SP3: A Flagellotropic Bacteriophage of Bacillus subtilis

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SUMMARY

Bacillus subtilis bacteriophage SP3 was shown to be flagellotropic. Mechanical deflagellation of host strain 168wt prevented phage adsorption but adsorption was normal when deflagellated 186wt was allowed sufficient time to regenerate flagella. Two host strains lacking flagella (130fla− and 19fla−) did not adsorb SP3. Two host strains with paralysed flagella (38mot− and 13mot−) adsorbed SP3 inefficiently. Revertants of fla− and mot− strains to the motile phenotype adsorbed SP3 normally. A temperature-sensitive host mutant, ts46, with reduced numbers of flagella at 46 °C was incapable of efficient SP3 adsorption at this temperature. However, transfection of ts46 by SP3 DNA occurred with virtually identical efficiency at either temperature. Adsorption of a non-flagellotropic phage, SP82, to all bacterial strains used was unaffected by absence of flagella. Isolated flagella adsorbed SP3 inefficiently. Spontaneously non-motile strains of 168wt had lost the ability to support growth of SP3 but SP82 grew normally. Revertants of these strains to the motile phenotype regained the ability to support SP3 growth. Strains derived from surviving bacteria in SP3 lysates of 168wt were non-motile.

Four bacteriophages, Chi of Escherichia coli, PBS1 of Bacillus subtilis, PBP1 of Bacillus pumilis and 7-7-1 of Rhizobium lupini, have been classified in the literature as flagellotropic. Such phage adsorb to the host's flagella and then proceed to the cell wall where the phage DNA is injected. This has been demonstrated by several methods. (i) Cells whose flagella have been mechanically sheared off do not adsorb phage (Lotz et al., 1977; Lovett, 1972; Raimondo et al., 1968) or do so at markedly reduced rates (Schade et al., 1967). (ii) Non-flagellated mutants of host bacteria do not adsorb their respective phages. Selection for resistant host strains often yields strains with no flagella or with chemically altered ones (Frankel & Joys, 1966; Joys, 1965; Lotz et al., 1977; Lovett, 1972; Meynell, 1961; Raimondo et al., 1968; Schade et al., 1967). (iii) Electron microscopic observations show the phages attached to host flagella. Chi, PBS1 and PBP1 attach to the flagella of their respective hosts by the long flexible phage tail fibres (Lovett, 1972; Raimondo et al., 1968; Schade et al., 1967). For phage 7-7-1, with short bushy tail fibres, the mechanism of flagellar attachment is not clear, but phage are characteristically observed on, or near, flagella (Lotz et al., 1977).

In the present study, evidence is presented that the B. subtilis bacteriophage SP3 is flagellotropic. Most of our experiments were done by standard procedures. Growth medium consisted of 8 g nutrient broth, 5 g yeast extract, 10−3 m-MnCl2, 1000 ml distilled water. Plating was performed using the soft agar overlay method on beef agar plates (6 g peptone, 3 g yeast extract, 1.5 g beef extract, 4 g casein hydrolysate, 15 g Bacto agar, 1000 ml distilled water; pH adjusted to 7.2 with NaOH). The soft agar overlay medium consisted of 10 g tryptone, 5 g yeast extract, 10 g NaCl, 2.5 × 10−4 m-CaCl2, 7 g Bacto agar, 1000 ml distilled water.

B. subtilis bacteriophage SP3 and SP82, used in this study, are those described by Eiserling & Romig (1962). B. subtilis hosts were 168wt, mutants 130fla−, 19fla−, 38mot−, 13mot− (generous gifts of G. Ordal, University of Illinois, Urbana, Ill., U.S.A.) and ts46, a temperature-sensitive

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Table 1. Adsorption of phages SP3 and SP82 to B. subtilis strains under various conditions

<table>
<thead>
<tr>
<th>Host bacterium</th>
<th>Motile</th>
<th>Temp. (°C)</th>
<th>Unadsorbed phage/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>None (phage control)</td>
<td>-</td>
<td>-</td>
<td>2.4 × 10⁶</td>
</tr>
<tr>
<td>168wt</td>
<td>+</td>
<td>37</td>
<td>3.1 × 10⁸</td>
</tr>
<tr>
<td>168wt</td>
<td>+</td>
<td>46</td>
<td>1.8 × 10⁸</td>
</tr>
<tr>
<td>Deflagellated 168wt</td>
<td>-</td>
<td>37</td>
<td>2.4 × 10⁶</td>
</tr>
<tr>
<td>Regenerated 168wt</td>
<td>+</td>
<td>37</td>
<td>2.6 × 10⁵</td>
</tr>
<tr>
<td>130fla⁻</td>
<td>-</td>
<td>37</td>
<td>2.2 × 10⁵</td>
</tr>
<tr>
<td>19fla⁻</td>
<td>-</td>
<td>37</td>
<td>2.4 × 10⁶</td>
</tr>
<tr>
<td>38mot⁻</td>
<td>-</td>
<td>37</td>
<td>2.0 × 10⁶</td>
</tr>
<tr>
<td>13mot⁻</td>
<td>-</td>
<td>37</td>
<td>9.0 × 10⁴</td>
</tr>
<tr>
<td>ts46</td>
<td>+</td>
<td>37</td>
<td>3.1 × 10⁵</td>
</tr>
<tr>
<td>ts46</td>
<td>-</td>
<td>46</td>
<td>9.0 × 10⁴</td>
</tr>
<tr>
<td>Deflagellated ts46</td>
<td>-</td>
<td>37</td>
<td>2.4 × 10⁶</td>
</tr>
<tr>
<td>Deflagellated ts46</td>
<td>-</td>
<td>46</td>
<td>2.2 × 10⁶</td>
</tr>
</tbody>
</table>

The adsorption of SP3 to mechanically deflagellated B. subtilis 168wt was drastically reduced as compared to the adsorption of the phage to a flagellated culture. A culture subjected to deflagellation and subsequently allowed sufficient time for flagellar regeneration (Raimondo et al., 1968) adsorbed SP3 as efficiently as the flagellated control culture. Flagella-less host mutants 130fla⁻ and 19fla⁻ lack flagella. 38mot⁻ and 13mot⁻ have paralysed flagella. To isolate motile revertants of these strains, isolated colonies grown on beef agar plates were transferred onto motility plates and incubated for 48 h at 37 °C. Swarming colonies were picked and tested for their ability to support phage growth. Mutant ts46 shows reduced numbers of flagella and is non-motile at 46 °C, while at 37 °C it is flagellated normally and motile.

For measuring phage adsorption phage were added at a multiplicity of 0.1. Two min were allowed for adsorption at the appropriate temperature. The cultures were then chilled on ice, centrifuged under refrigeration, and the supernatants assayed for unadsorbed phage. Bacteria were deflagellated mechanically by use of a Waring blender, according to the procedure of Lovett (1972). For flagellar regeneration, a culture of B. subtilis 168wt was deflagellated, centrifuged, resuspended, and diluted with broth to a cell density calculated to reach a concentration of 2 × 10⁸ cells/ml after 30 min incubation. At this time the culture was indistinguishable from a control culture as judged by phase-contrast microscopy.

The effect of the absence of flagella on the adsorption of SP3 is shown in Table 1. The adsorption of SP3 to mechanically deflagellated B. subtilis 168wt was drastically reduced as compared to the adsorption of the phage to a flagellated culture. A culture subjected to deflagellation and subsequently allowed sufficient time for flagellar regeneration (Raimondo et al., 1968) adsorbed SP3 as efficiently as the flagellated control culture. Flagella-less host mutants 130fla⁻ and 19fla⁻ adsorbed virtually no SP3. Adsorption of SP3 to non-motile mutants 38mot⁻ and 13mot⁻ was inefficient. In contrast, adsorption of SP82 to deflagellated 168wt and to the above mutants was approximately equivalent to that of controls (Table 1).

The adsorption of SP3 to ts46 grown at 37 °C was identical to adsorption to 168wt, but was reduced when ts46 was grown at 46 °C. 168wt adsorbed SP3 with equal efficiency at 37 °C and 46 °C (Table 1). We have shown ts46 to be non-motile at 46 °C (see also Grant & Simon, 1969); electron microscopy revealed a reduced number of flagella at 46 °C as compared to 37 °C. Deflagellation of ts46 grown at either temperature resulted in a reduced capacity to adsorb SP3, comparable to that observed with a deflagellated 168wt culture, but SP82 adsorbed to ts46 equally well at either temperature.

When 130fla⁻, 19fla⁻, 38mot⁻ and 13mot⁻ were used as indicator bacteria, turbid plaques were obtained but the efficiency of plating was the same as on a 168wt lawn. Revertants of these strains to a stable motile phenotype resulted in clear plaques. Even after five successive clonings, we were unable to obtain an isolated colony which after overnight incubation was free of motile revertants; this is consistent with the high reversion rate of these mutants (G. Ordal, unpublished data).

No plaques were obtained on ts46 lawns at 46 °C. If, however, the plates were incubated at 37 °C, an equal number of plaques was observed with ts46 and with 168wt. SP3 formed plaques...
Table 2. Transformation and transfection of B. subtilis ts46 by DNA isolated from B. subtilis 168wt, SP3 and SP82

<table>
<thead>
<tr>
<th>DNA</th>
<th>Temp. of incubation with DNA (°C)</th>
<th>Transformants or p.f.u./ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>168wt</td>
<td>37</td>
<td>1.7 x 10^5</td>
</tr>
<tr>
<td></td>
<td>46</td>
<td>4.0 x 10^4</td>
</tr>
<tr>
<td>SP3</td>
<td>37</td>
<td>2.2 x 10^3</td>
</tr>
<tr>
<td></td>
<td>46</td>
<td>2.9 x 10^3</td>
</tr>
<tr>
<td>SP82</td>
<td>37</td>
<td>7.7 x 10^4</td>
</tr>
<tr>
<td></td>
<td>46</td>
<td>1.2 x 10^3</td>
</tr>
</tbody>
</table>

* Competent, tryptophan-requiring ts46 bacterial cells were prepared according to the method of Spizizen (1959). Transformation experiments were done with DNA from B. subtilis 168wt at a concentration of 8 μg/ml and transformants assayed on tryptophan-deficient plates. Transfection experiments were performed by incubating competent cells with 15 μg/ml of either SP3 or SP82 DNA for 30 min at 37 °C or 46 °C. Five μg/ml DNase was added and the incubation was continued for an additional 60 min at the same temperature. Plaque assays were done on a lawn of 168wt bacteria and the plates were incubated at 37 °C. The phage DNA preparations were plated without dilution and were found to be devoid of any intact phage.

at 46 °C if 168wt was used as indicator, and SP82 formed equal numbers of plaques at 37 °C and 46 °C with either ts46 or 168wt.

The ability of ts46 to support the development of SP3 at 46 °C was tested by transfection experiments. Table 2 compares the results of transfection of ts46 at 37 °C and 46 °C by SP3 and SP82 DNA and the results of transformation by 168wt DNA at the two temperatures. Although the observed values for transformation and transfection by SP82 were considerably lower at 46 °C than at 37 °C, transfection by SP3 DNA was somewhat enhanced at 46 °C.

There is certainly no restriction of expression of the phage DNA at 46 °C as compared to that at 37 °C. It is evident that SP3 phage is not adversely affected by the restrictive temperature since its ability to adsorb is not diminished when 168wt is incubated at 46 °C.

SP3 adsorbed to isolated flagella, although not very efficiently (30% adsorption after 60 min to a preparation of flagella corresponding to 2 x 10^8 cells/ml). Based on this limited adsorption, and the inefficient adsorption to mutants possessing paralysed flagella, it is reasonable to conclude that motion of the flagella is an essential component of efficient infection.

Three SP3-resistant strains of 168wt were derived by prolonged incubation of separate cultures after lysis by SP3. These three strains were non-motile as judged by the inability of colonies to swarm on motility plates, and the lack of translational motility under phase-contrast microscopy. These strains were unable to support SP3 plaque formation when used as lawn bacteria; they did support the growth of SP82 normally.

Of six additional SP3-resistant strains of 168wt (derived from surviving colonies within lysed areas of plates spotted with SP3), five were non-motile as judged by the above criteria. These strains failed to support SP3 when used as plating bacteria, but supported SP82 growth normally. The remaining strain was motile, supported SP82 growth, but did not support growth of SP3. The reason for the resistance of this strain is not clear. Parallel experiments with survivors of lysates of SP82 failed to produce any correlation of SP82 resistance with loss of motility.

Three strains of 168wt, derived from non-swarming colonies, were unable to support SP3 when used as lawn bacteria but supported SP82 growth.

Motile revertants isolated from all of the above 12 non-motile strains of 168wt supported SP3 at levels equal to 168wt.

Electron microscopy of negatively stained SP3–168wt complexes revealed phage on or near flagella (data not shown). Preliminary examinations failed to elucidate the specific structural component by which the phage adhered to the flagella (e.g. by tail fibres, etc.). When a lysate containing a large number of defective incomplete phage particles was used, empty, tailless heads were consistently observed on or near flagella. Tails did not congregate in the vicinity of the flagella. No specialized structures, such as the capsid fibrils reported for B. subtilis phage Nf (Shimizu et al., 1970), were observed on the surface of the SP3 capsid; the mode of attachment of the capsid to the flagella was not determined.
Short communication

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REFERENCES


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