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ACTIVITY OF FUNGI ON OILS

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Plastic films, like other substances composed of organic materials, are subject to the degradative action of microorganisms. Under favorable conditions of moisture and temperature, microorganisms attack certain types of plasticizers although the plastic itself does not appear to be affected. Since the plasticizer is added to make the films flexible, microbial action results in a stiffening of the film (Harvey, 1949). Plasticizers vary in resistance from inert compounds such as tri-octyl-phosphate to extremely susceptible ones such as castor oil. For reasons of availability, cost or requirements of specific properties, it is frequently necessary to use plasticizers which are non-resistant. A study has been made in these laboratories of the breakdown of several homologous series of esters by the fungus *Aspergillus versicolor* and by the bacterium *Pseudomonas aeruginosa* (Stahl and Pessen, 1953). Results of tests conducted under the auspices of the National Defense Research Committee during World War II are summarized by Brown (1946). Most plasticizers are of the ester type. Those composed of naturally occurring oils and fatty acids (i.e. laurates, stearates, ricinoleates, etc.) are known to support microbial growth, while synthetic plasticizers of the phthalate and phosphate types are generally resistant. The present study was undertaken to determine the range of organisms capable of growing on typical plasticizers. It supplements a previous publication covering the activity of the same fungi on cotton and on wool (Reese, et al, 1950). The activities of some (358) of these organisms on coconut oil, methyl-acetyl-ricinoleate (or di-hexyl-sebacate) are being reported here.

Comparatively little information on fat utilization by fungi is available. Much more interest has been shown in fat *synthesis*. On the other hand degradative action by bacteria has been more fully covered. One of the best reviews is the 26 page chapter "Action of Microorganisms on Fat" by Jensen in his recent book (1945). Fats decompose slowly in the soil (Waksman, 1932). Fungi and aerobic bacteria are largely responsible. A comparative study of bacterial action on lipids (Castell and Garrard, 1941) showed *Pseudomonas aeruginosa*, *Alcaligenes viscosus*, and *Staphylococcus aureus* as possessing distinct lipolytic action. The factors affecting lipase production by two of these bacteria, *Alcaligenes viscosus* and *Pseudomonas aeruginosa,* were recently investigated (Cutchins, Doetsch, Pelczar, 1951). In the Actinomycetes, several of the antibiotic producing species of Streptomyces have been found to utilize animal and

vegetable oils. Replacement of all of the carbohydrate by lipids did not decrease the yields of antibiotic produced (Perlman and Wagman, 1952). Fungi, especially Aspergilli and Penicillia, have frequently been observed growing on margarine and butter. Undoubtedly many of those fungi found on paints, meats and other complex substrata are obtaining their nutrient from the *fatty* constituents therein.

We are not concerned here with the mechanism of breakdown of the compounds under consideration. Indeed, the manner of attack does not appear to have been clearly established for various types of esters. Probably the action revolves around hydrolytic breakdown — lipases attacking glycerides such as coconut oil; esterases breaking down the simple esters. This aspect of the enzymic hydrolysis of fats and esters has been reviewed recently by Ammon and Jaarma (1950).

METHODS

Cultures were set up in triplicate in 250 ml Erlenmeyer flasks containing 0.5g of coconut oil ("Moonstar," Procter and Gamble), di-hexyl sebacate (Hardesty Chemical Company) or methyl acetyl ricinoleate (P4, Baker Castor Oil Company) and 50 ml of nutrient solution (1.0 g NH_4NO_3; 1.36 g KH_2PO_4; 0.2 g $MgSO_4 7H_2O$; 0.1 g Difco yeast extract; 1000 ml distilled H_2O; pH adjusted to 6.4). The di-hexyl sebacate was purified by adsorption on activated silica, activated alumina and Fuller's earth. After autoclaving, the media were inoculated with a spore suspension, and placed on a reciprocal shaker at 30°C for 7 days. Dry weights of the mycelium produced were determined after filtering on sintered glass crucibles, extracting the residual oil with an excess of absolute ethanol and drying at 70°C. Results are based on the average dry weight of mycelium per flask.

RESULTS

Results are presented in detail in the appendix. The data are summarized in Table 1 in which 5 categories of growth have been set up. The percentage of the strains falling into each category is shown as well as the total number of strains tested. Sebacate was distinctly inferior as a substratum. Over

TABLE 1. GROWTH OF FUNGI ON FATTY MATERIALS

	Growth		% of Strains Tested		
Category	Wt/flask	as % wt. of oil	Coconut Oil 5%	Ricinoleate 10%	Sebacate 40%
0	0–10 mg	0–2%			
1	11–100	3–20	39	57	58
2	101–200	21–40	31	29	1
3	201–300	41–60	22	4	0
4	301+	60+	3	0	0
Number of strains tested			358	309	82

95% of all strains tested showed less than 50 mg growth/flask (i.e. less than 10% conversion of oil to mycelium). No effort was made to determine the cause. The other ester, ricinoleate, was a fairly good substratum for 90% of the test organisms. The glyceride, coconut oil, was by far the best substratum of those tested in that 95% of the organisms were able to grow on it, and the per cent conversion of substratum to fungus tissue was greatest. While a few cases have been found where the amount of growth on ricinoleate exceeded that on coconut oil, the reverse is usually true.

The organisms falling at either extreme, i.e., no growth (group 0) or very good growth (groups 3,4) are listed in Tables 2 and 3. Much of the screening done has been on Aspergilli. The results indicate that members of the following groups are usually very active: *A. terreus*, *A. niger* (the *A.*

TABLE 2. ORGANISMS GROWING LITTLE OR NOT AT ALL UNDER CONDITIONS OF THE TEST (GROUP 0) †

Acrostalagmus cinnabarinus	QM	320e	Colletotrichum sp.	QM	533
Amblyosporium botrytis	QM	971	Ctenomyces serratus	QM	256
Aspergillus repens	QM	44c	Phialophora lagerbergii	QM	267
Basidiomycete (conidial stage)	QM	592	Pholiota adiposa	QM	512
" " "	QM	870	Polyporus sulfureus	QM	509
Botryosporium pulchrum	QM	965	Sepedonium sp.	QM	913
Botryotrichum piluliferum	QM	991	Stereum purpureum	QM	1014

TABLE 3. ORGANISMS GROWING BEST ON OILS (Groups 3, 4) †

Alternaria tenuis	QM	73b	Beauveria bassiana	QM	972
Aspergillus carbonarius	QM	331	Brachysporium sp.	QM	70g
A. clavatus	QM	872	Chaetomium globosum	QM	38f
A. fischeri	QM	865	* Ch. spirale	QM	622
A. flavus	QM	63c	Circinella sydowi	QM	629
A. niger	QM	458	* Cunninghamella bertholletiae	QM	1021
A. niger mut. cinnamoneus	QM	326	* C. blakesleeana	QM	631
A. niger mut. schiemanni	QM	327	* C. echinulata	QM	154f
A. ochraceus	QM	880	Paecilomyces varioti	QM	823
A. oryzae	QM	82i	Penicillium citrinum	QM	1a
A. parasiticus	QM	884	Pestalotia royenae	QM	531
A. phoenicis	QM	1005	Pestalotia virgatula	QM	479
A. sydowi	QM	31c	* Phomaceae	QM	13e
A. tamarii	QM	75b	* Phomaceae	QM	699
* A. terreus	QM	72f	Phomaceae	QM	618
* A. ustus	QM	891	Phomaceae	QM	703
A. ustus var. laevis	QM	893	Phomaceae	QM	576
A. versicolor	QM	432	Rhizopus sp.	QM	1032
A. violaceo-fuscus	QM	335	* Septonema sp.	QM	818

* The asterisk indicates those organisms whose mycelial weight exceeded 60% of the initial weight of the coconut oil.

† See Table 1.

luchuensis series is only moderately active), *A. flavus-oryzae*, *A. fumigatus*, *A. clavatus*, *A. tamarii*, *A. versicolor*, *A. ustus*. Moderate activity is shown by *A. nidulans* group. Least activity is shown by members of the *A. repens*, and *A. wentii* groups. In the genus Chaetomium, as in the Aspergilli, the isolates of each species are quite uniform in activity. The relative activities of the several species are:

Very active: *Ch. globosum*, *Ch. spirale*, *Ch. mollipilium*

Moderate activity: *Ch. elatum*, *Ch. funicolum*, *Ch. cupreum*, *Ch. atrobrunneum*, *Ch. indicum*, *Ch. tortile*, *Ch. cochliodes*

Weakly active: *Ch. causiaeformis*, *Ch. velutinum*

Cladosporium herbarum isolates had medium to low activity on the oils used. This is odd since the isolates included two from sheepskin which Weston (1951) found to grow well on both animal and plant oils. *Cladosporium herbarum* and *Pullularia pullulans* are frequent causes of spotting of paints, yet neither fungus compares in activity with many others here tested on oils. This would indicate the difficulty of translating information obtained in shake flasks to other environmental conditions. Activity on the oils of paint, or of animal carcasses, evidently hinges on other factors.

Insect parasites appear to be very active consumers of oils. Thus, we find *Beauveria bassiana*, *Aspergillus parasiticus*, and *Aspergillus flavus*, in the very active group, but the common plant parasite *Botrytis cinerea*, on the other hand, has very little fat degrading ability. This observation is based on very few cases, and no generalization is intended.

The Phycomycetes vary in ability to metabolize oils. Best of the Phycomycetes tested are three species of Cunninghamella, *C. bertholletiae*, *C. blakesleeana*, *C. echinulata*. All three showed a mycelial weight equal to 65% of the initial weight of coconut oil, and to 20–40% of the initial ricinoleate. *Rhizopus spp.*, and *Circinella sydowi* are also very active, while *Absidia capillata* is rather weak. Of the Basidiomycetes tested, most appear to be relatively inactive, though this may be largely the result of the use of slow-growing vegetative inoculum. *Polyporus versicolor* had moderate activity on coconut oil.

SUMMARY

Of the fungus isolates in the Quartermaster Culture Collection tested for their ability to grow on fatty materials: 95 percent grew on coconut oil, 90 percent on methyl acetyl ricinoleate and 60 percent on di-hexyl sebacate. Ability of fungi to hydrolyze extracellularly the ester linkage appears to be extremely widespread.

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APPENDIX

Organism	QM Number	Relative Activity * on Coconut Oil	Ricinoleate
Absidia capillata van Tieghem	8b	1	1
Absidia sp.	579	2	
Acremonium sp.	1b	1	
"	89c	1	1
"	581	1	
"	582	1	1
"	583	1	
Acrostalagmus cinnabarinus Corda	320e	0	0
Aegerita sp.	566	2	
Alternaria oleracea Milbrath	280	1	1
Alternaria solani (Ellis and Martin) Sorauer	281	1	
Alternaria tenuis Nees	26a	2	2
"	73b	3	
"	85i	1	1
"	120m	2	
"	584	2	1
"	585	1	
"	586	2	2
Alternaria sp.	15a	2	
"	298	2	
"	587	2	

* See table 1.

Organism	QM Number	Relative Activity on Coconut Oil	Ricinoleate
Amblyosporium botrytis Fresenius	971	1	
Arthrobotrys arthrobotryoides (Berlese) Lindau	669	1	
"	1024	1	
"	1025	1	
Arthrobotrys superba Corda	670	1	
Arthrobotrys superba Corda var. **oligospora** Coemans	671	1	
Aspergillus caespitosus Raper and Thom	961	1	2
Aspergillus carbonarius (Bainier) Thom	331	3	1
Aspergillus chevalieri (Mangin) Thom and Church	52b	1	0
"	64c	1	
"	112a	1	
"	312	1	
Aspergillus chevalieri (Mangin) var. **intermedius** Thom and Raper	58b	1	0
"	914	1	
Aspergillus clavatus Desmazieres	862	1	1
"	872	3	1
"	863	2	
Aspergillus echinulatus (Delacr.) Thom and Church	962	1	1
Aspergillus fischeri Wehmer	864	3	2
"	865	3	
"	866	2	2
"	867	2	
Aspergillus flavipes (Bainier and Sartory) Thom and Church	24a	1	1
"	868	1	1
"	869	1	
Aspergillus flavus Link	4m	2	2
"	10e	3	
"	63c	3	3
"	70a	3	
"	138f	3	
"	380	2	
"	870	2	
Aspergillus flavus-oryzae series	871	3	2
Aspergillus foetidus Thom and Raper	328	2	1
Aspergillus fonsecaeus Thom and Raper	330	3	1
Aspergillus fumigatus Fresenius	6b	2	2
"	45h	2	1
"	445	2	
"	497	2	
Aspergillus giganteus Wehmer	620	2	2
Aspergillus luchuensis series	102d	1	1
"	155e	2	
"	873	2	
"	874	2	
"	21e		1

Organism	QM Number	Relative Activity on Coconut Oil	Relative Activity on Ricinoleate
Aspergillus montevidensis Talice and MacKinnon	401	1	1
Aspergillus nidulans (Eidam) Winter	25b	2	2
"	87c	2	1
"	875	1	
"	876	1	
Aspergillus niger van Tieghem	458	3	2
"	877	3	1
"	878	3	
Aspergillus niger mut. **cinnamomeus** (Schiemann) Thom and Raper	326	3	2
Aspergillus niger mut. **schiemanni** (Thom) Thom and Raper	327	3	3
Aspergillus niger series	4j	3	
"	38b	3	
"	45d	3	
"	50c	2	
"	154a	3	
"	198b	3	
"	386	4	
"	861	3	2
Aspergillus niveus Blochwitz	879	2	2
Aspergillus ochraceus Wilhelm	26b	3	1
"	58c	2	1
"	880	3	2
Aspergillus oryzae (Ahlburg) Cohn	22b	3	2
"	82i	3	2
Aspergillus panamensis Raper and Thom	882	3	1
Aspergillus parasiticus Speare	883	3	3
"	884	3	3
Aspergillus phoenicis (Corda) Thom and Currie	329	3	2
"	1005	3	2
Aspergillus repens (Corda) deBary	44c	0	0
"	56f	1	0
"	59g	1	1
"	210	1	0
"	360	1	0
"	361	1	0
"	364	0	0
"	564	3	2
Aspergillus restrictus G. Smith	885	2	0
Aspergillus rugulosus Thom and Raper	886	1	1
Aspergillus sclerotiorum Huber	661	2	2
Aspergillus sydowi (Bainier and Sartory) Thom and Church	4d	1	1
"	31c	3	1
"	41a	3	1
"	54a	1	1
"	96a	3	1
"	103g	3	1

Organism	QM Number	Relative Activity on Coconut Oil	Relative Activity on Ricinoleate
Aspergillus tamarii Kita	50b	3	2
"	75b	3	2
"	887	3	2
"	888	3	2
Aspergillus terreus Thom	72f	4	2
"	82j	3	2
"	91c	3	1
"	106g	4	2
"	442	3	2
"	889	3	2
Aspergillus unguis Emile-Weil and Gaudin	8f	2	1
"	30b	2	2
"	45e	2	1
"	53c	1	2
"	890	2	2
Aspergillus ustus (Bainier) Thom and Church	29c	2	2
"	89d	1	1
"	133f	3	3
"	137d	2	3
"	891	4	3
"	892	2	1
Aspergillus ustus (Bainier) var. **laevis** Blochwitz	24a–2	3	2
"	893	3	2
Aspergillus versicolor (Vuillemin) Tiraboschi	4g	2	1
"	17d	2	1
"	134c	2	1
"	432	3	1
"	894	2	1
Aspergillus violaceo-fuscus Gasperini	335	3	1
Aspergillus wentii Wehmer	44a	1	1
Basidiomycete (Conidial stage)	589	3	2
"	592	0	0
"	594	1	1
"	806	1	1
"	807	0	1
Beauveria bassiana (Bals.) Vuill.	972	3	2
Blakeslea trispora Thaxter	1019	1	1
Botryodiplodia theobromae Patouillard	78a	1	1
"	145h	2	1
"	166a	2	1
Botryophialophora sp.	571	2	2
Botryosporium pulchrum Corda	907	1	0
"	965	0	0
Botryotrichum piluliferum Sacc. & Marchal	336	1	1
"	337	1	0
"	991	0	1
Botrytis cinerea Persoon	520	1	1
Botrytis sp.	344	1	1
"	578	1	0

Organism	QM Number	Relative Activity on Coconut Oil	Relative Activity on Ricinoleate
Brachysporium oosporum (Corda) Saccardo	665	2	2
Brachysporium sp.	38d	3	2
"	70g	3	2
"	595	1	1
Byssochlamys sp.	438	1	1
Cephaliophora tropica Thaxter	596	1	1
Cephalosporium sp.	107a	1	1
"	124h	1	1
"	127e	1	2
"	597	1	1
"	598	1	1
Cephalothecium roseum Corda	599	1	1
"	936	1	1
Chaetomium atrobrunneum Ames	626	2	2
"	627	1	1
"	952	1	1
Chaetomium causiaeformis Ames	949	1	1
Chaetomium cochliodes Palliser	604	1	1
"	624	3	3
Chaetomium cupreum Ames	954	2	1
Chaetomium elatum Kunze and Schmidt	382	1	1
"	605	1	1
"	606	1	1
Chaetomium funicolum Cooke	33c	2	1
"	34d	1	1
"	35e	1	1
"	145k	1	1
"	155b	1	1
"	383	1	1
"	607	1	1
Chaetomium globosum Kunze	32b	3	2
"	38f	3	2
"	85n	3	1
"	104a	3	2
"	459	3	3
"	608	3	2
Chaetomium indicum Corda	46b	1	1
"	47c	1	1
"	156f	2	1
"	621	1	1
Chaetomium mollipilium Ames	1007	2	2
"	1008	3	2
Chaetomium spirale Zopf	622	4	2
Chaetomium tortile Bainier	895	2	1
Chaetomium turgidopilosum Ames	948	2	1
Chaetomium velutinum Ames	623	1	1
"	950	1	1
"	951	1	1
"	953	1	1
Chaetomium n. sp. of Ames	625	2	1
Circinella spinosa van Tieghem and Le Monnier	537	2	1

Organism	QM Number	Relative Activity on Coconut Oil	Ricinoleate
Circinella sydowi Lendner	629	3	1
Circinella sp.	902	2	1
Cladosporium herbarum Link	17b	1	1
"	52a	2	1
"	55b	1	1
"	71d	2	1
"	120g	1	1
"	121k	2	2
"	122e	2	1
"	279a	1	0
"	489	1	1
"	1027	1	1
"	1028	1	1
Cladosporium sp.	122c	1	1
"	146h	1	1
"	236	1	2
"	279b	1	1
Colletotrichum sp.	533	0	0
Collybia velutipes (Curt.) Lond.	1012	1	0
Coprinus sclerotigenus Ellis and Everhart	933	2	2
Corynespora sp.	569	0	0
Ctenomyces serratus Eidam	256	0	0
Ctenomyces sp.	199	0	
"	287	2	1
"	774	1	1
"	845	1	1
Cunninghamella bertholletiae Stadel	1021	4	2
Cunninghamella blakesleeana Lendner	631	4	3
Cunninghamella echinulata (Thaxter) Saccardo	154f	4	2
Cunninghamella elegans Lendner	634	2	1
Curvularia falcata (Tehon) Boedijn	77a	1	1
Dactylium dendroides (Bulliard) Fries	508	2	1
Fusarium roseum Link	38g	1	1
Humicola fuscoatra Traaen	580	1	1
Memnoniella echinata (Rivolta) Galloway	1c	1	1
Mucor genevensis Lendner	549	1	1
Mucor heterosporus Fischer	615	2	1
Myrothecium verrucaria (Alb. and Schw.) Ditmar ex Fries	460	2	1
Paecilomyces varioti Bainier	822	2	1
"	823	3	1
"	824		2
Penicillium capsulatum Raper and Fennell	2572	2	2
Penicillium chrysogenum Thom	943	2	1
Penicillium citrinum Thom	1a	3	1
Penicillium duclauxi Delacroix	1078	2	1
Penicillium frequentans Westling	2497	2	1
Penicillium funiculosum Thom	443	2	2

Organism	QM Number	Relative Activity on Coconut Oil	Relative Activity on Ricinoleate
Penicillium lilacinum Thom	4e	2	2
Penicillium luteum series	474	2	1
Penicillium martensii Biourge	50a	1	1
Penicillium palitans Westling	919	2	1
Penicillium pusillum Smith	137g	1	1
Pestalotia bicolor Ellis and Everhart	664	3	2
Pestalotia dichaeta Spegazzini	698	2	2
Pestalotia palmarum Cooke	381	2	2
Pestalotia royenae Guba	531	3	2
Pestalotia virgatula Klebahn	478	2	2
"	479	3	2
Pestalotia sp.	2d	3	2
"	119b	2	
"	121L	2	2
"	795	3	2
"	796	3	
Phialophora compacta (Carrion) Binford, Hess & Emmons	260	2	1
Phialophora fastigiata (Lagerberg and Melin) Conant	265	1	1
Phialophora jeanselmei (Langeron) Emmons	270	2	2
Phialophora lagerbergii (Melin and Nannfeldt) Conant	267	0	0
Phialophora pedrosoi (Brumpt) Binford, Hess & Emmons	259	1	1
"	261	1	1
"	262	2	1
Phialophora verrucosa Medlar	264	1	1
"	269	1	1
Phialophora sp.	645	1	1
Pholiota adiposa Fr.	512	0	0
Phomaceae	13e	4	2
"	29b	2	2
"	40c	1	1
"	106d	1	2
"	120k	2	1
"	534	2	2
"	568	0	
"	576	3	2
"	603	2	2
"	618	3	1
"	699	4	3
"	701	2	3
"	702	1	1
"	703	3	3
"	704	2	2
"	798	2	2
"	799	2	1
"	804	2	1
"	830	2	1
"	831	1	1

Organism	QM Number	Relative Activity on Coconut Oil	Ricinoleate
"	832	1	2
"	896	2	2
"	935	3	2
Phymatotrichum sp.	985	1	0
Polyporus sulfureus (Bulliard) Fries	509	0	0
Polyporus versicolor (L.) Fr.	1013	2	1
Pseudocoprinus sp.	801	1	1
Ptychogaster rubescens Boud.	1011	1	0
Pullularia pullulans (deBary) Berkhout	72c	2	2
"	279c	1	1
"	388	3	1
"	802	2	2
Rhizopus arrhizus Fischer	46c	2	2
"	187a	1	2
"	808	2	1
"	809	2	1
"	839	2	1
"	500	3	2
Rhizopus nigricans Ehrenberg	387	2	2
"	810	2	1
"	860	2	2
Rhizopus oryzae Went and Geerlings	811	3	1
Rhizopus sp.	231	1	1
"	1032	3	
Scopulariopsis brevicaulis (Saccardo) Bainier	609	1	1
"	773	1	1
"	813	1	1
"	814	1	1
"	815	1	1
"	816	1	1
Scopulariopsis repens Bainier	399	1	1
Sepedonium sp.	913	0	0
Septonema sp.	818	4	3
Spegazzinia tessarthra (Berk. and Curt.) Saccardo	373c	2	1
Sphaeropsis sp.	47a	2	1
"	104g	2	1
Spicaria violacea Abbott	1031	1	1
Spiroschisma sp. ?	708	2	1
Sporotrichum pruinosum Gilman and Abbott	168	1	1
"	244	1	1
"	303		1
"	591	1	1
"	593	2	1
"	825	1	1
Stachybotrys atra Corda	102a	1	1
Stemphylium botryosum Wallroth	544	2	1
Stereum purpureum Fr.	1014	0	0
Syncephalastrum racemosum (Cohn) Schroeter	57a	2	1

Organism	QM Number	Relative Activity on Coconut Oil	Ricinoleate
Torula sp.	986	2	1
Trichoderma viride Harz	6a	2	1
"	13b	2	1
Tritirachium roseum van Beyma	164	1	1
Ustilago zeae (Beckm.) Unger	990	1	2
Sporocytophaga myxococcoides (Krzemieniewska) Stanier	B482	1	0
Streptomyces sp.	B1549	1	0