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Cloning of the mating-type gene *MAT*A of the yeast *Yarrowia lipolytica*

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Summary. The mating type gene *MAT*A of the dimorphic yeast *Yarrowia lipolytica* was cloned. The strategy used was based on the presumed function of this gene in the induction of sporulation. A diploid strain homozygous for the mating type *B* was transformed with an integrative gene bank from an *A* wild-type strain. A sporulating transformant was isolated, which contained a plasmid with an 11.6 kb insert. This sequence was rescued from the chromosomal DNA of the transformant and deletion mapping was performed to localize the *MAT* insert. The *MAT* gene conferred both sporulating and non-mating phenotypes on a *B*/*B* diploid. A *LEU2* sequence targeted to this locus segregated like a mating type-linked gene. The *A* strain did not contain silent copies of the *MAT* gene.

Key words: Mating type – *Yarrowia lipolytica* – Protoplast fusion – Sporulation – Conjugation

Introduction

The dimorphic yeast *Candida lipolytica* was transferred from an imperfect species group to the perfect genus *Saccharomycopsis* (Wickerham et al. 1970) and later reclassified as *Yarrowia* (van der Walt and von Arx 1980) after the discovery of the sexual cycle. Sexuality comprises mating (conjugation) of haploid cells belonging to two different mating types *A* and *B*, and sporulation of the diploid, followed by formation in most cases of four haploid ascospores per ascus (Ogrydziak et al. 1978). The complete sexual cycle of *Y. lipolytica* has been established from these observations, and shown to be similar to that of *Saccharomyces cerevisiae*. However, there are several physiological and morphological differences between the sexual cycles of the two species, presumably due to the dimorphic growth of *Y. lipolytica* (Weber et al. 1988) *Y. lipolytica* has one mating-type locus at which either a *MAT*A or *MAT*B allele resides, determining the *A* and *B* mating type, respectively. Changes of the mating type, which are indicative of the existence of homothallic strains, have not been described. Normally the mating efficiency of *Y. lipolytica* is very low (~0.1%), and even after improvement by inbreeding the frequency does not exceed 10–12% (Weber and Kurischko 1989). Against this background no mutants for mating (changes in mating behavior, sterility) have been isolated.

There has been no genetic analysis of the structure and function of the mating type gene. We therefore decided to clone the *MAT* gene for further analysis. Several mating-type genes have already been cloned from the yeasts *S. cerevisiae* (Hicks et al. 1979; Nasmyth and Tatchell 1980), *Schizosaccharomyces pombe* (Kelly et al. 1988) and the filamentous fungi *Podospora anserina* (Picard et al. 1991), *Neurospora crassa* (Glass et al. 1988), *Coprinus cinereus* (Mutasa et al. 1990), *Ustilago maydis* (Schulz et al. 1990) and *Schizophyllum commune* (Giasson et al. 1989). Some of these species *(S. cerevisiae, S. pombe, U. maydis)* contain homeobox-like sequences in the mating-type genes, supporting the presumption that these genes code for DNA-binding proteins. In this paper we describe the strategy for cloning the mating type gene *MAT*A of *Y. lipolytica* and discuss its possible functions.

Materials and methods

Strains. Strain III–04 *(MAT*B *ura2 lys1 leu2–35)* from the INRA Grignon collection and 1–K297 *(MAT*A *lysB)* from the collection of ZIMET Jena were crossed. After three back-crosses with strains 12–K335 *(MAT*B *adeA ura3 hisA)* and 40–K354 *(MAT*B *lysA metA ura3)* of the ZIMET collection, strains 46–KF6 *(MAT*B *hisA leu2–35 lysA adeA)* and 52–KF6 *(MAT*B *leu2–35 lysA ura3)* were selected. These two strains were combined by protoplast fusion. Other strains used were E122 *(MAT*A *leu2–270 ura3–302 lys11–23)* and Pa *(MAT*B *his1)* from the INRA collection. The designations of the respective

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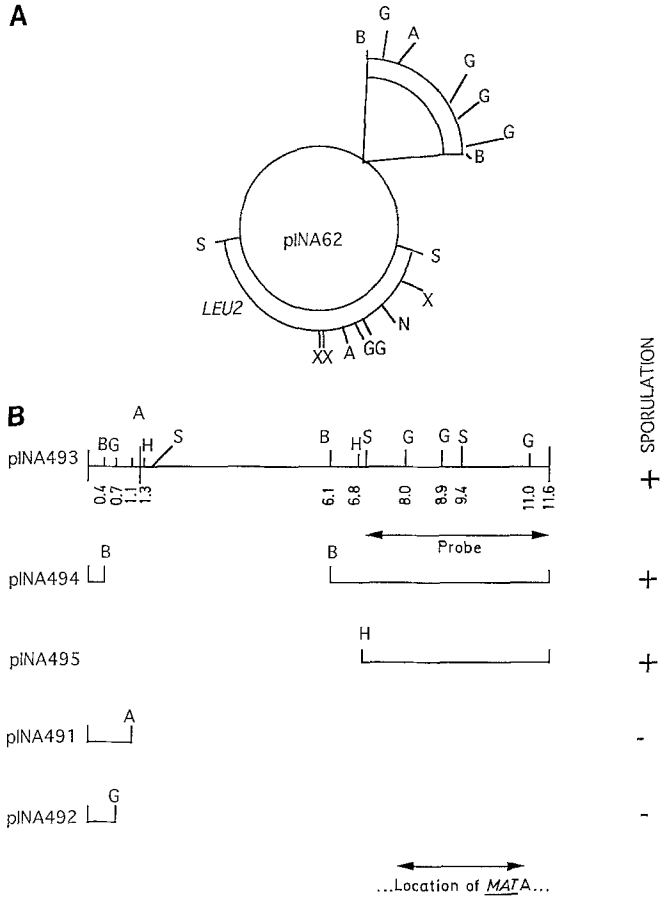

Fig. 1. A Restriction map of a typical plasmid contained in the gene bank. **B** Deletion mapping of the cloned insert, remaining sequences are shown. Plasmids pINA62, pINA493, pINA494 and pINA495 were integrated at the *leu2* locus after digestion at the *Not*I site in pINA62. Plasmids pINA491 and pINA492 were integrated after *Xho*I digestion. It should be noted that one of the *Xho*I sites present in the *LEU2* part of pINA62 was eliminated in pINA491 and pINA492 as these two plasmids were recloned from the genome by either an *Apa*I digestion or a *Bgl*II digestion. When these plasmids are linearized by *Xho*I the 30 bp sequence between the two very closely located *Xho*I sites is repaired upon integration at the *leu2* locus. The fragment used as probe is shown by an *arrow* and consisted of two 2.4 kb *Sal*I fragments isolated from pINA493 (one of the *Sal*I sites originates from pBR322). Restriction site abbreviations: A, *Apa*I; B, *Bam*HI; G, *Bgl*II; H, *Hin*dIII; N, *Not*I; S, *Sal*I; X, *Xho*I

genes reflect the different origins of the mutants and the absence of identification of complementation groups.

Plasmids. pINA62 contains the *LEU2* gene of *Y. lipolytica* on a 5.3 kb *Sal*I fragment cloned in pBR322 and has been described previously (Gaillardin and Ribet 1987) (Fig. 1A). The DNA gene bank has already been described (Xuan et al. 1988). The plasmids constructed in this work are shown in Fig. 1B.

Media. Yeast-malt-extract medium (YM) was used for matings (Wickerham 1951), and citrate medium (CSM) for sporulation (Barth and Weber 1985).

Protoplast fusion. The experiments were carried out as previously described (Weber and Spata 1981).

Yeast transformation. This was performed as described previously (Xuan et al. 1988). All DNA manipulations were as described by Maniatis et al. (1982). Standard genetic techniques were used for working with *Y. lipolytica* (Kurischko et al. 1983).

Results and discussion

In *S. cerevisiae* the mating-type genes *MAT***a** and *MAT* α were cloned by complementation of mutations *mat***a** (Hicks et al. 1979) and *mat*α (Nasmyth and Tatchell 1980). No mating-type mutants are available in *Y. lipolytica,* which could be used for cloning the *MAT* gene by complementation. A different approach was therefore used for the selection of mating-type transformants, based on the presumed function of the gene in the induction of sporulation. The idea was that a diploid strain homozygous for the mating type should sporulate if the complementary mating-type allele is introduced. Although Stahl (1978) described the sporulation of diploids homozygous for the mating type, this finding has not been confirmed by other workers (L. Spata, personal communication; J.M. Beckerich, pers. comm.) Diploid cells homozygous for the mating type *B* were constructed by protoplast fusion of strains 46–KF6 and 52–KF6. One fusion product called FP10 was tested for mating efficiency, sporulation ability and mitotic stability. More than 40 000 single colonies of FP10 were found to be unable to sporulate as assessed by the absence of change in colony pigmentation (*Y. lipolytica* changes from white to light brown when sporulating). Three hundred single colonies isolated from FP10 were of the mating type *B*. The mitotic stability of FP10 was tested by analysing the segregation of the heterozygous markers *ura3, hisA* and *adeA*. No mitotic segregant for any of the markers was detected among the 1035 cells tested.

FP10 was transformed with a *Y. lipolytica* gene library constructed in the plasmid pINA62 (Xuan et al. 1988) as follows. The plasmid library was divided into five pools of DNA. Each pool was digested with *Not*I, an enzyme for which sites are rare. It has a single site in the library vector, in the *LEU2* gene. Most of the recombinant plasmids of the library, therefore, were linearized, and the *Y. lipolytica* DNA insert in each case was flanked by parts of the *LEU2* gene. The linearized DNA was used to transform FP10, and Leu^+ transformants resulting from homologous integration at the *leu2* locus were selected (Davidow et al. 1985; Xuan et al. 1988). Leu^+ transformants were obtained at high frequency (50 000 transformants/μg DNA) and 10 000 were directly replica plated on CSM sporulation medium. Two brown colonies which sporulated were obtained and isolated. One yielded a 1:1 ratio of segregants in a random spore analysis. The 208 spore clones analysed fell into three classes: 108 were *B* Leu^-, 95 were Leu^+ and non-mater, and 5 were *B* Leu^+. This distribution of classes is consistent with the theoretically expected ratios assuming linkage of the *MAT* insert to the *leu2* marker after transformation. The first two classes had the phenotype expected of cells bearing a cloned gene required for mating (see Fig. 2). The last minor class could have originated by rare recombination events.