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THE ZONATION PHENOMENON AND STRUCTURE OF THE SWARM COLONY IN *PROTEUS MIRABILIS*

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PLATES XV AND XVI

IT is commonly believed that the formation of concentric zones of growth around a point of inoculation by swarming strains of *Proteus* is due to a rhythmic alternation of elongated, motile swarming bacteria with shorter, non-swarming ones at the leading edges of the swarm colony. The accepted physiological explanation is that swarming is a device to escape from concentrations of toxic metabolites produced by the growth of the sessile stage, and that the former reverts to the latter when that stimulus is removed (Hughes, 1957; see Grabow, 1972).

A recent study, by phase-contrast microscopy, of the motion of the swarm (Bisset, 1973) led me to the conclusion that the main agent of advance is growth rather than motility, because the movement of the swimmers is either random, or in the vicinity of the edge of the swarm frequently at right-angles to the direction of advance.

These observations gave no assistance in explaining the zonation phenomenon, because the anticipated alternation of growth phases was not, in fact, observed. Zonation did occur, but the margin was constantly in the swarming phase. The present work was undertaken for the purpose of clarifying this apparent anomaly.

MATERIALS AND METHODS

Six strains of *Proteus mirabilis*, isolated and identified in this laboratory or in the Bacteriology Department of the Queen Elizabeth Hospital, Birmingham, were examined. They were grown at 37°C and 25°C, on nutrient agar plates (BBL Trypticase Soy), either at full strength or diluted to $\frac{1}{2}$ or $\frac{1}{4}$ strength with 1.0 per cent. non-nutrient agar, for the experiments described hereafter.

Observations and photographs were made directly, or with the $\times 10$ and $\times 40$ lenses of a Vickers phase-contrast microscope. Plates were photographed with a Nikon F camera, Micronikkor Auto 55 mm lens, and Kodak Tri-X film. The best results were obtained with

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swarms that had been fixed by exposure to the vapour 40 per cent. formalin poured into the lid of the inverted plate and left at 25°C for several hours. Photographed by reflected, diffuse daylight, against a black background, the structure of these swarms could be resolved with exceptional clarity.

Electron micrographs were made from suspensions in distilled water, taken from minimal areas at marked points on the swarm colony, and shadowed by gold-palladium.

RESULTS

Swarms, produced by inoculation of plates of full strength agar at a central point, developed best when incubated initially for 3 or 4 hr at 37°C and thereafter at 25°C. Full growth took about 48 hr until the edge of the plate was approached, and by that time the medium usually became too dry for active swarming, although slow growth continued at the edges for several days thereafter.

Growth

The initial incubation at 37°C usually produced a small lobate colony, surrounded by a fringe of swimmers up to 1 cm in width. From then on the swarms were examined at intervals of 15 or 20 min. The cell-type and motility of the bacteria were observed by phase-contrast microscopy, and the edge of the growth and any developing rings were marked on the back of the plate. The conclusions drawn from this were that, in a swarm of what might be called classical appearance, producing three or four rings of alternate thick and thin growth in 20–30 hr (figs. 1 and 2), the marginal area was composed of bacteria in a constant state of active swarming. The speed of growth of the colony edge was irregular, and usually ceased at least once, when the colony was approximately half-grown. These halts usually resulted in the appearance of a thickened ring at the point, but similar rings appeared behind the growing edge when there had not been any observable halt of the advance of the swimmers that constituted the growing edge. The edge, although often appearing structureless to casual observation (fig. 1), was actually lobate (figs. 2 and 3). When viewed by the phase-contrast microscope it could be seen that these thickened lobes were composed of very active swimmers, behind which there was frequently a thinner area, often of an open mesh of growth (cf. Bisset), also in active swarming movement (fig. 4). Behind this active area, the growth thickened rapidly, and the movement of the swimmers was inhibited, although it did not cease immediately. Thus, the area 0.3–0.5 cm behind the swarming edge consisted of swimmers, becoming more and more constrained in their efforts at motility. Towards the centre of the colony, and after several hours (the actual time is difficult to estimate), a relatively thick growth of non-swarming elements is produced. As seen by electron microscopy the swimmers, whether or not derived from an actively motile area, were elongated and had very large numbers of flagella (fig. 5); whereas the non-swimmers were short, often coccoid, but still had from 2–12 flagella (fig. 6). These observations gave the impression that the thick, lobate outer edge of the swarm was able to achieve its rapid growth by advancing across fresh, unused medium (Bisset). It outdistanced the rest of the swarm, while behind it the organisms

SWARMING OF *PROTEUS*

FIG. 1.—*Proteus mirabilis*. Swarm at c. 36 hr showing very finely lobate margins of zones and apparently simple margin of thin, outer swarm. $\times 1.2$.

FIG. 2.—Swarm at c. 48 hr showing very markedly lobate margins of zones and at the edge of the colony. $\times 1.2$.

FIG. 3.—Swarm at c. 48 hr. Only the central zone and edge are clearly marked, but radial striations marking the progress of the lobes are clearly seen. $\times 1.2$.

FIG. 4.—Phase-contrast photomicrograph of lobes at the edge of a rapidly advancing swarm. The outer edge (appearing bright) is thick and in very active swarming movement. Behind it the growth is relatively scanty but still swarming. $\times 80$.

FIG. 5.—Electron micrograph; swarming element showing very profuse flagella. $\times 12,000$.

SWARMING OF *PROTEUS*

FIG. 6.—Electron micrograph; short, non-swarming element with flagella. $\times 12,000$.

FIG. 7.—Edge of swarm at c. 36 hr, showing edge of small lobes with zones synchronised across the spaces. $\times 1.2$.

FIG. 8.—Arborescent type of swarm. Natural size.

FIG. 9.—Portion of a young arborescent swarm showing large and small lobes with zones synchronised across the spaces. $\times 2.5$.

FIG. 10.—Small lobes at the edge of colony after swarming has ceased (c. 4 days). Arborescent growth proceeds at the edges and small synchronised zones still appear. The original colony was of the type shown in fig. 2. $\times 4$.

grew more slowly initially. They were thus deprived of free space to develop, and eventually collided with the existing growth in the outer area, producing the thickened ring with a thinner phase outside it, whilst the advancing edge was still retreating outwards.

Structure of the colony

The small number of strains of a single species of *Proteus* studied showed considerable variation in the appearance of the swarm colony. The lobate edge occurred both on the outer edge and on the thickened rings (fig. 2). In some cases

FIG. 11.—This illustration was composed by tracing in the lines of growth on a photograph of the swarm, and xerographing the result. A photograph of reproducible quality could not be made because the two halves of the plate were of unequal opacity. In the upper half the medium is at full strength ($1 \times \text{NA}$) and in the lower half it is at $\frac{2}{3}$ nutrient strength, but full agar strength ($\frac{2}{3} \times \text{NA}$). The upper part of the swarm has proceeded normally. The zones in the lower half are synchronised, although the advance has been much less rapid, presumably because of reduction in the available energy source.

there were large lobes, subdivided into smaller ones (fig. 7), or they were arborescent (figs. 8 and 9). Such arborescent projections were also seen at the edges of mature swarms, growing very slowly (fig. 10), this effect was therefore not associated only with swarming. The colonial appearances were consistent for each strain during the period of the observations.

Synchronisation

A feature of the lobate and arborescent swarms was the degree of synchronisation shown by the different parts of the colony in the production of rings, although they had no apparent contact (figs. 1-3, 7, 8). This is clearly seen in fig. 9, where the divisions between the four major zones of growth are accurately continued across the minor gaps between the small lobes and the major gaps between the larger ones. This can also be seen in fig. 10, although these lobes are part of an old colony, where swarming had ceased and growth was very slow.

Synchronisation was also shown in experiments made with plates in which one half of the medium had been removed with sterile precautions, and replaced with medium containing a $\frac{1}{3}$ or $\frac{2}{3}$ nutrient concentration. The plates were inoculated in the centre, at the point of junction of the two types of medium. Swarming proceeded normally, but took almost twice as long to cover the same area on the side containing $\frac{2}{3}$ -strength medium, and the growth was much thinner. In plates where zonation was clearly visible, however, the zone rings were approximately continuous on both sides of the plate, although those on the $\frac{2}{3}$ -strength medium were more closely spaced (fig. 11). The $\frac{1}{3}$ -strength medium did not support clear zonation rings; the swarm was small, very thin, and irregular in outline.

DISCUSSION

These observations suggest that the premises of current theories concerning the nature of the zonation phenomenon in the swarming of *Proteus* may be incomplete and not entirely accurate.

The assumption that alternation between the swarming and non-swarming phases occurs regularly at the edge of the growing colony, and that this edge advances and halts accordingly, could not be confirmed. Indeed, so long as conditions of space, moisture and nutrition were suitable, the swarming phase persisted at the edge, for most if not all of the period of active growth, which lasted 3 or 4 days. The edge changed its rate of advance, and even ceased to advance for several hours, but the formation of the visible zones of growth did not seem to depend upon such checking of the rate of swarming. Rather the reverse seemed to be the case, as a thickening was liable to be produced at the rear of the swarming edge when the latter advanced so rapidly as to leave an only partially filled area behind it. This was encroached upon by a new edge, advancing from the rear at a regular distance behind the true edge. It had the same morphology (whether composed of large or small lobes, or arborescent) as the true edge, and seemed to be no more than a new attempt, by the interior layers of the colony, to produce an edge of the standard type. But it failed to advance beyond a short distance because its path was blocked by the developing growth ahead of it. Thus the swarming motion does not cease because the bacteria change into the non-swarming phase, but because they are constricted, and probably also deprived of adequate supplies of energy. Movement ceases before the morphological change occurs; and it is significant that non-swarming elements are nonetheless flagellate and capable of a degree of motility.

Lastly, the structural complexity shown by the swarms, of which little notice has previously been taken, and the synchronisation of the zoning phenomenon in widely different areas, require an explanation. So also does the fact that a slowly-advancing, weakly-growing swarm, on dilute medium, retains its synchronisation with an adjacent, stronger growth. It seems possible that the timing of the zones is controlled by the comparative rates of growth of the bacteria of the two morphological phases, although a simulacrum of zonation can also be seen in growth at the edges of aged colonies, when swarming has long ceased.

SUMMARY

Zonation of the swarm colonies of six strains of *Proteus mirabilis* did not appear to be caused by an alternation of swarming and sessile phases. The margin was constantly composed of active swimmers; and, although a thickened ring could be caused by a check, it was more often due to the rapid advance of the edge, leaving a thinner area which permitted a new, interior edge to form behind it. Motility ceased when the bacteria became crowded; the change to the non-swarming phase took place later. The structure of the swarm colonies was lobate, and the zonation of the separate lobes was synchronised. Reduction of nutrients in the medium reduced the speed of advance of the swarm, but the timing of the zone formation was unaltered. Lobes, at the edges of old colonies, in which swarming had ceased, also showed synchronised zonation. These appearances are suggestive of genetic programming.

Addendum (2 Oct. 1973.) Since this work was completed, two papers published from the Cantacuzino Institute, Rumania, have been brought to my attention by the author, Dr S. A. Sturdza (*Arch. Roum. Path. Exp. Microbiol.*, 1973, 32, pp. 63 and 179). The observations reported in these papers, which were made by methods very similar to mine, and the conclusions drawn, conform with those recorded in my two papers on the swarming of *Proteus* in vol. 6 of this *Journal*.

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