ssbA, a suppressor of sbA in stock strains of Aspergillus nidulans.

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Recommended Citation

Kafer, E. (1986) "ssbA, a suppressor of sbA in stock strains of Aspergillus nidulans.," Fungal Genetics Reports: Vol. 33, Article 5. https://doi.org/10.4148/1941-4765.1579

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Abstract

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ssbA, a suppressor of sbA in stock strains of Aspergillus nidulans

Recent analysis of haploid and aneuploid segregants from a diploid with markers on all 16 chromosomes (diploid no. 26, Käfer 1984 Mutat. Res. 135:53-75) produced inconsistent segregation for sbA3 (inability to use sorbitol as a carbon source). Detailed testing of haploid mitotic segregants from this diploid revealed as the probable cause a mutation on chromosome IV of the sb^+ haploid component (M2468; Table I). This mutation apparently inhibits the expression of sbA3 and was given the symbol ssbA1 (suppressor of sbA). ssbA1 does not seem to have any other phenotype and has not been found to affect any other carbon source mutants.

To discover the origin of ssbA1 which would identify strains unlikely to contain it, and to construct ssbA free diploids for tests of environmentally induced chromosome malsegregation, parental and ancestor strains of M2468 were tested for the presence of ssbA1. Two replacement strains, free of ssbA, were constructed which are available from FGSC as A591 and A593. These strains, in combination with A592 and A594, respectively, produce the test diploids No. 29 and 30 (Käfer et al. 1986 Mutation Res. 167:9-34), which have all chromosomes marked.

To check for the ssbA suppressor in strains with a direct ancestor or progeny which showed this mutation, such strains were combined into diploids with suitable tester strains that carried sbA3 on chromosome VI and usually methG1 on chromosome IV (none of the suspect strains contained sbA3 and very few were methG but most carried pyroA4 on IV). From these heterozygous diploids, haploids which result from malsegregation of chromosomes were induced with benlate (on complete medium supplemented with methionine to improve the imperfect recovery of methG haploids; Table I). Haploid segregants were tested for markers on IV and VI. Observed ratios for sbA3 : + among methG haploids which cannot have ssbA1 if it is on chromosome IV, should be 1:1. On the other hand, met^+ haploids will show such 1 : 1 ratios only if ssbA is not present, but segregate 0 : 2 for sb : + phenotypes if ssbA1 is present since all met^+ haploids must have ssbA1. As expected, it was found that for each ssbA1 strain at least one parent carried ssbA1 (left half of Table I). In a few cases both parental strains showed the suppressor and it was assumed, without testing, that all progeny carried ssbA. Fortunately, most of the suspect FGSC strains were without the suppressor (right half and footnote of Table I.)

Table I.

Segregation of the "sb" phenotype in haploid segregants from diploids which combine strains that may carry ssbA1 with sbA3;metG1 tester strains.

| Tested strains with ssbA1* | Strains normal (ssb^+)** |
|---------------------------|-------------------------|
| Haploids: meth G vs. meth^+ | Haploids: meth G vs. meth^+ |
| FGSC no. | sb : + | sb : + | FGSC no. | sb : + | sb : + |
| A75 (M 391) | 40 : 30 | 0 : 68 | A271 (M2008) | 8 : 5 | 9 : 13 |
| A452 (M2162) | 20 : 10 | 0 : 31 | A283 (M1655) | 16 : 9 | 21 : 16 |
| A473 (M2233) | 13 : 14 | 0 : 17 | A363 (M1274) | 19 : 17 | 23 : 28 |
| A513 (M2308) | 53 : 8 | 0 : 62 | A426 (M2023) | 7 : 8 | 26 : 54 |
| (M2468) | 21 : 46 | 0 : 148 | A468 (M2216) | 11 : 23 | 11 : 21 |
| Not | | | A479 (M2243), 18 : 21 | 16 : 31 |
| FGSC 8 other | A514 (M2356) | 11 : 14 | 20 : 23 |
| M strains 144 : 169 | 447 | A550 (M2358) | 13 : 14 | 16 : 21 |
| 14 strains, combined total | 315 : 291 | 0 : 819 | A608 (M1996) | 15 : 9 | 21 : 19 |
| | | | (M2257) | 12 : 11 | 26 : 30 |
| 10 strains total | 130 : 131 | 189 : 256 |

* To replace with strains, which are unlikely to carry ssbA1;
proposed for A473: A614 (M2843), A615 (M2844) or A616 (M2485)
for A513: A617 (M2848), A618 (M2851)

** Other tested FGSC strains which are not ssbA1:
A55 (M892), A164 (M645), A213 (M1634), A348 (M921), A453 (2165), A474 (M2234), A477 (M2240), A487 (M2269), A502 (M2285), A511 (M2325)
To check for meiotic segregation and linkage, various ssbA strains were crossed to methG and pyroA. No meiotic linkage could be demonstrated (recombination over 40%). As expected, such crosses produced 25% sb- progeny when they were heterozygous for sbA3 (68/274 for ssbA X sbA, and 21/77 for ssbA;sbA X +;+) and 50% sb- in crosses homozygous for sbA3 (51/100); 50% sb- was found also in control crosses heterozygous for sbA3 (206/438).

The ssbA1 mutation was traced back through 15 generations to strain FGSC A75 (progeny of cross 362, top of Fig. 2 in Barratt et al. 1965 Genetics 52:233-246). Further tracing to the strain of origin of ssbA1 is not possible because few of the ancestral stocks are available by now. Exceptions are the original mutagenized strains in which the various mutants were induced by UV or X-rays that are in the pedigree of A75. Only two of these are fairly direct ancestors of A75 and probably worth testing (A1, choA1 and #5, riboB2) Please note that galD5 was prematurely assumed to be induced in the same strain as ssbA1, and contrary to the listing in NN 1985 32:41, strain A213 does not carry ssbA1.

### Table II

| Untested FGSC strains with an ancestor or progeny that carry ssbA1 |
|---------------------------------------------------------------|
| **a) Strains to be replaced**            | **Replacement strains**            |
| FGSC A342 (M771)          | FGSC A607 (M2878)                   |
| A494 (M2277)             | A608 (M1966)                       |
| A507 (M2295)             | --- (M2275)                        |
| **b) Strains to be discontinued**        | **c) Strains to be tested**         |
| A455 (M2175)             | A1 (M7)                            |
| A465 (M2212)             | A5 (M149)                          |
| A476 (M2239)             | A44 (M857)                         |
|                    | A108 (M455)                        |

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Construction of testers for reversion assay.

Dr. T. Ishikawa (University of Tokyo) had isolated and kept many ad-8 mutants in his laboratory. We screened revertability of seventy eight ad-8 mutants by the spot test method of Ong (1978, Mutation Res. 53:297-308) and selected two mutants on the basis of reversion specificity. One of them, ad-8(E193), was highly reverted by MNNG but not by ICR170 and the other, ad-8(E146) was highly reverted by ICR170 but not by MNNG. Each of these mutants was back-crossed to standard strain 74-OR31-14a(al-2 cot-1 pan-2 a) twice and two testers (T26 and T28) were constructed. Their genotypes are shown in Table 1. Responses of both testers to several mutagens are shown in Table 2. These data are from the plate test method of Ong. The results indicate that T26 responds to frameshift mutagens and T28 to basepair substitution mutagens. These testers may be useful along with Ong's strains N23 and N26, which had been constructed for the assay of environmental mutagens and are useful for quantitative and qualitative comparison of DNA repair ability and mutagen specificity.

### Table 1. Tester strains

| Tester Strain # | FGSC # | Genotype (allele) |
|-----------------|--------|-------------------|
| T26 C3-T26-14a  | 5071   | ad-8(E193) al-2(Y112M38) cot-1(C102(t)) pan-2(Y387-15.7) |
| T28 C3-T28-39a  | 5071   | ad-8(E193) al-2(Y112M38) cot-1(C102(t)) pan-2(Y387-15.7) |