Martinez-Rossi, N.M.^1 and J.L. Azevedo^2

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

Most of the fungicides based on the benzimidazole nucleus, including benomyl, thiabendazole and thiophanate, are systemic and because they have a broad action spectrum they can be used to control many important fungal diseases. Even though the chemical structures of benomyl and thiophanate may appear not to be similar, the two substances are metabolized to a common compound 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 of resistant mutants and also codes for ß-1 and ß-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 concentration 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 thiophanate-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 recombined 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. Furthermore, 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	ED50^a(ug/ml) benomyl^b	thiophanate-methyl^c
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	benA10 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-thioureido)-benzene

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