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Characterization of a Unique Methyl-Specific Restriction System in *Streptomyces avermitilis*

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Streptomyces avermitilis contains a unique restriction system that restricts plasmid DNA containing _N_^{6}-methyladenine or 5-methylcytosine. Shuttle vectors isolated from _Escherichia coli_ RR1 or plasmids isolated from modification-proficient _Streptomyces_ spp. cannot be directly introduced into _S. avermitilis_. This restriction barrier can be overcome by first transferring plasmids into _Streptomyces lividans_ or a modification-deficient _E. coli_ strain and then into _S. avermitilis_. The transformation frequency was reduced >1,000-fold when plasmid DNA was modified by _dam_ or _Taq_I methylases to contain _N_^{6}-methyladenine or by _Alu_I, _Hha_I, or _Hph_I methylases to contain 5-methylcytosine. Methyl-specific restriction appears to be common in _Streptomyces_ spp., since either _N_^{6}-methyladenine-specific or 5-methylcytosine-specific restriction was observed in seven of nine strains tested.

Streptomyces avermitilis produces avermectins, commercially important macrolide secondary metabolites with potent anthelmintic activities (4). Avermectins are active against almost all arthropod ectoparasites (10) and are effective in controlling numerous agricultural pests (30). Procedures to study the biosynthesis of secondary metabolites by using cloning vectors and recombinant DNA techniques have been developed for several *Streptomyces* species (for examples, see references 16, 18, and 33). However, certain procedures can be performed only with *Escherichia coli* (i.e., the use of lambda vectors, cosmids, M13 sequencing vectors, transposon mutagenesis, and regulated expression vectors). To take advantage of the procedures available with *E. coli*, *Streptomyces*-*E. coli* shuttle vectors have been made (20, 21, 29, 37, 38). Cloning systems developed for *S. avermitilis* include vectors derived from phage TG1 (12) and plasmid pVE1 (24) and an efficient transformation procedure (23). Unfortunately, *Streptomyces*-*E. coli* shuttle vectors cannot be introduced directly into *S. avermitilis* because *S. avermitilis* restricts the entry of DNA isolated from *E. coli*. This restriction barrier could also pose a problem in any attempt to produce hybrid antibiotics in *S. avermitilis* (15).

Restriction-modification systems are widespread in *Streptomyces* spp. (1, 6, 8, 17, 26, 35). Most restriction-modification systems are composed of a methylase and an endonuclease. The modification enzyme (methylase) modifies the host DNA at a specific sequence composed of four or more bases, and the restriction endonuclease cleaves unmodified foreign DNA at or near the specific sequence (for a review, see reference 17). More than 600 restriction endonucleases and 98 methylases are known (17). Three methyl-specific restriction systems have been described elsewhere (19, 31, 36). In strains with methyl-specific restriction systems, foreign methyl-modified DNA is restricted and the host does not modify DNA. *Diplococcus pneumoniae* restricts DNA containing *N*^{6}-methyladenine at the sequence GATC (19). Two other strains restrict DNA containing 5-methylcytosine, but these strains show little or no sequence specificity (31, 36). In this report, the methyl-specific restriction system of *S. avermitilis* is characterized.

(Part of this work has been presented previously [D. MacNeil, Abstr. Annu. Meet. Am. Soc. Microbiol. 1988, H71, p. 78].)

MATERIALS AND METHODS

Strains and plasmids. The sources for the strains and plasmids used in this paper are listed in Table 1. Plasmids used included two *amp* (ampicillin-resistant) *tsr* (thiostrepton-resistant) *Streptomyces*-*E. coli* shuttle vectors; pVE3 (24), a 15.4-kilobase-pair (kb) pVE1-pBR322 cointegrate formed by ligation at the unique *Eco*RI site in each; and pVE328 (to be described elsewhere), a 7.5-kb plasmid derived from pBR322 and pVE1. Other plasmids used during transformation experiments included pVE28, a 4.8-kb *tsr* derivative of pVE1 (24); pVE614, a 4.5-kb *tsr* derivative of pVE28; and pVE203, a 4.5-kb *neo* (neomycin-resistant) derivative of pVE1.

Media. *E. coli* was grown in LB (27). *Streptomyces* strains were grown as dispersed cultures in YEME medium (39) with 30% sucrose; cells grown for the preparation of protoplasts also contained 0.5% glycine. The regeneration medium for plating *Streptomyces lividans* protoplasts was R2YE (39); *S. avermitilis* was regenerated on RM14 (23). Putative transformants were purified on YD medium (12) supplemented with an appropriate antibiotic. Soft agar used in overlays was RM14 containing 0.01 M TES [*N*-tris(hydroxymethyl)methyl-2-aminoethanesulfonic acid] and 0.01 M MES [2-(*N*-morpholino)ethanesulfonic acid] with 3 g of agar per liter. Thiostrepton (E. R. Squibb & Sons, Princeton, N.J.) was added to give a final concentration of 10 μg/ml in solid media, 5 μg/ml in liquid media, and 15 μg/ml in soft agar. P medium (28), a 10% sucrose buffer, was used to prepare and dilute protoplasts. T medium (39) contained 25% polyethylene glycol and 2.5% sucrose. Polyethylene glycol 1000 was obtained from Sigma Chemical Co., St. Louis, Mo. TE buffer (10 mM Tris [pH 7.9], 1 mM EDTA) was used to store and dilute DNA.

DNA isolation. Miniprep plasmid DNA was isolated by rapid boiling procedures (13, 22). Large-scale DNA preparations were done by a Triton lysis procedure for *E. coli* (7) and a rapid boiling procedure for *Streptomyces* strains (22).

Transformation of *Streptomyces* protoplasts. *Streptomyces* cultures were converted to protoplasts and transformed by

TABLE 1. Bacterial strains and plasmids

Strain or plasmid	Genotype or characteristic	Source or reference
Strains		
Escherichia coli RR1	Transformable	2
E. coli GM272	*hsdS21 dam-3 dcm-6*	M. Marinus, University of Massachusetts
Streptomyces avermitilis	Original soil isolate	NRRL 8165
S. bikiniensis		ATTC 11062
S. coelicolor		ATTC 10147
S. cyanogriseus		NRRL 15773
S. fervens		NRRL 2755
S. griseus		ATTC 10137
S. griseoplanus		NRRL 3507
S. lividans TK21	SLP2⁻ SLP3⁻	D. Hopwood (14)
S. parvulus		ATCC 12434
S. rochei		ATCC 10739
Plasmids		
pVE3 (16.3 kb)	Shuttle, *tsr amp*	T. MacNeil (24)
pVE28 (4.9 kb)	*tsr*	T. MacNeil (24)
pVE203 (4.5 kb)	*neo*, pVE28 derivative	This laboratory
pVE328 (7.3 kb)	Shuttle, *tsr amp*	This laboratory
pVE614 (4.5 kb)	0.4-kb deletion of pVE28, *tsr*	This report

DNA in the presence of polyethylene glycol. The procedure for obtaining *Streptomyces* protoplasts was a modification of the previously described methods for obtaining *S. lividans* (39) and *S. avermitilis* (23) protoplasts. The modifications included growing *Streptomyces* cultures for 2 to 5 days, until they appeared densely grown. Both P medium and RM14 soft agar contained 0.01 M MES and 0.01 M TES at pH 7.0. Protoplasts were suspended at a concentration of 1×10^9 to 4×10^9 per ml in P medium containing 20% sucrose, quick-frozen in dry ice-ethanol, and stored at −80°C. For transformations, the protoplasts were thawed rapidly and then kept on ice. Samples (100 μl) of protoplasts were mixed with 100 ng of plasmid; 0.5 ml of T medium prepared as previously described (23) was added, and after 30 s, the transformation mixture was diluted in P medium containing 0.01 M MES and 0.01 M TES at pH 7.0 and was spread on R2YE or RM14 regeneration medium. After 18 h, the transformation plates were overlaid with 3 ml of RM14 soft agar containing an appropriate antibiotic. Transformants were scored 5 to 12 days later.

Recombinant DNA techniques. Enzymes were used according to the directions of the suppliers. Restriction enzymes were obtained from Bethesda Research Laboratories, Industrial Biological Laboratories Inc., and New England BioLabs, Inc., and methylases were obtained from New England BioLabs. The efficiency of the methylation reaction was determined by a test digestion of the methylated DNA with the corresponding restriction endonuclease. A 20-μl reaction mixture containing 0.5 μg of methylated DNA and 2 U of the corresponding restriction endonuclease was incubated for 2 h and then analyzed by agarose electrophoresis. Successfully methylated DNA showed no or very little evidence of nuclease digestion when compared with unmethylated control DNA. A previously described procedure was used to transform *E. coli* (25).

RESULTS

Restriction of shuttle vectors by *S. avermitilis*. Several derivatives of the broad-host-range *Streptomyces* plasmid pVE1 have been constructed (24), and some of these derivatives could be useful as cloning vectors in *S. avermitilis*. One small (4.9-kb) derivative of pVE1 encoding thiostrepton resistance, pVE28, can efficiently transform *S. avermitilis* to yield 10^7 transformants per μg of DNA (23). A potentially useful class of vectors for use in *S. avermitilis* comprises shuttle vectors composed of *E. coli* and *Streptomyces* plasmids. These vectors would allow many procedures developed in *E. coli* to be applied to *S. avermitilis* DNA. However, when shuttle vector pVE3 (a 15.4-kb cointegrate between pBR322 and pVE1) was isolated from *E. coli* and used to transform *S. avermitilis*, no transformants were obtained. This same vector replicates in *Streptomyces* spp., since *S. lividans* protoplasts can be transformed by pVE3 DNA. Moreover, when pVE3 DNA was isolated from *S. lividans*, a streptomycete described as lacking any significant restriction (39), the pVE3 DNA efficiently transformed *S. avermitilis* protoplasts. These results suggest that *S. avermitilis* contains a restriction system. Since restriction of DNA during transformation is dependent on the presence of recognition sites for the host restriction enzyme, smaller plasmids often can be used to transform a particular strain. However, when a smaller (7.5-kb) shuttle vector, pVE328 (to be described elsewhere), was isolated from *E. coli* and used to transform *S. avermitilis*, pVE328 also was restricted.

The nature of restriction among *S. avermitilis*, *S. lividans*, and *E. coli* was investigated. DNA from the shuttle vectors pVE3 and pVE328 was prepared from *E. coli*, *S. lividans*, and *S. avermitilis*, and 100 ng of DNA was used to transform the three species. Table 2 shows the results of these experiments. Both shuttle vectors showed a similar restriction pattern. *S. avermitilis* efficiently restricted DNA isolated from *E. coli*, reducing the transformation frequency more than 10^5-fold. *S. lividans* also restricted the entry of DNA from *E. coli*, but only by 10-fold. There was no restriction by *E. coli* of DNA isolated from either *S. avermitilis* or *S. lividans*.

Restriction by *S. avermitilis* of in vivo-modified DNA. Restriction by *S. avermitilis* was not limited to DNA isolated from *E. coli*. Restriction was also observed when DNA was isolated from modification-proficient *Streptomyces* spp. This was determined by isolating DNA from *Streptomyces griseus*. *S. griseus* is a known host for pVE1 derivatives (22), although it restricted the entry of DNA from *S. avermitilis* and *S. lividans* (Table 3). When *S. griseus* was transformed with pVE28, only a derivative, which was 0.4 kb smaller than pVE28, was isolated from the transformants. This deletion derivative was designated pVE614. When pVE614 was isolated from *S. griseus*, it was resistant to cleavage by *Sst*I. However, when pVE614 was isolated from *S. lividans*, it was cleaved by *Sst*I at a single site. The parental plasmid pVE28 contains two *Sst*I sites, but one site is within the 0.4-kb region deleted to form pVE614. Evidently, *S. griseus* contains a restriction-modification system which modifies the DNA that overlaps the *Sst*I site. When pVE614 was isolated from *S. griseus*, it could be used to transform *S. lividans* but not *S. avermitilis*. This was due to restriction rather than problems with the maintenance or replication of pVE614, because pVE614 DNA isolated from *S. lividans* efficiently transformed *S. avermitilis* (Table 3). Thus, *S. avermitilis* contains a restriction system which restricts the entry of DNA from *E. coli* RR1, known to contain the *dam*

TABLE 2. Transformation of shuttle vectors between *E. coli* and *Streptomyces* spp.

Plasmid and organism	Transformation frequency[a] in:		
	E. coli	*S. lividans*	*S. avermitilis*
pVE3			
E. coli	2×10^3	1×10^4	<10
S. lividans	2×10^3	2×10^5	8×10^5
S. avermitilis	2×10^3	1×10^5	2×10^6
pVE328			
E. coli	2×10^4	5×10^4	<10
S. lividans	2×10^4	4×10^5	3×10^6
S. avermitilis	2×10^4	4×10^5	5×10^6

[a] Number of transformants per 100 ng of DNA. Results are the averages of two experiments.

and *dcm* modification systems, and from *S. griseus*, shown in this work to modify DNA at or near a *Sst*I site in pVE614.

Restriction by *S. avermitilis* of in vitro-methylated DNA. Since *S. avermitilis* did not restrict DNA isolated from *S. lividans*, *S. lividans* must process DNA in one of two ways so that DNA is not cleaved by *S. avermitilis* restriction enzymes. *S. lividans*, a species unrelated to *S. avermitilis*, could coincidentally modify the sites in DNA recognized by a *S. avermitilis* restriction-modification system. Alternatively, *S. avermitilis* might contain a methyl-specific restriction system, and *S. lividans* might not methylate DNA. This latter possibility was tested by transforming *S. avermitilis* protoplasts with pVE28 that had been modified in vitro with various methylases. *S. avermitilis* strongly restricted DNA that was modified with *Alu*I, *dam*, *Hha*I, *Hph*I, and *Taq*I methylases, and the efficiency of transformation was reduced by 10^{-3}- and 10^{-4}-fold (Table 4). The 4- or 5-base-pair recognition sites for these methylases occur frequently in the DNA tested. pVE28 and other pVE1 derivatives with a maximum of only three sites for in vitro methylation were weakly restricted, since their efficiency of transformation was reduced only about 10-fold (Table 4). This indicates that the probability of a DNA molecule being restricted by *S. avermitilis* is proportional to the number of methylated bases it contains. There appears to be no sequence specificity to the methyl-specific restriction observed in *S. avermitilis*, since all eight methylases tested reduced the efficiency of transformation of the modified plasmids. *S. avermitilis* restricts both 5-methylcytosine- and N^6-methyladenine-modified DNA.

Restriction of *E. coli-Streptomyces* shuttle vectors is due to in vivo methylation. *E. coli* RR1, the host for the shuttle vectors listed in Table 2, contains two DNA methylases. The *dcm* product modifies the sequence GG(A/T)CC to produce 5-methylcytosine, and the *dam* product modifies the sequence GATC, yielding N^6-methyladenine. As explained above, *S. avermitilis* restricts modified DNA, and this could explain why *S. avermitilis* cannot be transformed by shuttle vectors isolated from *E. coli* RR1. To test if the *dcm* and *dam* modifications cause *S. avermitilis* to restrict shuttle vectors isolated from *E. coli*, shuttle vectors were isolated from methylase-deficient *E. coli* GM272 (*dcm dam hsd*). DNA from strain GM272 could be transformed directly into *S. avermitilis* (Table 5). However, the plasmids isolated from GM272 still showed a reduced efficiency of transformation compared with plasmids isolated from *Streptomyces* spp. The residual restriction of DNA isolated from GM272 may be because the *dam* and *dcm* mutations in GM272 do not completely eliminate all DNA methylation. This was tested by comparing the restriction enzyme digestion patterns of shuttle vector DNA which had been isolated from GM272 and *Streptomyces* spp. and cleaved with methylation-sensitive enzymes. When pVE3 and pVE328 DNAs isolated from GM272 were cleaved with *Mbo*I and *Eco*RII, faint bands resulting from only partial cleavage were visible. In contrast, the shuttle vector DNAs isolated from *Streptomyces* spp. were cleaved to completion. Thus, it is likely that some or all of the restriction seen when strain GM272 DNA was introduced into *S. avermitilis* was the result of the residual methylase activity in GM272.

TABLE 3. Restriction of pVE614 DNA

Plasmid and organism	Transformation frequency[a] in:		
	S. griseus[b]	*S. lividans*	*S. avermitilis*
pVE28			
S. avermitilis	1×10^2	6×10^5	2×10^6
S. lividans	1×10^2	8×10^5	3×10^6
pVE614			
S. griseus	3×10^5	1×10^5	<10
S. lividans	2×10^3	6×10^5	2×10^6
S. avermitilis	2×10^3	7×10^5	2×10^6

[a] Number of transformants per 100 ng of DNA. Results are the averages of two experiments.
[b] When *S. griseus* was transformed by pVE28, all *tsr* transformants contained deletion derivatives such as pVE614.

TABLE 4. Restriction of in vitro-methylated DNA by *S. avermitilis*

Plasmid	Methylase[a]	No. of methylase sites	Relative transformation[b] efficiency of *S. avermitilis*
pVE28	*Alu*I (AGmCT)	>15	2×10^{-3}
pVE28	*Hha*I (CmCGG)	>15	2×10^{-4}
pVE28	*Taq*I (TCGmA)	>15	2×10^{-4}
pVE28	*dam* (GmATC)	>15	2×10^{-4}
pVE28	*Hph*I (TmCACC)	>10	1×10^{-2}
pVE203	*Bam*HI (GGATmCC)	1	5×10^{-1}
pVE28	*Pst*I (CTGCmAG)	2	2×10^{-1}
pVE28	*Cla*I (ATCGmAT)	2	3×10^{-1}
pVE203	*Pst*I (CTGCmAG)	3	7×10^{-2}

[a] Sequences are indicated in parentheses.
[b] Relative transformation efficiency calculated as number of transformants with 100 ng of methylated DNA/number of transformants with 100 ng of unmethylated DNA. Results are the averages of at least two experiments.

TABLE 5. Transformation of shuttle vectors isolated from methylase-deficient bacteria

Plasmid and strain	Methylase	Transformation frequency[a] in:	
		E. coli RR1	*S. avermitilis*
pVE3			
E. coli RR1	*dam dcm*	2×10^3	<10
E. coli GM272	None	2×10^3	1×10^4
S. lividans	None	2×10^3	3×10^5
pVE328			
E. coli RR1	*dam dcm*	2×10^4	<10
E. coli GM272	None	2×10^4	4×10^2
S. lividans	None	2×10^4	4×10^6

[a] Number of transformants per 100 ng of DNA. Results are the averages of two experiments.

TABLE 6. Transformation of several *Streptomyces* spp. with methylated pVE28

Streptomyces sp.	No. of transformants untreated[a]	Relative transformation frequency with methylase[b]:			
		dam (GmATC)	*Taq*I (TCGmA)	*Alu*I (AGmCT)	*Hha*I (GmCGC)
S. avermitilis	2×10^6	0.0002	0.0002	0.001	0.0002
S. bikiniensis	3×10^5	1	1	1	1
S. coelicolor	3×10^6	0.02	0.02	1	1
S. cyanogriseus	3×10^5	1	1	0.0003	0.0003
S. fervens	5×10^6	1	1	1	1
S. griseoplanus	4×10^5	0.005	0.001	1	1
S. lividans	2×10^6	0.3	0.4	1	1
S. parvulus	2×10^6	1	1	0.0005	0.001
S. rochei	9×10^5	1	1	0.0002	0.001

[a] Per 100 ng of pVE28 DNA. Results are the averages of two experiments.
[b] Relative transformation frequency = number of transformants with 100 ng of methylated pVE28/number of transformants with 100 ng of untreated pVE28. Sequences are given in parentheses.

Common occurrence of methyl-specific restriction in *Streptomyces* spp. *S. avermitilis* is the first strain described which restricts DNA containing either 5-methylcytosine or N^6-methyladenine. To determine if methyl-specific restriction was unique to *S. avermitilis*, nine *Streptomyces* spp. were tested for methyl-specific restriction. These included eight other *Streptomyces* spp. known to be hosts for pVE1 derivatives (22, 24) and *Streptomyces cyanogriseus*, which produces avermectinlike compounds. Table 6 presents the methyl-specific restriction patterns of the nine *Streptomyces* spp. successfully transformed by pVE28. Most of the strains tested (seven of nine) had a methyl-dependent restriction system (Table 6). However, *S. avermitilis* was unique in restricting DNA modified at either A or C. Three strains restricted only N^6-methyladenine-containing DNA, and three strains restricted only 5-methylcytosine-containing DNA. Two strains, *Streptomyces bikiniensis* and *Streptomyces fervens*, showed no evidence of methyl-dependent restriction.

DISCUSSION

S. avermitilis possesses a unique methyl-specific restriction system. This system prevents the movement of shuttle vectors from most *E. coli* strains directly into *S. avermitilis*. *S. avermitilis* was shown to restrict in vitro-methylated DNA containing 5-methylcytosine or N^6-methyladenine modifications. The amount of restriction observed in *S. avermitilis* was correlated with the number of modified sites in DNA. Plasmids with 1 to 3 methylated bases per molecule were restricted only about 10-fold, but plasmids with more than 10 methylation sites were restricted >1,000-fold (Table 4). No evidence was found for sequence specificity, since plasmid DNA treated with each of the eight methylases tested (Table 4) was restricted by *S. avermitilis*. Restriction systems in *Streptomyces* spp. are common (1, 6, 8, 17, 26, 35), but this report is the first description of a methyl-specific restriction system in *Streptomyces* spp. Some bacteria methylate DNA to produce N^4-methylcytosine (5, 9). The *S. avermitilis* restriction system has not been tested with this form of modification.

Two other bacteria, *E. coli* (31) and *Acholeplasma laidlawii* (36), are known to restrict DNA containing 5-methylcytosine with little or no sequence specificity. In *E. coli*, two distinct genetic loci have been correlated with 5-methylcytosine-specific restriction (31). One locus, *mcrB*, restricts DNA methylated with many but not all methylases tested and may recognize the sequence GmC or purine mC. A second locus, *mcrA*, restricts only DNA methylated by the *Hpa*II methylase at the site CmCGG. In *A. laidlawii* (36), restriction was observed when DNA was modified with any of five 5-methylcytosine-specific methylases tested, but no sequence specificity was observed. Several bacteria, notably *D. pneumoniae*, contain a sequence-specific, N^6-methyladenine-specific restriction system (19, 32). This system restricts DNA containing GmATC (19). The GmATC modification is a result of *dam* methylases, which are common in members of the family *Enterobacteriaceae* and in *Haemophilus* spp. (3). *S. avermitilis* is unique in that it restricts both 5-methylcytosine- and N^6-methyladenine-modified DNA, with no apparent sequence specificity. Methyl-specific restriction occurred in seven of the nine *Streptomyces* strains tested (Table 6). However, it is unclear how frequently methyl-specific restriction occurs among all *Streptomyces* spp. This is because 8 of the 9 *Streptomyces* strains used in this study were previously selected from 75 strains for their ability to accept pVE1 derivatives introduced by conjugation (24). It is possible that the eight strains are hosts for pVE1 derivatives because they lack classical restriction-modification systems. Nonetheless, it is clear that methyl-specific restriction is widespread in bacteria.

The methyl-specific restriction system in *S. avermitilis* is more restrictive than the *E. coli* or *A. laidlawii* system. The *S. avermitilis* restriction system reduced the transformation frequency of in vitro-methylated DNA nearly 10,000-fold (Table 4). *E. coli* reduced the transformation frequency of methylated DNA 500-fold (31), while *A. laidlawii* reduced the transformation frequency of modified DNA only 50-fold (36). When shuttle vectors modified by *E. coli* in vivo (Table 2) were introduced into *S. avermitilis*, no transformants were obtained. This indicates that the *S. avermitilis* restriction system reduced the transformation frequency at least 10^6-fold.

The methyl-specific restriction system of *S. avermitilis* prevented the direct introduction of shuttle vectors grown in *E. coli* (Table 2) as well as of DNA isolated from other bacteria that modify DNA (Table 3). This restriction barrier can be either overcome by passage of DNA through nonmethylating hosts such as *S. lividans* (Tables 2, 3, and 5) or reduced by passage of DNA through methylase-deficient *E. coli* GM272 (Table 5). However, there are problems with using GM272 as a general host for *S. avermitilis-E. coli* shuttle vectors. First, although DNA isolated from GM272 can be introduced into *S. avermitilis*, the DNA is still restricted by *S. avermitilis*. This restriction may be caused by residual methylase activity in strain GM272. Evidence for

continued methylation by GM272 was observed, since plasmids isolated from GM272 are partially resistant to cleavage by methylation-sensitive restriction enzymes. It is also possible that *S. avermitilis* contains another restriction system. Second, GM272 has a very low transformation frequency, i.e., only 10 to 100 transformants per μg of plasmid DNA. Russell and Zinder also observed a very low frequency of transformation of GM272 (34). However, they reported that *dam* strains, including GM272, show an approximately 100-fold increase in transformation frequency when transformed with plasmids grown in a Dam^- host compared with DNA isolated from a Dam^+ host. Third, presumably because GM272 is a poor host for methylated DNA, I have observed Dam^+ revertants among the transformants of GM272.

The methyl-specific restriction system in *E. coli* is complex, involving at least two loci (31), while the *A. laidlawii* system appears to involve only one locus (36). The restriction system of *S. avermitilis* may also be complex. Attempts to isolate *S. avermitilis* mutants which do not restrict DNA modified in vitro or by *E. coli* have been unsuccessful (data not shown).

There is a continuing interest in developing new and modified secondary metabolites (11, 15). One way to do this is the creation of hybrid molecules by the introduction of biosynthetic genes from one pathway into a strain that produces a different compound (15). However, restriction barriers can limit this approach. Methyl-specific restriction barriers can be overcome by passage of the cloned genes through a strain like *S. lividans*, which apparently does not modify DNA (Tables 2 and 3). However, *S. lividans* is not an ideal host since it possesses a weak methyl-specific restriction system (Tables 2 and 6). A preferable host may be *S. fervens*. This strain does not possess a detectable restriction system and is very efficiently transformed.

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