Short Communication

POLYETHYLENEGLYCOL-INDUCED TRANSFORMATION OF *STREPTOMYCES* PROTOPLASTS BY CHROMOSOMAL DNA

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BIBB *et al.* (1) have developed an effective transformation system of *Streptomyces* strains, which involves the uptake of covalently closed circular DNA by protoplasts in the presence of polyethyleneglycol (PEG). The PEG-induced uptake of extracellular DNA has been successfully utilized for transfection of actinophage DNA by the authors (2) and SUAREZ and CHARTER (3). In this paper, we report that the PEG-induced DNA uptake can be used for chromosomal transformation of *Streptomyces parvulus*.

R2 medium (4) without casamino acids was used as a minimal regeneration medium. Protoplasts of *S. parvulus* 123 (*cysB1 his*) cells were prepared according to the method of OKANISHI *et al.* (4) with a slight modification (2). The transformation of chromosomal DNA was performed as follows: 0.2 ml of the protoplast suspension (*ca.* 2×10^8 cfu/ml) was mixed with 0.1 ml of P medium (4) containing varying amounts of wild-type DNA, and then with 0.3 ml of 40% (w/v) PEG 4000 (2). The mixture was incubated for 5 min at room temperature. After being diluted with an amount of P medium equal to 4 times its own volume, it was spun down by centrifugation and gently suspended in 0.8 ml of P medium. An aliquot of this solution was plated on the minimal regeneration medium supplemented with 50 μ g/ml of L-histidine or L-cysteine. After incubation at 28° for 10 days, colonies were counted. The effect of DNA amount or DNase treatment on the transformation frequency is shown in Table 1. The transformation frequency of Cys⁺ with DNA of wild-type *S. parvulus* was 1×10^{-6} with 2.5 μ g of DNA used. The reversion frequency of *cys* marker was 5×10^{-8} . Also, it was shown that the PEG-induced genetic transformation is sensitive to DNase treatment (Table 1). When spore suspensions of 35 Cys⁺ transformations were plated on the minimal medium (5) supplemented with L-cysteine or L-histidine, all clones turned out to be of

Table 1. Effect of amount of DNA on the transformation of *S. parvulus* 123 with chromosomal DNA.

DNA amount (μ g)	Cys ⁺ transformants among regenerants
2.5	1.0×10^{-6}
0.5	4.5×10^{-7}
0.13	3.5×10^{-7}
2.5 + DNase ^a	5×10^{-8}
0	5×10^{-8}

^a The chromosomal DNA was digested with 10 μ g/ml of DNase I in P medium at 37° for 60 min.

^b cfu: colony-forming units.

90 ml of S medium (4) plus 1.5% glycine supplemented with 50 μ g/ml of L-cysteine and L-histidine was inoculated with spores of *S. parvulus* 123, followed by cultivation for 2 days at 30° on a reciprocal shaker. Mycelia were harvested, washed with 0.3 M sucrose by centrifugation, and suspended in 3 ml of lytic mixture. The latter consisted of a P medium containing 2 mg/ml of lysozyme and 0.2 mg/ml of lytic enzyme No. 2 (Kyowa Hakko Co.). The mixture was incubated at 30° for 60 min. After the suspension of protoplasts, the mixture was washed with the P medium through centrifugation (2). The transformation was performed as described in the text. The regeneration number of protoplasts on the supplemented minimal regeneration medium was 2×10^8 cfu^b/ml.

Chromosomal DNA from wild-type *S. parvulus* 2266 was purified as follows. S medium plus 1.5% glycine was inoculated, followed by cultivation for 3 days at 30°. Mycelia were harvested and treated with lytic mixture. The DNA from protoplasts was purified by a combination of phenol extraction and RNase treatment.

Table 2. Effect of cell age on the transformation of *S. parvulus* 123 with chromosomal DNA.

Cell age (days)	DNA amount (μ g)	Transformants among regenerants	
		Cys ⁺	His ⁺
2	3.0	1.0×10^{-6}	3.0×10^{-7}
2	0	$<5 \times 10^{-8}$	$<5 \times 10^{-8}$
3	3.0	3.0×10^{-8}	1.0×10^{-8}
3	0	$<5 \times 10^{-9}$	$<5 \times 10^{-9}$

The transformation was performed as described in the text. The regeneration number at cell ages of *S. parvulus* 123 protoplasts on supplemented minimal regeneration medium were 2.1×10^8 cfu/ml at 2 days and 1.8×10^9 cfu/ml at 3 days cultivated in supplemented S medium plus 1.5% glycine.

Cys⁺His⁻ phenotype. Among the progeny of Cys⁺ transformants, essentially no Cys⁻ segregant appeared. These results indicate that the Cys⁺ colonies are true transformants produced by genetic recombination.

As shown in Table 2, protoplasts produced from a growing phase (2-day) culture were more efficient for transformation than those from the stationary phase (3-day) culture.

Streptomyces lividans could be used as the recipient of this transformation

system (data not shown). Although the transformation frequency is rather low, the results in this communication clearly indicate that *Streptomyces* strains have a recombination system with linear DNA. This system can be used for the genetic analysis of *Streptomyces*.

In general, the transformation frequency by linear form DNA is lower than that of closed form DNA. BIBB *et al.* (6) reported that the endonuclease-treated linear form DNA of SCP2 and SLP1.2 plasmid gave a 10–100 fold lower frequency of transformation than ligated DNA. This might be due to exonucleolytic degradation of linear molecules in the cells.

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