SENSITIVITY OF THERMOPHILIC CAMPYLOBACTERS TO R-TYPE PYOCINES OF *PSEUDOMONAS AERUGINOSA*

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SUMMARY. Strains of thermophilic campylobacters of human origin were examined for bacteriocine activity and for susceptibility to R-type pyocines of *Pseudomonas aeruginosa*. None of 50 strains inhibited the growth of any other strain, but 13 of 80 strains (16%) were sensitive to R-type pyocines. Absorption of one of the partially purified pyocine preparations with a sensitive strain resulted in the removal of pyocine activity and a decrease in viable count of the organism by 85%.

INTRODUCTION

The importance of campylobacters as animal pathogens has been recognised for many years. Human campylobacter infection was first reported by Vinzent, Dumas and Picard (1947), but during the next decade only 15 cases were reported (King, 1957). More recently, the thermophilic members of the genus have been shown to be commonly associated with diarrhoeal disease in man (Butzler *et al.*, 1973; Skirrow, 1977; Telfer Brunton and Heggie, 1977). Large outbreaks have been attributed to ingestion of contaminated water (Trehan and Vogt, 1978) and contaminated milk (Robinson *et al.*, 1979).

Studies of the epidemiology of campylobacter enteritis in man have been hindered by difficulties in classification and by the lack of simple methods for typing the causative organism. Biotyping has provided useful information but is time consuming and exacting (Skirrow and Benjamin, 1980). The development of serological methods has been hindered by difficulties in detecting somatic antigens (Butzler and Skirrow, 1979; Watson, Kerr and McFadzean, 1979), but they have now proved their value in epidemiology (Jones, Eldridge and Dale, 1980) and taxonomy (Penner and Hennessy, 1980). We have not found any reports of successful typing by means of bacteriocines or bacteriophages in the literature.

In this study we examined two possible methods for typing thermophilic campylobacters: examining strains for bacteriocine activity, and testing their sensitivity to partially purified preparations of rod-type (R-type) pyocines of *Pseudomonas aeruginosa*, which are of use for typing *Neisseria gonorrhoeae* (Blackwell, Young and Anderson, 1979), and non-serogroupable strains of *N. meningitidis* (Blackwell and Law, 1981).

MATERIALS AND METHODS

Bacteria. Thermophilic strains of *Campylobacter* were isolated from the faeces of patients or animals by the method of Skirrow (1977) and had the following characters: positive oxidase and catalase reactions; sensitive to nalidixic acid; growth at 43°C but not at 25°C. In all, 80 strains, each representing a separate isolation, were used in the following experiments. They were grown in an atmosphere containing *c.* 5% O₂ and 5% CO₂ (Skirrow, 1977).

Strains of *P. aeruginosa* that produce R-type pyocines, listed in table I, were obtained from Dr J. R. W. Govan of this department and Dr M. Kageyama, Mitsubishi-Kasei Institute of Life Sciences, Tokyo. Kageyama (1975) classified many R-type pyocines into groups on the basis of their spectra of activity; strains representing each of these groups were chosen for this study. *P.*

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aeruginosa strain ZD8/38, also from Dr Kageyama and sensitive to all the pyocines tested, was used as a positive indicator strain.

Screening of campylobacter strains for bacteriocine activity. Fifty strains of thermophilic campylobacters were tested against one another for the production of bacteriocines by the method of Gillies and Govan (1966). "Producer strains" were grown overnight (18 h) at 37°C on Tryptone Soya Agar (Oxoid) supplemented with 5% (v/v) of defibrinated horse blood. "Indicator strains" were grown overnight (18 h) in nutrient broth (Oxoid Nutrient Broth No. 2). This experiment was repeated with producer strains (1) that had been grown overnight on tryptone soya agar at 35°C, and (2) log-phase cultures of which had been grown for 18 h at 37°C in nutrient broth containing mitomycin C (1 µg/ml) before being plated on to tryptone soya agar and grown for 18 h at 37°C. Areas of inhibition of growth of the indicator strains by producer strains were taken as evidence of bacteriocine-like activity.

Assay of pyocine activity against campylobacter strains. The R-type pyocines were induced and partially purified as described previously (Blackwell *et al.*, 1979), after the method of Govan (1974). Sensitivity to R-type pyocines was determined by placing drops (15 µl) of a 1 in 2 dilution of each pyocine preparations on to plates of (1) tryptone soya agar supplemented with 5% (v/v) of defibrinated horse blood, (2) nutrient agar (Columbia Agar Base, Oxoid) containing agar 1% (w/v), and (3) similar nutrient agar containing agar 2% (w/v). After the drops had been absorbed into the agar, the plates were flooded with a 1 in 100 dilution of an overnight culture of a campylobacter strain in nutrient broth. Excess inoculum was removed by means of a sterile pipette, and the plates were then incubated overnight at 37°C in microaerobic conditions as described above. Results were recorded as + = inhibition, ± = inhibition with some overgrowth, - = no inhibition.

Preparation of electronmicrographs. A negative-staining technique described by Govan (1974) was used.

Titration of pyocine activity. Twofold dilutions of the pyocine suspension were made in Dulbecco's Buffered Salt Solution (BSS; Dulbecco A, Oxoid) with 0.2% phenol red. A drop (15 µl) of each dilution was placed on a dried plate of nutrient agar (agar 2% w/v). When the drops had been absorbed the plate was flooded with a suspension of *P. aeruginosa* strain ZD8/38 (10⁷ cfu/ml), and incubated at 37°C for 18 h. The pyocine titre was the highest dilution that produced a clear area of inhibition of the bacterial lawn.

Adsorption of pyocine activity by campylobacter cultures. A turbid suspension of the campylobacter containing 10⁸-10⁹ cfu/ml was made in BSS. Equal volumes of bacterial suspension and pyocine preparation were mixed, incubated at 37°C for 30 min and then

TABLE I
Pyocine-producing strains of Pseudomonas aeruginosa

Kageyama classification	Number of pyocine-producing strain
R1	IS4 IS6 IS8 ISD 2285
R2	ISB R21 pp-430
R3	ISE
R4	R4
R5	ISC ISA
Unclassified	R205 9579

centrifuged for 15 min at 5000 rpm. The supernatant fluid was titrated for pyocine activity as described above. The pellet was resuspended to the original volume in BSS and the viable count determined by the method of Miles, Misra and Irwin (1938).

RESULTS

Production of bacteriocines by campylobacter strains

None of the 50 strains inhibited the growth of any other strain, whether it had been grown at 37°C or 35°C or pre-incubated with mitomycin C. Thus, none of them gave evidence of bacteriocine-like activity in the conditions examined.

Sensitivity of campylobacter strains to R-type pyocines

Of the 80 strains examined, 13 (16%) were sensitive to one or more of the pyocine preparations. These were most often and most markedly sensitive to pyocines of strains 9579, ISA and ISC. Two isolates were sensitive to these three and also to R205 (unclassified) and three pyocines of group R1: ISD, IS6 and IS8. Sensitivity to any of the pyocines of groups R2, R3 or R4 was not observed (table II).

In addition to the initial sensitivity tests on blood agar, the experiments were repeated on 1% and 2% nutrient agar on which growth tended to be less mucoid, so that extracellular material would be less likely to interfere with the attachment of pyocines. Identical results were obtained on all three media.

Electronmicrographic studies

We were unable to demonstrate attachment of pyocine no. 9579 to campylobacter strains that were sensitive to its inhibitory activity.

Adsorption of pyocine activity by campylobacter strains

Absorption studies were performed with a preparation of pyocine no. 9579 (titre 256) and a pyocine-sensitive campylobacter strain (no. 13624) and a pyocine-resistant campylobacter strain (no. 01/T). After absorption with the resistant strain the titre of pyocine was 128 and after absorption with the sensitive strain it was less than 4. The viable count of the two suspensions used to adsorb the pyocine were determined before adding the pyocine suspension and at the end of the experiment. There was no decrease in the viable count of the resistant isolate during incubation with the pyocine but the viable count of the sensitive isolate was reduced by 85%, i.e., from 1.51×10^8 cfu/ml to 2.25×10^7 cfu/ml.

TABLE II
Inhibition of campylobacter strains by extracts containing R-type pyocines

Number of campylobacter strains	Inhibition of the stated number of campylobacter strains* by pyocines of Kageyama group													
	R1					R2			R3	R4	R5		Unclassified	
	ISD	2285	IS4	IS6	IS8	ISB	R21	pp-430	ISE	R4	ISA	ISC	R205	9579
67	—	—	—	—	—	—	—	—	—	—	—	—	—	—
4	—	—	—	—	—	—	—	—	—	—	—	—	—	+
2	—	—	—	—	—	—	—	—	—	—	+	—	—	+
5	—	—	—	—	—	—	—	—	—	—	+	+	—	+
2	±	—	—	±	±	—	—	—	—	—	+	±	±	±

+ = Inhibition of growth; ± = partial inhibition of growth; — = no inhibition of growth.

* Reference number of pyocine-producing strain (see table I).

DISCUSSION

Sensitivity to R-type pyocines has been found to be of value in typing strains of *P. aeruginosa* (Gillies and Govan, 1966), strains of *N. gonorrhoeae* (Blackwell *et al.*, 1979) and non-serogroupable *N. meningitidis* (Blackwell and Law, 1981). As far as we are aware, the sensitivity of thermophilic campylobacters to R-type pyocines has not been reported previously. We were unable to demonstrate the attachment of pyocines to campylobacter cells by electronmicroscopy, possibly because of the instability of the campylobacter cell envelope, but we were able to remove the pyocine activity by absorption with suspensions of pyocine-sensitive campylobacters but not of pyocine-resistant campylobacters.

Only a small minority of the cultures tested were sensitive to the pyocines used in this study. Similar results were obtained with capsulate serogroupable meningococcal strains (Blackwell and Law, 1981). The capsular material of campylobacter strains may prevent the pyocines from penetrating to the receptor sites. Among 13 campylobacter isolates sensitive to the pyocines there were four distinct patterns of sensitivity (table II).

The receptor site for R-type pyocines is in the lipopolysaccharide (LPS) of the cell envelope of *P. aeruginosa* (Ikeda and Egami, 1973; Govan, 1974; Koval and Meadow, 1977) and a similar location has been suggested in *Neisseria* (Sidberry and Sadoff, 1977; Sadoff, Zollinger and Sidberry, 1978; Blackwell *et al.*, 1979). Because campylobacter cell walls closely resemble those of other gram-negative organisms, similar receptors for R-type pyocines may exist in the LPS of campylobacters. Our results suggest that some campylobacter strains share similar cell-envelope components, and sensitivity to the various groups of pyocines may reflect this.

Sensitivity to pyocines occurs too infrequently among thermophilic campylobacters to form the basis of an independent typing scheme, but it might be of help in the further development of serological typing schemes, in that it may provide a means of detecting differences in somatic antigens. The different LPS types might be used to develop assay systems similar to the microbactericidal assay used to classify *N. meningitidis*, group B (Frasch and Chapman, 1972), or the haemagglutination inhibition (Mandrell and Zollinger, 1977) and radioimmunoassay methods (Zollinger and Mandrell, 1977) used to determine LPS serotypes among capsulate strains of meningococci.

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