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C. P. Selitrennikoff

R. E. Nelson

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Abstract

Putative transformation of Neurospora *os-1* protoplasts by a plasmid containing a G-481 phosphotransferase gene

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Selitrennikoff, C. P[1] and R. E. Nelson[2]

Putative transformation of Neurospora os-1 protoplasts by a plasmid containing a G-481 phosphotransferase gene.

Transformation of filamentous fungi (including Neurospora) using a number of plasmids has been reported (Stohl and Lambowitz, 1983 Proc. Natl. Acad. Sci. USA 80: 1058-1062; Case et al., 1979 Proc. Natl. Acad. Sci. USA 76: 5259-5263). This includes the use of plasmids carrying an appropriate gene into an auxotrophic host, as well as a variety of other selectable markers. Hosts typically are cells whose cell walls have been removed by treatment with wall-degrading enzymes and, after treatment, are allowed to regenerate cell-walls (e.g. Case et al., ibid).

In this communication we report the apparent transformation of a temperature-sensitive protoplast-forming os-1 strain of Neurospora crassa by plasmid pSV-3 neo. This plasmid contains a gene that codes for a phosphotransferase (Southern and Berg, 1982 J. Molec. Applied Genetics 1: 327-341) thus conferring resistance to Kanamycin or Antibiotic G-418. os-1 cells are sensitive to G-418 (see below), yet after treatment with purified pSV-3 neo plasmid DNA, G-418-resistant clones are recovered. The precise fate of the pSV-3 neo DNA is not known with certainty, yet available data are consistent with the idea that the plasmid (or at least the gene coding for the phosphotransferase) remains autonomous.

Protoplasts of os-1, nic-1 were formed and grown as described (Seltirennikoff et al., 1981 Exper. Mycol. 5 : 155-161). Cells (protoplasts) were harvested from log phase cultures by centrifugation (500 x g; 5 min) and washed twice with 50 mls ice-cold medium (SS: 7.5% (w/v) sorbitol, 1.5% (w/v) sucrose, IX Vogel's Salts, 10 µg/ml nicotinamide). Plasmid DNA was purified from an E. coli strain containing pSV3 neo by ethidium bromide - CsCl density centrifugation.

Purified supercoiled DNA (~ 4 µg) was mixed with ~ 15 µg high molecular weight salmon sperm DNA (carrier) in 250 µl buffer containing 145 mM NaCl, 21 mM HEPES, 0.7 mM NaH_2PO_4, pH 7.1. To this mixture 12.5 µl 2.5 M $CaCl_2$ was added and a precipitate allowed to form for 30 min at ambient temperature. This mixture was added to an os-1 cell-pellet containing ~ 5×10^7 cells, and incubated for 15 min at ambient temperature. Fresh medium (10 ml) was added and the culture shaken for 2 h at 25° C. Cells were shocked with glycerol (3 ml for 60 sec), 20 mls fresh medium added and cells harvested by centrifugation. Cell pellets were resuspended in warm (45° C) SS medium containing 1.0% agar and poured into petri dishes (final cell concentration 1×10^5 cells/ml). After 24 h incubation at 25° C, cells were overlayed with SS + 1.0% agar containing antibiotic G-418 (final concentration, 400 µg/ml). After seven days of incubation at 25° C, colonies were transferred to individual plates containing 200 µg/ml antibiotic G-418. No G-418 resistant colonies were recovered in control experiments (< 1 per 106 cells). About 40 colonies were recovered using 4 µg plasmid DNA. In each case resulting cultures had formed hyphae and macroconidia. To convert resultant conidia to protoplasts, conidia were harvested and germinated in Nelson's liquid medium A (10% (w/v) sorbose, 2% (w/v) sucrose) containing 200 µg/ml Polyoxin B and 200 µg/ml G-418 at 37° C. After 2 days of incubation with shaking, resulting protoplasts were transferred and maintained in SS + 200 µg/ml G-418 at 37° C.

TABLE I

Effect of G-418 on growth of os-1 protoplasts[a]

STRAIN	G-418 Concentration μg/ml	Time of incubation (Hours) 2	6	18	22
Control	0	6[b]	11	103	123
	50	5	10	45	63
	100	6	9	21	28
	200	4	7	18	23
Transformed	0	5	15	113	123
	200	4	10	84	96
	400	3	9	58	72
Transformed -G418	0	3	16	114	130
	200	2	6	28	37

[a]protoplasts of os-1,nic-1 were inoculated at about x 10^5 cells/ml and their growth (Klett units) recorded at the indicated times of incubation (SS medium containing the indicated final concentration of filter-sterilized G-418) at 37°C with shaking (140 rpm).

[b]klett units

Putative transformed cultures were tested for their sensitivity to G-418. The results presented in Table I show that control cultures were inhibited by as little as 50 μg/ml (50% inhibition) while 100 and 200 μg/ml inhibited growth by ~ 80%. The results using a putatively transformed strain are shown in Table I (Transformed). In contrast to the untreated controls, these cells were about 4-fold more resistant to G-418. When resistant cells were cultured for 7 days in the absence of G-418 and then retested for sensitivity, they were found to have lost their resistance (Table I, transformed-G-418). Further, the G-418-resistant phenotype was not recovered in the progeny of backcrosses to 74-OR23-1A wild-type (five putative transformed strains tested).

Overall, our data suggest successful transformation of os-1 protoplasts by pSV-3 neo DNA. The definitive proof will, of course, be the demonstration of plasmid sequences in Neurospora. We hope that our preliminary work will encourage others to examine this system - - -. **[1]Department of Anatomy, Health Sciences Center, Denver, CO 80262 and [2]Molecular Biology Institute, University of California at Los Angeles, Los Angeles, CA 90024.**