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A study on bacteriocin typing of avian strains of *Pasteurella multocida*

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SUMMARY

In two groups, each containing 56 *Pasteurella multocida* strains of avian origin, the percentage of multicin-producing strains was 80·4 and 71·4. In two groups containing 46 and 58 isolates tested for sensitivity to multicins, 82·6% and 62·1% respectively were listed as indicators. *P. multocida* strains producing bacteriocins were active on a range of 1–33 indicators. A preliminary multicin typing system was set up with the use of eight selected indicators; 52% of multicin-producing strains acted on one indicator only. *P. multocida* serotypes and serologically untypable strains were subdivided into multicin types.

INTRODUCTION

Bacteriocins, especially those derived from gram-negative bacteria, have a restricted range of activity, limited mainly to the same or closely related species. Bacteriocin typing schemes have been established for many species of bacteria (Reeves, 1965; Tagg, Dajani & Wannamaker, 1976).

Studies on bacteriocin activity within the genus *Pasteurella* are scanty. It was reported that *P. pestis* produced a bacteriocin-like material and bacteriocins for which the name 'pesticin' was proposed (Ben-Gurion & Hertman, 1958; Brubaker & Surgalla, 1961). Bacteriocin activity was shown by bovine and bison strains of *P. multocida* (Chengappa & Carter, 1977).

The present communication refers to bacteriocins produced by *P. multocida* strains isolated from poultry in Israel (Mushin, 1979). Serotyping of somatic antigens of these strains, using sixteen diagnostic sera, pointed to the predominance of two serotypes, 1 and 3. This limited the usefulness of the typing scheme, which was meant to indicate the source of infection and the geographical distribution of the isolates. A study was therefore carried out on *P. multocida* strains as potential bacteriocin-producers to utilize these agents for a finer resolution of the serotypes. The naming of bacteriocins has been haphazard (Tagg *et al.* 1976) but the designation 'multicins' seems to be appropriate in reference to *P. multocida* species and is introduced in the present paper.

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MATERIALS AND METHODS

Pasteurella multocida *cultures*

The sources of the organisms were turkeys, chickens and geese affected with fowl cholera. The strains used had been in storage for periods of a few days to four years, kept in nutrient broth at room temperature or in a lyophilized state. They were identified on the basis of morphological, cultural and biochemical characteristics and were serotyped by the gel diffusion precipitin test (Mushin, 1979).

Multicin typing methods

Two methods were compared:

(i) A lawn of a *P. multocida* strain serving as an indicator was grown on a tryptose agar plate and strains tested for bacteriocin activity were spotted on the surface (Chengappa & Carter, 1977).

(ii) The results of the present study were based on the method of deferred antagonism and will be described briefly.

Media and temperature

The composition of the medium, the temperature and the time of incubation of cultures are known to be critical factors for the production of bacteriocins. In the preliminary tests dextrose starch agar (Difco) with 5% sheep blood or Columbia agar (Oxoid) were used. However, in the course of this study other media proved to be more suitable and these were as follows. In the preparation of agar plates a layer of saline (0·85%) peptone (0·5%) agar (1·5%) served as a base, followed by a layer of tryptose agar (Difco) with the addition of 0·3% yeast extract, 0·01 M-$CaCl_2$ and 5% sheep blood. The liquid cultures were in 5 ml tryptose broth.

The temperature of 30 °C and the period of incubation of 16 h proved to be suitable for the demonstration of multicin production and for testing the sensitivity of indicator strains. These conditions were introduced after preliminary screening tests involving incubation at 37 °C, 32 °C and 30 °C for periods of 8 h, 16 h and 18 h.

Multicin typing

The procedure was similar to that employed for pyocine typing (Gillies & Govan, 1966; Tagg & Mushin, 1971). A tryptose broth culture of a *P. multocida* strain, used as a potential producer of multicin, was streaked across a plate. The growth was removed and the plate was exposed to chloroform. Tryptose broth cultures of the potential indicator strains were diluted 10^4 and were streaked at a right angle to the original band of growth, using a simple instrument with eight prongs for the delivery of the indicators.

Table 1. *Frequency of multicin producing and indicator strains amongst isolates of* Pasteurella multocida

	Producer strains			Indicator strains		
		Positive			Positive	
Group	No. tested	No.	%	No. tested	No.	%
A	56	45	80·4	46	38	82·6
B	56	40	71·4	58	36	62·1

Table 2. *Patterns of multicin production amongst strains of* P. multocida

Group A – 56 strains			Group B – 56 strains		
Potential producer strains		Inhibition of a stated number of indicator strains	Potential producer strains		Inhibition of a stated number of indicator strains
No.	%		No.	%	
15	26·8	1	11	19·6	1
7	12·5	2	4	7·1	2
5	8·9	3	9	16·1	3
4	7·1	4	4	7·1	4
3	5·3	6	2	3·6	5
2	3·6	7	1	1·8	6
2	3·6	8	1	1·8	7
2	3·6	9	1	1·8	8
1	1·8	12	3	5·3	9
1	1·8	21	2	3·6	10
1	1·8	24	1	1·8	12
1	1·8	27	1	1·8	27
1	1·8	33	16	28·6	0
11	19·6	0			

RESULTS

Multicin producers and indicators

Table 1 shows the frequency of multicin-producing strains amongst a total of 112 *P. multocida* strains. The strains were placed at random in two groups A and B, each containing 56 strains; the percentage of multicin producers was 80·4 and 71·4. The percentage of indicator strains was 82·6 and 62·1 in two groups of 46 and 58 strains respectively. The two groups shared 17 selected indicators.

Table 2 presents the pattern of multicin activity on the indicator strains. The range of activity varied; thus some strains were active on a variable number of indicators, from 1 to 33, while other strains were inactive (19·6% and 28·6%).

The above data were used for a preliminary multicin typing scheme (Table 3). With eight selected indicator strains it was possible to type 50 (45%) of *P. multocida* strains, allocating them to ten multicin types. Table 4 indicates the subdivision of *P. multocida* serotypes into multicin types, using the scheme adopted in Table 3.

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Table 3. *Patterns of inhibition of eight indicator strains by ten multicin types of 50 strains of* P. multocida

Multicin type	Inhibition of indicator strains no. 1	2	3	4	5	6	7	8	Strains belonging to a stated multicin type No.	%
1	−	+	−	−	−	−	−	−	20	40
2	−	−	−	+	−	−	−	−	6	12
3	+	+	+	+	+	+	+	+	3	6
4	+	+	+	−	+	+	+	+	2	4
5	+	+	+	−	+	+	+	−	2	4
6	+	+	−	−	+	+	+	−	2	4
7	+	+	−	−	−	+	+	−	3	6
8	+	+	−	+	+	−	−	−	5	10
9	−	+	−	+	+	−	−	−	4	8
10	−	+	+	−	−	−	−	−	3	6

Table 4. *Distribution of multicin types amongst serotypes of* P. multocida

Serotype	No. of strains of each serotype	No. of producer strains	No. of multicin types
1	64	32	10
3	30	13	5
Various	13	8	4
Untypable	5	5	4

Reproducibility of multicin typing results

Tests were carried out using several strains originating from the same flocks, and usually the results were consistent. However, occasionally resistant colonies appeared amongst the sensitive indicator strains, thus giving a less distinct or different pattern. A higher dilution of the indicators or a shorter time of incubation often gave the original pattern.

U.v. radiation

During the course of this study some *P. multocida* strains were exposed to u.v. radiation to induce or enhance multicin activity. Some weak multicin producers were activated, however a variability in the results was encountered and therefore u.v. treatment was not applied in the routine procedure.

DISCUSSION

Two groups of *P. multocida* strains examined for bacteriocin production contained 80·4 % and 71·4 % showing an inhibitory activity. Exposure to u.v. radiation activated some weak producers but the results were not stable. In a survey of 33 *P. multocida* bovine and bison strains exposed to u.v. radiation, 42·4 % strains showed bacteriocin activity (Chengappa & Carter, 1977).

The critical factors in the tests proved to be the dilution of the indicator strains, the type of media used, the temperature and the time of incubation of cultures. Thus the laboratory conditions should be strictly controlled. The validity of a bacteriocin typing scheme rests on the reproducibility of the results, but occasional discrepancies were encountered.

The bacteriocin producers within the large groups of *P. multocida* serotypes 1 and 3 acted on a variety of indicators, thus providing a subdivision in the serological classification, useful in epidemiological surveys. The subdivision of the untypable strains pointed to their different origin.

It is expected that the selection of additional sensitive indicators will increase the percentage of typable strains, and provide a wider range of susceptible strains to serve as indicators.

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