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ANTIBIOTICS FROM ASPERGILLI.

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Received for publication February 18, 1949.

A SUMMARY of the work of a number of investigators on the antibiotics produced by Aspergilli has recently been made (Florey, Chain, Heatley, Jennings, Sanders, Abraham and Florey, 1949). During the compilation of the tables for this summary it became apparent that some gaps remained to be filled. The object of the present work was to investigate named and identified species which had not, so far, been examined for their capacity to produce antibiotics. The work completes the examination, under certain conditions of growth, of strains of all known species of Aspergilli with the exception of a few so far unobtainable.

Though the examination of fungi for the production of antibiotics is essentially straightforward, it should be emphasized that the statement that a given fungus does or does not produce an antibiotic must be received with a certain reserve, as conditions of growth and testing may greatly affect the result.

Clearly, in a preliminary examination of a number of different cultures it is not practicable to carry out exhaustive experiments on each species. In the following work a certain set of conditions was selected, and each culture tested under approximately the same conditions of growth.

EXPERIMENTAL.

The fungi examined were each grown on all the nine media shown in Table I, which were chosen because they have been found by many observers to allow good growth of many fungi.

TABLE I.—*Media Used.*

1. Potato dextrose	200 g. chopped potato, steamed for one hour with 600 ml. water. Supernatant liquid drawn off and 10 g. glucose added. Made up to 1000 ml. with water.
2. Modified Sabouraud	10 g. peptone. 40 g. maltose. 26 g. malt extract. Made up to 1000 ml. with water.
3. Modified glucose Sabouraud	10 g. peptone. 40 g. glucose. 26 g. malt extract. Made up to 1000 ml. with water.
4. Malt extract	50 g. malt extract. Made up to 1000 ml. with water.
5. Czapek-Dox	3 g. $NaNO_3$. 1 g. KH_2PO_4. 0·5 g. KCl. 0·5 g. $MgSO_4.7H_2O$. ·01 g. $FeSO_4.7H_2O$. 40 g. glucose. Made up to 1000 ml. with water.
6. Czapek-Dox + 5% corn steep	Same as Medium 5, with the addition of 50 ml. corn steep liquor, neutralized and filtered, before making up to 1000 ml.
7. Czapek-Dox + 5% corn steep, neutralized	Same as Medium 6, but brought to pH 7.
8. Beer wort	50 ml. wort, made up to 1000 ml. with water.
(This medium was little used as the beer wort was difficult to obtain.)	
9. Yeast medium	20 g. dried autolysed yeast stirred into 100 ml. warm water. Centrifuged and supernatant liquid poured off. 50 ml. of the supernatant liquid and 40 g. glucose made up to 1000 ml. with water.

Distilled water was used to make up all the above media.

The first experiments were done by growing the fungi at 24° C. in 10 ml. of sterile medium contained in hard glass bottles which were roughly 3 cm. in diameter and $5\frac{1}{2}$ cm. tall. Testing could only be carried out for 12 days, as at the end of that time no medium remained. For investigations involving more prolonged sampling the cultures were planted in 250 ml. Erlenmeyer flasks containing 70 ml. of sterile medium, and were tested for 35 days. Some slow-growing members of the *Aspergillus glaucus* group were grown in hard glass bottles with extra sugar in the media, 20 per cent glucose in Czapek-Dox, potato dextrose, or yeast medium. Such fungi were allowed to grow at room temperature and were tested for up to 50 days.

The metabolism fluid at various stages of growth was tested by the cylinder-plate method (Abraham, Chain, Fletcher, Florey, Gardner, Heatley and Jennings, 1941 ; Heatley, 1944) with arbitrarily selected bacteria ; strains of *Bact. coli*, *Staph. aureus*, *C. xerosis* and *Myco. phlei* were used. Clearly, production of very specific antibiotics, such as those produced by *Bact. coli* (Heatley and Florey, 1946), may have remained unobserved if an organism sensitive to them was not included.

RESULTS.

The results are set out in Table II.

TABLE II.—*Results.*

Aspergillus.	Strain.	Test organism. *Bact. coli*	*Staph. aureus.*	*Myco. phlei.*	*C. xerosis.*	References from Table 1 of media on which activity was produced.
A. GLAUCUS group.						
A. repens series.						
A. pseudo-glaucus Bloch	Baarn	−	−	−	−	..
A. ruber series.						
A. proliferans G. Smith	NCTC 6546	−	−	−	−	..
A. chevalieri series.						
A. chevalieri (Mang.) Thom and Church	NRRL 78	−	−	−	−	..
A. chevalieri (Mang.) Thom and Church, var. *intermedius* Thom and Raper	NRRL 82	−	+	+	+	2, 3, 4.
A. amstelodami series.						
A. itaconicus Kinoshita	Baarn	−	−	−	−	..
A. herbariorum series.						
A. carnoyi (Biourge) Thom and Raper	NRRL 126	−	−	−	−	..
A. mangini n. comb.	NRRL 117	−	+	+	+	9 with 20% glucose after 50 days.
A. echinulatus (Delacr.) Thom and Church	Baarn	−	−	−	−	..
A. niveo-glaucus Thom and Raper (syn. *A. glaucus*)	NRRL 127	−	+	−	+	5 with 20% glucose after 35 days.
A. umbrosus Bainier and Sartory	Baarn	−	−	−	−	..
A. restrictus series.						
A. gracilis Bainier	Baarn	−	+	+	+	5 with 20% glucose after 50 days.
A. NIDULANS group.						
A. caespitosus Thom and Raper	NCTC 6972	−	+	+	+	5, 6, 7.
A. nidulans (Eidam) Wint. var. *latus* Thom and Raper	NRRL 200	−	−	−	−	..
A. quadrilineatus Thom and Raper	NRRL 201	−	+	+	+	5, 6, 7.
A. rugulosus Thom and Raper	NRRL 206	−	−	−	−	..
A. unguis (Emile-Weil and Gaudin) Thom and Raper	Baarn NRRL 216	−	+	+	+	5 or 1.
A. variecolor (Berk. and Br.) Thom and Raper	Baarn	−	−	−	−	..

TABLE II (cont.).—*Results.*

Aspergillus.	Strain.	Test organism. *Bact. coli.*	*Staph. aureus.*	*Myco. phlei.*	*C. xerosis.*	References from Table I of media on which activity was produced.
A. USTUS group.						
A. granulosus Raper and Thom	NCTC 6973	−	−	−	−	..
A. VERSICOLOR group.						
A. janus Raper and Thom	NCTC 6970	−	+	+	+	1, 5, 6, 7, 8.
A. janus var. *brevis* Raper and Thom	NCTC 6971	−	+	+	+	1, 5, 6, 7.
A. TERREUS group.						
A. terreus series.						
A. terreus Thom var. *aureus* n. var.	NRRL 1923	−	+	+	+	1 or 4.
A. terreus Thom var. *boedijni* n. var.	NRRL 680	+	+	+	+	1, 2, 3, 4.
A. terreus Thom var. *floccosus* Shih	Baarn	−	+	+	−	4.
A. carneus series.						
A. carneus (v. Tiegh.) Bloch	NRRL 527	−	+	+	+	1, 2, 6.
A. NIGER group.						
A. niger series.						
A. awamori Nakazawa	NCTC 2044	−	−	−	−	..
A. foetidus n. sp.	Baarn	−	−	−	−	..
A. phoenicis (Cda.) Thom	Baarn	−	(+)	(+)	(+)	..
A. carbonarius series.						
A. atropurpureus Zimmerman	Baarn	−	−	−	−	..
A. fonsecaeus n. sp.	NRRL 67	−	(+)	(+)	(+)	..
A. luchuensis series.						
A. japonicus Saito	NCTC 5604*b*	−	−	−	−	..
A. WENTII group.						
A. alliaceus Thom and Church	Baarn	+	+	+	+	4.
A. avenaceus G. Smith	NCTC 6545	−	−	−	−	..
A. panamensis Raper and Thom	NCTC 6974	−	+	+	+	1 or 8.
A. TAMARII group.						
A. terricola series.						
A. lutescens (Bain.) Thom and Church	NRRL 425	+	+	+	+	all.
A. FLAVUS-ORYZAE group.						
A. micro-virido citrinus Cost and Lucet	NRRL 48	−	−	−	−	..
A. OCHRACEUS group.						
A. sulphureus series.						
A. quercinus (Bain.) Thom and Church	NCTC 6979	±	+	+	+	3, 6.
A. sulphureus (Fres.) Thom and Church	Baarn	±	+	+	+	3, 2, 4, 6.
A. ochraceus series.						
A. butyraceae Bainier	Baarn	−	−	−	−	..
A. elegans Gasperini.	Baarn	−	−	−	−	..
A. melleus Yukawa	Baarn	±	+	+	+	2, 3, 4, 6
A. sclerotiorum Huber	Baarn	−	−	−	−	..
A. sparsus Raper and Thom	NCTC 6975	−	−	−	−	..

Source of culture ; NCTC = National Collection of Type Cultures, England. Baarn = Centraalbureau voor Schimmelcultures, Baarn, Holland. NRRL = Northern Regional Research Laboratory, Peoria, U.S,A.

Activity ; + active; − inactive; ± very slightly active; (+) activity probably due to low pH as on neutralisation no activity was demonstrable.

The following species of Aspergilli have not been examined, as cultures could not be obtained: (1) *Aspergillus miyakoensis* Nakazawa; (2) *Aspergillus montevidensis* Talice and Mackinnon; (3) *Aspergillus humicola* Chaudhuri and Sachar; (4) *Aspergillus delacroixii* (Sacc.) Thom and Church. Thom and Raper (1945) considered that "it is possible that some old material of a strain of *A. oryzae* might have furnished the type."

SUMMARY.

Thirty-seven species of Aspergilli, comprising 42 strains, which are not known to have been investigated before, have been examined for their ability to produce antibiotics in surface culture on 9 different liquid media. Twenty strains produced metabolism solutions with some antibacterial activity.

I am indebted for the supply of organisms to Dr. K. B. Raper, of the Northern Regional Research Laboratory, Peoria, Professor Dr. Johanna Westerdijk, of the Centraalbureau voor Schimmelcultures, Baarn, and the National Collection of Type Cultures.

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