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Transformation of *Streptomyces avermitilis* by plasmid DNA

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SUMMARY

Polyethylene glycol (PEG) efficiently mediated the transformation of *Streptomyces avermitilis* protoplasts by plasmid DNA to yield 10^7 transformants per μg of plasmid DNA. Under conditions in which the maximum transformation frequency was observed, the cotransformation frequency exceeded 10%. The number of transformants increased linearly with the amount of DNA and number of *S. avermitilis* protoplasts. Relaxed and supercoiled, but not linear DNA transformed protoplasts efficiently. Dimethyl sulfoxide (DMSO)-mediated transformation of protoplasts was 1000-fold less efficient. PEG and, less efficiently, DMSO also mediated the transformation of whole cells of *S. avermitilis* by DNA.

INTRODUCTION

Streptomyces avermitilis produces avermectins, which are commercially important in the control of animal parasites. Avermectins are potent anthelmintic compounds [2] which are active against many endoparasites of animals and humans, including *Onchocerca volvulus*, the agent of 'river blindness'. The avermectins are also active against almost all arthropod ectoparasites [3] and are effective in controlling numerous agricultural pests [18].

Avermectins are an example of secondary metabolites produced during the stationary phase of growth in *Streptomyces*. In order to study the biosynthesis of secondary metabolites, cloning vectors and recombinant DNA techniques have been developed for several *Streptomyces* species [7,9,19]. In order to isolate and study the genes involved in avermectin biosynthesis, we are developing cloning systems for *S. avermitilis*. Vectors have been developed which include those derived from phage TG1 [4] and plasmid pVE1 [13]. To use vectors for cloning in *S. avermitilis*, a technique for the efficient transformation of DNA into *S. avermitilis* must be developed. Since Bibb et al. [1] first described a high-frequency transformation procedure for *S. coelicolor* protoplasts using polyethylene glycol (PEG) and dimethyl sulfoxide (DMSO), many reports of PEG-mediated transformation of *Streptomyces* by plasmid DNA have been published (for example, see Refs. 11,14,15 and 17). In this report we describe and characterize the efficient PEG-mediated transformation of *S. avermitilis* protoplasts

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by DNA. We also describe two alternative procedures to transform *Streptomyces* with DNA. One procedure uses DMSO instead of PEG and the second procedure involves whole cells instead of protoplasts. A preliminary report of this work was presented at the 1986 annual meeting of the Society for Industrial Microbiology, P68.

MATERIALS AND METHODS

Media, solutions and chemicals

The original soil isolate of *S. avermitilis*, MA4680, was grown as a dispersed culture for protoplasting in YEME medium [22] with 30% sucrose and 0.5% glycine. Regeneration media for plating protoplasts included R2YE [22] and RM14 media (C. Ruby, personal communication). RM14 medium is similar to R2YE but contains MES buffer pH 6.5 instead of TES, 20% sucrose instead of 10% sucrose, and 3 g/l of Bacto oatmeal agar. Putative transformants were purified on YD medium [4] supplemented with the appropriate antibiotic. Soft agar used in overlays was RM14 with 3 g/l of agar. The following antibiotics were used: thiostrepton (Squibb, New Brunswick, NJ) 10 μg/ml in solid media, 5 μg/ml in liquid media, and added to a final concentration of 15 μg/ml when added in soft agar; and neomycin (Sigma, St. Louis, MO) 20 μg/ml in solid media, 10 μg/ml in liquid medium, and added to a final concentration of 50 μg/ml when added in soft agar. P medium [16], a 10% sucrose buffer which contained MES pH 6.5 instead of TES, was used to prepare and dilute protoplasts. T medium [22] contained 25% PEG and 2.5% sucrose. Some transformations were done in the presence of polybrene (Aldrich, Milwaukee, WI). PEG 1000 was obtained from Sigma, and DMSO was obtained from Aldrich.

Strains and plasmids

The original soil isolate of *S. avermitilis*, MA4680, was obtained from S. Curry, Merck & Co., and used in transformation experiments. It is not known to contain any plasmids. Plasmids used in transformation experiments were isolated from *S. lividans* strain 1326. Plasmid pIJ350 (encoding thiostrepton-resistance, *tsr*) [9] was obtained from D. Hopwood. Plasmids pVE28 (*tsr*) and pVE203 (encoding neomycin resistance, *neo*) are derivatives of pVE1 [13]. All three plasmids are high-copy-number plasmids, transfer-deficient, and about 5 kb in size.

Plasmid preparation

Plasmid DNA was prepared from *S. lividans* cultures by a rapid boiling procedure [12]. For minilysates 6-ml cultures were grown, and for large-scale preparations 500-ml cultures were grown.

Transformation of S. avermitilis protoplasts

The procedure for obtaining *S. avermitilis* protoplasts and introducing plasmid DNA into the protoplasts using PEG is a modification of the method of Hopwood et al. [6]. 30 ml of YEME were inoculated with 5×10^7 spores and grown for 3 days at 28°C, the mycelium was washed once in P medium, resuspended in 10 ml of P medium with 1 mg/ml lysozyme and incubated at 37°C for 1 h with slow shaking. The resulting protoplasts were filtered through glass wool, centrifuged at 4000 $\times$ *g* for 10 min, resuspended in 2 ml of P medium modified to contain 20% sucrose, and 100-μl aliquots (1×10^8– 4×10^8 viable protoplasts) were used in each transformation. Viable protoplast titers were determined by measuring colony-forming units on regeneration agar. The total number of protoplasts was determined microscopically using a Petroff-Hausser counting chamber viewed under phase-contrast at $\times$1000. DNA was added in 10 μl of TE (10 mM Tris, pH 7.9, 0.5 mM EDTA). T medium, the buffer containing 25% PEG 1000, was prepared, the pH was adjusted to 9.0, and the buffer was filtered through 0.45-μm filters and autoclaved. Immediately before the transformation experiments, three additions were made to T medium resulting in the following concentrations: 0.4 mM KH_2PO_4, 50 mM Tris-maleate pH 8.0, and 100 mM $CaCl_2$. 0.5 ml of T medium was added to the protoplasts and DNA. After 30 s, the transformation mixture was diluted, aliquots were mixed with 3 ml of RM 14 soft agar, and the mixture was

Table 1

Factors important for the efficient transformation of *S. avermitilis*

The procedure of Hopwood et al. [6] was modified to increase the transformation of plasmid DNA into *S. avermitilis* protoplasts. In each experiment, 100 ng of pVE28 was added to 0.1 ml of protoplast solution as described in the Materials and Methods

Procedural change	Transformants (per μg)	
	S. avermitilis	*S. lividans*
Method of Hopwood et al. [6]	2×10^3	4×10^7
Grow cells in 30% sucrose	1×10^4	4×10^7
Regenerate transformants on RM14	7×10^5	1×10^7
Spread transformants in RM14 soft agar	2×10^7	1×10^7

spread on RM14 regeneration plates. Some transformations were done in the presence of 30 mM polybrene, or with DMSO replacing PEG in T medium. After 18 h incubation at 28°C, the regeneration plates were overlayed with 3 ml of RM 14 soft agar containing antibiotics. After 10 days incubation at 28°C the plates were scored for transformants. Putative transformants were purified and minilysates were made from 6-ml cultures to test for the presence of the appropriate plasmid.

Enzyme treatments

Plasmid DNA was treated with topoisomerase I (BRL Bethesda, MD), gyrase (BRL), *Bal*I restriction endonuclease (BRL), *Bgl*II restriction endonuclease (IBI New Haven, CT) and *Sst*II restriction endonuclease (BRL) according to the suppliers directions. The identity of the plasmid in transformants was confirmed by *Sst*II endonuclease digestion followed by agarose gel electrophoresis [13].

RESULTS

The standard transformation procedure in *Streptomyces* is a PEG-mediated transformation of protoplasts by DNA [6] which was developed by Bibb et al. [1] and refined by Thompson et al. [22]. Initial attempts to apply this procedure to *S. avermitilis* yielded only 10^3 transformants per μg of plasmid pVE28 (see Table 1). In order to develop an efficient transformation system for *S. avermitilis* we sought modifications of the PEG procedure and we explored the possibility of developing alternative procedures.

PEG-mediated transformation

When *S. avermitilis* was grown for the preparation of protoplasts in YEME medium containing 34% sucrose and 1% glycine [6], the cultures became only faintly turbid (OD_{600} less than 0.7) after 3 or even 4 days. Consequently, only a small amount of protoplasts, about 10^8 per 30 ml culture, was obtained. Hopwood et al. [6] recommend that the concentration of glycine, which is present in YEME to partially inhibit cell wall synthesis, be chosen which just inhibits growth. We eliminated the glycine and found that this did not significantly enhance mycelial growth. However, we found that by lowering the sucrose level from 34% to 30% (or 25%), cultures became very turbid (OD_{600} greater than 1.4) after 3 days. Lowering the glycine to 0.5% yielded more protoplasts, as judged by the size of the protoplast pellet. When an aliquot of these protoplasts was transformed with pVE28, about 5-times more transformants were obtained than from cells grown with 1% glycine. Neither increasing the concentration of lysozyme, increasing the time of lysozyme treatment, nor altering the temperature of lysozyme treatment to 0°C or 30°C yielded more protoplasts or protoplasts that were more effective in DNA uptake. The protoplasts of *S. avermitilis*

have an unusual appearance. When viewed in a phase contrast microscope, *S. avermitilis* protoplasts often do not appear spherical; rather, one side appears flattened. It is possible that some cell wall material remains attached to the protoplasts.

The uptake of DNA by *S. avermitilis* protoplasts occurs in T medium, a 25% PEG/2.5% sucrose buffer, which we prepare differently than Hopwood et al. [6]. We have found that slowly adding 1 M NaOH or in KOH to T medium buffer before autoclaving, to bring the pH up to 9.0, produces a buffer that is a consistent and efficient mediator of transformation. The buffer remained effective when stored over 1 year at room temperature. Small liposomes have been shown to enhance transfection in *S. lividans* [20]. We prepared liposomes from lecithin by hand, as described [20], and we had uniform liposomes of 0.3 μm prepared (courtesy of Microfluidics Corp., Newton, MA). Addition of 50 μl of the hand-prepared liposomes had no effect on transformation, while addition of 50 μl of the commercially prepared liposomes resulted in protoplast lysis. We also tried to enhance DNA uptake by adding the polycation polybrene to the DNA-protoplast mixture at a final concentration of 30 mM. Polybrene has been found to enhance DNA uptake in yeast [8], but it had no effect on transformation of *S. avermitilis* protoplasts.

S. avermitilis regenerates poorly on the standard R2YE regeneration medium; only 0.01% of the total protoplasts regenerated on this medium. Better regeneration, between 1 and 10%, was obtained when protoplasts were regenerated on RM14, an alternative regeneration medium. The transformation frequency also increased, to nearly 10^6 transformants per μg of pVE28 DNA, when the transformed protoplasts were plated on RM14 (see Table 1). There are several differences between RM14 and R2YE. To determine whether the buffer or pH was responsible for the improved transformation, eight regeneration media were made. These included both RM14 and R2YE media with MES or TES buffer at pH 6.5 or pH 7.2. In pair-wise comparisons in which only the base medium (R2YE vs. RM14) or the buffer (MES vs. TES) were different, no significant difference in the number of transformants was observed. However, in the four comparisons between media differing only in pH, the transformation frequency was 20–30-fold higher at pH 6.5 than at pH 7.2. Thus, pH, rather than the buffer, was the most important factor. The maximum transformation frequency, 2×10^7 per μg, was obtained by spreading transformation mixtures on RM14 in a 3-ml RM14 soft agar overlay, rather than directly with a glass rod spreader. The regeneration of *S. avermitilis* protoplasts appeared to occur in two phases. About 1% of the transformants appeared on regeneration plates after 4 days, the other 99% of the transformants appearing after 10 days. The reason for this is unknown, but both types of transformants appeared identical upon subculturing and both contained plasmid DNA.

Table 1 summarizes the factors which are important in achieving an efficient transformation of *S. avermitilis*. Maximal transformation was obtained by growing *S. avermitilis* in YEME containing 30% sucrose and 0.5% glycine, and by plating transformation mixtures as soft agar overlays on RM14 regeneration medium. Table 1 also shows the result of applying the conditions which enhanced the transformation of *S. avermitilis* to the transformation of *S. lividans*. The only change observed was that the use of RM14 regeneration medium reduced *S. lividans* transformation by 75%. Although the method described here has reproducibly yielded transformation frequencies of about 10^7 per μg, occasionally the maximum transformation observed in an experiment will be 10–50-fold lower. This lower transformation frequency is always correlated with a lower viable titer of the protoplasts. However, we have been unable to identify why this lower viability is observed. This protoplast viability difference affects the absolute number of transformants obtained in any experiment. However, the relative differences reported here, for example between the different conditions in Table 1, show a less than 2-fold variation when repeated with different protoplast preparations.

DNA concentration and protoplast number

We tested the effect of varying plasmid DNA concentrations on the frequency of PEG-mediated

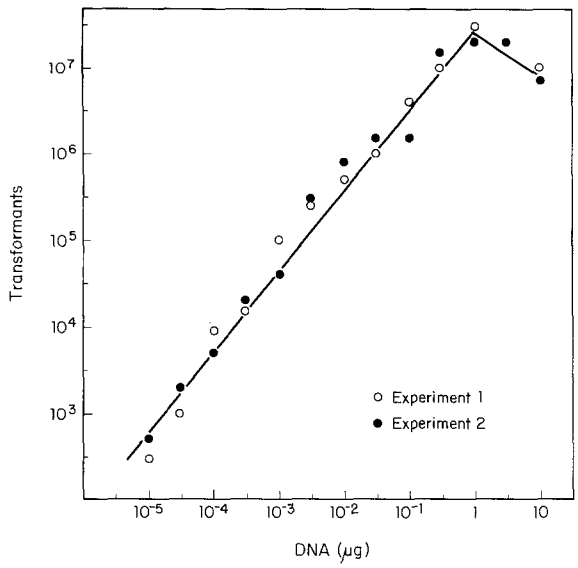

Fig. 1. The effects of plasmid concentration on transformation of 2×10^8 viable protoplasts of *S. avermitilis*.

transformation of protoplasts by serially diluting a preparation of pVE28 in TE such that 10 μl would contain between 10 pg and 10 μg of DNA. These preparations were then added to about 4×10^9 protoplasts. As Fig. 1 shows, the number of transformants increased linearly with DNA until 1 μg was added. A small decrease was found when higher amounts of DNA were added to the protoplasts. Under these conditions, in which the transformation of *S. avermitilis* protoplasts is saturated, only 10% of the viable protoplasts are transformed. This indicates that there is a subpopulation of viable protoplasts competent for DNA uptake. When the DNA added was held constant at 100 ng and the number of protoplasts that was added was varied from 10^3 to 10^9, the number of transformants increased linearly (see Fig. 2).

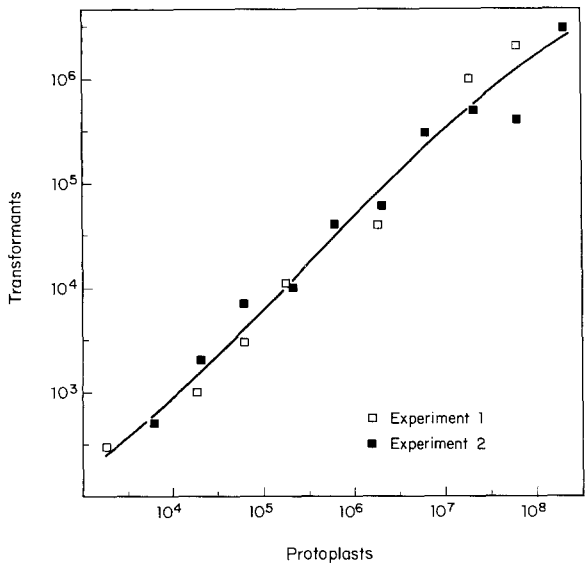

Fig. 2. The effects of protoplast concentration on transformation of *S. avermitilis* with 100 ng of pVE28 DNA.

Cotransformation

The process by which the subpopulation of viable *S. avermitilis* protoplasts takes up DNA can be elucidated by analysis of the cotransformation of two plasmids. Cotransformation could occur under three conditions. Firstly, the same competent subpopulation may independently take up both plasmids. In this case, the probability of a cell taking up two plasmids is the product of the probability of one cell in the competent population taking up one plasmid times the probability of it taking up the second plasmid. Secondly, a population of protoplasts may preferentially take up more than one DNA molecule, a process termed congression. In this case, the frequency of cotransformation would be a constant fraction of the transformation frequency for one plasmid. Thirdly, although the data presented above indicate that there is a competent subpopulation of protoplasts, a different subpopulation may be involved in taking up a second plasmid. The cotransformation frequency would be similar to that expected in the first case, except that the frequency would depend on the probability of a cell in the viable population, rather than the competent subpopulation, taking up DNA.

We determined the cotransformation frequency at various DNA concentrations by preparing a solution containing equal amounts of two plasmids, pIJ350 (*tsr*) and pVE203 (*neo*). These two plasmids are compatible, have a high copy number, and are about 4.3 kb in size [9,13]. Starting with a solution containing 0.5 μg of each plasmid per 10 μl, serial dilutions were made and used to transform *S. avermitilis* protoplasts. After DNA uptake, the trans-

Table 2

Cotransformation of *S. avermitilis* by pVE203 and pIJ350

2×10^8 viable protoplasts of *S. avermitilis* were transformed with 10 μl of various DNA solutions containing equal amounts of pIJ350 (*tsr*) and pVE203 (*neo*) DNA. The number of transformants was determined by overlaying transformation plates with the appropriate antibiotics. The cotransformation frequencies are calculated as indicated in the text.

DNA	Transformants			Cotransformation frequency		
(μg)	Neo^r	Tsr^r	Neo^rTsr^r	relative	competent	predicted
1.0	3×10^6	3×10^6	3×10^5	1×10^{-1}	3×10^{-2}	2.3×10^{-2}
0.3	1×10^6	2×10^6	6×10^4	4×10^{-2}	6×10^{-3}	5.0×10^{-3}
0.1	3×10^5	7×10^5	1×10^4	2×10^{-2}	1×10^{-3}	5.3×10^{-4}
0.03	2×10^5	4×10^5	2×10^3	7×10^{-3}	2×10^{-4}	2.0×10^{-4}
0.01	1×10^5	1×10^5	3×10^2	3×10^{-3}	3×10^{-5}	2.5×10^{-5}
0.003	4×10^4	5×10^4	2×10^1	4×10^{-4}	2×10^{-6}	5.0×10^{-6}

formation mixtures were titered in triplicate on regeneration media. One plate was overlaid with thiostrepton-containing medium (to assay for pIJ350), one with neomycin-containing medium (to assay for pVE203), and one with medium containing both drugs (to assay for both plasmids). Table 2 details the results of a cotransformation experiment. The data were used to calculate three different cotransformation frequencies. The relative cotransformation frequency is the number of cotransformants relative to the number of singularly transformed protoplasts and is calculated as ($2 \times Neo^rTsr^r$ transformants)/(Neo^r + Tsr^r − Neo^rTsr^r transformants). The competent cotransformation frequency is the number of cotransformants relative to the subpopulation of competent protoplasts and is calculated as ($2 \times Neo^rTsr^r$ transformants)/(total competent protoplasts). These two formulas account for cotransformants containing two *neo* or two *tsr* plasmids, as well as the measured transformants containing a *neo* and a *tsr* plasmid. If cotransformation is the result of competent protoplasts randomly taking up DNA, then from the transformation data one would calculate the predicted competent cotransformation frequency as [(Neo^r transformants)/(total competent protoplasts)] × [(Tsr^r transformants)/(total competent protoplasts)]. The latter two frequencies depend on the number of competent protoplasts. As shown in the transformation studies above, the competent subpopulation is approximately 10% of the viable protoplasts. The titer of the viable protoplasts for the experiment in Table 2 was 2×10^8, indicating the competent population was 2×10^7.

As Table 2 shows, the relative cotransformation frequency at the highest DNA concentration tested, 1 μg, is about 0.1. The relative cotransformation frequency declines with lower amounts of DNA, indicating that there is not a subpopulation of protoplasts which is competent to take up multiple DNA molecules. The close agreement between the competent and predicted competent cotransformation frequencies shown in Table 2 indicates that the same competent subpopulation was involved in the uptake of both plasmids.

DMSO-mediated transformation

Because of the initial low transformation frequency obtained when we used the protocol of Hopwood et al. [6], we investigated the use of DMSO to mediate transformation. DMSO has been shown to induce uptake of DNA by whole cells of *Escherichia coli* and yeast [8]. The transformation frequency of protoplasts by pVE28 when mixed with T medium containing DMSO instead of PEG was low. When the concentration of DMSO was varied from 0 to 40%, the transformation frequency was maximal at 40%, yielding 3×10^4 trans-

Table 3

Transformation of *S. avermitilis* mediated by PEG or DMSO

10^9 cells were transformed with 100 ng of pVE28. Transformations were performed as described in Materials and Methods with various amounts of DMSO substituted for PEG. The control treatment lacked PEG and DMSO. Spores were germinated for 5 h at 28°C to form germlings. Germling transformants were recovered on RM14 regeneration plates and also on rich medium (YD).

	Number of transformants (per μg)		
Treatment	protoplasts	germlings	mycelia
25% PEG	5×10^6	3×10^4	< 10
20% DMSO	1×10^4	5×10^2	< 10
30% DMSO	2×10^4	4×10^3	< 10
40% DMSO	3×10^4	5×10^3	< 10
Control	< 10	< 10	< 10

formants per μg. When the time of contact with DMSO was varied from 30 s to 16 min, maximal transformation was observed at 30 s. We also pretreated the cells with a 30 μg/ml solution of the polycation polybrene, which enhanced DMSO-mediated transformation in yeast [8]. The polybrene-treated protoplasts showed no enhancement when either DMSO or PEG solutions were used. A comparison of DMSO- and PEG-mediated transformation is shown in Table 3.

A rapid and simple transformation system would be useful for introducing a particular plasmid into many strains, even if the frequency of transformation was low. Thus we tested the ability of DMSO and PEG to mediate transformation of 3-day-old mycelium, prepared for protoplasting as described in Materials and Methods. No transformants were detected. However, PEG, and to a lesser extent DMSO, mediated the transformation of *S. avermitilis* germlings by pVE28. *S. avermitilis* germlings resulted from incubating spores in YD medium for 5 h, after which time nearly all the spores had germinated. As shown in Table 3, 3×10^4 transformants were obtained with PEG, and 5×10^3 with DMSO. Transformants were obtained on regeneration medium (RM14) and on rich medium (YD).

Simplifying transformation

In addition to identifying conditions for the efficient transformation of *S. avermitilis* by plasmid DNA, we are interested in simplifying the transformation procedure. We have found that two steps recommended by Hopwood et al. [6], the washes with NaCl before protoplasting and the wash with

Table 4

Transformation of *S. avermitilis* with pVE28

Each transformation used 100 ng of pVE28 and 100 μl of protoplasts (1×10^8 to 4×10^8 viable protoplasts). pVE28 was purified from CsCl gradients and appeared as an approximately 1:1 mixture of supercoiled and relaxed forms on agarose gels. Supercoiled pVE28 DNA was made by treating plasmid DNA with gyrase, relaxed pVE28 DNA was made by treating pVE28 with topoisomerase I. Linearized plasmid was made by treating pVE28 DNA with the indicated restriction enzyme.

Cells	DNA	Transformants (per μg)
Fresh protoplasts	Purified	5×10^6
Frozen protoplasts	Purified	4×10^6
Frozen protoplasts with DMSO	Purified	5×10^6
Frozen mycelia	Purified	2×10^6
Fresh protoplasts	Supercoiled	7×10^6
Fresh protoplasts	Relaxed	7×10^6
Fresh protoplasts	*Bal*I linearized	3×10^3
Fresh protoplasts	*Bgl*II linearized	6×10^3

P medium after transformation, can be eliminated. The latter step is used to dilute away the PEG, which can be toxic. However, we saw no loss of viability if cells remain in PEG for up to 4 min, perhaps because we adjust the pH of the T medium before autoclaving.

We also tested several ways to prepare and store *S. avermitilis* protoplasts. Protoplasts prepared from fresh mycelium, or from mycelium that were washed in P medium and then frozen at −20°C, both yielded about 10^7 transformants per μg of pVE28. Similarly, protoplasts in P medium or in P medium with 10% DMSO could be quick-frozen in a solid CO_2/ethanol bath and stored at −80°C. Upon thawing, these protoplasts were about as effective in taking up DNA as freshly prepared protoplasts (see Table 4).

Physical state of the DNA for transformation

The pVE28 DNA used to characterize transformation appears as a mixture of supercoiled and relaxed circles when run on agarose gels. We treated pVE28 with a variety of enzymes to see whether the physical state of the DNA affected transformation. pVE28 was treated with topoisomerase I and gyrase, which resulted in predominantly relaxed circles and supercoiled molecules, respectively, when analyzed by agarose gel electrophoresis. However, as shown in Table 4, neither of these preparations had any detectable effect on the transformation frequency. As expected, when pVE28 was cleaved with restriction enzymes which linearize pVE28, the transformation frequency decreased at least 1000-fold. The two enzymes tested, *Bgl*II, which leaves a 4-base extension, and *Bal*I, which leaves blunt ends, each cleave pVE28 once, in regions that are not essential for replication.

DISCUSSION

In this report we describe conditions for the efficient transformation of *S. avermitilis*, a commercially important streptomycete, by plasmid DNA. Several modifications to the procedure of Hopwood et al. [6] were necessary to achieve transformation frequencies of 10^7 per μg. The most important changes leading to efficient transformation were to alter the growth and regeneration media. Reducing the sucrose in the YEME medium yielded better cell growth and subsequently larger numbers of protoplasts. Changing the buffer in the regeneration medium from TES pH 7.2 to MES pH 6.5 increased the regeneration frequency more than 20-fold. The addition of polybrene, or small liposomes, both reported to increase DNA uptake in some systems [8,14,20], had no effect on the transformation of *S. avermitilis* protoplasts by DNA.

We characterized several factors to determine their role in the transformation of *S. avermitilis* by plasmid DNA. Previously it was reported that transformation increased linearly with increasing DNA over the range of 10 ng to 10 μg in *Streptomyces* strain C5 [11] and over the range 1 ng to 100 ng in *S. fradiae* [14]. We found that the number of transformants increased linearly in *S. avermitilis* with increasing DNA from 10 pg to 1 μg. No increase in transformation was observed when 3 or 10 μg of DNA were added to about 10^8 viable protoplasts. When the number of protoplasts was varied from 10^3 to 4×10^8 viable cells, and 100 ng of plasmid DNA were added, the number of transformants also increased linearly. Apparently, protoplasts are limiting when over 1 μg of DNA is added to a transformation mixture. However, only a subset of the viable protoplasts take up DNA, since 90% of the viable protoplasts remained untransformed under conditions which gave the maximum number of transformants. Similar observations of a competent subpopulation of viable protoplasts have been observed in other *Streptomyces* species [14,21]. Our study of the cotransformation of *S. avermitilis* protoplasts by two compatible plasmids helps elucidate the transformation process. There was no evidence of congression, that is, the ability of a subpopulation of cells to preferentially take up multiple DNA molecules. If such a population had existed, one would expect the number of cotransformants to remain a constant fraction of the number of transformants. Table 2 clearly shows that, with decreasing DNA, the relative cotransformation frequency declined. The competent and pre-

dicted cotransformation frequencies from Table 2 have very similar values at the six DNA concentrations tested. These results indicate that the same subpopulation of protoplasts is competent to take up each of the two compatible plasmids, and takes them up independently of each other. Suarez and Chater observed a similar result in *S. lividans*, where the same subpopulation was competent to take up DNA of two different phages [21].

The cotransformation results in Table 2 show that at the highest DNA level tested, 1 μg, 10% of the transformed protoplasts took up two plasmid molecules. Under these conditions, 1 μg of plasmid DNA corresponds to about 1000 molecules per protoplasts. Thus, only a minority of the added DNA is taken up by protoplasts. If one wishes to avoid transformants with more than one plasmid, which can be important when constructing gene libraries, less than 100 ng of plasmid DNA should be used per transformation. Linearized plasmid molecules transformed *S. avermitilis* protoplasts poorly, but supercoiled or relaxed circular plasmids were equally effective in transforming *S. avermitilis*.

We have found that DMSO promotes transformation of *S. avermitilis*, the first time that this chemical has been shown to mediate transformation in a streptomycete. In the initial paper by Bibb et al. [1] describing transformation of *S. coelicolor* protoplasts by plasmid DNA, the buffer used to mediate transformation contained both PEG and DMSO. Later papers indicated that PEG alone was effective. In this report we show that DMSO alone is also an effective mediator of transformation, although at a 1000-fold lower frequency than PEG. We also tested to see whether DMSO (or PEG) might mediate the transformation of whole cells by plasmid DNA. We obtained no transformants when 3-day-old mycelium was used as the recipient. However, we observed transformation of 5-h germlings with plasmid DNA, mediated by DMSO and PEG, to yield 3×10^3 and 5×10^4 transformants per μg, respectively. There are two other reports describing the transformation of whole cells of *Streptomyces* by DNA [10,23]. Konvalinkova et al. described the transfection of *S. virginiae* mycelium by phage S1 DNA, observing the maximal transfection frequency with early stationary phase mycelium after a 24-h incubation with phage DNA [10]. Zenghui et al. reported maximal transformation of *S. griseus* early log phase mycelium after a 1-h incubation of cells with plasmid DNA [23]. Neither group investigated the effects of DMSO or PEG on transformation. In *S. avermitilis*, we only observed transformation of whole cells in the presence of DMSO or PEG, although we used a relatively short incubation time of 30 s. The whole cell transformation procedure described here could be especially useful for introducing a particular plasmid into many different strains. Although the transformation frequencies reported for germlings and for protoplasts mediated by DMSO are relatively low, no attempt has been made to optimize these procedures.

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