Direct Transfer of Plasmid DNA between *Streptomyces* spp. and *E. coli* by Electroduction

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We describe a simple and reliable method which allows the direct transfer of shuttle plasmids between *Streptomyces* spp. and *E. coli*. The method is based on the fact that plasmid DNA molecules can be released or taken up from cells under conditions of electroporation. When a suspension of a plasmid-containing *Streptomyces* spp. is mixed with electroporation-competent *E. coli* and submitted to an electric pulse, plasmid DNA transfer to the *E. coli* recipient takes place. Two different *Streptomyces* spp. (*S. lividans* TK23, or TK24 and *S. rimosus* 554w) were effective donors, and the method was successfully employed to transfer four different bifunctional vectors (pPM801, pFD666, pRL270X and pZG5) varying in size from 5.2~14.4 kb, to *E. coli*. This provides a convenient method for the analysis of Streptomycete transformants.

Genetic manipulation in Streptomycetes frequently requires plasmid analysis of recombinants. While *E. coli-Streptomyces* spp. bifunctional vectors are commonly used for DNA manipulations in *E. coli*, the isolation of plasmid DNA from Streptomycetes is sometimes difficult; yields are low and the product often contaminated with chromosomal DNA. Therefore a technique which permits direct transfer of plasmid DNA to *E. coli*, bypassing plasmid DNA purification, could allow significantly faster analysis of Streptomycete transformants.

Electroduction is a process that can be used for the direct transfer of plasmid DNA from donor to recipient cells under conditions of electroporation. The procedure depends on transient permeability as a result of the formation of small pores in cell membranes induced by short and intense electric pulses[1], and the fact that plasmid DNA can be successfully released[2] or taken up[3] during this process. Recent reports have demonstrated that simultaneous electroporation of two different bacteria can facilitate intra-[4] or inter-species[5,6] transfer of plasmid DNA. We have applied this procedure to the direct transfer of shuttle vectors between *Streptomyces* spp. and *E. coli*.

Materials and Methods

Electroporation

Electroporation was performed with a Gene Pulser unit, equipped with Gene Pulse Controller (Bio Rad), adjusted to a voltage of 12.5 kV/cm or 10 kV/cm, capacitance of 25 μF and 200 ohms or 400 ohms resistance as required. The time constants were typically 4.5 ms and 8.5~9 ms respectively.

Microorganisms, Plasmids and Culture Conditions

Bacterial strains and plasmids used in this study are shown in Table 1. TCA agar contains (in g/liter); Bactopeptone (Difco) 2; yeast extract 2; casamino acids 2.5; glucose 10; $MgCl_2$ and agar 1; pH 7.0. YEME medium used for *Streptomyces* spp. was as described[7]. Growth conditions, on LB-agar plates, for *E. coli* were as described[8]. Media were supplemented with kanamycin (25 μg/ml) or ampicillin (50 μg/ml) and proline or methionine (20 μg/ml) as required. SOC medium was added immediately after pulsing the cells[3].

Plasmid Isolation and Analysis

Qiagen Plasmid Isolation Kits (Qiagen Inc., Chadworth, Calif.) were used according to the manufacturer's instructions. Restriction enzymes (Gibco BRL, Life Technologies, Inc.) were used as recommended by the supplier. Agarose gel electrophoresis was as described[7].

Preparation of Strains for Electroduction

Donor

A loopfull of Streptomycete cells was removed from an agar plate, suspended in 1 ml of 10% glycerol, washed, centrifuged at 5000 rpm and resuspended in 40 μl of 10% glycerol.

Recipient

E. coli cells (Table 1) were prepared for electroporation as described (BioRad). Both strains used showed similar electroporation efficiencies of 2 to 2.5×10^6 transformants per 1 μg of pPM801.

We are pleased to dedicate this paper in recognition of the outstanding contributions of our good friend, SATOSHI ŌMURA to the science of secondary metabolites.

Table 1. Strains and plasmids used in this study.

Donor	Vector	Markers	Size (kb)	Ref.
Streptomyces lividans TK23	pPM801	Kan/Neo^R	14.4	(10)
Streptomyces lividans TK24	pRL270X	Kan/Neo^R	7.2	(11)
	pFD666	Kan/Neo^R	5.2	(12)
Streptomyces rimosus 554w	pZG5	Amp^R, Tsr^R	7.4	(13)

Recipient	Markers	Comments
Escherichia coli S17.1	F$^-$, *pro*, Sm^R, Tmp^R	Both strains lack restriction
Escherichia coli WA802	F$^-$, *met*	endonuclease activity

Table 2. Electroduction procedure.

Direct Plasmid Transfer from *Streptomyces* spp. to *E. coli*

1. 40 μl or 80 μl of donor suspension was placed in a chilled electroporation cuvette (0.2 cm).
2. The cuvette was pulsed at 10 kV/cm, 25 μF and 400 ohm, and cooled in ice.
   Alternatively, the pulsed donor suspension (80 μl) was centrifuged (6,000 rpm, at 4°C for 10 minutes), in a cold sterile microfuge tube (500 μl), and the supernatant was transferred to a prechilled cuvette.
3. 40 μl of recipient cells prepared for electroporation were added to the cuvette with the donor suspension (or donor supernatant) and mixed.
4. The mixture was exposed to an electric pulse (12.5 kV/cm, 25 μF, and 200 ohms).
5. 1 ml of SOC was added and the mixture incubated for one hour at 37°C.
6. The cell suspension was plated on selective media.

Table 3. Electroduction results using various procedures.

Donor	Vector	Method	Electroductant	
			WA802	S17.1
Streptomyces lividans TK23	pPM801	One pulse	7	8
		Two pulses	40	30
		p-cs-p[a]	286	n.d.[b]
Streptomyces lividans TK24	pRL270X	Two pulses	12	n.d.
		p-cs-p	15	n.d.
	pFD666	Two pulses	8	n.d.
		p-cs-p	83	n.d.
Streptomyces rimosus 554w	pZG5	Two pulses	20	n.d.

[a] pulse - cold spin - pulse method, [b] not done.

Results and Discussion

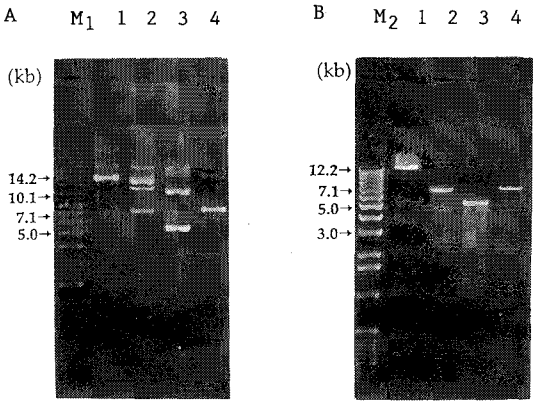
Bacterial donor and recipient cells (Table 1) were prepared as described in Methods. In most experiments donor strains were harvested directly from the agar plate; electroduction with liquid cultures, was successfully performed with cells in late stationary phase (not shown), but the efficiency was significantly lower.

Field strength intensity and pulse length were varied to obtain the maximum number of electrotransductants. Frequent arcing occurred if the maximum field (12.5 kV/cm) was applied, therefore 10 kV/cm was routinely applied to the donor mycelium. Pulse length (time constant) was varied by selecting resistors in a range from 200 ohms to 800 ohms, with constant capacitance of 25 μF and field strength of 10 kV/cm. Maximum yields of electroductants were obtained with a resistance of 400 ohms. The time constant (pulse length) was typically between 8.5 ~ 9 ms (data not shown).

The method for direct transfer of plasmid DNA from *Streptomyces* spp. to *E. coli* is summarised in Table 2. This procedure permitted transfer of shuttle vectors varying in size from 5.2 kb to 14.4 kb from *Streptomyces lividans* TK23 or TK24 and *Streptomyces rimosus* 554w directly to *E. coli* S17.1 or WA802 (Table 1). Electroduction results are shown in Table 3. Three different techniques were employed: a) one pulse: a mixture of donor and recipient was pulsed once under conditions optimized for *E. coli* (step 4-Table 2); b) two pulse: a suspension of donor cells was pulsed as described, and

Fig. 1. Plasmid DNA analysis of electroductants.

(A) Plasmid DNA isolated from recipient cells: M_1, supercoiled ladder DNA (GibcoBRL); 1, pPM801 (14.4 kb); 2, pRL270X (7.2 kb); 3, pFD666 (5.2 kb); 4, pZG5 (7.4 kb); (B) Plasmid DNAs were digested at the unique *Hind* III site: M_2, 1 kb DNA ladder (GibcoBRL), lanes from 1~4, order as described for panel A.



a suspension of recipient cells was added directly to the same cooled cuvette and pulsed again; or c) pulse-cold spin-pulse: a pulsed suspension of donor cells was centrifuged at 4°C/6000 rpm for 10 minutes (alternative protocol, Table 2.) Mixing the donor with recipient without an electric pulse or shocking the recipient alone and mixing with donor did not yield electroductants. The highest frequencies of electroduction were obtained with the pulse-cold spin-pulse method. In the first few milliseconds after electrical pulsing, newly formed pores expand to 20~120 nm in diameter[1]; once formed they shrink rapidly (in milliseconds) at temperatures between 30° and 42°C, but only slowly (minutes to hours) below 10°C[9]. We suggest that centrifugation of the pulsed mycelium enhances transformation efficiency by more efficient release of plasmid DNA. Alternatively, centrifugation may remove interfering cell debris from the DNA-containing supernatant used to electroporate *E. coli*.

Plasmid DNA was isolated from several electroductants (Fig. 1-A) and examined by restriction analysis (Fig. 1-B). Although the mechanism of electroporation and transfer of plasmid DNA through the bacterial cell membrane remains unclear, we confirm and extend earlier observations that electroduction is an effective way to promote gene transfer between microorganisms[4~7]; such gene transfer mechanisms may take place under natural conditions[14].

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