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# Production of mutations in the white-spore strain

## Abstract

Production of mutations in the white-spore strain

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## Auxotrophic and visible mutations in white-spore (ws-1).

Previous studier of the white-spore mutant of N. crassa (Phillips and Srb 1967 Can. J. Genet. Cytol. 9:766) indicated that ascospores carrying the ws-1 marker germinate with a very low frequency. Therefore, the introduction of additional genetic markers into the ws-1 strain is difficult but may be accomplished by isolating spores from the rare asci that contain more than four black spores. The exceptional black spores will be genotypically ws-1, barring mutation or gene conversion, and may carry the additional genetic markers. Another means of introducing new genetic markers into the wr-I strain is through mutagenic treatment. The purpose of this investigation was to induce by UV-irradiation adenine and methionine mutations in the wr-I strain. An auxotrophic ws-1 strain war needed for studier involving wr-I in balanced heterocaryons.

The procedure was to subject approximately $10^7$ conidia of the wr-I strain (in 20 ml of minimal medium) to UV-irradiation for 90 seconds and then follow the filtration procedure of Woodward et al. (1954 Proc. Natl. Acad. Sci. U. S. 40:192). Aliquots (0. Iml) of the filtered conidial suspension were spread over 20 plater each with 20 ml of solid minimal medium supplemented with adenine, methionine and sorbose. Approximately 500 slow-growing colonies were selected for further testing. From the 500 colonies, one albino, three adenine and 11 methionine mutants were obtained.

Albino mutant: The albino mutant ( isol. #RP100 ) was found to be independent of T(I;II) 4637, al-1; T(IV;VI) 45502, pyr-3 (IVR); T(I;VII) 17084, thi-I; vel (IIIR); tryp-1 (IIIR); and ylo-1 (VIL). Linkage was detected in crosses with at (V cent), arg-4 (VR), arg-7 (VR), arg-8 (VR), T(V;VI) 46802 inos, and inos (VR) The recombination data indicate that the albino locus is in the right arm of LG V, less than one map unit from arg-8 and inos (Table 1 ). Complmentation data from R. E. Subden (personal communication) also indicate that the new albino locus is discrete from al-I and al-2; we are suggesting the designation al-3.

The al-3 mutant is on excellent visible marker; although obviously albino, it doer have a very light pink color. The strain shows a high degree of crossability to all Neurospora strains tested, when used either as the protoperithecial or conidial parent. The al-3 ascospores germinate readily and grow well on minimal media.

Adenine mutants. The three adenine mutants (isol. #'s RPIOlad, RPIOZ and RP103 ) have been mapped and the linkage results are presented in Table 2. Linkage war not detected between RP101ad (a purple adenine mutant) and fl (IIR) but was evident with m. t. (IL), ad-5 (IL) and ad-3B (IR). No recombinants were recovered from crosses of RPIOlad to ad-5 or ad-3B. Since RPIOlad complements with od-5 but not ad-38 and is phenotypically similar to ad-3B, the adenine mutation in RP101ad appears to be allelic to ad-3B. The lack of recombination between RPIOlad and ad-5 is probably the result of scoring an insufficient number of progeny.

The second adenins mutant (RPIOZ) showed independence with T(I;II)4637 al-1; T(III;VI) 1, ylo-1; al-3 (RP101) (VR); and pl (VR). Linkage was observed in the crosses of RPIOZ to pyr-1 (IVR); ad-6 (IVR); and T (IV;V) R2355 and cot-I (IVR). No recombinants were recovered in the cross RP102 x ad-6 nor was any complementation detected between these two mutants. RPIOZ is presumably allelic to cd-6.

The third adenine mutant (RP103) is independent of T(I;II) 4637 al-I; T(IV;V) R2355; cot-I (IVR) and ylo-I (VIL). Linkage was detected between RP103 and T(III;VI)1, ylo-1; ad-2 (IIIR); tryp-1 (IIIR); and vel (IIIR). Crosses with ad-2 have not been successful. No complementation was observed, however, between ad-2 and RP103. ad-4 is also located on IIIR.

The above adenine mutants respond well to adenine. The fertility with other strains and spore germination rates also are quite satisfactory.

Table 1. Tests between al-3 (RP100) and linked genetic markers.

| Tester strain | FGSC# | Linkage group(s) | Parental spores | Recombinant spores | % Germination | % Recombination |
|---|---|---|---|---|---|---|
| at (M111) | 1884 | V cent. | 72 | 35 | 70 | 32.7 |
| arg-4* | | VR | 110 | 7 | 71 | 4.3 |
| arg-7** | | VR | 72 | 3 | 50 | 4.0 |
| arg-8 (44207 ) | 1311 | VR | 137 | 1 | 81 | 0.7 |
| T( V; VI )46802 inos | 1199 | VR, VIL | 74 | 0 | 50 | 0 |
| inos (37401 ) | 406 | VR | 45 | 0 | 35 | 0 |

* Strains kindly provided by Val Woodward

Table 2. Tests between the adenine mutants (RP101 ad, RP102 and RP103) and linked genetic markers.

| Tester strain | FGSC# | Linkage group(s) | Parental spores | Recombinant spores | % Germination | % Recombination |
|---|---|---|---|---|---|---|
| RP101ad | | | | | | |
| m.t. (Em.) | | IL | 256 | 14 | 90 | 5.2 |
| ad-5 (Y175M253) | 678 | IL | 69 | 0 | 23 | 0 |
| ad-3B (35203) | 360 | IR | 2236 | 0 | 75 | 0 |
| RP102 | | | | | | |
| pyr-1 (H263) | 72 | IVR | 104 | 7 | 74 | 6.3 |
| "alcoy" • | 998 | IVR, VR | 108 | 4 | 77 | 3.5 |
| ad-6 (Y175M221) | 663 | IVR | 375 | 0 | 83 | 0 |
| RP103 | | | | | | |
| "alcoy" A • | 997 | IIIR, VIL | 29 | 5 | 50 | 14.7** |
| tryp-1 • | | IIIR | 143 | 15 | 53 | 9.5 |
| vel**** | | IIIR | 145 | 13 | 53 | 8.2 |

• "alcoy" linkage tester = T(I;II)4637, al-1; T(IV;V)R2355, cot; T(III;VI)1, ylo-1.
** Albino progeny were excluded from this calculation.
• ** Strain was vel, tryp-1; ylo-1 synthesized by crossing vel, tryp-1 (B18, 10575) x tryp-1; ylo-1 (10575; Y30539) (FGSC# 173 x FGSC# 1207).

Table 3. Tests between os (RP101os) and linked genetic markers.

| Tester strain | FGSC# | Linkage group(s) | Parental spores | Recombinant spores | % Germination | % Recombination |
|---|---|---|---|---|---|---|
| m.t. (STA4) | 262 | IL | 141 | 75 | 72 | 35.5 |
| m.+. fl (P605) | 297 | IL | 49 | 21 | 78 | 30.1 |
| ad (RP101 ad) | | IR | 58 | 33 | 60 | 36.3 |
| al-2 (15300) | 99 | IR | 83 | 11 | 63 | 11.7 |
| os-1 (8135) | 951 | IR | 154 | 27 | 81 | 17.5 |

RP101 also carried a separate, visible marker morphologically similar to os-1 and unable to grow in complete medium supplemented with KCl (4%). Linkage and complementation tests indicate that RP101os is not allelic with or-1 (IR), although they are linked (Table 3). Linkage was also detected between RP101os and m.t. (IL), RP101ad (IR) and al-2 (IR). os-4 is also located in IL (Mays 1969 Genetics 63: 781). or-3 and os-5 are also in I but in the right arm.

Methionine mutants. None of the 11 methionine mutants have been mapped. Preliminary complementation tests revealed that eight of the mutants are in distinct complementation groups, while three overlap the others to varying extents.

In summary, the ws-1 mutant is now available with ad-38 (RP101 ad), ad-6 (RP102), ad (RP103), al-3 (RP100), the unidentified os (RP101 os), and the unidentified me mutants. These strains may be obtained from R.L. Phillips.

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