

Dr KENNETH E. PAPA'S COLLECTION

40 CHRIS ALBERTIN (RECENTECH III)

DEPT of PLT PATH

-UGA - ATHENS GA 30602

Tulane

Department of Biology
2000 Percival Stern Hall
Tulane University
New Orleans, Louisiana 70118
(504) 865-5546

August 3, 1986

Dear Chris:

I'm taking my kids on a vacation next week and am afraid this won't get off in the mail before I leave if I give the letter to a typist. So I'm typing it myself.

I'm enclosing copies of the various letters that have gone to All Concerned in the business of closing down Dr. Papa's Culture Collection. Also enclosed is a copy of the Bennett and Papa paper.

I have spent rather a lot of time trying to find out what strains need to go to ATCC. I have generated two lists: Aspergillus parasiticus and A. flavus. These give the mutants in the sequence they appear in Tables 2 and 3, respectively, of the Bennett and Papa chapters. For the A. parasiticus strains, I could not find several of the genotypes listed in the 1978 paper in the silica gel log. I list what I could find -- I looked pretty hard and don't think I missed anything, but you are more familiar with the collection than I, so you are welcome to double check.

The A. flavus list seems to be more complete. It lists the mutant gene symbols in alphabetic order (which is the sequence of Table 4, Bennett and Papa). I have checked the genotypes which are already at ATCC on the master list. Of the remainder, I have given the silica gel numbers for everything but afl-20, afl-22, arg-2, arom (on linkage group IV), ts, and ylo. Here I'm not as certain that I excluded everything as I was with the A. parasiticus stocks. I suspect you can find these somewhere. My big problem is that I'm not sure the various "ylo's" or "arom's" I see listed in many places are the same ones that Dr. Papa mapped.

In any event, the two lists I have prepared tell you what strains go to ATCC. The contact person there is:

Dr. S. C. Jong
American Type Culture Collection
12301 Parklawn Drive
Rockville, MD 20852 301/881-2600

*Also need
chloroform*



American Type Culture Collection

12301 PARKLAWN DRIVE · ROCKVILLE, MARYLAND 20852-1776 USA · (301) 881-2600 · TELEX: ATCCROVE 908-768

December 15, 1986

Dr. J.C. Albertin *RSCH TECH III*
(Dr. K.E. Papa's Lab)
Department of Plant Pathology
University of Georgia
Athens, GA 30602

Dear Dr. Albertin:

The cultures which you kindly deposited have been assigned the following ATCC accession numbers:

<u>Aspergillus flavus</u>	71-31a-10	ATCC 62627
<u>Aspergillus flavus</u>	88-A14-1	ATCC 62628
<u>Aspergillus flavus</u>	100 5t-8	ATCC 62629
<u>Aspergillus flavus</u>	170 5t-8	ATCC 62630
<u>Aspergillus flavus</u>	241 T37-10	ATCC 62631
<u>Aspergillus flavus</u>	244 T37-17	ATCC 62632
<u>Aspergillus flavus</u>	650 5T-18-6	ATCC 62633
<u>Aspergillus flavus</u>	651 5T-18-7	ATCC 62634
<u>Aspergillus flavus</u>	653 16-7-8	ATCC 62635
<u>Aspergillus flavus</u>	654 A17-19	ATCC 62636
<u>Aspergillus flavus</u>	655 A14-1-3	ATCC 62637
<u>Aspergillus flavus</u>	656 A14-1-4	ATCC 62638
<u>Aspergillus flavus</u>	657 A14-1-5	ATCC 62639
<u>Aspergillus flavus</u>	658 9-2	ATCC 62640
<u>Aspergillus flavus</u>	659 9-6	ATCC 62641
<u>Aspergillus flavus</u>	660 9-7	ATCC 62642
<u>Aspergillus flavus</u>	1020 (19-8+579)-16	ATCC 62643
<u>Aspergillus flavus</u>	1025 (T44-7+592)-20	ATCC 62644
<u>Aspergillus flavus</u>	1028 (31a-18-1+579)-33	ATCC 62645
<u>Aspergillus flavus</u>	1060 81-46-193	ATCC 62646
<u>Aspergillus flavus</u>	1063 81-6-160	ATCC 62647
<u>Aspergillus parasiticus</u>	617 B43-23	ATCC 62648
<u>Aspergillus parasiticus</u>	619 B144-17	ATCC 62649
<u>Aspergillus parasiticus</u>	621 A43-7	ATCC 62650
<u>Aspergillus parasiticus</u>	624 B144-26	ATCC 62651

AFFILIATED ORGANIZATIONS

AMERICAN ASSOCIATION OF IMMUNOLOGISTS · AMERICAN INSTITUTE OF BIOLOGICAL SCIENCES · AMERICAN PHYTOPATHOLOGICAL SOCIETY · AMERICAN SOCIETY OF BIOLOGICAL CHEMISTS
AMERICAN SOCIETY FOR CELL BIOLOGY · AMERICAN SOCIETY FOR MICROBIOLOGY · AMERICAN SOCIETY OF PARASITOLOGISTS · AMERICAN SOCIETY OF ZOOLOGISTS
AMERICAN SOCIETY OF TROPICAL MEDICINE AND HYGIENE · CANADIAN FEDERATION OF BIOLOGICAL SOCIETIES · GENETICS SOCIETY OF AMERICA · INFECTIOUS DISEASES SOCIETY OF AMERICA
MYCOLOGICAL SOCIETY OF AMERICA · NATIONAL RESEARCH COUNCIL · NATIONAL ACADEMY OF SCIENCES · SOCIETY OF PROTOZOOLOGISTS · TISSUE CULTURE ASSOCIATION

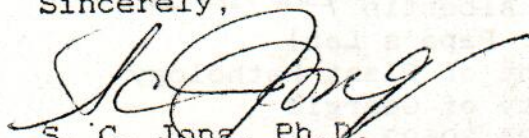
Dr. J.C. Albertin

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December 15, 1986

We also received a tube of Aspergillus flavus strain 652 16-7-4 labelled "will not grow" on it. Would it be possible for us to receive a replacement culture?

Thank you very much for your assistance.

Sincerely,



S. C. Jong, Ph.D.
Head, Mycology & Botany Department

SCJ/psk

Dr. Maren Klich of the Southern Regional Research Labs has agreed to curate the remainder of the collection, as described in the various letters enclosed. I think she should also have reference stocks of what goes to ATCC. Her address and phone:

Dr. Maren Klich
Southern Regional Research Center
P. O. Box 19687
New Orleans, LA 70179 504/589-7597

Since ATCC will be lyophilizing their stocks, they should be sent some living material. Dr. Klich will just keep the silica gel stocks as is.

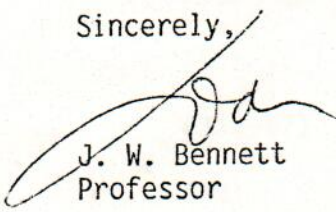
I'll be out of town Aug. 5-17th, but back here after that. Please write or call if you have any questions. I know this is super-tedious, but we are almost finished.

Finally, a very big personal thank you for all you have done. I don't think I could have made very much progress if you hadn't prepared the "Blue Book" with all the silica gel log information typed up.

I'm only going to send copies of this to Dick Hanlin and Louise Miller since they are the most concerned.

Best regards.

Sincerely,


J. W. Bennett
Professor

cc: R. Hanlin
M. Klich
L. Miller

DR DAVID WICKLOW
PHONE - 309/685-4011 EXT 384
NORTHERN RESEARCH CENTER
1815 N UNION ST
PEORIA, IL 61604

LIST OF ATCC ACQUISITIONS

<u>ATCC #</u>	<u>SILEICA Gel #</u>	<u>DESCRIPTION</u>
-	1	"PC-7" wild type
44617	(34-4) 234	t pdx bi B ₂ /B ₁ > 1 (HB2)
44617	649	afl 1 leu t
44618	241	afl 4 pdx t
46107	(2) 470	pdx w (NG/UV)
- 08	(17) 460	pab w (NG)
- 09	(6) 804	his w (NG)
- 10	(32) 516	IV w (NG)
- 11	(31a-22) 83	pro w (NG)
- 12	(31a-6) 176	phe w
- 13	(31a-18) 79	ad w (NG)
- 14	(58) 434	met w (NG)
- 15	(31a-17) 78	thi w
- 16	(19) 271	pdx w B ₂ /B ₁ > 1 (NG/UV/NG)
60040	(1-N) 827	nor lys w
- 1	(31a-25) 86	arg-7 w
- 2	(86-1) 796/797	afl 15 arg-7 w
- 3	(5t-18) 109	leu t
- 4	(118-1) 118 (T44)	afl 16 arg-2 t
- 5	(271-1) 774 (19-1)	afl 21 pdx w
- 6	491	leu pdx t
- 7	506	leu pdx w
- 8	579	t pro met pab his
- 9	592	w pro met pab his

Aspergillus flavus

The following genetic loci were cited in Table 3 of the Bennett and Papa paper. They exclude loci already represented in stocks at the ATCC.

In listing genotypes, I have placed the spore color mutant first.

Available on silica gel:

- ✓ #650. 5T-18-6 t leu afl-2
- ✓ #651. 5T-18-7 t leu afl-3
- ✓ #241. T37-10 pdx afl-4 Ts-1
- ✓ #244. T37-17 afl-5
- ✓ #652. 16-7-4 ylo pab afl-6
- ✓ #653. 16-7-8 lgt ylo pab afl-7
- ✓ #655. A14-1-3 w met afl-8
- ✓ #656. A14-1-4 w met afl-9
- ✓ #657. A14-1-5 w met afl-10
- ✓ #658. 9-2 w nic afl-11
- ✓ #659. 9-6 w nic afl-12
- ✓ #660. 9-7 w nic afl-13
- ✓ #654. A17-19 fawn arom afl-14
- ✓ #1028. (31a-18-1 + 579)-33 t met his afl-17
- ✓ #1025. (T44-7 =592)-20 w his afl-19
- ✓ #1020. (19-8 + 579)-16 t pab met his afl-25
- ✓ #1034. 31-7 cnx
- ✓ #170. 5T-f fwn
- ✓ #100. 5t-8 lys (lys-3)
- ✓ #88. A14-1 met (met-4)

Aspergillus parasiticus

Published strains available on silica gel:

- ✓ #621. A43-7 or nic afla-1
- ✓ #617. B43-23 ylo pro afla-1
- ✓ #619. B144-17 gb lys-2 afla-2
- ✓ #624. B144-26 w met afla-2

Citation: Papa, K. E. 1978.
The parasexual cycle in Aspergillus parasiticus.
Mycologia 70: 766-773.

cit 7
cit 11

Other strains listed in this paper could not be located in Dr.
Papa's silica gel log.

- 2 -

A. flavus con't

- ✓ #1063. 81-6-160 nia NO₃ hc group 3 tester
- ✓ #71. 31a-10 nic
- ✓ #1060. 81-46-193 nii NO₂ hc group 3 tester

used as it to construct ...
 the genes which are already in stocks at ATCC. The list I send Chris Liberson
 consists of those strains not already represented at ATCC.

Genetic Loci of Aspergillus flavus

"MASTER LIST"

Gene symbol	Linkage group	Properties	References
✓ <u>ad</u> ATCC 4613	II	adenine req.	2, 4, 10
✓ <u>afl-1</u> ATCC 44617	VII	aflatoxin ⁻ , dominant (dominance expressed in diploids but, in heterokaryons)	9, 10, 13
✓ <u>afl-4</u> ATCC 44618	II	aflatoxin ⁻ , recessive	9, 10
<u>afl-2</u> <u>afl-3</u> <u>afl-5</u> <u>afl-6</u> <u>afl-7</u> <u>afl-8</u> <u>afl-9</u> <u>afl-10</u> <u>afl-11</u> <u>afl-12</u> <u>afl-13</u> <u>afl-14</u>	?	aflatoxin ⁻ , recessive, and all appear to be nonallelic	9
✓ <u>afl-15</u>	VII	aflatoxin ⁻ , recessive	13
✓ <u>afl-16</u>	VII	aflatoxin ⁻ , recessive	13
<u>afl-17</u>	VII	aflatoxin ⁻ , recessive	13
<u>afl-19</u>	VII	aflatoxin ⁻ , recessive	13
<u>afl-20</u>	V VII	aflatoxin ⁻ , recessive	13
✓ <u>afl-21</u> b2	VII	aflatoxin ⁻ , recessive	13
<u>afl-22</u>	VII	aflatoxin ⁻ , recessive (allelic to <u>afl-20</u>)	13
<u>afl-25</u>	VII	aflatoxin ⁻ , recessive	13
✓ <u>afl-B2</u> ATCC 34081	VIII	higher B ₂ than B ₁ accumulation, recessive (originally referred to as HB2)	5, 6, 7, 10
✓ <u>arg-2</u>	II	arginine req.	2, 4
<u>arg-4</u>	IV	arginine req.	4
✓ <u>arg-7</u>	VII	arginine req.	13
<u>arom</u>	IV	aromatic metabolites req.	4
✓ <u>bi</u>	III	biotin req.	4

Genetic Loci of Aspergillus flavus (Continued)

Gene symbol	Linkage group	Properties	References
<u>cnx</u>	?	nitrate and hypoxanthine non-utilizing	14
<u>fwn</u>	II	fawn conidia	4
✓ <u>his</u>	VIII	histidine req.	7, 10
✓ <u>iv</u>	VII	isoleucine/valine req.	4, 10
✓ <u>leu</u>	VII	leucine req.	2, 4
<u>lys-3</u>	III	lysine req.	4
✓ <u>lys-4</u>	IV	lysine req.	4
✓ <u>met-3</u>	III	methionine req.	2, 4, 10
<u>met-4</u>	IV	methionine req.	4
<u>nia</u>	II	nitrate non-ut.	14
<u>nic</u>	IV	nicotinic acid req.	4
<u>nii</u>	VI	nitrate non-ut.	14
✓ <u>nor</u>	VII	norsolorinic acid accumulation	12, 13
✓ <u>pab</u>	V	p-aminobenzoic acid req.	4, 10
✓ <u>pdx</u>	VI	pyridoxin req.	2, 4, 10
✓ <u>phe</u>	III	phenylalanine req.	10
✓ <u>pro</u>	I	proline req.	2, 4, 10
✓ <u>t</u>	IV	tan conidia	1, 2, 4
✓ <u>thi</u>	I	thiamine req.	4, 10
<u>ts</u> (see # 241-fig ²)	VIII	temperature sensitive	
✓ <u>w</u>	II	white conidia	1, 2, 4
<u>ylo</u> (see # 152) ²	III	yellow conidia	4

1. Leaich, Laurie and K. E. Papa. 1974. Aflatoxins in mutants of Aspergillus flavus. Mycopathologia et Mycologia Applicata 52: 223-229.
2. Papa, K. E. 1973. The parasexual cycle in Aspergillus flavus. Mycologia 65: 1201-1205.
3. Leaich, Laurie L. and K. E. Papa. 1975. Identification of Diploids of Aspergillus flavus by the nuclear condition of conidia. Mycologia 67: 674-678.
4. Papa, K. E. 1976. Linkage groups in Aspergillus flavus. Mycologia 68: 159-165.
5. Papa, K. E. 1977. A mutant of Aspergillus flavus producing more aflatoxin B₂ than B₁. Applied and Environmental Microbiology 33: 206.
6. Papa, K. E. 1977. Genetic analysis of a mutant of Aspergillus flavus producing more aflatoxin B₂ than B₁. Mycologia 69: 556-562.
7. Papa, K. E. 1977. Genetics of aflatoxin production in Aspergillus flavus: Linkage between a gene for a high B₂:B₁ ratio and the histidine locus on linkage group VIII. Mycologia 69: 1185-1190.
8. Papa, K. E. 1978. The parasexual cycle in Aspergillus parasiticus. Mycologia 70: 766-773.
9. Papa, Kenneth E. 1979. Genetics of Aspergillus flavus: Complementation and mapping of aflatoxin mutants. Genetical Research 34: 1-9.
10. Papa, K. E. 1980. Dominant aflatoxin mutant of Aspergillus flavus. J. Gen. Microbiol. 113: 279-282.
11. Foudin, L. L., K. E. Papa, and R. T. Hanlin. 1981. Nuclear behavior during conidiogenesis in Aspergillus flavus. Canadian Journal of Botany 59: 2116-2120.
12. Papa, K. E. 1982. Norsolorinic acid mutant of Aspergillus flavus. J. Gen. Microbiol. 128: 1345-1348.
13. Papa, K. E. 1984. Genetics of Aspergillus flavus: Linkage of aflatoxin mutants. Can. J. Microbiol. 30: 67-73.
14. Papa, K. E. 1986. Heterokaryon incompatibility in Aspergillus flavus. Mycologia 78: in press.

For: Genetics of Pathogenic Fungi

(G. S. Sidhu, ed). Vol. 6

Advances in Plant Pathology

Genetics of Aflatoxigenic Aspergillus species

1

2

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University of Georgia, Athens, Georgia 30602

I. INTRODUCTION

The genus Aspergillus is both common and cosmopolitan. The many known species are classified within eighteen "Groups" by Raper and Fennell (1965) in the major taxonomic treatment of the genus. The "A. flavus Group" consists of a number of closely related yellow-green molds; the precise number of distinguishable species is a matter of dispute among taxonomists.

Economically the four most important species within the "A. flavus group" are A. flavus, A. parasiticus, A. oryzae and A. sojae. A. oryzae and A. sojae are "good" species. Sometimes known as the "koji molds" these fungi are used in the preparation of Oriental condiments and drinks such as soy sauce, saké and miso. A. oryzae is on the GRAS list ("Generally Regarded as Safe") of the U. S. Food and Drug Administration. Several major industrial amylases and proteases are isolated from A. oryzae fermentations (Bennett, 1985 a,b).

A. flavus and A. parasiticus are "bad" species. They produce aflatoxins, a group of pharmacologically active secondary metabolites which are highly toxic, carcinogenic, and mutagenic. \rightarrow

\rightarrow Chemically the aflatoxins are substituted coumarins containing the reactive difuran moiety. The major aflatoxins are called B₁, B₂, G₁, and G₂ based on their respective blue and green fluorescence under long wave ultraviolet light and their relative chromatographic mobilities. Typically, aflatoxin B₁ is the major metabolite produced

by aflatoxigenic strains, and most studies concerning the biological activity of aflatoxin have concentrated on this metabolite. The early history of aflatoxin research is thoroughly reviewed in Goldblatt (1969).

In the mycotoxin and plant pathology literature, the term "A. flavus group" has been used loosely in a collective sense for the various isolates and strains that are potentially aflatoxigenic, not to encompass the formal taxonomic unit delineated in Raper and Fennell (1965). Many researchers do not distinguish between A. flavus and A. parasiticus, choosing to call all aflatoxigenic molds "A. flavus". We will follow the practice of using "A. flavus group" in this limited sense, to describe strains and isolates of A. flavus and A. parasiticus.

II. AFLATOXIGENIC MOLDS AS PATHOGENS

The A. flavus group fungi are extremely common molds that can grow on a wide variety of substrates, under a wide range of conditions. In general, A. flavus group fungi are associated with damage to seed crops (grains, legumes, nuts) rather than with fruits and vegetables. The number of substrates from which the mold/and or aflatoxin has been isolated is long and impressive (Ciegler, 1977).

For convenience, the fungi that infest grains have been divided into: 1) field fungi, those which invade crops before harvest, and 2) storage fungi, those associated with post-harvest damage. The storage fungi are primarily saprophytes of the genera Aspergillus and Penicillium (Christensen and Kaufman, 1974). A. flavus

has been traditionally viewed as a storage fungus, and this categorization influenced the direction of much of the early research. As we have learned more and more about the A. flavus group it has become obvious that these molds can and do invade crops in the field, and that aflatoxins can accumulate prior to harvest, especially in peanuts (McDonald and Harkness, 1967; Hanlin, 1985; Hill et al., 1985), cottonseed (McMeans and Ashworth, 1966) and corn (Lillehoj et al., 1976; Payne, 1983). Such field invasion is frequently, but not always, associated with insect damage, drought or other environmental stress. Some evidence suggests that field contamination is more common in warm, wet climates, particularly in regions of subtropical and tropical agriculture (Williams and McDonald, 1983). Attempts to breed cultivars resistant to A. flavus invasion and aflatoxin damage have been made (Mixon and Rodgers, 1973; Mixon, 1981; Widstrom and Zuber, 1983).

One cannot help but respect the metabolic versatility and ecological adaptability of the A. flavus group. In addition to their nearly ubiquitous presence as saprophytes and their success as facultative plant pathogens, these molds are among the agents involved in aspergillosis, a frequently lethal opportunistic infection of Homo sapiens (Rippon, 1982) and are also aggressive insect pathogens (Steinhaus, 1949).

III. SOME TAXONOMIC AND PHYSIOLOGICAL CONSIDERATIONS

Not all wild type strains of A. flavus and A. parasiticus produce aflatoxins. In a literature survey of published studies encompassing 3343 isolates, a total of 1847 or 56% were aflatoxigenic (Bennett, 1982). The amount of aflatoxin produced by aflatoxigenic strains also varies widely. In general, isolates of A. parasiticus are highly toxigenic and produce both B and G aflatoxins, while A. flavus isolates produce only B aflatoxins and contain a greater percentage of non-toxigenic strains (Klich and Pitt, 1985; Hesseltine et al., 1970). Some taxonomists recognize a third aflatoxigenic species, A. toxicarius (Murakami, 1971) but others subsume A. toxicarius within A. flavus or A. parasiticus (Klich and Pitt, 1985). The 16th Edition of the American Type Culture Collection Catalogue (Jong and Gantt 1984) no longer lists a separate heading for A. toxicarius. Early reports that other species of Aspergillus, or species of Penicillium and Mucor produce aflatoxin, have never been confirmed (Detroy et al., 1971).

Substrate influences the amount of aflatoxin produced by toxigenic isolates. Nearly all natural substrates tested allow some aflatoxin formation, with high carbohydrate substrates being the best. In natural habitats, moisture content is the most important limiting variable. A seed moisture content of 15-30% and a relative humidity of 85% or higher are required for aflatoxin production. Optimal temperature range for aflatoxin synthesis is narrower than that required for growth; highest levels of aflatoxin are produced between 25-30°C. See Diener

and Davis (1969) for a review of environmental parameters affecting aflatoxin production.

The Aspergilli are not particularly fastidious and are easy to handle in the laboratory. Members of the A. flavus group are no exception. Recipes for media, and descriptions for single spore isolations, transfer and storage methods, and other routine microbiological maneuvers for aspergilli are given in Raper and Fennell (1965). The most commonly employed minimal medium used in both taxonomic identification and in genetic studies is Czapek-Dox formulated as follows (per liter of water): Na NO₃, 3.0 g; K₂HPO₄, 1.0 g; Mg SO₄ · 7 H₂O, 0.5 g; KCl, 0.5 g; Fe SO₄ · 7 H₂O, 0.01 g; sucrose, 30.0 g; agar 20.0 g.

Most complete media devised for molds will support satisfactory growth of A. flavus and A. parasiticus. In our genetic studies, the complete medium used by Bennett is potato dextrose agar supplemented with 0.5% yeast extract. The complete medium used by Papa is Czapek-Dox supplemented with 0.75% malt extract and 0.25% yeast extract. Sodium laecynholate (0.08-0.096%) may be added to complete medium to restrict colony size. p-Fluorophenylalanine (0.1%) or benlate (3.5-4.0 mg ml⁻¹) may be added to complete medium to induce haploidization in parasexual diploids.

Aspergillus nidulans is one of the best characterized model systems for eukaryotic genetic analysis. Experimental protocols for mutagenesis, enrichment, culture practices, and biochemical analyses developed for A. nidulans usually can be modified for use with species

of the A. flavus group. Strategies developed for studying genetic phenomena in A. nidulans are reviewed in Smith and P. E. (1977). Several textbooks devoted to fungal genetics also provide relevant methodologies and references (Esser and Kuenen, 1967; Burnett, 1975; and Fincham et al., 1979).

Many media have been devised for the laboratory production of aflatoxins. Highest yields are obtained with natural substrates (peanuts, rice, wheat) or with complex liquid media containing supplements such as yeast extract or corn steep liquor. Because Czapek-Dox medium is a poor substrate for aflatoxin production, two defined media have been devised especially for this purpose. The medium developed by Adye and Mateles (1964) is formulated as follows (per liter of water): ~~(do not leave space)~~

KH_2PO_4 , 10.0 g; $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 2.0 g; $\text{Na}_2\text{B}_4\text{O}_7 \cdot 10\text{H}_2\text{O}$, 0.7 mg; $(\text{NH}_4)_6\text{Mo}_7\text{O}_{24} \cdot 4\text{H}_2\text{O}$, 0.5 mg; $\text{Fe}_2(\text{SO}_4)_3 \cdot 6\text{H}_2\text{O}$, 10.0 mg; $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$, 0.11; $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$, 17.6 mg; and sucrose, 50.0 g; $(\text{NH}_4)_2\text{SO}_4$, 3.0 g.

The medium developed by Reddy et al., (1971) is formulated as follows (per liter of water): ~~(do not leave space)~~

(NH_4SO_4) , 3.5 g; KH_2PO_4 , 0.75 g; $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 0.35 g; $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$, 0.75 g; $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$, 10.0 mg; $\text{MnCl}_2 \cdot 4\text{H}_2\text{O}$, 5.0 mg; $(\text{NH}_4)_2\text{Mo}_7\text{O}_{24} \cdot 9\text{H}_2\text{O}$, 2.0 mg; $\text{Na}_2\text{B}_4\text{O}_7$, 2 mg; $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$, 2 mg; sucrose, 85.0 g; L-asparagine, 10.0 g.

Several "quick screening" media facilitate plate assays of putative aflatoxin - producing ability. These are reviewed in Bennett and Deutsch (1985). The most useful one for genetic studies is "Aflatoxin Producing Ability Medium" developed by Hara et al., (1974).

It is a modified Czapek-Dox medium and contains (per liter of water): $(\text{NH}_4)_2\text{H}_2\text{PO}_4$, 10 g; K_2HPO_4 , 1 g; $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 0.5 g; KCl , 0.5 g; $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$, 0.01 g; sucrose, 30 g; corn steep liquor, 0.5 g; and HgCl_2 , 5×10^{-4} M. Inverted Petri plates are viewed under long wave ultraviolet light after 2-10 days of growth. Blue fluorescence in the agar surrounding the colonies is a putative test for aflatoxin production, which is then confirmed by an official method using thin layer chromatography or high performance liquid chromatography, and authentic standards (Walker, 1983).

IV. GENETIC STUDIES

A. General considerations

Both A. flavus and A. parasiticus are imperfect (anamorphic) species with multinucleate conidiospores. As such, neither species offers an advantage over A. nidulans for basic research in fungal genetics. They are of interest because they produce aflatoxins.

The parasexual cycle has been elucidated in both A. flavus (Papa, 1973; Gussack et al., 1977) and A. parasiticus (Papa, 1978; Bennett, 1979), and the genetics of aflatoxigenic molds has been reviewed by Bennett (1982), Bu'Lock (1985) and Bennett and Deutsch (1985). Parasexual analysis has been hampered by the non-random recovery of certain genotypes among segregants from diploids, and by a uniform diameter of haploid and diploid conidiospores which makes classification of segregant ploidy difficult (Leach and Papa, 1975; Bennett et al., 1980; Foudin et al., 1981). Both fluoro-phenyl alanine and benlate are successful haploidization agents.

B. Aspergillus parasiticus

Three major classes of mutants have been described in A. parasiticus:

- 1) anthraquinone-accumulating mutants blocked in the aflatoxin pathway,
- 2) spore color and auxotrophic mutants used as markers in elucidating the parasexual cycle and generating linkage maps and
- 3) two peculiar classes of variants with lost or attenuated aflatoxigenicity which have been dubbed fan and fluff.

1. Anthraquinone-accumulating mutants. These mutants are blocked in the aflatoxin pathway and accumulate anthraquinone intermediates (Table 1). They all have brightly colored mycelia which can be used as a visual screen in parasexual studies. These mutants have been exploited even more extensively in studies of aflatoxin biosynthesis (Steyn, 1980; Bennett and Christensen, 1983). Each of these anthraquinone-accumulating strains has been deposited in several culture collections, and each one has been designated by a series of slightly different gene symbols in the many published reports on aflatoxin biosynthesis. Bennett confesses to being one of the worst offenders in this proliferation of symbols, and by way of penance, has compiled a comprehensive summary of synonymy in Table 1.

2. Spore color and auxotrophic mutants. Published spore color and auxotrophic mutants of A. parasiticus are listed in Table 2. Mutants from Bennett's and Lennox's laboratories were isolated from different transfers of A. parasiticus SU-1 (ATCC 56775 and ATCC 56856) by ultraviolet light mutagenesis (Bennett, 1979; Lennox and Davis, 1983). Mutants from Papa's laboratory were isolated from a strain of

A. parasiticus found on Georgia pecans by NTG mutagenesis (Papa, 1978).

After parasexual analysis, Papa (1978) assigned seven loci to four linkage groups: I pro; II. nic; III. lys-1, afl-1; IV. gb, lys-2, afl-2. By similar parasexual methods, ten loci were assigned to six linkage groups by Bradshaw et al., (1983): I. yeA II. brA III. whA cysA IV. lysA lysB; V. norA verA; and VI. adeA metA pdxA. Lennox and Davis (1983) performed complementation tests between aflatoxin-deficient mutants and proposed seven complementation groups: adm-4,7; adm-5,8,9; adm-10; adm-11, bwn-1; ver-1; and nor-1. No attempt to cross mutants from Papa's strain of A. parasiticus with the mutants derived from SU-1 have been made, so the rudimentary linkage groups proposed in Papa (1978) and Bradshaw et al., (1983) must be viewed as independent entities.

Each laboratory has followed different nomenclatural conventions for gene symbols. In some cases, the gene symbols are unique, e. g. independently isolated white-spored mutants are designated, respectively, w, wh-1 and w-1 by Papa, Bennett, and Lennox. In other cases there is overlapping usage; e. g. both Papa and Bennett have named different lysine requiring auxotrophs "lys-1", and both Lennox and Bennett have named different adenine-requiring auxotrophs "ade-1".

According to the conventions for gene symbols in A. nidulans (Clutterbuck, 1973), an italicized three letter symbol is used to describe the specific locus that produces a phenotype (e. g., pdx for pyridoxine- requirement); hyphenated numbers designate unmapped mutants (pdx-1); a capital letter immediately following the three

letter symbols designates a mapped gene (e. g., pdxA); and unhyphenated numbers designate alleles (e. g., pdxA1). Bradshaw et al., (1983) modified some of the gene symbols of A. parasiticus to conform to the A. nidulans conventions. Because there are virtually no collections of multiple alleles and because there have been almost no collaborative studies between laboratories, we now feel that these modifications were premature. We recommend, for the present, retention of original gene symbols, or use of the conventions for unmapped genes. For example, we prefer the symbol ver-1 (Bennett, 1979) rather than verA1 (Bradshaw et al., 1983) for the versicolorin-accumulating mutant (see Table 1).

We recognize that a uniform system for nomenclature should be adopted at some point and hope that as more workers become interested in the genetics of aflatoxigenic molds, sufficient numbers of mutants and crossing data will be available to justify revision of published gene symbols. The system adopted for the yeast Saccharomyces cerevisiae offers some advantages to the A. nidulans system and before a whole-sale change of gene symbols is proposed this system should be considered. For those interested in the subtleties of genetic nomenclatural conventions fungi, see Bennett and Lasure (1983) for a comparative summary.

3. Attenuated strains. Experimental induction of attenuated and aflatoxin-negative strains of A. flavus and A. parasiticus can be achieved by successive transfers of mycelial macerates in defined medium (Bennett, 1981; Bennett et al., 1981a, 1986). These peculiar

variants display altered morphology as well as lowered or lost aflatoxigenicity. The characteristic phenotypes are called fan and fluff. The pleiotropic fan or fluff phenotype can be introduced into wild type strains as well as into auxotrophs and marked diploids (Bennett et al., 1981b, 1986). We originally reported that both fan and fluff were non-aflatoxigenic (Bennett et al., 1981a,b; Bennett, 1982); more recent data indicate that the fluff isolates are unstable and revert to aflatoxigenicity. (Bennett et al., 1986). The high frequency with which fan and fluff variants are isolated, the pleiotropic nature of their respective phenotypes, and the anomalous behavior of anthraquinone genes (nor-1 and ver-1) in crosses involving fan and fluff are consistent with a model involving genetic transpositions.

C. Aspergillus flavus

Three major classes of mutants have been described in A. flavus: 1) high aflatoxin-B₂ accumulating strains, 2) spore color and auxotrophic mutants used as markers in elucidating the parasexual cycle and generating linkage map data, and 3) aflatoxin-negative mutants. The formal genetics of A. flavus is more developed than that of A. parasiticus and over thirty genes have been mapped to eight linkage groups.

1. High aflatoxin B₂-accumulating strains.

A number of natural isolates of A. flavus accumulate an excess of aflatoxin B₂ over aflatoxin B₁. Van Walbeek et al., (1968) isolated six of these strains from commercial food products (ATCC strains 18166-18171). (do not leave space)

Schroeder and Carlton (1973) isolated a similar variant from black

pepper (ATCC 24109; SRRC 141; Papa 5642), and Gunasekaran (1981) reported a high aflatoxin B₂ strain from the facial scar of a leukemia patient. The strain isolated by Schroeder and Carlton (ATCC 24109) has been used to study the late stages of aflatoxin biosynthesis (Dutton *et al.*, 1985).

Papa (1977a) induced a mutant with a high aflatoxin B₂/ B₁ ratio using nitrosoguanidine. This mutant (afl-B2, originally referred to as HB2) has been linked to the histidine locus on Linkage Group VIII (Papa, 1977a, b, c; 1980).

2. Spore color, auxotrophic and aflatoxin negative mutants.

Published genetic loci of A. flavus, including known linkage assignments, are listed in Table 3. Because this work was all done in Papa's laboratory, there is less confusion with gene symbols than for A. parasiticus. Papa's conventions have been followed in both Tables 3 and 4.

In addition to the loci listed in Table 3 a series of aflatoxin negative mutants has been isolated and mapped. The mutants afl-2, afl-3, afl-5, afl-6, afl-7, afl-8, afl-9, afl-10, afl-11, afl-12, afl-13, and afl-14 are all recessive and non-allelic; no linkages have been determined for these markers (Papa, 1979). Another recessive aflatoxin-negative locus (afl-4) maps to Group II (Papa, 1979; 1980), while afl-15, afl-16, afl-17, afl-19, afl-20, afl-21, afl-22, and afl-25 are all recessive and on Group VII; of these, afl-20 and afl-22 are allelic (Papa, 1979). Finally, afl-1 also maps to Linkage Group VII.

This marker acts as a dominant in diploids, but the dominance is not expressed in heterokaryons (Papa, 1979; 1980; 1984). As noted above, the high aflatoxin-B₂ accumulating mutant, afl-B₂, maps to Group VIII (Papa, 1977a, b, c; 1980).

The sequence of markers on Group II is: afl-4 w ad arg-2 (Papa, 1973, 1976, 1979). The sequence of markers on Group VII is: (nor afl-1) leu afl-15 arg-7 afl-17 centromere. It has not been possible to determine whether nor or afl-1 is the terminal locus (Papa, 1979; 1982; 1984). A collection of mutant strains containing mapped loci has been deposited with the American Type Culture Collection (Table 4).

D. Interstrain and Interspecific Crosses

Heterokaryon incompatibility is widespread in the *Aspergilli* and the *A. flavus* group. No successful interstrain or interspecific crosses have been reported. Successful heterokaryons are only formed among complementing auxotrophs derived from the same ancestral wild type strain.

One study has specifically addressed heterokaryon incompatibility. Papa (1986) sampled strains of *A. flavus* from corn collected in fifteen Georgia counties. Auxotrophs of each of 32 strains were used to test all possible pair-wise combinations; 22 different heterokaryon compatibility groups were detected. Strains within the same heterokaryon compatibility group were not restricted to the same geographical area.

In an earlier study, six combinations of auxotrophs from *A. flavus* PC-7 and *A. flavus* NRRL 5565 failed to grow, as did twelve interspecific mixtures of auxotrophs of *A. flavus* and *A. parasiticus* (Gussack *et al*,

1977). Protoplast fusions between A. flavus and A. parasiticus auxotrophs were also unsuccessful (Leong et al., 1981).

Workers interested in isolating new mutants of aflatoxigenic molds would do well to select Papa's strain of A. flavus (PC-7) or the SU-1 strain of A. parasiticus. This would help insure that new mutants can be crossed with existing stocks of genetically marked strains.

The American Type Culture Collection contains an extensive lyophilized collection of wild type and mutant A. flavus and A. parasiticus strains; historical information on each strain is listed in the "Sixteenth Edition of the ATCC Catalogue of Fungi/Yeasts" (Jong and Gantt, 1984). All of these reference strains were recently tested on three different substrates for aflatoxin production: 41% of the A. flavus strains and 85% of the A. parasiticus strains were toxigenic (Wei and Jong, 1986). The variability in aflatoxin-producing potential from wild type isolates remains an important enigma. Our inability to cross different aflatoxigenic strains make it difficult to design experiments to probe the genetic basis of this phenomenon.

V. THE FUTURE

Given the relatively rudimentary level of classical genetic studies on A. flavus and A. parasiticus, it is not surprising that there have been few studies at the molecular level. In fact, the only published molecular studies conducted to date have been taxonomic in intent. Using DNA hybridization, Kurtzman (1985 a, b) reported 100% complementarity between A. flavus and A. oryzae, and 91% complementarity between A. sojae and A. parasiticus. The complementarity between A. flavus and A. parasiticus was 70%. More recently, Klich and Mullaney (1986) found that different restriction patterns were produced after digestion of total DNA from A. flavus and A. oryzae with SmaI. A. parasiticus was not tested.

Out of these taxonomic surveys have come rapid methods for the isolation of DNA from A. flavus-group molds, and it is hoped that these techniques will hasten molecular genetic studies. Several laboratories are attempting to develop transformation systems for A. flavus or A. parasiticus, but at the writing of this paper (July, 1986) no one has yet reported a success. Nevertheless, it is almost inevitable that the power of modern genetic analysis will soon be brought to bear on aflatoxigenic molds. We predict further clarification of the taxonomic relationship of the koji molds to the aflatoxigenic species, and the cloning of an aflatoxin pathway gene in the near future.

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Dr. Kenneth E. Papa died May 10, 1986, at the age of 54.

Although he had prepared Tables 3 and 4 before his untimely death, he was unable to contribute to the final writing of the manuscript. Every effort has been made to give a complete and accurate rendition of his pioneering work on the genetics of aflatoxigenic molds, but there may be errors and omissions. For these I take sole responsibility.

Many people helped me go through Dr. Papa's culture collection and laboratory notes. Particular thanks go to Chris Albertin, Wiley Garrett, Richard Hanlin, Branch Howell, Louise Miller and Libby Papa. Arrangements have been made for Dr. Papa's culture collection, including strains not mentioned in this chapter or published elsewhere, to be deposited with the American Type Culture Collection, Rockville, Maryland, the Fungal Genetics Stock Center, University of Kansas Medical Center, Kansas City, Kansas, or The Southern Regional Research Laboratory, New Orleans, Louisiana.

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Table 1. Characterized Anthraquinone-Accumulating Mutants of Aspergillus parasiticus Blocked in Aflatoxin Production

Mutant Genotype	Description of mutant stock ^a	Published stock and culture collection numbers ^b
<u>nor-1</u>	Accumulates norsolorinic acid and low levels of aflatoxin. Isolated by UV treatment of brown spored mutant of SU-1 (Bennett and Goldblatt, 1973; Lee <u>et al.</u> , 1971) ^c	<u>1-11-79 br-1 red-1</u> , <u>br-1 nor-1</u> ; <u>nor-1</u> ; NOR-1, SRRC 162; NR A-17996; ATCC 24690
<u>avr-1</u>	Accumulates averufin and low levels of aflatoxin. Isolated by UV treatment of wild type ATCC 15517 (Donkersloot <u>et al.</u> , 1972; Lin and Hsieh, 1973)	W 49; <u>av-1</u> ; <u>avr-1</u> ; AVR-1; SRRC 165; ATCC 24551
<u>ver-1</u>	Accumulates versicolorin A, versicolorin C, and trace levels of aflatoxin. Isolated by UV treatment of a white spored mutant of SU-1 (Bennett and Goldblatt, 1973; Lee <u>et al.</u> , 1975, 1976)	<u>1-11-105 wh-1</u> ; <u>wh-</u> <u>wh-1 ver-1</u> , <u>ver-1</u> ; VER-1; SRRC 169; NR 16462; ATCC 36537
<u>avn-1</u>	Accumulates averantin and no detectable aflatoxin. Isolated by NTG treatment of <u>1-11-105 wh-1 (=ver-1)</u> (Bennett <u>et al.</u> , (1980). This a "double mutant", with two distinct blocks in the aflatoxin pathway.	<u>ver-mu-39</u> ; <u>avn-1</u> ; SRRC 163; ATCC 567

^a Mutagens were ultra-violet light (UV) or nitrosoguanidine (NTG)

^b SRRC= Southern Regional Research Center; NRRL= Northern Regional Research Center; ATCC= American Type Culture Collection

^c In addition, several norsolorinic acid-accumulating mutants designated NOR were isolated by Detroy et al., (1973) by NTG treatment of NRRL 2999. A norsolorinic-acid accumulating mutant was also isolated from A. flavus (See Tables 3 and 4 this paper).

p-24 (ATCC 56858)	<u>Y-11 glu-1 adm-7</u>	yellow	glutamic acid	-	Lennox and Davis (1983)
p-25 (ATCC 56859)	<u>w-1 ade-1 adm-8</u>	white	adenine	-	Lennox and Davis (1983)
p-26 (Atcc 56860)	<u>W-1 ade-1 adm-9</u>	white	adenine	-	Lennox and Davis (1983)
p-27 (ATCC 56861)	<u>w-1 ade-1 adm-10</u>	white	adenine	-	Lennox and Davis (1983)
p-28 (ATCC 56862)	<u>w-1 ade-1 adm-11</u>	white	adenine	-	Lennox and Davis (1983)
-1	<u>Ye-1 lys-2</u> <u>Yea1 lysA2b</u>	yellow	lysine	Tr	Bradshaw <u>et al.</u> , (1983)

+ = producer, - = non-producer; Tr = trace

convention used in Bradshaw et al., (1983)

listed as br-1 red-1 ade-1 in Gussack et al., (1977)

listed as Ap-23 in ATCC update 1985

Table 2. Mutants of Aspergillus parasiticus

Strain number	Genotype	Spore color	Phenotype Nutritional requirement	Aflatoxin ^a	Citation
A43-1	<u>or nic</u>	orange	nicotinic acid	+	Papa, 1978
A43-7	<u>or nic afa-1</u>	orange	nicotinic acid	-	Papa, 1978
B-11-2	<u>yg lys-1</u>	yellow green	lysine	+	Papa, 1978
B43-21	<u>yl_o pro</u>	yellow	proline	+	Papa, 1978
B43-23	<u>yl_o pro afa-1</u>	yellow	proline	-	Papa, 1978
B144-17	<u>gb lys-2 afa-2</u>	green buff	lysine , lysine	-	Papa, 1978
B-144-26	<u>w met afa-2</u>	white	methionine	-	Papa 1978
A44-16	<u>w pab</u>	white	paraminobenzoic acid	?	Leong <u>et al.</u> , 1981
yl-1	<u>ye-1</u>	yellow	none	+	Bennett (1979)
1-1-6	<u>ye-1 lys-1</u>	yellow	lysine	Tr	Bennett (1979) Bennett <u>et al.</u> , 1980b
2-1-6	<u>ye-1 lys-4</u>	yellow	lysine	Tr	Bennett 1979
w1-2	<u>wh-1</u>	white	none	+	Bennett 1979
1-13-18	<u>wh-1 met-1</u> = <u>whA1 metA1b</u>	white	methionine	+	Bennett (1979) Bennett <u>et al.</u> , 1980b
1-14-22	<u>wh-1 cys-1</u>	white	cysteine	+	Bennett (1979) Bennett <u>et al.</u> , (1980b)

14-24	<u>wh-1 pdx-2</u> (= <u>whAI pdxA2</u>) ^b	white	pyridoxine	+	Bennett (1979); Bennett <u>et al.</u> , 1980
23-114	<u>wh-1 ver-1 lys-7</u>	white	lysine	-	Bennett (1979); Bennett <u>et al.</u> , 1980
22-103	<u>wh-1 ver-1 cys-5</u>	white	cysteine	-	Bennett (1979); Bennett <u>et al.</u> , 1980
23-110	<u>wh-1 ver-1 arg-2</u>	white	arginine	-	Bennett (1979); Bennett <u>et al.</u> , 1980
22-102	<u>wh-1 ver-1 ser-1</u>	white	serine	-	Bennett (1979); Bennett <u>et al.</u> , 1980
-2	<u>br-1</u>	brown	none	+	Bennett (1979); Bennett <u>et al.</u> , 1980
11-7	<u>br-1 pdx-1</u> (= <u>brAI pdxA1</u>) ^b	brown	pyridoxine	+	Bennett (1979); Bennett <u>et al.</u> , 1980
19-78	<u>br-1 nor-1 lys-5</u>	brown	lysine	Tr	Bennett (1979); Bennett <u>et al.</u> , 1980
16-51	<u>br-1 nor-1 ade-1</u> ^c (= <u>brAI norAI adeAI</u>) ^b	brown	adenine	+	Bennett (1979); Bennett <u>et al.</u> , 1980
19-80	<u>br-1 red-1 arg-1</u>	brown	arginine	+	Gussack <u>et al.</u> , (1977)
22-100	<u>wh-1 yel-1 lys-6</u> (= <u>whAI ver AI lysB6</u>) ^b	white	lysine	-	Gussack <u>et al.</u> , (1977)
9-14	<u>y-11 glu-1</u>	yellow	glutamic acid	+	Lennox and Davis (1983)
9-16	<u>w-1 ade-1</u>	white	adenine	+	Lennox and Davis (1983)
9-11	<u>bwn-1</u>	green	none	-	Lennox and Davis (1983)
9-22 (ATCC 56857) ^d	<u>y-11 glu-1 adm-4</u>	yellow	glutamic acid	-	Lennox and Davis (1983)
9-23	<u>y-11 glu-1 adm-5</u>	yellow	glutamic acid	-	Lennox and Davis (1983)

Table 3. Genetic Loci of Aspergillus flavus

Gene symbol	Linkage group	Phenotype	Citations
<u>ad</u>	II	adenine requirement	Papa (1973, 1978, 1980)
<u>arg-2</u>	II	arginine requirement	Papa (1973, 1978)
<u>arg-4</u>	IV	arginine requirement	Papa (1976)
<u>arg-7</u>	VII	arginine requirement	Papa (1984)
<u>arom</u>	IV	aromatic metabolites requirement	Papa (1976)
<u>bi</u>	III	biotin requirement	Papa (1976)
<u>cnx</u>	?	nitrate and hypoxanthine non-utilizing	Papa (1986)
<u>fwn</u>	II	fawn conidia	Papa (1976)
<u>his</u>	VIII	histidine requirement	Papa (1977c, 1978)
<u>iv</u>	VII	isoleucine/valine requirement	Papa (1976, 1978)
<u>leu</u>	VII	leucine requirement	Papa (1973, 1978)
<u>lys-3</u>	III	lysine requirement	Papa (1976)
<u>lys-4</u>	IV	lysine requirement	Papa (1976)
<u>met-3</u>	III	methionine requirement	Papa (1973, 1978, 1980)
<u>met-4</u>	IV	methionine requirement	Papa (1976)
<u>nia</u>	II	nitrate non-utilizing	Papa (1986)
<u>nic</u>	IV	nicotinic acid requirement	Papa (1976)
<u>ni1</u>	VI	nitrate non-utilizing	Papa (1986)
<u>nor</u>	VII	norsolorinic acid accumulation	Papa (1982, 1983)
<u>pab</u>	V	P-aminobenzoic acid requirement	Papa (1976, 1978)
<u>pdx</u>	VI	pyridoxin requirement	Papa (1973, 1978, 1980)
<u>phe</u>	III	phenylalanine requirement	Papa (1980)

<u>pro</u>	I	proline requirement	Papa (1973, 1976, 1980)
<u>t</u>	IV	tan conidia	Leaich and Papa (1974) Papa (1973, 1976)
<u>thi</u>	I	thiamine requirement	Papa (1976, 1980)
<u>ts</u>	VIII	temperature sensitive	
<u>w</u>	II	white conidia	Leaich and Papa (1974) Papa (1973, 1976)
<u>ylo</u>	III	yellow conidia	Papa (1976)

Table 4. Mutant Strains of Aspergillus flavus Deposited with the American Type Culture Collection (ATCC)

ATCC number	Linkage group markers							
	I	II	III	IV	V	VI	VII	VIII
36081	-	<u>bi</u>	-	<u>t</u>	-	<u>pdx</u>	-	<u>afl-B2</u>
44617	-	-	-	<u>t</u>	-	-	<u>afl-1 leu</u>	-
44618	-	<u>afl-4</u>	-	<u>t</u>	-	<u>pdx</u>	-	-
46107	-	<u>w</u>	-	-	-	<u>pdx</u>	-	-
46108	-	<u>w</u>	-	-	<u>pab</u>	-	-	-
46109	-	<u>w</u>	-	-	-	-	-	<u>his</u>
46110	-	<u>w</u>	-	-	-	-	<u>iv</u>	-
46111	<u>pro</u>	<u>w</u>	-	-	-	-	-	-
46112	-	<u>w</u>	<u>phe</u>	-	-	-	-	-
46113	-	<u>w ad</u>	-	-	-	-	-	-
46114	-	<u>w</u>	<u>met-3</u>	-	-	-	-	-
46115	<u>thi</u>	<u>w</u>	-	-	-	-	-	-
46116	-	<u>w</u>	-	-	-	<u>pdx</u>	-	<u>afl-B</u>
60040	-	<u>w</u>	-	<u>lys-4</u>	-	-	<u>nor</u>	-
60041 ³¹⁰⁻²⁵	-	<u>w</u>	-	-	-	-	<u>arg-7</u>	-
60042 ⁸⁶⁻¹	-	<u>w</u>	-	-	-	-	<u>arg-7 afl-15</u>	-
60043	-	-	-	<u>t</u>	-	-	<u>leu</u>	-
60044 ^{T44}	-	<u>arg-2</u>	-	<u>t</u>	-	-	<u>afl-16</u>	1
60045	-	<u>w</u>	-	-	-	<u>pdx</u>	<u>afl-21</u>	-
60046	-	-	-	<u>t</u>	-	<u>pdx</u>	<u>leu</u>	-
60047	-	<u>w</u>	-	-	-	<u>pdx</u>	<u>leu</u>	-
60048	<u>pro</u>	-	<u>met-3</u>	<u>t</u>	-	-	-	<u>his</u>
60049	<u>pro</u>	<u>w</u>	<u>met-3</u>	-	<u>pab</u>	-	-	<u>his</u>

- ✓ 9. Leach, Laurie and K. E. Papa. 1974. Aflatoxins in mutants of Aspergillus flavus. Mycopathologia et Mycologia Applicata 52: 223-229.

Stock culture: PC7

produced 183 morphological + 33 auxotrophic mutants by UV.

10. Papa, K. E. 1973. The parasexual cycle in Aspergillus flavus. Mycologia 65: 1201-1205.

Probably PC7 stock: white & tan mutants (UV)

Diploids identified: arg + t + / + ad + w

met = t + / + ad + w

met + t + / + prol + w

leu + T + / + pdx + w

11. Leach, Laurie L. and K. E. Papa. 1975. Identification of Diploids of Aspergillus flavus by the nuclear condition of conidia. Mycologia 67: 674-678.

Wild type PC7 from pecans - R. T. Hanlin

developed 7 haploid mutants identified by white 1-4 and tan 1-4.

- ✓ 12. Papa, K. E. 1976. Linkage groups in Aspergillus flavus. Mycologia 68: 159-165.

Stock culture PC 7 (R. T. Hanlin: UGA) 16 different auxotrophs not identified (UV).

- ✓ 13. Papa, K. E. 1977. A mutant of Aspergillus flavus producing more aflatoxin B₂ than B₁. Applied and Environmental Microbiology 33: 206.

Original stock: PC7

NG

tan bi pdx → HB2: B₂/B₁ = 7.1
ATCC #236081:234 (34-4)

14. Hendrix, F. F., Jr. and K. E. Papa. 1974. Taxonomy and Genetics of the Genus Pythium. Proc. Amer. Phytopath. Soc. 1: 200-207.

no isolates identified

15. Papa, K. E. 1977. Genetic analysis of a mutant of Aspergillus flavus producing more aflatoxin B₂ than B₁. Mycologia 69: 556-562.

used: pdx bi t HB2 ATCC 36081: 234 (34-4)

crosses: a) nic w #264

b) pab w

c) pro w

d) leu w #205

e) pab ylo[#]777

- ✓ 16. Papa, K. E. 1977. Genetics of aflatoxin production in Aspergillus flavus: Linkage between a gene for a high B₂: B₁ ratio and the histidine locus on linkage group VIII. Mycologia 69: 1185-1190.

See Bk III p5 6/18/79 (HB2 = Hi B2 Afl production)

- 1) pro w met pob
- 2) w pdx leu (#271?)
- 3) t his (#116:T38)
- 4) tHB2 (36081:234) X w pdx (46107:470) → w pdx HB2 (46116:271)

17. Papa, K. E. 1978. The parasexual cycle in Aspergillus parasiticus. Mycologia 70: 766-773.

"A" and "B": original stock cultures of Pecan Group. Isolates - mutants per NG

orange	nic	+	A43-1	fr 5325-43
or	nic	Afl-1	A43-7	#621 -
ylo gr	lys-1	+	B11-2	fr 5319-11
ylo	pro	+	B43-21	fr 5319-43
ylo	pro	Afl-1	B43-23	#617 -
gr buff	lys-2	Afl-2	B144-17	#619 -
w	met	Afl-2	B144-27	fr 5319-144 #624

Diploids: A43-7/B144-17 B11-2/B42-23
 (#610) B43-23/B144-17 B43-23/B144-26 (#609)
 B43-23/B144-26 B43-1/B43-21
 A43-7/B43-23

18. Papa, Kenneth E. 1979. Genetics of Aspergillus flavus: Complementation and mapping of aflatoxin mutants. Genetical Research 34: 1-9.

Testers: #700 pro w met t pab his

w t leu

#827 ATCC 60040 nor lys w

Parents: t leu 60043:109 (5T-18) w met 88 (A-14-1)
 t pdx 241 (T37-10) w nic 425 (w nic 5)
 ylo pab 177 (16-7) fwn arom 178 (a-17)

Mutants:

t leu afl 1 #649	t pdx afl 4 #241	w met afl 8 #655	w nic afl 11 658
t leu afl 2 650	t pdx afl 5 244	w met afl 9 656	w nic afl 12 659
t leu afl 3 651	ylo pab afl 6 652	w met afl 10 657	afl 1 44617:649
fwn arom afl 14 654	ylo pab afl 7 653		afl 4 44618:241

19. Papa, K. E. 1980. Dominant aflatoxin mutant of Aspergillus flavus. J. Gen. Microbiol. 118: 279-282.

Mutants produced by NG

afl 1	leu	t	ATCC 44617:649	IV w	ATCC 46110:516
afl 4	pdx	t	44618:241	pro w	46111:83
afl B2	pdx	w	46116:271	phe w	46112:176
	pdx	w	46107:470	ad w	46113:79
	pab	w	46108:460	met w	46114:434
	his	w	46108:804	thi w	46115:78

20. Foundin, L. L., K. E. Papa, and R. T. Hanlin. 1981. Nuclear behavior during conidiogenesis in Aspergillus flavus. Canadian Journal of Botany 59: 2116-2120.

Continued PC7 and white and tan mutants.

21. E. J. Mullaney and K. E. Papa. 1982. Heterokaryons among strains of Neurospora crassa with different linear growth rates. The Journal of Heredity 73: 245-245.

Isolates from these crosses: HON 1a/HON3A
77a/74a
HON 3a/77a

- ✓ 22. Papa, K. E. 1982. Norsolorinic acid mutant of Aspergillus flavus. J. Gen. Microbiol. 128: 1345-1348.

Strains used:

NG

- 1) w lys → 1 N (w lys nor) ATCC 60040: 827
Diploids: 2) t pdx leu 60046:491
3) t leu 60043:109 (5t-18)
4) t leu afl-1 44617:649 (5T-18-2)
5) t arg 118 (T44)

- ✓ 23. Papa, K. E. 1984. Genetics of Aspergillus flavus: Linkage of aflatoxin mutants. Can. J. Microbiol. 30: 68-73.

Testers: pro w met pab his 60049: 592
pro met t pab his 60048: 579
w pdx leu 60047: 506
t pdx leu 60046: 491
t leu 60043: 109 (5T-18)
w arg-7 60041: 86 (31a-25)
w lys nor 60040: 827 (1-N:31a-6-2)
t leu afl 1 44617: 649 (5T-18-2)

Aflatoxin Mutants:

w arg-7 <i>afl 15</i>	15	60042: 86-1
t arg-2	16	60044: 118-1 (T44)
w ad	17	60044: 79-1 (31a-18)
t arg-2	18	60044: 118-2 (T44-7) [log bk I 10.22.70]
w ad	19	60044: 79-4 (31a-18) ?
w pdx	20	60045: 271-1
w ad	21	60045: 79-3 (31a-18) ?
w pdx	22	60045: 271-2 (#19 from 34-4 + 16-7)

Haploid Segregants:

lys t nor	1017:(1N/109)-22	pro t	(79-2/579)-20
w pab his	1025:(118-1/592)-20	pro t	1020:(271-1/579)-3
met t	1028:(79-1/579)-33	met t pab his	(271-2/579)-16
w his	1029:(118-2/592)-10		

24. Papa, K. E. 1986. Heterokaryon incompatibility in Aspergillus flavus. Mycologia 78: 98-101.

No isolates identified.

TABLE OF CONTENTS - SILICA GEL LOG

# 1-60	in container	I	(silica gel 11/16/71)	auxotrophs
# 61-118		II	(11/16/71)	auxotrophs
# 119-170		III	11/16/71	morphologicals
# 171-204		IV	9/10/74	auxotrophs
# 205-233		V		auxotrophs
# 234-261		VI	34-4(B ₂ /B ₁)>1, other mutants	
# 262-285		VII	Diploids involving 34-4, segregants	
# 286-308		VIII	Diploids	
# 309-332		IX	Diploids	
# 333-358		X	Diploids	
# 359-388		XI	Diploids	
# 389-421		XII	Selections	
# 422-444		XIII	Selections	
# 445-472		XIV	Selections	
# 473-496		XV	Selections	
# 497-520		XVI	Selections	
# 521-544		XVII	Selections	
# 545-569		XVIII	Selections	
# 570-605		XIX	Selections	
# 606-630		XX	Selections	
# 631-662		XXI	Selections	
# 663-691		XXII	Selections	
# 692-722		XXIII	Selections	
# 723-750		XXIV	Selections	
# 751-779		XXV	Selections	
# 780-810		XXVI	Selections	
# 811-840		XXVII	Selections	

# 841-892	In container	XXVIII	Selections
# 893-940		XXIX	Selections
# 941-986		XXX	Selections
# 987-1029		XXXI	Selections
# 1030-1056		32	Selections
# 1057-1085		33	Selections
# 1086-1149		34	Selections
# 1150-1204		35	Selections
# 1205-1262		36	Selections
# 1263-		37	Selections

- end -

Nutritional requirements - vitamins or amino acids

pdx - pyroxidine
ribo - ribonucleic acid
cys - cystine
met - methionine
ser - serine
paba - para-amino benzoic acid
arg - arginine
lys - lysine
bi - biotin
ad - adenine
his - histidine
leu - leucine
nic - nicotinamide
Hx - hypoxanthine
pan - pantothenate
prol - proline
cit - L citrulline
arom - aromatics
tryp - tryptophane
glu - glucose
thi - thiamine
iv - isoleucine/valine
anthr - anthranilic acid
tyr - tyrosine
orn - ornithine
comp - complete media
I-1 - Isoleucine
Øal or phe - phenalalanine

Colors

Lg - lg light green

t - tan

ylo - yellow

ylo-or - yellow orange

A - albino

W - white

fwn-f -fawn

gt - golden tan

or - orange

Rf - running front of spot on TLC plates

nor - norsolorinic acid

NG - nitrosoguanidine

(np) NP - non producer of aflatoxin

su - suppressor mutant - possibly prevented aflatoxin production - blocked pathway?

af1 1-4 - aflatoxin 1 etc. 2, 3, 4

- end -

1-208 all derived from PC-7

1-60 in container I (silica gel 11/16/71)

- * 1. PC-7 original isolates from pecans (R. T. Hanlin)
2. PC3-76 cys⁻ met⁻ green
3. PC3-234 ser⁻ g
4. PC3-241 paba⁻ g
5. PC3-264 arg⁻ g
6. Lg-80 Lg = light green prototroph
7. Lg-81 lys⁻
8. Lg-82 arg⁻
9. Lg-83 cys-hist?
Lg-84 cys + met
10. Lg-85 cys + met
11. Lg-86 leu
12. Lg-87 met
13. Lg-88 met
14. Lg-89 met
15. Lg-90 Lys
16. Lg-91 bi leaky
17. Lg-91-1 multiple requirements
18. Lg-91-2 bi
19. Lg-91-3 casein
20. Lg 91-4 bi
21. Lg 91-5 bi
22. Lg 91-6 met
23. Lg 92 cys + met
24. Lg 93 lys
25. Lg 94 ad
26. Lg 96 ad

27. Lg 97 ?
28. Lg 98 ser leaky
29. Lg 99 ?
30. Lg 100 ?
31. Lg 101 leu
32. Lg 102 ribo
33. Lg 103 ad leaky
34. 3-255 light green prototroph
35. 3-255-1 nic
36. 3-255-2 ?
37. 3-255-3 paba (green)
38. 3-255-4 multiple requirements
39. 3-255-5 lys (very leaky)
40. 3-255-6 paba
41. 3-255-7 lys albino (fluffy)
42. 3-255-8 paba, green leaky
43. 3-255-9 ?
44. 3-255-13 paba green
45. 3-255-14 paba green
46. 3-255-15 nic very leaky
47. 3-255-16 cys-met
48. 3-255-17 paba g
49. 3-255-18 paba g
50. 3-255-19 paba g
51. 3-255-20 bi g leaky
52. 3-255-21 paba g
53. 3-255-22 paba g
54. 3-255-23 paba g

55. 3-255-24 paba t
56. 3-255-25 bi, g, leaky
57. 3-255-26 paba, g
58. 3-255-27 paba g
59. 3-255-28 paba g
60. 3-255-29 paba g I
61. 31a albino prototroph I
62. 31a-1 met-cys
63. 31a-2 met
64. 31a-3 multiple requirements
65. 31a-4 pdx
66. 31a-5 met (leaky)
67. 31a-6 lys (lys-4)
68. 31a-7 ad
69. 31a-8 ad
70. 31a-9 multiple requirements
71. 31a-10 nic
72. 31a-11 pan leaky
73. 31a-12 bi leaky
74. 31a-13 paba
75. 31a-14 paba
76. 31a-15 nic
77. 31a-16 nic
78. 31a-17 thi
79. 31a-18 ad
80. 31a-19 bi
81. 31a-20 ?
82. 31a-21 met

83. 31a-22 prol (slight growth on arg)
84. 31a-23 prol (slight growth on arg)
85. 31a-24 lys
86. 31a-25 (arg-7) arg (no compl with #18) on VII # 18 = arg-7
grows on orn, cit, + arg
87. A29-10 met A = albino
88. A14-1 met (met-4)
- ~~89. A19-3 met LOST~~
90. A30-6 cys-met met-2
91. A7-2 ?
92. A13-3 arg (arg-4)
- ~~93. 5t tan prototroph LOST~~
94. 5t-1 met (met-3)
95. 5t-2 bi
96. 5t-3 ad
97. 5t-4 met
98. 5t-5 ad
99. 5t-7 pdx
100. 5t-8 lys (lys-3)
101. 5t-9 asparagine (slight on glu-may be inhibited on comp.)
102. 5t-10 uracil leaky
103. 5t-12 ?
104. 5t-13 nic
105. 5t-14 ad
106. 5t-15 ad
107. 5t-16 nic
108. 5t-17 paba
109. 5t-18 leu

110.	5t-19	ad	
111.	T41	bi?	T = tan
112.	T37	pdx	
113.	T39	ad?	
114.	T53	?	
115.	T40	lys	
116.	T38	hist	
117.	T45	?	
118.	T44	arg (arg-2) grows on orn, cit, and arg	
119.	Lg-71	morphological mutants 119-139 all Lg	
120.	-64		
121.	-32		
122.	-62		
123.	-65		
124.	-22		
125.	-46		
126.	-43		
127.	-42		
128.	-68		
129.	-61		
130.	-27		
131.	-70		
132.	-39		
133.	-44		
134.	-38		
135.	-20		
136.	-58		
137.	-17		
138.	-41		
139.	-51		

			140-147 all PC
140	PC 26-1		
141.	3-125		
142.	58-2		
143.	67-8		
144.	5b		
145.	23-4		
146.	210-42		
147.	3-216		
148.	21-3	albino	
149.	26-4	albino	
150.	11-3	albino	
151.	28-7	albino	
152.	T54	tan	
153.	* T19		
154.	T-6		
155.	T-5		
156.	T-24		
157.	T-13		
158.	T-48		
159.	T-51		
160.	<u>31a</u> M ₁		
161.	M ₂		
162.	M ₃		
163.	M ₄		
164.	31a-29		
165.	5T-60		
166.	5T-51		
167.	31a-30		
168.	5T-52		

169.	16-5	greenish ylo from (5T-16 ylo) from 5t	
170.	5T-f	"FAWN"	
171.	5T-38	(2, arom ⁻)	
172.	31a-9	paba	w
173.	31a-10	paba	w
174.	a-10	paba	fawn from 5t
175.	31a-5	∅ al	w
176.	31a-6	∅ al	w
177.	16-7	paba ⁻ ylo	from 5t (via 5t-16 ylo)
178.	a-17	a-17	arom ⁻ f
179.	5T-38	arom	t
180.	a-25	arom	f
181.	a-13	paba	f
182.	5t-10	met + cys	t
183.	31-55	bi	w
184.	5t-1	pdx	t
185.	31-49a	lys	w
186.	5t-33	lys	t
187.	31-36b	nic tryp	w
188.	a-15a	nic	f
189.	31a-25	met	w
190.	31-59b	ad	w
191.	a-1	nic	f
192.	a-26a	paba	f
193.	5T-35	amino acid(s)?	t
194.	31-50	nic	w
195.	31-23b	leaky arg, glu prol, asp.	w
196.	31a-21	nic	w

- 8
- | | | | | | |
|------|------------|---|-----------------------|---------------------------|-------------------|
| 197. | 16-27 | bi ⁻ | ylo | from (5t) | |
| 198. | 31a-51a | arg + prol | w | | |
| 199. | 31-8 | lys | w | | |
| 200. | 31-48 | met cys | w | | |
| 201. | a-20 | nic | f | | |
| 202. | 31-19 | pdx | w | | |
| 203. | 5t-7 | nic | t | | |
| 204. | a-28b | nic | f | | |
| 205. | 5T-36 | met | t | | |
| 206. | 5T-37 | amino acid(s)? | t | | |
| 207. | 5T-38 (1) | arom | t | | |
| 208. | 5T-40 | nucleate? | | | |
| 209. | 28-1 | red pigment | w | from 5642 | |
| 210. | 114-1 (67) | prol ⁻ | leaky green | from 5642 | |
| 211. | 79-1 (110) | prol ⁻ | t | from 5642 | |
| 212. | 5642 | produces only B ₂ - from Schroader & Carlton (Appl. Microbiol.)
(probably <u>A. parasiticus</u>) | | | 25: 146-148, 1973 |
| 213. | lys-2 | ylo | <u>A. parasiticus</u> | from Joan Bennett, Tulane | |
| 214. | pdx-1 | br-1 | " | " | " |
| 215. | ad-1 | br-1, red-1" | " | " | " norsolorinic |
| 216. | 5319 | prototrophs from R. T. Hanlin | | | |
| 217. | 5325 | | | | |
| 218. | 5329 | | | | |
| 219. | 5351 | | | | |
| 220. | 5332 | | | | |
| 221. | 5337 | | | | |
| 222. | 5350 | | | | |
| 223. | 5359 | | | | |

224. 5350-14 white prototroph from 5350
225. 5350-39 ylo prototroph B > G
226. 5350-14 (1) ad⁻¹ w
227. 5350-14 (3) met⁻ w
228. 5350-14 (27) met⁻ w
229. 5350-39 (2) ad⁻ ylo-w
230. 5350-39 (14) met⁻ org
231. Diploid 5350-14 (1) + 5350-39 (14)
232. 5350-39 (22) ad⁻ ylo-w
233. 5350-39 (58) met⁻ org
234. 34-4 (1) pdx⁻ bi⁻ from #34 [isolate from (31a-17 + 5T-9)]
B₂/B₁ > 1 (NG as mutagen)
235. 34-4 (2) B₂/B₁ > 1
236. 34-4 (3) B₂/B₁ > 1
237. 34-18 low B₁ (~0) from #34
- ~~238. 34-26 high B₁ (~16,000) from #34 LOST~~
239. 35-6 thi⁻ from #35 [isolate from 31a-17 + 5T-17]
240. T37-3 low B₁ from T37 (NG as mutagen)
241. T37-10 B₁ = 0 pdx afl-4 Ts 1
242. T37-13 pdx⁻
243. T37-14
244. T37-17 B₁ ~0 afl-5
245. T37-17a
246. T37-17b
247. T37-18 high B₁
248. #40 from (T37-13 + 31a-22) pdx⁻ low B₁ red pigment
249. 34-8 (B₁ - 14,000) from #34
250. Diploid (T37-17 + 31a-22)
251. Diploid (T37-13 + 31a-22)

fox 

252. Diploid (T37-17 + 31a-14)
253. Diploid (T37-17 + 31a-19)
254. 34-15 high B_1 (10,000) from #34
- ~~255. 34-8 low B_1 from #35 LOST~~
256. Diploid (T37-14 + 31a-22)
- ~~257. #4 from (T37-13 + 31a-22) low B_1 red pigment LOST~~
258. Diploid (#4 above + 31a-22)
259. Diploid (#4 + 31a-14)
260. Diploid (#4 + #18, leu⁻)
261. Diploid (#4 + #9, nic⁻)
- 261a. Diploid (# + 31a-19)
262. Diploid (34-4 + 31a-14)
263. Diploid (34-4 + 31a-19)
264. Diploid (34-4 + #9, nic⁻)
265. Diploid (34-4 + #18, leu⁻)
- ~~266. Diploid (34-4 + 31a-22) LOST~~
267. Diploid (34-4 + 16-7)
268. #5 leu⁻ $B_2/B_1 > 1$ from (34-4 + #18) W
269. #34 pdx⁻ leu⁻ $B_2/B_1 > 1$ from (34-4 + #18) W
270. #13 paba⁻ pdx⁻ $B_2/B_1 > 1$ from (34-4 + 31a-14)
271. #19 pdx⁻ $B_2/B_1 > 1$ from (34-4 + 31a-14)
272. #109 pdx⁻ $B_2/B_1 > 1$ from (34-4 + 31a-22)
273. #25 pdx⁻ bi⁻ $B_2/B_1 > 1$ from (34-4 + 31a-19)
274. #31 bi⁻ t $B_2/B_1 > 1$ " "
275. #38 Bi⁻ t $B_2/B_1 > 1$ " "
276. #28 pdx⁻ bi⁻ $B_2/B_1 > 1$ " "
277. #40 pdx⁻ nic⁻ $B_1 = B_2 = 0$ from (34-4 + #9)
278. #50 nic⁻ t $B_2/B_1 > 1$ ($B_1 + B_2 = 0$) (34-4 + #9)

279. Diploid (34-4 + #5, above)
280. Diploid (#25 + #5)
281. Diploid (#40 + #5)
282. Diploid (#50 + #5)
283. Diploid (#31 + #5)
284. Diploid (#19 + #50)
285. #42 leu^- w $B_2/B_1 > 1$ from (#50 + #5, above)
286. Diploids: T44 + 31a-22
287. 5T-2 + met^- $prol^-$ pdx^-
288. T37 + 31a-22
289. 5T-18 + 31a-14
290. 5T-1 + 31a-19 (2)
291. 5T-1 + 31a-17
292. 5T-1 + 31a-18
293. T37 + 31a-17
294. 31a-22 + 5T-2
295. 5T-17 + met^- $prol^-$ pdx^-
296. 31a-22 + 5T-13
297. 31a-15 + 5T-18
298. 5T-1 + A30-6
299. T44 + met^- $prol^-$
300. 31a-22 + 5T-17
301. T44 + pdx^- met (t)
302. D5 = 5T-1 + 31a-22
303. D17 = T44 + A30-6
304. D25 = T44 + A30-6
305. D7 = 5T-1 + 31a-22
306. D28 = T44 + Lg 82
307. D16 = T44 + A30-6

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- V 11
308. Diploids: D26 = T44 + A30-6
309. 31a-22 + T44
310. D15 = Lg 82 + A30-6
311. 5T-13 + met⁻ prol⁻ pdx⁻
312. 31a-15 + 5T-17
313. 5T-13 + 31a-22
314. D8 = T44 + A30-6
315. 5T-17 + A13-3
316. met⁻ prol⁻ + pdx⁻ cys (t)
317. 5T-2 + 31a-17
318. 31a-17 + met⁻ prol⁻ pdx⁻
319. 31a-15 + T37
320. 5T-9 + 31a-17
321. 5T-13 + 31a-17
322. 5T-9 + A14-1
323. 5T-15 + met⁻ prol⁻ pdx⁻
324. Diploids: 5T-17 + 31a-22
325. 5T-18 + 31a-17
326. 5T-9 + 31a-22
327. 31a-15 + 5T-1
328. 5T-18 + A14-1
329. 5T-1 + 31a-19 (w)
330. T37 + 31a-19
331. 31a-19 + met⁻ prol⁻ pdx⁻
332. •31a-17 + #1 (w) #1 (f16rom 5T-1 + 31a-22) prol⁻
333. 5T-1 + 31a-22
334. 31a-19 + T44
335. 5T-1 + 31a-19 (1)

336. Diploids: 5T-18 + #40 #40 pdx⁻ from T37 + A30-6
337. 31a-17 + 5T-17 (w), #25
338. 5T-9 + #40 (t) #40 pdx⁻ from T37 + A30-6
339. 31a-22 + 5T-18
340. 5T-9 + 31a-14
341. T37 + A14-1
342. D27 = T44 + Lg82
343. 31a-17 + 5T-17 (w) #35
344. T44 + A14-1
345. 5T-1 + A13-3
346. 5T-38 + A13-3
347. D12 = Lg82 + A30-6
348. D5 prol + pdx⁻ met⁻ (t)
349. 31a-13 + T37
350. 5T-18 + A13-3 (1)
351. 16-7 + a-17 (2)
352. a-17 + T37
353. 5T-38 + 31a-19
354. 31a-19 + #34 #34 = thi from 5T-9 + 31a-17
355. 16-7 + T37
356. 5T-9 + A13-3
357. 5T-18 + A13-3 (2)
358. 31a-19 + 16-7
359. 5T-38 + 31a-22
360. #10 + 5T-8 #10 = paba⁻ prol⁻ met⁻
361. 5T-9 + #4 #4 = leu⁻ from 5T-18
362. 16-7 + A14-1
363. 5T-18 + 31a-19

please

34

Diploids

34

364. Diploids: 16-7 + a-17 (1)
365. 5T-9 + 31a-9
366. 5T-38 + #9 #9 = nic⁻
367. 31a-6 (Ø a1) + #1 #1 prol⁻
368. #18 + 5T-9 #18 = leu⁻
369. a-17 + A14-1
370. 31a-6 (Ø a1) + T37
371. a-17 + 5T-18
372. 5T-18 + #9 #9 = nic⁻
373. 31c-18 + T44
374. #5 + 31a-18 #5 =
375. #1 + 31a-18
376. 5T-38 + 31a-14
377. #44 + 31a-18 #44 arg⁻ cys⁻
378. 5T-38 + 31a-18
379. T44 + a-17
380. #18 + 5T-38 #18 = leu⁻
- = PARENTS =
381. Diploids: 5T-9 + #9
382. 9-17 + 5T-9
383. 5T-38 + #32 #32 = iv⁻
384. #13 + a-25 #13 = paba⁻ prol⁻ met⁻
385. 5T-38 + #40 #40 = pdx⁻
386. a-17 + 5T-1
- ~~387.~~ ~~a-17 + 5T-18~~ *lost*
388. 31a-18 - 16-7
389. Isolates: #21 ad⁻ cit⁻ w from 31a-18 + T44 → ad⁻ + NG → cd⁻ cit⁻
(No linkage) leaky

390. Isolates: #18 ad⁻ arg⁻ w " " ad⁻ arg⁻
 (No linkage) arg⁻ linked to leu = (arg-7) NG → ad⁻ orn⁻
391. #14 ad⁻ orn w " " " ad⁻ orn⁻
 (No linkage) - leaky
392. #4 (A14-1 + 16-7) B₁ = 500
393. #40 (T37 + 16-7) B₁ = 0
394. #6 (A14-1 + 16-7) B₁ = 2500
395. #32 (5T-38 + #18) B₁ = 4500
396. #34 (31a-19 + T37) B₁ = 4500
397. #48 leu⁻ t⁻ B₁ = 2000
398. #22 (5T-38 + 31a-18) B₁ = 4000
399. #55 (A14-1 + 16-7) B₁ = 0
400. #10 (5T-38 + 31a-18) B₁ = 2500
401. #45 (5T-9 + #18) B₁ = 2800
402. #35 (T37 + 16-7) B₁ = 4500 paba⁻
403. #40 paba⁻ iv t (5T-9 + 31a-14)
404. #20 prol iv w (5T-9 + 31a-22)
405. #15 leu paba t (5T-18 + 31a-14)
406. #37 leu met w (5T-18 + A14-1)
407. #1 pdx iv w (5T-9 + #40) #40 = pdx⁻
408. #40 arom⁻ bi⁻ t (5T-38 + 31a-19)
409. #28 arom⁻ prol w (5T-38 + 31a-22)
410. #56 arom⁻ t (a-25 + #13)
411. #6 pdx⁻ ylo (16-7 + T37)
412. #7 paba ylo (16-7 + T37)
413. 69 lys prol paba met t (#10 + 5T -8)
414. 33 arom prol t (5T-38 + 31a-22)
415. 54 iv⁻ bi⁻ t (5T-9 + 31a-19)
416. 16 pdx⁻ f (a-17 + T37)

417. OMIT

418. 8 arom f (a-17 + T37)
 419. 43 arom⁻ t (#13 + a-25)
 420. 35 pdx⁻ arom⁻ t (a-17 + T37)
 421. 8 arom⁻ bi⁻ w (5T-38 + 31a-19)
 422. 1 arg pdx t (T44 + pdx⁻ met⁻)
 423. 52 arg pdx t (T44 + pdx⁻ met⁻)
 524. 70 arg pdx met (slow) t (T44 + pdx⁻ met⁻)
 425. 9 nic w (5T-13 + 31a-19)
 426. T-1 met⁻ prol⁻ (5T-1 + 31a-22)
 427. 4 prol nic w (31a-22 + 5T-13)
 428. 8 prol leu t (31a-22 + 5T-18)
 429. 1 nic t (31a-15 + 5T-1)
 430. 22 paba met w (met prol pdx + 5T-17)
 431. 2 bi nic w (5T-13 + 31a-19)
 432. W-2 met⁻ prol⁻ (5T-1 + 31a-22)
 433. 31 met bi prol pdx w (met prol pdx + 31a-19)
 434. 58 met w (31a-15 + 5T-1)
 435. w-1 met prol (5T-1 + 31a-22)
 436. 10 paba prol met w (met prol pdx + 5T-17)
 437. 13 paba prol met t (met prod pdx + 5T-17)
 438. 76 arg⁻ met⁻ t (T44 + pdx⁻ met⁻)
 439. 16 paba prol t (met prol pdx + 5T-17)
 440. 18 ad met w (5T-1 + 31a-18)
 441. 2 arg met t (T44 + A14-1)
 442. 100 nic met t (nic #1 + #37 met⁻ bi⁻)
 443. 53 arg met t (T44 + pdx⁻ met⁻)
 444. 10 met pdx t (met⁻ prol⁻ + T37)
 445. 2 prol t (5T-1 + 31a-22)

446. } omit
 447 }
 448 }
 449 }

450.	59	thi t	(5T-18 + 31a-17)
451.	1	pdx w	(T37 + A14-1)
452.	34	thi t	(5T-9 + 31a-17)
453.	1	prol t	(5T-1 + 31a-22)
454.		met t	[(T44) + (T37 + ?)]
455.	82	met g	(5T - 18 + A14-1)
456.	23	prol pdx w	(T37 + 31a-22)
457.	115	arg pdx met w	(T44 + pdx met)
458.	37	met bi w	(5T-1 + 31a-19)
459.	24	prol cys w	[(T37 + A30-a) + (5T-1 + 31a-22)]
460.	17	paba w	(met prol pdx + 5T-17)
461.	55	bi t	(5T-13 + 31a-19)
462.	47	paba t	(5T-18 + 31a-14)
463.	1	bi t	(5T-1 + 31a-19)
464.	9	prol leu w	(31a-22 + 5T-18)
465.	15	paba prol w	(met prol pdx + 5T-17)
466.	1	ad ⁻ w ⁻ t ⁻	(31a-18 + T44)
467.	18	leu w	(5T-18 + #40)
468.	21	bi prol w	(met prol pdx + 31a-19)
469.		bi paba w	(5T-17 + 31a-19)
470.	2	pdx w	(T37 + A30-6)
471.	79	leu thi t	(5T-18 + 31a-17)
472.	9	leu paba w	(5T-18 + 31a-14)
473.	1	met w	(5T-1 + 31a-22)
474.	20	pdx met w	(T37 + A30-6)
475.		pdx met w	" "
476.	17	prol pdx t	(met prol + 37)
477.	6	bi met prol pdx w	(met prol pdx + 31a-19)

Box X.1

478.	44	nic met t	(mic #1 + met ⁻ ki ⁻ #37)
479.	37	bi nic t	(5T-13 + 31a-19)
480.	9	met arg t	(met prol + T44)
481.	10	bi w	(5T-2 + 31a-17)
482.	104	prol cys w	[(T37 + A30-6) + (5T-1 + 31a-22)]
483.	1	pdx met w	(T37 + A14-1)
484.	85	met lt g.	(5T-18 + A14-1)
485.	86	met lt t	" "
486.	8	thi nic w	(5T-13 + 31a-17)
487.	1	prol nic t	(31a-22 + 5T-13)
488.	4	prol pdx t	T37 + 31a-22
489.	76	thi nic t	5T-13 + 31a-17
490.	37	thi pada t	
491.	6	leu pdx t	(5T-18 + #40)
492.	2	bi thi w	(5T-2 + 31a-17)
493.	t-2	met prol	
494.	34	paba met w	(met prol pdx + 5T-17)
495.	40	pdx w	(T37 + A30-6)
496.	14	bi pdx t	(met prol pdx + 31a-19)
497.	3	prol paba t	(31a-22 + 5T-17)
498.	27	ad met w	(5T-1 + 31a-18)
499.	6	leu w	(5T-18 + 31a-14)
500.		pdx pale g	[(Lg 82) + (T37 + A30 - 6)]
501.	9	w ⁻ t ⁻ ad	31a-18 + T44
502.	9	nic paba w	31a-15 + 5T-17
503.	4	prol paba w	31a-22 + 5T-17
504.	9	pdx thi w	met prol pdx + 31c-17
505.	1	bi prol t	met prol pdx + 31a-19

Bx XV

506.	22	leu pdx w	5T-18 + #40
507.	2	pdx bi w	T37 + 31a-19
508.	44	bi paba t	5T-17 + 31a-19
509.	1	pdx bi t	T37 + 31a-19
510.	4	leu w	5T-18 + 31a-17
511.	35	leu met	5T-18 + A14-1
512.	26	bi pdx w	met prol pdx + 31a-19
513.	2	leu thi w	5T-18 + 31a-17
514.	4	thi I- $\bar{1}$ /v w	5T-9 + 31a-17
515.	35	iv/thi t	5T-9 + 31a-17
516.	32	iv w	5T-9 + 31a-17
517.	10	paba prod pdx met w	(met prol pdx + 5T-17)
518.	17	ad pdx or ad pdx prol met w	(met prol pdx + 5T-15)
519.	3	paba iv w	5T-9 + 31a-14
520.	46	prol iv t	(5T-9 + 31a-22)
521.	7	arom ⁻ met w	9-17 + A14-1
522.	66	lys prol met paba t	(#10 + 5T-8)
523.	39	lys prol w	" "
524.	9	arom pdx f	a-17 + T37
525.	5	leu arg w	5T-18 + A13-3
526.	45	leu arg w	" "
527.	15	arom ylo	16-7 + a-17
528.	39	\emptyset al t	(31a-6 = \emptyset + # 1)
529.	4	arom w	5T-38 + 31a-22
530.	18	arom w	5T-38 + A13-3
531.	16	\emptyset al pdx ⁻ w	(31a-6 = \emptyset + T37)
532.	44	arg cys t	D26
533.	5	arom w	5T-38 + 31a-18

See Table

534.	9	paba f	(16-7 + a-17)
535.	15	arom g	5T-38 + 31a-18
536.	66	arg g	31a-18 + T44
537.	10	nic iv w	(5T-9 + #9 nic)
538.	44	arg g	D25
539.	63	arg ltg	31a-18 + T44
540.	7	arom ad w	(5T-38 + 31a-18)
541.	37	nic iv t	(5T-9 + #9 nic)
542.	33	∅ al pdx t	(31a-6 = ∅ + T37)
543.	30	∅ al t	" "
544.	12	pdx arom w	(5T-38 + #40)
545.	34-4 (1)	high B ₂	(from 3 single spore transfers) Feb. 1977
546.	34-4 (2)	"	"
547.	34-4 (3)	"	"
548.	34-4 (4)	"	"
549.	34-4 (5)	"	"
550.	34-4 (7)	"	"
551.	34-4 (9)	"	"
552.	34-4 (11)	"	"
553.	#35	met pro pdx w	(10 X 6)
554.	#70	leu met pdx t	(10 X 6)
555.	#28	high B ₂	(T44 X #19)
556.	#39	high B ₁	(T44 X #19)
557.	D #13 X 31a-6	(∅ al)	
558.	D #13 X #22	(1)	
559.	D #13 X #22	(2)	
560.	D #13 X 31a-5	(∅al) (1)	
561.	D #6 X 31a-5	(∅al) (1)	

Box FDK

Box is numbered
you check

562. D #6 X 31a-5 (Øal) (2)
563. D T38 + #22 (1)
564. D T38 + #10 (2)
565. D T38 + #10 (1)
566. D T38 + #22 (2)
567. #8 leu paba pdx t (10 X 6)
568. #27 leu paba pdx w (10 X 6)
569. #31 leu paba pro w (10 X 6)
570. D #10 X #6 (1)
571. D #10 X #6 (2)
572. D #13 X 31a-5 (2)
573. A83-7 his
574. A83-6 his
575. A83-4 his
576. A83-3 his
577. *OMIT*
578. A83-1 his
579. #51 t (T38 X #10) pab⁻ pro⁻ met⁻ his⁻ t
580. #4 leu pab prol t (10 X 6)
581. B188-9 anthr lgt.t
582. B188-2 anthr w
583. B188-1 anthr w
584. A118 tyr
585. #20 (#13 X #22)
586. D 31a-18 X #39 (Øal)
587. D 34-4 X 31a-5
588. C65-31 B1 only-his
589. A103 thi

590. #5 Øal paba pro w
591. #62 w (#32 X #22)
592. #1 w pab pro met his (T38 X #10)
593. B144-30 (A)
594. D B144-26 X B43-21
595. A122-2 or amino acid
596. *HERE*
597. D #1 pro X 31a-5
598. D T38 X #19
599. D #40 X 31a-22
600. A43-27 nic
601. B11-1
602. C65-16 his
603. C66-29
604. A43-9
605. A108-5
606. D #40 + 16-7
607. D 31a-19 X #39 (2)
608. D B144-17 X B43-21
609. D B144-26 X B43-23
610. D B144-17 + B43-23
611. B11-5
612. A45-14 met
613. *HERE*
614. B11-3
615. A43-26
616. C53-6 pab
617. B43-23
618. *omit*

619. B144-17 lys
620. #9 (#40 + 31a-22) nic⁻ t
621. A43-7 ——— *orange nic afl-1*
622. C65-15
623. B43-2
624. B144-26 ——— *w-met Afl-2*
625. A43-8
626. B144-30 (C)
627. D 5T-18 X 31a-5 (Øal)
628. B144-30 (B)
629. B144-30 (D)
630. B50-2
631. B27-14
632. C53-30 paba lemon buff low producer (close to NP)
633. 24551-4 pab
634. 18168-46 ad
635. 18168
636. 24551
637. *OMIT*
638. Diploid 1-17 + A14-1
639. *OMIT*
640. A44-26 pab t np
641. A43-9 nic ylo-or np —
642. C53-5 pab or np
643. C53-23 pab or np
644. C53-26 pab w np
645. C56-12 ad or buff np
646. C66-30 pro olive np

647.	B50-20	leu	gn buff	np		
648.	B21-4	arg	or	np		
649.	5T-18-2	leu	t	np	<u>afl-1</u>	<u>ts4</u>
650.	5T-18-6	leu	t	np	afl-2	
651.	5T-18-7	leu	t	np	afl-3	
652.	16-7-4	pab	ylo	np	afl-6	
653.	16-7-8	pab	lgt ylo	np	afl-7	
654.	A17-19	arom	fawn	np	afl-14	
655.	A14-1-3	met	w	np	afl-8	}
656.	A-14-1-4	met	w	np	afl-9	
657.	A14-1-5	met	w	np	afl-10	
658.	9-2	nic	w	np	afl-11	
659.	9-6	nic	w	np	afl-12	
660.	9-7	nic	w	np	afl-13	
661.	18168-46-10	ad	t	B ₂		
662.	18168-46-2	ad	w	B ₂		
663.	24551-4-4	pab	w	low B & G	averufin	
664.	24551-4-10	pab	ylo	low B & G		
665.	24551-2	pdx				
666.	24551-6	bi				
667.	18168-27	nic				
668.	18168-68	met				
669.	18168-67	lys				
670.	<i>omit</i>					
671.	18168-33	leu				
672.	18168-45	met				
673.	75 (C66-17 X C70-46)	g buff	pro	met		
674.	54 (C65-24 X C70-46)	or buff	his	met		

675. 1 (C65-20 X c70-46) or his met
676. 96 (Cgg-17 X C70-46) or buff pro met
677. 7 (C66-17 X C70-46) or pro met
678. 28 (B43-6 X B11-2) W pro lys
679. 61 (B43-6 X B11-2) or pro lys
680. 34 (C65-24 X C70-46) buff his met
681. 61 (A43-1 X B43-21) ylo nic pro
682. 5 (A43-1 X B43-21) or nic pro
683. 2 (A26-7 X A45-13) or ad met
684. 10 (A43-7 X B144-17) lys⁻ nic⁻
685. 86 (A26-7 X A45-13) or⁻ met⁻ ad⁻
686. 78 (A26-5 X A45-13) or ad met
687. 6 (A26-5 X A45-13) or ad met
688. 43 (B11-6 X B12-8) buff lys⁻ met⁻
689. 39 (B11-6 X B12-8) buff lys⁻ met⁻
690. 17 (C65-31 X C53-30) pab⁻ his⁻
691. 1 (A26-3 X A45-5) w⁻ ad⁻ met⁻
692. B144-30-D54 ad NP
693. B144-30-D48 leu NP
694. B144-30-C37 pab NP
695. B144-30-B27 arg NP
696. B144-30-A6 pad NP
697. 29 (C66-17 X C7046) arg⁻ met⁻
698. 46 (C53-7 X C70-11) w met⁻ pab⁻
699. #56 (T37-10 + 592) pro⁻ his⁻ pdx⁻ np
700. #32 (T37-10 + 592) pab pro met his np
701. #33 (T37-10 + 467) pdx⁻ leu⁻ t np
702. #35 (T37-10 + 467) pdx⁻ leu⁻ t np

703.	#92 (T37-10 + 592)	pro	his	pdx	t	np		
704.	#58 (T37-10 + 592)	pab	pro	met	his	np		
705.	#53 (5 X 6)	ad ⁻	met ⁻	nic ⁻	or			
706.	#42 (5 X 6)	ad ⁻	met ⁻	nic ⁻	or			
707.	#43 (5 X 6)	ad ⁻	met ⁻	nic ⁻	or			
708.	#88 5T-18-2 + 592		his	leu	pro	w		
709.	#91 5T-18-2 + 592		his	leu	pro	w		
710.	#139 5T-18-2 + 592		leu	pro	w			
711.	#50 " "		his	leu	pab	pro	t	
712.	#49 42 X 61 (706 X 679)	ad	met	nic	lys	or		
713.	#53 42 X 48 (706 X ?)	ad	met	nic	his	or		
714.	#74 42 X 48 (700 X ?)	ad	met	nic	his	or		
715.	#33 42 X 49 (706 X ?)	ad	met	nic	his	or		
716.	#29 " "	ad	met	nic	his	or		
717.	1-11-105-13	requirement?		mutant of #722				
718.	1-11-105-4	"		"				
719.	1-11-105-9	met ⁻ or pab ⁻		"				
720.	1-11-105-7	met ⁻ or pab ⁻		"				
721.	1-11-105-6	nic ⁻		"				
722.	1-11-105- wh-1	A parasiticus from J. Bennett, 1978 -accumulates versicolorim A						
723.	P3	mutants induced in 700 P = PFA resistant						
724.	P6							
725.	P9							
726.	P13							
727.	P14							
728.	P15							
729.	P16							
730.	P17							

731. P19
732. P20
733. P21
734. P22
735. *OMIT*
736. P24
737. P25
738. P29
739. P35
740. P36
741. T14-2 mutants reduced in 700 T = thiophanate resistant
742. T14
743. T14-9
744. T14-9
745. T14-10
746. T14-4
747. T14-3
748. T14-13
749. T14-15
750. T18-16
751. T18-18
752. T18
753. T19
754. T20
755. B1 good growth on benlate up to 5 $\mu\text{g/ml}$
756. B2 fair growth up to 5 $\mu\text{g/ml}$
757. B12 fair growth up to 2.5 $\mu\text{g/ml}$
758. B14 fair growth up to 2.5 $\mu\text{g/ml}$

759. B15 " "
760. B17 fair growth up to 2.5 $\mu\text{g/ml}$
761. C22
762. C30
763. P28
764. 700-59
765. 700-510
766. 700-5-13
767. 86 vol (650 X 700-5) np leu pab w (af1-
768. Diploid (C53-26 + B144-17)
769. #13 ad arg pdx leu w
770. #13 A26-11 + 49
771. #59 A26-11 + 49
772. #68 his arg met w
773. #47 (14 X 491) ad arg pdx leu w
774. 19-1 pdx w af1-b2⁻ np from #19
775. 19-5 " " prod " " golden pigment
Rf-.33
776. 19-8 " " np " "
777. 700-1 af1-4 w (see 700) = same requirements
778. 4-16 leu w np (trace) from #4
779. 16-2 pdx fwn spot within B₁ spot?
780. 16-5 pdx fwn orange spot below B₂ high B₁
none on benzene-acetic acid
781. 16-6 pdx fwn " "
no orange spot on benzene acetic acid
782. 17-2 pab w red pigment above B₁ to below B₂ none on
benzene
783. 31a-608 B₂ > B₁ Ø al w + another requirement
pab met w allelic to (#19) 34-4 since
red spot diploid w/34-4 is B₂ > B₁

✓ = requirement tested

784. ~~31a-18-1~~ ~~ad~~ ~~w~~ ~~nap~~ ^{LOST} ~~af1~~ 17 (can be obtained from 1028 leaky (LOST)
784-797 tested on benzene acetic acid
785. 31a-18-2 ad w np leaky ✓
786. 32-2 iv w np ✓
787. 32-3 iv w np ✓
788. 4n-1 nic w np leaky ✓
789. 4n-7 nic w np leaky ✓
790. 59-6 thi t np leaky ✓
791. 59-18 thi t np (trace) ✓
792. 31a-17-8 thi w np ✓
793. 31a-17-11 thi w np ✓
794. 5T-38-12 nic t prod (red pigment) ✓
no red pigment on benzene acetic acid
795. 5T-38-13 nic t np (trace) ✓
796. 31a-25-3 arg w np ✓ *af1=15*
797. 31a-25-6 arg w np ✓
798. 8 bi t selected from (5T-18 + 31a-19)
799. 17 bi t " " "
800. 38 (C66-7 + C70-27) pro met or
801. 67 (" ") pro met ylo
802. 1 (C66-7 + C70-11) pro met w
- 805-815 tested on benzene acetic acid 817-836 retested
803. 28 (C65-14 + C66-24) his pro ylo
804. #6 his w (from dip T38 + 22)
805. 4n-12 nic w np (leaky) ✓
4n-12 has three faint yellow spots on benzene-acetic acid
806. 4n-5 nic w np ✓
807. 5T-38-10 nic t afl⁺ pigment ✓
808. 30-22 Øal t np leaky ✓

809.	5T-17-12	<u>pab</u>	<u>t</u>	trace	leaky	✓
810.	5T-17-10	<u>pab</u>	<u>t</u>	afl ⁺		
811.	5T-17-4	<u>pab</u>	<u>t</u>	afl ⁺	trace of B ₁	✓
812.	59-7	<u>thi</u>	<u>t</u>	afl ⁺		✓
813.	59-9	<u>thi</u>	<u>t</u>	afl ⁺		✓
814.	T44-13	<u>arg</u>	<u>t</u>	np		✓
815.	T44-7	<u>arg</u>	<u>t</u>	afl ⁺	(strange spots on TLC) yellow spot Rf. 16	✓
816.	#17	afl-1 ⁻	afl-b2 ⁻	<u>leu</u>	<u>t</u>	✓
817.	31a-18-8	<u>al</u>	<u>w</u>	np		✓ (leaky)
818.	31a-18-5	<u>al</u>	<u>w</u>	np		✓
819.	4n-16	<u>nic</u>	<u>w</u>	np		✓
820.	31a-25-9	<u>arg</u>	<u>w</u>	np	not tested on benzene-acetic acid	✓
821.	31a-18-10	<u>ad</u>	<u>w</u>	tr	on retest produced 765 µg/B ₁ /g	✓
822.	32-10	<u>iv</u>	<u>w</u>	low toxin		✓
823.	32-9	<u>iv</u>	<u>w</u>	np		✓
824.	31a-22-11	<u>pro</u>	<u>w</u>	np		✓
825.	31a-22-25	<u>pro</u>	<u>w</u>	np		✓
826.	31a-22-26	<u>pro</u>	<u>w</u>	np		✓
827.	31a-6-2	<u>lys</u>	<u>lys</u>	<u>w</u>	produces red spot (norsolorinic acid) on benzene acetic acid red spot has Rf 43	
828.	2-22	<u>pro</u>	<u>t</u>	low toxin		✓
829.	5T-17-14	<u>pab</u>	<u>t</u>	np	trace B ₁ on retest	✓
830.	5T-17-16	<u>pab</u>	<u>t</u>	np		✓
831.	5T-17-20	<u>pab</u>	<u>t</u>	trace w/red spot no red spot on benzene acetic acid		
832.	T38-4	<u>his</u>	<u>t</u>	low toxin		
833.	8-1	<u>nic</u>	<u>w</u>	high B ₁	(ylo, blue, red spots) on retest benzene acetic acid only light yellow and blue	✓
834.	5T-8-5	<u>lys</u>	<u>t</u>	low toxin		

862. D1 31a-17-8 + 5T-38-10
863. D1 31a-18-10 + afl-1
864. D1 31a-25-3 + afl-1 ✓ 864
865. D1 19-1 + 5T-18-2
866. D1 31a-25-6 + afl-4
867. D1 31a-25-6 + afl-1 867
868. D1 30-22 + 710
869. D 4-16 + 5T-17-12
870. D1 31a-25-9 + afl-4
871. D1 5T-17-10 + 710
872. D1 4-16 + 30-22
873. D2 T44-13 + 19-5
874. D2 31a-18-5 + afl-4
875. D1 19-8 + 5T-17-12
876. D1 19-5 + 704
877. D2 31a-18-10 + afl-4
878. D1 31a-17-8 + 30-22
879. AF10 y1 ben 1 benlate 5 ppm - no success (growth) From
Makins in
London
↓
880. AF11 w³ teo² met teoquil chloride (Hedoquinium chloride)
0.125 mg/ml
881. AF12 w³ thi² met thiram (tetramethyl thiuran disulfide)
0.125 mg/ml
882. 16-4 pdx fwn low toxin (2 µg B₁)
883. 16-5 pdx fwn low toxin (21 µg B₁)
884. 5T-19-2 ad t low toxin (15 µg B₁)
885. T38-5 his t low toxin (33 µg B₁)
886. D1 2-22 + 31a-18-1
887. D1 793 + 4 (afl)

888. D1 31a-6-2 (lys) + 4 (afl)
889. D1 31a-6-2 (lys) + afl-1 ✓
890. D1 31a-22-26 + afl-4
891. D1 31a-22-11 + afl-4
892. D 700-1 + 794
893. D1 4 + 782
894. D1 4 + 783 (781 704)?
895. D1 779 + 704
896. D1 776 + 704
897. D1 T38-4 + 700-1
898. D1 774 + 704
899. D1 4 + 31a-22-13
900. D1 4 + 820
901. D1 4 + 31a-22-25
902. D1 700-1 + 807
903. D1 700-1 + 795
904. D1 5T-17-12 + 710
905. D1 808 + 700-1
906. D1 700-1 + T38-2
907. D1 780 + 704
908. Af15 y nys1 12 units/ml mycostatin from Mackins (London)
909. D1 4 + 787
910. D1 4 + 786
911. D1 700-1 + 2-27
912. D1 781 + 704 (44783)?
913. D1 4 + 32-9
914. 31a-22-27 (dark pigment-slow growing)
915. D1 30-22 + 506

944. su 1-27
945. su 4-19
946. su 4-20
947. su 4-21
948. su 4-23
949. su 4-9
950. su 4-27
951. su 4-28
952. su 4-29
953. su 4-30
954. su 4-32
955. su 4-33
956. su 4-39
957. D (su 1-14 + 592)
958. D9 827 + T44-7
959. D8 827 + T44-7
960. D6 827 + T44-7
961. D4 (827 + 5T-18)-22 + 31a-18-1
962. D2 " "
963. D1 " "
964. 73 (827 + 491) nor lys t
965. 59 (827 + 491) nor lys w
966. 22 (827 + 5T-18) nor lys t
967. 61 (827 + T44) arg t nor
968. 62 (927 + T44) arg lys t nor
969. 17 (659 + 491) afl-12 leu t
970. D T44-70 + 710
971. D3 (827 + 5T-18-2) afl-1
+ nor +/+ nor +

972. D4 () + nor +/- nor leu (contamin. ?)
973. D (827 + 5T-18)-22 + 31a-18-1 nor/+ het diploid
974. D (827 + 5T-18)-22 + 31a-25-3
975. D (827 + 491)-73 + 31a-25-3
976. D (31a-18-5 + [827 + 5T-18]-22) nor/nor (20-N)
977. 31a-18-5 + (827 + 491)-73 nor g (1-5)
978. 31a-18-5 + (827 + 5T-18)-22 nor w (3-9)
979. D 30-22 + 31a-18-1
980. D 19-1 + T44-7
981. D 31a-25-3 + 31a-18-5
982. D #19 + 5T-18-2
983. D T44-13 + 31a-25-6
984. D 19-8 + T44-13
985. D T37 + 31a-25-6
986. D 31a-25-6 + (31a-18-5 + 579)-20
987. D T44-7 + 31a-25-6
988. D 30-22 + (T44-7 + 592)-20
989. D T44-13 + 19-1
990. D 16-4 + (31a-18-1 + 579)-33
991. D 31a-25-6 + (31a-18-1 + 579)-33
992. D 31a-25-3 + (19-1 + 579)-14
993. D 16-4 + (31a-18-5 + 579)-20
994. D 16-4 + 5T-18-2
995. D 16-4 + T44-7
996. D T44-7 + 31a-18-2
997. D 31a-18-2 + (31a-18-5 + 579)-20
998. D 31a-25-3 + (19-1 + 579)-3
999. D 30-22 + 31a-18-5

- 1000. D 19-8 + 30-22
- 1001. D 19-1 + 5T-18
- 1002. D T44-7 + 31a-18-5
- 1003. D 19-8 + T44-7
- 1004. D 30-22 + 31a-25-6
- 1005. D 5T-18-2 + 19-8
- 1006. D 19-8 + 5T-18
- 1007. D 19-8 + (31a-18-5 + 579)-20
- 1008. D 19-1 + (31a-18-5 + 579)-20
- 1009. D 19-8 + (31a-18-1 + 579)-33
- 1010. D 19-1 + (31a-18-1 + 579)-33
- 1011. D 16-4 + T44-13
- 1012. D 31a-10-2 + (19-1 + 579)-3
- 1013. D 31a-18-2 + (19-1 + 579)-14
- 1014. D T44-7 + 31a-18-1
- 1015. D 31a-18-5 + T44-13
- ~~1016. D 31a-25-3 + (31a-18-5 + 579)-20~~ *LOST*
- 1017. (827 + 5T-18)-22 lys t nor
- 1018. (31a-25-6 + 491)-6 arg t afl-23
- 1019. (31a-25-3 + 579)-22 arg his t afl-15
- 1020. (19-8 + 579)-16 pab met his t afl-25
- 1021. D 31a-18-1 + (31a-18-5 + 579)-20
- 1022. (16-4 + 5T-18)-28 leu t afl-24
- ~~1023. (659 + 491)-17 leu t afl-12~~ *LOST*
- 1024. (31a-25-3 + 579)-16 arg his t afl-15
- 1025. (T44-7 + 592)-20 his w afl-19
- 1026. (19-1 + 579)-3 pab pro t afl-21
- 1027. *OMIT*

1028. (31a-18-1 + 579)-33 met his t afl-17
1029. (T44-7 + 592)-10 his w afl-19
1030. D [31-1 (No₃⁻ w) + afl-4]
1031. D [31-1 (No₃⁻ w) + T44]
1032. 31-1 No₃⁻ w
1033. 31-4 No₂⁻ w
1034. 31-7 (H8) hypoxanthine w (from 31a) + PC7
 ↓
 hc group 17 tester
1035. 81-12-17 No₃
1036. 81-12-18 HX
1037. 81-12-33 No₂
1038. 81-1-24 No₃
1039. 81-1-38 No₂
1040. 81-1-41 HX
 ↓
 hc group 1 tester
1041. 81-15a-44 No₃ w
1042. 81-15a-55 No₂ w
1043. 81-156-121 No₃
1044. 81-156-123 No₂
1045. 81-156-124 HX
 ↓
 hc group 5 tester
1046. 81-8-129 No₃
1047. 81-8-131 HX
 ↓
 hc group 4 tester
1048. 81-30a-136 HX
1049. 81-30a-137 No₃
1050. 81-30a-139 No₂
1051. 81-37a-141 HX
 ↓
 hc group 6 tester

1052. 81-32a-142 No₂
1053. 81-13-157 No₃
1054. 81-13-158 No₂
1055. 81-47-213 No₂
1056. 81-47-211 HX
 ↓
 hc group 21 tester
1057. 81-28a-210 HX
1058. 81-28a-208 No₂
1059. 81-28a-207 No₃
1060. 81-46-193 No₂ hc group 3 tester
1061. 81-51-174 HX
1062. 81-51-171 No₃
1063. 81-6-160 No₃
 ↓
 hc group 3 tester
1064. 81-6-159 No₂
1065. 81-49-223 No₂
1066. 81-33b-227 HX
1067. 83-76-269 No₃
1068. 83-96-271 No₃
1069. 83-6-282 No₃
1070. 83-6-284 No₂
1071. 83-50-386 No₃
1072. 83-75-428 No₂
1073. 83-52-456 No₂
1074. 83-52-457 No₃
1075. 84-59c-574 No₂ A. clavatus (formed hets only with 631)
1076. 84-59c-629 No₂ A clavatus (formed hets well with 631; poorly w/630)
1077. 84-59c-630 No₃ " "
1078. 84-59c-631 HX " "

1079. 83-50 wild-type isolates from which nitrate mutants were obtained
1080. 83-52
1081. 83-76
1082. 83-90
1083. 83-6
1084. 83-75
1085. 83-96
1086. 81-1
1087. 81-15a
1088. 81-30a
1089. 81-13
1090. 81-15b
1091. 81-12
1092. 81-28b
1093. 83-47
1094. 81-37a
1095. 81-51
1096. 81-33b
1097. 81-28a
1098. 81-49
1099. 81-8
1100. 81-47
1101. 81-6
1102. 81-46
1103. 81-5
1104. 81-4
1105. 83-14
1106. 83-11
1107. 84-24

1108. 84-23
 1109. 83-23
 1110. 83-72
 1111. 83-89
 1112. 81-30b
 1113. 81-4-93
 1114. 81-4-96 HX hc group 2
 1115. 81-5-152
 1116. 81-5-154 No₃
 1117. 81-30b-177
 1118. 83-30b-180
 1119. 81-28b-214
 1120. 81-28b-216
 1121. 83-47-260
 1122. 23-47-261
 1123. 83-14-373
 1124. 83-11-374
 1125. 84-24-583

1941

The *A. clavatus* was
 in preparation for a
 graduate student's study
 on parasituality. If
 you ever need any
clavatus we have 190
 isolates, incl. diploids, which
 are clearly identified.
 ger

1130-1136 all *A. clavatus* collected from
 maize weevils by Doug Dix

1126. 84-24-584 ✓ HX
 ↓
 hc group 23
 1127. 83-89-618
 1128. 83-72-626 ✓ No₃
 1129. 84-23-588
 1130. 10-50 ✓ *A. clavatus* surface contaminant of maize weevil
 collected 10-22-82, Midville, Ga
 row 10 in corn field, plant 50
 1131. 10-40-1 " "
 1132. 22-20 " "

1133.	104-20	maize weevils collected 8-31-82 Griffin, Ga.		
1134.	126-60	"	"	"
1135.	4-10	"	"	"
1136.	26-25	"	10-27-82	"
1137.	47-1	<u>arg</u> ⁻	<u>w</u>	(from 31-1 + T44)
1138.	1114-1	<u>w</u>	<u>HX</u>	white
1139.	1114-2	<u>or</u>	HX	leiteous 19b
1140.	1114-3	<u>ol</u>	HX	lt. olive buff 21''d
1141.	1114-7	<u>ylo</u>	HX	amber 21'b
1142.	1068-9	<u>w</u>	No ₃	lt. olive buff 21''d
1143.	1068-4	<u>ol</u>	No ₃	lt. olive buff 21''d
1144.	1068-3	<u>ylo</u>	No ₃	luteous 19b
1145.	1068-2	<u>w</u>	(dirty) No ₃	pale buff 19''d*
1146.	1068-1	<u>or</u>	No ₃	luteous 19b
1147.	1045-1	<u>ol</u>	HX	primrose 23''d
1148.	1045-2	<u>or</u>	HX	lt. sienna 13i
1149.	1045-5	<u>ylo</u>	HX	amber 21'b
1150.	1047-1	<u>cr</u>	HX	white
1151.	1047-2	<u>ol</u>	HX	primrose 23''d
1152.	1047-3	<u>w</u>	HX	white
1153.	1047-4	<u>ylo</u>	HX	luteous 19b
1154.	1047-8	<u>or</u>	HX	luteous 19b
1155.	1128-4	<u>or</u>	No ₃	luteous 19b
1156.	1128-5	<u>ylo</u>	No ₃	lt. luteous 19b
1157.	1128-7	<u>w</u>	No ₃	white
1158.	116-1	<u>al</u>	No ₃	primrose 23''d
1159.	1116-4	<u>or</u>	No ₃	dk. luteous 19d
1160.	1116-5	<u>w</u>	No ₃	white

*refers to location on A Mycological Colour Chart Rayner

1161.	1126-1	<u>ylo</u>	HX	amber 21'b
1162.	1126-3	<u>w</u>	HX	white
1163.	1126-4	<u>ol</u>	HX	olive buff 2''d
1164.	1126-5	<u>or</u>	HX	luteous 19b
1165.	1126-6	<u>ol-ylo</u>	HX	dk. amber 21'b
1166.	1126-7	<u>t-g</u>	HX	citrine 21K
1167.	1126-8	<u>w</u> (dirty)	HX	lost
1168.	1040-1	<u>w</u>	HX	white
1169.	1040-4	<u>glo-g</u>	HX	dk. amber 21'b
1170.	1040-5	<u>cr</u>	HX	primrose 23"d
1171.	1117-1	<u>or</u>	No ₃	lt. sienna 13i
1172.	1117-7	<u>t</u>	No ₃	citrine 21K
1173.	1117-8	<u>ylo</u>	No ₃	dk. amber 21'b
1174.	1059-1	<u>ol</u>	No ₃	primrose 23"d
1175.	1059-2	<u>or</u>	No ₃	lt. amber 21'b
1176.	1069-4	<u>w</u>	No ₃	pale primrose 23"d
1177.	1069-3	<u>al</u>	No ₃	primrose 23"d
1178.	1056-1	<u>or</u>	HX	luteous 19b
1179.	1056-5	<u>ylo</u>	HX	lt. luteous 19b
1180.	1056-6	<u>ol</u>	HX	sulfur yellow 25F
1181.	1056-7	<u>ol-g</u>	HX	olive buff 21''d
1182.	1056-8	<u>w</u>	HX	white
1183.	1051-4	<u>w</u> (dirty)	HX	very pale buff 19"f
1184.	1051-3	<u>w</u>	HX	white
1185.	1051-2	<u>t</u>	HX	citrine 21K
1186.	1062-3	<u>ylo</u>	No ₃	amber 21'b
1187.	1062-5	<u>w</u>	No ₃	white

1188.	1062-7	<u>yl</u> -g	No ₃	amber 21'b
1189.	1135-775	No ₂	<u>A. clavatus</u>	
1190.	1130-777	No ₃		
1191.	1130-778	NX		
1192.	1134-781	No ₃		
1193.	1134-783	HX		
1194.	1136-784	No ₂		
1195.	1136-786	HX	lost	
1196.	1133-788	No ₂		
1197.	1133-789	HX		
1198.	1132-794	No ₃		
1199.	1132-797	No ₂		
1200.	1131-791	No ₃		
1201.	1131-793	No ₂		
1202.	1117-1 + 506 (2n)			
1203.	1117 + 506 (2n)			
1204.	1117-8 + 506 (2n)			
1205.	1060-1	No ₂		
1206.	1060-2	No ₂		
1207.	1060-3	No ₂		
1208.	1060-6	No ₂		
1209.	1035-5	No ₃		
1210.	1035-6	No ₃		
1211.	84-78 (725)	No ₂		
1212.	1117-4	<u>w</u>	<u>No₃</u>	
1213.	1035-7	<u>w</u>	No ₃	
1214.	1064-7	<u>w</u>	<u>No₃</u>	
1215.	1064-4	<u>or</u> -g	No ₃	

1216. 1064-2 w No₃ (dirty w)
1217. [506 + 1117-1]-8 or pdx No₃
1218. [506 + 1117-8]-67 or pdx No₃ leu
1219. [506 + 117-8]-19 ylo pdx No₃
1220. [506 + 117-8]-36 ylo pdx No₃
1221. A flavus
1222. A. flavus A266 36
1223. " 40
1224. " 23
1225. " 25
1226. " 30
- NRRL 3357 from Shane Tucker supplied by
D. T. Wicklow - isolated from subterranean
seed caches of the desert kangaroo rat -
Dipodomys spectabilis - produce tan-beige
sclerotia
1227. A. clavatus 1131-7 arg ? or ang + ?
1228. 1130-15 arg
1229. 1130-4 arg
1230. 1130-1 arg
1231. 1130-8 arg ? or arg + ?
1232. 1132-10 ad ? or ad + ?
1233. 1132-24 arg
1234. 1131-2 arg
1235. 1131-16 arg
1236. 1131-12 arg
1237. 1130-14 leu
1238. 1130-16 leu
1239. 1132-7 arg
1240. 1132-13 arg
1241. 1134-1 ad
1242. 1132-15 leu
1243. 1431 t

1244.	1132	light blue
1245.	1131	<u>w</u>
1246.	1130	<u>w</u>
1247.	1134	<u>w</u>
1248.	1134	<u>t</u>
1249.	1132	<u>w</u>
1250.	1130	golden tan (gt)
1251.	1134-8	<u>arg</u>
1252.	1134-3	<u>arg</u>
1253.	1134-6	<u>arg</u>
1254.	1133-	<u>w</u>
1255.	1133-28	ad ? or ad + ?
1256.	1131-12	<u>red</u> <u>arg</u>
1257.	1131-12	<u>w</u> <u>arg</u>
1258.	1131-12	off <u>w</u> <u>arg</u> (bluish <u>w</u>) ?
1259.	1131-12	<u>w</u> <u>arg</u>
1260.	1134-1	<u>ad</u> <u>w</u>
1261.	1135	<u>ad</u>
1262.	1135	<u>arg</u>
1263.	1132	<u>lt</u> <u>bl</u> leu? or double mutant
1264.	1134	<u>leu</u>
1265.	1131	ad
1266.	1133	<u>w</u> <u>arg</u>
1267.	1136	<u>arg</u>
1268.	1132	met? or double mutant no. 1
1269.	1132	met? or double mutant no. 2
1270.	1130	met
1271.	1136	leu? or double mutant no. 1
1272.	1136	" " " no. 2

1273.	1131	<u>met</u>		
1274.	1130	<u>ad</u>		
1275.	1131	<u>leu</u>	leaky	
1276.	1132-24	<u>ad</u>	<u>w</u>	
1277.	1134	met		
1278.	1133	<u>leu</u>	<u>w</u>	
1279.	1132	lt. blue met?	or double mutant	
1280.	1133	<u>w</u>	met?	(" ")
1281.	1136	<u>met</u>		
1282.	81	81-18	<u>w</u>	h-c group 13 tester
1283.	58	81-17	<u>w</u>	" 12
1284.	46	81-17	<u>w</u>	" 12
1285.	91	81-16		" "
1286.	118	81-11		" 10
1287.	109	81-9		" 9
1288.	107	81-9		" 9
1289.	133	81-7		" 8
1290.	75	81-2	<u>w</u>	" 7
1291.	67	81-2	<u>w</u>	" 7
1292.	92	81-18	<u>w</u>	" 13
1293.	23	81-20		" 14
1294.	21	81-20		" 14
1295.	128	81-29a		" 15
1296.	186	81-37b		" 20

1297.	187	81-37b	h-c group 20
1298.	196	81-36	" 19
1299.	197	81-36	" 19
1300.	163	81-32	" 18
1301.	204	81-32	" 18
1302.	201	81-29b	" 16
1303.	200	81-29b	" 16
1304.	102	81-52	" 22
1305.	103	81-52	" 22

~~1306.~~

end

1307.

1308.

1309.

1310.

1311.

1312.

