

Selection of a new auxotrophic mutant by transformation-mediated gene disruption in *Podospira anserina*.

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A mutant auxotroph for methionine was isolated in *Podospira anserina* during a transformation experiment. The transforming plasmid (pPAaURA5) consisted of a 1.55 kb nuclear DNA fragment of *Podospira* containing the *URA5* gene (Begueret et al. 1984, Gene 32:487-492; Turq and Begueret 1987, Gene 53:201-209), and most (2.06 kb) of the *Podospira* intronic alpha mitochondrial sequence (Osiewacz and Esser 1984, Curr. Genet. 8:299-305) cloned in the pUC18 vector. The recipient strain carried the mutant *ura5-6* allele (Razanamparany and Begueret 1986, Curr. Genet. 10:811-817) and the mating plus locus (*mat+*). [In *Podospira*, transformation with plasmids occurs by integration of the vector mainly outside the resident locus (Brygoo and Debuchy 1985, Mol. Gen. Genet. 200:128-131; Razanamparany and Begueret 1986, Curr. Genet. 10:811-817).]

After transformation of the *ura5-6 mat+* strain with plasmid pPAaURA5 and selection of primary (*ura+*) transformants, the transformants were crossed to a *ura5-6 mat-* strain in order to purify the transformant nuclei through meiosis. In most cases, 50% *ura+* and 50% *ura-* spores were obtained in the progeny. However, one primary transformant gave different results: of 46 monocaryotic spores tested, 20 were auxotrophic for uracil, the 26 others were methionine auxotrophs.

Three purified *ura+* *met-* transformants were crossed with wild-type. The *met-* phenotype segregated as a single recessive gene. The percentage of second division segregation was about 60%. *ura-* spores were obtained in the progeny indicating that the parental transformant strain contained the *ura5-6* allele. Furthermore, the presence of tetrads containing 2 dicaryotic spores (*ura-* *met+*) and 2 dicaryotic spores (*ura+* *met-*) indicated the integration event of the *URA5* gene occurred in a chromosome different from that carrying the *URA5* locus. Three purified transformants (*ura+* *met-*) were crossed with the *ura5-6* strain. In the progeny, the two phenotypes (*ura+* and *met-*) were associated and segregated together.

These results indicate that the *met-* phenotype of the primary and purified transformants resulted from the integration of the *URA5* gene of plasmid pPAaURA5 in a gene involved in methionine biosynthesis. Analysis of the genomic DNA of this transformant has not been completed; we do not know if the entire plasmid or only the *URA5* gene has been integrated. The strain has been called *met1*. It is quite fertile and stable through vegetative growth as well as through meiosis. It grows on minimal medium supplemented with methionine, cysteine or homocysteine, but is not complemented by O-acetyl-homoserine. It constitutes the first example in *Podospira* of the isolation of mutants by transformation-mediated gene disruption.