RESEARCH LETTER

The expression of the *cerato-platanin* gene is related to hyphal growth and chlamydospores formation in *Ceratocystis platani*

Ivan Baccelli[1], Cecilia Comparini[1], Priscilla P. Bettini[2], Federica Martellini[3], Michelina Ruocco[4], Luigia Pazzagli[3], Rodolfo Bernardi[5] & Aniello Scala[1]

[1]Dipartimento di Biotecnologie Agrarie, Università di Firenze, Florence, Italy; [2]Dipartimento di Biologia Evoluzionistica 'Leo Pardi', Università di Firenze, Florence, Italy; [3]Dipartimento di Scienze Biochimiche, Università di Firenze, Florence, Italy; [4]Istituto per la Protezione delle Piante, CNR, Portici (NA), Italy; and [5]Dipartimento di Biologia delle Piante Agrarie, Università di Pisa, Pisa, Italy

Correspondence: Priscilla P. Bettini, Dipartimento di Biologia Evoluzionistica 'Leo Pardi', Università di Firenze, Via Romana, 17-19, 50125 Florence, Italy. Tel.: +39 055 2288238; fax: +39 055 2288250; e-mail: p.bettini@unifi.it

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Abstract

Cerato-platanin (CP) is a protein produced by *Ceratocystis platani*, the causal agent of canker stain disease of plane trees. CP is the first member of the 'cerato-platanin family', and its role as a pathogen-associated molecular pattern (PAMP), inducing defence responses both in host and nonhost plants, is established. However, the primary role of CP and its homologues in the fungal life remains unknown. In the present work, we investigated the regulation of the *cp* gene during the *in vitro* growth of *C. platani* in different conditions and under the effect of potential stress factors. Fungal growth and conidiogenesis were also analysed. Results showed that *cp* is a single-copy gene whose expression level is strictly associated with hyphal growth and with chlamydospores formation. The analysis of a 1368 bp 5′-flanking region revealed putative motifs that could be involved in the regulation of gene expression in response to stress and developmental cues. Taking into account the localization of CP in the fungal cell wall and the recently published 3D structure of the protein, our results support a role for CP in growth and developmental processes of *C. platani*.

Introduction

Cerato-platanin (CP) is a 12.4 kDa noncatalytic protein firstly isolated by Pazzagli *et al.* (1999) from culture filtrates of the ascomycete *Ceratocystis platani* (Walter) Engelbrecht & Harrington, the causal agent of canker stain disease of plane trees (*Platanus orientalis* L., *Platanus occidentalis* L. and their hybrid *Platanus acerifolia* (Ait.) Willd.) (Panconesi, 1999; Engelbrecht & Harrington, 2005). Mature CP consists of 120 amino acids, with four cysteines forming two disulphide bonds, and it is a stable component of the fungal cell wall (Pazzagli *et al.*, 1999; Boddi *et al.*, 2004). The protein is secreted when the fungus grows both in axenic culture and on plane leaves; in the latter condition, the *cp* gene is expressed earlier (Scala *et al.*, 2004; Bernardi *et al.*, 2011). CP elicits defence-related reactions from both host and nonhost plants; in plane leaves, it causes cell plasmolysis, programmed cell death, production of hydrogen peroxide, nitric oxide and phenolic compounds, localized resistance and overexpression of defence-related genes (Pazzagli *et al.*, 1999; Scala *et al.*, 2004; Bennici *et al.*, 2005; Fontana *et al.*, 2008; Lombardi *et al.*, 2010). According to the zig-zag model of resistance development in plants, as described by Jones & Dangl (2006), CP seems to behave as a pathogen-associated molecular pattern (PAMP) able to trigger the basal defence system.

CP is the first member of the cerato-platanin family (Pfam PF07249) (Pazzagli *et al.*, 1999), a family of secretion proteins that so far contains more than 40 proteins belonging to the ascomycetes and the basidiomycetes. Some members of this family have been studied in detail, and their role as PAMPs is emerging (Wilson *et al.*, 2002; Djonović *et al.*, 2006, 2007; Seidl *et al.*, 2006; Jeong *et al.*, 2007; Vargas *et al.*, 2008; Yang *et al.*, 2009; Zaparoli *et al.*, 2009), while others, instead, are allergenic in humans (Pan & Cole, 1995; Kurup *et al.*, 2002). However, not much work has been aimed to study the regulation of the genes encoding cerato-platanins and to highlight their primary role in fungal life.

A clue to address this question can be provided by the recently published 3D structure of CP, which revealed that the protein has a double-ψβ-barrel fold similar to that occurring in endoglucanases, in the plant-defence protein barwin and in domain I of expansins (de Oliveira *et al.*, 2011). As CP lacks lytic activity and is located in the fungal cell wall, the authors suggested that its similarity to expansins might indicate a role in the remodelling and enlargement of the cell wall.

In the present work, we investigated the regulation of *cp* during the *in vitro* growth of *C. platani* exposed to many potential abiotic and biotic stresses. The promoter region of *cp* was also isolated and studied.

Materials and methods

Fungal strains and culture

Ceratocystis platani Cf AF 100, *Trichoderma harzianum* T22 and *Trichoderma atroviride* P1 were used in previous studies (Pazzagli *et al.*, 1999; Tucci *et al.*, 2011). Solid or liquid cultures of *C. platani* were prepared with potato dextrose agar (PDA) or broth (PDB) (Difco, Detroit, MI), respectively. An autoclaved cellophane disc was placed on the surface of the solid cultures. For the establishment of fungal cultures, conidia were obtained as described in Bernardi *et al.* (2011) and inoculations were performed with about 6×10^4 conidia.

Stress conditions

Ceratocystis platani was exposed to the following stresses: high and low temperature, ionic and nonionic osmotic stress, matric stress, oxidative stress, addition to the culture medium of sawdust from different sources or of the plane tree phytoalexin umbelliferone, and co-culture with mycoparasitic fungi. Still or shake liquid cultures were also prepared. Unless specified otherwise, cultures were grown on PDA or PDB for 3 days in the dark at 25 °C.

To test the effect of temperature, *C. platani* was grown at 15 or 32 °C for 3 days on PDA. The influence of water potential was assessed by adding to PDA the ionic solute NaCl (Lang, 1967), the nonionic solute glycerol (osmotic stress) (Dallyn & Fox, 1980) or PEG 8000 (matric stress) (Steuter *et al.*, 1981). Theoretical water potentials of −1.5 MPa with NaCl and glycerol, or −5.5 MPa with PEG 8000 were obtained (Michel & Kaufmann, 1973).

Sawdust-agar media were prepared with 15 g L^{-1} of agar (Sigma-Aldrich, St Louis, MO) and 100 g L^{-1} of sawdust from susceptible *P. acerifolia*, from the resistant *P. acerifolia* clone 'Vallis clausa' (Vigouroux & Olivier, 2004) and from the nonhost plant *Ulmus* spp.

Co-cultures of *C. platani* with the mycoparasitic fungi *T. harzianum* and *T. atroviride* were set up by growing *T. harzianum* and *T. atroviride* in PDA plates on sterile cellophane discs for 1 day at 25 °C before the discs bearing the mycoparasitic fungi were removed and placed on 4-day-old cultures of *C. platani*. As a control, *C. platani*/*C. platani* co-cultures were prepared. The co-culture plates were incubated at 25 °C for 1 day.

To produce an oxidative stress to the fungus, *C. platani* was grown in 10 mL of PDB in 20-mL airtight vials containing H_2O_2 at a final concentration of 200 μM and incubated for 6 days at 25 °C in the dark on a rotary shaker at 100 r.p.m.

The phytoalexin umbelliferone (Sigma-Aldrich), dissolved in distilled water and autoclaved at 120 °C for 15 min, was added to 100-mL flasks each containing 20 mL of PDB to a final concentration of 150 μM. The flasks were sealed with aluminium foil and parafilm and incubated for 6 days at 25 °C in the dark at 100 r.p.m.

Still cultures were grown at 25 °C in the dark in 100-mL flasks containing 20 mL of PDB each. The shake cultures (100 r.p.m.) were incubated in the same growth chamber as a control. The flasks were sealed as described earlier and incubated for 6 days.

For each experiment, six replicates were prepared for the solid cultures and twelve for the liquid cultures.

Growth assessment and microscopic analysis

The mycelium was collected from the cellophane discs and weighed and its RNA extracted. For the liquid cultures, six replicates were processed to assess the dry weight by incubating at 60 °C for 24 h, whereas RNA was extracted from the remaining replicates.

Fresh mycelium was also examined with an optical microscope equipped with a USB camera (Konus #5829 CMOS Camera USB Plug, Konus, Italy) to evaluate both conidia and chlamydospores presence. The amount of chlamydospores produced over time was determined as number per field of view (FOV) at 250× magnification, examining 20 FOVs per time-point.

Southern blot analysis

Genomic DNA (20 μg per sample) of *C. platani* was extracted with the DNeasy Plant Mini Kit (Qiagen, Valencia, CA) and digested overnight at 37 °C with the restriction enzymes EcoRI or HindIII, which did not cut within the *cp* sequence. The digested DNA was fractionated by 0.7% agarose gel electrophoresis, transferred onto a positively charged Hybond-N^+ nylon membrane (GE Healthcare, UK) and hybridized with a digoxigenin-labelled probe obtained by PCR amplification of a 356-bp fragment

of the *cp* cDNA sequence using the following primers: *cp*-for 5′-TCTCTTATGACCCTATCTAC-3′, *cp*-rev 5′-CT AATTAGCGCCGTTAATGC-3′. Probe labelling, hybridization and chemiluminescence detection were performed following the DIG Application Manual for Filter Hybridization (Roche Applied Science, Switzerland).

Quantitative RT-PCR analysis

RNA extraction from *C. platani*, DNase treatment and reverse-transcription of total RNA (400 ng per sample) were performed as described by Bernardi *et al.* (2011). The amount of *cp* transcript was determined by real-time PCR with TaqMan® MGB probes (Applied Biosystems, Foster City, CA) using the *18S* rRNA gene as endogenous control. FAM™ dye-labelled probes and primers were designed by the Custom TaqMan® Gene Expression Assay Service on the *C. platani cp* and *18S* sequences (GenBank accession nos. EF017218 and U43777). The resulting 20× assay mix for the *cp* transcript analysis contained the primers *cp*-for 5′-GAAGTTCTCTATCCTACCCATGATT GC-3′, *cp*-rev 5′-TCAGGTCAGCGGCGTAGATA-3′, and the probe spanning the exon–exon boundary, 5′-CCGTC TCGATCTCTTATGAC-3′. The 20× assay mix for *18S* transcript analysis contained the primers *18S*-for 5′-GGA ACAATTGGAGGGCAAGTCT-3′, *18S*-rev 5′-CAACTACG AGCTTTTTAACCACAACA-3′, and the probe 5′-TTGGA GCTGGAATTAC-3′. The amplifications of the target gene and the endogenous control were run in triplicate on the same plate in separate tubes. Reactions (25 μL) were carried out with 20 ng of cDNA, 1× TaqMan® Gene Expression Assay mix and 1× TaqMan® Gene Expression Master Mix following the manufacturer's instructions. Amplifications were performed in an Applied Biosystems 7300 Real-Time PCR System using the recommended thermocycling conditions. The size of the amplification products was verified on agarose gel. The relative amount of *cp* gene transcript in each sample was determined using the comparative C_T method as described in the ABI PRISM 7700 Sequence Detection System User Bulletin #2 (Applied Biosystems). Before the quantification, a validation experiment was performed to ensure that the amplification efficiencies of the target gene and the reference gene were approximately equal.

Promoter isolation and analysis

The Universal GenomeWalker™ Kit (Clontech Laboratories Inc., Palo Alto, CA) was used to isolate the upstream region of the *cp* gene following the manufacturer's instructions. The genomic DNA of *C. platani* was digested with the blunt-end enzymes DraI, EcoRV, PvuII and StuI, respectively, and the resulting fragments were ligated to a GenomeWalker™ adaptor. Genome walking was then performed by two rounds of PCR with gene-specific primers: GW *cp* 1rev 5′-TCAGCGGCGTAGATA GGGTCATAAGAG-3′ for the first PCR and GW *cp* nes-t2rev 5′-GCGCTGGCAATCATGGGTAGGATAGAG-3′ for the nested PCR.

Putative binding sites for transcription factors in the 5′-flanking region of the *cp* gene were investigated with the programmes PATCH™ 1.0 (http://www.gene-regulation.com/pub/programs.html), based on the TRANSFAC® database release 7.0 and MATINSPECTOR 8.0.5 (Cartharius *et al.*, 2005). Only binding sites with a high matrix similarity ($\geq$ 0.85) were retained.

Results

Effect of stress factors on *cp* gene expression and growth of *C. platani*

The *cp* gene in *C. platani* is a single-copy gene as demonstrated by the Southern blot analysis after hybridization of the fungal genomic DNA digested with the enzymes HindIII and EcoRI with the *cp* probe (Fig. 1).

The expression of *cp* was quantified by real-time PCR using the *18S* gene as endogenous control (Bernardi *et al.*, 2011). Before the analysis, the *18S* gene was assessed for stability between samples (treated vs. control) under the growth conditions studied in the present work, and it did not show significant differences in the transcriptional level (data not shown).

The *cp* transcript level turned out to be highly regulated by the growth conditions and the stress factors here

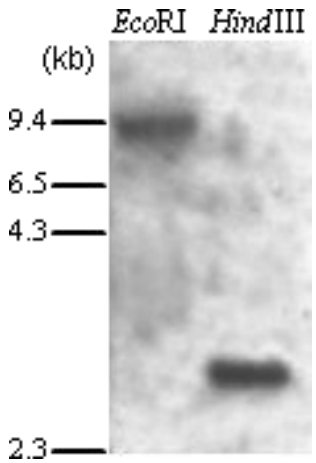

Fig. 1. Southern blot analysis of the *cerato-platanin* (*cp*) gene in the *Ceratocystis platani* genome. Genomic DNA was digested with the restriction enzymes EcoRI and HindIII, which had no restriction sites within the *cp* sequence, transferred to a membrane and hybridized with a gene-specific probe for *cp*.

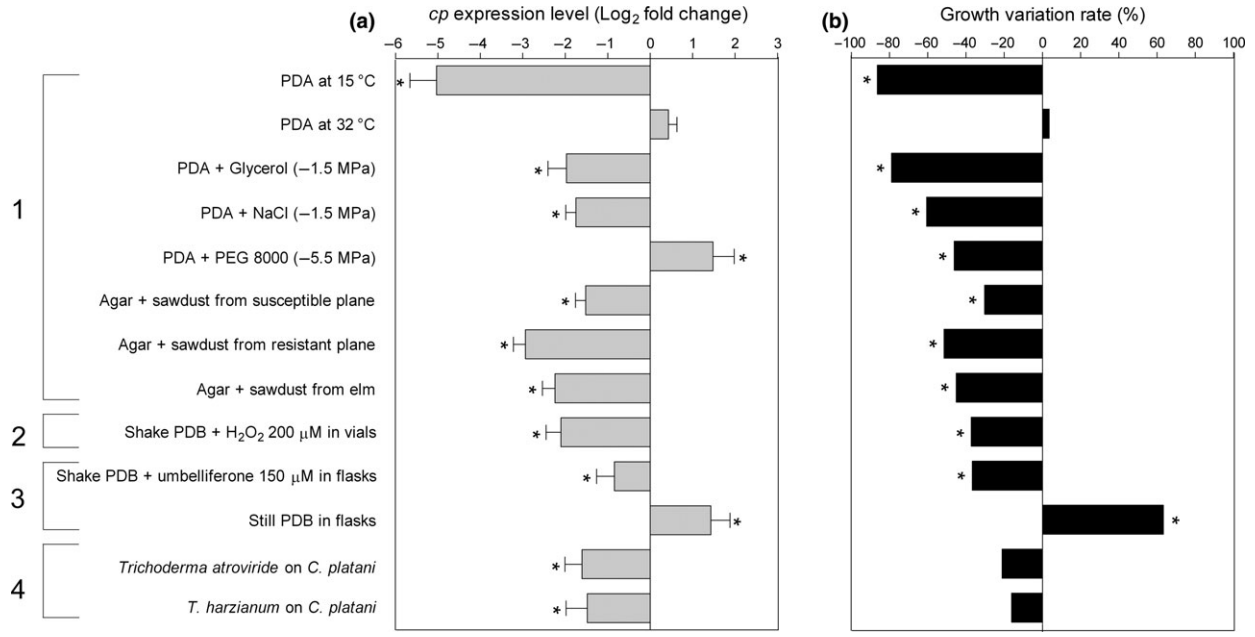

Fig. 2. Effect of various stress factors on the expression of the *cerato-platanin* (*cp*) gene (a) and growth (b) of *Ceratocystis platani*. In (a) the *x*-axis represents the expression level of *cp* as a fold change (Log_2), each compared with its control (= 0). The controls were represented by the growth on PDA at 25 °C (1), by the growth in vials (2) or flasks (3) containing PDB and by the growth of *C. platani* on itself for the co-culture tests (4). The data represent the means ± SD of three repeated samples. Negative or positive values represent a lower or higher amount, respectively, of *cp* transcript compared with the respective control. In (b) the growth variation percentage compared with the corresponding control was used to graph the results; values as mg of fresh weight (blocks 1 and 4) or dry weight (blocks 2 and 3) of fungal biomass ($n = 6$) were used to perform the statistical test. *Significantly different compared with control at $P \leq 0.05$.

tested (Fig. 2a). The *cp* transcript amount was lower in almost every condition compared with the corresponding control, showing a down-regulating effect of the stress factors on the expression of the *cp* gene. Specifically, when compared with the growth on PDA at 25 °C (control 1), the *cp* gene expression was down-regulated by low temperature (15 °C), osmotic water stress (caused by NaCl or glycerol added to PDA) and growth on the sawdust-agar media. It was also down-regulated when H_2O_2 or umbelliferone was added to the medium in comparison with the respective controls 2 and 3 (growth in PDB in vials or flasks, respectively) and finally during the co-culture with *T. atroviride* or *T. harzianum* compared with the *C. platani*/*C. platani* co-culture (control 4). On the other hand, the *cp* transcript amount was higher than control under matric water stress caused by PEG 8000 and when the culture was maintained static, whereas at 32 °C the increase was not significant.

At the same time, most conditions also reduced the growth of *C. platani* as compared with the respective controls (Fig. 2b). Fungal growth increased only at a temperature of 32 °C and in static culture, although the latter increase was again not significant.

Therefore, the amount of *cp* transcript was strictly related to the growth level of the fungus: in all those conditions that reduced the growth of *C. platani*, the *cp* transcript level was lower than the control. The only exception was represented by the matric water stress, where the *cp* transcript level increased while fungal growth was reduced.

Relation between conidiogenesis and *cp* gene expression

The effect of the different growth conditions on conidiogenesis in *C. platani* was evaluated by analysing the production of both conidia and chlamydospores (Table 1).

Conidia were generally formed in all the conditions studied, although in different amounts; with NaCl or PEG 8000, however, no conidia were present. In particular, they were produced in large amount on the sawdust-agar media where the *cp* transcript level was reduced and not formed under matric stress where *cp* was up regulated. No relation could therefore be found between conidia formation and *cp* gene expression.

On the other hand, the highest production of chlamydospores was observed where *cp* was up regulated, including the matric water stress (Fig. 3 and Table 1), suggesting that the *cp* transcript level could be related to chlamydospores production, despite the reduction in

Table 1. Conidiogenesis of *Ceratocystis platani* exposed to various stresses

Stress factor	Presence of chlamydospores	Presence of conidia
PDA (control 1)	+++	+++
PDA at 15 °C	–	+
PDA at 32 °C	+++	+++
PDA + glycerol (−1.5 MPa)	–	+
PDA + NaCl (−1.5 MPa)	–	–
PDA + PEG 8000 (−5.5 MPa)	+++	–
Agar + sawdust from susceptible plane	–	+++
Agar + sawdust from resistant plane	–	+++
Agar + sawdust from elm	–	+++
Shake PDB in vials (control 2)	++	++
Shake PDB + H_2O_2 200 μM in vials	++	++
Skake PDB in flasks (control 3)	++	++
Shake PDB + umbelliferone 150 μm in flasks	++	+
Still PDB in flasks	+++	++

The presence of conidia or chlamydospores is expressed as relative units according to Fig. 3.
The colonies were grown at 25 °C, except where indicated. For the other growth conditions, see Materials and methods.

growth. As chlamydospores could not be detached from hyphae, to test this hypothesis, *C. platani* was inoculated on PDA plates amended with PEG 8000, and chlamydospores differentiation, *cp* gene expression and fungal growth were investigated at 2, 3 and 4 days post-inoculation.

The presence of chlamydospores and hyphal growth was monitored in the course of the experiment: at day 0, only conidia of the inoculum could be detected; at day 2, hyphae and the first chlamydospores (5 per FOV); at day 3, hyphae and a consistent presence of chlamydospores (about 70 per FOV); and at day 4, hyphae and the highest presence of chlamydospores, whose number remained constant also in the following days (more than 300 per FOV). During the same period, it is important to emphasize that the daily increase in mycelial mass remained constant.

A positive correlation between *cp* gene expression and chlamydospores formation was found (Fig. 4). The transcript level increased from the conidium status to the second day of growth, where hyphae were present and chlamydospores just began to be formed. The highest increase occurred at day 3 (Log_2 fold change = 6.15), which preceded the maximum increase in chlamydospores concentration observed at the fourth day post-inoculation.

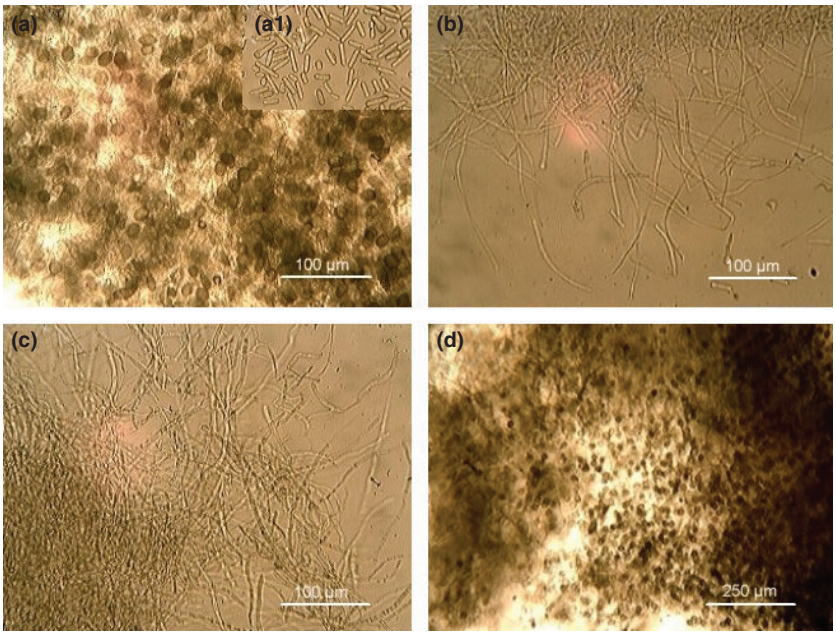

Fig. 3. Microscopic analysis of *Ceratocystis platani* grown at 25 °C on PDA (a, a1), on PDA amended with glycerol (b), on PDA amended with NaCl (c) or on PDA amended with PEG 8000 (d). Chlamydospores can be observed on PDA and on PDA amended with PEG 8000 (a, d), whereas conidia are present on PDA (a1) and in very small amounts on PDA amended with glycerol (b). The amounts of chlamydospores and/or conidia observed in (a), (a1) and (d) are representative of the relative value '+++' in Table 1.

Conidia used to inoculate the plates were known to have a low level of *cp* transcript (Bernardi *et al.*, 2011) and were used as calibrator (time zero).

Promoter analysis

The analysis of a 1368 bp region upstream of the ATG codon for the presence of putative regulatory motifs revealed a putative TATA box at position −167 and putative CCAAT boxes at positions −634 and −817 (Fig. 5). Moreover, putative motifs involved in the regulation of gene expression in response to stress and developmental cues were identified. Two CATTCY sites bound by transcription factor of the TEA/ATTS family, such as yeast Tec1p (Köhler *et al.*, 2002) and *Aspergillus nidulans* AbaA, were located at positions −297 and −1258. In *A. nidulans*, the *abaA* gene controls the expression of the genes involved in morphogenesis and developmental regulation and is required in the final stages of conidiophore development and in spore maturation (Andrianopoulos & Timberlake, 1994). Three stress response elements (STRE) were found at positions −293, −415 and −782 (Marchler *et al.*, 1993) together with a putative binding site for the Nrg1/Nrg2 Zn finger repressors at position −400. In yeast, these two regulatory sequences are associated with the promoters of many genes that respond to a variety of stress conditions (Vyas *et al.*, 2005). Finally, two recognition sites for the yeast Skn7 regulators involved in the response to stress such as oxidative stress and high osmolarity stress were found at positions −713 and −963 (Morgan *et al.*, 1997; Izumitsu *et al.*, 2007).

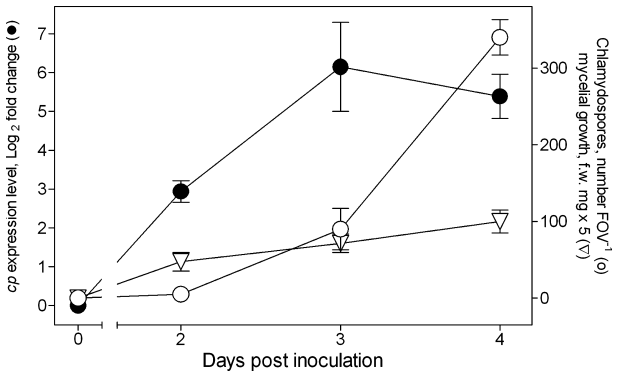

Fig. 4. Relation between *cp* gene expression and chlamydospores formation in *Ceratocystis platani*. The fungus was grown for 4 days under matric water stress caused by PEG 8000 added to PDA. Gene expression is shown as fold change (Log_2) ± SD (n = 3) with respect to conidia used to inoculate the plates (time zero), used as calibrator (= 0). The concentration of chlamydospores is expressed as number per FOV ± SD (n = 20) at 250× magnification.

Discussion

The present work showed for the first time a significant correlation between regulation of the *cp* gene and growth of *C. platani*: when fungal growth was reduced, the *cp* gene expression was down-regulated; when the growth

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-1368 GTTGGGTGTCTACTTTGTGGTTGTCTAATGCCATGCATGCATACATAGCATATACAAAAC
-1308 ACATGACAGACGATCATCTCCAAAGCCGGGCAGGGAGCATTACGAGAAAGCATTCCGAGG
-1248 CCATTAGTTCCGTTTACGAGAGAAAAAAAAGGTAAGTGGCTAAGCAGAGAGGGAAAAGGG
-1188 AGGGAAGAGGACGAACTCTTACTCTGGTTTTGGCTTATGCTGGATGGAGGCTGTTTGTTC
-1128 ATGGTGAAGCCAGCCTAGCCCGGCTCAGCCCCAAGATCGCTTCTTACACTCCCTTCCTAC
-1068 CTGTTTGCCTTTGGGTATTTTTGGTATTTTTCTTTTTTGCTTCCACCCTTGGCCTGGCTT
-1008 GGCTCCTTTTGGTCTGTCGACCCTTGAAACATTTCATGCCCGGCCACTCACTGGAACATC
- 948 TCATACGAGCGAAAACGTCGTTGTCACCCGGCAAACAGCCGGGGTTTGCGGGTGTTTTTT
- 888 AGGGACATTTGGGACATCTCGTGTGGGCGGCCTGGTACATGACAGGCGGAAATAATAACT
- 828 GGACTGATTTTATTGGTGCTTTTATTCATCTTTTGCCGTCCTATAGAGGGGTGGTGCCAT
- 768 GAGGCACTTACTACCTGTGTGTTCATGTATCAACACCCACTGGATGGATAGCTTAGGGCT
- 708 GGCCTCGGTCACTGGTAGCATGCTGTGCCTTTGCATCTTTCTACGCCTGGAATCAAGCTG
- 648 ACGCCCCATGTAGACCAATGGAGGGCTCGTTTTCATTGCCTAGTGTGGGTTGAAGTGCTT
- 588 CGCTCCTCTCCCGAGCCTACCGTGCTTAATTCCAAGGACTACTCTTCATACAGAGCATTT
- 528 GGCCCCCGCGCTTGCATTGATGCTCGTTGTGATTCCAACTCTCGTATATGTGCGTTTCGG
- 468 ACGGCTACTAACTTTAGTCAACACTACGTCGGTACCCAAGCTCCCCACCCCACCCCTCC
- 408 CAACAAGACCCTGTGGGGCCAGGCAAAGGACAAGGCCAAAGGAGCCGAAGCGAAGTGGTT
- 348 TGCTAGGTCTAAGTGCCGGGAAGGGCAAAGCTAGCAGCAGGCAGATAGATACATTCCCCT
- 288 GTACAGTACAGCACACGACAAACGATCCCGAAGCAGTATGGCTACAGCAGCAGCAATGTT
- 228 GAAGGAGGCTTGAGCAACCTTTGGGTTTTCCGTTCCCGCCCCAAGCCCATATCAGAAGTT
- 168 GTATAAAGAGCCCGACCTGCCCGCCTCCAAGATGGGCTTTTCTTGTTCTTCTCCTCATCA
- 108 CATTCAAGCATCTCTCTTCTCTTCTAATAGCTTCTCTACCACTACTCTACTGCATCTTCC
-  48 ACCTAGTTACTCTAGACATCTCTTTCATACTTCCACCAACCTTTCAAAATGAAGTTCTCT
                                                          +1
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Fig. 5. Nucleotide sequence of the 1368 bp region upstream to the *cp* gene (GenBank acc. no. JN383983). The entire nucleotide sequence of the *cp* gene is not shown as it has been already published (GenBank acc. no. EF017218). Putative regulatory motifs described in the Results are indicated as follows: TATA box, light grey; CCAAT boxes, dark grey; STRE elements, black; recognition sites for Skn7 regulators, underlined; binding site for Nrg1/Nrg2 repressor, bold underlined; TEA/ATTS factors DNA binding domain, italics underlined. The first nucleotide of the *cp* ORF is indicated with +1.

level was increased, it was instead up regulated. In addition, the expression of the *cp* gene appeared to be positively correlated with the differentiation process of chlamydospores.

The modulation of transcription had already been analysed in some studies concerning *cp* and other cerato-platanins, but without taking into account the growth level of the fungus and not so extensively as in the present work (Wilson *et al.*, 2002; Chagué *et al.*, 2006; Djonović *et al.*, 2006; Seidl *et al.*, 2006; Rincones *et al.*, 2008; Yang *et al.*, 2009; Zaparoli *et al.*, 2009; Bernardi *et al.*, 2011). The relation here found, and the fact that these widely different stresses and growth conditions all had much the same down-regulating effect on the transcription of *cp*, suggest that the regulation of *cp* was most likely not caused directly by the particular factor tested, but was a more general response to the growth level of the fungus. This hypothesis is supported by the similarity of the 3D structure of CP to expansins (de Oliveira *et al.*, 2011), proteins mainly found in plants where they have various roles in growth and in developmental processes involving cell wall modifications (McQueen-Mason & Cosgrove, 1994; Cosgrove *et al.*, 2002; Li *et al.*, 2003; Choi *et al.*, 2006). A small number of expansin-like proteins has also been found in fungi (Saloheimo *et al.*, 2002; Bouzarelou *et al.*, 2008; Brotman *et al.*, 2008; Chen *et al.*, 2010; Wang *et al.*, 2010; Quiroz-Castañeda *et al.*, 2011). Expansins cause cell wall loosening and cellulose disruption even though they do not have any cellulose-hydrolytic activity. Like expansins, CP is localized in the cell wall, has a double-ψβ-barrel fold, lacks lytic activity and has the ability to bind oligosaccharides. Moreover, the residues involved in carbohydrate binding are conserved among the members of the CP family, suggesting that the biological function of these proteins could be related to polysaccharide binding (de Oliveira *et al.*, 2011).

In conclusion, our results strengthen the functional similarity between CP and expansins and allow us to propose the involvement of CP in the remodelling and enlargement of the cell wall that occur during hyphal growth and in the formation and differentiation process of chlamydospores.

Acknowledgements

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