Characterization of a Family of Temperate Actinophages of *Faenia rectivirgula*

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Six temperate phages of the thermophilic actinomycete *Faenia rectivirgula* were characterized by restriction analysis and found to be closely related: (1) three phages (φFR114, φFR371, φFR114) isolated from lysogenic strains, (2) a deletion-derivative of φFR114 (φFR755R) and (3) two phages (φFR747 and φFR9) isolated from soil. φFR371 differs from φFR114 by a deletion not overlapping with that in φFR755R. The restriction maps of φFR114 and φFR113 are very similar. φFR747 and φFR9 belong to the same family as shown by the comparison of their restriction fragment patterns.

**INTRODUCTION**

Actinophages have been investigated as taxonomic tools for phage-typing of actinomycetes (Kutzner, 1961; Korn et al., 1978) or as suitable vectors for molecular cloning techniques (Chater et al., 1985). Most of the work on actinophages deals with those of the genus *Streptomyces* (Lomovskaja et al., 1980), whereas little is known about the phages of other actinomycete genera.

Recently Greiner-Mai et al. (1987a) described phage-typing of the thermophilic actinomycete *Faenia rectivirgula*. It was demonstrated that lysogenization by any of the five temperate phages (φFR114, φFR113, φFR371, φFR747 and φFR9) which had been used for phage-typing mediated resistance to all phages but one, even including the two lytic phages Mp1 and P517. The latter was originally isolated for *Streptomyces erythraeus* by F. Korn-Wendisch in our laboratory; phages of this industrially important species have recently received great attention as sources for cloning vectors (Donadio et al., 1986; Brzezinski et al., 1986). P517 is the only phage of our set which infects *S. erythraeus*. Only the lytic phage P113 is able to lyse the lysogenic as well as the non-lysogenic strains. These observations indicated that most of the phages discussed might be genetically related.

In this paper we describe the restriction maps of three temperate phages (φFR114, φFR113 and φFR371) which were isolated from lysogenic strains of *F. rectivirgula*. A derivative (φFR755R) of φFR113 obtained by re-isolation from a lysogenized host was also studied, and two temperate phages of this group (φFR747 and φFR9) which were isolated from soil were compared with the others regarding their restriction fragment pattern.

**METHODS**

*Bacteria and phages. Faenia rectivirgula* DSM 43114, DSM 43113 and DSM 43371 are the original lysogenic hosts of the temperate phages φFR114, φFR113 and φFR371 respectively. The non-lysogenic strain DSM 43755 was used for the propagation of all phages. The temperate phages φFR747 and φFR9 had been isolated from soil (Greiner-Mai et al., 1987a). Handling and conservation of bacteria and phages was as described by Greiner-Mai et al. (1987b) and Hopwood et al. (1985).
**J. SCHNEIDER, I. AGUILERA GARCIA AND H. J. KUTZNER**

*F. rectivirgula* was grown in GPHF medium pH 7.2 at 50 °C. GPHF broth contained glucose 10 g l⁻¹, peptone 5 g l⁻¹, yeast extract 5 g l⁻¹, meat extract 5 g l⁻¹, MgCl₂ 0.74 g l⁻¹. For phage propagation, GPHF was prepared as for the broth but with 1 g glucose l⁻¹ and 3 or 12 g agar l⁻¹ as top-layer or base-layer respectively.

*Escherichia coli* JM83 was used as the host for pUC18 recombinants (Yanisch-Perron et al., 1985). It was cultured at 37 °C in LB broth (Maniatis et al., 1982). For the selection of recombinant clones, ampicillin (50 µg ml⁻¹) and the chromogenic substrate 5-bromo-4-chloro-3-indolyl β-D-galactoside (X-Gal, Stehelin, 20 µg ml⁻¹) were incorporated into LB broth with 12 g agar l⁻¹.

**DNA manipulations.** Phage DNA was prepared as described by Hopwood et al. (1985) using GPHF medium. Plasmids were isolated from *E. coli* by the method of Birnboim & Doly (1979).

Restriction analysis was done according to Fuchs & Blakesley (1983) with enzymes from Anglia and New England Biolabs. For ligation, transformation and agarose gel electrophoresis the methods described by Maniatis et al. (1982) were used. Individual restriction fragments (for cloning) were isolated from agarose gels by the DEAE-cellulose paper method of Dretzen et al. (1981).

**Analysis of the phage genomes.** The total length of the phage genomes was calculated by combining the length of overlapping restriction fragments, using phage λ DNA cut with *PstI* or *BstEII* as molecular mass standards. Restriction mapping was done several times using different combinations of restriction enzymes and comparing total phage DNA with parts of it cloned in pUC18. Cohesive ends (cos-sites) were demonstrated by comparing restricted phage DNA loaded on agarose gels with or without prior heat-treatment (70 °C, 10 min).

**Southern blots.** After separation of DNA fragments by agarose gel electrophoresis the gel was treated as described by Hopwood et al. (1985). For transfer to a nitrocellulose filter the Immunoblot Transfer Apparatus (kindly provided by Chemie + Werkstoff-Technik GMBH, Idstein) was used with 6× SSC (0.9 M-NaCl, 0.09 M-trisodium citrate) as transfer buffer. Transfer of DNA was complete after 1 h with 0.8 mA per cm² of gel (LKB Macrodriev 1 power supply). After baking the filter (2 h, 85 °C) and prehybridization at 65 °C in 6× SSC with 0.7% (w/v) dry skimmed milk (Johnson et al., 1984), the labelled DNA-probe was added. The nick-translation was done as described by Hopwood et al. (1985) using Biotin-11-DUTB (Gibco-BRL). The hybridization was performed at 65 °C overnight. Afterwards the filter was consecutively washed with 2× SSC + 1% SDS, 0.2× SSC + 1% SDS (2× 15 min, 55 °C), 2× SSC + 0.1% SDS and finally with 6× SSC + 0.7% (w/v) dry skimmed milk (20 min, 40 °C). The biotinylated DNA was detected using the ‘ENZO DETEK 1 alk’ system (Ortho Diagnostic Systems).

**RESULTS**

**Restriction maps of φFR113 and φFR114**

The two genomes were mapped by restriction analysis with seven restriction enzymes which cut them up to nine times. In addition fragments were cloned with pUC18 to confirm the mapping data and for more detailed mapping. φFR113 and φFR114 have linear genomes of 43 and 42 kb with cohesive ends (cos-sites). Comparison of the two restriction maps revealed considerable similarity (Fig. 1). However, within the central region several differences are found (Fig. 2a, b).

**Derivatives of φFR113 caused by deletions**

The temperate phage of DSM 43371 (φFR371) turned out to be a deletion variant of φFR113 being 2 kb shorter than this phage. Another variant of φFR113 was discovered when comparing the phage progeny from several φFR113-resistant (lysogenized) cells of DSM 43755: 24 colonies were picked from different plaques of φFR113 and used for re-isolation of the temperate phage. Four of the 24 phages had a genome 1 kb shorter than that of φFR113. Comparison of restriction fragment patterns of these four phages did not reveal any differences; one of the phages (φFR755R) was studied in detail.

Eleven restriction enzymes cutting the phages up to 18 times were used to detect differences in fragment length between φFR113 and its variants. In addition the genomes of φFR755R and φFR371 were mapped in the same way as φFR113. The three phages had the same restriction map with the exception of the central part of the genomes. This was cloned as a *BamH1–PstI* fragment and the resulting pUC18 recombinants pJS50 (φFR113), pJS60 (φFR371) and pJS70 (φFR755R) were used for detailed analysis. Fig. 2(b, c, d) shows that both variations are due to independent, non-overlapping deletions which are adjacent to each other on the φFR113 genome.
Temperate phages of *F. rectivirgula*

Two temperate phages from soil (*φFR747 and φFRG9*)

The attempt to find more phages for *F. rectivirgula* resulted in the isolation of the temperate phages *φFR747* and *φFRG9*, which were identified as relatives of *φFR113* and *φFR114*: although neither phage has been mapped in detail, comparison of restriction fragments with

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**Fig. 1.** Restriction maps of *φFR114* and *φFR113* (abbreviation of enzymes as in Fig. 2). The linear restriction maps of the two phages cut with seven restriction endonucleases are compared with the cos-sites indicated at both ends. The approximate fragment sizes are given in kb. A wider range of enzymes was used to demonstrate similarity between several cloned fragments; three examples which are indicated at the bottom of this figure are shown in Fig. 2(a, b).

**Fig. 2.** Comparison of the central region of *φFR114*, *φFR113*, *φFR755R* and *φFR371*. The central region of the phages (indicated in Fig. 1) was cloned in pUC18 and compared by digestion with eleven restriction endonucleases: A, ApaI; B, BamHI; Bg, BglII; Bs, BseII; E, EcoRV; K, KpnI; P, PsI; PvuII; S, SacI; Sl, SalI; X, XhoI (note that in (a) KpnI is not completely mapped).
Fig. 3. Fragments of \( \phi FR747 \) and \( \phi FRG9 \) assumed to be homologous to fragments of \( \phi FR114 \) (and \( \phi FR113 \)) (abbreviation of enzymes as in Fig. 2; \( E^* \) is an \( EcoRV \) site found on \( \phi FR113 \) but not on \( \phi FR114 \)). The migration of restriction fragments smaller than 8 kb from \( \phi FR114 \), \( \phi FR113 \) (not shown), \( \phi FR747 \) and \( \phi FRG9 \) was compared on agarose gels under different conditions (0.6-1.0% agarose, Tris/acetate or Tris/borate buffers). Fragments of \( \phi FR747 \) and \( \phi FRG9 \) which always comigrated with fragments of \( \phi FR114 \) (or \( \phi FR113 \) in the case of \( E^* \)) are shown in the figure.

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\text{Fig. 4. DNA-hybridization of } \phi FR114 \text{ with } \phi FR747 \text{ and } \phi FRG9. (a) DNA from } \phi FR114 \text{ (A), } \phi FR747 \text{ (B) and } \phi FRG9 \text{ (C) was digested with } BsrEII \text{ and the fragments were separated by agarose gel electrophoresis. (b) After transfer to a nitrocellulose filter they were hybridized with biotin-labelled DNA from } \phi FR114. \end{array} \]

\( \phi FR114 \) and \( \phi FR113 \) demonstrated considerable similarity between these four phages (Figs 3 and 4a). This is supported by DNA–DNA hybridization: on Southern blots all four phages hybridize with each other (Fig. 4 and data not shown). \( \phi FR747 \) and \( \phi FRG9 \) had cos-sites and a length of approximately 42 kb.
DISCUSSION

Our results clearly demonstrate the existence of a family of temperate bacteriophages which are closely related as far as this can be seen by cross-immunity and comparison of the phage genomes by restriction analysis and DNA–DNA hybridization.

Two types of variation can be distinguished within this group of phages. (1) Specific deletions which can be caused by a single mutational event. φFR113 readily undergoes deletion of two regions of its genome without any obvious phenotypic changes. The resulting variants φFR755R or φFR371 can be regarded as identical with the exception of the deleted regions. It would be interesting to find a viable derivative which has lost both fragments together. The deletions might be substituted by foreign DNA, thus making the phages suitable for vector development. (2) Various differences along the whole phage genome. The differences in restriction site pattern between φFR114, φFR113, φFR747 and φFRG9 can only be explained by a large number of mutations. In fact, a diverging development from a common ancestor might be responsible for the different genome structures. These changes may cause differences within the phenotypes of the phages which have not been observed yet, e.g. host specificity, molecular mass and function of phage proteins. Therefore, this group of phages seems to be an interesting subject for studying phage evolution.

Another aspect of phage variation is the existence of some lytic phages which seem to belong to the same genetic family. Preliminary restriction mapping and DNA–DNA hybridization strongly indicates a close relationship between the temperate phages and the lytic phages Mp1 and P517. Phage P517, which was originally isolated for S. erythraeus, has a different host range than the other members of the family known so far, making host recognition another interesting aspect to be studied.

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REFERENCES


