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Assimilation of Alkanes and Alkenes by Fungi

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A group of filamentous fungi were assayed for their ability to utilize a series of *n*-alkanes and 1-alkenes as the sole source of carbon. Although strains of *Cunninghamella* exhibited profuse growth on most of the hydrocarbons tested, the majority of fungi tested were able to produce definite growth on one or more of the compounds. The hydrocarbons with a 14-carbon chain length appeared to be more consistently utilized than any other. Strains of *Aspergillus* appeared to differ in their capacity to utilize individual members of the hydrocarbon series. Thin-layer chromatographic analyses of ether extracts from *C. blakesleeana* grown on *n*-tetradecane and 1-tetradecene were similar and revealed the presence of a monocarboxylic acid, a primary alcohol, and a secondary alcohol.

Beerstecker (1) listed several older references indicating that certain *Aspergillus* and *Penicillium* species assimilate paraffin. Recently (9), paraffin has been used in a soil-baiting method which has yielded several fungi (of the genera *Aspergillus*, *Chaetomium*, *Penicillium*, *Syncephalastrum*, and *Cunninghamella*) which can utilize paraffin as the sole carbon source for growth. Fergus (3) has also shown that 3 of 10 thermophilic fungi tested were able to utilize paraffin as the sole source of carbon (only 1 of the 3 organisms that grew also sporulated well).

Krynitsky and McClaren (7) observed vigorous growth of *Hormodendrum*-type fungi when jet fuels were inoculated with a mixture of four different strains. *Fusarium moniliforme* isolated from diesel fuel (4) was found to grow only on *n*-decane and *n*-dodecane when a series of *n*-alkanes of 6 through 13 carbons was tested. Three *Fusarium* species were shown by Krause and Lange (6) to grow rapidly in soil after addition of long-chain *n*-alkanes and one α-olefin.

Foster (5) assayed a number of bacteria and fungi for their ability to use *n*-tridecane as the sole C source. The following fungi grew at the expense of the *n*-alkane substrate: *Acremonium potronii*, *Aspergillus alliaceus*, *Cephalosporium roseum*, *Colletotrichum atramentarium*, *Fusarium bulbigenum*, and *Monilia bonordenii*.

Assimilation patterns of *n*-alkanes and 1-alkenes by yeast led us to suggest that this type of information may be of taxonomic value (8). To initiate our work on the oxidation of hydrocarbons by filamentous fungi, we studied the ability of a group of these organisms to assimilate *n*-alkanes and 1-alkenes. This report presents our findings on assimilation patterns as well as preliminary results on oxidative studies.

MATERIALS AND METHODS

A series of five *n*-alkanes and five 1-alkenes (C_{10}, C_{12}, C_{14}, C_{16}, and C_{18}) were employed as substrates in the growth survey. The hydrocarbons used were the best materials commercially available. Oxygenated impurities present in the hydrocarbons were removed by passing the substrates through a column containing Adsorbosil-1 (Applied Science Laboratory, State College, Pa.). Prior to use in the growth survey, the hydrocarbons were passed through a 0.45-μ membrane filter (Millipore Corp., Bedford, Mass.).

Assimilation tests were carried out in 250-ml De Long culture flasks with stainless-steel culture tube closures (Bellco Glass Inc., Vineland, N.J.). Each flask contained 100 ml of a basal salts solution (2) to which 1.0 ml of the test hydrocarbon was added. The cultures were incubated at 25 C, and after 3 weeks the amount of growth in each flask was estimated visually. Since the basal salts solution alone would not support growth of the fungi, the following arbitrarily chosen scale was used to estimate the relative amount of growth in each flask: 1+ = barely discernible growth; 2+ = definite growth; 3+ = moderate growth; 4+ = good growth; and 5+ = heavy growth.

After the survey of hydrocarbon assimilation, diethyl ether was added to the cultures to extract degradative products arising from the substrate. The solvent layer was allowed to remain in contact with the culture for 24 hr. During this period, the flasks were subjected to several periods of vigorous mixing. The ether layer was removed, reduced in volume, and assayed for various classes of organic compounds by thin-layer chromatography on plates spread with Adsorbosil-1. To move residual hydrocarbon to the top of the plate, the chromatograms were first de-

TABLE 1. *Growth response of various fungi to n-alkanes and 1-alkenes*[a]

Organism	Hydrocarbons									
	Alkanes					Alkenes				
	C-10	C-12	C-14	C-16	C-18	C-10:1	C-12:1	C-14:1	C-16:1	C-18:1
Phycomycetes										
Basidiobolus ranarum	—	—	—	—	—	—	—	*	—	—
Cunninghamella sp.	***	***	***	***	***	*	***	***	***	***
C. blakesleeana (+)	***	***	***	***	***	*	***	***	***	***
C. blakesleeana (−)	***	***	***	***	***	**	***	***	***	***
Mucor sp.	—	—	*	*	*	—	—	*	—	*
Rhizopus nigricans	—	—	—	—	—	—	*	—	*	—
Syncephalastrum sp.	—	—	*	—	*	—	—	*	—	*
Ascomycetes and Deuteromycetes										
Allescheria boydii	*	—	*	*	*	*	—	*	*	—
A. boydii (*M. apiospermum*)	—	—	*	**	**	—	—	*	*	*
Alternaria sp.	—	—	—	—	—	—	—	—	—	—
Alternaria sp.	—	—	—	—	—	—	—	—	—	—
Aspergillus sp.	—	—	—	—	—	—	—	—	—	—
A. fumigatus	*	**	**	*	*	*	*	**	*	*
A. niger	—	—	—	*	—	—	—	—	—	—
A. terreus	*	**	**	**	**	—	—	**	**	**
Botrytis sp.	—	*	—	—	—	—	—	*	—	—
Botrytis sp.	—	*	*	—	—	—	—	*	—	—
Cephalosporium sp.	*	**	**	**	**	*	**	**	**	**
Cephalosporium sp.	**	**	**	**	**	—	**	**	**	**
Chaetomium sp.	*	—	*	*	—	*	*	*	—	—
Cladosporium sp.	*	—	*	*	—	*	*	*	—	—
Epidermophyton floccosum	—	—	—	—	—	—	—	—	—	—
Fusarium sp.	*	**	*	*	*	*	*	*	*	*
Fusarium sp.	*	**	**	**	**	*	*	**	**	*
F. solani	*	*	*	*	*	*	*	*	—	*
Geotrichum sp.	—	—	*	—	—	—	—	*	—	*
Gliocladium sp.	*	*	**	*	*	*	—	*	*	**
Gliocladium sp.	*	*	**	—	*	*	*	*	—	*
Helminthosporium sativum	—	*	*	*	—	—	—	*	—	—
H. tercicum	*	**	**	*	*	*	*	**	**	—
Hormodendrum sp.	—	—	—	—	—	—	—	—	—	—
Keratinomyces ajelloi	—	—	*	—	—	—	—	—	—	—
Microsporum gypseum	—	*	**	—	—	—	—	—	—	—
Nigrospora sp.	—	—	—	—	—	—	—	—	—	—
Oospora sp.	*	—	*	—	—	*	—	*	—	*
Penicillium sp.	—	**	**	**	*	—	*	**	**	*
P. chrysogenum	—	*	*	*	*	—	—	*	*	*
P. notatum	*	**	**	**	*	—	—	*	*	*
Phialophora compactum	*	*	*	*	—	*	*	*	*	*
P. pedrosoi	*	*	*	—	—	*	—	*	*	*
P. verrucosa	*	*	*	*	—	*	*	*	*	—
Phoma sp.	*	—	*	—	—	—	—	*	—	—
Pullularia pullulans	*	*	—	—	—	—	—	—	—	*
Scopulariopsis sp.	—	—	*	*	*	—	—	—	—	—
Sepedonium sp.	*	—	*	**	*	*	—	—	—	—
Spicaria sp.	*	*	**	*	—	*	—	*	*	—
S. elegans	*	*	*	*	*	*	*	*	*	*
S. violacea	**	**	**	**	**	*	*	**	**	**
Trichoderma sp.	*	**	*	*	*	—	*	*	*	*
Trichophyton rubrum	—	—	*	—	—	—	—	—	—	—
T. tonsurans	—	—	—	—	—	—	—	—	—	—
T. violaceum	—	—	*	—	—	—	—	*	—	—
Basidiomycetes										
Polyporus circinata	*	—	*	*	—	*	*	*	*	—

[a] Growth is indicated as follows: —, scanty or no growth; *, definite (2+) growth; **, moderate to good (3+ and 4+) growth; ***, heavy (5+) growth.

veloped with *n*-hexane, and then the plates were redeveloped with *n*-hexane-diethyl ether-acetic acid (80:20:2, v/v). Spots corresponding to various classes of compounds were detected by spraying the plate with 0.2% ethanolic 2',7'-dichlorofluorescein and observing the plate under ultraviolet light (254 mμ).

RESULTS AND DISCUSSION

The relative ability of a diverse group of filamentous fungi to utilize a series of *n*-alkanes and 1-alkenes as the sole carbon source for growth is shown in Table 1. All readings were made at the end of a 3-week incubation period carried out at room temperature. In general, the data show (i) that when an organism was able to grow at the expense of an *n*-alkane it was not necessarily able to assimilate the 1-alkene of the corresponding carbon chain length, and (ii) that the fungi tested were able to assimilate the hydrocarbon with a 14-carbon chain length more consistently than any of the others.

The three strains of *Cunninghamella* exhibited, by far, the best growth response to the series of hydrocarbons tested. A few of the fungi exhibited moderate to good growth on the hydrocarbons, but most organisms simply showed evidence of definite growth. Several of the fungi were unable to initiate growth under the conditions of the experiment. No attempt was made to determine whether failure to initiate growth resulted from inability of the organism to assimilate the hydrocarbon, or whether the medium was simply deficient in specific growth factors.

In some instances, where more than one member of the genus was tested, certain similarities and differences in the ability of various species to assimilate hydrocarbons were apparent. Species of *Cunninghamella*, *Alternaria*, and *Cephalosporium* demonstrated, within the respective genera, a consistent response in their ability to assimilate the series of hydrocarbons. Species of *Penicillium*, *Fusarium*, and *Spicaria*, while showing some evidence of differing in their quantitative ability, demonstrated similar patterns in their qualitative ability to use the hydrocarbons. Striking differences in the hydrocarbon assimilation patterns occurred within the genus *Aspergillus*. These results suggest that the ability to assimilate hydrocarbons may be a useful adjunct in the study of the taxonomy of aspergilli. Whether this technique will be useful with aspergilli or with other filamentous fungi, however, will be known only after more extensive investigation of a larger series of specific organisms.

The widest spectrum of assimilation patterns occurs in the group of Phycomycetes. With the limited number of Phycomycete genera and species tested, however, it is difficult to evaluate the significance of these findings.

Thin-layer chromatographic analyses of ether extracts from *Cunninghamella blakesleeana* (−strain) grown on *n*-tetradecane and 1-tetradecene were quite similar. Definite spots corresponding to the monocarboxylic acid and primary and secondary alcohol standards were observed. Tentative designations have been assigned to the following spots: β-hydroxy acid, dicarboxylic acid, and 1,2-diol. A spot moving in approximately the same manner as the methyl-ketone class was also observed. Work is in progress to determine the chain lengths of the compounds observed on the thin-layer chromatograms, in attempts to assign these compounds a place in the catabolic sequence of alkane and 1-alkene oxidation by *C. blakesleeana*.

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