GENE CONTROL OF RESISTANCE VS. SENSITIVITY TO ACTIDIONE IN SACCHAROMYCES¹

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Actidione was shown by WHIFFEN (1948) to have marked antibiotic activity against yeasts but little or no activity against bacteria. The observation that sensitivity vs. resistance to copper (BRENES-POMALES *et al.*) and cadmium (MIDDLEKAUFF *et al.*, is under gene-control in *Saccharomyces* led to an attempt to discover if sensitivity vs. resistance to antibiotics is also gene-controlled.

MATERIALS AND METHODS

A modified Burkholder's medium (table 1) autoclaved at 15 lbs. pressure for 15 minutes after being adjusted to pH 5 was used in 10 ml amounts in broth tubes, or 3% agar was added for use in petri dishes. Actidione stock solution (10 mg of actidione in 100 ml of water) was sterilized by Seitz filtration, kept in a refrigerator until used, and added aseptically to the medium after autoclaving.

One loopful of cells from a 24 hour culture was transferred to a 6 ml water blank to make a final turbidity of about 150 Klett units. One loopful of this suspension containing about 60,000 cells was used to inoculate a broth tube or planted on a spot about 1 cm in diameter on an agar plate. Tubes and plates were incubated at 30°C. Results of growth in tubes were recorded after each 24-hour incubation (up to 96 hours) with a Klett Summerson Photoelectric colorimeter, using a 420 m μ blue filter, with the zero point adjusted against an uninoculated broth tube. Growth on agar was recorded at four different quantitative levels; 0, no growth; 1, discrete colonies; 2, irregularly confluent growth; and 3, fully confluent growth.

EXPERIMENTAL RESULTS

Ten cultures of *Saccharomyces* from the Carbondale breeding stock were tested on agar plates containing various concentrations of actidione from 0.7 to 1.0 parts per million. Two cultures showed weak but confluent growth on 0.7 ppm and a few small colonies on 1.0 ppm; the others failed to grow. A resistant colony of culture 18159 (18159R) was picked from a 1.0 ppm plate. It grew vigorously at 1.2 ppm. Family I (fig. 1) was established by crossing 18159R to a sensitive strain.

Tests on solid and liquid medium (table 2) revealed that yeast cells were inhibited by lower concentrations of actidione in liquid than in solid medium, especially when the inoculum on solid medium was large and many cells were planted in a restricted area. This was interpreted to indicate that the local concentration of actidione was probably reduced by the absorption of the toxic agent by cells which had died, when

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TABLE 1
Modified Burkholder's medium (per liter)

SALTS	
(NH ₄) ₂ SO ₄	4 g
KH ₂ PO ₄	1.5 g
MgSO ₄	0.5 g
CaCl ₂	0.33 g
FeSO ₄ ·7H ₂ O.....	2.5 mg
ZnSO ₄ ·7H ₂ O.....	3.1 mg
CuSO ₄ ·5H ₂ O.....	0.4 mg
KI.....	10 mg
MnSO ₄ ·4H ₂ O.....	0.4 mg
(NH ₄) ₆ Mo ₇ O ₂₄ ·4H ₂ O.....	0.18 mg
Na ₂ B ₄ O ₇ ·10H ₂ O.....	0.88 mg
SUGAR SOURCE	
glucose.....	20 g
AMINO ACIDS	
Proline, arginine, leucine, lysine, isoleucine, histidine, methionine, phenylalanine and anthranilic acid, each in 0.0002 M final concentration.	
Asparagine.....	2 g
N-BASES	
Adenine sulfate.....	40 mg
Uracil.....	20 mg
VITAMINS	
Thiamine-HCl.....	200 mcg
Nicotinic acid.....	200 mcg
Paba.....	50 mcg
Pyridoxine.....	200 mcg
Ca pantothenate.....	200 mcg
Inositol.....	1000 mcg
Biotin.....	2 mcg

many cells had been planted on solid medium. The diffusion of more toxin into the region was probably slow and the surviving cells were exposed to concentrations of actidione lower than the original. A series of tests were designed to discover the tolerances of actidione by resistant and sensitive cultures and to determine the influence of cell numbers upon the inhibitory effect of actidione. The four cultures of an ascus in which regular segregation had occurred for sensitivity and resistance were inoculated into broth tubes and agar plates at concentrations from 0 to 3.0 ppm. Loops of cell suspensions containing numbers ranging from 3.7×10^3 to 2.5×10^5 cells were used as inocula.

The plus (+) and minus (−) signs in table 2 (instead of density readings) record the results of the tests in *liquid* medium. The lowest density readings in the (+) categories were about 150 as compared to over 900 for the tubes in which abundant growth occurred, but the (−) categories uniformly showed no growth. Although culture 18718 is clearly a sensitive culture, it grew at 0.2 ppm when the inoculum was heavy. Culture 18720 (which is also sensitive) failed to grow in every instance in liquid medium at 0.2 ppm. At cell concentrations ranging from 2.5×10^5 to $3.0 \times$

TABLE 2
Diagnosis in liquid and in solid medium

Cultures	Numbers of Cells	Actidione Concentration in PPM.							
		0	0.2	0.5	0.8	1.0	1.5	2.0	3.0
18717	2.5×10^5	+/3	+/3	+/3	+/3	+/3	+/3	+/3	-/2
18718	2.5×10^5	+/3	+/2	-/1	-/0	-/0	-/0	-/0	-/0
18719	2.5×10^5	+/3	+/3	+/3	+/3	+/3	+/3	+/3	-/2
18720	2.5×10^5	+/3	-/3	-/1	-/0	-/0	-/0	-/0	-/0
18717	1.2×10^5	+/3	+/3	+/3	+/3	+/3	+/3	-/3	-/2
18718	1.2×10^5	+/3	+/2	-/1	-/0	-/0	-/0	-/0	-/0
18719	1.2×10^5	+/3	+/3	+/3	+/3	+/3	-/3	-/3	-/2
18720	1.2×10^5	+/3	-/3	-/1	-/0	-/0	-/0	-/0	-/0
18717	6.0×10^4	+/3	+/3	+/3	+/3	+/3	-/3	-/2	-/1
18718	6.0×10^4	+/3	+/2	-/1	-/0	-/0	-/0	-/0	-/0
18719	6.0×10^4	+/3	+/3	+/3	+/3	+/3	-/3	-/3	-/1
18720	6.0×10^4	+/3	-/3	-/0	-/0	-/0	-/0	-/0	-/0
18717	3.0×10^4	+/3	+/3	+/3	+/3	+/3	-/3	-/2	-/1
18718	3.0×10^4	+/3	+/1	-/0	-/0	-/0	-/0	-/0	-/0
18719	3.0×10^4	+/3	+/3	+/3	+/3	+/3	-/3	-/2	-/0
18720	3.0×10^4	+/3	-/3	-/0	-/0	-/0	-/0	-/0	-/0
18717	1.5×10^4	+/3	+/3	+/3	+/3	+/3	-/1	-/2	-/1
18718	1.5×10^4	+/3	-/1	-/0	-/0	-/0	-/0	-/0	-/0
18719	1.5×10^4	+/3	+/3	+/3	-/3	-/3	-/1	-/2	-/0
18720	1.5×10^4	+/3	-/2	-/0	-/0	-/0	-/0	-/0	-/0
18717	7.5×10^3	+/3	+/3	+/3	-/3	-/3	-/1	-/1	-/0
18718	7.5×10^3	+/3	-/1	-/0	-/0	-/0	-/0	-/0	-/0
18719	7.5×10^3	+/3	+/3	+/2	+/3	-/3	-/1	-/2	-/0
18720	7.5×10^3	+/3	-/1	-/0	-/0	-/0	-/0	-/0	-/0
18717	3.7×10^3	+/3	+/3	+/3	-/3	-/3	-/1	-/1	-/0
18718	3.7×10^3	+/3	-/1	-/0	-/0	-/0	-/0	-/0	-/0
18719	3.7×10^3	+/3	+/3	+/2	-/3	-/3	-/1	-/2	-/0
18720	3.7×10^3	+/3	-/1	-/0	-/0	-/0	-/0	-/0	-/0

10^4 and at concentrations of actidione ranging from 0.5 ppm to 1.0 ppm, the gene-pair principally responsible for differentiating sensitivity and resistance are clearly distinguished. The regularity of segregation indicates (fig. 1) that the principal genes are stable.

The numbers (table 2) show the results of tests on *solid* medium. Diagnosis is possible in every instance at 2 ppm even with the smallest number of cells. Growth occurred on every spot at concentrations of 0.2 ppm. There is general agreement between tests in liquid and solid media, but cells grow on solid medium at concentrations of actidione which are inhibitory in liquid medium. Discrete colonies are

TABLE 3
Replicate tube tests

Cultures	Days	Turbidity Readings						
		1	2	3	4	5	6	7
18717	2	10	45	15	45	15	15	30
	3	220	130	300	105	240	300	375
18718	2	0	0	0	0	0	0	0
	3	0	0	0	0	0	0	0
18719	2	35	50	75	70	55	115	95
	3	240	240	260	260	230	300	290
18720	2	0	0	0	0	0	0	0
	3	0	0	0	0	0	0	0

formed in the area of the drop when sensitive cultures are planted on low concentrations of actidione, indicating that a few cells from a sensitive culture can grow on low concentrations of actidione. Discrete colonies also appear when resistant cells are planted on high concentrations of actidione. This suggests that the populations of both resistant and sensitive inocula are usually mixed. As long as the cultures are tested, without previous contact to actidione, in liquid medium containing 1 ppm with an inoculum of about 6×10^4 cells per tube, a differentiation between the genes controlling sensitivity and resistance can be demonstrated.

Table 3 records the results of a series of replicate tube tests read after two and three days in which 1 ppm of actidione was used with an inoculum of 6×10^4 cells per tube. The two sensitive cultures failed to grow; all resistant cultures grew in every tube with minor variations in amounts of growth. This finding supports the use of the tube test for distinguishing the two members of this gene-pair.

Six heterozygous families (I to VI, fig. 1) were analyzed from crosses made between actidione-resistant and actidione-sensitive cultures. The expected segregations occurred in 68 of the 71 tetrads from the heterozygotes. The actidione-resistant parental cultures for Families II, IV, V, and VI were descended from the resistant mutant 18159R which was the actidione-resistant parent in Families I and III. The actidione-sensitive parental cultures for the first five families were all obtained from the breeding stock (outside the pedigree). The actidione-sensitive parental culture of Family VI was selected from Family IV.

A homozygous mating (Family VII of two actidione-sensitive strains, chosen from Family II) produced segregants all of which were sensitive to actidione.

Family VIII arose from a cross made between an actidione-sensitive strain from Family II and an actidione-sensitive strain selected from outside the pedigree. It produced offspring which were all sensitive to actidione.

A homozygous mating (Family IX) of two actidione-resistant cultures, both of which were obtained from Family II, yielded tetrads all resistant to actidione.

Every family was heterozygous for from two to six other genes which generally segregated regularly. There was no evidence of linkage between AC and other genes.

FIGURE 1.—Pedigree of Actidione Sensitivity and Resistance. The symbols AC and ac signify resistance and sensitivity respectively. The inscribed symbols indicate cultures selected from the breeding stock. The numerals represent the number of different types of tetrads resulting from each mating.

Three diploid zygotes of the same genotype as the hybrid from which Family I (fig. 1) originated:

<u>17911</u>	<u>α</u>	<u>su</u>	<u>GA ME</u>	<u>ma</u>	<u>CD MG</u>	<u>ac</u>
18159R	a	su	GA me	MA	cd mg	AC

were tested for fermentative ability and found to be suc GAL MAL MGS MEB. The two-letter symbols indicate genotypes (small letters for recessive and capitals for dominants): *SU*, sucrose-fermentation; *GA*, galactose-fermentation; *MG*, methyl alpha-D-glucopyranoside-fermentation; *ME*, melibiose-fermentation; *CD*, cadmium-resistance. Three-letter symbols indicate phenotypes. Capitals indicate fermentative ability (or resistance) and small letters inability to ferment (or sensitivity).

This confirms the view that the isolates were diploid. The diploid cultures were tested under the conditions which discriminate resistance and sensitivity of haploid cultures to cadmium and actidione and found to be "resistant." It was inferred that the genes controlling resistance to cadmium and actidione are dominant although all the implications of this inference have not yet been explored completely.

A series of transfers on agar containing high concentrations of actidione yielded a few discrete colonies which grew on agar containing 50 ppm of actidione. The discrete colonies were all restricted to a single drop and presumably originated from a single clone.

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SUMMARY

A single gene-pair in *Saccharomyces* controlling sensitivity versus resistance to actidione has been described. The optimal conditions for diagnosis of the genotype is liquid culture at 1 ppm of actidione inoculated with 6×10^4 cells. The relations between the size of the inoculum and the concentration of actidione (as well as the effect of testing on solid and liquid medium) were observed.

LITERATURE CITED

- BRENES-POMALES, A., C. C. LINDEGREN, and G. LINDEGREN, 1955 Gene control of copper-sensitivity in *Saccharomyces*. *Nature* **176**: 841-842.
- MIDDLEKAUFF, J. E., S. HINO, G. LINDEGREN, and C. C. LINDEGREN. Gene control of resistance vs. sensitivity to cadmium in *Saccharomyces*. *J. Bacteriol.* (in press).
- WHIFFEN, A. J., 1948 Actidione, an antibiotic produced by *Streptomyces griseus*. *J. Bacteriol.* **56**: 283-291.