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Efficient transformation of germinating Neurospora conidia using total nuclear DNA fragments.

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Abstract

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We have developed a simple, reliable transformation method that does not require enzymic digestion of cell walls. This method exploits the tendency of *inl* and *os* strains to form swellings resembling sphaeroplasts on germ tubes when macroconidia are germinated in the absence of inositol in media of high osmolarity. Prototrophic transformants have been obtained from several auxotrophs of low or zero spontaneous reversion frequency, including the well defined deletion strain am132. Donor DNA was prepared from nuclear pellets by essentially the method of Hautala *et al.* (1977 J. Bacteriol. 130:704) and was usually used as linear fragments of average molecular weight at 2 x 10^7 without further shearing. DNA sheared to an average molecular weight of 5 x 10^6 gave similar results. Restriction fragments and single stranded DNA (the latter obtained by melting and rapid cooling of unsheared preparations of nuclear DNA as above) have also been used successfully. Detailed results will be reported elsewhere (submitted for publication).

In a typical experiment, 2 x 10^8 conidia of the recipient strain were germinated in 1 ml of Vogel's minimal medium containing 20% sucrose (w/v) and all required supplements except inositol for 3 to 5 hours at 30°C an an orbital shaker. When the majority of germ tubes showed swellings indicating weakened cell walls, the conidia were harvested by centrifugation, washed 3 times with Vogel's minimal liquid medium containing 1M-mannitol and suspended in 0.4 ml of this medium. Donor DNA (1 to 5 µg) was precondensed by mixing with 0.1 ml of 500 mM-$CaCl_2$ and added to the suspension of germinated conidia. Incubation was continued for 1 h at 30 C on an orbital shaker. Treated suspensions were diluted in 0.8M-mannitol for plating (either by spreading or in thin layers of soft agar) on sorbose medium containing 0.6M-mannitol and supplements as appropriate for selection of prototrophs or viability measurements. Viability of partially sphaeraplasted germinated conidia obtained by this method is usually greater than 90%. Control treatments were also included using DNA prepared from the recipient, DNase-digested DNA, and $CaCl_2$ alone.

Transformation frequencies obtained by this method using *inl* recipients ranged from 0.5 to 12.3 transformants per µg of DNA (1.5 x IO-7 to 4.2 x IO-E per viable recipient conidium), with a mode around 5 transformants per µg of DNA (1.5 x 10^{-6} per viable conidium). *os* recipients have given similar results. For comparisons, the same recipients have been used in transformations employing the mycelial fragment method described by Mishra and Tatum (1973, Proc. Natl. Acad. Sci. 70:3875) and a protoplasting method similar to that of Hinnen *et al.* (1978 Proc. Natl. Acad. Sci. 75:1929). Both of these methods gave transformation frequencies in the 0.04 to 0.12 transformants per µg of DNA (0.8 x 10^{-7} to 2.9 x 10^{-7} per viable fragment or protoplast plated), approximately 50-fold lower frequencies than those obtained by our method using germinating conidia.

The choice of *inl* strains may be important. Best results have been obtained with recipients carrying *inl* 37401 (FGSC 2145) and *inl* R233 crossed from an isolate in our collection originally obtained from S. R. Gross. However, the properties of *inl* R233 stocks changed after 3 backcrosses into STA4 background, and very low transformation frequencies were subsequently obtained. Presumably the kinetics of the lytic processes involved in inositol-less death are important for successful partial sphaeroplasting and may be influenced by genetic background.

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