SHORT COMMUNICATIONS

A Common Host Specificity in the Restriction and Modification of a Bacteriophage by Three Distinct *Streptomyces* Species

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INTRODUCTION

Restriction/modification of bacteriophage is a well characterized phenomenon in many bacterial species and has been identified among streptomycetes in *Streptomyces albus* G (Chater & Wilde, 1976), *Streptomyces griseus* Kr. 15 and *Streptomyces coelicolor* (Lomovskaya *et al.*, 1977).

Direct correlation between bacterial restriction of bacteriophage and a specific endonuclease has only been demonstrated for a few species, including one *Streptomyces* species (Chater & Wilde, 1976). The discovery of extrachromosomal elements in *Streptomyces* (Kirby *et al.*, 1975; Freeman *et al.*, 1977; Kirby, 1978) opened up the possibility of detecting such an element carrying restriction/modification, as has been shown to occur in eubacteria (Arber, 1974).

METHODS

Strains CA221 (*Streptomyces cattleya* NRRL 8057), LP326 (*Streptomyces lipmanii* NRRL 3584), OL328 (*Streptomyces olivaceus* ATCC 31126) and AL322 (*Streptomyces albus* CMI52766) were obtained from various culture collections. Actinophage FF4 was isolated from soil using the specific enrichment method of Dowding (1973). Nutrient broth (NB), nutrient broth agar (NBA) and soft nutrient broth agar (SNBA) were as used by Dowding (1973). TE buffer contained 10 mM-Tris/HCl pH 7.5 and 1 mM-EDTA.

General methods for the *Streptomyces* species were those used by Hopwood (1967), and for the bacteriophage those used by Dowding (1973). The efficiency of plating (e.o.p.) is calculated throughout the paper assuming an e.o.p. of 1 on *S. albus*.

Large scale confluent lysates were prepared as follows. Two 300 × 300 mm sterile glass bioassay trays were prepared containing approximately 150 ml NBA with appropriate additions. A top layer of SNBA (40 ml) was added; this contained a single plaque soak-off of bacteriophage, the spores from half a slope of the streptomycete and appropriate additions. The trays were incubated overnight and then flooded with 30 ml NB containing 4 mM-calcium nitrate. The slurry was scraped off into a flask. After 2 h, the agar was sedimented at 12000 rev. min⁻¹ in an MSE HS18 centrifuge for 15 min. The supernatant was treated with deoxyribonuclease and ribonuclease (each at 20 µg ml⁻¹) for 1 h and then centrifuged at 30000 rev. min⁻¹ and 5 °C in an MSE SS50 for 1 h. The pellet was resuspended in 2 ml Tris/HCl buffer pH 7.0 containing 4 mM-calcium nitrate and filtered through a Millipore filter (2 µm pore size). The titre of actinophage was between 10¹⁰ and 10¹² plaque-forming units ml⁻¹.

RESULTS

The bacteriophage FF4 was isolated during routine screening of soil samples for bacteriophage of *Streptomyces* and conformed in behaviour with other bacteriophages of *Streptomyces*.

FF4 was initially grown on SNBA overlays containing *S. albus* as host as this gave the highest titre of bacteriophage. FF4 grown on *S. albus* had very low e.o.p. on *S. olivaceus*,

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Table 1. Restriction/modification of FF4 by *Streptomyces olivaceus* (OL328), *Streptomyces cattleya* (CA221) and *Streptomyces lipmanii* (LP326)

First host	Second host	Third host	Strain assayed against	Efficiency of plating relative to AL322
AL322	—	—	OL328	3.0×10^{-8}
AL322	OL328	—	OL328	7.1×10^{-2}
AL322	OL328	AL322	OL328	$< 9.1 \times 10^{-5}$
AL322	—	—	CA221	1.8×10^{-7}
AL322	CA221	—	CA221	1.1×10^{-1}
AL322	CA221	AL322	CA221	$< 5.9 \times 10^{-7}$
AL322	—	—	LP326	8.3×10^{-8}
AL322	LP326	—	LP326	5.5×10^{-3}
AL322	LP326	AL322	LP326	$< 2.6 \times 10^{-6}$

Table 2. Similarity of restriction/modification of FF4 in *Streptomyces olivaceus* (OL328), *Streptomyces cattleya* (CA221) and *Streptomyces lipmanii* (LP326)

First host	Second host	Strain assayed against	Efficiency of plating relative to AL322
AL322	OL328	OL328	2.4×10^{-1}
AL322	OL328	CA221	1.0×10^{-1}
AL322	OL328	LP326	4.1×10^{-3}
AL322	CA221	OL328	2.7×10^{-1}
AL322	CA221	CA221	3.5×10^{-2}
AL322	CA221	LP326	4.2×10^{-2}
AL322	LP326	OL328	2.2×10^{-1}
AL322	LP326	CA221	2.3×10^{-1}
AL322	LP326	LP326	2.8×10^{-2}

S. lipmanii and *S. cattleya* (Table 1), suggesting that these hosts were restricting the bacteriophage. Once grown on these strains, the e.o.p. on the respective hosts rose by a factor of about 10^6 . This suggests that host-specific modification was occurring in these species. These indications of restriction and modification were supported by the finding that after growth again on *S. albus*, FF4 was restricted to about the original extent by the three strains.

FF4, modified on any one of the three hosts which showed restriction/modification, gave a high e.o.p. on the other two hosts (Table 2). This indicates that all three strains possess restriction/modification systems of similar specificity.

No evidence for restriction/modification with these three *Streptomyces* species was found with eight other bacteriophages of *Streptomyces*: VP11, VP12, Pal6 and A2 (kindly provided by K. F. Chater, John Innes Institute, Colney Lane, Norwich) and FF1, FF2, FF3 and FF5 (isolated from soil samples by F. Flett).

DISCUSSION

Restriction/modification has been identified in three species of *Streptomyces*: *S. olivaceus*, *S. lipmanii* and *S. cattleya*. The restriction/modification acted on one bacteriophage FF4 and the results clearly showed that no mutational event was involved as the phenomenon was reversible. The similarity between the restriction/modification systems of the three species was unexpected. It implies that the target sites for any restriction enzymes are similar and that the host-specific modification protects the sites from all three enzymes. Whether the differences in the e.o.p. of the modified bacteriophages in Table 2 are due to slight differences in specificity between the restriction/modification systems or to differences in bacteriophage attachment efficiency or stability cannot be elucidated from this data.

Restriction/modification in *Streptomyces* seems to be relatively bacteriophage-specific (K. F. Chater, personal communication) and not to act generally over a wide spectrum of

bacteriophages. This was confirmed by our work. It could be due to a selection for loss of restriction sites in the natural environment.

The three species seem to be taxonomically distinct. They have very different morphologies, produce different antibiotics and have different sensitivity patterns to the eight bacteriophages tested. As there is evidence to support a relationship between restriction/modification systems and plasmids in some cases (Arber, 1974), a possible explanation for the similarity would be a common extrachromosomal element coding for restriction/modification.

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