are well adapted to a terrestrial existence by their creeping type of motility which requires a mechanical support. The highest evolutionary development in this group can be seen in the myxobacteria where the microcysts are grouped into fruiting bodies. In *Sporocytophaga*, the microcysts, although very resistant to drying and inanition, are formed and distributed singly. It is possible, therefore, that they may be the ancestors of the higher myxobacteria.

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