Additive effect involving a new locus of benomyl resistance in Aspergillus nidulans

N. M. Martinez-Rossi
J. L. Azevedo

Follow this and additional works at: https://newprairiepress.org/fgr

This work is licensed under a Creative Commons Attribution-Share Alike 4.0 License.

Recommended Citation
Martinez-Rossi, N. M., and J.L. Azevedo (1989) "Additive effect involving a new locus of benomyl resistance in Aspergillus nidulans," Fungal Genetics Reports: Vol. 36, Article 9. https://doi.org/10.4148/1941-4765.1506

This Regular Paper is brought to you for free and open access by New Prairie Press. It has been accepted for inclusion in Fungal Genetics Reports by an authorized administrator of New Prairie Press. For more information, please contact cads@k-state.edu.
Additive effect involving a new locus of benomyl resistance in Aspergillus nidulans

Abstract
Most of the fungicides based on the benzimidazole nucleus, including benomyl, thiabendazole and thiophanate, are systemic and because they control many important fungal diseases.
Martinez-Rossi, N.M.¹ and J.L. Azevedo²

Additive effect involving a new
locus of benomyl resistance in
Aspergillus nidulans

which is probably the cause of cross-resistance between them. Benomyl-resistant mutants
are of interest from at least two points of view: the understanding of the genetic bases
that govern this resistance and the study of microtubulins using a genetical-biochemical
approach, since benomyl is an antimicrotubular drug.

Three loci of benomyl resistance have been described in A. nidulans: benA (Hastie
and Georgopoulos 1971 J. Gen. Microbiol. 67:371-373, benB and benC (van Tuyl 1977 Ph.D.
Thesis, Agricultural University, Wageningen, The Netherlands). The benA locus maps on
linkage group VIII, confers high resistance to benomyl even though it does not permit
conidiation resistant mutants and also codes for β-1 and β-2 tubulin (Sheir-Neiss, G.
et al. 1978 Cell 15:639-647). The other two loci map elsewhere and make the fungus
resistant to low fungicide levels.

To determine the possible existence and interrelationship of other loci responsible
for this resistance, conidia of the proA1 pabaA6 ya2 strain were irradiated with UV light
and inoculated at 37°C in complete medium dishes containing thiophanate-methyl fungicide
(40 ug/ml). This concentration inhibits the growth of sensitive strains. A mutant
obtained under these conditions (BEN-35) proved to be resistant to benomyl and showed
normal conidiation in complete medium containing up to 10 ug/ml benomyl or 50 ug/ml
thiophanate-methyl. In contrast FGSC A524 (benA10 biA1 fwA1) did not conidiate at a con-
centration exceeding 5 ug/ml benomyl or 10 ug/ml thiophanate-methyl.

Genetic analyses of this new mutant (BEN-35) carried out by cross with the Master
Strain E (MSE) showed that a single gene mutation which also mapped on linkage group VIII
was responsible for the benomyl and thiophanate-methyl resistance. About 300 segregants
obtained from a cross between BEN-35 and FGSC A524 were tested for allelism between these
resistant genes by incubation in several concentrations of benomyl. Two of the segregants
were as sensitive as the wild type strain and another one showed high resistance to the
fungicide. Thus, it seems that a new locus (benD), located about 1.0 unit from benA and
probably centromere-proximal to it, is responsible for the resistance.

The ED50 values of the BEN-35, FGSC A524, sensitive strains, heterozygous diploids
and double mutant (Table 1) obtained from dose-response curves for benomyl and thiophan-
ate-methyl showed: a) cross resistance between benomyl and thiophanate-methyl, b) a
higher level of resistance to both fungicides in the BEN-35 mutant as compared to the
FGSC A524 strain, c) the resistance of the BEN-35 strain to thiophanate-methyl was about
60 times higher than to benomyl. For FGSC A524 and sensitive strains the ratio between
the two fungicides was only 10, d) intermediate resistance of the diploid heterozygous
for benD1/+ indicating a semidominant trait, e) the double mutant obtained by crossing
the BEN-35 strain (benD1) with FGSC A524 (benA10) has a high level of resistance to
benomyl. This interaction has also been detected among the benA, benB and benC loci of
A. nidulans (van Tuyl 1977 Ph.D. Thesis, Agricultural University, Wageningen, The
Netherlands). The double mutant, benA benB, showed an intermediate type of resistance to
benomyl and somewhat higher resistance to thiabendazole. When benB and benC were recomb-
ined into one strain, only a slight increase in resistance was observed. Thus, it seems
that benomyl (or benzimidazole) resistance is governed by a multigenic system. Further-
more, the additive effect and the physical closeness of the benA and benD loci suggests
that the latter might be responsible for the synthesis of another tubulin polypeptide.
Table 1. ED50 values from dose-response curves of benomyl and thiophanate-methyl for various strains

| Strains          | Relevant genotype | Benomyl a (ug/ml) | Thiophanate-methyl b (ug/ml) |
|------------------|------------------|-------------------|-----------------------------|
| MSE              |                  | 0.7               | 7.0                         |
| proA1 pabaA6 yA2 |                  | 0.7               | 7.0                         |
| benA10 biA1 fwA1 (A524) | benA10 | 7.1 | 80.0 |
| BEN-35           | benD1           | 31.3              | >2000.0                     |
| BEN-35/MSE       | benD1           | 19.4              | 794.3                       |
| Double resistant | benD1           | >100.0            | -                           |

a - Concentration reducing the colony radial growth by 50%
b - Methyl-1-butylcarbamoyl-2-benzimidazole carbamate
c - 1,2-bis (3 methoxy-carbonyl-2-thiourea)-benzene

^1 Dept. de Genetica, Universidade de Sao Paulo, 14049 Ribeirao Preto, SP, Brazil; ^2 Inst. de Genetica, ESALQ-USP, Caixa Postal 83, Sao Paulo, Brazil

Metzenberg, R.L. and J. Grotelueschen

Restriction polymorphism maps of Neurospora crassa: update.

When a gene or other fragment of DNA is cloned, it is often useful to identify the chromosomal region from which it arose. This is conveniently done with a set of progeny from one or more reference crosses in which many polymorphic differences are segregating. Data which allow the mapping of cloned genes have been published (Metzenberg et al. 1984, Neurospora Newsl. 31:35-39; ibid. Proc. Nat. Acad. Sci. U.S. 1985, 82:2067-2071; Metzenberg and Grotelueschen, 1988, Fungal Genetics Newsl. 35:30-35). The following is an update of the 1988 article. As noted previously, 38 segregants from the first cross were taken from ordered ascis, and provide somewhat more information than can be obtained from the 18 segregants which represent random spores from the second cross. Both crosses, however, have been used in a number of laboratories, and data from both are presented. The scoring of segregants is coded in the same way as before: "M" or "0" indicate segregants that are like the Mauriceville parent or like the Oak-Ridge-derived parent, respectively; "-" indicates that the scoring was not done or was equivocal for technical reasons; and (0) in Isolate 1 and (M) in Isolate 6 for all lanes of the second cross means that these are not progeny but are the parental strains of the cross, and are 0 and M by definition. Loci which were previously identified in the 1988 article, or are in the Compendium (Perkins et al. 1982, Microbiol. Reviews 46:426-570) are not further identified. All loci corresponding to 5S RNA-coding regions, previously given only as Arabic numerals starting with 1, are now identified as Fsr-1, etc. All of the telomeres (Tel) which have been included are due to the efforts of Michael Schechtman, whose article in this issue should be consulted. A number of loci whose identities were being kept confidential are now shown with informative names, instead of coded numbers beginning with a string of zeroes. COXVIII is subunit VIII of cytochrome oxidase, and is coded by a cloned, but unnamed, gene (M. Suarez/U.L. RajBhandary, in preparation). The following are newly named or unpublished loci, with the name(s) of the person or persons who should be consulted about them: cyt-21 (M. Kuiper/A. Lambowitz); sod-1 superoxide dismutase (D. Natvig); pma-1, plasma membrane ATPase (K. Hager/C.W. Stayman/B. Bowman); lys-6, (Ming Chou/U.L. RajBhandary); cyt-2, (A. Kubelik/A. Lambowitz); cyt-8 and cya-2, (M. David/U.L. RajBhandary); cat-3, (D. Natvig); ccc-1 and ccc-2, (J. Lorey/S. Dunlap); SPIAE, an anonymous DNA fragment, (M. Schechtman); frq and for, (J. Dunlap). pho-4 was formerly called van (B. Bowman). Finally, the scoring of rDNA (LG V) of isolate C4 has been changed from M to 0. The previous result may have been in error, but inspection of the original blots suggests it may not have been. Initially, C4 was an exceptional strain having both M and 0 types of rDNA, with a large preponderance of M, but subsequent preparations have been pure 0. The preponderant rDNA form of the strain may thus have changed during mitotic events, and should be considered questionable. The other genes of this strain seem to behave conventionally.

If you have found these data useful, please pass on the favor by pencilling in any results of your own onto a copy of this table and sending it to RLM. You may ask that it be assigned a number which preserves confidentiality.

Supported by NIH Grant GM 08995. - - - Dept. of Physiological Chemistry, University of Wisconsin, Madison, WI 53706.