mts(MN9), a cpc-1 allele involved in a translocation

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Abstract
Among several amino acid analogue sensitive mutants, D.E.A. Catcheside selected mts(MN1) and mts(MN9) via their 5-methyltryptophan sensitive phenotype (1966, Ph.D thesis, University of Birmingham).

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Sterile, 24-well tissue culture plates are filled with melted minimal agar medium at 1.0 ml per well using a repeating syringe. After the medium has solidified, the plates can be used immediately or stored for several weeks in plastic bags at 5°C. To initiate complementation tests, each well of a plate is inoculated with a drop of spore suspension from an auxotrophic mutant (usually nitM) of a VCG standard strain. A sterile Pasteur pipette is used for inoculations. A drop of spore suspension from a complementary auxotrophic mutant (usually nit1 or nit3) of a field isolate is then added to the inoculated wells. Thus, 24 different field isolates can be paired with a VCG standard on one multiwell plate. As a positive, one well per VCG standard is inoculated with a compatible, complementing auxotrophic strain. The plates are incubated seven days at 25°C in a plastic bag to prevent drying. Pairings giving wild type growth are repeated on minimal agar plates (Correll et al. 1987) to confirm the complementation reaction. Complementation reactions are more definitive when the auxotrophic mutants are separated on a plate and meet to form a line of heterokaryotic growth. By screening field isolates in multiwell plates and retesting positive reactions, we save time in labeling, inoculating, and scoring pairings, and dramatically reduce the space required to conduct hundreds of pairings simultaneously.

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mts(MN9), a cpc-1 allele involved in a translocation

Among several amino acid analogue sensitive mutants, D.E.A. Catcheside selected mts(MN1) and mts(MN9) via their 5-methyltryptophan sensitive phenotype (1966, Ph.D thesis, University of Birmingham). While mts(MN1) was located to the right of ylo-1 of linkage group VI by Catcheside, mts(MN9) was not assigned to any locus.

Both in Neurospora crassa and in yeast, mutants defective in general or cross-pathway control of amino acid synthesis display amino acid analogue sensitivity. mts(MN1) and mts(MN9) were therefore tested for their regulatory capacity. Both turned out to be defective in cross-pathway control (for mts(MN1) see Koch and Barthelmess, 1987, FGN 33:30-32).

Mutant strains carrying the mts(MN9) mutation failed to derepress the ornithine carbamoyltransferase of arginine synthesis, the leucine aminotransferase of leucine synthesis and the saccharopine dehydrogenase of lysine synthesis under arginine, asparagine, histidine, methionine, threonine, tyrosine and tryptophan limitation, respectively. In this respect, mts(MN9) resembled a typical cpc-1 allele (Barthelmess, 1982, Genet. Res. 39:169-185). The effect on the remaining basal enzyme activity, however, was not as pronounced as found for most cpc-1 alleles, e.g. j5 (Barthelmess, 1982), CD15 or CD86 (Davis, 1979, Genetics 93:557-575), but resembled j9, a less stringent cpc-1 allele (Barthelmess, 1982). In agreement with this, the mts(MN9) mutant was able to grow like cpc-1 (j9) on medium supplemented with 0.03% glycine, a condition that does not allow growth of cpc-1 alleles j5 or CD15 (Barthelmess, 1986, Mol. Gen. Genet. 203:533-537).

Crosses with mts(MN9) produced many unpigmented ascospores. This was a first hint that the mts(MN9) strain might carry a chromosomal aberration. When mts(MN9) was crossed with cpc-1 (CD86 or CD15) only three classes of segregants were observed: the two parental classes and a new class of slow germinating ascospores with slow vegetative growth. The latter is presumed to be a duplication bearing class, while the unpigmented ascospores probably represent the corresponding segregants carrying a deficiency. The wild type did not segregate. This suggests that mts(MN9) is involved in a translocation and is linked with cpc-1. Since further mapping studies indicated linkage of mts(MN9) with pan-1 as well as met-5 on linkage group IV, it is assumed that linkage groups IV and VI are affected by the translocation. Marker studies to find out which of the two is the donor chromosome were not performed. Very helpful discussions with Dr. Perkins made us aware of the fact that the data so far available do not allow us to draw any conclusions on the precise nature of the translocation.
Complementation studies including mts(MN9), cpc-1 (CD86 and j5) and mts(MN1) were performed as already described for mts MNl) (Koch and Bartheless, 1987). The mutants were recessive to their respective wild type alleles, but complementation of the amino acid analogue sensitive phenotype was not observed in heterocaryons carrying mutant alleles simultaneously. These findings suggest that cpc-1, mts(MN9) as well as mts(MN1) belong to the same complementation group.

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Lee, S.B., M.G. Milgroom # and J.W. Taylor

Fungal genetic studies require a rapid method of isolating DNA from a large number of samples for restriction enzyme analysis. Previous methods we have used are limited by relatively low yield of 50 ug DNA/0.1g lyophilized mycelium (Zolan, M.E. and P.J. Pukkila 1986. Mol. Cell. Biol. 6:195-200) or tedious gel exclusion column chromatography (Biel, S.W. and F.W. Parrish 1986. Anal. Biochem. 154:21-25). In addition, these two methods yielded no readily digestable DNA from Phytophthora cinnamomi.

The following method facilitates rapid isolation of large quantities of easily digested total, genomic DNA from several species of Phytophthora, including P. cinnamomi, and several species of Boletus, Chroogomphus vinicolor, Gomphidius glutinosus, Leccinum manzanitae, Magnaporthe grisea, Neurospora crassa, N. tetrasperma, Omphalotus olivascens, and Talaromyces flavus. Yield was increased to 200 ug DNA/0.1 g lyophilized mycelium and isolation of DNA from two to three times as many samples can be achieved using this rapid method (current record is 64 isolates in one day versus 24 using previous methods). DNA has been successfully cut with all restriction enzymes tried to date.

Solutions needed:
1. Lysis buffer: 50 mM Tris-HCl 50 mM EDTA 3% SDS 1% 2-mercaptoethanol (add just before use)
2. Chloroform:phenol (1:1)
3. SEVAG (chloroform:isoamyl alcohol, 24:1)
4. 3 M NaOAc (pH 8.0)
5. Isopropanol
6. Ethanol (100%, -20°C)

Protocol:
1. Fill a 1.5 ml eppendorf microcentrifuge tube 2/3 to the joint with ground lyophilized mycelium (60-100 mg dry, or 0.5-1.0 g wet, ground in liquid nitrogen).
2. Add 750 ul of lysis buffer, stir with a dissecting needle and/or vortex so the mixture is homogenous. Incubate at 65°C, 1 hr.
3. Add 700 ul of chloroform:phenol; 1:1 and vortex briefly. Microcentrifuge at 12,000 x g for 10 minutes or until the aqueous (top) phase is clear.
4. Remove 600-650 ul aqueous phase to a new tube -- be careful not to take any cellular debris from the interface. Don't get greedy!
5. Add 700 ul of SEVAG, vortex briefly. Be careful, as the caps are loosened by chloroform. Microcentrifuge as above for 5 minutes.
6. Remove the aqueous phase to a new tube (approx. 550-600 ul). Add 20 ul of 3 M NaOAc. Top off the eppendorf tube with isopropanol. Invert gently several times. You should see DNA "ropes" precipitate.
7. Microcentrifuge as above for 30 seconds to pellet the DNA. Pour off the supernatant. Invert the tubes for 1 minute to drain.
8. Add 300 ul TE and place in a heat block at 65° C for 10-15 minutes. Finger vortex to resuspend the pellets.