How to choose wild type laboratory strains of *N. crassa* for reference and for special uses.

Background

Oak Ridge (OR) wild types are preferred for most purposes (Perkins 2004). These were derived from wild types designated 'Standard' (ST), which had been selected originally by Patricia St. Lawrence (1953) on the basis of high fertility and properties which made them favorable for cytology -- good chromosome pairing at pachytene and asci predominantly with eight viable black spores. Differences in heterokaryon-compatibility led to replacement of the original St. Lawrence standards by derivatives designated OR (Oak Ridge) (Case *et al*. 1965). Additional backcrosses and reisolations were made to minimize remaining heterogeneities and obtain largely coisogenic strains of opposite mating type, 74-OR23-1V A (FGSC 2489) and 74-ORS-6a (FGSC 4200) (Mylyk *et al*. 1974). For pedigree and references, see Newmeyer *et al.* (1987) and Gavric and Griffiths (2004). The Oak Ridge wild types and mutant derivatives in the OR (ST) genetic background came to be widely used. Strain 74-OR23-1V A (FGSC 2489) was chosen as the source of DNA for genome sequencing (Galagan *et al.* 2003).

There have been notable exceptions to the use of Oak Ridge strains as standards. Tatum and his associates (Slayman *et al.* 1964) derived *mat A* and *mat a* reference strains from Lindegren wild types and designated them Rockefeller Lindegren (RL). D. G. Catcheside *et al.* used Emerson (Em) wild types as a standard. These RL, Em, and OR wild type strains differed from one another in heterokaryon incompatibility genotypes and in many other respects (Newmeyer *et al.* 1987).

Early work in the Beadle and Tatum laboratories (1945) employed strains from several wild sources. The mutant markers they obtained in these backgrounds continued in circulation. As a result, abundant genetic polymorphisms were present in laboratory stocks that continued to be used because they contained needed markers. The polymorphisms were both a blessing and a curse. Some of the differences proved valuable, leading to the discovery of *het* genes , which control heterokaryon incompatibility (Garnjobst 1953, Holloway 1953), and rec genes, which determine the frequency of meiotic ecombination (Catcheside *et al.* 1964). Often, however, heterogeneities present in laboratory strains were disadvantageous, complicating mapping and genetic analysis and impeding the formation of heterokaryons needed for determining allelism or studying complementation.

Natural populations provide a rich source of variability, and strains of *N. crassa* that are markedly polymorphic relative to the OR laboratory wild types have been sought out for special purposes. Strain Mauriceville-1c A (FGSC 2225), which differs from OR at thousands of sites in the genome, was adopted for use in RFLP mapping (Metzenberg *et al* 1984). Strain Panama CZ30.6 A (FGSC 1131) was found to differ from OR in alleles at most of the known heterokaryon-incompatibility (*het*) loci (Mylyk 1975, Newmeyer *et al.* 1987, Perkins *et al*. 1993). Dominant suppressors of Repeat-Induced Point Mutation were obtained by screening the global collection of >400 wild-collected *N crassa* isolates that is available from FGSC (Bhat *et al.* 2003).

Crosses between highly inbred or closely related *N. crassa* strains may be characterized by a high rate of nonselective abortion of entire asci, within which all eight ascospores shrink until they resemble tiny hyaline bubbles ("bubble asci". Raju *et al.* 1987). This is true, for example, of crosses between the laboratory wild types OR A and OR a or RL A and RL a. The frequency of bubble asci is greatly reduced when OR and RL strains are intercrossed or outcrossed to strains of different genetic background (see *How to minimize the occurrence of bubble asci*). Because entire asci are aborted in the crosses between inbred strains, bubble ascus production does not distort genetic ratios among the survivors. Bubble asci are disadvantageous, however, when rosettes of asci are being observed or demonstrated, or when the effects of chromosome rearrangements on ascospore development are being examined.

Ascus development is impaired in most interspecific crosses. However, strains of *N. intermedia* show excellent pairing of homologous chromosomes at pachytene in crosses by *N. crassa* OR strains, even though few viable ascospores are produced in the interspecific cross (Barry, personal communication; see *How to prepare aceto-orcein squashes*). Markers introgressed into *N. crassa* from other species and markers transferred between other combinations of species are listed in the FGSC catalog.

Procedure

Recommended Oak Ridge standards are 74-OR23-1VA (FGSC 2489) and 74-ORS-6a (FGSC 4200), and the OR-compatible *fluffy* testers *fl* (OR) A (FGSC 4317) and *fl* (OR) a (FGSC 4347) (Perkins *et al*. 1989). For outcrossing to minimize bubble-ascus abortion, crossing strains of OR background by RL strains is effective. RL3-8 A (FGSC 2218), RL 21 a (FGSC 2219), or *fl* (RL) A, (FGSC 6682), *fl* (RL) a (FGSC 6683) are recommended (Perkins and Pollard 1989).

The FGSC catalog lists field-collected strains of *N. crassa* and other species from many localities throughout the world. Many additional Neurospora strains from collections described by Turner *et al.* (2001) are not listed in the catalog but are available through FGSC. For a database, go to http://www.fgsc.net/ncrassa.html. For diagnostic species-tester strains representing all five conidiating Neurospora species, see *How to determine the species of a wild-collected isolate*.

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