SHORT ARTICLES

OBSERVATIONS ON THE GROWTH AND MOVEMENT OF *ACINETOBACTER* ON SEMI-SOLID MEDIA

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PLATE XLII

MEMBERS of the genus *Acinetobacter* are normally regarded as non-motile, although some workers have described gliding movements by some of them. Lautrop (1961) observed gliding motility with strains of *Moraxella lwoffii* and *Bacterium anitratum*, and these observations were confirmed by Piéchaud (1963) and Halvorsen (1963). Lautrop found that the gliding movement was poorly reproducible, and so concluded that it was of no use diagnostically but only of taxonomic significance. Because members of these species showed gliding motility and did not form fruiting bodies he transferred them to the genus *Cytophaga*. Cowan and Steel (1965) preferred to allocate them to the genus *Acinetobacter*, because they considered the techniques required to demonstrate gliding motility were not well suited to routine diagnostic work.

In the present investigation, a rapid surface movement or swarming was observed with strains of *Acinetobacter* grown on plates of commercially available semi-solid media marketed as " motility test media "; some strains produced channels or " ditches " in the semi-solid agar. The swarming and ditching phenomena have been further studied in an attempt to learn something of their nature and to determine whether they have any practical relevance to the identification of the genus *Acinetobacter*.

MATERIALS AND METHODS

Organisms. A total of 29 strains of *Acinetobacter* were studied. These included three strains obtained from the National Collection of Type Cultures: *Acinetobacter* (*Achromobacter*) *anitratus* (nos. NCTC7844 and NCTC8102) and *Acinetobacter* (*Moraxella*) *lwoffii* (no. NCTC7976). The remaining strains were clinical isolates comprising 24 strains of *A. anitratus* and two strains of *A. lwoffii*; these were identified according to the scheme of Cowan and Steel (1965). Some of the strains were sent to us from Birmingham General Hospital and Birmingham Public Health Laboratory. *Klebsiella aerogenes* (no. NCTC8172) was used as a non-motile control organism, and clinical isolates of *Escherichia coli* and *Proteus mirabilis* as motile controls.

Media. The following commercial (semi-solid) motility test media were used: Difco Motility Test Medium, Difco Cystine Tryptic Agar and Baltimore Biological Laboratories' (BBL) Motility Test Medium. These were prepared according to the manufacturers' instructions but dispensed in plates instead of tubes. We prepared our own media with Bacto-Peptone (Difco) and one of the following agars: Oxoid Agar No. 1, Oxoid Agar No. 3, Oxoid " Ionagar " and Davis Standard New Zealand Agar (Davis Gelatine Ltd, Upper Grove Street, Leamington Spa, Warwickshire, UK).

Except where otherwise specified, the plates were dried for 15 min., inoculated from cultures grown at 30°C for 18 h in Tryptone Water (Oxoid Ltd) by stabbing with a straight wire to the bottom of the petri dish, and then incubated at 30°C for 18 h.

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RESULTS

Fourteen of the 29 strains of *Acinetobacter* studied exhibited the strange phenomenon of producing " ditches " when grown on plates of semi-solid motility test media (figs. 1 and 2). The phenomenon was first noticed when one of us (J. B.) was working at the Public Health Laboratory, Birmingham. The typical appearance is shown in fig. 1. Here the organism was stab-inoculated at the centre of the plate; during incubation for 18 h at 30°C, fissures formed in the agar, extending from the centre in a serpentine fashion and apparently at random. The fissures containing bacterial growth and fluid are referred to as " ditches ". Nineteen of the 29 strains showed surface swarming and 11 produced spreading growth at the bottom of the petri dish beneath the medium, extending to a maximum diameter of 10 mm. The strains of *A. lwoffii*, including the NCTC strain, exhibited only the latter type of spreading. The strains that produced ditches in the medium also showed surface swarming and were all strains of *A. anitratus*, including the NCTC strains. The size of the area of swarming after 18 h at 30°C varied, with an average diameter of about 20 mm, but up to 40 mm with some strains. *Klebsiella aerogenes* did not show surface swarming on any of the media. *E. coli* produced a diffuse area of growth within the medium—the typical appearance of motile, flagellate organisms (fig. 4). None of the *Acinetobacter* strains spread within the medium.

The effect of different media, inocula and varying conditions of growth on swarming and ditching

Ditching and swarming took place on Difco Cystine Tryptic Agar and BBL Motility Test Medium which contain 0·35% (w/v) and 0·4% (w/v) of agar respectively. The ditching phenomenon was most reproducibly obtained with the BBL medium. Only swarming was observed on Difco Motility Medium which contains 0·5% agar. The constituents of our own medium were varied as follows: 1% peptone water was solidified with agar at concentrations ranging from 0·1% to 0·7%. We found that the optimum concentration of agar necessary to induce either phenomenon was 0·3%, and Davis Agar 0·3% with 1% peptone proved to be the most suitable medium for showing the ditching phenomenon. At concentrations lower than this the agar was too sloppy, and at higher concentrations the number of strains swarming and ditching decreased; there was complete inhibition of these effects on media containing 0·6% agar. The optimum peptone concentration for surface swarming and ditching was 1%. At concentrations below this, swarming and ditching were significantly reduced, and at 0·1% only five strains produced narrow, short channels.

The addition of sodium chloride 0·5% to the medium had no significant effect on ditching or swarming. 2,3,5-Triphenyl tetrazolium chloride (TTC) at final dilutions ranging from 0·001% to 0·05% was added to BBL Motility Medium. The growth of *A. lwoffii* was inhibited by TTC at a concentration of 0·0025%. Swarming by strains of *A. anitratus* was reduced as the concentration of TTC was increased, with complete inhibition at 0·05%.

The use of different types of liquid and solid media for the culture of the inoculum had little influence on subsequent ditching or swarming activity; cultures grown at 30°C for 18 h in tryptone water were adopted for our experiments. We found that very few strains would show the phenomena immediately on primary isolation; most required storage on artificial media for at least 7 days, and some required storage for several months before they would react in these ways.

The use of undried plates or those dried for up to 55 min. at 37°C did not influence the ditching effect, but the wetter the plate the larger the area of swarming. Plates that had been dried for 15 min. were the most suitable for demonstrating swarming. Plates were prepared with 10-ml, 14-ml, 25-ml, and 30-ml amounts of motility media: the resulting variations in plate depth had no consistent effect on either phenomenon.

The optimum temperature range for ditching and swarming was between 30°C and 37°C. At 20°C all the strains grew, but ditching was not marked and no swarming was seen.

We made knife cuts in the agar to determine the effect of presenting the organisms with artificial faults. Strains of *A. anitratus* grew along the faults, transformed them into wide

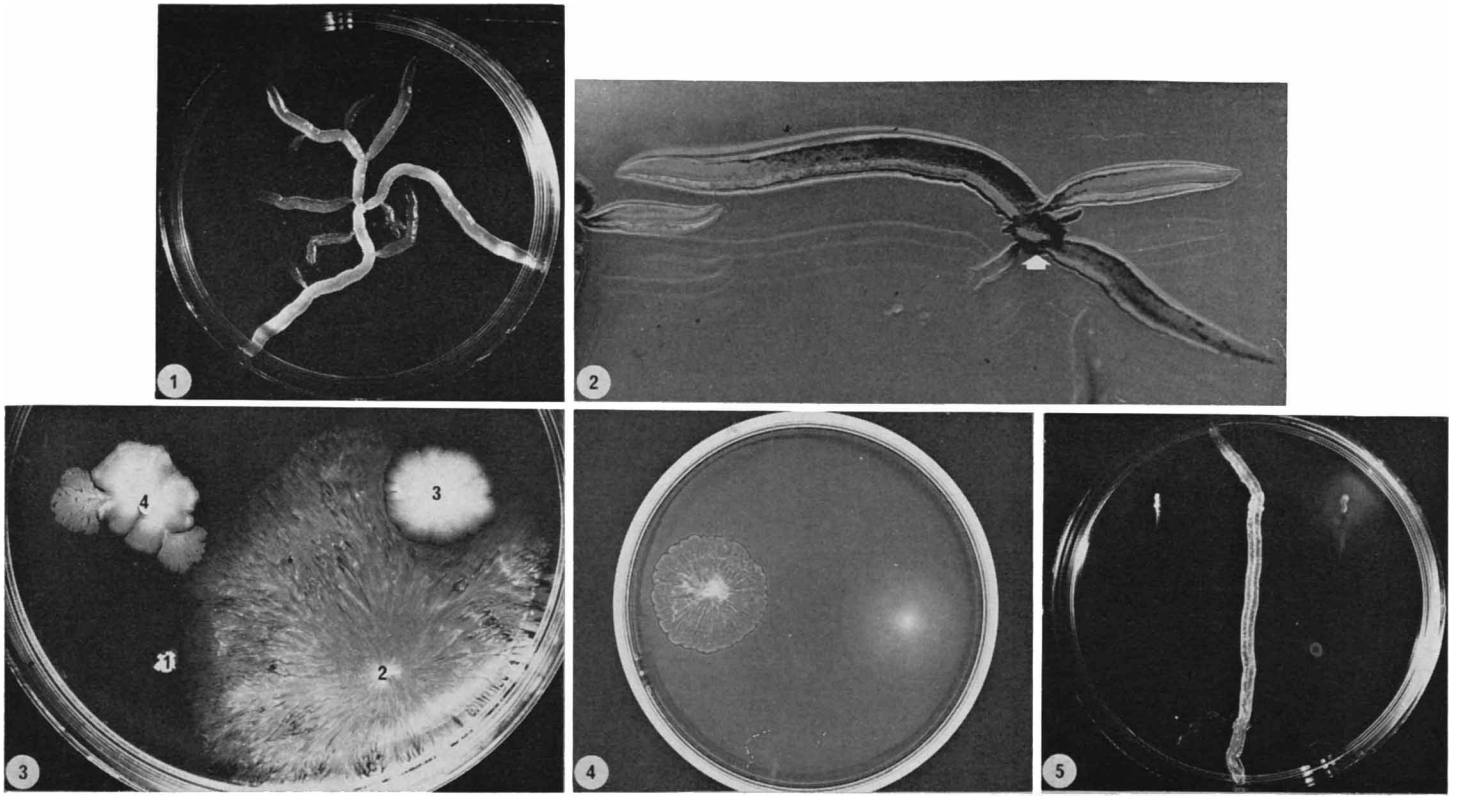

FIG. 1.—Ditching by a strain of *A. anitratus* on a medium containing 0·3% Davis Agar and 1% peptone. The organism was stab-inoculated at the plate centre and during incubation for 18 h it produced the sinuous fissures containing fluid which are referred to as ditches. ×0·5.

FIG. 2.—Ditching by *A. anitratus*; the channels are about 2 mm deep. The organism was stab-inoculated on BBL motility medium at the point indicated and has produced three large ditches and a small one. ×2·25.

FIG. 3.—Four strains of *A. anitratus* growing on 0·3% Oxoid "Ionagar" and 1% peptone; the numbers indicate the points of inoculation of the different strains. Strain no. 1 shows no swarming, whereas nos. 2, 3 and 4 show various types of swarm. Note the inhibition of swarming between strains 2 and 3. ×0·7.

FIG. 4.—*Left*: *A. anitratus* swarming from a stab-inoculum. *Right*: *E. coli* showing typical diffuse growth of motile organisms from a stab inoculum. The medium was 0·3% Oxoid "Ionagar" with 1% peptone. Contact print of the petri dish. ×0·5.

FIG. 5.—Three different organisms were inoculated at the top of three knife-cut faults in BBL motility medium. *Centre*: *A. anitratus* which spread down the cut, extended it at both ends and widened it into a ditch. *Right*: motile *E. coli* which extended only about 1·5 cm down the cut and spread within the medium in the usual way of a motile organism. *Left*: *Klebsiella aerogenes* which shows minimal spread from the inoculation site. ×0·5.

channels full of liquid and extended them beyond the ends of the cuts. The control organisms, both motile and non-motile, produced no such effect on the faults (fig. 5).

Agar-liquefaction studies

BBL Motility Medium and Davis Agar 0·3% without added nutrients were separately prepared in capped bottles and used for stab cultures that were incubated at 30°C for 3 months. The organisms failed to grow in the agar without nutrients, but grew in the BBL Motility Medium without liquefaction of the agar.

Microscopical study of the swarming phenomenon

Microscope slides were covered with a thin layer of motility medium; they were seeded with *Acinetobacter* strains and incubated for 2 h at 30°C. The edge of the swarming colony was observed by ordinary light and phase-contrast microscopy. Swarming of *Proteus mirabilis* on nutrient agar was observed in the same way for comparison. The swarm of the *Acinetobacter* strains advanced in the following way: chains of cells extended from the edge of the colony, some breaking away from the main body of growth. As multiplication continued, lobes were formed with gaps between them that were filled gradually with new growth. This irregular movement resulted in the arborescent appearance of the swarm (fig. 3, no. 2). No flexing or jerking movements were observed. The swarming was slower than that of *Proteus*, and streaming of the cells could be seen in the latter. The swarms of *Proteus* showed marked zonation; this was not seen with the *Acinetobacter* strains.

Electron microscopy

Electron-microscopic studies, made at the Public Health Laboratory, Birmingham, with one strain of *A. anitratus*, revealed no flagella. This confirmed the findings with " hanging drop " preparations that the organisms are non-motile.

Discussion

As far as we are aware, the production of channels or ditches by bacteria in semi-solid agar has not been described previously. It is a capricious phenomenon; often a single strain seeded twice to separate areas on the same plate produced ditches from one inoculum and not the other. This unreliability, together with the need to store some of the strains before they demonstrated this effect, limit the use of this observation as an aid to identification. As the organisms fail to liquefy agar in stab cultures and follow artificial faults cut in the agar, we postulate that the organisms follow naturally occurring faults in the agar gel and produce a physical effect that is influenced by the type and percentage of the agar used.

The ditch phenomenon was first observed on BBL Motility Test Medium containing triphenyl tetrazolium chloride at the concentration (0·005%) recommended by the manufacturers for its incorporation as an indicator of bacterial growth in their medium; however the growth of the three *A. lwoffii* strains we tested was inhibited by this amount of TTC and we do not recommend its use with these organisms.

The strains that produced ditches also showed surface swarming which we consider to be a distinct phenomenon and possibly a manifestation of gliding movement. Gliding motility has been observed with this group of organisms by Lautrop (1961), Piéchaud (1963) and Halvorsen (1963); this took the form of flexing and jerking movements—a feature that we did not observe—and these authors made no mention of swarming. A reason for this discrepancy may be that the agar concentrations used in their studies were at least twice those used by us. Swarming growth of non-motile marine flavobacteria was observed by Hayes (1963), who suggested that any non-flagellate bacterium that shows spreading growth should exhibit some form of gliding.

When comparing this type of movement with swarming by *Proteus* spp., a number of differences were evident. The *Acinetobacter* strains were not affected by the addition of 0·5% sodium chloride to the medium. The swarming of *Acinetobacter* is slower than that of

Proteus; streaming of the *Acinetobacter* cells was not observed and no zonation was seen. However, arborescent swarms were produced by many strains and the appearances were similar to those described by Bisset (1973) in studies with *Proteus mirabilis*. In addition, we noted that some strains of *Acinetobacter* showed inhibition of swarming similar to the phenomenon described by Dienes (1946) with *Proteus* spp. (fig. 3).

The organisms were stab-inoculated to the bottom of the petri dish and this may account for the spreading growth beneath the agar seen with some strains. The relationship of this movement to surface swarming is not clear because less than half of the " surface swarmers " produced this effect.

We consider that the ability of *Acinetobacter* spp. to swarm has no practical application for their prompt identification because it has the same limitations as ditch production. However, swarming is a characteristic not previously associated with this group of organisms, although gliding motility has been observed, and it is possible that the swarming we have described is a manifestation of gliding movement; this could be of significance in relation to the classification of the group. We are unable to attribute any taxonomic significance to the ditching phenomenon.

Summary

The growth of 29 strains of *Acinetobacter* spp. on semi-solid media was studied; 19 showed surface swarming and 14 produced channels (" ditches ") in the agar that do not seem to have been described previously. An attempt was made to define the cultural and physical conditions for the demonstration of these phenomena. Possible taxonomic implications are discussed.

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