Bacteriocinogeny in the Athiorhodaceae

By J. R. GUEST

*Department of Microbiology, University of Sheffield,
Sheffield S10 2TN*

(Received 24 September 1973)

INTRODUCTION

A wide variety of Gram-negative and Gram-positive bacteria produce bacteriocins which are active against strains of the same or related species, but the occurrence of the phenomenon amongst the photosynthetic bacteria does not appear to have been reported (Reeves, 1972). However, a bacteriophage which lyses a strain of *Rhodopseudomonas palustris* has been discovered (Freund-Mölbart, Drews, Bosecker & Schubel, 1968). Several years ago, during an unsuccessful attempt to find either lysogenic strains of Athiorhodaceae or phage active against members of this family, specific inhibitory interactions resembling bacteriocinogeny were discovered. This communication reports these findings.

METHODS

Organisms. The following organisms were obtained from Dr June Lascelles: *Rhodospirillum rubrum* (NCIB8255); *Rhodopseudomonas spheroides*, strains L (NCIB8253), 2.4.1 and 2.4.3; *Rhodopseudomonas capsulatus*, strains OX, CAM, 2.3.1, 2.3.11 and 2.3.13; and *Rhodopseudomonas palustris*, strains 2.1.7, 2.1.9 and 2.1.12. The other strains of *Rhodopseudomonas spheroides* (2.4.7, 2.4.9 and 2.4.15), *Rhodopseudomonas capsulatus* (2.3.2, 2.3.3, 2.3.6, 2.3.8, 2.3.9, 2.3.10 and 2.3.14), and of *Rhodopseudomonas palustris* (2.1.14, 2.1.23, 2.1.26, 2.1.30 and 2.1.35), were obtained from Professor C. B. van Niel. The cultures were maintained as stabs developed photosynthetically according to Lascelles (1956).

Medium. The malate-glutamate medium of Lascelles (1956, 1959) supplemented with Difco yeast extract (0.2 %, w/v) was used throughout and this was solidified with 1.5 % (w/v) Difco agar or with 0.6 % (w/v) for soft agar overlays. Liquid cultures (8 ml) were inoculated from fresh stab or agar slants and grown without aeration in narrow tubes in the light for approximately 40 h at 30 °C.

Detection of bacteriocin production. The most satisfactory procedure for detecting inhibitory interactions between strains consisted of inoculating plates of solid medium with small drops of liquid cultures of 12 different potential producer strains. After incubating for 2 or 3 days at 30 °C in the dark, the bacteria were killed by irradiation for 10 min 25 cm from a Hanovia Chromatolite u.v. source (15 W, without filter). The plates were then covered with soft agar overlays (2.5 ml), seeded with different indicator strains (approximately 4×10^8 bacteria) and incubated up to 6 days with daily examination for zones of growth inhibition around the original colonies. If sufficient numbers of bacteriophages survived the irradiation step then this procedure would not distinguish between bacteriocin or bacteriophage production by bacteriocinogenic or lysogenic strains respectively.

Fig. 1. Interactions between members of the family Athiorhodaceae. Strains were tested as potential bacteriocin producers and indicators. The degree of inhibition, estimated visually from the size and transparency of the zone around producer strains is represented thus: ■, good inhibitory activity with clear zones extending 3 to 6 mm from the producer colonies; ▨, weak inhibition; ▩, traces of inhibition just detectable; □, no inhibition; □, corresponding pairs were not tested.

RESULTS AND DISCUSSION

Several attempts to enrich for phage active against representative strains of Athiorhodaceae using streptomycin-resistant hosts and material from a variety of environments, failed to yield plaque-forming or lytic agents. A further screen for lysogenic or bacteriocinogenic strains involving simultaneous growth of six cultures spotted on plates spread with the same strains indicated that *Rhodospseudomonas capsulatus* 2.3.11 inhibited the growth of *R. capsulatus* CAM. However, no plaque-forming activity could be detected when culture filtrates of the former were plated with cultures of the sensitive strain. This suggested that the rather specific antibiotic activity of strain 2.3.11 was due to bacteriocinogeny rather than lysogeny.

A more sensitive screening procedure was developed (see Methods) and the number of strains tested was increased to 27; the results for the tests between 432 pairs are shown in Fig. 1. It can be seen that *Rhodospirillum rubrum* neither inhibited nor was inhibited by any

of the other strains tested. Within the *Rhodopseudomonas spheroides* species group, intra-specific inhibitory interactions were few and weak but all the strains inhibited at least one strain of *Rhodopseudomonas capsulatus*. For the strains of *Rhodopseudomonas pallistris*, intra-specific interaction was also very limited and only half of the strains were significantly active with *R. capsulatus* strains as indicators. The greatest inhibitory activity, both intra-specific and inter-specific, was exhibited by the *R. capsulatus* group. Strains 2.3.2, 2.3.3, 2.3.6, 2.3.10, 2.3.11, 2.3.13, and 2.3.14 were particularly active against most strains of *R. spheroides* and the remaining strains of *R. capsulatus*. Tests between all possible pairs were not included partly because some of the strains of *R. capsulatus* (2.3.6, 2.3.10 and 2.3.11) grew poorly in soft agar overlayers and died rapidly. Several variations in technique yielded similar results. These included omitting the irradiation of producer strains, separating the producer and indicator strains by a layer of unseeded soft agar, and illumination during both periods of growth.

No plaque-forming activity or lytic activity could be demonstrated in the culture filtrates of inhibitory strains. In this respect, strains *R. spheroides* L, *R. capsulatus* 2.3.2, 2.3.11 and 2.3.13, and *R. palustris* 2.1.9 were examined most thoroughly using several indicators. Attempts to induce plaque-forming and lytic activity in culture filtrates by treating log phase cultures with sublethal doses of u.v. light or mytomycin C (0.5 µg/ml) were also unsuccessful. It is appreciated that special conditions may be required for detecting plaque formation and that the survey is limited. Nevertheless, the simplest interpretation of the observed inhibitory interactions is that they are probably due to a low level and uninducible production of bacteriocins. A suitable name for this family of antibiotic agents would be 'rhodopseudomonacins'.

I wish to acknowledge support from a Guinness Research Fellowship in Microbiological Biochemistry (Oxford) at the time of this work.

REFERENCES

- FREUND-MÖLBERT, E., DREWS, G., BOSECKER, K. & SCHUBEL, B. (1968). Morphologie und Wirkkreis eines neu isolierten *Rhodopseudomonas palustris*-Phagen. *Archiv für Mikrobiologie* **64**, 1-8.
- LASCELLES, J. (1956). Synthesis of porphyrins and bacteriochlorophyll by cell suspensions of *Rhodopseudomonas spheroides*. *Biochemical Journal* **62**, 78-93.
- LASCELLES, J. (1959). Adaptation to form bacteriochlorophyll in *Rhodopseudomonas spheroides*: changes in activity of enzymes concerned in pyrrole synthesis. *Biochemical Journal* **72**, 508-118.
- REEVES, P. (1972). *Molecular Biology, Biochemistry and Biophysics*, vol. 11, *The Bacteriocins*. London: Chapman and Hall.