

Parasexuality in Aspergillus awamori

NRRL 3112 and interspecific

hybridization with A. niger

Although A. awamori is widely used in industry, we were not able to find a description of the parasexual cycle for that species in the literature. So we decided to start from strain NRRL 3112 which has been used in several research programs aiming to isolate improved glucoamylase producing strains (Banks et al. 1976-Prog. Ind. Microbiol. 6:95-139).

In conidial platings, 20% of the progeny were auxotrophic for proline and attempts to isolate a prototrophic strain by selection of monosporic colonies, or by purification of sectors were unsuccessful. One strain, M16, which segregated only 0.6% of pro1 conidia was selected for analysis. All pro1 segregants were stable in respect to the requirement with a reversion frequency of $<10^{-8}$. Auxotrophic and morphological mutants were induced in strain M16 with ultraviolet light. The frequency of auxotrophic mutants was 0.1% but filtration enrichment techniques improved the frequency of recovery to 2%. One reason for such a low frequency could be multinucleate conidia.

Heterokaryons were made between mutant strains complementary for auxotrophic markers, and diploids were isolated at a frequency of 10^{-5} . Diploid strains showed a high sensitivity to Benlate fungicide and could easily be distinguished from haploids by conidial diameter measurements. Four linkage groups have been detected from analyses involving 9 markers. A master, and single mutant strains have been deposited with the Fungal Genetics Stock Center. Genotype of the master strain is:

Linkage group	I	II	III	IV
MSW1	bwm1	mor1	leu1 mor1	pab1

Additional information about linkage was obtained following interspecific hybridization with an A. niger strain, using protoplast fusion methods. The A. niger strain used was proline prototrophic and unable to form a detectable halo on starch medium (shh). The hybrid was normal in respect to sporulation and growth and the segregation pattern was very similar to intraspecific diploids. The shh (starch hydrolysis halo) trait cosegregated with proline prototrophy allocating shh to mitotic linkage group I.

In general, interspecific heterokaryons were difficult to maintain on minimal medium. They grew slowly and had an abnormal morphology. Fusions were more easily maintained on Pontecorvo's complete medium when typical A. awamori sectors and mixed growth sectors, which segregated up to 2% of A. niger conidia, were observed. After transfer to fresh medium, vigorous sectors, which showed complementation of mutations for conidial color emerged. These were shown to be interspecific hybrids. It appears that incompatibility is stronger at the heterokaryon level than at the nuclear (chromosomal) level. These results, coupled with the isolation of recombinants which were the result of haploidization following mitotic crossing over, indicate the phylogenetic closeness of these species.

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