DEVELOPMENT OF CONCENTRIC ZONES IN THE *PROTEUS* SWARM COLONY

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PLATES XXX AND XXXI

IN the past, the zonation of growth in the swarming of *Proteus* has been studied mainly at the cellular level (Russ-Münzer, 1935; Lominski and Lendrum, 1947; and others). A cyclical change from the long, swarming form to the short, non-swarming form, after cessation of motion at the leading edge of the colony, has been assumed to be the cause of the formation of the series of rings of growth on solid media.

Results from this laboratory have indicated that this explanation is not invariably accurate (Bisset 1973*a* and *b*; Bisset and Douglas, 1976). The bacteria at the leading edge may be constantly in the swarm phase, even when motion ceases. The main agency of outward movement is growth, and the zones seem to be the result of internal collisions within the colony as growth proceeds.

These conclusions were largely extrapolative, and the present study employs a technique for observing the phenomenon continuously at the colonial level.

MATERIALS AND METHODS

Strains. Four *Proteus vulgaris* and 10 *P. mirabilis* strains were isolated either in this department or in the Bacteriology Laboratory of the Queen Elizabeth Hospital, Birmingham. One strain of *P. mirabilis* (no. P11) was kindly provided by Dr J. P. Armitage, Department of Botany and Microbiology, University College, London.

Media. BBL Trypticase Soy Agar plates were used, with 15–20 ml of medium per petri dish. The plates were dried for 10–15 min. at 55°C to remove the excess surface moisture.

Observations of swarming colonies. Nutrient-agar plates were seeded centrally, either from an overnight plate culture or from an 18-h broth culture. They were incubated on the stage of the microscope, which was maintained at 35°C by heating with a 60-watt lamp. The lid was kept in place at all times, except during photography, to prevent the agar from drying too rapidly.

Observations were made, against a dark background, by means of a dissecting microscope with one of the eye-pieces removed and a microscope lamp directing light down the tube upon the plate. A Vickers camera was fitted to the left-hand tube. If the lighting and the angle between the two optical paths was critically adjusted, this method highlighted the irregularities of the surface, and gave a very clear *quasi*-three-dimensional picture of the swarm colony. Sequential hourly observations allowed the study of the development of concentric rings.

Each strain was examined on numerous occasions; two *P. vulgaris* strains and two *P. mirabilis* strains (including no. P11) were examined in greater detail and photographed. Composite photographs showing a segment of the colony were made by overlapping individual exposures of adjacent fields. Photographs of plate cultures fixed with formaldehyde vapour were made with oblique rear lighting against a dark background.

Morphological observations of the component bacteria were made *in situ* on or excised agar blocks, according to the methods described previously (Bisset, 1973*a*; Bisset and Douglas, 1976).

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RESULTS

Observations with the incident lighting system described revealed that ring formation did not always follow the same pattern with every strain, nor with the same strain on different occasions.

The first main type of growth was that described by Bisset (1973*b*) and Bisset and Douglas (1976); it is illustrated in figs. 1, 2 and 5). The edge of the swarm colony advanced fairly continuously, and the great majority of component bacteria retained the elongated "swarmer" morphology. Variations in the speed of advance, however, appeared to result in crowding of the growth behind the edge.

The rings could be seen to form within the colony, well behind the leading edge, producing a ripple in the surface which later developed into one of the concentric rings. In some rapidly spreading strains, the rings could be seen to form after the swarm had covered almost the entire plate, by essentially the same mechanism. These rings, which are effectively folds or wrinkles, were less sharply defined than those produced by the classical phenomenon described below; they were usually produced by the strains of *P. vulgaris* examined, and upon occasion by *P. mirabilis* also (figs. 2 and 4), but not normally by the P11 strain (Armitage, Rowbury and Smith, 1974), in accordance with these workers' findings.

The classically described method of ring formation was also confirmed by this technique. A thin, even film of growth first progressed over the medium. Motion at the leading edge then slowed and ceased, but growth continued, resulting in a thickening over the whole area. The edge of this thickened layer comprised the edge of the ring, from which a renewed thin film emanated to repeat the cycle (figs. 3 and 6), the growth being composed of sharply defined steps (fig. 4). As described by Bisset and Douglas (1976), the leading edge consisted of bacteria in the elongated, swarm phase; even when at rest, this morphology was retained by the great majority of bacteria at the edge, although some broke down to a slightly shorter, intermediate form. Usually, the short, almost cocco-bacillary, typical non-swarmer phase was developed *behind* the leading edge, in conditions of active growth and congestion.

A range of variations between these types occurred, even within the same strain on different occasions. For example, if the advancing edge ceased to move, but only for a very short period, the build-up of growth was small, so that the rings were relatively thin, and sometimes undiscernible.

DISCUSSION

The observations recorded here indicate that ring formation in the swarm colony of *Proteus* may not invariably coincide with the cessation of advance at the edge, nor is it necessarily associated with a change in morphological phase of the component bacteria. This may be the result, rather than the cause, of congestion and overgrowth at the sites of ring formation, and eventually throughout the colony as movement ceases.

Most workers have assumed that the morphological change occurring at the edge of the swarm is the actual cause of ring production, but it is now shown that rings are not always produced at the edge of the colony, or always in the same manner. These observations support previous work from this laboratory (Bisset 1973*a* and *b*; Bisset and Douglas, 1976), which suggested that the rings are due to crowding of growth, often caused by collision of two expanding areas. This seems to be equally true, whether the classical start-and-stop mechanism is observed, or whether the ring is formed by a fold, at some distance behind the edge; as in the second and first methods described in the preceding section. A whole spectrum of variation may be seen, and is probably caused by small changes in such conditions as moisture of the agar, humidity, incubation temperature etc., as well as by the behavioural tendencies of the bacterial strains.

The fact that these observations show deviations from the accepted norm brings into question the validity of previous explanations of the swarming process. Both the theory of local depletion of nutrients (Moltke 1927; Russ-Münzer, 1935) and the theory of a toxic metabolite (Lominski and Lendrum, 1947; Hughes, 1957; Grabow, 1972) as stimuli for elongation and subsequent swarming, explain adequately only the classically described type

SWARMING GROWTH OF *PROTEUS*

FIG. 1.—Sequence of composite photographs taken at intervals of *c.* 1 h, commencing *c.* 5 h after inoculation at the centre of the plate, showing the growth of a segment of the spreading colony of a *Proteus vulgaris* strain during ring formation: (a) the growth is a relatively even film; (b) thickening of this layer occurs when the leading edge has slowed down; (c) a fold is produced in the surface behind the edge; (d, e) the fold develops into one of the concentric rings. $\times 20$.

FIG. 2.—*P. vulgaris*, *c.* 20-h growth at 37°C on agar, showing relatively ill-defined rings associated with the mode of formation illustrated in fig. 1.

SWARMING GROWTH OF *PROTEUS*

FIG. 3.—Photographs at intervals of *c.* 1 h, commencing *c.* 5 h after inoculation; showing the edge of a spreading colony during the classically described type of ring formation. Initially the edge is a thin film of growth (*a*), which gradually thickens when it ceases to advance (*b* and *c*). From this edge a new thin swarming film emanates to repeat the cycle (*d*), hence forming sharply defined concentric step-like regions of growth. (Compare fig. 4.) $\times 35$.

FIG. 4.—Photograph of an overnight plate culture of a *P. mirabilis* strain showing the sharply defined step-like growth characteristic of the classically-described mechanism of ring formation illustrated in fig. 3. (Compare with fig. 6.) $\times \frac{1}{4}$.

FIG. 5.—Diagram representing the sequence of events in the method of ring formation shown in fig. 1. Each segment depicts the condition of the colony, the straight lines representing thin even growth and the rippled lines thickened regions (drawn after original photomicrographs). Numbers indicate hours since inoculation.

FIG. 6.—Diagram representing the sequence of events in the method of ring formation shown in fig. 3. The growth spreads first as a thin even film and then thickens when the edge ceases to advance. The heavy black lines depict the position of a ring, from which a new edge is seen to emanate (drawn after original photomicrographs). Numbers indicate hours since inoculation.

of ring formation, and assume that morphological phase change is synonymous with cessation or recommencement of movement. They do not explain the other observations presented here. The relatively attractive, visual clarity of the rings produced by the classical method has almost certainly caused them to be regarded as typical, so that a degree of unintentional selection for these characters may have occurred in the past in the choice of strains for study (for example, see Jones and Park, 1967).

SUMMARY

Two main mechanisms of concentric ring formation in cultures of *Proteus* species are described. One of these is the classically accepted method, wherein rings are produced as the advance of the swarm stops and recommences. In the other, the ring is produced as a fold inside the colony. These are considered to be extremes of a spectrum of growth types, the common feature being that rings are formed by piling-up of growth, and this process is not always directly correlated with morphological change in the individual bacteria, or with cessation of advance of the swarm. The validity of the presently accepted theories of ring formation and swarming in general are thus called into question.

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