The Adsorption of Phage by *Staphylococcus* spp.

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**SUMMARY**

Phages for coagulase-negative staphylococci were adsorbed to heat-killed cells. The phages showed equal affinities for all the cells, which appeared to have an equal number of binding sites for all the phages tested. This number is estimated at $1.2 \times 10^6$ sites/cell. Competition for binding sites could be demonstrated between a pair of phages. It is concluded that coagulase-negative staphylococci have only a single series of binding sites for phage, probably the outer 20% or so of the wall teichoic acids. These organisms therefore bind all 'coagulase-negative' phages whether or not they are sensitive to them.

Recently, Schumacher-Perdreau *et al.* (1978) presented phage adsorption as a simple test for discriminating between *Staphylococcus* and *Micrococcus* spp. Micrococci do not usually have wall teichoic acids (Schleifer & Kocur, 1973) and the phage receptor sites in staphylococci are thought to be part of the wall teichoic acids (Chatterjee, 1969). This supposition is supported by the work of Rogers *et al.* (1978) with *Bacillus subtilis*. In *Staphylococcus aureus* the number of teichoic acid chains/cell is estimated to be $7.8 \times 10^6$ (J. Barrie Ward, personal communication). By analogy with *Bacilluslicheniformis* (Burge *et al.*, 1977), wall teichoic acids are probably distributed uniformly throughout the murein.

It has already been found that any *Staphylococcus epidermidis* phage will strongly bind to any *S. epidermidis* strain, with or without lysis (Verhoef *et al.*, 1972), but not to *S. aureus* strains (Verhoef *et al.*, 1971, 1972). Their results were presented as standard adsorption curves showing exponential binding to live cells over a period of 1 h. No appreciable binding to *S. aureus* was observed even with phages propagated on *S. aureus* and then adapted to *S. epidermidis* strains.

It is likely that different phages bind to the same cells with different affinities, binding to different binding sites which may be present in different numbers on the cell wall. Therefore, we determined the relative binding affinities for different phage-cell pairs and the total number of binding sites in each case. As adsorption was the only parameter of immediate interest, the cells used were killed by minimum heat treatment. Six phage-cell combinations were tested, using three phages isolated from *S. epidermidis* biotype 1 (Baird-Parker, 1974), and two strains of *S. epidermidis* biotype 1 and one strain of *S. aureus*. The bacterial combinations with phage 200F were *S. epidermidis* 83 (the propagating strain), *S. epidermidis* 188 (not sensitive to 200F but the propagating strain for 103F and 116F) and *S. aureus* 33. Phage 116F was used in combination with *S. epidermidis* 188 and *S. aureus* 33. Phage 103F was used in combination only with *S. epidermidis* 188.

The adsorption of fixed titres of the phages to varying concentrations of autoclaved cells was examined. The bacteria were subcultured after successive incubation at 37 °C for 18, 12 and 6 h to ensure the maximum proportion of active cells and the viable numbers determined by Miles Misra counts on Oxoid nutrient agar no. 2. The 6 h cultures were autoclaved for 10 min at 4.5 kg and 0.1 ml phage suspension was added to 0.9 ml dilutions (1:8, 1:64, 1:500, 1:2000, 1:4000) of this cell suspension. The mixtures were incubated at 37 °C for 3 h before centrifugation (3000 g, 10 min) and the residual phage in the supernate titred. These figures were used to calculate the adsorption index (p.f.u. adsorbed/ml ÷ number of cells/ml) for each cell concentration. The log of the adsorption index was plotted against the log of the cell
Fig. 1. The adsorption of phage particles by *S. epidermidis* cells. ○, 200F adsorbed by strain 33; △, 116F adsorbed by strain 188; □, 200F adsorbed by strain 83; ●, 200F adsorbed by strain 188; ■, 103F adsorbed by strain 108; ■, 116F adsorbed by strain 33.

concentration. The resulting straight line was extrapolated to give the number of phage binding sites/cell.

The adsorption of a mixture of phages 9f (also isolated from *S. epidermidis* biotype 1) and 116F at 500 routine test dilution (RTD) by heat-killed *S. epidermidis* 83 at a concentration of $1.35 \times 10^5$ was tested by the same method.

Fig. 1 shows that most of the points obtained by plotting log cell concentration against log adsorption index fall on a single line. Two points on a higher line are due to 103F at 500 RTD, and two on a lower line of 116F at 50 RTD. Since contact of a phage with a bacterium occurs by passive diffusion, the higher initial phage concentration gave higher adsorption indices. The main line crosses the ordinate at a log adsorption index of 6.05. This limits the theoretical maximum number of tail ends which could bind to a single cell to $1.1 \times 10^6$ to $1.2 \times 10^6$. The true value must be less since the larger heads would not allow such close packing. Only two points were available for 103F bound to strain 188, since this phage was adsorbed at a greater starting concentration, and only two points were obtained for 116F bound to strain 33. These additional points, while not part of established series, conform with lines crossing the ordinate at about the same value. Using 1.5 μm as the average cell size for *S. epidermidis* it can be calculated that the phage binding sites must be a maximum of 2.4 nm across, assuming they are all in the same plane. However, the head size restricts the maximum number of phages binding to a single cell to about 6000 (L. Barnard & A. Seaman, unpublished data). In other words, at most, only about one in 200 receptor sites may be used at any one time and in practice probably fewer. This figure of $1.2 \times 10^5$ binding sites applies to all the phages and cells tested. As all the phages bind to all the strains (Verhoef *et al.*,...
Table 1. Titre results after binding two *S. epidermidis* phages, in combination, to a single *S. epidermidis* strain 83 with a cell concentration of $1.35 \times 10^5$*

<table>
<thead>
<tr>
<th>Nominal titre</th>
<th>Neat</th>
<th>1:10</th>
<th>1:10²</th>
<th>1:10³</th>
<th>Starting titre RTD</th>
<th>Loss in RTD</th>
<th>P.f.u. adsorbed index</th>
</tr>
</thead>
<tbody>
<tr>
<td>Standard</td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>116F 100 RTD</td>
<td>CFL</td>
<td>RTD</td>
<td>RTD</td>
<td>2p†</td>
<td>15</td>
<td>100</td>
<td>85</td>
</tr>
<tr>
<td>116F 200 RTD</td>
<td>CFL</td>
<td>CFL</td>
<td>RTD</td>
<td>1p</td>
<td>50</td>
<td>200</td>
<td>150</td>
</tr>
<tr>
<td>9F 100 RTD</td>
<td>CFL</td>
<td>RTD</td>
<td>RTD</td>
<td>2p</td>
<td>10</td>
<td>95</td>
<td>85</td>
</tr>
<tr>
<td>9F 200 RTD</td>
<td>CFL</td>
<td>RTD</td>
<td>RTD</td>
<td>5p</td>
<td>50</td>
<td>190</td>
<td>140</td>
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<td>Unadsorbed control</td>
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<tr>
<td>9F 100 RTD</td>
<td>CFL</td>
<td>RTD</td>
<td>9p</td>
<td>neg</td>
<td>95</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>116F 100 RTD</td>
<td>CFL</td>
<td>CFL</td>
<td>RTD</td>
<td>3p</td>
<td>100</td>
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<td>—</td>
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<td>Mix. on 83‡</td>
<td>CFL</td>
<td>RTD</td>
<td>RTD</td>
<td>4p</td>
<td>50</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Mix. on 188§</td>
<td>CFL</td>
<td>RTD</td>
<td>6p</td>
<td>neg</td>
<td>50</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Adsorbed control</td>
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<tr>
<td>Mix. on 83</td>
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<td>RTD</td>
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<td>RTD</td>
<td>RTD</td>
<td>3p</td>
<td>50</td>
<td>100</td>
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</tr>
</tbody>
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* Abbreviations are as follows: CFL, confluent lysis; neg, no plaques seen; RTD, routine test dilution; this is $1.473 \times 10^4$ p.f.u./ml (L. Barnard & A. Seaman, unpublished data).
† Number × p = average number of plaques/dilution.
‡ 9F titred in the presence of 116F.
§ 116F titred in the presence of 9F.

1972 b), a complete set of binding sites for each page involves the consideration of an infinite number of series of sites of $1.2 \times 10^6$ each. Clearly the implication is that biotype 1 *S. epidermidis* strains carry only a single set of binding sites which are used by all *S. epidermidis* biotype 1 phages and these are most probably the outer 20% or so of the wall teichoic acids (Chatterjee, 1969; J. Barrie Ward, personal communication). Binding was observed with these phages to a single *S. aureus* strain (33). This contrasts with the results of Verhoef *et al.* (1971, 1972 a). However, if, as has been suggested (Nordström & Forsgren, 1974), protein A acts as a block for phage receptor sites in *S. aureus* then the fact that these cells were autoclaved could well account for the present observations, since heat killing removes protein A.

Table 1 shows the results of binding two phages simultaneously to a single strain in an attempt to demonstrate competition for binding sites which would occur if these sites are non-specific. The available experimental evidence suggested that the adsorption index of any phage at 200 RTD would be greater than, but not double, that at 100 RTD, and that the adsorption indices at the same initial titre would be the same for all the phages. In competition, the sum of the adsorption indices of the two phages would equal the control adsorption index for 200 RTD of either phage and the adsorption index for each phage at 200 RTD, measured in dual binding conditions, should therefore be depressed. The results showed this to be the case. However, adding the 100 RTD indices did not give a figure as great as that for 200 RTD of either phage. It is possible that undetected phage (from the propagating strains, for instance) are responsible. Such phage would only exert detectable effects in dual binding conditions. It should also be noted that, in the unadsorbed controls, titrating one phage in the presence of the other resulted in a depressed titre compared with the single controls. One phage appears to interfere with the detection of the other. Adsorption was to dead cells and relatively few phage remained for subsequent detection; therefore, the observed effect is probably because of phage competition for space on the cell surface rather than multiplicity of infection.

Verhoef *et al.* (1971) demonstrated the existence of at least three host modification systems for *S. epidermidis*, the evidence suggesting that each phage acquired the host specificity
dictated by its last host. Within one host-specific system, the lytic differences observed appear to be due not to the phages themselves, but to the phenotypic effects of a combination of restriction, immunity due to lysogeny and possession of plasmids in the host staphylococci. An international typing set for these organisms may therefore need to contain a large number of phages, each propagated in different hosts. This would probably be unmanageable and it may well be that a series of regional sets, corresponding to variations of host within the region, will prove to be an alternative to a truly international set of typing phages such as exists for *S. aureus*.

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### REFERENCES


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