Fungal Genetics Reports

Volume 49 Article 10

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Recommended Citation
Metzenberg, R. L., and M. Sachs (2002) "Neurospora heterokaryons involving a thymidine kinase-positive "Helper": use in storing poorly viable strains or crossing strains of limited fertility," *Fungal Genetics Reports*: Vol. 49, Article 10. https://doi.org/10.4148/1941-4765.1192

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Abstract

Many of the most interesting and useful strains encountered in research readily die in storage and/or are semi-sterile. If such strains are Oak Ridge-compatible, they can be carried and even crossed as heterokaryons with the sterile but vigorous strain from the Griffiths lab, *a*^{m1} *ad-3B cyh-1* (FGSC #4564 - Perkins, Neurospora Newsl. 31: 41-42, 1984).

Neurospora Heterokaryons Involving a Thymidine Kinase-positive "Helper": Use in Storing Poorly Viable Strains or Crossing Strains of Limited Fertility

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Many of the most interesting and useful strains encountered in research readily die in storage and/or are semi-sterile. If such strains are Oak Ridge-compatible, they can be carried and even crossed as heterokaryons with the sterile but vigorous strain from the Griffiths lab, *a^{ml} ad-3B cyh-1* (FGSC #4564 - Perkins, Neurospora Newsl. 31: 41-42, 1984). This strain has a mutation in the mating type gene that prevents it from engaging directly in fertilization and also eliminates the heterokaryon incompatibility that is associated with a difference in mating type. We call this strain "Helper 1" and have used it extensively for improving the fertility of crosses. However, it has one shortcoming as an aid for promoting abundant conidiation and thus preservation of poorly viable strains. When one wants to retrieve a homokaryotic culture of the fragile strain for purposes other than crossing, it is at best a nuisance and often even a challenge to isolate it from the heterokaryon.

We have deposited at the FGSC three new strains, Helpers 2, 4, and 5, that have a deletion of the *mat* locus and, in addition, contain an insertion of the herpes simplex thymidine kinase gene, *tk^+*. The encoded enzyme causes 5-fluorouracil-2'-deoxyriboside (FUDR) to be phosphorylated, turning a minimally toxic pro-drug into a powerful inhibitor. Thus the *tk^+* can be regarded as a dominant sensitivity gene (Sachs, M. S., E. U. Selker, B. Lin, C. J. Roberts, Z. Luo, D. Vaught-Alexander and B. S. Margolin, Nucleic Acids Res. 25: 2389-2395, 1997), and this can be used to select strongly against the outgrowth of heterokaryotic conidia or germlings. The new helpers are:

FGSC #8745	*mat^D tk^+ cyh-1; Bml pan-2; inl*	"Helper 2"
FGSC #8746	*mat^D his-2 tk^+ cyh-1; Bml pan-2; inl*	"Helper 4"
FGSC #8747	*mat^D his-3; hyg^R tk^+ Bml pan-2*	"Helper 5"

In our hands, optimal conditions for isolating strains not bearing *tk^+* from heterokaryons with a strain that is *tk^+* (sensitive) are as follows: FUDR, 2 micromolar, and uracil, 1 millimolar. The rationale for adding uracil is as follows. Wild type is itself appreciably inhibited by FUDR, especially in germination rather than growth, though not nearly as severely as are *tk^+* strains. This fact limits the robustness of the method. The inhibition of wild type by FUDR has long been known, as is evident by the existence of a mutant, *ud-1*, which is resistant to it by virtue of its inability to take up the FUDR (Perkins, Radford, and Sachs, The Neurospora Compendium, 2001). However, it is obviously not practical to build *ud-1* into every strain that one would wish to separate from a *tk^+* strain. Our operating hypothesis is that the toxicity of FUDR to wild type is <u>not</u> due to traces of thymidine kinase, but to the successive action of nucleoside phosphorylase on FUDR to give the free base, 5-fluorouracil (FU) followed by salvage of FU with 5-phosphoribose-1-pyrophosphate (PRPP) to give pyrophosphate and fluorouridine-5'-phosphate (FUR-5'-phosphate). The latter would probably be toxic in itself by incorporation into RNA. In addition, it would almost certainly be reduced by ribonucleotide reductase to the toxic FUDR-5'-phosphate, which of course is what is made from FUDR in *tk^+* strains. A simple remedy, if this rationale is correct, would be to add a relatively large supply of uracil (<u>not</u> uridine) to compete with the FU for salvage via PRPP. That this does very substantially increases the resistance of wild type to FUDR while leaving the *tk^+* strains fully sensitive suggests that the rationale may be correct.

Whatever the explanation, streaking or plating a water-suspension of conidia from such a heterokaryon to appropriately-supplemented sorbose-glucose-fructose agar medium results in colonies which are homokaryons of the component that does not carry *tk^+*. In our hands, 1-2 micromolar FUDR is the minimum concentration which gives only homokaryons, but at least 10 micromolar FUDR works equally well and may be preferred for the most exacting work. The uracil concentration is 1 millimolar. The method works about equally well at 23°C or 34°C with either the salts base of Vogel or of Westergaard-Mitchell.