Transformation of filamentous fungi by electroporation

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Transformation systems have been reported for a number of filamentous fungi (1) based on the procedure perfected for Neurospora crassa by Case et al. employing sphaeroplasts (2). While efficient transformation is achieved with sphaeroplasts, their preparation demands a careful monitoring of the individual steps, along with optimization of conditions for each batch of cell wall-degrading enzymes. Therefore, a new efficient transformation system was developed involving direct electroporation of germinating conidia of N. crassa. Initially we employed a recombinant plasmid harboring the qa-2 + gene, encoding the catabolic dehydroquinase (2) in conjunction with a double mutant (qa-2 arom-9) recipient strain, devoid of both the biosynthetic and catabolic dehydroquinase activities. Fifteen-day-old conidia were grown in 0.5 × Fries’ medium (3) with aromatic amino acid supplements, for 4 h at 30°C, while shaking. Germinated conidia were washed and suspended in 1 mM HEPES buffer (pH 7.0)-50 mM mannitol and 100-μl (6 × 10^6) samples were mixed with 2 to 7 μg of plasmid DNA and subjected to electroporation using the Bio-Rad Gene Pulser apparatus. After electroporation, 1 ml Vogel’s minimal medium, Vm (3) was added and incubation resumed at 30°C for 3 h. Transformants were selected by plating on Vm-1.0% sorbose-0.1% glucose-0.1% fructose-1.5% agar.

Virtually no transformants were recovered when field strength ranging from 3.0 to 9 KV/cm was employed, with capacitance values of 1 and 3 μF. On increasing the field strength to 12.5 KV/cm, at 25 μF capacitance and 5 msec pulse length, 2 to 3 stable transformants per μg DNA were obtained. Raising the pulse length to 25 msec resulted in suppression of transformation efficiency. Assuming that the cell wall impedes the uptake of DNA, we added 1 mg/ml β-glucuronidase (Sigma: from Helix pomatia) to the growth medium for the final 2 h of germination. With this treatment a dramatic improvement in the efficiency of transformation was achieved: yields averaging 21 transformants per μg DNA. Following a slight modification of this procedure, conidia of another filamentous fungus, Penicillium urticae, were transformed to hygromycin resistance at an efficiency of 2.8 × 10^3 per μg DNA. We have described a rapid and effective means of transformation, without prior sphaeroplast formation, that will be readily applicable to other conidial as well as mycelial fungi.

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REFERENCES


Figure 1. Southern blot hybridization of genomic DNA of N. crassa transformants digested with EcoRI. The blot was first hybridized with a 2.2 kb BamHI fragment encoding the qa-2 + gene (A) and (after removal of the probe) with Bluescript vector DNA (B). In panels A and B, the lanes contain DNA (5 μg) as follows: 1, untransformed recipient strain; 2 to 5, DNA from transformants E-17, E-20, E-21 and E-22, respectively. Numbers on the left show position of size markers in kb.