

## Fungal Genetics Reports

---

Volume 38

Article 17

---

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

A. Sainsard-Chanet

*C.N.R.S.*

O. Begel

*C.N.R.S.*

Follow this and additional works at: <https://newprairiepress.org/fgr>



This work is licensed under a [Creative Commons Attribution-Share Alike 4.0 License](https://creativecommons.org/licenses/by-sa/4.0/).

---

#### Recommended Citation

Sainsard-Chanet, A., and O. Begel (1991) "Selection of a new auxotrophic mutant by transformation-mediated gene disruption in *Podospora anserina*," *Fungal Genetics Reports*: Vol. 38, Article 17.  
<https://doi.org/10.4148/1941-4765.1463>

This Regular Paper is brought to you for free and open access by New Prairie Press. It has been accepted for inclusion in Fungal Genetics Reports by an authorized administrator of New Prairie Press. For more information, please contact [cads@k-state.edu](mailto:cads@k-state.edu).

---

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

### Abstract

A mutant auxotroph for methionine was isolated in *Podospora anserina* during a transformation experiment. The transforming plasmid (pPAaURA5) consisted of a 1.55 kb nuclear DNA fragment of *Podospora* 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 *Podospora* intronic alpha mitochondrial sequence (Osiewacz and Esser 1984, Curr. Genet. 8:299-305) cloned in the pUC18 vector.

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

A. Sainsard-Chanet and O. Begel. Centre de Génétique Moléculaire, C.N.R.S., Allée de la Terrasse, 91198 Gif-sur-Yvette, France.

A mutant auxotroph for methionine was isolated in *Podospora anserina* during a transformation experiment. The transforming plasmid (pPAaURA5) consisted of a 1.55 kb nuclear DNA fragment of *Podospora* 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 *Podospora* 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 *Podospora*, 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 *Podospora* of the isolation of mutants by transformation-mediated gene disruption.