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LYSOGENY IN COAGULASE-NEGATIVE STAPHYLOCOCCI

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COAGULASE-NEGATIVE staphylococci (*Staphylococcus epidermidis*) are generally considered non-pathogenic. Recently, however, they have been associated with an increasing number of infections complicating cardiac surgery and ventriculo-atrial shunt operations for the treatment of hydrocephalus (Quinn, Cox and Fisher, 1965; Holt, 1969). In most of these cases the route of infection has not been established, nor is it clear whether the hospital is the source of the causative organisms.

To obtain a better understanding of the host-parasite relation and the epidemiology in the hospital environment of coagulase-negative staphylococci, a typing system to characterise individual strains seems highly desirable. Classification by means of biochemical tests according to the system proposed by Baird-Parker (1963) has recently given encouraging results in distinguishing between strains of *Staph. epidermidis* (Bentley *et al.*, 1968; Holt).

Phage-typing proved to be of considerable value in the study of *Staph. aureus* infections. The typing phages were isolated from lysogenic *Staph. aureus* strains and in general do not lyse coagulase-negative staphylococci. Phages active on coagulase-negative staphylococci have been described (Rountree, 1947; Rippon, 1956; Comtois, 1962), but lysogeny in coagulase-negative staphylococci has been demonstrated only rarely (Karska *et al.*, 1960; Comtois). The occurrence of lysogenic *Staph. epidermidis* strains was studied systematically to evaluate the possibility of obtaining phages suitable for typing purposes.

MATERIALS AND METHODS

Strains

Coagulase-negative staphylococci were collected from staff, patients and air in the Department of Thoracic Surgery, University Hospital, Utrecht, from medical students that had not yet been in contact with the hospital environment, and from a sample of the population of the village of Vleuten, near Utrecht.

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TABLE I
Production of phages by 14 strains of *Staphylococcus epidermidis* biotype 1—of 39 strains tested—and by one strain of *Staph. aureus* (no. 108) that were active against one or more of the same 39 *Staph. epidermidis* strains or against *Staph. aureus* no. 57

Indicator strain no.	Strength of lytic reaction, on the indicator strain shown, of supernatant of strain no.											
	48	51	71	82	95	99	127	130	159	171	178	234
41	+	+	—	—	—	—	—	—	—	—	—	—
71	—	+	—	—	—	—	—	—	—	—	—	—
81	+	—	—	—	—	—	—	—	—	—	—	—
86	+	—	—	—	—	—	—	—	—	—	—	—
87	—	—	—	—	—	—	—	—	—	—	—	—
92	—	+	—	+	—	—	—	—	—	—	—	—
98	—	+	—	—	—	—	—	—	—	—	—	—
128	—	+	—	—	—	—	—	—	—	—	—	—
132	—	—	—	—	—	—	—	—	—	—	—	—
154	—	—	—	—	—	—	—	—	—	—	—	—
159	—	—	—	—	—	—	—	—	—	—	—	—
171	—	—	—	—	—	—	—	—	—	—	—	—
202	—	—	—	—	—	—	—	—	—	—	—	—
213	—	—	—	—	—	—	—	—	—	—	—	—
240	—	+	—	—	—	—	—	—	—	—	—	—
242	—	—	—	—	—	—	—	—	—	—	—	—
57*	—	—	—	+	+	+	+	+	+	+	+	+

* *Staph. aureus*.
+++ = Semiconfluent and confluent lysis; ++ = more than 50 plaques to semiconfluent lysis; + = 20-50 plaques; — = less than 20 plaques.

Coagulase-negative staphylococci were isolated from nasal swab cultures, from settle-plates exposed during the operations in the operating theatre, and from clinical specimens. Those from the latter, i.e., blood, pus, wound swabs and cerebrospinal fluid, were incorporated in the study only when the specimens yielded coagulase-negative staphylococci repeatedly in pure culture. All primary isolations were made on 5 per cent. sheep blood agar plates. After incubation for 24 hr at 37°C single colonies were subcultured twice in succession on tryptone yeast-extract agar. Strains were examined microscopically for morphology and Gram reaction, and tested for the production of coagulase (Bacto-coagulase Plasma, Difco Laboratories, Detroit, Michigan, USA) and catalase. Cultures were maintained in agar stab cultures and stored at room temperature. The strains were numbered consecutively, and will be referred to by these numbers.

The Gram-positive, catalase-positive and coagulase-negative cocci were examined for production of phosphatase and acetoin, for aerobic and anaerobic fermentation of glucose and mannitol, and for aerobic fermentation of lactose and maltose. The methods used were essentially the same as those described by Baird-Parker (1963). Strains that fermented glucose anaerobically were considered to be staphylococci and were allotted to one of the five biotypes in the modified scheme of classification proposed by Baird-Parker in 1965. Originally, Baird-Parker (1963) recognised six subgroups of staphylococci of which subgroup I corresponded to *Staph. aureus*. In the later scheme the remaining subgroups II-VI were renumbered as *Staph. epidermidis* biotypes 1-5 respectively. Staphylococci belonging to biotype 2 were not encountered in this investigation.

Phage induction

Induction was carried out with Mitomycin C (Sigma Chemical Corporation, St Louis, Missouri, USA). Preliminary experiments showed that the following procedure yielded reproducible and maximal phage production. A 10 per cent. inoculum of an overnight broth culture was made into 5 ml of broth containing 0.5 µg Mitomycin C per ml. After incubation for 4 hr at 37°C, the cultures were centrifuged (20 min., 1600g) and the supernatant fluids were spotted on indicator strains spread on the surface of agar plates. Unless otherwise stated, each strain tested for lysogeny was used in turn as indicator strain. In addition, the non-lysogenic *Staph. aureus* strain no. 57 (Winkler *et al.*, 1965) was used as indicator strain. When employed as indicators, strains were grown in broth for 24 hr at 37°C before being spread on plates. The plates were examined after 18 hr at 37°C. The media used throughout the phage studies were Nutrient Broth or Nutrient Agar (Difco) with calcium chloride added to a final concentration of 0.008M. Strain no. 108, erroneously considered as *Staph. epidermidis* on first isolation, was included in the induction experiments. On further identification it appeared to be a *Staph. aureus*. As the phage obtained from this strain proved very useful, strain no. 108 appears in some of the tables.

RESULTS

In a series of separate screening tests for lysogeny, each member of a group of *Staph. epidermidis* strains was tested against each other member of the same group after induction with Mitomycin C.

Biotype 1

The first group included 39 strains of *Staph. epidermidis* biotype 1; in addition, one strain of *Staph. aureus* (no. 108) was tested for lysogeny and another (no. 57) was included among the indicators. The results, recorded as supernatant fluids that contained phage and the strains on which phage activity could be detected, are shown in table I. Fourteen of the *Staph. epidermidis* strains (36 per cent.) released phage active on one or more of 16 of the biotype-1

TABLE II
Production of phages by 14 strains of *Staphylococcus epidermidis* biotype I—of a further 51 tested—that were active against 15 selected indicator strains that were also members of biotype I*

Indicator strain no.	Strength of lytic reaction, on the indicator strain shown, of supernatant of strain no.														
	400	420	429	436	448	456	459	489	540	554	654	820	935	967	108*
41	+	-	-	-	-	-	-	+	-	-	-	+	-	-	-
48	+	-	-	-	-	-	-	+	-	-	-	+	-	-	-
76	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-
81	+	-	-	+	-	-	-	-	-	-	-	-	-	-	-
87	+	-	-	+	-	-	-	+	-	-	-	+	-	-	-
98	+	-	-	+	-	-	-	+	-	-	-	+	-	-	-
141	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-
202	-	-	-	-	-	-	-	+	-	-	-	+	-	-	-
240	+	-	-	-	-	-	-	+	-	-	-	+	-	-	-
330	-	-	-	-	-	-	-	+	-	-	-	+	-	-	-
368	-	-	-	-	-	-	-	+	-	-	-	+	-	-	-
380	+	-	-	-	-	-	-	+	-	-	-	-	+	-	-
382	+	-	-	-	-	-	-	+	-	-	-	-	+	-	-
407	+	-	-	-	-	-	-	+	-	-	-	-	+	-	-
548	+	-	-	-	-	-	-	+	-	-	-	+	+	+	+
57*	-	+	+	-	+	+	+	-	+	+	+	+	+	+	+

* *Staph. aureus* no. 108 tested for lysogeny and *Staph. aureus* no. 57 used as indicator strain.
Symbols: see table I.

indicator strains, or on *Staph. aureus* no. 57, or on both. In addition, *Staph. aureus* no. 108 yielded a phage active on four of the *Staph. epidermidis* indicators as well as on *Staph. aureus* no. 57. Phages released by eight of the biotype-1 strains gave a strong reaction on *Staph. aureus* no. 57; those from four of these strains lysed no. 57 only, the rest also lysed some of the *Staph. epidermidis* strains.

Further experiments and preliminary phage-typing of biotype-1 strains led to the identification of a series of strains that were sensitive to many of the

TABLE III

Production of phages by Staphylococcus epidermidis strains of biotypes 3, 4 and 5 that were active against other members of the same set of strains, or against Staph. aureus no. 57*

Indicator strain		Strength of lytic reaction, on the indicator strain shown, of supernatants of							
		biotype-4 strain							biotype-5 strain
no.	biotype	no. 35	no. 91	no. 138	no. 157	no. 170	no. 200	no. 211	no. 195
121	3	—	+	—	—	—	—	—	+++
33	4	—	+	—	—	—	—	—	+
35	4	—	+	—	—	—	—	—	—
70	4	—	—	—	—	—	—	+	—
157	4	—	+	—	—	—	—	—	+++
170	4	—	++	—	—	—	—	—	++
195	5	—	++	—	—	—	—	—	—
37	5	—	+	—	—	—	—	—	+++
57	†	+++	+++	+++	±	±	++	—	+++

* 41 strains tested: 8 of biotype 3; 24 of biotype 4; 9 of biotype 5.

† *Staph. aureus*.

Symbols as in table I, except that $\pm$ = weak reaction (less than 20 plaques) and — = no plaques.

phages obtained from biotype-1 strains and were subsequently propagated on other members of this biotype. These strains appeared to be particularly useful as indicator strains. Eleven of these were used as indicators in an induction experiment with 51 additional biotype-1 strains. Four strains which had been used as propagating strains were included in the indicators together with *Staph. aureus* no. 57 (table II). *Staph. aureus* no. 108 was again tested for lysogeny. Phage was detected in lysates of 14 *Staph. epidermidis* strains. Five lysates were active only on *Staph. aureus* no. 57 and the remaining nine were active on *Staph. epidermidis* indicators. In contrast with the results of the first experiments, however, only *Staph. aureus* no. 108 yielded a lysate active on both *Staph. aureus* no. 57 and on *Staph. epidermidis* strains.

Other biotypes

Preliminary experience with phage-typing had shown that phages derived from biotype 1 were rarely active on strains of biotype 3 or biotype 5. To obtain phages active against other biotypes, separate induction experiments were set up.

A group of 41 *Staph. epidermidis* strains, which comprised eight of biotype 3, 24 of biotype 4 and nine of biotype 5, were tested for lysogeny in a similar manner (table III). Again, *Staph. aureus* no. 57 was included among the indicator strains. Phage activity was demonstrated in the supernatant fluids of seven of the biotype-4 strains (29 per cent.). Phage from five of those strains was active only on *Staph. aureus* no. 57; one phage was active on several

TABLE IV

Production of phages by Staphylococcus epidermidis strains of biotypes 3 and 5 that were active against other members of the same set of strains*

Indicator strain		Strength of lytic reaction, on the indicator strain shown, of supernatants of								
		biotype-3 strain	biotype-5 strain							
no.	biotype	no. 1499	no. 576	no. 1427	no. 1431	no. 1433	no. 1537	no. 1548	no. 1597	no. 1640
1405	3	—	—	—	+++	—	+++	—	+++	—
1491	3	—	—	—	++	—	+++	—	—	—
1525	3	—	—	—	—	—	+++	—	+++	—
571	5	—	+++	—	—	—	—	—	—	—
1407	5	—	+++	+	++	+	+++	—	+++	+++
1518	5	+++	+++	+++	+++	+++	+++	+++	—	+++
1520	5	+++	—	+++	—	+++	—	+++	—	+++
1640	5	—	—	—	+++	—	+++	—	+++	—
407	1	—	—	—	—	—	—	—	—	—
57	†	—	—	—	—	—	—	—	—	—

* 42 strains tested: 15 of biotype 3; 27 of biotype 5.

† *Staph. aureus*.

Symbols as in table I.

biotype-4 strains and on one strain of biotype 3 and two of biotype 5; and one was active only on a single biotype-4 strain. One of the nine biotype-5 strains produced phage action on several biotype-4 strains, on one strain of biotypes 3 and 5, and on *Staph. aureus* no. 57. None of the biotype-3 strains appeared to be lysogenic in these tests.

A further induction experiment was made with larger numbers of biotype-3 and biotype-5 strains (table IV). Eight of 27 biotype-5 strains, but only one of 15 biotype-3 strains, yielded phage. Phage from each of the biotype-5 strains was active on several biotype-5 strains and that from three of them also lysed some biotype-3 strains. The only phage obtained from a biotype-3 strain was active on biotype-5 strains but not on biotype-3 strains. Unlike the phages obtained in earlier experiments, none of these phages lysed *Staph. aureus* no. 57. All of the lysates obtained in this experiment were also tested against a strain of *Staph. epidermidis* biotype 1 (no. 407), which—in phage-typing experiments—appeared to be a general indicator for phages from biotype-1 strains (see table II), but no lysis was seen.

DISCUSSION

Several workers have isolated temperate phages from coagulase-negative staphylococci (Burnet and McKie, 1929; Rountree, 1947; Rippon, 1956; Comtois, 1962), but lysogenic strains were thought to be rare (Comtois) and some authors failed to demonstrate them (Fisk, 1942; Karska *et al.*, 1960). Karska *et al.* suggested a correlation between coagulase production and lysogeny in staphylococci. We found lysogeny in a considerably greater proportion of coagulase-negative staphylococci than has previously been reported, but less often than it occurs in *Staph. aureus*. The ability to detect lysogeny is limited by experimental conditions such as the effectiveness of the method of induction and the availability of sensitive indicator strains. We used Mitomycin C as inducer. In preliminary experiments in which we used the original cross-culture technique of Fisk, lysogenic strains could not be detected. Later, however, some known lysogenic strains were tested by this technique on sensitive indicator strains and lysogenicity was readily demonstrated. Mitomycin C probably enhanced the ease with which latent phage could be detected without increasing the actual number of detectable lysogenic strains. We do not know whether induction by Mitomycin C is more effective than induction by radiation with ultraviolet light, as used by previous workers (Karska *et al.*; Comtois).

Phage could be detected in 28 of 90 strains of biotype 1, in seven of 24 strains of biotype 4, in nine of 36 strains of biotype 5 and in one of 23 strains of biotype 3. Of the lysates obtained from these 45 staphylococcal strains, 25 were active on one or more strains of *Staph. epidermidis*, 14 could be detected on *Staph. aureus* no. 57 only and six were active on *Staph. epidermidis* and on *Staph. aureus* no. 57. The latter six lysates might have been mixtures (as was indeed shown in a few cases), and this suggested that at least two groups of phages might be involved. Again, *Staph. aureus* no. 57 is sensitive to 19 of 35 phages derived from strains of biotype 1 and 4 (tables I, II, III) and rarely (1 of 10) to phages derived from biotypes 3 and 5. The possibility that phages from various biotypes indeed differ will be discussed in a later paper.

SUMMARY

Lysogenicity in coagulase-negative staphylococci was tested by incubating the strains in the presence of Mitomycin C. Among 173 strains examined, 45 were found to release phage. Twenty-eight of these phages were obtained from 90 strains belonging to *Staphylococcus epidermidis* biotype 1 (Baird-Parker, 1965).

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