Genetic Transformation of an argB Mutant of Aspergillus oryzae

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An argB mutant of Aspergillus oryzae NRRL 492 has been genetically transformed with the Aspergillus nidulans argB gene. Protoplasts were generated with a combination of Novozyme 234 and β-glucuronidase and regenerated on sucrose-stabilized minimal medium without arginine as described for A. nidulans. A frequency of 5 to 10 transformants per μg of DNA was obtained; however, most transformants appeared abortive. The A. nidulans argB gene and vector sequences appeared to be integrated into the A. oryzae chromosome.

Aspergillus oryzae is a filamentous fungus used for the production of certain fermented foods and as a source of enzymes used in food manufacturing. Recently, protocols have been reported for the transformation of Aspergillus nidulans by using several complementation (1, 5, 9, 11) and dominant (2, 3, 10) selectable markers. These approaches have also been used to transform Aspergillus niger (7). Iimura et al. (4) have recently reported transformation of A. oryzae by complementation of a met mutant. Using methodology similar to that developed for A. nidulans, we have successfully transformed an argB mutant of A. oryzae NRRL 492.

The vector pILJ16 (kindly provided by A. J. Clutterbuck, University of Glasgow, United Kingdom) is a pUC8 derivative carrying the A. nidulans argB gene (6). A corresponding argB mutant of A. oryzae NRRL 492 (kindly provided by the Northern Regional Research Center, U.S. Department of Agriculture, Peoria, Ill.) was obtained with UV irradiation at a killing rate of 99.9%. Putative argB mutants were screened by selection for the ability to grow on Czapek medium with DL-citrulline (200 mg/liter) but not on Czapek medium alone or supplemented with ornithine. Two argB mutants with stable phenotypes and with reversion rates of less than 1 in 10^9 were obtained from 5 x 10^7 survivors. One A. oryzae argB mutant was designated YTH-1 and used for transformation studies.

A. oryzae YTH-1 was transformed with the protocol described by Yelton et al. (11) for A. nidulans. A. oryzae YTH-1 protoplasts were produced with a combination of Novozyme 234 (kindly provided by Novo Laboratories, Inc.) and β-glucuronidase (Sigma Chemical Co.). Protoplasts were harvested after centrifugation on a 0.6 M sorbitol cushion at 4,000 x g. Cesium chloride gradient-purified pILJ16 (10 μg) was added to the protoplasts, and after polyethylene glycol fuson, transformants were selected on minimal medium containing 1.0 M sucrose as an osmotic stabilizer. Transformants arose after 3 to 5 days at 37°C at a frequency of approximately 5 to 10 transformants per μg of input pILJ16. Presumptive transformants were transferred to minimal medium (without arginine) for further purification. Approximately 90% of the initial transformants failed to grow, which suggested that they were abortive (5). Abortive A. nidulans transformants have been reported, although the exact mechanism resulting in the abortive event is not known (5).

To confirm the presence of integrated pILJ16 sequences, hybridization analysis of total chromosomal DNA was performed with pILJ16 as a probe. Chromosomal DNA was isolated from mycelia (8), digested with BamHI, and separated electrophoretically on a 0.8% agarose gel in TBE (98 mM Tris, 89 mM borate, 2 mM EDTA [pH 8.0]) buffer. After capillary transfer to nylon membrane (Gene Screen Plus; Du Pont Co.), the membrane was probed with pUC8 (or pILJ16) labeled with ^32P by nick translation (8). One 6-kilobase BamHI fragment, which hybridized to pUC8 (or pILJ16), corresponded in size to that expected for pILJ16 and appeared exclusively in the lane containing chromosomal DNA isolated from a transformant (Fig. 1 and 2). A second 8-kilobase band of lesser intensity was also observed and probably represents the junction fragment flanking a tandemly repeated copy of pILJ16 (Fig. 1 and 2). No positive hybridization was observed in either the original wild-type A. oryzae NRRL 492 or its argB derivative, A. oryzae YTH-1. This suggests that the A. nidulans argB gene is not homologous to the argB gene in A. oryzae under the stringent conditions used (2× SSC [1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate], 0.1% sodium dodecyl sulfate).

To confirm the integration event, total undigested chromosomal DNA isolated from A. oryzae YTH-1 transformed

FIG. 1. Southern hybridization analysis of total DNA isolated from A. oryzae parental strains and transformants. Lane 1, A. oryzae NRRL 492; lane 2, A. oryzae YTH-1; and lane 3, A. oryzae YTH-1 transformed with pILJ16. Filters were probed with ^32P-labeled pUC8 (A) and pILJ16 (B). Molecular weight markers noted on the left are λ DNA digested with HindIII.

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with pILJ16 was subjected to Southern hybridization analysis (Fig. 2). Hybridization with the pILJ16 probe was observed with the bulk migrating chromosomal DNA; no satellite band was observed to hybridize with pILJ16. The presence of pILJ16 in transformed A. oryzae YTH-1 was confirmed by recloning the integrated plasmid back into Escherichia coli HB101. Total A. oryzae YTH-1(pILJ16) was digested with HindIII, which liberates the entire pUC8 portion of pILJ16 along with a small 200- to 300-base-pair fragment from the argB gene. The restricted DNA was digested, ligated, and transformed into competent E. coli HB101; transformants were selected on the basis of ampicillin resistance. Restriction endonuclease analysis of the plasmids recovered from these E. coli HB101 transformants revealed that they contained the predicted regions of pILJ16 (data not shown). No transformants were obtained when undigested chromosomal DNA was used to transform E. coli HB101.

To date, all transformation events reported for Aspergillus spp. involve integration of the vector (9). The only exception is the report by Limura et al. (4), who reported that the A. oryzae transformants obtained by complementation of a met auxotroph with an A. oryzae met gene carried the vector in an extrachromosomal state. Their evidence was based upon the observation that the vector could be recovered by retransformation of E. coli with undigested total DNA isolated from A. oryzae transformants. In all other reports of transformation of Aspergillus spp., the vector integrates either at the homologous site of its marker or elsewhere in the chromosome. Since no extensive homology was observed between the A. nidulans argB marker and the A. oryzae argB gene, it is not possible to determine the location of integration. Given the absence of detectable homology, it is not possible to determine the nature of the integration event observed in our A. oryzae transformants.

LITERATURE CITED