SHORT ARTICLES

A BACTERIOCIN TYPING SCHEME FOR *BACTEROIDES*

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SUMMARY. Epidemiological studies of *Bacteroides* spp. have been hindered because a suitable typing method is not available. In preliminary studies, 50 strains of *Bacteroides* were screened against each other for bacteriocin production and sensitivity; 54% of them produced bacteriocin(s) and more than 90% were sensitive to at least one bacteriocin. After calculation of similarity values for these 50 isolates, a typing set of six bacteriocinogenic strains was selected for a typing method based on bacteriocin sensitivity. With this typing set *c.* 90% of strains could be typed and tests of reproducibility suggested that acceptable accuracy and discrimination could be obtained without applying any one-reaction or two-reaction difference rules. Isolates from four hospitals gave a similar spectrum of typing patterns with 18 bacteriocin types being demonstrated. There was no correlation between bacteriocin type and species of *Bacteroides*. Repeat isolates from the same patient gave identical typing patterns.

INTRODUCTION

Epidemiological studies with bacteroides isolates have been hindered by the lack of readily available, standardised typing methods. For epidemiological purposes, other bacteria have been classified by serotyping, phage typing, biotyping and bacteriocin typing. Serological classification of *Bacteroides* spp. by direct agglutination has been proposed by Elhag, Bettelheim and Tabaqchali (1977) and Lambe and Moroz (1976); this may be a reliable method but it is time consuming and requires the production of a large range of antisera, and hence is not readily available. Fluorescent-antibody techniques have limited value as a means of classification but may be useful in the rapid diagnosis of bacteroides infections (Kasper, Fiddian and Tabaqchali, 1979). Bacteriophages active against *B. fragilis* have been reported by Keller and Traub (1974), and Booth *et al.* (1979) suggest that this may form the basis for a typing scheme. Biotyping has been used successfully as an epidemiological tool with some members of the Enterobacteriaceae such as *Escherichia coli* (Crichton and Old, 1979), but this method of typing has never been suggested for *Bacteroides* spp.

There have been few reports on the production of bacteriocins by *Bacteroides* spp. (Beerens and Baron, 1965; Booth, Johnson and Wilkins, 1977; Mossie *et al.*, 1979) and none has described a method of typing based on production of, or sensitivity to, bacteriocins. The aim of this report is to investigate the phenomenon and to develop a reliable and discriminatory bacteriocin typing system for clinical isolates of *Bacteroides*.

MATERIALS AND METHODS

Bacterial strains. One hundred and twenty strains of *Bacteroides* were obtained from the Department of Microbiology Anaerobe Laboratory, University of Western Australia. They represented those *Bacteroides* spp. isolated most commonly from human infections at four major metropolitan hospitals (Sir Charles Gairdner Hospital, Repatriation General Hospital,

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Princess Margaret Hospital, and King Edward Memorial Hospital) in Perth, Western Australia. These hospitals provide a wide range of services including general medicine and surgery, geriatrics, paediatrics, and obstetrics and gynaecology. All isolates from clinical specimens were identified to genus level on the basis of growth characteristics, morphology in gram-stained smears and the detection of the end products of glucose fermentation by gas-liquid chromatography, as described by Holdeman, Cato and Moore (1979). They were assigned to species on the basis of their biochemical reactions tested by the Microbact 24A Anaerobe System (Disposable Products, South Australia). The test strains were *B. fragilis* (43 strains), *B. capillosus* (16), *B. ruminicola* (16), *B. disiens* (8), *B. oralis* (6), *B. melaninogenicus* (5), *B. multiaacidus* (5), *B. distasonis* (4), *B. asaccharolyticus* (4), *B. vulgatus* (4), *B. bivius* (4), *B. ovatus* (2), *B. thetaiotaomicron* (2) and *B. uniformis* (1).

Bacteriocin-typing method. Fifty strains of *Bacteroides* chosen randomly were screened for sensitivity to bacteriocins against the same 50 strains as producers by the membrane-filter method of Riley and Mee (1981).

Selection of a typing set. From the results of the initial screening, a similarity matrix was produced. A dendrogram was constructed from the similarity matrix by the method of Sneath (1957) and a typing set of six producers, one from each of the major sets of isolates, was selected from the relationships exposed in the dendrogram. The mnemonic code devised by Farmer (1970) was used to denote typing patterns: the reactions of the bacteriocin producers are divided into triplets and each triplet is given a number from 1 to 8. Because only six producer strains were used, each bacteriocin type was coded as a two-digit number.

Tests for reproducibility of the typing method. The reproducibility of the typing system was examined in three ways: (i) a collection of 20 unrelated *Bacteroides* strains, including the six standard producer strains, was typed on four consecutive weeks; (ii) 10 colonies of a bacteroides isolate were picked from a pure culture and each used as an indicator strain; (iii) repeat isolates of *Bacteroides* from infections in three patients were typed; 11 isolates were obtained during several weeks.

TABLE I
Bacteriocin-sensitivity typing patterns of 20 unrelated strains of Bacteroides

Test strains		Inhibition by bacteriocin from producer strain						Code
		P1	P2	P3	P4	P5	P6	
<i>B. fragilis</i> ,	strain A76	—	+	—	—	—	—	41
<i>B. fragilis</i> ,	strain A11	—	+	+	+	+	+	38
<i>B. capillosus</i> ,	strain A71	+	+	—	—	+	+	73
<i>B. distasonis</i> ,	strain A64	—	+	—	—	+	+	43
<i>B. fragilis</i> ,	strain QE27	—	+	—	+	+	+	48
<i>B. fragilis</i> ,	strain A74	—	—	—	—	—	—	11
<i>B. capillosus</i> ,	strain PMH4	—	+	—	—	—	+	42
<i>B. ruminicola</i> ,	strain QE35	+	+	+	+	+	+	88
<i>B. vulgatus</i> ,	strain RGH5	—	+	—	—	+	—	44
<i>B. fragilis</i> ,	strain QE23	—	+	—	—	+	+	43
<i>B. fragilis</i> ,	strain A45	—	+	+	+	+	+	38
<i>B. capillosus</i> ,	strain PMH2	—	+	—	—	+	+	43
<i>B. capillosus</i> ,	strain PMH1	—	+	—	—	+	+	43
<i>B. capillosus</i> ,	strain PMH5	—	+	—	+	+	+	48
<i>B. fragilis</i> ,	strain A69 (P1)	—	+	—	+	—	—	46
<i>B. fragilis</i> ,	strain A55 (P2)	+	—	+	+	+	+	58
<i>B. fragilis</i> ,	strain PMH3 (P3)	+	—	—	—	—	+	62
<i>B. capillosus</i> ,	strain RGH9 (P4)	+	—	+	—	—	+	52
<i>B. fragilis</i> ,	strain A67 (P5)	+	+	+	+	—	+	85
<i>B. capillosus</i> ,	strain RGH7 (P6)	—	—	—	—	—	—	11

+ = Inhibition; — = no inhibition.

RESULTS

Selection of a typing set of bacteriocin producers. Of the 50 strains screened originally 27 (54%) were bacteriocinogenic; sensitivity to at least one bacteriocin was found in 45 (90%) while 42 (84%) were sensitive to two bacteriocins, and 40 (80%) were sensitive to three or more bacteriocins. Hence by sensitivity testing it should have been possible to type all, or most, isolates of *Bacteroides*. The six producer strains chosen were *B. fragilis* strains A69 (P1), A55 (P2), PMH3 (P3), A67 (P5) and *B. capillosus* strains RGH9 (P4) and RGH7 (P6).

Tests for reproducibility of the typing method. The typing reactions given by 20 unrelated bacteroides isolates are shown in table I. No significant differences in typing pattern were found during the 4-week period although slight variations in the intensity of some reactions occurred occasionally. All 10 colonies used as indicator strains gave the same typing reactions. The isolates in each set from the different patients were of the same bacteriocin type.

Typing patterns in clinical isolates of Bacteroides. The typing patterns given by 120 clinical isolates of *Bacteroides* are shown in table II; 18 bacteriocin types out of a possible maximum of 64 were found. The most common pattern amongst the 120 strains (type 38) represented 21.6% of the strains. Fourteen isolates (11.7%) were insensitive to the set of six bacteriocins produced and hence were untypable. Table III shows the relationship between the species of *Bacteroides* and the bacteriocin type.

TABLE II
Frequency of typing patterns among 120 clinical isolates of *Bacteroides*

Bacteriocin type	Number (and percentage) of isolates	Bacteriocin type	Number (and percentage) of isolates
38	26 (21.7)	44	3 (2.5)
43	22 (18.3)	46	2 (1.7)
48	12 (10.0)	52	2 (1.7)
41	8 (6.7)	56	2 (1.7)
73	6 (5.0)	58	2 (1.7)
86	6 (5.0)	62	2 (1.7)
88	4 (3.3)	76	2 (1.7)
71	3 (2.5)	85	1 (0.8)
42	3 (2.5)	11	14 (11.7)
		(untypable)	

TABLE III
Relationship between the species of *Bacteroides* and bacteriocin type

Species	Number of isolates of bacteriocin type																		
																			(untypable)
	38	43	48	41	73	86	88	71	42	44	46	52	56	58	62	76	85	11	
<i>B. fragilis</i>	10	11	5	2	2	0	2	0	0	0	1	0	0	1	2	0	1	6	
<i>B. capillosus</i>	0	8	2	0	3	0	0	0	0	0	1	0	0	0	0	0	0	2	
<i>B. ruminicola</i>	9	0	2	1	1	0	2	0	0	0	0	1	0	0	0	0	0	0	
<i>B. disiens</i>	2	2	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	3	
<i>B. oralis</i>	0	0	2	1	0	0	0	0	0	0	0	1	0	0	0	2	0	0	
<i>B. melaninogenicus</i>	0	0	0	0	0	2	0	3	0	0	0	0	0	0	0	0	0	0	
<i>B. multiaacidus</i>	0	1	0	1	0	0	0	0	2	0	0	0	0	0	0	0	0	1	
<i>B. distasonis</i>	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	2	
<i>B. asaccharolyticus</i>	0	0	0	0	0	4	0	0	0	0	0	0	0	0	0	0	0	0	
<i>B. vulgatus</i>	0	0	1	0	0	0	0	0	1	2	0	0	0	0	0	0	0	0	
<i>B. bivius</i>	2	0	0	0	0	0	0	0	0	1	0	0	0	1	0	0	0	0	
<i>B. ovatus</i>	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
<i>B. thetaiotaomicron</i>	1	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	
<i>B. uniformis</i>	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	

DISCUSSION

Bacteriocinogeny and sensitivity to bacteriocins have been applied successfully as epidemiological tools with several species of bacteria, e.g., *Shigella sonnei* (Abbott and Shannon, 1958), *Pseudomonas aeruginosa* (Gillies and Govan, 1966), *E. coli* (Linton, 1960). Most typing schemes are based on the production of bacteriocins; sensitivity to bacteriocins is used only occasionally. Results of the initial screening suggested that a typing scheme based on sensitivity to bacteriocins would be the more rewarding approach. These results also demonstrated some relationships between some producer strains and were confirmed by statistical analysis. By the use of nine or more producer strains it would have been possible to derive a larger number of types, each comprising fewer members. The six producer strains chosen showed a wide range of activity and preliminary tests of the typing method demonstrated that most strains of *Bacteroides* were typable and that various patterns were obtained.

With typing schemes of this nature it is important to assess the reproducibility of the method before applying it in any epidemiological investigation. We tested 20 unrelated *Bacteroides* isolates during a 4-week period, and also groups of related *Bacteroides* strains, and obtained complete reproducibility. Because only six producer strains were used it was found unnecessary to apply any two-reaction or three-reaction difference rules which have been introduced for bacteriocin typing schemes of *P. aeruginosa* (Rampling, Whitby and Wildy, 1975) and *Serratia marcescens* (Anderhub *et al.*, 1977) to obtain acceptable reproducibility. Good discrimination was still obtained and only 11.7% of isolates could not be typed, a figure that compares favourably with the 16.8% untypable reported by Edmondson and Cooke (1979) for their bacteriocin typing of *Klebsiella*. There was no obvious correlation between the bacteriocin type and the species of *Bacteroides*. Because the 120 isolates used in this study had come from four hospitals differing in their patient population, it was thought that differences in bacteriocin type might have been noticeable depending on the origin of the isolate. However this was not the case and various bacteriocin types were distributed evenly throughout the isolates from the four hospitals studied. Whether or not a relationship exists between the serological type and bacteriocin type has not been determined.

Investigations into the epidemiology of bacteroides infections have been hampered by lack of a readily available typing method. The bacteriocin-typing method described in this paper is easier to perform than serotyping and its discriminatory power and reproducibility suggest that it may be a valuable diagnostic and epidemiological tool.

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