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Purification, Characterization, and Amino Acid Sequence of Cerato-platanin, a New Phytotoxic Protein from *Ceratocystis fimbriata* f. sp. *platani**

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A new phytotoxic protein (cerato-platanin) of about 12.4 kDa has been identified in culture filtrates of the Ascomycete *Ceratocystis fimbriata* f. sp. *platani*, the causal agent of canker stain disease. The toxicity of the pure protein was bioassayed by detecting the inducing necrosis in tobacco leaves. The pure protein also elicited host synthesis of fluorescent substances in tobacco and plane (*Platanus acerifolia*) leaves. We purified the protein from culture medium to homogeneity. Its complete amino acid sequence was determined; this protein consists of 120 amino acid residues, contains 4 cysteines (S—S-bridged), and has a high percentage of hydrophobic residues. The molecular weight calculated from the amino acid sequence agrees with that determined by mass spectrometry, suggesting that no post-transnational modification occurs. Searches performed by the BLAST program in data banks (Swiss-Prot, EBI, and GenBank™) revealed that this protein is highly homologous with two proteins produced by other Ascomycete fungi. One, produced during infection of wheat leaves, is codified by the *snodprot1* gene of *Phaeosphaeria nodorum* (the causal agent of glume blotch of wheat), whereas the other is the rAsp f13 allergen from *Aspergillus fumigatus*. Furthermore, the N terminus of cerato-platanin is homologous with that of cerato-ulmin, a phytotoxic protein belonging to the hydrophobin family and produced by *Ophiostoma (Ceratocystis) ulmi*, a fungus responsible for Dutch elm disease.

The European plane tree *(Platanus acerifolia)* is an ornamental plant species of the urban environment. A great number of plane trees in the parks and towns of southern Europe have been destroyed by *Ceratocystis fimbriata* (Ell. and Halst.) Davidson f. sp. *platani* Walter, the Ascomycete responsible for canker stain disease (1). This disease is characterized by foliar wilting and spreading lesions that involve phloem, cambium, and extensive regions of sapwood (2, 3). The pathogen spreads from tree to tree by means of root grafts of closely spaced plants and, more frequently, through wounds caused by pruning (4).

The American species *Platanus occidentalis* has been shown to contain a source of resistance to *C. fimbriata* f. sp. *platani* that could prove of great interest in the genetic improvement of the European plane (5). Known post-infection host defense mechanisms involve physical factors such as the occlusion of the xylematic vessels and the compartmentalization of infected tissue areas as well as the production of flavans, umbelliferone, and scopoletine phytoalexins (6–9). Unfortunately, only resistant *P. occidentalis* clones, not yet acclimatized to Europe, localized the disease (7, 8). Recent papers (10, 11) have shown that *C. fimbriata* f. sp. *platani* displays an array of phytotoxic metabolites possibly involved in determining some of the symptoms of canker stain.

In the present paper we report on the purification procedure, the amino acid sequence, and the characterization of the biological activity of a new protein (named cerato-platanin) from the culture filtrate of *C. fimbriata* f. sp. *platani*.

EXPERIMENTAL PROCEDURES

Materials—Trypsin, chymotrypsin, Asp-N endoproteinase, and thermolysin (all sequencing grade) were obtained from Sigma. Activated Sequelon-1,4-phenylenediisothiocyanate and Sequelon-AA polyvinylidene difluoride membranes were purchased from PerSeptive Biosystems. Bio-Gel P-10 was from Bio-Rad, and Sephadex LH-60 was from Amersham Pharmacia Biotech. The peptide and protein C18 HPLC[1] column (4.6 × 250 mm, 5 μm) was from Vydac. All other reagents were the purest commercially available.

Fungus Culture—The virulent strain Cf AF 100 of *C. fimbriata* f. sp. *platani* was isolated from a naturally infected plane tree in Versilia (Tuscany, Italy) (2). The fungus was inoculated in 500-ml flasks containing 150 ml of sterilized Sigma potato dextrose broth medium and incubated on the Gerhardt RO30 rotary shaker (Gerhardt, Bonn, Germany) at 100 revolutions/min for 21 days at 25 °C. Culture filtrates were obtained by removing mycelia and spores from the medium after filtration through a 0.45-μm Millipore membrane (Millipore Co., MA).

Bioassays—Before bioassays, all samples were dialyzed against distilled water. For the routine determination of the biological activity of samples, a few quantities (50–200 μl) were infiltrated into tobacco leaf mesophyllum by means of a hypodermic syringe; control tobacco leaves were infiltrated with distilled water. The samples capable of inducing tissue cell necrosis were considered biologically active. Sometimes tobacco leaves were detached and observed on a transilluminator (Bio-Rad) at UV 312-nm light; the infiltrated part of the leaf appeared slightly fluorescent.

A 100-μl aliquot of 80 μM cerato-platanin was added to the lower surface of the plane leaf and maintained in a moisture chamber at room temperature. At various times after treatment the leaves were observed under incident 254-nm UV light, and fluorescence was evaluated.

Purification Procedure—The culture filtrate (1 liter, 800 mg of proteins) was lyophilized, redissolved in water to have a 60-fold concentrated solution, and stored at −30 °C. Before starting the purification procedure, this concentrated solution was clarified by centrifugation at

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The amino acid sequence reported in this paper has been submitted to the Swiss Protein Database under Swiss-Prot accession number P81702.

The nucleotide sequences reported in this paper (snodprot1 and rAsp f13) have been submitted to the DDBJ/GenBank$_{TM}$/EBI Data Bank with accession numbers AF074941 and AJ002026, respectively.

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[1] The abbreviations used are: HPLC, high performance liquid chromatography; MALDI-TOF, matrix-assisted laser desorption ionization-time of flight spectroscopy; CM, carboxymethylated.

8,000 × *g* for 30 min. The supernatant was dialyzed against 1% acetic acid and successively filtered through a 0.45-μm Millipore membrane. A 10-ml aliquot of concentrated filtrate was loaded onto a Bio-Gel P-10 column (2.5 × 70 cm) equilibrated with 1% acetic acid and eluted at a flow rate of 0.2 ml/min. Five-ml fractions were collected and assayed for their ability to induce tobacco leaf cell necrosis and for optical density at 280 nm. The toxic fractions were pooled, concentrated to 10 ml by freeze-drying, dialyzed against 60% ethanol, and chromatographed twice on a Sephadex LH-60 column (2.4 × 90 cm) previously equilibrated with 60% ethanol. The column was eluted with 60% ethanol at a flow rate of 0.2 ml/min; 5-ml fractions were collected and assayed for optical density at 280 nm and phytotoxic activity. All purification procedures were performed at 4 °C. The final yield was 4 mg of pure protein starting from 1 liter of filtrate.

Polyacrylamide Gel Electrophoresis—Sodium dodecyl sulfate-polyacrylamide gel electrophoresis was performed on 15% polyacrylamide according to Laemmli (12). Electrophoresis was run at constant 200 V for about 30 min. Proteins were stained with Bio-Rad silver stain.

Mass Spectrometry—The pure protein (2 nmol) was dissolved in aqueous 50% acetonitrile containing 0.1% trifluoroacetic acid and mixed with a 2,5-dihydroxybenzoyc acid matrix. Spectra were acquired by a matrix-assisted laser desorption ionization-time of flight (MALDI-TOF) Reflex mass spectrometer, equipped with a Scout ion source (Bruker-Franzen Analytik, Bremen, Germany). Ions formed by a pulse UV laser beam were accelerated at 20 KeV. Mass spectra were obtained averaging 100–200 shots.

Amino Acid Analysis—The pure protein (1–2 nmol) was hydrolyzed in sealed evacuated tubes with 0.2 ml of 6 N HCl containing 0.1% phenol at 110 °C for 20 and 70 h. Amino acid analysis was performed by the procedure of Spackman *et al.* (13) using an amino acid analyzer (model 3A29, Carlo Erba, Italy). Amino acids were detected by a post-column derivatization with *o*-phthaldialdehyde and 2-mercaptoethanol or with ninhydrin (14).

Cysteine was determined as cysteic acid after oxidation with performic acid (15). Alternatively, cysteine was determined as carboxymethylcysteine. Tryptophan was determined by a spectrophotometric method (16) or alternatively by the method of Penke *et al.* (17).

Determination of Free Thiol Groups—Reaction of Ellman's reagent (18) with thiol compounds causes the release of 1 eq of the 2-nitro-5-thiobenzoate anion, thus providing the basis for determining the concentration of thiols in aqueous solution. The determination was performed on 5 nmol of native protein.

Carboxymethylation—Cerato-platanin (30 nmol) was dissolved in 0.2 ml of 0.1 M Tris-HCl buffer, pH 8.5, containing 6 M guanidinium chloride and then treated with 10 μl of 2-mercaptoethanol overnight at 40 °C in a nitrogen atmosphere. Successively, 0.2 ml of a solution of iodoacetic acid (freshly recrystallized, 120 mg/ml) in 3 M guanidinium chloride adjusted to pH 8.7 with Tris base was added. After 15 min at room temperature the carboxymethylated (CM) protein was separated from reagents by dialysis.

Enzymatic Digestions and Peptide Separation—Before protease addition, the CM protein solution was incubated for 3 min in a boiling water bath and then chilled in ice.

Trypsin digestion was performed as follows. 20 nmol of CM cerato-platanin was dissolved in 80 μl of 0.05 M ammonium bicarbonate, the the protease (3.2% w/w) was added, and the mixture was incubated at 37 °C for 4 h. Asp-N endoproteinase digestion was carried out on 30 nmol of CM protein dissolved in 0.1 ml of 50 mM sodium phosphate buffer pH 8.0. The protease (2 μg) was added to this solution, and the mixture was incubated at 37 °C for 18 h. Thermolysin was added to 20 nmol of CM protein dissolved in 0.2 ml of 0.1 M 4-ethylmorpholine buffer, pH 7.8, and the protein was digested at 37 °C for 2 h. The protease/protein molar ratio was 1/50. Chymotrypsin digestion was performed as described previously (19).

The determination of disulfide bonds was achieved by treating 40 nmol of the native protein, dissolved in 120 μl of 50 mM sodium phosphate buffer, pH 8.0, containing 1.0 M guanidinium chloride, with 6 μg of endoproteinase Asp-N (in aliquot of 2 μg). The solution was kept at 30 °C for 72 h.

Peptides produced after each enzymatic digestion were purified by reverse-phase HPLC on a C18 Vydac (5 μm, 4.6 × 250 mm) column using a trifluoroacetic acid/acetonitrile gradient. The peaks containing more than one peptide were rechromatographed using different elution gradients.

Sequence Determination—Edman degradation was carried out using the Milligen Protein Sequencer model 6600, which operates the solid phase Edman degradation on peptides or proteins covalently immobilized on polyvinylidene difluoride membranes derivatized with 1,4-phenylene diisothiocyanate (Sequelon DITC) or arylamine groups (Sequelon AA) (20). The C-terminal region sequence was obtained using the HP G1009A C-terminal protein sequencing system (Hewlett-Packard, Germany) (21).

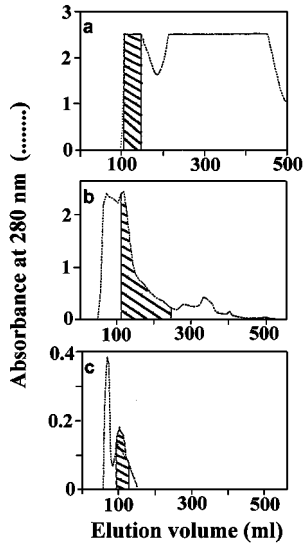

FIG. 1. **Purification of cerato-platanin from *C. fimbriata* f. sp. *platani* isolate Cf AF 100**. Bio-Gel P-10 chromatography of the concentrated culture filtrate (*a*) and the pooled biologically active fractions from Bio-Gel P-10 chromatography (*b*) were dialyzed against 60% ethanol and then applied to the first Sephadex LH-60 column (2.4 × 90 cm). The column was eluted with 60% ethanol at a flow rate of 0.2 ml/min. The pooled biologically active fractions from this column were rechromatographed on the same Sephadex LH-60 column (*c*). The *hatched areas* indicate the active fractions.

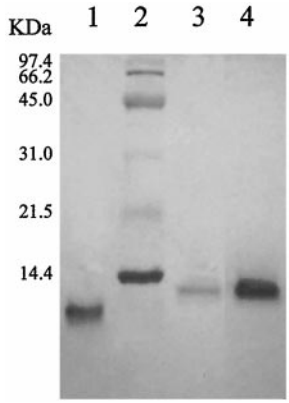

FIG. 2. **Sodium dodecyl sulfate-polyacrylamide gel electrophoresis.** *Lane 1*, pure cerato-ulmin; *lane 2*, Bio-Rad silver stain SDS-polyacrylamide gel electrophoresis standards, low molecular range; *lane 3*, 1 μg of cerato-platanin; *lane 4*, 5 μg of cerato-platanin. Proteins were stained with Bio-Rad silver stain.

RESULTS

Protein Purification—The elution profile of the Bio-Gel P-10 chromatography is shown in Fig. 1*a*. The *hatched area* indicates fractions eluted with the column void volume that contained biological activity. These fractions, which were nearly free of the brown-to-black pigments abundantly present in the concentrated culture filtrate, were able to induce tobacco leaf cell tissue necrosis (Fig. 3*A*). We found that the protein solution in 60% ethanol maintained a stable toxic activity for several weeks (data not shown).

The successive purification steps performed with Sephadex LH-60 chromatography permitted us to isolate a protein that was completely free of pigments (Fig. 1, *b* and *c*; the *hatched areas* indicate biologically active fractions). This protein, which

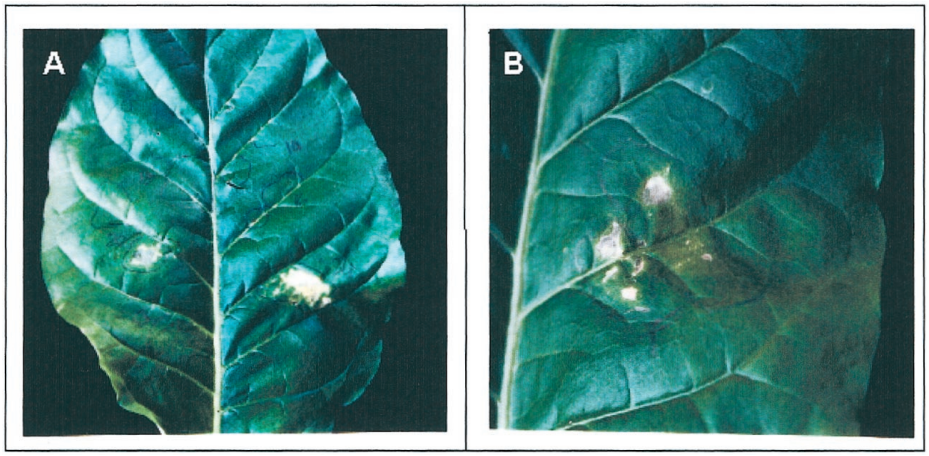

FIG. 3. **Cerato-platanin induces tobacco leaf cell necrosis.** Leaf cell necrosis induced by active fractions from the Bio-Gel P-10 column (*A*) and pure cerato-platanin (*B*).

elicits toxic activity, shows a single band in the SDS-polyacrylamide gel electrophoresis analysis (15% gel), indicating that it is a pure protein. It shows an apparent molecular mass of about 12 kDa (Fig. 2). The mass spectrum obtained with the native protein by a MALDI-TOF spectrometer revealed a single peak at 12,383.6 *m/z*. The amino acid composition reveals an high percentage of hydrophobic residues, four cysteines and two tryptophans.

Biological Activity of Cerato-platanin—When infiltrated in tobacco leaf mesophyllum, pure cerato-platanin induced leaf cell necrosis (Fig. 3*B*) and fluorescence (Fig. 4*A*). We found that the minimum dose able to cause leaf necrosis is 0.8 nmol. Furthermore, drops containing about 8 nmol of purified cerato-platanin became fluorescent just 24 h after addition to the lower leaf surface of *P. acerifolia* (Fig. 4*B*), suggesting the release of phytoalexins from leaves.

Amino Acid Sequence—Automated Edman solid-phase degradation was first performed on whole CM cerato-platanin to obtain the N terminus sequence (residues 1–36, Fig. 6). The amino acid sequence at the C terminus (residues 117–120) was determined by using the C-terminal sequencing system (Fig. 6).

Aliquots of the CM cerato-platanin were separately digested with trypsin, chymotrypsin, thermolysin, and Asp-N proteinase. The peptide fragments were purified by reverse phase HPLC (Fig. 5), and their amino acid compositions were determined. All peptides from trypsin and Asp-N proteinase digestions were sequenced to obtain most of the sequence. Among the chymotryptic and thermolytic peptides, only the overlapping ones were subjected to automated Edman solid-phase degradation. All the information obtained enabled us to reconstruct the complete amino acid sequence of cerato-platanin (Fig. 6). It consists of 120 amino acid residues and contains 4 cysteines and more than 40% hydrophobic residues. No blocking groups are present at the N terminus, as indicated by the accessibility of the N-terminal residue to Edman degradation. The isoelectric point, calculated by the ProtParam program at the Expasis server, is 4.33, indicating that cerato-platanin is an acidic protein. The molecular weight calculated from sequence is 12,399.79 (this molecular weight was calculated by the Peptide Mass program at the Expasy server, Switzlerland using average isotopic masses), a value that agrees with that determined by MALDI-TOF mass spectrometry (12, 383.6), suggesting that no post-translational modifications occur.

Disulfide Bond Pattern—Cerato-platanin contains four cysteines at positions 20, 57, 60, and 115, which are present as two disulfide bonds in the native protein. In fact, we have found no free SH groups by the method of Ellman (18). To determine the actual disulfide bond arrangement in cerato-platanin, we proceeded to isolate and analyze the disulfide-bonded peptides

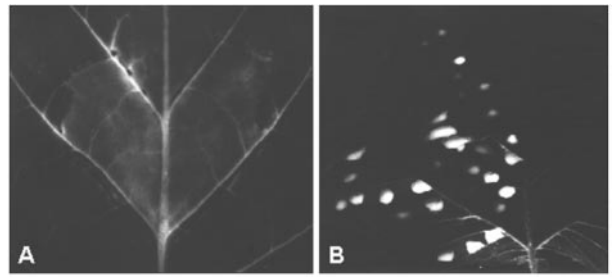

FIG. 4. **Cerato-platanin induces the host release of fluorescence substances.** Fluorescence was induced by 8 nmol of pure cerato-platanin following infiltration in tobacco leaves (*A*) or addition on the lower surface of *P. acerifolia* (*B*).

from the protein. This was achieved by digesting the native protein with Asp-N endoproteinase and separating peptides, as described under "Experimental Procedures." Amino acid analysis, after performic acid oxidation, of the HPLC peaks and the amino acid sequencing of the cysteine-containing peptide were performed. The peak eluting at about 33.5% acetonitrile in the reverse phase HPLC gave three sequences, which represent fragments 12–23 (DLSMGSVA**C**SNG), 53–76 (DSPS**C**GT**C**-WKVTIPNGNSIFIRGV), and 111–120 (DLSN**C**INGAN), held together by two intramolecular disulfide bonds. This clearly excludes that the vicinal Cys-57 and Cys-60 are S—S-bridged to each other. The actual disulfide bonding pattern was determined as previously suggested by Seetharam *et al.* (22) by analyzing the cycles that yield diphenylthiohydantoin-cystine during N-terminal sequencing. We have found diphenylthiohydantoin-cystine in cycles 8 and 9, indicating the disulfide bonding pattern Cys-20—Cys-57 and Cys-60—Cys-115.

DISCUSSION

Several extracellular low M_r proteins produced by phytopathogenic fungi (such as a necrosis-inducing protein from *Pyrenophora tritici-repens* (23), a protein from *Fusarium solani* (24), and cerato-ulmin from *Ophiostoma novo-ulmi* (25, 26)) have phytotoxic activity. In addition, a number of other low M_r peptides (<1 kDa) were characterized, and some had phytotoxic or antimicrobial activity (27, 28). In contrast, the AVR4 and the AVR9 proteins from *Cladosporium fulvum* (29, 30), the necrosis-inducing peptides of varying molecular weights from *Rhynchosporium secalis* (31, 32), and the family of acidic α- and basic β-elicitins from various species of *Phytophthora* and *Pythium* (33, 34) all behaved as avirulence gene products or elicitors. These elicitors are produced by microorganisms and act as host defense mechanism-inducing molecules; elicitins are proteic elicitors. Recently, Templeton *et al.* (35) proposed the following criteria for the classification of proteins from

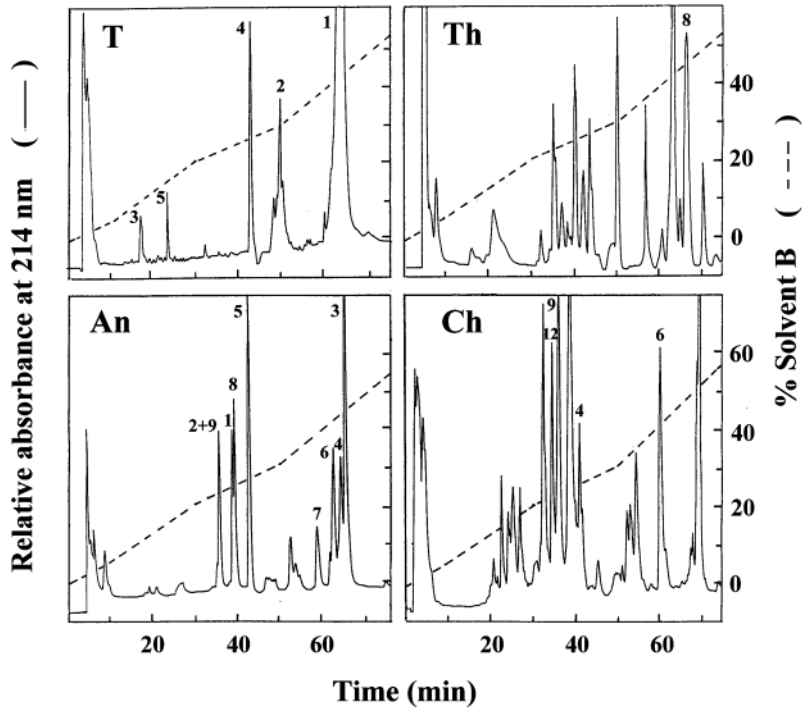

FIG. 5. **The HPLC separations of peptides produced by enzymatic digestions.** All digestions were performed on 30 nmol of CM cerato-platanin. Column: Vydac C18, 5 μm, 4.6 × 250 mm. Solvent A: 10 mM trifluoroacetic acid in water. Solvent B: 10 mM trifluoroacetic acid in acetonitrile. Flow rate: 0.8 ml/min. *Solid line*, absorbance; *dashed line*, elution gradient. The overlapped peptides were rechromatographed using different conditions. *T*, tryptic peptides; *Th*, thermolytic peptides; *An*, endoproteinase Asp-N peptides; *Ch*, chymotryptic peptides.

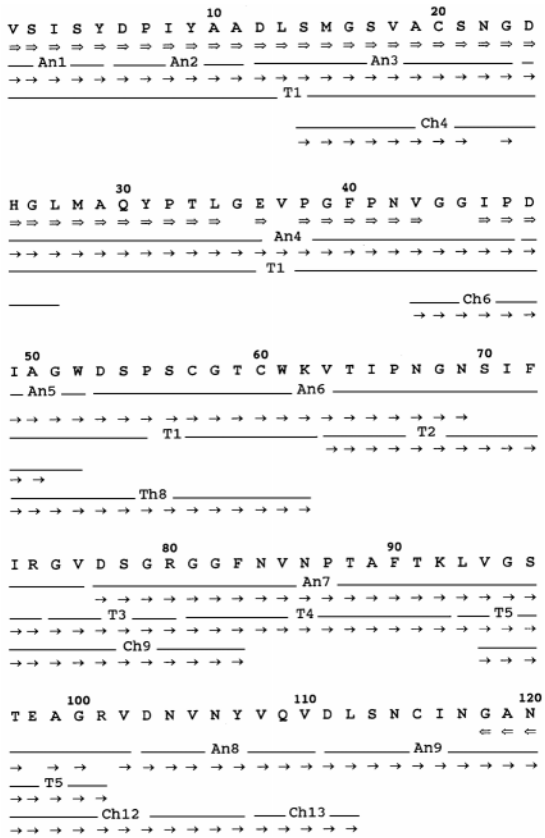

FIG. 6. **The complete amino acid sequence of cerato-platanin.** *An*, endoproteinase Asp-N peptides; *T*, tryptic peptides; *Ch*, chymotryptic peptides; *Th*, thermolytic peptides. →, sequence results determined on peptides. ⇒, sequence results from the whole protein by Edman degradation. ⇐, sequence results obtained by C-terminal sequencing. Cys-20—Cys57 and Cys-60–Cys-115 are S—S-bonded.

pathogenic fungi: (i) the number of cysteine residues (6–10 or more); (ii) the relatively small molecular weight (<150 amino acids); and (iii) the role of these proteins in the action specificity and in the pathogenicity. Hydrophobins are an interesting subgroup of the cysteine-rich proteins described by Templeton. These all contain eight cysteine residues, the consensus sequence CCN, and show moderate to strong hydrophobicity (35–38). Hydrophobins play important physiological roles in the morphogenesis and/or the pathogenicity and host specificity of some saprophytic and/or pathogenic fungi (39).

This paper deals with a protein of about 12.4 kDa called cerato-platanin, which is produced by *C. fimbriata* f. sp. *platani* in axenic culture. We have demonstrated that it induces both cell necrosis and fluorescence after infiltration into tobacco leaf mesophyllum; in addition, the treatment of the lower surface of *P. acerifolia* leaves causes the host production of fluorescent substances (phytoalexins) (see Figs. 3 and 4). Other authors (40) also report that this protein behaves as a fungal toxin that rapidly causes plane cuttings to wilt when they are immersed in a solution of this fungal protein. These behaviors do not define the exact role of cerato-platanin in the biology of *C. fimbriata* f. sp. *platani* but rather suggest a potential involvement in the host plane-fungus interaction. Host reactions like those described in this paper are indicated as effects of activity of elicitors, a property also possessed by elicitins; the minimum active dose of 0.8 nmol of cerato-platanin is comparable with the α-elicitins (41, 42).

Starting from culture filtrates of a *C. fimbriata* strain isolated from plane trees in the Marseille area, Ake *et al*. (10) purified a protein called fimbriatan, which is able to inhibit the growth of plane (*P. acerifolia*) callus cultures. This protein has an apparent molecular mass of 15 kDa and contains a high percentage of hydrophobic residues. However, both its amino acid composition and the presence of a blocking group at the N terminus render fimbriatan different from the protein purified by us.

Our cerato-platanin consists of 120 amino acid residues and contains four cysteines and a high percentage (more than 40%) of hydrophobic amino acids; the grand average of hydropathicity (43) is 0.02. Compared with hydrophobins, this value lies in the lower part of the hydrophobin grand average of hydropathicity index range.

We aligned the amino acid sequence of cerato-platanin with that of cerato-ulmin, a phytotoxic protein belonging to the

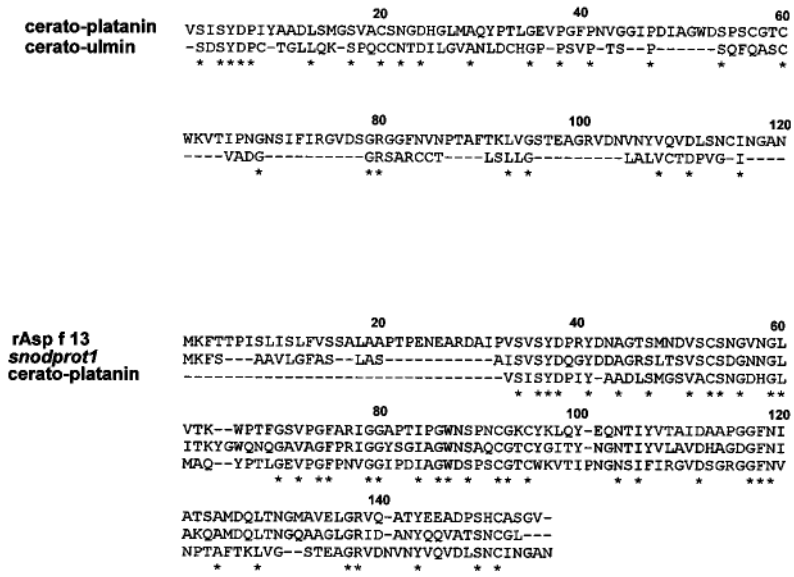

FIG. 7. **Sequence alignment.** *Top*, alignment of cerato-platanin and cerato-ulmin (accession number Q06153). *Bottom*, alignment of cerato-platanin with two homologous proteins found in data banks using the BLAST program; *snodprot1* (accession number AF074941) is the product of a gene from *P. nodorum* (the causal agent of glume blotch of wheat) (45), whereas rAsp f13 (accession number AJ002026) indicates an allergen from *A. fumigatus*. Alignments were obtained by the CLUSTALW program. The *asterisk* indicates conserved residues. CLUSTALW and BLAST were run using the server at the Pôle BioInformatique Lyonnais, in Lyon, France).

hydrophobin family and produced by *O.* (*Ceratocystis*) *ulmi*, a fungus responsible of Dutch elm disease (25, 44). The identity between cerato-ulmin and cerato-platanin is only 17.5% (Fig. 7, *top*). It can be noted that most of the homologous residues in the two sequences are clustered at the N terminus: in the 1–25 stretch, the identity value increases to 40%.

Considering that cerato-platanin does not contain the eight-cysteine pattern characteristic of hydrophobins, this new protein should not be included in the hydrophobin family. Nevertheless, some features of cerato-platanin link this protein to hydrophobins. In fact, cerato-platanin elicits a quite high hydrophobicity and possesses an N terminus sequence highly homologous to cerato-ulmin (Fig. 7, *top*); in addition, it contains the sequence Cys-Ser-Asn-22 aligned with the cerato-ulmin Cys-Cys-Asn sequence, which is the signature sequence of hydrophobins. This sequence contains the conservative substitution Cys → Ser, but we can note that two other hydrophobins, such as Eas from *Neurospora crassa* (consensus sequence = CCQ) (44) and Hfb1 from *Thricoderma reesei* (consensus sequence = CCA) are exceptions to the consensus rule (45). Furthermore, some recent findings obtained by immunolocalization experiments[2] have indicated that cerato-platanin accumulates abundantly on the fungal cell surface, as hydrophobins do.

Searches performed using the BLAST program in data banks (Swiss-Prot, EMBL, and GenBank™) revealed that this protein is highly homologous with two other proteins produced by different Ascomycete fungi. One, expressed during infection of wheat leaves, is codified by the *snodprot1* gene of *Phaeosphaeria nodorum*, which is the causal agent of glume blotch of wheat (46), and the other is the rAsp f13 allergen from *Aspergillus fumigatus* (Fig. 7, *bottom*). These three proteins could represent the first members of a new family of fungal proteins that possess biological activity. The *snodprot1* gene product is produced by a plant pathogen fungus during infection, and cerato-platanin has direct necrotic and eliciting effects on the *P. acerifolia* plants.

Although the molecular mechanism of cerato-platanin in the plant pathogenesis remains to be clarified, the amino acid sequence gives us the possibility of new experimental approaches. One possibility is to synthesize short peptides designed on the basis of homologous regions present in fungal phytotoxic proteins. For example, the N termini of cerato-platanin and cerato-ulmin (residues 1–25, Fig. 7, *top*) are highly homologous, suggesting that they could be involved in the biological effects of these fungal proteins. Combinatorial chemistry approaches could be devised to construct peptide libraries that will be tested for induced host defense reactions.

The knowledge of the cerato-platanin-host plant interaction will enable us to understand whether this protein is involved as a signal molecule in the activation of the plane defense response (such as phytoalexin production) and, successively, to obtain host genotypes more timely and effective in defense response.

Furthermore, a synthetic gene coding for cerato-platanin can be easily constructed, and the recombinant wild type or the mutants proteins can be produced to reveal the molecular site(s) involved in the biological activities of this phytotoxic protein. Finally, inhibitor substances can be found that could be employed in the control of the *P. acerifolia* fungal disease.

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Purification, Characterization, and Amino Acid Sequence of Cerato-platanin, a New Phytotoxic Protein from *Ceratocystis fimbriata* f. sp. *platani*

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