The Length of the Filamentous *Pseudomonas aeruginosa* Bacteriophage Pf

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**SUMMARY**

The length of the *Pseudomonas aeruginosa* filamentous bacteriophage Pf was found to be 1915 ± 77 nm, as measured in the electron microscope using the Kleinschmidt spreading technique. Pf is thus the longest filamentous phage so far isolated. Coliphage If, the I-specific filamentous phage, is nearest to it with a length of 1300 nm.

Bacteriophage Pf, which is the only filamentous bacterial virus so far isolated for *Pseudomonas aeruginosa*, has not been studied in any detail. However, it is known that it contains 12% single-stranded DNA and has a similar appearance to other filamentous phages (Takeya & Amako, 1966). While the length of the virus particle is not known, electron micrographs suggest that Pf may be considerably longer than the I- and F-specific phages of *Escherichia coli* (Bradley, 1972). If this were so, it would be the longest filamentous bacterial virus found so far. This communication describes the accurate measurement of Pf using the electron microscope and the Kleinschmidt spreading technique (Kleinschmidt et al. 1962.)

A high titre suspension of Pf (ATCC 25102B) was prepared by infecting a broth culture of the host organism (*Pseudomonas aeruginosa* strain K: ATCC 25102) and incubating it with shaking at 37 °C for 20 h. The phage was separated by salting out with ammonium sulphate. The floc was suspended in 0.1 M-neutral ammonium acetate solution, and dialysed against the same solution overnight. Final purification was achieved by differential centrifuging (100000 g for 5 h and 7000 g for 15 min).

For electron microscopy the phage was suspended in neutral 0.1 M-ammonium acetate + 0.005 M-EDTA and processed as follows. The suspension was diluted tenfold with 0.25% (v/v) formaldehyde + 0.5 M-ammonium acetate solution, and mixed with an equal vol. of a 0.5 mg/ml solution of cytochrome C. One drop was placed on a coverslip and allowed to run on to a water surface previously cleaned for spreading. The resulting surface film was picked up immediately on carbon-coated specimen grids. Contrast was produced by rotary shadow-casting with platinum at an angle of 6°.

Electron micrographs of well-separated Pf particles were taken at random at about ×10000 magnification in a Siemens Elmiskop 1. Several calibration micrographs were taken of a diffraction grating replica, previously measured optically to 31308 ± 750 (2.4%) lines per inch, at the same session. The photographic plates of both grating and virus particles were placed in an enlarger (magnification about ×5) and an accurate magnification was obtained from the image by measuring the spacing between the centre 5 of the 7 grating lines visible on each of 4 plates, using a steel ruler. The image of each bacteriophage filament was traced on to paper and measured with the ruler if straight, and if not, with an electronic measurer. This consisted of a wheel, as used on a map measurer, geared to a sliding resistance which was connected to a digital voltmeter. The device was calibrated to ± 1% on a 10 cm line drawn on the same paper used for tracing the virus particles. It measured curved filaments about as accurately as the ruler measured straight ones. No bias was therefore
Table 1. Sources of operational errors in phage length measurements

<table>
<thead>
<tr>
<th>Item</th>
<th>Cause of error</th>
<th>How determined</th>
<th>± %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Electron microscope</td>
<td>Specimen level</td>
<td>Estimated</td>
<td>1.0</td>
</tr>
<tr>
<td>Electron microscope</td>
<td>Pincushion distortion</td>
<td>Estimated</td>
<td>2.0</td>
</tr>
<tr>
<td>Diffraction grating</td>
<td>Optical calibration</td>
<td>Six measurements</td>
<td>2.4</td>
</tr>
<tr>
<td>Diffraction grating</td>
<td>Measurement of image under enlarger</td>
<td>Determined experimentally</td>
<td>0.2</td>
</tr>
<tr>
<td>Diffraction grating</td>
<td>Local errors in line spacing</td>
<td>Measured from 10 lines in various areas</td>
<td>1.5</td>
</tr>
<tr>
<td>Steel ruler</td>
<td>Scale error introduced to grating measurement</td>
<td>Estimated</td>
<td>0.5</td>
</tr>
<tr>
<td>Pf filaments</td>
<td>Tracing image onto paper</td>
<td>Determined experimentally</td>
<td>0.3</td>
</tr>
<tr>
<td>Pf filaments</td>
<td>Measurement of trace with ruler or electronic measurer</td>
<td>Includes ruler scale error and measurer calibration error</td>
<td>1.5</td>
</tr>
<tr>
<td>Expected standard deviation on length measurements (geometric sum)</td>
<td></td>
<td></td>
<td>3.95</td>
</tr>
</tbody>
</table>

introduced by selecting only straight phage particles. Such a measuring procedure introduces a series of errors (Lang et al. 1967) which are listed above (Table 1).

The accuracy of individual length measurements is determined by the operational errors listed in Table 1, some of which require clarification. In the electron microscope, a magnification error of 1% is introduced by slight changes in the specimen level within the objective lens (bending of the grid or support film, etc). Pincushion distortion, which is best described as an increase in magnification relative to the distance of an object from the optical axis of the instrument, was estimated at 2% by measuring the spacing of diffraction grating lines at the edge and in the centre of the field of view. This error compensates for variations in the distance of particles from the optical axis. The optical calibration of the diffraction grating includes an error for the stage micrometer used in the light microscope. The enlarger used for projecting the grating and virus particle images had no detectable pincushion distortion. As stated by Lang et al. (1967), these errors may have a positive or negative sign so that the expected total sample standard deviation is their geometric sum (the square root of the sum of the squares). The value of 4% is close to that given by Lang et al. (1967) in their measurements of DNA molecules.

A length distribution histogram of all the 113 measurements is shown in Fig. 1. It can be seen that, apart from the sharp peak around 1900 nm, there is a more or less even scatter of measurements over the whole range. These are considered to be contaminating pili, a few of which are always present in Pf preparations. They are indistinguishable from Pf filaments in the absence of antibody-labelling, which could not be used here because of interference with the spreading process. Some of the shorter particles may also be broken phages. It is notable that there are no peaks at half or double the modal length, showing that Pf has a single unit length, and that it is not readily halved by shear, nor does it form any double-length particles. A modal value of 1890 nm was obtained from the length distribution curve derived from the histogram. The arithmetical mean and standard deviation must be calculated from those measurements falling within the major portion of the frequency peak. The limits, which are necessarily somewhat arbitrary, are considered to be between 1750 nm and 2100 nm (Fig. 1, arrows A and B). The 80 observations within these limits give a mean of 1915 ± 77 nm (4%), which is taken to represent the absolute length of the virus particle. The standard deviation is the same as the estimated total operational error, as might be expected.

The most accurate measurement available for the F-specific coliphages is represented by d at 883.5 ± 15 nm when spread on water (Frank & Day, 1970). This is less than half the
Fig. 1. Length distribution histogram of 113 measurements of particles from a suspension of phage Pf, class interval 50 nm. A and B mark the estimated limits of the frequency peak, the mean of the values between them being 1915 ± 77 nm. The estimated modal length is 1890 nm.

length of Pf. The I-specific filamentous coliphages have a modal length of 1300 nm (Meynell & Lawn, 1968) so that Pf is nearly 1.5 times their length. The Xanthomonas oryzae filamentous phage Xf, which might be taxonomically closer to Pf than the coliphages, is only 977 nm long (Kuo, Huang & Chow, 1969). In fact, Pf is the longest filamentous bacterial virus so far isolated. The possibility exists that Pf might form preferred heterozygotes, which are filaments containing two separate DNA molecules, each the same length as the bacteriophage. Such particles are formed by coliphage M13 under certain conditions (Salivar, Henry & Pratt, 1967). However, preliminary measurements of the single-stranded DNA of Pf (D. E. Bradley, unpublished observations) indicate that it is about twice the length of the phage, which is therefore likely to contain only one molecule. This is similar to the situation with fd particles where the single DNA molecule is about 2.5 times the virus length (Marvin & Hohn, 1969). It seems that Pf is basically similar to other filamentous phages save that it is much longer, and its DNA presumably has a correspondingly higher mol. wt. The length of filamentous phages is considered of importance in their classification (Marvin & Hohn, 1969), so that Pf represents a new class of this type of bacterial virus.

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REFERENCES


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