A Bacteriophage-lysing Strain of *Staphylococcus* Employed in the Manufacture of Dry Sausage

H. G. GYLLENBERG AND C. R. HACKMAN

Department of Microbiology, University of Helsinki, Helsinki, Finland

Received for publication February 13, 1961

**ABSTRACT**

GYLLENBERG, H. G. (University of Helsinki, Helsinki, Finland), AND C. R. HACKMAN. A bacteriophage-lysing strain of *Staphylococcus* employed in the manufacture of dry sausage. Appl. Microbiol. 9:480–483. 1961.—A bacteriophage of a certain *Staphylococcus* (a strain of *Staphylococcus lactis*) employed in the manufacture of dry sausage has been characterized. The host range of this bacteriophage is wide. In addition to the original host, 15 other strains (out of 40 strains tested) were found to support reproduction of the phage. The sensitive strains represented *Staphylococcus saprophyticus* and different types of *S. lactis*.

The growth rate of the bacterial host did not influence the rates of phage adsorption, nor the maximal reproduction rate of new particles. With increasing bacterial growth rate, the “lag” observed before phage reproduction started was distinctly decreased. This phase was shorter with the original host strain than with other sensitive strains.

Resistant cultures of the original host strain were easily obtained. These cultures grew as rapidly and gave as good yields of cell mass as the original phage-sensitive host. However, phage resistance was frequently lost.

Although rather extensive information is available concerning the bacteriophages of medically important staphylococci, very little is known about the phages of those staphylococci which are specifically connected with food technology. Certain strains of *Staphylococcus lactis* [the nomenclature proposed by Shaw, Stitt and Cowan (1951) is used in this report] are particularly important in the processing of dry sausage varieties that are dependent upon nitrate reduction for color development (Pohja, 1960b), and may thus be added at high levels to sausage. In experiments on large-scale culture of a suitable strain of *S. lactis* by continuous methods, sudden lysis of the cultures was frequently observed. It was realized that this phenomenon was due to the occurrence of a specific bacteriophage, the general characteristics of which will be described in this report.

**MATERIALS AND METHODS**

The lytic effect was first observed in experiments carried out by R. Müller, Rudolph Müller and Company, Hamburg, Germany, and the active phage was isolated from a sample of lysed culture obtained from Mr. Müller. The original host strain (strain 132) together with 40 other strains representing different types of staphylococci of meat origin were received from M.S. Pohja, Research Laboratory of the Farmers’ Cooperative Packinghouses, Hämeenlinna, Finland.

For cultivation of the staphylococci, the following substrate was employed: Yeast extract (Difco), 3 g; glucose, 1 g; sodium citrate, 3 g; sodium chloride, 25 g (in some experiments 50 g); potassium nitrate, 1 g; in 1,000 ml of tap water (final pH 7.2). To give a solid medium, this substrate was supplemented with 15 g of agar. Continuous cultivation was carried out employing the equipment of Gyllenberg and Hackman (1960). For ordinary batch cultures, a 1-liter fermentor was used. In both cases, intense aeration and agitation of the culture was applied.

The bacteriophage was regenerated in cultures of

---

**LITERATURE CITED**


strain 132 grown in the liquid medium given above. Phage titers after filtration of the culture through glass sinter (G 5, Jena) were determined by plaque counts, which were carried out after incubation at 27 °C for 24 hr. To test the host range relationships of the bacteriophage, a drop test on solid medium and tests in the liquid medium were performed in addition to plaque counts. In the drop test, cells of the strain of Staphylococcus to be tested were seeded in a top layer of the solid medium. A drop of undiluted phage suspension was then placed on the surface of the medium and, after incubation, the plates were examined for bacterial growth at the place of the drop. In the liquid culture tests, the strain to be tested was inoculated together with the bacteriophage to the substrate. The tube cultures, which were incubated at 23 to 25 °C, were strongly aerated and examined for lysis during a period of 12 to 18 hr after inoculation.

Quantitative data concerning the adsorption of phage particles and the rate of phage reproduction were recorded in experiments where the bacterial host strain was grown continuously. The host strain was grown at steady state at growth rates (= dilution rates) ranging from 0.08 to 0.3 doublings hr⁻¹ for a few days before phage suspension was added to give a phage titer of about 10⁸ to 5 × 10⁹ per ml. From samples taken during the experiments the following determinations were made: (i) ordinary colony count, (ii) total plaque count, and (iii) count of free phage particles (after filtration of the sample through a bacterial membrane filter). On the basis of the data obtained, the time required for adsorption of the free phage particles added, and the maximal rate of phage reproduction were calculated.

Results

General characteristics of the bacteriophage. Strain 132 was found very susceptible to phage attack during the exponential phase of growth. The phage titers of filtrates from such lysed cultures were in the range of 10⁵ to 10⁶ lytic particles per ml. The plaques were close to 1 mm in diameter, and developed well at room temperature (20 to 22 °C) and at 27 but not at 37 °C. According to observations made with the aid of electron microscope (Fig. 1), the total length of the phage particles seemed to be somewhat less than 100 mμ, the diameter of the hexagonal head being about one third of the total length and the tail constituting two thirds. The lytic activity of filtrates was easily maintained by cold storage. During 6 weeks at refrigerator temperature (± 4 °C), no decrease in the titer of lytic particles could be observed. The bacteriophage in question was found to be highly sensitive to heat. Heating to 80 °C completely destroyed the lytic activity in less than 60 sec. At 55 °C, the decimal reduction time was found to be 7.5 min; at 60 °C, 2.5 min; and at 65 °C, about 1 min (Fig. 2).

Host range. The question concerning the host range of this bacteriophage is of particular interest. As shown by Pohja (1960a), strains of S. lactis which may favor the processing of dry sausage are rather rare. As every screening procedure for favorable strains is laborious and time consuming, it would be valuable for this purpose to have at disposal a bacteriophage specific for potential "sausage staphylococci." Accordingly, we tested the bacteriophage against 40 other strains of staphylococci of meat origin.

It was found that 15 out of these 40 strains were affected by the bacteriophage in one or more of the tests applied for studying the host-range relationships. Except for strain 132, the material included 2 other strains which were regarded useful in the manufacture

![Image](http://aem.asm.org/Downloadedfromhttp://aem.asm.org/)

**Fig. 1.** Ultrathin section of a cell with adsorbed phage particles (strain 132). Fixation: 2% osmic acid. Embedment: methyl metacrylate. Magnification, 94,000X.

**Fig. 2.** Effect of heating on phage titers.
of dry sausage. Both these strains were sensitive to the particular phage, but as 13 further strains were found susceptible, it may be concluded that this characteristic seems to be of minor significance in the screening for industrially useful strains.

In a recent work, Pohja (1960b) has divided the cocci of S. lactis type originating from meat products into several subgroups. It was interesting to note that in our experiments no one of the strains representing Pohja's subgroups a (2 strains were tested), b (3 strains), e (1 strain), i (6 strains), and f (1 strain) were attacked by the phage. On the other hand, the strains of subgroups d (5 strains), g (1 strain), and k (1 strain) were all susceptible. Among 8 strains belonging to Pohja's subgroup e, 3 were lysed by the particular bacteriophage. In addition, the 5 strains representing Staphylococcus saprophyticus were all sensitive to the phage, which suggests a close relationship between S. saprophyticus and certain subtypes of S. lactis. The single strain which represented Staphylococcus aureus was resistant, and 6 strains belonging to nonfermentative types of micrococci showed no sensitivity.

Quantitative experiments on phage adsorption and reproduction. Preliminary observations suggested that even cultures of susceptible strains might remain unlysed if the actual growth rate of the host was low at the time of introduction of phage material into the culture. It was also recognized that, although several strains in addition to the original host (strain 132) were sensitive to the phage, cultures of these strains even under similar conditions were lysed much slower than those of strain 132. To obtain more exact information on these points, some experiments employing continuous culture were performed.

The results presented in Table I indicate that no significant differences were found in the adsorption rate of the phage particles with the two bacterial host strains employed, nor were differences observed at varying growth rates of the hosts. A similar conclusion seems to hold true also when the highest rates of phage reproduction observed during the tests are evaluated. There occurred, however, distinct differences in the time elapsing (from the introduction of phage) before actual reproduction of the phage was demonstrable. The general conclusion was that this "latent period" was considerably shorter for the original host (strain 132) than for the other strain tested (strain 115), thus resulting in earlier lysis of the cultures. It also was found that this "lag" in phage reproduction decreases with increasing growth rate of the host. This phenomenon was observed with both the strains tested.

Characteristics of phage-resistant cultures of strain 132. Secondary growth due to the development of phage resistance was observed frequently with strain 132. With certain other strains, secondary growth occurred regularly and often so early that the clearing up of the substrate due to phage activity could be observed only during a few hours. The phage-resistant cultures thus obtained were compared with the original phage-sensitive cultures, especially as to the growth rate and the total yields of cells. These figures were found to be rather similar with both phage-sensitive and phage-resistant cultures, respectively (Table 2). Only with regard to the changes in pH in the growth medium a clear-cut difference was found in experiments with strain 132. Whereas a rapid rise in the pH up to final values of 7.8 to 8.2 was recognized for the original phage-sensitive culture, a distinct initial fall in the pH was characteristic for the phage-resistant culture (Fig. 3). This may

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Culture</th>
<th>Yield (dry weight)</th>
<th>Colony count</th>
</tr>
</thead>
<tbody>
<tr>
<td>I: 24 C/48 hr</td>
<td>Sensitive</td>
<td>0.49</td>
<td>490</td>
</tr>
<tr>
<td></td>
<td>Resistant</td>
<td>0.60</td>
<td>410</td>
</tr>
<tr>
<td>II: 24 C/27 hr</td>
<td>Sensitive</td>
<td>1.12</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td>Resistant</td>
<td>1.10</td>
<td>—</td>
</tr>
<tr>
<td>III: 32 C/27 hr</td>
<td>Sensitive</td>
<td>1.12</td>
<td>1100</td>
</tr>
<tr>
<td></td>
<td>Resistant</td>
<td>1.23</td>
<td>1170</td>
</tr>
</tbody>
</table>

**Table 1. Quantitative data concerning the adsorption of free phage particles and phage reproduction in experiments with two different host strains and at different growth rates of the hosts.**

<table>
<thead>
<tr>
<th>Host strain</th>
<th>Growth rate (= dilution rate) during experiment</th>
<th>Time required for the adsorption of the phage particles added</th>
<th>Maximal rate of phage reproduction</th>
<th>Time elapsing before actual phage reproduction was observed</th>
</tr>
</thead>
<tbody>
<tr>
<td>132</td>
<td>0.08 hr⁻¹</td>
<td>56 min</td>
<td>1.72 hr⁻¹</td>
<td>1-2 hr</td>
</tr>
<tr>
<td>132</td>
<td>0.20 hr⁻¹</td>
<td>58 min</td>
<td>1.55 hr⁻¹</td>
<td>&lt;1 hr</td>
</tr>
<tr>
<td>115</td>
<td>0.08 hr⁻¹</td>
<td>56 min</td>
<td>1.34 hr⁻¹</td>
<td>&gt;4 hr</td>
</tr>
<tr>
<td>115</td>
<td>0.17 hr⁻¹</td>
<td>52 min</td>
<td>0.88 hr⁻¹</td>
<td>2-4 hr</td>
</tr>
<tr>
<td>115</td>
<td>0.27 hr⁻¹</td>
<td>53 min</td>
<td>0.52 hr⁻¹</td>
<td>1-2 hr</td>
</tr>
</tbody>
</table>

**Fig. 3. Changes in the pH of medium during growth of phage-sensitive and phage-resistant cultures of strain 132.**
point to differences in the utilization of citrate by the cultures.

The results presented above indicate that phage-resistant cultures of the "sausage staphylococci," compared with the original phage-sensitive cultures, give equally good yields of cell mass, and may obviously be used in industrial large-scale culture to avoid losses in yields caused by bacteriophage contamination. It was found, however, that a reversion to phage sensitivity frequently took place in phage-resistant stock cultures during conventional storage.

Discussion

In the present investigation mainly practical aspects concerning the topical bacteriophage were taken into consideration. In large-scale propagation of bacteria, phage contamination may originate either from the substrate and utensils or introduced with the air needed for aeration. As the heat resistance of this particular bacteriophage is weak, no extraordinary treatment of substrates or utensils may be necessary to control contamination. Control of contamination due to the air seems to be a much more difficult problem. It is known (Humphrey, 1960) that, above a critical point, increased air velocity in fibrous filters improves the efficiency of the filtration process when the removal of bacterial cells is concerned. On the contrary, however, smaller amounts of phage particles are removed when the air velocity increases. Filtration at high air velocities, although otherwise effective, thus introduces an actual danger of phage contamination.

As the host range of this particular phage is wide, the possibilities of overcoming contamination by changing the bacterial strains from one batch or run to the next may be limited. On the other hand, phage-resistant cultures are easily obtained and may be used as they give yields similar to those of ordinary phage-sensitive cultures. When the propagation is carried out continuously, phage activity can be prevented to some extent by regulating the bacterial growth rate to a low level, but slow bacterial growth rates also would diminish the gain of any propagation process.

Acknowledgments

The authors are indebted to R. Müller for the original sample of lysed culture. We also wish to thank M. S. Pohja, who kindly placed the cultures of different Staphylococcus types at our disposal. M. G. Nyholm, Department of Electron Microscopy, University of Helsinki, has given us very valuable advice in connection with the use of the electron microscope.

Literature Cited


