A New, Wide Host-range, Temperate Bacteriophage (R4) of *Streptomyces* and its Interaction with some Restriction–Modification Systems

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A new temperate phage, R4, of *Streptomyces* was isolated from soil on a restriction-deficient mutant of *S. albus* G. In its morphology, adsorption properties and growth kinetics R4 resembled other temperate phages of *Streptomyces* though its requirements for Ca^{2+} and Mg^{2+} were higher than usual. It was unable to form plaques above 34.5 °C. R4-mediated transduction was not detected. Unlike other *Streptomyces* temperate phages, R4 had a wide host-range, which correlated better with the absence of detectable class II restriction enzymes than with conventional taxonomic divisions. Many of the sensitive strains [but not, apparently, *S. coelicolor* A3(2)] could be lysogenized.

With the wild-type R4, plaques were obtained on *S. albus* G only after growth on a restriction-deficient, modification-proficient mutant, and then only at a very low efficiency of plating. All of these plaques were of a mutant type (R4G) which (unlike the parental R4 phage) showed conventional patterns of restriction–modification in the *S. albus* G (*Sal*GI) and *S. albus* P (*Sal*PI) systems. R4G mutants, but not R4, were sensitive to a restriction–modification system present in two *S. rimosus* strains (2251 and NRRL 2234). DNA from *Sal*GI-unmodified (but not from modified) R4 or R4G was cleaved by *Sal*GI into more than 30 fragments (mean size 1.35 kilobases; summed molecular weight 30.02×10^6). R4 DNA was cleaved at one site by *Eco*RI, at one site by *Sal*PI ($\equiv$ *Pst*I), and not at all by *Hind*III or *Bam*HI.

INTRODUCTION

The most detailed study of a temperate bacteriophage of *Streptomyces* has been of ϕ C31, which originated from *Streptomyces coelicolor* A3(2) where it is apparently normally present as a defective prophage (Lomovskaya *et al.*, 1971). A group of temperate phages of *S. coelicolor* A3(2), homoimmune to each other but heteroimmune with respect to ϕ C31, was isolated from soil by Dowding & Hopwood (1973), and one of these (VP5) has been investigated further (Chater & Sykes, 1976). A third immunity group of *S. coelicolor* temperate phages is represented by ϕ 448 (isolated by N. D. Lomovskaya, Moscow) and S14, recently isolated in this laboratory (C. Stuttard & K. F. Chater, unpublished results). All three groups share the feature of relatively narrow host-range, while most virulent phages of *S. coelicolor* isolated from soil formed plaques on most wild-type *Streptomyces* spp. (Dowding, 1972).

We report here the isolation from soil of a temperate *Streptomyces* bacteriophage (R4) which represents a fourth immunity group and has an unusually wide host-range. Some features of R4 relevant to our interest in restriction in *Streptomyces* are described.

METHODS

Strains and microbiological techniques. The wild-type strains used are listed in Table 2. Complete medium (CM), minimal medium (MM), the growth of streptomycetes and the preparation of spore suspensions were as in Hopwood (1967). Adsorption, one-step growth experiments, propagation and assay of bacteriophages were done with Difco-Bacto nutrient broth (NB) or nutrient agar (NA) as described by Dowding (1973), except that 25 mM- $\text{Ca}(\text{NO}_3)_2$ and 10 mM- MgSO_4 were added for best results with R4 (see Results), and adsorption prior to one-step growth experiments was done in the presence of 0.1 mM-KCN (Chater & Sykes, 1976).

DNA. The preparation of phage DNA, its digestion with restriction enzymes and subsequent analysis by agarose gel electrophoresis were as in Chater & Wilde (1976) and Chater (1977). Guanosine plus cytosine content of phage DNA was calculated from buoyant density in CsCl density gradients as described by Bibb *et al.* (1977). Internal standards were coliphage λ DNA (49 mol % guanosine plus cytosine; MacHattie & Thomas, 1968) and *S. albus* G DNA (73 mol % guanosine plus cytosine; K. Chater, unpublished data).

Restriction enzymes. *Sal*GI was prepared as in Chater & Wilde (1976) and *Sal*PI as in Chater (1977); *Eco*RI, *Hind*III and *Bam*HI were from Miles Laboratories. Examination of cell-free extracts of various wild-type streptomycetes for class II restriction enzyme activity was done as in Chater & Wilde (1976) using λ DNA as substrate unless stated otherwise.

Electron microscopy. Phage particles sedimented by centrifugation at 40000g for 90 min were resuspended in 200 mM-sodium acetate and negatively stained with uranyl acetate before examination in a Siemens Elmiskop electron microscope.

RESULTS

Storage and assay conditions

R4 was first seen as a turbid plaque obtained by plating a nutrient broth extract of local soil on strain J1074, a mutant of *Streptomyces albus* G defective in the *Sal*GI restriction and modification system (Chater & Wilde, 1976). R4 showed little loss in viability when stored for 2 h in nutrient broth, distilled water, or 10 mM-Tris/acetate pH 7.0 and 4 mM- $\text{Ca}(\text{NO}_3)_2$, but lost about 50% viability after 24 h in T2 buffer (Hershey & Chase, 1952) or SM buffer (Chater & Wilde, 1976). R4 was slowly inactivated by the addition of 1% chloroform (survival 3×10^{-3} at 24 h) and was sensitive to 15 mM-sodium citrate (in 150 mM-NaCl, pH 8.0) (1% survival after 10 min at room temperature).

The efficiency of plating (e.o.p.) and plaque morphology of R4 were considerably influenced by the divalent cation content of the medium (Fig. 1). The highest e.o.p. was obtained with 25 mM- $\text{Ca}(\text{NO}_3)_2$. MgSO_4 was less effective: the highest e.o.p. (with 10 mM- MgSO_4) was about 40% of that obtained with 25 mM- $\text{Ca}(\text{NO}_3)_2$. Addition of 10 mM- MgSO_4 slightly increased the e.o.p. with a range of $\text{Ca}(\text{NO}_3)_2$ concentrations and resulted in much larger plaques. All the experiments described below were done with 25 mM- $\text{Ca}(\text{NO}_3)_2$ and 10 mM- MgSO_4 .

Morphology of R4

In overall dimensions and appearance R4 was similar to bacteriophage lambda (Kellenberger & Edgar, 1971), having a head with a hexagonal outline measuring 64 nm both lengthways and laterally and a laterally striated flexible tail of 190 nm length and 12.5 nm diameter (Fig. 2). No specialized structures at either end of the tail were seen.

Adsorption and one-step growth curve

Adsorption to freshly germinated spores of J1074 was somewhat more efficient (95% adsorption in 35 min, adsorption constant 1.7×10^{-9} ml min⁻¹) than adsorption of other *Streptomyces* phages to spores of *S. coelicolor* A3(2). The extent to which this was a function of the host was not determined.

Using a clear-plaque mutant of R4 (R4c1; see below) the one-step growth kinetics (Fig. 3) were similar to those for temperate phages ϕ C31 (Lomovskaya *et al.*, 1972) and VP5 (Chater & Sykes, 1976) and for virulent phages VP11 (Dowding, 1973) and VP12 (K. F. Chater,

Fig. 1. Effect of Mg^{2+} and Ca^{2+} on the efficiency of plating of R4 on *S. parvulus* ATCC 12434: ○, $Ca(NO_3)_2$; ●, $MgSO_4$; □, 10 mM- $MgSO_4$ plus $Ca(NO_3)_2$ as indicated.

Fig. 2. Electron micrograph of bacteriophage R4 negatively stained with uranyl acetate. Bar marker represents 100 nm.

unpublished results). The latent period was 45 to 60 min and the burst size was 10 to 50. Similar results were obtained with either J1074 or *S. parvulus* as host.

Heat sensitivity of R4 development

R4 did not form plaques on J1074 or *S. parvulus* at temperatures above 34.5 °C, although both hosts grew well at temperatures several degrees higher. This was not due to temperature-sensitivity of the intact particle, since no loss of viability was observed when R4 was maintained at 37 °C for 100 min. Modified one-step growth experiments (Fig. 4) incorporating temperature shift-up indicated that phage development became heat-resistant only late in the latent period (30 to 35 min). Mutants able to form plaques at 35.5 °C were not found either spontaneously (frequency less than 2.5×10^{-9}) or after ultraviolet irradiation to 1% survival (frequency less than 4×10^{-5}).

R4 is a temperate phage

Upon extensive purification on CM containing 10 mM-sodium citrate (which should have greatly reduced the amount of free phage), cultures derived from the turbid centres of R4 plaques on J1074 often proved to be completely resistant to R4 infection. To test whether these strains had become lysogenic for R4, spore suspensions washed in 10 mM-sodium citrate were plated at various dilutions on CM for viable counts and on NA base plates in soft agar overlays containing J1074 indicator spores to detect the presence of plaque-forming units. Colony counts on CM were about 100-fold higher than plaque counts.

Fig. 3

Fig. 4

Fig. 3. One-step growth curve on *S. parvulus* ATCC 12434 of a clear-plaque mutant (R4c1) of bacteriophage R4. Counts of plaque-forming units (p.f.u.) derived from plating samples from an undiluted flask (○) and from a flask containing a 10-fold dilution of the infected cells (●) are shown.

Fig. 4. Modified one-step growth curve incorporating temperature shifts. A one-step growth experiment (■, □) was carried out at 30 °C (using J1074 as host) as in Fig. 3. In addition, samples were transferred to NB at 37 °C at intervals, incubated until 100 min after the start of the experiment, and then rapidly plated to assay plaque-forming units (●, ○). Results from plating 10⁻¹ dilutions (■, ●) and 10⁻² dilutions (□, ○) are shown. The presence of low counts in the early samples subjected to temperature shift was attributable to the presence of a low level of unadsorbed phage.

However, most colonies could be shown to contain free phage by replication to NA followed 24 h later by overlaying with soft agar containing J1074 indicator. Thus the colonies were lysogenic and R4 was therefore a temperate phage. Unlike other temperate phages of *Streptomyces*, clear-plaque mutants of R4 were rare: after ultraviolet irradiation to 1% survival, none was found among 92000 survivors. However, a single spontaneous clear-plaque mutant (R4c1) was obtained during routine platings. R4c1 was unable to form plaques on R4 lysogens.

Attempted transduction with R4

An R4 mutant (R4G2; see below) propagated on strain J1074 was used as a potential transduction agent at multiplicities of infection of 1 to 20 with germinated spores of a restriction-defective mutant of *S. albus* G, requiring adenine and arginine plus uracil (Ade⁻Aur⁻) for growth, in a number of experiments involving continued incubation of the mixture in NB before spreading on media (MM plus adenine, MM plus arginine plus uracil) selecting Aur⁺ or Ade⁺ transductants, or on CM to allow a round of non-selective growth before harvesting spores and plating them on selective media. In other experiments, germinated spores were spread on selective media and phage was then added. No evidence of transduction was obtained in any of these experiments.

Host-range

Of 36 wild-type *Streptomyces* spp. tested, plaques were formed initially on 14 with quite high efficiency, on four with much lower efficiency and were not detected with 18 (Tables 1 and 2). No plaques were seen with *Nocardia mediterranei* (ATCC 13685) or *Corynebacterium glutamicum* (GAB 18000).

Table 1. *Interaction of unmodified wild-type R4 phage with sensitive Streptomyces strains*

Efficiency of plating (e.o.p.) on putative lysogens is given in relation to that on the non-lysogenic parent strain. The column 'p.f.u./c.f.u.' gives the ratio of plaque numbers with the non-lysogen as indicator to the number of colony forming units on CM. R⁻ and R⁺ refer to restriction deficiency and proficiency, respectively.

Strain	R4 e.o.p.	Plaque morphology	Ease of isolation of lysogens from plaques	Test of putative lysogens	
				R4 e.o.p.	p.f.u./c.f.u.
<i>S. albus</i> G R ⁻ J1074	1 × 10 ⁰	Large	Easy	< 2 × 10 ⁻³	1 × 10 ⁻²
<i>S. albus</i> G R ⁺	> 1 × 10 ⁻⁸	—	—	—	—
<i>S. albus</i> P CMI 52766	1 × 10 ⁻⁵	Large	NT	—	—
<i>S. antibioticus</i> CUB 471	1 × 10 ⁻³	—	—	—	—
<i>S. azureus</i> ATCC 14921	1 × 10 ⁰	Small	NT	—	—
<i>S. coelicolor</i> A3(2)	1 × 10 ⁻²	Small	None detected	—	—
<i>S. coelicolor</i> (Müller)	2 × 10 ⁻⁷	Medium	NT	—	—
<i>S. erythreus</i> CUB 92	9 × 10 ⁻¹	Small	Each plaque mixed	< 3 × 10 ⁻⁴	1.5 × 10 ⁻²
<i>S. griseus</i> CUB 294	8 × 10 ⁻¹	Medium	Easy	< 3 × 10 ⁻⁴	5 × 10 ⁻²
<i>S. griseus</i> CUB 625	6 × 10 ⁻¹	Small	Each plaque mixed	< 1 × 10 ⁻²	4 × 10 ⁻³
' <i>S. hygroscopicus</i> ' IMET 20664	1 × 10 ⁻¹	Small	None detected	—	—
<i>S. lividans</i> 66 (Lomovskaya)	1 × 10 ⁰	Large	Very rare	< 1 × 10 ⁻⁸	5 × 10 ⁻⁶
<i>S. parvulus</i> ATCC 12434	1 × 10 ⁰	Large	Each plaque mixed	< 2 × 10 ⁻⁴	1 × 10 ⁻³
<i>S. rimosus</i> NRRL 2234	1 × 10 ⁻¹	Small	None detected	—	—
<i>S. rimosus</i> 2251	1 × 10 ⁻¹	Small	Easy	< 1 × 10 ⁻²	5 × 10 ⁻³
<i>S. venezuelae</i> CUB 104	6 × 10 ⁻¹	Medium	Easy	< 2 × 10 ⁻⁴	4 × 10 ⁻³
<i>S. viridochromogenes</i> CUB 539	4 × 10 ⁻¹	Large	Each plaque mixed	NT	1 × 10 ⁻²
<i>S. viridochromogenes</i> CUB 608	1 × 10 ⁻³	Small	NT	—	—
<i>S. virginiae</i> ATCC 13161	1 × 10 ⁰	Small	Very rare	< 1 × 10 ⁻⁸	< 1 × 10 ⁻⁸
Unidentified 1158	1 × 10 ⁻¹	Small	NT	—	—

NT, Not tested.

Most of the strains giving high e.o.p. could be lysogenized by R4, although the ease with which lysogens were obtained varied greatly (Table 1). We never were able to obtain lysogens of *S. coelicolor* A3(2): indeed, some derivatives of *S. coelicolor* A3(2) were very poor hosts for R4. This was independent of the status of the derivatives with respect to plasmids SCP1 and SCP2 (Hopwood *et al.*, 1973; Bibb *et al.*, 1977). We were unable reliably to score this difference in susceptibility by replica-plating, so that attempts to locate genes controlling it on the *S. coelicolor* linkage map failed.

A remarkable feature of the host-range of R4 is its independence of the host's taxonomic status (Table 2). Interestingly, however, among strains showing initial high sensitivity to R4, 0/6 tested had detectable class II restriction enzyme activity in cell-free extracts, whereas among those initially resistant to R4, 9/12 tested showed class II restriction enzyme activity in extracts. In the case of *S. albus* G, initial resistance to R4 was certainly due to restriction since R4 had a high e.o.p. on restriction-deficient mutants (e.g. J1074). Further work on the interaction of R4 with *S. albus* G is discussed in the following section.

The possible involvement of host-controlled restriction-modification systems in the low e.o.p. of R4 on *S. coelicolor* (Müller) and *S. albus* P was also examined. Phages soaked out from the rare plaques on each host had acquired high e.o.p. on the respective strains. This was retained after subsequent propagation on strain J1074, suggesting that either (i) host-range mutants, perhaps with changed adsorption characteristics, had been selected or (ii) the DNA of the phages had originally possessed a single target for restriction by the respective hosts, and mutants lacking this target were being selected. In the case of *S. albus* P the latter hypothesis proved true (Chater & Carter, 1978), the high apparent level of

Table 2. *Sensitivity of various Streptomyces strains to R4*

The strains are arranged according to their taxonomic status (after Arai *et al.*, 1976). Strains on which R4 or its mutant derivatives can form plaques, whatever the circumstances, are considered sensitive (see Table 1 and text for further details).

Group	Sensitive to R4	Resistant to R4
Rectiflexibiles, Yellow	<i>S. coelicolor</i> (Müller) <i>S. griseus</i> CUB 294 <i>S. griseus</i> CUB 625	<i>S. coelicolor</i> (Müller) UC5240 <i>S. griseus</i> CUB 609 * <i>S. griseus</i> ATCC 23345 <i>S. griseus</i> ATCC 10137
Rectiflexibiles, Grey	<i>S. antibioticus</i> CUB 471	* <i>S. achromogenes</i> ATCC 12767
Rectiflexibiles, Red/violet	† <i>S. venezuelae</i> CUB 104	
Retinaculiaperti, Red/violet	<i>S. virginiae</i> ATCC 13161	† <i>S. fradiae</i> CUB 305
Spirales, White	* <i>S. albus</i> G * <i>S. albus</i> P CMI 52766 † <i>S. rimosus</i> 2251 † <i>S. rimosus</i> NRRL 2234	* <i>S. albus</i> A138 (Lacey) * <i>S. albus</i> A343 (Lacey) * <i>S. albus</i> A505 (Lacey) † <i>S. albus</i> A647 (Lacey) † <i>S. rimosus</i> ATCC 14827 † <i>S. rimosus</i> 2249 † <i>S. rimosus</i> 2252
Spirales, Yellow		* <i>S. varsoviensis</i> CUB 308
Spirales, Grey	† <i>S. coelicolor</i> A3(2) * <i>S. hygroscopicus</i> IMET 20664 <i>S. lividans</i> 66 † <i>S. parvulus</i> ATCC 12434	<i>S. matensis</i> ISP 5188 <i>S. violaceoruber</i> S199 (Bradley) <i>S. violaceoruber</i> K673 (Kutzner)
Spirales, Blue/green	<i>S. azureus</i> ATCC 14921 <i>S. viridochromogenes</i> CUB 539 <i>S. viridochromogenes</i> CUB 608	<i>S. viridochromogenes</i> 93 (Lechevalier)
Spirales, Red/violet	† <i>S. erythreus</i> CUB 92	

* These strains contain site-specific endodeoxyribonucleases (Chater, 1978*a*; E. Clare & K. F. Chater, unpublished data; Arrand *et al.*, 1978; J. R. Arrand, P. A. Myers & R. J. Roberts, personal communication).

† These strains lacked detectable site-specific endodeoxyribonucleases (in single extractions using coliphage λ DNA as substrate; K. F. Chater & E. Clare, unpublished data).

restriction being accounted for by undermodification of the progeny DNA molecules in cells where the initial infection had escaped restriction. However, for *S. coelicolor* (Müller) we have not been able to demonstrate the involvement of restriction, since a cell-free extract contained no detectable restriction endonuclease activity on λ DNA; an R4 mutant (R4G2) with increased *in vivo* susceptibility to modification in the *S. albus* P and *S. albus* G systems (see below, and Chater & Carter, 1978) did not show increased e.o.p. on *S. coelicolor* (Müller) compared with wild-type R4 phage; and an R4 mutant with high e.o.p. on *S. coelicolor* (Müller) was unaffected in its responses to restriction by *S. albus* G or *S. albus* P (data not shown). Possibly the adaptation of R4 to *S. coelicolor* (Müller) is due to a simple kind of host-range mutation affecting adsorption efficiency.

Restriction of R4 by S. albus G

Although R4 formed plaques efficiently on J1074, it normally gave no plaques at all on wild-type *S. albus* G, the restriction-proficient strain from which J1074 was derived. R4 was then propagated on an independent restrictionless mutant (*sal-40*) of *S. albus* G which (unlike J1074) retained modification activity (Chater & Wilde, 1980) in the expectation that modified phage would be obtained, which should then have a high e.o.p. on wild-type *S. albus* G. Instead, an e.o.p. of about 10^{-6} was obtained. Phages from five such plaques

Table 3. Restriction-modification of R4 and its derivatives by *S. albus* G strains

E.o.p. values on *S. albus* G were determined after pregrowth of the various R4 derivatives on *S. albus* G wild-type (restriction and modification proficient, i.e. R⁺M⁺), J1074 (*S. albus* G R⁻M⁻) or *sal-40* (*S. albus* G R⁻M⁺; Chater & Wilde, 1980). R4G1 to R4G5 were obtained independently from single plaques on *S. albus* G (R⁺M⁺) of R4 last grown on *sal-40* (see text).

R4 derivative used	*E.o.p. on <i>S. albus</i> G		
	Last host J1074 (R ⁻ M ⁻)	Last host <i>sal-40</i> (R ⁻ M ⁺)	Last host <i>S. albus</i> G (R ⁺ M ⁺)
R4	< 1 × 10 ⁻⁹	ca 1 × 10 ⁻⁶	—
R4G1	1 × 10 ⁻⁵	2 × 10 ⁻²	1 × 10 ⁻¹
R4G2	1 × 10 ⁻⁵	5 × 10 ⁻¹	7 × 10 ⁻¹
R4G3	7 × 10 ⁻⁵	NT	1 × 10 ⁰
R4G4	8 × 10 ⁻⁶	NT	6 × 10 ⁻¹
R4G5	5 × 10 ⁻⁵	NT	1 × 10 ⁰

NT, Not tested.

* Assuming an e.o.p. of 10⁰ on J1074.

obtained independently on *S. albus* G were found to give high e.o.p. on both *S. albus* G and its restrictionless derivatives. The subsequent e.o.p. of two of these phages was tested on *S. albus* G, and depended upon the modification status of their last host (Table 3). Thus, high e.o.p. on *S. albus* G was always obtained after propagation of the phages on modification-proficient strains, but after their propagation on a modification-defective *S. albus* G mutant (e.g. J1074) the e.o.p. on *S. albus* G fell to about 10⁻⁵. We concluded that these phage derivatives were mutants (which we termed R4G) affected in their *SalGI* restriction-modification sensitivity.

In vitro cleavage of R4 and R4G2 DNA by *SalGI*

Since the restriction-deficient mutant J1074 lacks the endonuclease *SalGI* present in the restriction-proficient parent strain (Chater & Wilde, 1976), we concluded that *SalGI* was the agent of R4G restriction in *S. albus* G. In confirmation of this, DNA isolated from R4G2 grown on *S. albus* G (and therefore modified) was not cleaved by *SalGI* *in vitro*, whereas that isolated from either R4 or R4G2 grown on a non-modifying host was cleaved into more than 30 fragments (Fig. 5). No differences in the R4 or R4G2 cleavage patterns were detected on agarose gels, though it was not excluded that there might be differences among the lower molecular weight fragments. Microdensitometry of a number of gels showed that the band of highest molecular weight (3.4 × 10⁶) was present in variable, but always less than equimolar, amounts and that the brighter this band, the fainter were two other bands (1.97 × 10⁶ and 1.45 × 10⁶). This may indicate that the two smaller bands are the ends of the linear phage DNA molecule and that these [as in coliphage λ and 886 DNA (Kaiser & Wu, 1968) and *Streptomyces* phage φC31 DNA (Sladkova *et al.*, 1977)] have complementary single-stranded extensions allowing hydrogen bonding between two fragments. This is assumed to be the case in the summing of all the fragment molecular weights to obtain an estimated molecular weight for R4 DNA (30.02 × 10⁶; Fig. 5). The mean frequency of *SalGI* sites in this DNA is about one per 1.35 kilobases. A similar high frequency of *SalGI* target sites was seen for DNA of the virulent phage VP12 (Chater & Wilde, 1980) and, like VP12 DNA, R4 DNA had a high (67 mol %) content of guanosine plus cytosine, based on its buoyant density in CsCl density gradients (Fig. 6).

Fig. 5. Cleavage of DNA from various R4 preparations by *SalGI*. About $1 \mu\text{g}$ DNA prepared from R4 propagated on a non-modifying host (tracks B and E) or from R4G2 propagated on a modifying host (track A) or a non-modifying host (track D; less DNA used) was incubated with *SalGI*, electrophoresed in 1.4% (w/v) agarose, stained with ethidium bromide, and photographed under irradiation at 365 nm. Electrophoresis was from top to bottom. Molecular weights ($\times 10^{-6}$) were estimated by comparison with standards (track C) obtained by digestion of λ DNA with *SalPI* (Chater, 1977). Relative molar amounts of fragments in each band were estimated by microdensitometry (lower figures). In calculating the total molecular weight, the fragment of 3.4×10^6 molecular weight is excluded (see Results). The microdensitometer tracings shown are of tracks D and E. Molecular weights ($\times 10^{-6}$) are given for some peaks on the tracings in order to facilitate cross-reference with the gels. The peak marked X is an artifact caused by a scratch on the negative.

Fig. 6. CsCl density gradient centrifugation of DNA from R4G2. The relative position in a CsCl density gradient (at equilibrium) of DNA of R4G2 and two standards (DNA of phage λ and of *S. albus* G) was recorded by photographing under ultraviolet light (260 nm) during centrifugation on a Spinco Model E centrifuge.

Fig. 7. Agarose gel electrophoresis of R4G2 DNA cleaved by *SalPI* and *EcoRI*. Molecular weights ($\times 10^{-6}$) of standards are given. Electrophoresis was from left to right.

Cleavage of R4 DNA by other restriction enzymes

R4 DNA was not cleaved at all by *Bam*HI or *Hind*III but was cleaved at single sites by *Eco*RI (4.5 kilobases from one end of the molecule) and *Sal*PI (12.75 kilobases from one end of the molecule) (*Sal*PI is an isoschizomer of *Pst*I; Chater, 1977). Figure 7 shows that the two enzymes cut at opposite ends of the molecule, since the 12.75 kilobase *Sal*PI fragment is not reduced in size after cleavage with *Eco*RI.

Restriction of R4G mutants by two *S. rimosus* strains

Unmodified R4 (i.e. last grown on J1074) gave quite high e.o.p. on two *S. rimosus* strains (2251 and NRRL 2234; Table 1). In the case of 2251 (NRRL 2234 was not tested), this was not changed by *passage* through the *S. rimosus* strains, suggesting that restriction and modification of R4 did not take place (Table 4). Surprisingly, R4G2 phage had a 10-fold lower e.o.p. on both strains, and on further examination this proved to depend upon the last host; for example, prior growth on 2251 gave a relatively high e.o.p. on 2251, but subsequent *passage* through J1074 resulted in a return to the lower e.o.p. on 2251. Similar results were obtained with NRRL 2234 (data not shown). Thus the R4G2 mutant, but not its parental phage R4, was subject to host-controlled restriction and modification in the two *S. rimosus* strains. Identical specificity of the two systems was established by finding that when 2251 was the last host a relatively high e.o.p. was obtained on NRRL 2234, and vice versa (Table 5). Further studies were therefore confined to 2251.

Table 4. *Restriction-modification of R4G2 by S. rimosus 2251*

Previous hosts (in sequence)	*E.o.p. on 2251	
	R4	R4G2
J1074	3×10^{-1}	2×10^{-2}
J1074, 2251	3×10^{-1}	2×10^{-1}
J1074, 2251, J1074	3×10^{-1}	2×10^{-2}

* Assuming an e.o.p. of 10^0 on J1074.

Table 5. *Shared specificity in the restriction-modification systems of S. rimosus 2251 and S. rimosus NRRL 2234*

Previous hosts (in sequence) of R4G2	*E.o.p. on NRRL 2234	*E.o.p. on 2251
NRRL 2234, J1074	3×10^{-2}	4×10^{-2}
NRRL 2234, NRRL 2234	3×10^{-1}	3×10^{-1}
2251, J1074	3×10^{-2}	4×10^{-2}
2251, 2251	3×10^{-1}	3×10^{-1}

* Assuming an e.o.p. of 10^0 on J1074.

The e.o.p. on 2251 of the four other R4G mutants mentioned earlier was also tested, and in all cases it was 10- to 100-fold lower than was obtained with R4. Two of the phages (R4G1 and R4G4) were examined further. In the case of R4G4, which had the lowest initial e.o.p. (1×10^{-3}) on 2251, subsequent behaviour was identical to that described for R4G2. In the case of R4G1, however, a plaque taken from the first plating on 2251 subsequently proved insensitive to 2251, like the parental phage R4. Since the other R4G characteristics (restriction-modification characteristics in the *S. albus* G and *S. albus* P systems; Table 5 and Chater & Carter, 1978) remained unchanged in this derivative of R4G1 (i.e. it had not reverted from R4G to R4), the simplest (but untested) explanation would be that a mutant lacking any target site for the 2251 restriction system had been selected.

Despite the anomalous behaviour of R4G1, it therefore appears that mutations of R4 leading to the R4G phenotype invariably confer sensitivity to a restriction-modification system present in two *S. rimosus* strains. However, attempts to find type II restriction enzyme activity in cell-free extracts of both strains failed, using either λ DNA or unmodified R4G2 DNA (from phage grown on J1074) as the substrate.

DISCUSSION

Four groups of heteroimmune temperate bacteriophages attacking *Streptomyces coelicolor* A3(2) have been discovered and they share certain features. VP5, S14 and R4 are fairly similar to lambda phage in morphology and dimensions (Fig. 2; Dowding & Hopwood, 1973; C. Stutter, unpublished data) and all are similar in the molecular weights of their DNA (Chater, 1978*b*). Their one-step growth curves are almost superimposable. However, R4 differs from the other groups of phages in several ways. It has unusually high divalent cation requirements for optimal adsorption; it is unable to form plaques above 34.5 °C; clear-plaque mutants are very rare; and, most importantly, it forms plaques on a wide range of *Streptomyces* hosts, and lysogenizes many of them (though it does not appear to lysogenize *S. coelicolor*).

Thanks to its wide host-range, R4 is proving useful in studies of several *Streptomyces* restriction-modification systems. We have previously described its sensitivity to the SalPI system of *S. albus* P (Chater & Carter, 1978) and here we have shown that it is also subject

to restriction-modification systems operating in *S. albus* G (the *Sal*GI system; Chater & Wilde, 1976) and in two *S. rimosus* strains. In none of the cases was restriction initially obvious. For the two *S. albus* strains, modification of phages which had evaded the initial restriction was apparently so inefficient that plaques were formed only by phage mutants. In the case of *S. albus* P these were invariably mutants (R4P) lacking the single *Sal*/PI target site (Chater & Carter, 1978) whereas in the case of *S. albus* G the mutants (R4G) were apparently altered in their ability to be modified, for reasons that are not understood. With many R4G mutants, modification is also more efficient in the *Sal*/PI system, and these mutants reveal 'classical' patterns of restriction-modification in the two systems (Table 3; Chater & Carter, 1978). R4G also reveals restriction-modification systems of shared specificity in two *S. rimosus* strains, but here the wild-type R4 phage, instead of being more sensitive to restriction than R4G, is unrestricted. This might be due to the presence of a restriction site in R4G DNA which is absent from R4 DNA (if, for example, R4G mutants contained inserted DNA absent from the parental phage; however, no differences in restriction enzyme cleavage patterns have been noted between R4G2 DNA and R4 DNA). Another testable possibility is that R4 might specify a non-site-specific modification system that alters *Sal*GI and *Sal*/PI target sites in such a way that the *Sal*GI and *Sal*/PI modification enzymes are prevented from effectively carrying out the specific modification needed for protection against the respective restriction enzymes. This same non-specific system might protect R4 DNA against the *S. rimosus* restriction enzyme. Thus the loss of non-specific modification would result in the acquisition of sensitivity to the *S. rimosus* system.

Further study of these interesting interactions between R4 and various restriction-modification systems may lead both to insights into the biological roles of restriction-modification and to a better understanding of factors affecting host-range in bacteriophages.

Recent work has led to real hopes that plasmid vectors for the cloning of DNA fragments into *Streptomyces* will soon be available. For various purposes, bacteriophage vectors may also be of value. R4 appears to offer certain advantages here: it is temperate; it has a wide host-range; its DNA can quite easily be obtained in reasonable quantity; and it has single target sites for two restriction enzymes, *Eco*RI and *Pst*I ($\equiv$ *Sal*/PI), widely used in DNA cloning in other bacteria. A critical requirement for this use of R4 is the development of a transfection system. The polyethylene glycol-dependent system used by Bibb *et al.* (1978) to transform *Streptomyces* protoplasts with SCP2* plasmid DNA has also proved effective in transfection with VP5 DNA (Chater, 1978*b*), and has recently been extended to several other phages including R4 (J. E. Suarez, unpublished results).

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