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The role of lysogeny in the modification of phage typing patterns of *Staphylococcus aureus* isolated from dairy cows

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SUMMARY

Strains of *Staphylococcus aureus* were isolated at a single sampling from cows in a dairy herd. When typed by phage most showed a complex pattern of lysis with group III and IV phages. Cross-spotting of strains showed great lytic activity and ten phages were isolated. The parent strains were lysogenized with these phages and the effect on the phage typing pattern of 202 daughter strains from 25 parent cultures was studied. Phage action was often blocked completely, with up to four being blocked on the one strain. Those most frequently blocked were phage 101, 117, 367 and 42D.

INTRODUCTION

Probably most if not all staphylococci are lysogenic (Wentworth, 1963), and although it is well known that lysogeny may affect the phage typing pattern, most have concluded that it is of little significance in interpreting phage typing patterns for epidemiological investigations. Williams Smith (1948*a*, *b*) carried out the classic early study on lysogeny of bovine strains and both he and Rountree (1949) considered that acquired phage resistance was of importance in susceptibility to the typing phages. Attention was later focused on epidemic hospital strains of lytic group I. Such strains later spread throughout the world. Suspicion that lysogeny might be responsible for variation in the typing pattern (Rountree, 1956) was finally confirmed for these '80/81' strains (Rountree & Asheshov, 1961).

Some authors who isolated phages from lysogenic cultures and used them to type bovine strains have commented on the role of lysogeny in modifying the typing pattern. Davidson (1961) considered it an uncommon event in nature that would not interfere with typing, and this is implied in the collaborative study on bovine phages (Davidson, 1972). Nakagawa (1960) also concluded that lysogenicity was not an important factor in acquired phage resistance.

When the phage patterns of strains isolated from a single dairy herd are examined it is common to find a limited number of basic patterns, but within each there may be often considerable variation. Often this would be sufficient, on the basis of the rules generally accepted (Blair & Williams, 1961), to determine that the strains are dissimilar. However, it is considered that in the environment of the dairy herd, opportunity might be frequent for contact with phage from other

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Table 1. *The lytic spectrum of four new phages*

	Propagating or testing strains	7/2	7/4	14/11	26/26
	29	—	—	—	3
	52	—	3	—	5
	52A/79	—	—	—	—
	80	1	—	—	—
	2009	4	—	3	—
	71	—	—	1	2
	8719	—	—	—	—
	42C	—	3	—	3
	42E	—	3	—	3
	47	—	—	—	—
	53	—	—	—	—
	54	—	4	—	3
	75	—	—	—	—
	77	—	1	—	—
	31B	—	—	1	—
	42D	1	—	1	—
	78	1	—	3	—
PS of Davidson's (1961) phages	101	—	5	—	5
	102	—	—	2	—
	105/107	—	—	5	—
	108	5	—	5	—
	110	5	5	4	5
	111	4	4	4	5
	115	—	—	—	—
PS of Brisbane phages	10	—	1	5	3
	11/12	5	4	4	5
	13	—	—	—	—
	186	5	4	—	4
	367	—	—	—	—
	373	—	3	5	5
	425	—	—	4	—
	600	—	—	4	—

PS, Propagating strain

strains, and that if lysogeny resulted, the typing pattern of the parent culture might alter. In this paper, strains isolated at a single sampling of a dairy herd were examined and the effect of phages carried by lysogenic organisms on the typing pattern of these organisms was studied.

MATERIALS AND METHODS

Bacterial strains

A large herd was chosen from which quarter samples had been cultured regularly and the staphylococci phage-typed. The major pattern was of group III/IV, with considerable variation. Thirty-four isolates were chosen from a single sampling of the herd, each from a single infected quarter.

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Isolation of phage

The 34 strains were cross-spotted (Fisk, 1942) and the reactions recorded. Twenty phages were isolated, purified, propagated and the lytic spectrum was determined on 32 propagating or testing strains (Table 1). On the basis of the lytic activity of the phages on these strains, 10 were selected for further study.

Lysogeny. The ten new phages were all used at ten times the routine test dilution (RTD $\times$ 10) to 'type' the 25 strains (see below). Incubation was continued until secondary growth occurred. A colony from each area was picked with a straight wire, purified and phage-typed.

Phage typing. The basic techniques were as described by Blair & Williams (1961). A selected set of phages was used as follows: 29, 80, 71, 6, 42E, 47, 53, 77, 31B, 81, 42D; 101, 102, 107, 110 (Davidson, 1961); 117 (Davidson, 1972); 10, 13, 186, 367, 373, 425, 600. The last seven phages were isolated in Brisbane from bovine strains of *S. aureus* (Frost, 1967).

The parent strains were each typed at RTD on four different occasions; the lysogenized cultures were each typed twice on the same day. Eight strains were deleted on the basis of identical lytic patterns and one because of an irregular typing pattern, leaving a total of 25.

RESULTS

Cross-spotting

This yielded 459 reactions from a possible 1122, i.e. 40·9 %. The total possible was 34^2-34. This meant an average of 13·9 reactions on each strain; the range was from 6 to 29. It was clear that phages from some strains were very active, and a selection was made of 20 areas of lysis from which to isolate phage.

Lytic spectrum

Twenty phages were isolated, including one which arose as spontaneous lysis on strain 26. The lytic spectrum was not determined precisely for all these phages as activity on some strains was not carried to the end-point; some were almost certainly related or identical. The ten phages chosen appeared to differ sufficiently to warrant further study. The complete lytic spectrum of each of four new phages is shown in Table 1. They were coded on their origin as follows: 7/2, 7/4, 8/5, 10/25, 11/13, 14/11, 14/25, 29/4, 29/5, 26/26: i.e. the propagating strain of the first phage was strain 2, the phage donated by strain 7 and so on.

Phage patterns of parent strains

The patterns shown by the parent strains are summarized in Table 2, where consistent strong reactions are shown; weak reactions are not included. The repeat typings showed minor variations, usually a change from weak to trace, or strong to weak reaction of an occasional phage.

Table 2. *The lytic pattern of the* 26 *parent strains of* S. aureus

	Strong lytic reactions*													
Phage	6	10	42E	47	77	42D	81	101	102	107	367	600	117	373
1†	+	+	+	+	+	+	+	+	+	+	+	+	+	+
2	+	+	+	+	+	+	+	+	+	+	+	+	+	+
3	+	+	+	+	+	+	+	+		+	+	+	+	+
4	+	+	+	+	+	+	+	+		+	+	+	+	+
6	+	+	+	+	+		+	+		+	+	+	+	
8	+	+	+					+		+	+	+	+	
9		+	+			+		+			+	+	+	+
10	+	+	+					+		+		+	+	
11	+	+	+	+	+		+	+		+	+	+	+	
12	+	+	+	+	+		+	+		+	+	+	+	
13	+	+	+	+	+		+	+				+	+	+
14	+	+	+	+	+		+	+				+		
15	+	+	+	+	+	+	+	+		+	+	+	+	+
16	+	+	+			+		+		+		+	+	+
18		+	+					+		+	+	+	+	
19		+	+			+				+	+	+	+	+
21	+	+	+					+		+		+	+	
22	+	+	+	+			+	+		+	+	+	+	
23	+	+	+	+	+	+	+	+		+	+	+	+	+
24	+	+	+	+		+	+	+	+	+	+	+	+	+
25	+	+	+	+	+	+	+	+	+	+	+	+	+	+
26	+	+	+	+	+	+	+	+		+	+	+	+	+
27	+	+	+			+		+		+	+	+	+	+
28	+	+	+	+		+	+	+		+	+	+	+	+
29		+	+					+		+	+	+	+	+

* These reactions were consistent over four typings.
† Strain numbers.

The effect of lysogeny on parent strains

When the ten phages were used to 'type' the parent strains, lysis occurred on 202 of the possible 625 spots, and cultures, presumed lysogenic, were isolated from the secondary growth. The number of strains made lysogenic by each phage differed, depending on whether or not lysis occurred on the parent strain. No attempt was made to infect strains that were not lysed by the relevant phage.

These 202 cultures were then typed in duplicate with the bovine set of phages at RTD, and it was apparent that some lytic effects were lost. The effects on the lysogenic compared with parent strains are shown in Table 3, and the contribution of the ten phages to the changed typing pattern is shown in Table 4. There were some occasions when the lysogenized culture appeared to gain a strong reaction, but this could not be confirmed except for culture 9, which gained lysis to typing phage 425 when lysogenized with phage 7/2. Though some of the typing phages used were originally isolated from local strains (10, 13, 367, 373, 425, 600), the major and consistent effects of lysogeny were on phages isolated elsewhere – 42D, 101, 117. Up to four strong reactions were often lost (Table 3), sufficient to assume non-identity with any reasonable criteria used. Multiple deletion did not always occur; most common were due to phages 14/11 and 14/25, which accounted for

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Table 3. *The effect of lysogeny on the typing pattern of* 25 *strains of* S. aureus

Strain no.	L.D.* Total	L.D.* Pattern changed	Phage action lost in lysogenic compared with parent culture. ( ) number of strains	Combination of phage action lost
1	10	10	101 (3); 77 (4); 42D (5); 373 (6); 117 (2)	77/42D/373; 7/117/373
2	10	7	101 (3); 42D (2); 367 (3); 117 (3); 373 (3)	
3	10	8	47 (2); 101 (3); 373 (3); 42D (1); 367 (2); 117 (2)	367/117/373
4	10	8	101 (4); 42D (4); 367 (2); 373 (2); 117 (2)	42D/367/117/373
6	6	5	101 (4); 367 (3); 117 (2)	101/367; 367/117
8	6	6	101 (2); 6 (2); 117 (3)	6/101
9	10	10	101 (3); 373 (7); 42D (5); 10 (1)	42D/117/373
10	6	6	101 (3); 117 (3)	
11	6	3	101 (1); 367 (2); 117 (2)	367/117
12	6	5	101 (3); 367 (2); 117 (2)	
13	8	4	101 (3); 373 (3); 117 (3)	
14	6	4	101 (2); 600 (2); 77 (1)	
15	10	6	101 (2); 42D (1); 367 (2); 117 (2); 373 (3)	367/117/373
16	10	9	101 (2); 42D (6); 373 (5); 117 (2)	42D/117/373
18	6	4	101 (4); 107 (1); 117 (1)	101/107/117
19	7	4	600 (2); 117 (2); 373 (3); 42D (2)	600/117/373
21	6	6	101 (3); 117 (3)	
22	6	4	101 (3); 367 (1); 117 (2)	117/101
23	10	8	101 (3); 42D (3); 367 (2); 117 (2); 373 (3); 77 (1)	367/117/373
24	7	6	101 (3); 42D (1); 117 (2); 600 (1); 107 (1); 367 (3)	367/42D/107/117
25	10	4	101 (2); 42D (2)	
26	10	8	101 (3); 42D (3); 367 (3); 117 (3); 373 (3); 81 (1)	42D/367/117/373
27	10	7	101 (2); 42D (4); 367 (2); 117 (2); 373 (4); 6 (2)	42D/367/117/373
28	10	6	101 (2); 42D (2); 117 (2)	
29	6	5	101 (3); 117 (2); 10 (2); 42E (1)	10/101/42E

* Lysogenic derivatives isolated after lysogeny of parent strain with ten phages.

Table 4. *The action of ten new phages on* 25 *parent cultures and the effect of lysogeny on the phage pattern of the lysogenic daughter cultures*

Phage	No. of parent cultures lysed	Phage pattern modified	Phage action blocked on lysogenic daughter isolates
7/2	24	18*	101 (18);† 47, 81, 367 (1)
7/4	24	22	101 (22); 10 (3); 6 (2); 47, 42E (1)
8/5	14	10	42D (8); 373 (4); 101 (2); 600, 117, 77 (1)
10/25	24	17	42D (8); 373 (7); 367 (6); 600, 77 (1); 117 (10)
11/13	15	12	42D (10); 373 (6); 77 (2)
14/11	24	20	117 (18); 367 (10); 373 (8); 42D (4); 101 (2), 77 (1)
14/25	24	19	117 (19); 373 (11); 367 (9); 42D (1); 600 (1)
20/4	14	7	42D (6); 373 (3)
20/5	13	6	42D (4); 373 (6); 367 (1)
26/26	24	23	101 (22); 6 (2); 600 (2); 117 (1); 77 (1)

* Of the 24 parent cultures lysed by 7/2, 18 of the lysogenic daughter strains showed a pattern different from the parent strain.

† Number of daughter strains affected shown in parentheses.

Table 5. *Effects of the new phages in blocking lytic action of typing phages*

Phage action blocked	No. of strains	Phages producing loss of lytic activity
101	66	7/2; 7/4; 26/26
117	49	14/11; 14/25; 10/25
373	45	14/25; 14/11; 10/25; 11/13; 29/5; 29/4
42D	41	8/5; 10/25; 11/13; 29/4; 29/5; 14/11; 14/25
367	27	14/11; 14/25; 10/25
77	6	11/13; 8/5; 10/25; 14/11; 26/26
600	5	26/26; 14/25; 10/25; 8/5
6	4	7/4; 26/26
47	2	7/2; 7/4
81	1	7/2

14 of the 20 occasions in which three or more phage actions were deleted from the basic pattern.

The major effect on the action of the typing phages is shown in Table 5.

DISCUSSION

The stability of the phage type as exhibited by the typhoid bacillus (Anderson & Williams, 1956) does not extend to the staphylococcus; thus although 'type' is often used to describe the sensitivity of a strain to the typing phages (e.g. 'type 80/81'), it is preferable to use the term 'pattern' (Parker, 1962).

Williams & Rippon (1952) found that strains lysed by phages of group III showed greater variation in pattern than did those lysed by phages of group I or II; they allowed change of one strong reaction for such group III patterns, but none for the others in determining identity. After considerable experience with human strains suggesting this was too restrictive, the subcommittee on Phage Typing of Staphylococcus (Blair & Williams, 1961) advanced a new criterion: two cultures differed in phage type only if one is lysed strongly by two phages that produce no lysis on the other to any degree.

Perhaps the most detailed study of the reproducibility of phage patterns was that of Wentworth, Romig & Dixon (1964). They typed 10 colonies from each of 10 cultures on 10 different days. They were thus able to relate variability to the lytic group, the strength of the reaction with each phage and to the day of typing. There was considerable variation found, particularly with weak reactions. Variation was not well correlated with phage group or with complexity of phage pattern; there was some association with day of typing, but this was not consistent and was not entirely a matter of technique. In the collaborative study of the typing of bovine strains of *S. aureus* (Davidson, 1972), some estimate of the stability of phage patterns was made. Although there was some instability of phage patterns, most cultures varied only slightly or not at all. No phage was markedly more variable than the others in this respect. However, staphylococci isolated from a single herd may often show complex patterns, usually to groups III and/or IV phages, and even with the caution in interpretation stressed by Blair & Williams (1961) the identity of these cultures may be difficult to determine. The

lytic patterns shown by the parent strains (Table 2) were such that, given their origin, some workers might consider them variants of one or two original strains; however, they could equally well be interpreted as up to ten distinct patterns.

Only a single colony was isolated from each area of secondary growth, and while each was assumed to be lysogenic to the relevant phage it is known that secondary growth may consist of both resistant and sensitive variants (Lowbury & Hood, 1953). RTD × 10 was used to ensure that all organisms were exposed to free phage. More intensive investigation would undoubtedly have revealed more effects on the typing pattern, and perhaps on other attributes of the staphylococci.

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