INFRARED SPECTRA OF THE ETHER EXTRACTS OF CULTURES OF SOME WOOD DECAY FUNGI

by

A. A. LOMAN

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ABSTRACT

Infrared spectra of ether extracts were compared of ten-day-old liquid cultures of wood decay fungi. Replicates of one parent culture of Armillaria mellea (Vahl. ex Fr.) Kummer, as well as different cultural isolates of this species from lodgepole pine (Pinus contorta Dougl. var. latifolia Engelm.), had identical spectra in the "fingerprint" region (7 to 11μ). Infrared spectra of A. mellea cultures isolated from lodgepole pine in 1953 and maintained on 2% malt extract agar differed from those of A. mellea cultures isolated from lodgepole pine in 1968. Infrared spectra of A. mellea isolates from Abies amabilis (Dougl.) Forb. and Abies lasiocarpa (Hook.) Nutt. were identical, but differed from those of A. mellea isolates from lodgepole pine. Isolates of different species of Fomes, and of different genera, had different infrared spectra. Infrared spectroscopy is a potentially useful technique for the identification of unknown fungal cultures and for physiological and genetic studies of fungi in culture.

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* Research Scientist, Northern Forest Research Centre, Canadian Forestry Service, Department of the Environment, Edmonton 70, Alberta, Canada.

INTRODUCTION

Standard procedures for the identification of cultures of wood decay fungi involve the determination of physiological and morphological characteristics and of host relations. The physiological characters include effects on malt agar medium containing gallic or tannic acid, color changes induced in malt agar, pigmentation of the mycelial mat, and growth rates. The morphological characters include formation of fruit bodies, types and septation of hyphae, and the occurrence of secondary spores, cystidia, and setae. Nobles developed an excellent key that incorporated several variations of each of the above characteristics of 126 species of wood decay fungi (Nobles, 1948). The traditional methods of isolation and identification have been reviewed by Grant and Savory (1968). O'Connor et al. (1957) compared infrared spectra of acetone extracts of bacterial cultures.

In this paper, I describe a potential method of differentiating cultures of wood decay fungi by the infrared spectra of ether extracts of liquid cultures. The infrared spectrophotometer identifies functional groups on a molecule. The range of overlap for stretching (2.5 to 12.5μ) and bending (6 to 16μ) vibrations of molecular bonds is known as the "fingerprint" region, and it is in this range (about 7 to 11μ) that molecules have specific infrared spectra. The most powerful function of infrared spectroscopy is to establish the identity of two samples that have identical spectra in the "fingerprint" region (Dyer, 1965).

I assumed that enzyme-catalyzed transformations of substrate molecules reflect the genetic relations among decay fungi. Continuing

or periodic measurements of substrate transformations are therefore potentially useful for the differentation of fungal cultures. Infrared spectroscopy can be used here.

Infrared spectra with sharp and well-resolved absorption bands were obtained by selecting solvents which extract only a few of the many types of substrate molecules found in the aqueous nutrient medium.

MATERIALS AND METHODS

Fourteen fungal isolates were plated on 10 ml of 2% malt extract agar in 50-mm petri plates. The fungi, 6 genera and 8 species, originated from 5 tree-species in Alberta and British Columbia (see Table 1). After 10 days of growth on malt agar, the inoculum and substrate were transferred to 500-ml Erlenmeyer flasks containing 200 ml of the following liquid culture medium: KH_2PO_4, 0.5 g; $MgSO_4.7H_2O$, 0.5 g; NH_4Cl, 0.5 g; $FeCl_3$ (1% aqueous solution), 10 drops; glucose, 5.0 g; malt extract, 5.0 g; distilled water to 1000 ml. The liquid medium of one group of cultural isolates included ferulic acid (0.2mM). The flasks were swirled a few times three times a day. After 10 days of growth, the cultures were acidified with 0.5 ml of concentrated H_2SO_4. The cultures were homogenized in a Waring Blendor for 1 min. The homogenate was washed two times with 50 ml of diethyl ether, the aqueous fraction was then removed and the ether fraction was centrifuged at 10,000 rpm for 5 min. The ether fraction was removed by pipetting, and evaporated to dryness in vacuo. The residue was dissolved in 5 ml of spectroanalyzed chloroform, transferred to a centrifuge tube, and the water was removed with a little anhydrous $MgSO_4$. This

solution was allowed to stand overnight. It was then centrifuged in a clinical centrifuge at 2000 x g for 5 min. and the clear supernatant was transferred to a clean, dry test tube. The supernatant was evaporated to dryness, the residue was dissolved in 3 drops of spectroanalyzed chloroform, and an infrared spectrum was obtained of the solution in a Perkin-Elmer 237B infrared spectrophotometer from 2.5 to 16μ. The region of 7.0 to 11.0μ was used for comparisons.

RESULTS AND DISCUSSION

Results are summarized in Figs. 1-5. All comparisons were made in the "fingerprint" (7.0 to 11.0μ) region only.

Infrared spectra of different isolates of Armillaria mellea (Vahl. ex Fr.) Kummer isolated from lodgepole pine (Pinus contorta Dougl. var. latifolia Engelm.) were identical (Fig. 1). Infrared spectra of two replicates of A. mellea isolated from lodgepole pine in 1953 and maintained on 2% malt extract agar were identical (Fig. 2), but differed from the infrared spectra of cultures of this species isolated from lodgepole pine in 1968.(Figs. 1 and 2). Infrared spectra of A. mellea isolated from Abies amabilis (Dougl.) Forb. and Abies lasiocarpa (Hook.) Nutt. were identical (Fig. 3), but differed from A. mellea isolates from lodgepole pine (Figs. 1 and 3). Infrared spectra of different species of Fomes isolated from different hosts, differed (Fig. 4). Infrared spectra of different genera of fungi isolated from different hosts and grown on a liquid medium containing ferulic acid (0.2mM) differed.

The reproducibility of infrared spectra of A. mellea isolates having similar origins and histories suggests that this technique may be used for the determination of genetic relations of fungal cultures. The differences in infrared spectra of A. mellea isolates from different hosts suggest that active enzyme-systems of a fungal species are dependent on the presence of specific host-substrate molecules. The differences in infrared spectra between A. mellea cultures freshly isolated from lodgepole pine and A. mellea cultures isolated from the same host but grown on 2% malt extract agar for 16 years demonstrate the sensitivity of the infrared method in determinations of active enzyme-systems.

Infrared spectra can be manipulated by the addition of compounds known to influence fungal metabolism, and are soluble in the extracting solvents of choice. The phenyl-propanoid ferulic acid, a precursor in the biosynthesis of lignin, and which is slightly soluble in diethyl ether and chloroform, was added to the liquid medium to a concentration of 0.2 mM in the tests of different genera (Fig. 5). Three of the genera tested, Peniophora, Stereum, and Coryne, produce laccase (p-diphenol: oxygen oxidoreductase EC 10. 3.2.), an enzyme that catalyzes the transformation of phenolic substances in the substrate (Florkin and Stotz, 1965). Coniophora puteana Schum. ex Fr. was the only fungus not capable of producing laccase. The infrared spectrum of C. puteana was, predictably, similar to that of the control. The spectra of the genera that do synthesize laccase were unique for each genus (Fig. 5).

The results show that infrared spectroscopy is a potentially useful technique for physiological and genetic studies of fungi in culture. It has considerable practical potential as a tool for the identification of

unknown fungal cultures. For identification, the following conditions are essential: a) the host substrate of the unidentified fungus must be known, b) unidentified and reference cultures must originate from the same host substrate, c) unidentified and reference cultures must be grown on the same batches of malt extract agar and liquid media under identical environmental conditions, preferably simultaneously, and d) reference cultures must be rejuvenated on their substrate of origin.

ACKNOWLEDGMENT

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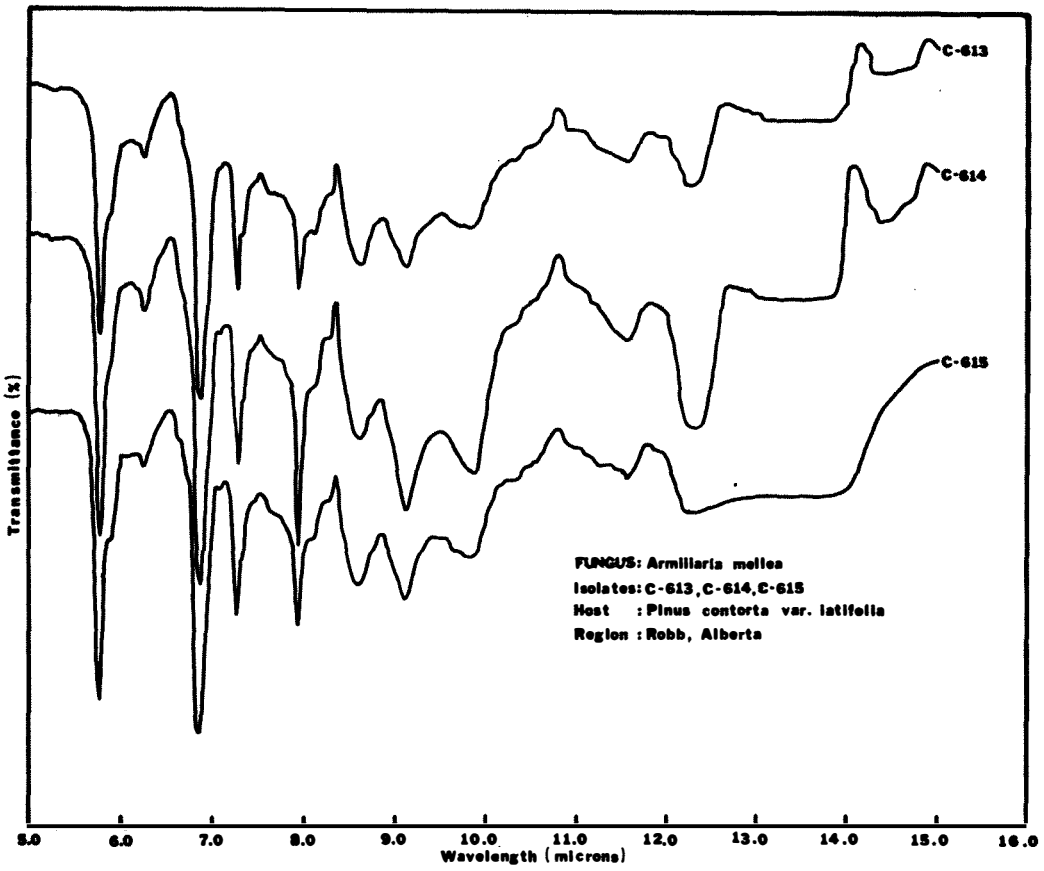

Fig. 1. Infrared spectra of diethyl ether extracts in chloroform, of homogenates of three 10-day-old liquid cultures of Armillaria mellea, isolated from lodgepole pine.

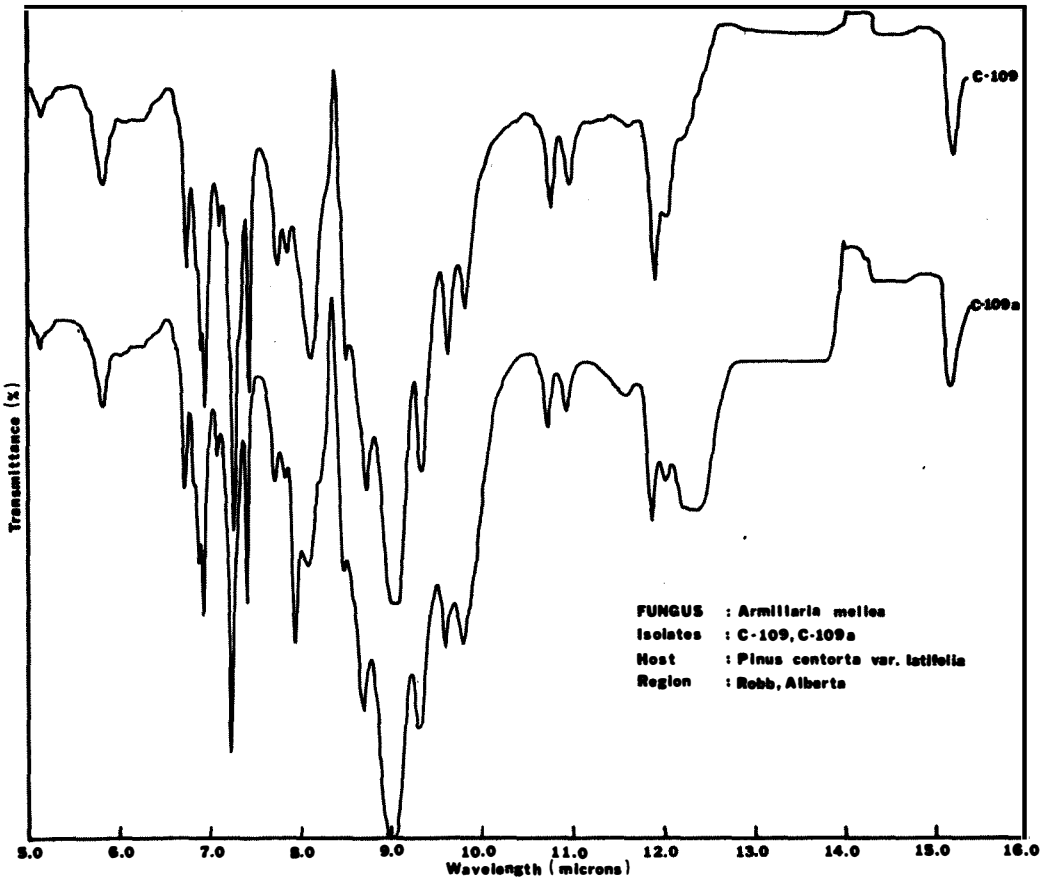

Fig. 2. Infrared spectra of diethyl ether extracts in chloroform, of homogenates of two 10-day-old liquid cultures of Armillaria mellea, isolated in 1953 from lodgepole pine.

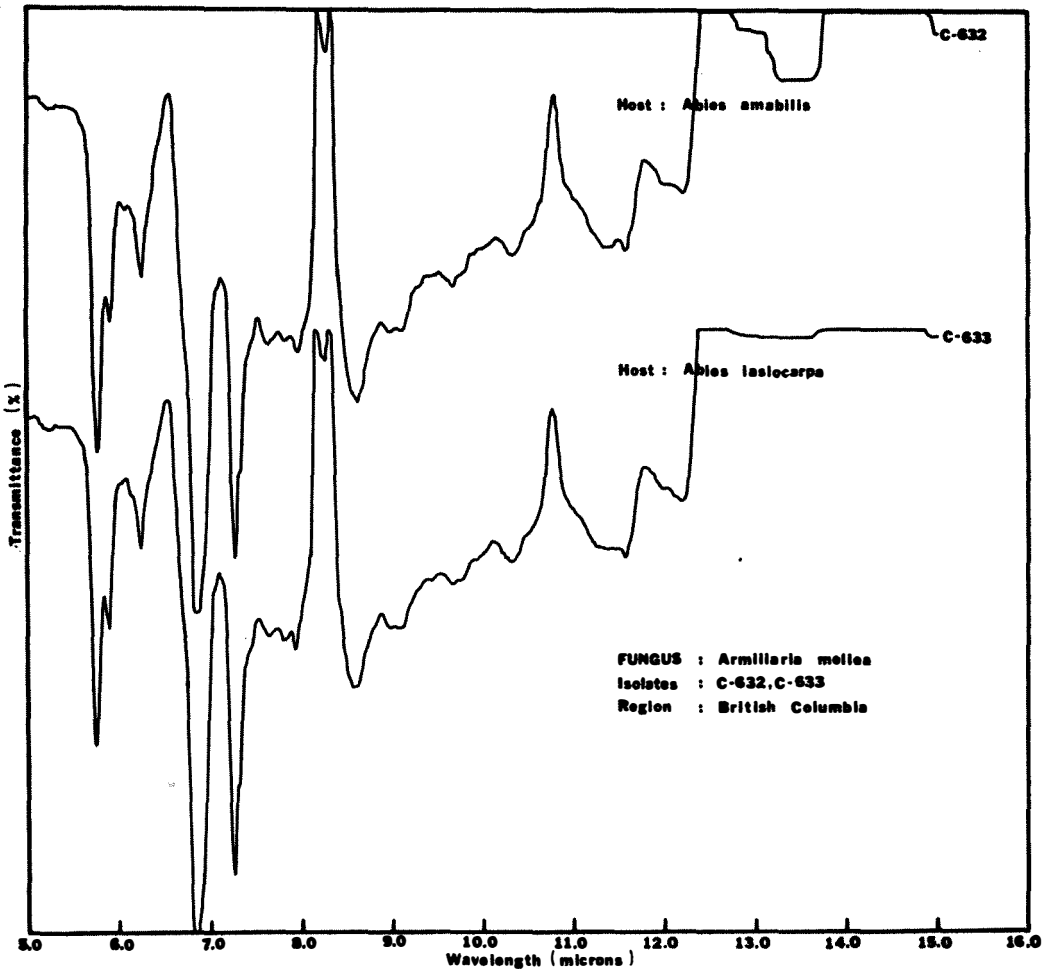

Fig. 3. Infrared spectra of diethyl ether extracts in chloroform, of homogenates of two 10-day-old liquid cultures of Armillaria mellea, isolated from Abies amabilis and Abies lasiocarpa.

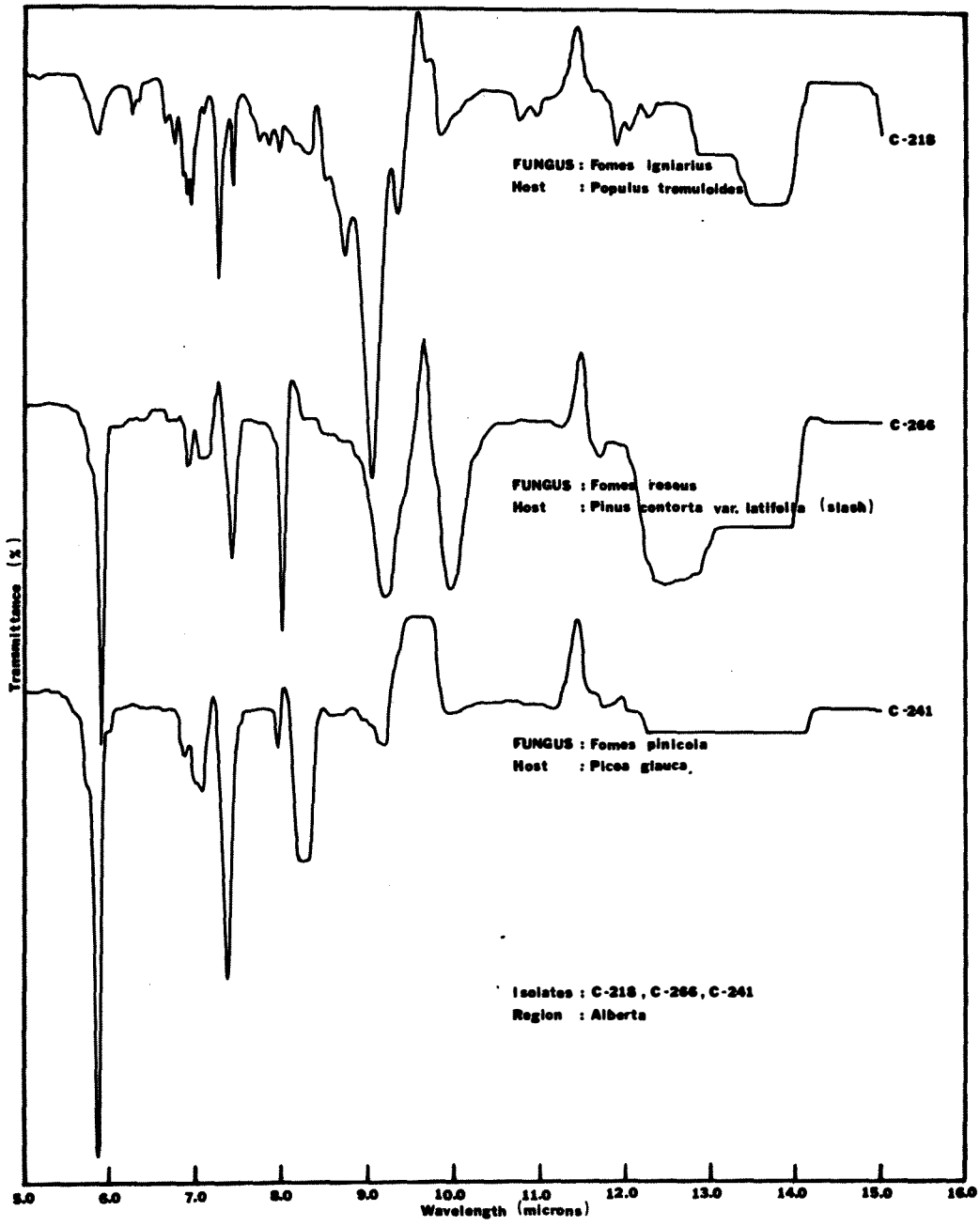

Fig. 4. Infrared spectra of diethyl ether extracts in chloroform, of homogenates of three 10-day-old liquid cultures of three species of Fomes isolated from three different hosts.

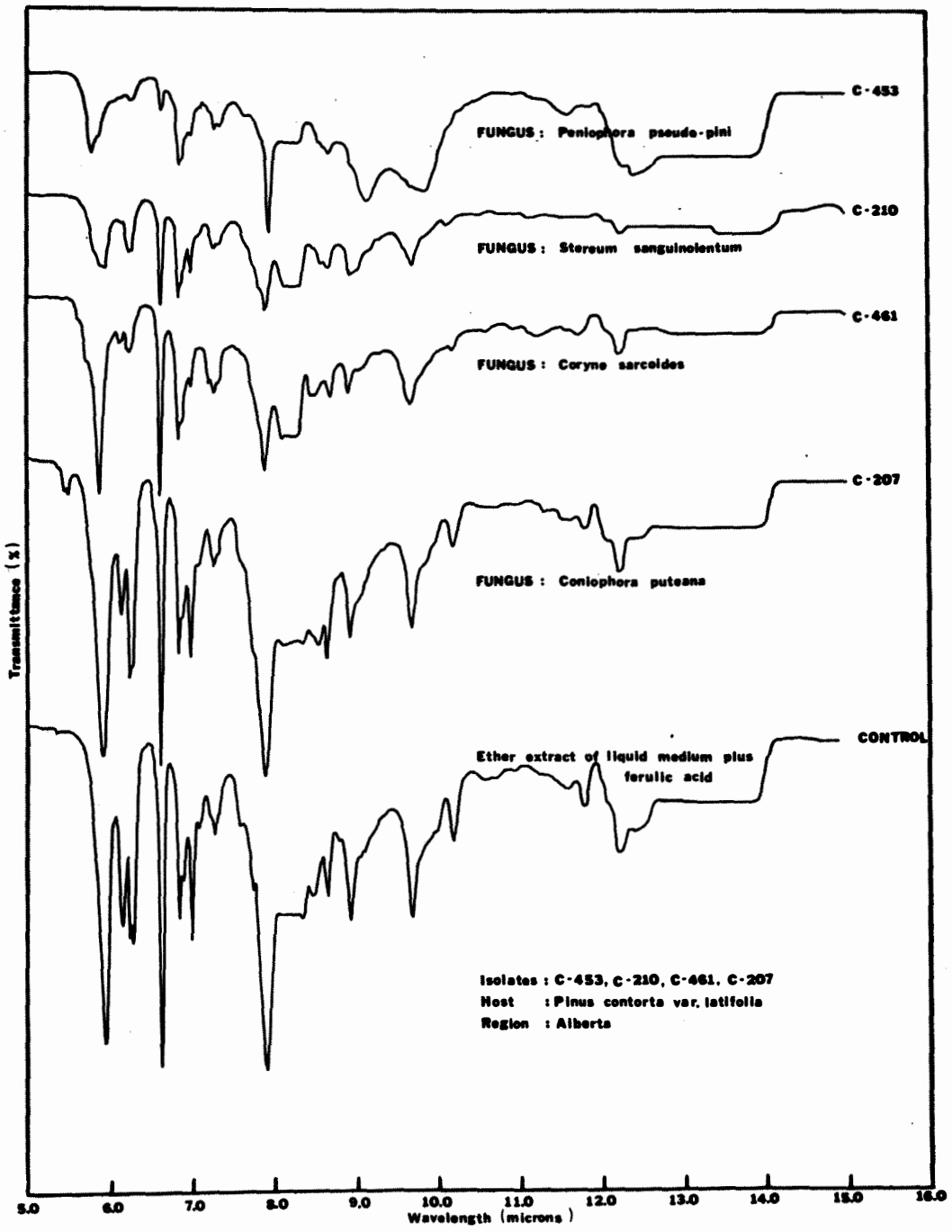

Fig. 5. Infrared spectra of diethyl ether extracts in chloroform, of homogenates of four 10-day-old liquid cultures of four genera of stain and decay fungi isolated from lodgepole pine.

TABLE I

Summary of fungal isolates, hosts, region of origin, and stock culture number

Fungus	Host	Region	Culture Number
Armillaria mellea (Vahl. ex. Fr.) Kummer	*Pinus contorta* Dougl. var. *latifolia* Engelm.	Alta.	C-613
"	"	"	C-614
"	"	"	C-615
"	"	"	C-109
"	"	"	C-109a
"	*Abies amabilis* (Dougl.) Forb.	B.C.	C-632
"	*Abies lasiocarpa* (Hook.) Nutt.	B.C.	C-633
Fomes igniarius (L. ex Fr.) Kickx.	*Populus tremuloides* Michx.	Alta.	C-218
Fomes roseus (Alb. and Schw. ex Fr.) Cooke	*Pinus contorta* Dougl. var. *latifolia* Engelm.	"	C-266
Fomes pinicola (Swartz.) Cooke	*Picea glauca* (Moench) Voss	"	C-241
Peniophora pseudo-pini Weres. and Gibson	*Pinus contorta* Dougl. var. *latifolia* Engelm.	"	C-453
Stereum sanguinolentum Alb. and Schw. ex. Fr.	"	"	C-210
Coniophora puteana Schum. ex Fr.	"	"	C-207
Coryne sarcoides (Dicks. ex Fr.) Bon.	"	"	C-461

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