CYCLOHEXIMIDE SENSITIVITY AS A TAXONOMIC CHARACTER IN *CERATOCYSTIS*

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SUMMARY

The effect of 100 ppm cycloheximide on the linear growth of 53 species of *Ceratocystis sensu amplo* and related fungi was determined on malt extract agar at 25 C. Generally, only those species with *Chalara*-like anamorphs (*Ceratocystis sensu stricto*) were inhibited by 100 ppm cycloheximide. Growth of other *Ceratocystis* species (*Ophiostoma sensu* von Arx), *Europhium* spp., and related anamorphs was generally unaffected by cycloheximide.

The genus *Ceratocystis* Ellis & Halst. as recognized by Hunt (**8**) comprises a very large and heterogeneous group of Ascomycetes with evanescent asci and hyaline ascospores. Olchowecki and Reid (**11**) delineated four groups within the genus based on ascospore morphology. Upadhyay and Kendrick (**15**) erected the genus *Ceratocystopsis*, the equivalent of Olchowecki and Reid's Minuta group, for members with falcate ascospores. Parker (**12**) erected the genus *Europhium* to accommodate astomatous species. Von Arx (**1**) and DeHoog (**4**) favored partitioning *Ceratocystis sensu amplo* by restricting *Ceratocystis* to species with *Chalara*-like anamorphs (enteroblastic conidium production) (**10**) and placing the remaining species in *Ophiostoma* H. & P. Sydow. Species in the latter group generally have holoblastic conidium production and, in contrast to *Ceratocystis sensu stricto*, cellulose (**9**) and rhamnose (**14**) as cell wall components (**17**). A numerical taxonomy study (**3**) also grouped species with *Chalara*-like anamorphs exclusive of other *Ceratocystis* spp.

Fergus (**5**) noted that species of *Ceratocystis* varied in their sensitivity to cycloheximide (Actidione), an antibiotic that prevents protein synthesis in most eukaryotes. Several selective media utilizing cycloheximide have been developed for *Ceratocystis* spp. and related fungi (**6, 13, 16**). My study explored the use of cycloheximide tolerance in *Ceratocystis* as a potential taxonomic character.

MATERIALS AND METHODS

Fifty-six isolates representing 53 species were acquired from the collections of T. E. Hinds (CO isolates) and R. W. Davidson (RWD isolates), U. S. Forest Service, Rocky Mountain Forest and Range Experiment Station, Fort Collins, Colorado; A. D. Partridge (ADP isolates), University of Idaho, Moscow; the American Type Culture Collection (ATCC); and the Centraalbureau voor Schimmelcultures, Baarn (CBS). Other isolates (TCH isolates) were those of the author. Original descriptions and illustrations of ascospores were used to categorize the species into one of the four groups of Olchowecki and Reid (11). Conidial states were identified based on examinations of the isolates used.

The effect of different concentrations of cycloheximide (1, 5, 25, 50, 100, and 1000 ppm) was tested on three species with *Chalara*-like anamorphs and three other species. Subsequently, all 53 species were tested only on 100 ppm cycloheximide. The appropriate amount of cycloheximide (Sigma Chemical Co.) was added to autoclaved malt extract agar (MEA; 15 g malt extract, 15 g agar, and 970 ml distilled water). Media were dispensed into 90 mm diam plastic Petri dishes (15 ml/plate). The center of each plate was inoculated with a 4 mm diam mycelial plug from the advancing margin of a MEA-grown culture and incubated at 25 C. All treatments were replicated three times.

RESULTS

Linear growth of *C. pilifera* (CO 432), *C. huntii,* and *E. aureum* on MEA with 1000 ppm cycloheximide, the highest concentration tested, did not differ from that on unamended MEA after 48 h. *Ceratocystis fimbriata* and *C. coerulescens* (RWD 431) failed to grow on MEA with 5 ppm cycloheximide. Linear growth of *C. paradoxa* was reduced at 5 and 25 ppm cycloheximide, and this species failed to grow at 50 ppm cycloheximide.

Extent of linear growth on MEA with 100 ppm cycloheximide of all but eight of the *Ceratocystis* and *Europhium* species tested was not significantly different than that on unamended MEA after 5 da (TABLE I). Six of the eight cycloheximide-sensitive species are members of the Fimbriata group with *Chalara*-like anamorphs. Although most of the investigated species of *Ceratocystis* and *Europhium* have been shown to possess cellulose and rhamnose as cell wall constituents, previous workers have failed to detect these compounds in species with *Chalara*-like anamorphs (TABLE I).

Data on cell wall constituents are not available for the anamorphs tested for cycloheximide tolerance. Of the nine anamorphs tested six were tolerant. Growth of *Leptographium engelmannii* Davidson (RWD 971), *L. lundbergii* Lagerb. (ATCC 22735), *L. pyrina* Davidson (RWD 1227), *Verticicladiella abietina* (Peck) Hughes (TCH OL1), *V. procera* Kendrick (ADP 1003), and *V. wageneri* Kendrick (TCH CA2) was unaffected by 100 ppm cycloheximide. However, *Chalara elegans* Nag Raj & Kendrick [= *Thielaviopsis basicola* (Berk. & Br.) Ferr.] (TCH CA1), *V. antibiotica* Kendrick (ADP 1), and *V. brachiata* Kendrick (ATCC 22059) failed to grow on MEA with 100 ppm cycloheximide.

DISCUSSION

The partitioning of *Ceratocystis* into two genera as proposed by von Arx (1) and DeHoog (4) tends to be supported by the results presented herein. Cycloheximide sensitivity was found in those members of Olchowecki and Reid's (11) Fimbriata group which have *Chalara*-like anamorphs. These species have enteroblastic conidium production and, in the investigated cases, lack detectable levels of cellulose and rhamnose. They comprise *Ceratocystis sensu stricto* (4). In contrast, other members of the Fimbriata group as well as members of the Ips, Pilifera, and Minuta (*Ceratocystopsis*) groups and the genus *Europhium*, generally have holoblastic conidium production, possess cellulose and rhamnose as cell wall constituents in the investigated cases, and are tolerant of 100 ppm cycloheximide. They comprise the genus *Ophiostoma* as recognized by von Arx (1) and DeHoog (4). Cycloheximide tolerance in *Europhium* further indicates its affinities with the *Ophiostoma* group and *Ceratocystopsis* (4).

The unique cell wall structure of members of the *Ophiostoma* group may be responsible for cycloheximide tolerance. Perhaps cycloheximide becomes bound to these cell walls and is prevented from entering the cell. Alternatively, the factors in question may be independent but coincidental. However, a few inconsistencies are apparent in the correlations of cycloheximide tolerance with the presence of cellulose and rhamnose. Jewell (9) failed to detect cellulose in *C. serpens, C. microspora* (=*C. perparvispora* Hunt), and *C. minuta* which were each tolerant of cycloheximide. However, rhamnose was found in *C. serpens* and *C. minuta* (14). Perhaps cellulose was present in these cases, but was too low in concentration to be detected. Although *C. galeiformis* does not have a *Chalara*-like anamorph (2, 8)

TABLE I

CYCLOHEXIMIDE TOLERANCE AND DISTRIBUTION OF CELLULOSE AND RHAMNOSE IN SOME SPECIES OF *Ceratocystis* AND *Europhium*

Species	Cycloheximide Tolerance[a]	Cellulose[b]	Rhamnose[c]
Fimbriata group			
With *Chalara*-like anamorphs			
Ceratocystis adiposa (Butler) C. Moreau (RWD 1024)	−	−	−
C. coerulescens (Münch) Bakshi (CO 381, RWD 431)	−	−	−
C. fagacearum (Bretz) Hunt (TCH MNB)	−	−	
C. fimbriata Ellis & Halst. (CO 301, CO 491)	−		−
C. moniliformis (Hedg.) C. Moreau (CO 411)	−		−
C. paradoxa (Dade) C. Moreau (ATCC 24271)	−	−	−
With other anamorphs			
C. brevicollis Davidson (CO 465)	+		
C. europhioides Wright & Cain (TCH 1312)	+	+	+
C. dryocoetidis Kendrick & Molnar (CO 565)	+	+	+
C. galeiformis Bakshi (CO 74)	−	−	+
C. huntii Robinson (RWD 776)	+	+	+
C. olivacea (Mathieson) Hunt (RWD 757)	+	+	+
C. olivaceapini Davidson (RWD 548)	+		
C. serpens (Goid.) C. Moreau (CBS 141.36)	+	−	+
Europhium aureum Robinson-Jeffrey & Davidson (ATCC 16936)	+	+	
E. clavigerum Robinson-Jeffrey & Davidson (CO 453)	+	+	
E. robustum Robinson-Jeffrey & Davidson (RWD 157)	+	+	
Ips group			
C. adjuncta Davidson (RWD 1227)	+		
C. bicolor (Davidson & Wells) Davidson (RWD 587)	+	+	
C. ips (Rumb.) C. Moreau (RWD 1196)	+	+	+
C. montia (Rumb.) Hunt (ADP 304)	+		
Pilifera group			
C. abiocarpa Davidson (RWD 494)	+	+	
C. ambrosia Bakshi (CO 436)	+	+	+
C. californica De Vay, Davidson & Molnar (RWD 633)	+	+	

TABLE I—*Continued*

Species	Cycloheximide Tolerance[a]	Cellulose[b]	Rhamnose[c]
C. denticulata Davidson (ATCC 38087)	+		
C. distorta Davidson (RWD 597)	+		
C. eucastanea Davidson (RWD 1231)	+		
C. fraxinopennsylvanica Hinds (ATCC 26664)	−		
C. gossypina Davidson (RWD 600)	+	+	+
C. microspora (von Arx) Davidson (RWD 413)	+	−	
C. minor (Hedg.) Hunt (CO 415)	+	+	+
C. perfecta Davidson (RWD 486)	+	+	+
C. picea (Münch) Bakshi (CO 586)	+	+	+
C. pilifera (Fries) C. Moreau (CO 432, CO 483)	+	+	+
C. pluriannulata (Hedg. & Davidson) Hunt (RWD 799)	+	+	+
C. ponderosa Hinds & Davidson (RWD 899)	+	+	+
C. populina Hinds & Davidson (ATCC 24094)	+	+	+
C. stenoceras (Robak) Nannf. (RWD 905)	+		+
C. tenella Davidson (RWD 563)	+	+	
C. tremulo-aurea Davidson & Hinds (ATCC 24095)	+	+	+
C. ulmi (Buisman) C. Moreau (TCH AM1)	+	+	+
Minuta group			
C. crassivaginata Griffin (CO 498)	+	+	
C. minuta (Siemaszko) Hunt (RWD 527)	+	−	+
C. retusi Davidson & Hinds (RWD 882)	+		

[a] Extent of linear growth on malt extract agar with 100 ppm cycloheximide equal to that on malt extract agar without cycloheximide (+) or failing to grow on it (−).
[b] Data from Jewell (**9**).
[c] Data from Spencer and Gorin (**14**).

and contains rhamnose (**14**), it was sensitive to cycloheximide. It should be noted, however, that this isolate has been in culture over 25 yr. Differences in the isolates used or misidentifications could account for some of the above stated discrepancies among the studies. On the other hand, these anomalous species could represent intermediates and the factors in question may not always coincide.

Ceratocystis fraxinopennsylvanica has ascospores characteristic of

the Pilifera group, but forms conidia enteroblastically (**7**). The suggestion that this species may not belong in *Ceratocystis* (*Ophiostoma*) on the basis of ascus development (**7**) may be supported by its sensitivity to cycloheximide.

Many species of *Verticicladiella* and *Leptographium* are anamorphs of *Europhium* and *Ceratocystis* spp. As expected, they were generally tolerant of cycloheximide, but two of them were sensitive. Interestingly, *V. brachiata* was not grouped with the other *Verticicladiella* in the numerical taxonomic scheme of Dabinett and Wellman (**3**) and was sensitive to cycloheximide. *Chalara elegans* has no known perfect state (**10**), but as in the *Ceratocystis* spp. with *Chalara* anamorphs, it was sensitive to cycloheximide.

In addition to supporting taxonomic groupings, cycloheximide tolerance greatly facilitates isolation of these fungi for ecological studies (**6**, **13**, **16**). Addition of 100 ppm cycloheximide and 100 ppm streptomycin to autoclaved MEA enables easy isolation of *Ophiostoma*-type fungi from insects. Isolation from colonized host tissue is readily accomplished on 1.5% water agar to which the antibiotics have been added.

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