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On the subdivision of the genus *Ceratocystis*

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The desirability is discussed of a subdivision of the genus *Ceratocystis* into a group characterized by the presence of a phialidic conidial state, and a group in which the conidia are usually produced exogenously. Corroborative evidence for this arrangement is found in the carbohydrate constitution of the cells: in all examined species of the former group (*Ceratocystis* s.str.) rhamnose and cellulose are absent, whereas in the latter both components are present (*Ophiostoma* H. et P. Sydow).

INTRODUCTION

Many attempts are recorded in the literature to subdivide the genus *Ceratocystis* Ellis et Halst., using a variety of methods and criteria. A concise review and evaluation was given by de Hoog (1974). Most of the applied parameters have appeared to be impracticable, either due to a lack of discontinuity, or to a lack of correlation with other characters. Only three characters show a distribution pattern applicable for a rough grouping of the species, viz. the properties of ascospores, the conidial states, and the carbohydrate constitution.

Properties of ascospores have proved to be of great practical value for the construction of keys to the species (Hunt, 1956; Griffin, 1968). Olchowecki and Reid (1974) stressed the importance of such characters by creating four groups within the genus, viz. the '*Minuta-group*', characterized by elongated, usually curved ascospores with terminally attenuated sheaths, the '*Ips-group*', characterized by cylindrical or dumbbell-shaped ascospores with gelatinous sheaths, the '*Pilifera-group*', characterized by curved, ovoidal or cylindrical ascospores without sheaths, and a heterogeneous '*Fimbriata-group*', comprising the remaining *Ceratocystis* species.

The conidial states can be classified in at least 10 described form-genera. In many cases two conidial states can be observed within the same species: *Chalara* (Corda) Rabenhorst is obligately found with *Chalaropsis* Peyronel and *Thielaviopsis* Went; *Sporothrix* Hektoen et Perkins ex Nicot et Mariat may be accompanied by *Pesotum* Crane et Schoknecht. *Graphium* Corda, *Phialographium* Upadhyay et Kendrick and *Pesotum* are almost identical in their general morphology. They are distinguished merely on the basis of the different modes of conidium ontogeny; their conidiogenous cells are annellidic, phialidic or sympodial respectively. *Pesotum* is also similar to *Verticicladiella* Hughes and *Raffaelea* v. Arx et Hennebert, which genera all have sympodial conidium formation but differ by the rate of differentiation and aggregation of the conidiophores. *Leptographium* Lagerberg et Melin is the annellidic counterpart of *Verticicladiella*. Consequently, *Graphium*, *Leptographium*, *Pesotum*, *Phialographium*, *Raffaelea* and *Verticicladiella* compose a rather homogeneous group in which no sharp lines between genera can be drawn, or only in part by regarding the hardly traceable difference in conidium ontogeny as the diagnostic character.

From this condensed account can be concluded that the only discontinuity in conidial state morphology is between species with *Chalara* states and those with *Graphium*-like states (de Hoog, 1974). Mature conidial states of both groups can be easily distinguished by their conidiogenesis and the shape and size of the conidia. It should be stated, however, that when comparing young states of some representatives of these groups, there may be a striking resemblance in conidiophore structure and pigmentation (Fig. 1). The nature of the conidial states alone should therefore be regarded as insufficient for a splitting of the perfect state genus.

The carbohydrate constitution of cells is generally considered a useful criterium in the taxonomy of fungi, since Wettstein (1921) divided the kingdom of fungi into a cellulose-containing group and a chitin-containing group. Usually, chemical data are only applicable to larger taxonomic groups, due to rather big fluctuations in quantitative cell wall composition (Hall, 1969), although some techniques allow differentiation down to the species level. Bartnicki-Garcia (1968) erected 8 groups within the fungi, based on cell wall carbohydrate composition. *Ceratocystis* and related genera were included in the chitin–glucan group. In addition to chitin and non-cellulosic glucans, other cell wall carbohydrates can serve as useful tools in subdividing this particular group, as indicated by Gorin and Spencer (1970) and Travassos, Gorin and Lloyd (1973).

For this reason we decided to study *Ceratocystis* cellular carbohydrates, in order to obtain additional information, supporting or rejecting a subdivision of the genus.

MATERIALS AND METHODS

Isolation and purification of fungal cell walls is a very laborious procedure. Chromatograms of intact cells deviate from those of purified cell-wall materials

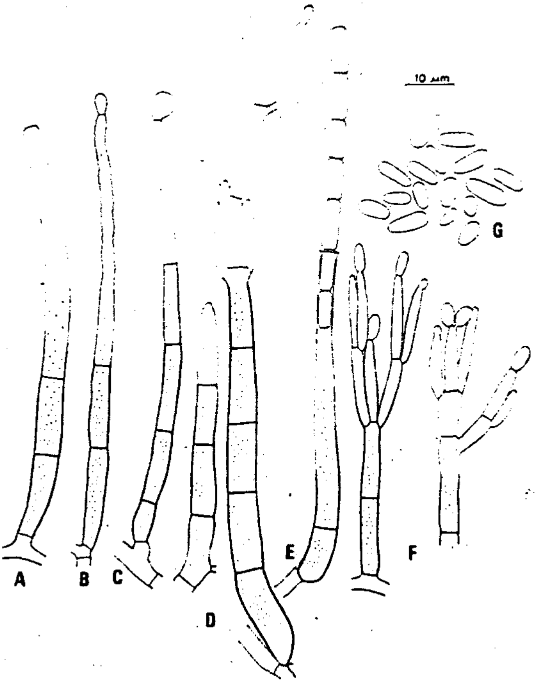

Fig. 1. Fertile structures of *Ceratocystis* conidial states and related species. a. *Ceratocystis fimbriata* Ellis et Halst.; b. *Europhium clavigerum* Robinson et Davidson; c. *Raffaelea ambrosiae* v. Arx et Hennebert; d. *Ceratocystis europhioides* Wright et Cain; e. sporulating phialide of *Ceratocystis fimbriata*; f-g, *Europhium aureum* Robinson et Davidson, f. conidiophores, g. conidia..

in the quantity of detected glucose, since this component not only originates from cell walls but also from cytoplasm (trehalose, glycogen). Renouncing glucose, gas chromatographical analysis of intact-cell monosaccharides, released by acid hydrolysis, proved to be a rapid alternative technique in screening a lot of strains. Recently the "intact-cell approach" has been successfully applied to characterizing bacteria by Jantzen et al. (1972).

All strains studied were cultured on glucose (2 %)–peptone (1 %)–yeast extract (0.5 %) medium in erlenmeyer flasks for 10 days at 25 C on a rotatory shaker, operated at 100 rpm. Cells were harvested by filtration or centrifugation, washed with 0.9 % NaCl and deionized water respectively, lyophilized, powdered and stored in a desiccator with silica gel. The powder obtained was hydrolyzed in M HCl (6 ml) for 12 hr at 100 C in Pyrex tubes. After filtration the hydrolyzate was dried in a rotatory evaporator at 40 C. Residual HCl was removed by 2 evaporations with deionized water. Carbohydrates were analysed by gas-liquid chromatography (GLC) as trimethylsilyl ethers, according to Sweeley et al. (1963), using a commercial preparation of HDMS (hexamethyldisilazane) and TMCS (trimethylchlorosilane) in pyridine (Tri.Sil.; Pierce Chem. Co.). Dried products released by hydrolysis from 1.25 mg cells were trimethylsilylated with 0.25 ml Tri.Sil., with a minimum reaction time of 15 min.

Analyses were performed with a Becker Model 420 gas chromatograph equipped with a flame ionization detector and dual column system. N_2 was used as carrier gas at a flow rate of 25 ml/min. The glass U-shaped columns (length 1.7 m, i.d. 4 mm) were packed with 80–100 mesh Chromosorb W(HP) coated with 3% OV-1 (Pierce Chem. Co.). Column temperature: programmed from 140–220 C with 2.5°C/min or isothermally at 180 C. Injection port temperature: 225 C. Detector temperature: 250 C. One µl of the reaction mixture was injected into the gas chromatograph. Retention times were calculated relative to α-glucose with the aid of a Hewlett-Packard Model 3370-B electronic digital integrator. Chromatograms were recorded on a Kipp 1 mV recorder at a chart speed of 1 cm/min.

RESULTS

The strains listed in Table 1 were analyzed by GLC as described above. In all strains fluctuating quantities of glycerol, myo-inositol, mannose, galactose and glucose could be detected, whereas hexitol was demonstrated in the majority of the strains. Glycerol and myo-inositol are supposed to be of lipid origin, mannose and galactose probably are restricted to the cell walls (Harris and Taber, 1973). Hexitol is of intracellular origin.

The distribution of rhamnose appeared to be stable in each species: it was absent from all examined strains of *Ceratocystis adiposa*, *C. coerulescens*, *C. fimbriata* (GLC Fig. 2A), *C. major*, *C. moniliformis*, *C. paradoxa* and *C. radicicola*, but present in *C. narcissi*, *C. nigrocarpum*, *C. perfecta*, *C. piceae*, *C. piliferum*, *C. stenoceras* (GLC Fig. 2B) and *C. ulmi*.

Furthermore, the ability to synthesize pentitol (probably arabitol; pentitols cannot be separated from each other by means of the applied method) seemed to be absent in strains which are lacking rhamnose.

Table 1. Distribution of rhamnose in some species of *Ceratocystis* Ellis et Halst. (sensu lato)

Spec	CBS-number	Rhamnose	Conidiogenesis	Hexoses[1]	Cellulose[2]
adiposa	127.27	−	endogenous	Man, g.	−
	136.34	−	endogenous		
	600.74	−	endogenous		
coerulescens	137.34	−	endogenous		−
	142.53	−	endogenous	Man, g, gal.	
fimbriata	123.39	−	endogenous		−[3]
	103.40	−	endogenous		
	600.70	−	endogenous		
	740.70	−	endogenous		
major	138.34	−	endogenous	Man, g.	−
	154.62	−	endogenous		
moniliformis	155.62	−	endogenous	Man, tr.g.	
paradoxa	150.55	−	endogenous		−
	601.70	−	endogenous		
	893.70	−	endogenous		
radicicola	114.47	−	endogenous	Man, g.	−
narcissi	138.50	+	exogenous	Man, rh.	+
	414.72	+	exogenous		
	774.73	+	exogenous		
nigrocarpum	637.66	+	exogenous	Rh, man, tr.gal.	+
perfecta	636.66	+	exogenous	Man, rh.	+
piceae	108.21	+	exogenous	Man, rh, g.	+
	236.32	+	exogenous	Man, rh, tr.gal.	
	263.35	+	exogenous	Man, rh.	
	180.69	+	exogenous		
	799.73	+	exogenous		
piliferum	129.32	+	exogenous		+
	158.74	+	exogenous		
stenoceras	237.32	+	exogenous	Rh, man, tr.gal.	
	139.50	+	exogenous		
	104.66	+	exogenous		
	146.71	+	exogenous		
	360.71	+	exogenous		
ulmi	152.55	+	exogenous		+
	212.64	+	exogenous		
	374.67	+	exogenous	Man, rh, tr.gal.	
	427.71	+	exogenous		

[1] Data from Gorin and Spencer (1971)
Abbreviations: Mannose (Man.), Glucose (g.), Galactose (Gal.), Rhamnose (Rh.), Trace (tr.).

[2] Data from Jewell (1974)
Data related to strains not examined in this study.

[3] Data from Smith et al. (1967)
Data related to strains not examined in this study.

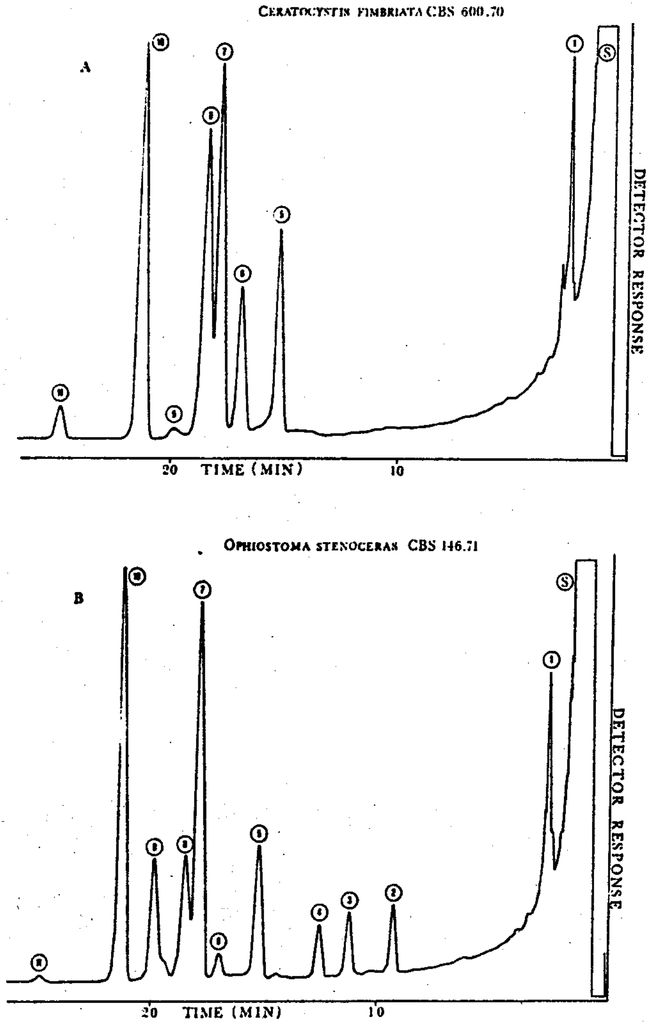

Fig. 2. Gas chromatograms of carbohydrates (TMS derivatives) released from intact fungal cells by acid hydrolysis. a. *Ceratocystis fimbriata* CBS 600.70, gas chromatograph operated with a temperature programme of 140–200 C at 2.5°C/min.; b. *Ophiostoma stenoceras* CBS 146.71, gas chromatograph operated with a temperature programme of 140–200 C at 2.5°C/min. S solvent peak; 1 glycerol; 2 α-rhamnose; 3 β-rhamnose; 4 pentitol; 5 α-mannose; 6 α-galactose; 7 α-glucose; 8 β-mannose and β-galactose; 9 hexitol; 10 β-glucose; 11 myo-inositol.

DISCUSSION

Rosinski and Campana (1964) first provided evidence of the simultaneous occurrence of chitin and cellulose in *Ceratocystis ulmi* cell walls, a unique phenomenon among fungi (LéJohn, 1971). This evidence was extended to other species of the genus (Smith, Patik and Rosinski, 1967; Jewell, 1974). The occurrence of cellulose is correlated with the presence of cell-wall rhamnose, as can be deduced from studies of Gorin and Spencer (1970), Spencer and Gorin (1973), and from the above results.

The distribution of cellulose and rhamnose is also correlated with the morphology of the conidial states (Hall, 1969). Table 1 clearly indicates that species without cellulose and rhamnose all have *Chalara* states (endogenous, phialidic conidium formation), whereas the species in which both compounds are present have *Sporothrix* and/or *Graphium*-like conidial states (usually exogenous conidium formation).

Thus the biochemical data obtained in our and previous studies strongly support the bipartition of the genus *Ceratocystis*, as proposed by von Arx (1974) and de Hoog (1974): the species with *Chalara* states were treated as *Ceratocystis* sensu str., those with *Graphium*-like states as *Ophiostoma* H. et P. Sydow. This subdivision is not contradictory to the grouping by Olchowecki and Reid (1974), as their '*Fimbriata-group*' fits *Ceratocystis* s. str. and their other groups compose *Ophiostoma*. For a further subdivision of the latter genus other criteria can be laid, such as the properties of the ascospores.

Little is known about the distribution of polyols in fungi (Lewis and Smith, 1967). Our results on pentitol distribution within *Ceratocystis* have to be treated with caution, because the metabolism of storage compounds is strongly dependent of the physiological state of a culture. Nevertheless the distribution pattern of pentitol shows the same correlation.

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