UDC 579.252.5:579.873.71:579.842.11
ISSN 1330–9862

preliminary comunication

(FTB-1039)

High Efficiency of Direct Plasmid Transfer between Two *Streptomyces* spp., and between *Streptomyces* spp. and *E. coli*

*Goran Gregorović and Dušica Vujaklija**

Department for Molecular Genetics, Institute Ruđer Bošković, Bijenička c. 54, 10000 Zagreb, Croatia

Received: June 9, 2000
Accepted: October 26, 2000

Summary

Electroduction is a method which allows direct transfer of plasmid DNA between donor and recipient under conditions of electroporation. The application of this technique allows significantly faster analysis of streptomycetes transformants by omitting plasmid DNA purification. Here, we describe improved procedure with substantial modifications which allows direct plasmid shuttling between *Streptomyces* spp. and *E. coli*. Three different *Streptomyces* spp. (*S. lividans*, *S. rimosus* and *S. coelicolor*) and three *E. coli* strains were effective donors or recipients. The method was successfully applied to transfer three different bifunctional vectors: pFD666, pZG5 and pRM5, varying in size from 5 kb to 21 kb. The efficiency of plasmid transfer by novel method was significantly increased in comparison to our previously published results. In addition, we succeeded for the first time: a) to transfer plasmid of 21 kb, and b) to transfer plasmid DNA directly from *S. lividans* to *S. rimosus*.

Key words: electroduction, *Streptomyces*, *E. coli*, plasmid transfer, electroporation, horizontal gene transfer

Introduction

Electroduction is a useful technique that permits direct transfer of genetic material between bacteria. Though simple, this method is still not widely exploited. Under conditions of electroporation two processes occur independently. Cells exposed to high voltage could release *(1)* or accept (*2)* DNA molecules. Electroduction basically involves cellular suspension of donor and recipient exposed to a short-duration, high-amplitude electrical pulse, resulting in the reversible permeabilization of the cell membrane. Transfer of DNA molecules can occur both intraspecifically (*3)* or interspecifically (*4)*. The effect of the external electrical field on the creation of the non-specific pores in the cell membrane was observed on all kinds of organelles and cells. A systematic investigation of *E. coli* electrotransformation was described in a series of scientific papers (*5,6)*. Although actual mechanism by which large molecules as DNA pass through cell membrane is still not completely understood, recent studies describe this process as a similar multistep pathway always observed on prokaryotic and eukaryotic cells in spite of significant differences between cell types (*7)*. Some biological and physical parameters must be examined with respect to the species. Nevertheless, pulsing the cells has become convenient way to mediate direct gene transfer from donor to recipient cells.

We previously reported application of the electroduction method to transfer plasmid DNA molecules directly from *Streptomyces* spp. to *E. coli* bypassing plas-

* Corresponding author; Phone: ++385 (0)1 4561 111/ext. 145; Fax: ++385 (0)1 4561 177; E-mail: vujaklij@rudjer.irb.hr

mid DNA purification *(8)*. The basic method described earlier is useful, but it is limited for some *Streptomyces* species and unsuccessful in transferring plasmids larger than 15 kb in size. The extensive use of this method could simplify analysis of *Streptomyces* transformants considerably. This prompted us to examine different parameters in order to broaden the application of this method. Here, we describe a novel electroduction method. We examined the substantial modifications that allowed transfer of plasmid DNA from *E. coli* to *Streptomyces rimosus* and between different *Streptomyces* species. We also succeeded to transfer plasmid over 20 kb in size.

Materials and Methods

Bacterial Strains and Plasmids

S. lividans TK24 (*9*), *S. rimosus* R6–593 (*10*), *S. coelicolor* CH999 (*11*), *E. coli* GM119 (*12*), *E. coli* XL1-Blue (*13*) and *E. coli* S17.1 (*14*) were used as donors or recipients in this study. Bifunctional plasmids pFD666 of 5.2 kb (*15*), pZG5 of 7.4 kb (*16*) and pRM5 of 21 kb (*11*) were used for direct transfer by electroduction.

Media and Growth Conditions

Two different solid media were prepared for the growth of *Streptomyces*. TCA medium that contains (in g/L): bactopeptone 2; $MgCl_2$ 1; yeast extract 2; glucose 10; casamino acids 2.5; and agar (*17*); and TSB that contains 30 g/L triptone soya broth. *Streptomyces* spp. were regenerated in CRM medium as previously described (*17*). Growth conditions for *E. coli* were as described (*18*). SOC medium (*19*) was added for regeneration of *E. coli* immediately after pulsing the cells. Media were supplemented with kanamycin (25, 50 or 350 μg/mL), ampicilin (100 μg/mL), tetracycline (50 μg/mL) or thiostrepton (20 μg/mL) as required.

Plasmid Isolation and Analysis

Plasmids were isolated with Qiagen Isolation kit (Qiagen Inc., Chadworth, USA) according to the manufacturer's instructions or by standard alkaline lysis method as previously described (*18*). The presence of plasmids in electroductants was analysed by standard gel electrophoresis method (*18*).

Preparation of Donor and Recipient for Electroduction

Donor

Streptomyces strains were grown on solid media containing the required antibiotic. A loopfull of mycelial fragments obtained by scraping small area was carefully removed from the plate to avoid contamination with agar. Therefore in some of our experiments *Streptomyces* spp. were grown on agar plates covered with cellophane membrane. Collected cell biomass was suspended in 80 μL of 10 % ice cold glycerol with no further treatment or it was treated with lysozyme (200 μg/mL) at 37 °C for 30 min. Treated cells were washed twice by centrifugation at 5000 rpm with 1 mL of 10 % glycerol and finally resuspended in 80 μL of ice cold 10 % glycerol.

E. coli colonies were used directly from LB plates supplemented with the required antibiotic. The cells were resuspended in 80 μL of 10 % ice cold glycerol and used as donors in electroduction experiments.

Recipient

Electrocompetent mycelia of *S. rimosus* R6–593 were prepared as described previously *(17)*. Transformation efficiency was $6.4 \cdot 10^4$ transformants per 1 μg of pFD666. *E. coli* cells were prepared for electroporation according to BioRad manual. The strain *E. coli* XL1-Blue showed electrotransformation efficiency of $1.5 \cdot 10^7$ transformants per 1 μg of pFD666, twice as high as the efficiency of *E. coli* GM119, when transformed with the same plasmid.

Electroporation

Gene Pulser Unit equipped with Gene Pulse Controller (BioRad) was used in all experiments. Electroporation cuvettes were EUROGENETEC with interelectrode distance of 2 mm. Gene Pulser parameters for pulsing *Streptomyces* spp. were set at 10 kV/cm, 400 Ω and 25 μF and for pulsing *E. coli* strains were set at 12.5 kV/cm, 200 Ω and 25 μF.

Electroduction procedure

Transfer of plasmid DNA from
Streptomyces spp. to *E. coli*

Donor biomass was resuspended in 10 % ice cold glycerol and treated with lysozyme when required as previously described. 80 μL of donor suspension was placed in prechilled cuvette and pulsed twice. Two times pulsed donor suspension was frozen at –80 °C for 30 min then thawed at 4 °C, transferred to prechilled cuvette and centrifuged at 13000 rpm for 15 min at 4 °C. Donor supernatant obtained as described was mixed with 40 μL of recipient cells prepared for electroporation and pulsed again. 1 mL of SOC medium was added and the mixture was incubated for 1 h at 37 °C and then plated on selective plates.

Transfer of plasmid DNA from *S. lividans* to *S. rimosus*

S. lividans (pFD666) was grown on selective solid medium supplemented with 50 μg/mL kanamycin. The mycelium to be used as donor was prepared and pulsed as described above. Electrocompetent mycelium of *S. rimosus* was mixed with donor supernatant and pulsed again. 1 mL of ice cold CRM medium was added, the cells were grown for 3 h at 30 °C and then plated on selective plates (*17*).

Transfer of plasmid DNA from
E. coli to *Streptomyces rimosus*

E. coli GM119 harbouring pFD666 was used as a plasmid donor. The cells were pulsed under conditions as described in *Electroporation*. Supernatant was mixed with electrocompetent mycelium of *S. rimosus* R6–593 and pulsed again. The cells were regenerated and plated on selective medium as described above.

Results and Discussion

Direct transfer of plasmid DNA between *Streptomyces* spp. and *E. coli* were performed as described in

Methods. Due to our previous data (*8*), electrical parameters were not varied. Fields strength intensity and pulse length were kept at optimum values for our model system, as described in electroduction procedure. The time constants for *E. coli* electroporation were typically 4–4.5 ms and for *Streptomyces* electroporation 7–9 ms. From the recent reports (*6*), it is known that the pulse duration, and consequently cell permeabilization, is a critical parameter for the gene transfer.

Several attempts to transfer plasmid pRM5 of 21 kb from *S. coelicolor* CH999 to *E. coli* S17.1 by already published electroduction method (*8*) have been unsuccsessful. This prompted us to examine the effect of freezing and thawing on the pulsed donor cells as an improvement of the basic protocol. Only by applying this additional step it was possible to transfer plasmid of 21 kb in size directly from *S. coelicolor* to *E. coli* (data not shown). We suggest that this process acts synergistically with electrical pulse, probably by affecting the integrity of the cell wall and thus enhancing the plasmid release from the cell.

In order to examine the effect on the efficiency of this and some other modifications in comparison to our basic protocol (*8*), the same donor strain, *S. lividans* TK24 harbouring pFD666 was used as a referent strain throughout this study. With previously published protocol, we were not able to get more than 100 electroductants, though the method was used routinely. With the same strain and by applying two additional steps we were able to get significantly increased number of electroductants. Table 1A, lane 1 shows the result of our typical experiment with *S. lividans* TK24 harbouring pFD666, when freezing and thawing of the donor cells were applied to the basic protocol. Furthermore, four times higher efficiency was obtained (Table 1A, lane 6) when donor cells were additionally pre-treated with lysozyme. The modified procedure described above was also applied to transfer plasmid pFD666 from *S. rimosus*. The number of electroductants was much lower (Table 1A, lane 4 compared to lane 1) when *S. rimosus* was used as donor, nevertheless the method was reproducible. This discrepancy could be ascribed to the different cell wall structure of these two *Streptomyces* species.

When pre-treatment with lysozyme was applied to *S. rimosus*, electroduction efficiency (Table 1A, lane 7) was slightly decreased. From our experience, fluctuation of transformation efficiency up to 50 % is not significant and it could be due to the amount of biomass taken by loop. In order to test whether the collection of micelia from the solid media would be easier, and to minimise the possibility of arcing during electrical pulsing, *S. rimosus* harbouring pFD666 was grown on cellophane. We obtained up to four times higher number of electroductants in comparison to results discussed above (data not shown). We suggest that this approach could be applied for the improvement of our electroduction method.

The electrodution procedure was also applied to transfer plasmid pZG5 from the same donors (*S. lividans* and *S. rimosus*). The result of our typical experiment when pZG5 was transferred from *S. lividans* is presented in Table 1A, lane 2. When *E. coli* XL1-Blue was used as recipient the result was two times better in comparison to the number of electroductants obtained with *E. coli* GM119. This result nicely correlates with the transformation efficiencies (see Methods) of these two recipients. Application of freezing and thawing for transferring pZG5 from *S. rimosus* was fallible. Few electroductants were obtained in one out of five performed experiments and therefore we consider this result negative. Successful and reliable method was achieved when pre-treatment of donor mycelia with lysozyme was included as described in Methods (Table 1A, lane 8). Microscopy of mycelia after treatment with lysozyme in 10 % glycerol revealed no presence of protoplasts, however the release of the plasmid was still sufficient for obtaining the electroductants in all performed experiments. Thus with minor modifications this method was successfully applied to *S. rimosus* strain. Although we assume that by pulsing the protoplasts more plasmid DNA could be released, taking into account the time needed for protoplast preparation with standard procedure and additional exchange of the buffer in order to avoid arcing (overheating), we suggest that protoplast preparation should be applied only to special cases.

Application of lysozyme treatment and freeze thawing of the donor cells were substantial for successful transfer of plasmid DNA from *S. lividans* to *S. rimosus*. This is the first example of plasmid shuttling between two *Streptomyces* species by electroduction. Five times higher efficiency was obtained when donor cells were pre-treated with lysozyme. The results are presented in Table 1B. The described procedure was also successfully applied to transfer plasmid from *E. coli* to *S. rimosus* (data not shown). *E. coli* GM119 (*dam-dcm-*) strain, defi-

Table 1. (A) Results of typical electroduction experiments using differently prepared donor suspensions. The plasmid DNA was transferred from *Streptomyces* spp. to different strains of *E. coli*

Procedure	Donor	Plasmid	Recipient	Number of electroductans
2p, –80 °C[a)]	*S. lividans* TK24	pFD666	XL1-Blue	422
		pZG5	XL1-Blue	650
			GM119	355
	S. rimosus R6–593	pFD666	XL1-Blue	27
		pZG5	XL1-Blue	n.r.
lys, 2p, –80 °C[b)]	*S. lividans* TK24	pFD666	XL1-Blue	1742
	S. rimosus R6–593	pFD666	XL1-Blue	18
		pZG5	XL1-Blue	9

Table 1. (B) Results of electroduction of plasmid DNA transfer from *S. lividans* TK24 to *S. rimosus* R6–593

Procedure	Donor	Plasmid	Recipient	Number of electroductans
2p, –80 °C[a)]	*S. lividans* TK24	pFD666	*S. rimosus* R6–593	430
lys, 2p, 80 °C[b)]	*S. lividans* TK24	pFD666	*S. rimosus* R6–593	2270

[a)] »two pulses – freeze donor – pulse« method
[b)] »lysozyme treatment – two pulses – freeze donor – pulse« method
n.r. – not reliable

cient in DNA methylation was used as plasmid DNA donor to avoid restriction barrier in *S. rimosus*.

Evidence for the presence of pFD666 in electroductants achieved in experiments described above (Table 1A,B) was also obtained directly. Whenever *Streptomyces* were transformed by electroduction, the plasmid was then transferred directly to *E. coli* to avoid tedious plasmid purification from *Streptomyces* as well as to get the results faster (Fig. 1).

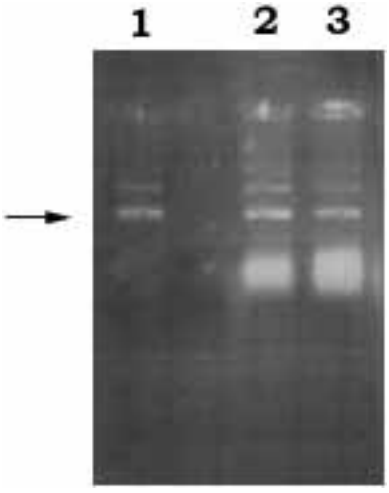

Fig. 1. Isolation of plasmid pFD666 from *E. coli*. Lane 1 – control pFD666; lane 2 – pFD666 isolated after shuttling from *E. coli* GM119 to *S. rimosus* R6–593 and back to *E. coli* XL1-Blue; lane 3 – pFD666 isolated after shuttling from *S. lividans* TK24 to *S. rimosus* R6–593 and then to *E. coli* XL1-Blue

These fast shuttling of plasmid DNA from *Streptomyces* to *E. coli* and back, or from *S. lividans* to *S. rimosus* reveals the power of the method. It can be routinely used for manipulation with bifunctional vectors and moreover, it is significantly time saving in analysis of *Streptomyces* transformants.

Conclusions

A novel electroduction method significantly improved direct transfer of plasmid DNA between distantly and closely related bacteria. Essential steps include freeze thawing of donor cells and pre-treatment with lysozyme. Described modifications enabled successful transfer of large size plasmid (21 kb). The method was also successfully applied to transfer plasmid directly from *S. lividans* to *S. rimosus*. This is the first example of shuttling bifunctional vector between two *Streptomyces* spp.

Acknowledgements

We thank Dr. Vera Gamulin and Dr. Jasenka Pigac for critical reading of the manuscript and for many useful discussions. We also thank Dr. Andreja Mikoč for the supply of *S. rimosus* electrocompetent cells. This work was supported by Croatian Ministry of Science and Technology, grant No. 00981003.

References

1. D. Heery, R. F. Powell, F. Gannon, L. K. Dunican, *Nucleic Acids Res. 17* (1989) 10131.
2. W. J. Dower, J. F. Miller, W. Ragsdale, *Nucleic Acids Res. 16* (1988) 6127–45.
3. D. K. Summers, H. K. Withers, *Nucleic Acids Res. 18* (1990) 2192.
4. R. Marcil, D. R. Higgins, *Nucleic Acids Res. 20* (1992) 917.
5. T. D. Xie, T. Y. Tsong, *Biophys. J. 63* (1992) 28–34.
6. N. Eynard, F. Rodriguez, J. Trotard, J. Teissie, *Biophys. J. 75* (1998) 2587–2596.
7. N. Eynard, M. P. Rols, V. Ganeva, B. Galutzov, N. Sabri, J. Teissie, *Bioelectroch. Bioener. 44* (1997) 103–110.
8. D. Vujaklija, J. Davies, *J. Antibiot. 48* (1995) 635–637.
9. D. A. Hopwood, M. J. Bibb, K. F. Chater, T. Kieser, C. J. Bruton, H. M. Kieser, D. J. Lydiate, C. P. Smith, J. M. Ward, H. Schrempf: *Genetic Manipulation of Streptomyces: A Laboratory Manual*, The John Innes Foundation, Norwich (1985).
10. B. Gravius, D. Glocker, J. Pigac, K. Pandža, D. Hranueli, J. Cullum, *Microbiology, 140* (1993) 2271–2277.
11. R. McDaniel, S. Ebert-Khosala, D. A. Hopwood, C. Khosla, *Science, 262* (1993) 1546–1550.
12. H. W. Boyer, D. Roulland-Dussoix, *J. Mol. Biol. 41* (1969) 459–472.
13. W. O. Bullock, J. M. Fernandez, J. M. Short, *BioTechniques, 5* (1987) 376.
14. P. Mazodier, R. Petter, C. Thompson, *J. Bacteriol. 171* (1989) 3583–3585.
15. F. Dennis, R. Brzezinski, *Gene, 111* (1992) 115–118.
16. S. Durajlija, J. Pigac, V. Gamulin, *FEMS Microbiol. Lett. 67* (1991) 317–321.
17. J. Pigac, H. Schrempf, *Appl. Environ. Microbiol. 61* (1995) 352–356.
18. J. Sambrook, E. F. Fritsch, T. Maniatis: *Molecular Cloning: A Laboratory Manual*, 2nd ed., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York (1989).
19. W. J. Dower, J. F. Miller, C. W. Ragsdale, *Nucleic Acids Res. 16* (1988) 6127–6145.

Velika učinkovitost izravnog prijenosa plazmida između dviju streptomicetnih vrsta te između bakterija *Streptomyces* spp. i *E. coli*

Sažetak

Elektrodukcija je metoda koja omogućuje izravan prijenos plazmidne DNA između donora i recipijenta, pod uvjetima elektroporacije. Stanična suspenzija donora ili recipijenta, izložena kratkom i intenzivnom električnom pulsu, dovodi do reverzibilne propusnosti stanične stijenke i membrane, što stanicama omogućuje slobodno otpuštanje ili primanje plazmidnih DNA molekula. Tako uzgoj stanične biomase i pročišćavanje plazmidne DNA postaju nepotrebni, pa zbog toga ta metoda omogućuje puno bržu analizu streptomicetnih transformanata. U ovom je radu prikazana poboljšana metoda koja s dodatnim izmjenama omogućuje izravan prijenos plazmidne DNA između bakterija *Streptomyces* spp. i *E. coli*. Tri različite streptomicetne vrste (*S. lividans*, *S. rimosus* i *S. coelicolor*) te tri soja bakterije *E. coli* služili su kao djelotvorni donori ili recipijenti. Metoda je uspješno primijenjena u prijenosu triju različitih dvojnih vektora: pFD666, pZG5 i pRM5, koji se razlikuju po veličini od 5 do 21 kb. Djelotvornost prijenosa plazmidne DNA ovom metodom bitno je povećana u usporedbi s dosada objavljenim rezultatima autora. Štoviše, po prvi su put autori uspjeli: a) izravno prebaciti plazmid veličine 21 kb i b) prebaciti plazmid izravno iz *S. lividans* u *S. rimosus*.