Characterization of SE-3, a Virulent Bacteriophage of *Saccharopolyspora erythraea*

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SE-3 is a virulent bacteriophage isolated from a large-scale culture of *Saccharopolyspora erythraea*, an erythromycin producer. The host range of the phage is narrow, limited to some strains of this species. Another strain of *Sac. erythraea*, and a strain of *Sac. hirsuta*, are able to adsorb phage particles but do not sustain their complete multiplication. SE-3 is closely related to the phage SE-5 as shown by DNA restriction mapping. The differences between SE-3 and SE-5 genomes are apparently limited to two DNA segments flanked by short inverted repeats, visualized by electron microscopy.

INTRODUCTION

The actinomycete *Saccharopolyspora erythraea* (formerly *Streptomyces erythraeus*) is the producer of erythromycin, a clinically important antibiotic. The phage SE-3 was isolated together with four other phages (SE-1 to SE-5) from large-scale cultures of this organism (Ostrowska-Krysiak *et al.*, 1971; Ostrowska-Krysiak, 1974). By their morphological structure, biological and serological properties, the phages were shown to be related among themselves and to the phage 121 isolated and described earlier by Retinskaya & Rautenstein (1960).

Phages 121 and SE-5 have a narrow host range, limited to *Sac. erythraea* strains, show intense cross-hybridization between their DNA and have similar restriction maps (Brzeziński *et al.*, 1986). This 'phage family' is a good model for studies of DNA rearrangements in phage genomes. Here we describe another phage from this group, SE-3, and show that it is closely related to SE-5.

METHODS

Strains. Phages SE-3 and SE-5, as well as the strains ISP 5517 and 64-575, were provided by Drs B. Ostrowska-Krysiak and A. Blońska (Institute of the Pharmaceutical Industry, Warsaw, Poland). Strains NRRL 2359, NRRL 2360, NRRL B-5616 and NRRL B-5792 were obtained from Dr A. J. Lyons (US Department of Agriculture, Northern Utilization Research and Development Division, Peoria, Ill., USA). Phage ϕ SE-6 and ϕ SE-60 DNAs were obtained from Dr C. R. Hutchinson (University of Wisconsin, Madison, Wis., USA).

Media and culture conditions; DNA extraction and analysis. The techniques used were described previously (Brzeziński *et al.*, 1986). The G + C content of DNA was estimated by determination of the thermal denaturation temperature (Mandel & Marmur, 1968) and by UV spectrophotometry (Ulitzur, 1972).

Adsorption. The adsorption rates were determined by free phage titration (Adams, 1959). Spores (10^8 c.f.u.) were suspended in 1 ml fresh liquid medium (Brzeziński *et al.*, 1986) supplemented with 1 mM-NaCl and 1 mM-Ca(NO₃)₂, briefly (10 min) heat-shocked at 55 °C and incubated at 30 °C for 6 h for germination. Then, such

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suspensions were mixed with 10^7 SE-3 p.f.u. and kept at 30 °C with gentle shaking. Samples were withdrawn every 30 min, centrifuged at 12000 r.p.m. and free phage was titrated on NRRL 2360 as indicator strain.

Electron microscopy. DNA heteroduplexes of purified SE-3 and SE-5 phages were prepared according to Davis *et al.* (1971), using a formamide concentration of 60% (v/v) and a renaturation time of 90 min, at room temperature. Photographs were taken with a Philips EM 300 electron microscope at a 12500-fold enlargement.

RESULTS

Plaque morphology and host range

Phage SE-3 formed large (6–8 mm diameter) clear plaques on many different media after incubation (48 h, 30 °C) with either spores or mycelium of *Sac. erythraea* used as indicator cells. A zone of secondary growth, invading the plaque from the circumference, was seen after longer incubation (3–7 d). This zone seemed to be formed by old mycelium, refractory to phage adsorption or lysis, rather than by lysogenized or resistant cells, as material taken from these areas could support phage plaque formation after liquid culture. We therefore consider SE-3 as a virulent phage.

We have determined the host range of SE-3 using five strains sent to us under the name of '*Streptomyces erythraeus*', one strain of *Saccharopolyspora hirsuta* and 19 other *Streptomyces* strains. Recently, Labeda (1987) has reclassified *Streptomyces erythraeus* as *Saccharopolyspora erythraea*, and this new name is used here. However, only the strain NRRL 2338 (the new type strain) was described in Labeda's article. Among other commonly used strains cited as '*Streptomyces erythraeus*' (Seno & Hutchinson, 1986), we determined, by restriction enzyme analyses of total DNA (data not shown), that strains ISP 5517, NRRL 2359 and NRRL 2360 should also be classified as *Sac. erythraea*, as well as strain 64-575 from the Warsaw collection. In contrast, strain NRRL B-5616 gave a different DNA digestion pattern and, according to Labeda (1987) should be considered as a different species, designated *Streptomyces labedae* (Lacey, 1987).

The host range of SE-3 seems to be limited to certain *Sac. erythraea* strains. Among these, the highest plating efficiency was observed on strain 64-575 (Ostrowska-Krysiak *et al.*, 1971). Plating efficiencies on the other strains, such as ISP 5517, NRRL 2360 and NRRL 2338, were between 0.8 and 0.9. No plaques were formed on strain NRRL 2359; however, a 'clearing effect' (Prauser, 1984) was observed. The same results were also observed with *Sac. hirsuta* NRRL B-5792. No lysis or clearing effects were observed (after plating up to 10^9 p.f.u. per plate) on *Str. labedae* NRRL B-5616, or on the other 19 *Streptomyces* species.

Strains giving the clearing effect (NRRL 2359 and B-5792) adsorbed phage SE-3 with kinetics similar to NRRL 2360, which is a good host for this phage (Fig. 1). The calculated adsorption

Fig. 1. Free phage titration during infection of different hosts by SE-3. N_0 , phage titre before adsorption; N , free phage titre in each sample. Phage was added, as described in Methods, to germinated spores of: NRRL 2359 (▲), NRRL 2360 (◆), NRRL B-5616 (■) and NRRL B-5792 (●).

rates were rather low ($0.85\text{--}1.5 \times 10^{-9}$ ml min $^{-1}$) but similar to values obtained for other actinophage-host systems (Dowding, 1973). Under the same conditions, SE-3 was unable to adsorb to germinated spores of the *Str. labedae* strain NRRL B-5616. Viable phage progeny became noticeable after 3 h incubation with NRRL 2360 host cells (Fig. 1). As in plate assays, no viable progeny were detected with either NRRL 2359 or NRRL B-5792 hosts, even after 6 h incubation.

DNA studies

Estimation of the G+C content. The G+C content was evaluated from the thermal denaturation temperature as 58 mol% (assuming that there is not extensive base modification). A slightly higher value (60 mol%) was obtained by the spectrophotometric method. The G+C content is very similar to that of 121 (59–62.5 mol%) and SE-5 (57.5–59.5 mol%) phage DNAs (Brzeziński *et al.*, 1986) and quite different from that of host DNA (70 mol%, Tewfik & Bradley, 1967).

Restriction enzyme mapping. A detailed restriction map is presented for five enzymes in Fig. 2. The map was deduced from double and partial DNA digestions, except for fragments *Bg*/II-D, E and F which, being similar in size, could not be positioned by these means. We therefore subcloned the *Pvu*II-A fragment in the *Sma*I site of the pUC18 vector (Yanish-Perron *et al.*, 1985). Mapping the *Bg*/II sites in this subclone showed that fragment *Bg*/II-F is excluded from it and is indeed adjacent to fragment B and that fragment *Bg*/II-D is separated by fragments I and C from the fragment E. This gave the arrangement shown in Fig. 2.

The total length of SE-3 DNA is 43.9 kb. The linear genome is flanked by cohesive ends, as shown by the disappearance of some larger bands in restriction digests after heating (5 min, 80 °C) and quick cooling of the DNA samples (data not presented). SE-3 DNA was not cut by *Bam*HI, *Xho*I, *Sph*I, *Aha*III, *Nco*I or *Sac*I.

Comparison with SE-5 and other phages. The cleavage maps of SE-3 and SE-5 DNAs appear identical except for the presence of an extra 1.7 kb of DNA, containing a *Bg*/II site, near the centre of the SE-3 map (Fig. 2). In order to obtain more precise data about the DNA rearrangements that may have occurred in this portion of the genome, the *Bg*/II-C and E fragments of SE-3, and the *Bg*/II-C fragment of SE-5 DNA, were subcloned in the *Bam*HI site of pUC18 and more detailed restriction maps were established (Fig. 3). The additional DNA in SE-3 compared with SE-5 is located in two segments of 1.2 kb and 0.5 kb respectively, separated by a common small segment of 0.3 kb.

Fig. 2. DNA restriction maps of phages SE-3 and SE-5. The SE-5 map is redrawn from Brzeziński *et al.* (1986). Hatched bars indicate major areas of hybridization between SE-3 and ϕ SE60 DNA.

Fig. 3. DNA restriction maps of the central areas of SE-3 and SE-5 genomes. ★, *AatII*; □, *AvaI*; ▲, *BamI*; ◇, *BglI*; *, *ClaI*; ■, *EcoRI*; ▽, *HaeII*; ○, *HindII*; ▼, *HindIII*; ●, *KpnI*; ◆, *PvuI*.

Fig. 4. Electron microphotograph of loop-and-stem structures observed in an SE-3/SE-5 DNA heteroduplex. Bar, 100 nm.

Heteroduplex analysis by electron microscopy confirmed these findings. The two phage DNAs hybridized along their whole length (not shown), except for two single-strand loops (Fig. 4), as predicted by restriction mapping. These single-stranded segments form loop-and-stem structures, indicative of inverted-repeat DNA sequences. These repeats were estimated to be 25 ± 5 bp long for the bigger loop and 45 ± 5 bp long for the smaller loop (mean of six measurements, $\pm$ SD).

Restriction fragments of SE-3 DNA were blotted and probed with ϕ SE6 and ϕ SE60 DNAs (Grund & Hutchinson, 1987). No homology was detected with the ϕ SE6 DNA probe, while some SE-3 segments hybridized with ϕ SE60 DNA (Fig. 2). This showed that ϕ SE60 was also in some way related to SE-3 (as well as phages 121 and SE-5).

DISCUSSION

Phage SE-3, with some other phages described previously (Retinskaya & Rautenstein, 1960; Ostrowska-Krysiak *et al.*, 1971; Ostrowska-Krysiak, 1974; Donadio *et al.*, 1986; Grund &

Hutchinson, 1987), is highly specific for the erythromycin producer *Sac. erythraea*. The adsorption properties of this phage seem to confirm, although indirectly, the wisdom of transferring these strains from the genus *Streptomyces* to the genus *Saccharopolyspora* (Labeda, 1987). In fact, phage SE-3 and/or phages related to it were unable to adsorb on all the *Streptomyces* strains examined to date (this work; Brzeziński *et al.*, 1986), while they were able to adsorb efficiently on germinated spores of *Sac. hirsuta* NRRL B-5792. This species is not very similar to *Sac. erythraea* but it has the same type of cell wall and the same phospholipid pattern (Labeda, 1987), which in many cases is necessary, although not always sufficient, for successful phage adsorption (Prauser, 1984). However, the phage ϕ SE60, related to SE-3, SE-5 and 121, is unable to form plaques on NRRL B-5792, while other phages of *Sac. erythraea*, such as ϕ SE6, ϕ SE45, ϕ SE57 and ϕ SE69, can develop on this strain (Grund & Hutchinson, 1987).

The inability of the *Sac. erythraea* strain NRRL 2359 to support phage SE-3 multiplication cannot yet be fully explained. The observed phenomenon is best described as 'lysis from without': efficient phage adsorption; no plaque formation and severe host cell mortality (clearing effect) at high m.o.i. However, contribution of other mechanisms, such as restriction, cannot be excluded. It should be noted that the phages G3, G4 and G5 described by Donadio *et al.* (1986) were also unable to infect this strain. In contrast, phage ϕ SE-6 (Grund & Hutchinson, 1987) was able to form plaques on this strain.

The physical structure of the SE-3 genome is very similar to that of phage SE-5. The differences are apparently limited to two DNA segments (deletions or insertions) flanked by short inverted repeats. The occurrence of some other differences, such as point mutations, or short insertions or deletions, could not, of course, be ruled out. These differences are not purely structural, but have important biological implications: phage SE-5 consistently gave 5–10-fold higher titres than SE-3 in large-scale amplification, either on Petri plates or in liquid cultures (data not shown), so we consider SE-5 as a more virulent derivative of SE-3. Furthermore, a host mutant strain, lysed by SE-5 but resistant to SE-3, has been described (Gurkau & Ostrowska-Krysiak, 1972; Ostrowska-Krysiak, 1974). This mutant is thought to be damaged in the phage-receptor systems since other related phages (SE-1 and V-II) were unable to adsorb on its cells.

The loop-and-stem DNA sequences of SE-3, visualized by heteroduplex analysis, are similar to other such 'transposon-like' structures present in actinophage (Sladkova *et al.*, 1980; Klaus *et al.*, 1982) or plasmid DNA (Pel'ts *et al.*, 1983; Klaus *et al.*, 1984). The sequence discovered in phage ϕ C43 DNA (Sladkova *et al.*, 1980) was able to transpose between phage and host genomes (Lomovskaya *et al.*, 1986). An insertion sequence, IS110, was detected in phage ϕ C31 derivatives after transposition from *S. coelicolor* A3(2) genomes (Chater *et al.*, 1985; Bruton & Chater, 1987).

The pair of phages, SE-3 and SE-5, can be considered as a starting and final point of recombinational events involving inverted-repeat sequences; therefore they furnish a model for further studies of such mechanisms as a facet of the more general problem of genetic instability in actinomycetes.

Recent experiments have extended the host range of phages 121, SE-3 and SE-5 to the thermophilic actinomycete *Faenia rectivirgula*, which is able to propagate them at temperatures not exceeding 43 °C (this laboratory and J. Schneider, Darmstadt, personal communication).

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