Colony Incompatibility among New Zealand Isolates of *Salmonella typhimurium*

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(Received 6 February 1978; revised 5 April 1978)

Strains of *Salmonella typhimurium* representing various phage-types were permitted to swarm towards each other on soft agar to determine their compatibility. Some strains, regardless of phage-type, were totally compatible, whereas others regularly formed incompatibility zones. The widths of these zones varied from less than 1 mm to 12 mm. A detailed study of 30 strains of phage-types 1 and 42 showed that they belonged to a variety of incompatibility groups, while four strains of phage-type 179, which was only recently found in New Zealand, all belonged to one incompatibility group.

INTRODUCTION

The Dienes phenomenon of *Proteus* (Coetzee, 1972; Senior, 1977) was considered to be peculiar to this group of bacteria until Bettelheim & Carlile (1976) showed that, with suitably adjusted media, a similar phenomenon of colony incompatibility could be demonstrated among other enterobacteriaceae. In a preliminary study, Bettelheim (1977) described various incompatibility reactions, and showed that in *Salmonella typhimurium* the H antigen played no role in this phenomenon.

Of the *S. typhimurium* strains isolated in New Zealand, phage-types 1 and 42 are most common and a method of subdividing them was sought. Phage-type 179 had not been described in New Zealand before 1977 (S. M. Harding, personal communication) and it was considered possible that all four strains found represented a single clone.

This paper describes the compatibility testing of a random selection of strains isolated in New Zealand during 1977 and a detailed study of strains representing phage-types 1, 42 and 179.

METHODS

Bacterial strains and medium. The three type strains for phage-types 1, 42 and 179 were from the Hospital Crossinfection Section of the National Health Institute and are designated T01, T42 and T179, respectively. Wild strains of *S. typhimurium* were isolated in many parts of New Zealand and sent to the National Health Institute in Wellington for identification. The strain numbers and their sources are listed in Table 1.

The medium used for all these studies consisted of (g l⁻¹ in distilled water): Bacto Tryptone (Difco), 10; NaCl, 5; Bacto agar (Difco), 3.5. Petri dishes were surface-dried by leaving them at 4 °C for 2 to 3 d.

Full motility was induced in all the strains by passage through semi-solid motility agar and the cultures were subsequently stored at room temperature on Dorset egg slopes. Regular subculture in broth followed by microscopic examination showed that during the course of these studies the strains maintained their full motility.

Test for compatibility. Two test strains were inoculated at either side of a Petri dish which was then incubated at 37 °C for 4 d. Dishes were examined daily in a darkened room under oblique lighting for incompatibility reactions, and the zones of growth were marked on the bottom of the dish. Tests of each strain against itself were included. The different reactions between strains were scored as degrees of incompatibility (Table 2). Being based on subjective observations, complete reproducibility of scoring in successive experiments

Table 1. *Strains of Salmonella typhimurium, their regions of isolation in New Zealand and their incompatibility groups*

Strain no.	Phage type	Region of isolation	Incompatibility group (see Table 3)
T01	1	Type strain	III
X185	1	Nelson	III
X232	1	Christchurch	III
X317	1	Ruakura	VII
X397	1	Tauranga	III
X407	1	Christchurch	III
X428	1	Auckland	III
X483	1	Dunedin	III
X484	1	Dunedin	III
X521*	1	Dunedin	III
X522*	1	Dunedin	III
X523	1	Auckland	III
X532	1	Gisborne	III
B44	1	Hastings	IV
B647	1	Wellington	IV
B173	26	Wellington	II
B179	26	Auckland	II
B798	26	Otahuhu	I
T42	42	Type strain	III
X63	42	Christchurch	VIII
X134	42	Napier	III
X166	42	Wellington	III
X242	42	Auckland	V
X323	42	Ruakura	III
X324	42	Auckland	III
X332	42	New Plymouth	III
X334*	42	Auckland	V
X335*	42	Auckland	III
X392	42	Christchurch	IX
X398*	42	Tauranga	V
X399*	42	Tauranga	V
X405	42	Christchurch	V
B356	42	Wellington	III
B43	101	Whangarei	XI
X427†	135	Auckland	XI
T179	179	Type strain	X
X414†	179	Auckland	XI
X600	179	Auckland	XI
B1123	179	Auckland	XI
B1162	179	Auckland	XI
B175	NT‡	Auckland	VI

* The three pairs of strains, X521/X522, X334/X335 and X398/X399, were isolated from pairs of members of the same families.

† The pair of strains X427/X414 was isolated from the same patient who had a mixed infection.

‡ NT, Not typable.

with the same pairs of strains was not always achieved, but the differences were within one arbitrary unit. All tests were done more than once and the range of scores is given.

Test for bacteriocin production. Selected strains were tested against each other for the production of bacteriocins by the method of Abbott & Shannon (1958).

RESULTS

The types of reactions which were observed between strains varied from a complete merging of two apposing strains, as was seen with two identical inocula, to the formation of a clear zone more than 10 mm wide between the two bacterial swarms (Fig. 1 *a, b*).

Table 2. *Scoring of observed interaction of swarms of Salmonella typhimurium*

Score	Observation
0	Total or partial merging of swarms, strains compatible
1	'Bunching' of one strain, no clear zone between swarms
2	Pronounced 'bunching', clear zone less than 1 mm between swarms
3	Pronounced 'bunching', clear zone 1 to 10 mm wide
4	Pronounced 'bunching', clear zone more than 10 mm wide

Fig. 1. (a) Complete merger of strains; score 0. (b) Pronounced incompatibility, with bunching, and a clear zone over 10 mm wide; score 4.

Table 3. *Incompatibility reactions between strains of Salmonella typhimurium*

For details of scoring, see Table 2 and Fig. 1.

Groups of strains	Incompatibility reaction between groups of strains										
	I	II	III	IV	V	VI	VII	VIII	IX	X	XI
I	0										
II	0	0									
III	0	0	0								
IV	0-1	0	0	0							
V	0-1	1-2	1-2	1-2	0						
VI	2	2	2	2	2	0					
VII	1	1	2	2	0-2	2	0				
VIII	3	3	3	3	3	2-3	2-3	0			
IX	3	3	3	3	3	2-3	3	3	0		
X	3	0-2	4	4	4	3	4	4	4	0	
XI	0	1	4	4	4	2	4	4	4	3	0
	I	II	III	IV	V	VI	VII	VIII	IX	IX	XI

The results of testing all strains against each other are given in Table 3. Pairs of strains, other than those giving reactions of types 3 and 4, developed their characteristic reaction within 1 d, by which time the swarms had come close enough to give either reactions of full compatibility or incompatibility reactions of types 1 or 2. When two strains normally giving a reaction of type 3 or 4 were inoculated, they approached each other to within 1 mm but did not meet. One strain then gave the impression of pushing the other one backwards; often it advanced to cover the area previously occupied by the other strain, which retreated and the density of bacteria nearest the demarcation point increased. The area between the two colonies remained clear despite being previously covered by organisms of the retreating colony.

Strains T01, T42, T179, X63, X232, B356 and X414 were tested against each other for bacteriocin production. They all grew equally well across the line of the killed strain, showing that the incompatibility could not be explained by bacteriocin production.

DISCUSSION

The cause of the phenomenon described here is unknown. Bettelheim (1977) has shown that antigenic differences are not responsible for it. The antagonism between strains of the same species may be due to phage production by one member of the pair, but this seems unlikely as some strains of different phage-types were not antagonistic whilst some pairs of strains belonging to the same phage-type were antagonistic. Bacteriocin production by one of the pair of antagonistic organisms also seems to be an unlikely cause of the antagonism in view of the present findings. This is somewhat surprising as Senior (1977) showed that, in the Dienes phenomenon of *Proteus*, bacteriocins play a crucial role in many, but not all, examples of incompatibility.

A broad explanation of the phenomenon may be that some strains produce a substance or substances to which some other strains are either insensitive, and thus form a compatible pair, or produce a negative chemotactic response and form an incompatible pair. Sturdza (1977) recently suggested such a reaction as an explanation of *Proteus* swarming. If it is shown that substances eliciting negative chemotactic responses in related strains really exist, it would partially explain why, in an ecological situation, certain strains can displace one another.

The photography of Mr G. Bruggemans of the Clinical Photography Department of Wellington Hospital is gratefully acknowledged. I am most grateful to Miss S. M. Harding for providing strains T01, T42 and T179 as well as all the wild strains designated X, and to Miss D. M. Norris for providing the wild strains designated B. Helpful discussions with Miss S. M. Harding, Professor N. P. Markham and Dr D. R. Martin are gratefully acknowledged. Published with the authority of the Director-General of Health, Department of Health, Wellington, New Zealand.

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