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## Abstract

We report the transformation of swollen A. nidulans conidia by electroporation. With this method, transformation frequencies were similar to those obtained by using protoplast fusion. The methodology employed is simple, requiring no enzymes nor osmotic stabilizers. The effects of conidial age, DNA topology/concentration and electric field strength are presented

# Efficient Transformation of *Aspergillus nidulans* by Electroporation of Germinated Conidia

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We report the transformation of swollen *A. nidulans* conidia by electroporation. With this method, transformation frequencies were similar to those obtained by using protoplast fusion. The methodology employed is simple, requiring no enzymes nor osmotic stabilizers. The effects of conidial age, DNA topology/concentration and electric field strength are presented.

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*A. nidulans* transformation by protoplast fusion is highly dependent on a good enzyme-mediated protoplast preparation (Ballance et al. 1983 Biochem. Biophys. Res. Commun. **112**:284-289, Tilburn et al. 1983 Gene **26**:205-221, Yelton et al. 1984 Proc. Nat. Acad. Sci. USA **81**:1470-1474). During this process, there is a serious compromise between protoplast yield and viability, which are greatly affected by variations in different enzyme lots, mycelial age and osmotic medium composition.

Electroporation as an alternative transformation method has proven successful for different types of fungi (Sánchez et al. 1993 Appl. Environ. Microbiol. **59**:2087-2092, Xoconostle-Cázares et al. 1996 Microbiology **142**:377-387, Vann, C., 1995 Fungal Genet. Newsl. **42A**:53). However, the only method reported for *A. nidulans* (Richey et al. 1989 Phytopathology **79**:844-847) still required protoplast production. We have adapted a previously reported electroporation method (Sánchez et al. 1993 Appl. Environ. Microbiol. **59**:2087-2092), to transform germinated conidia at frequencies similar to those obtained by protoplast fusion.

Conidia from strain RMS011 (*pabaA1, yA2, argB::trpC B, veA1, trpC801*; M. Stringer), washed 5 times with 10 ml of distilled water were used to inoculate 400 ml of Kafer's minimal-nitrate medium plus supplements, at a density of 1 X $10^7$ conidia/ml and incubated at 37 C in a rotary shaker (300 rpm) for 0, 2 or 7 h. Conidia recovered by centrifugation were resuspended in 400 ml of ice-cold sterile water, centrifuged again, resuspended in 25 ml of ice-cold pretreating buffer YED (1% yeast extract, 1% glucose) plus 12.5 mM DTT and 20 mM HEPES (adjusted to pH 8.0 with 100 mM Tris) and incubated for 1 h at 30 C in a rotary shaker at 100 rpm. Although the experiments reported here included DTT, we later found that it had no effect on transformation frequency. After this 60 min incubation, conidia were centrifuged and resuspended in 2.5 ml (about 1.6 X $10^9$ conidia/ml final) of ice-cold electroporation buffer (10 mM Tris-HCl [pH 7.5], 270 mM sucrose, 1 mM lithium acetate) and kept on ice. For electroporation, 1 ug of dialyzed DNA was added to 50 ul of the ice cold conidial supension. The final volume was adjusted to 60 ul with distilled water, the mixture was incubated on ice for 15 min and then transferred to a 0.2-cm cuvette. Electroporation was performed using the Bio-Rad Gene Pulser and Pulse Controller Apparatus. Voltage was adjusted to 1,000 V, capacitance to 25 uF and resistance was 400 Ohms (pulse length varied between 5.1 and 5.8 ms). Under these conditions, about 35 % of the conidia were killed.

Following electroporation, 1 ml of ice-cold YED was added to the cuvette and the cell suspension was transferred to a sterile 10 ml tube, kept on ice for 15 min and incubated at 30 C for 90 min in a rotary shaker at 100 rpm. Conidia subjected to electroporation were spread on supplemented minimal plates lacking arginine (250 ul/plate) and incubated at 37 C. Most of the plated conidia germinated on selective medium but failed to grow further; the actual transformants being evident after 48 h. Transformant stability was tested by velvet-replica plating in selective medium. Only healthy growing, well sporulated colonies were counted as transformants.

**Table 1**. Effect of conidial germination time and freezing-thawing on transformation frequency by electroporation[a].

| | Number of Transformants / ug DNA | | | |
|---|---|---|---|---|
| Plasmid | 0 h | 2 h | 7 h | 2h thawed * |
| None | 0 | 0 | 0 | 0 |
| pDHG25 | 30 | 551 | 97 | 372 |

[a]Conidia were germinated for the indicated times and electroporated, using

1 ug of the autonomous replicating plasmid pDHG25.

* 250 ul aliquots of 2 h germinated electrocompetent conidia were transferred to -70 C, stored for several days, thawed by incubating on ice and electroporated. Salt traces in DNA were removed by spin-filtration trough water-equilibrated Sephadex-G25-80 minicolumns. Except for 0 h, numbers represent mean values from two independent experiments, with a maximum variation of 10 % about the mean.

**Table 2**. Effect of DNA topology and replication type on transformation frequency by electroporation [a].

| Type of DNA | No. of Transformants/ ug DNA |
|---|---|
| pDHG25, circular | 623 |
| pDHG25, linear (BamHI) | 1,189 |
| pREN2, circular | 11 |
| pREN2, linear (KpnI) | 19 |

[a] DNAs digested with the indicated enzymes were filtered through Sephadex and stored at 4 C (Navarro et al. 1996 Curr. Genet. **29**:352-359). For pDHG25, numbers represent mean values

from four independent experiments. For pREN2, numbers are mean values from duplicates. Maximum variations were 9 and 26 % about the mean, respectively.

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Using this protocol, and the autonomous replicating plasmid pDHG25, which carries the *argB* gene as a selective marker (Gems et al. 1991 Gene **98**:61-67), we evaluated the effect of germination time on transformation frequency. Results in Table 1 show that in the 2 h germination time the number of transformants per microgram of DNA was about 18 and 5 times more than those obtained after 0 and 7 h germination times, respectively. Only 2 h germinated conidia were used in all further experiments. The number of transformants obtained with pDHG25 using the 2 h germinated conidia is slightly higher than what we routinely obtain using protoplast fusion; 400-500 transformants/ ug DNA.

When frozen electrocompetent conidia were electroporated with pDHG25, transformation frequency was about 30 % lower than with unfrozen conidia (Table 1). The convenience of having frozen stocks from different strains ready to be transformed could compensate for this reduction. We used frozen conidia to test the effects of plasmid concentration and field strength parameters on transformation efficiency. Within the DNA range tested (100 to 2,000 ng) we observed a non-linear response and a plateau in the number of transformants after 500 ng of DNA (Figure 1). With Regards to the effect of field strength, we found that 5 kV/cm (1000 Volts/0.2 cm) resulted in the highest number of transformants (Figure 2).

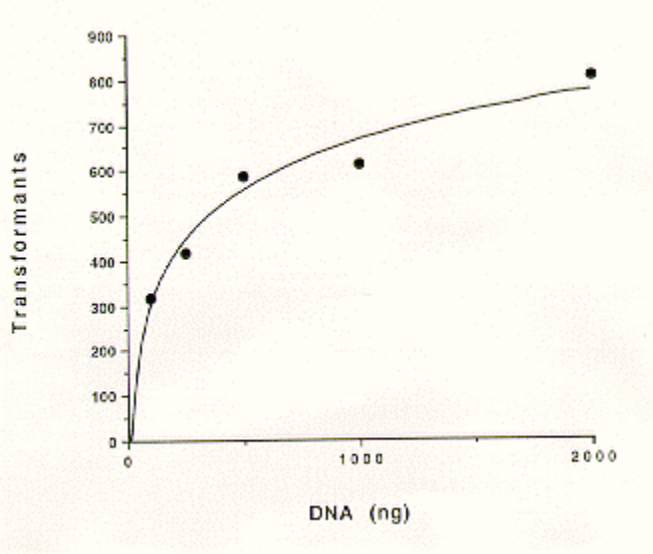


**Figure 1**. Effect of plasmid concentration on transformation efficiency. Frozen electrocompetent conidia were thawed, incubated with the indicated amounts of circular pDHG25 and electroporated. The results are means from duplicates with a maximum variation of 4 % about the mean.

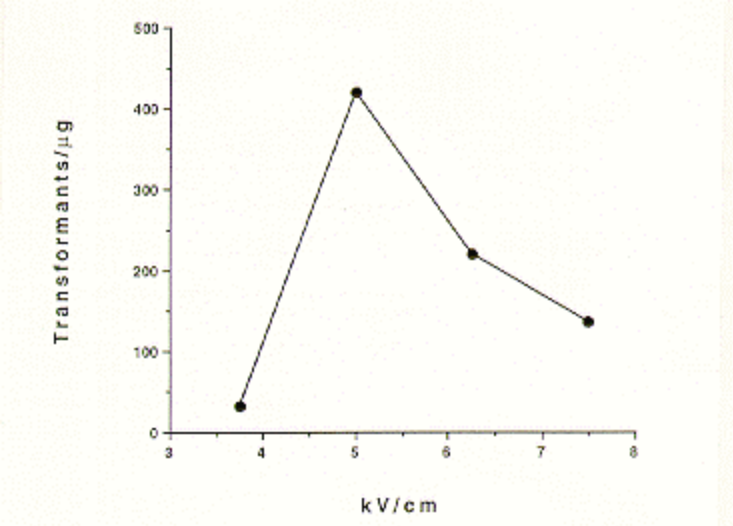


**Figure 2**. Effect of Field Strength on Transformation Frequency.50 ul aliquots from a frozen conidial pool were incubated with 1 ug of circular pDHG25 and electroporated using the indicated kV/cm.

DNAs with different topology and replication types were also tested for electroporation efficiency. Results in Table 2 show that transformation frequency for the integrative plasmid pREN2 was much lower than for the self replicating plasmid pDHG25. However, the number of transformants obtained with pREN2 is also similar to those obtained by us and others when using protoplast fusion (5-10 transformants/ ug; Upshall, A. 1986 Curr. Genet. **10**:593-599). Using linear forms of both types of plasmids increased transformation frequency about 2 times, perhaps reflecting an increased frequency of DNA entrance to the cells. Southern blot analysis of several transformants using *argB* and *catA* as probes showed that 8 out of 8 PDHG25-derived transformants contained *argB* sequences as part of the self replicating plasmid, whereas from 9 pREN2-derived transformants, 1 contained pREN2 sequences integrated at *catA* , 7 contained a single copy of pREN2 integrated at other genomic regions and 1 contained multiple integrations. Although we have not tested other electroporation apparatus, conditions described here are not too different from those reported for yeast (Becker and Guarente 1991 Methods in Enzymology **194**:182-187). Therefore, yeast protocols for other electroporators should be a good starting point, provided the use of germinated conidia and a self replicating plasmid to optimize conditions.

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