

GENE 09425

The *chsA* gene, encoding a class-I chitin synthase from *Ampelomyces quisqualis*

(Fungal cell wall; biocontrol agent; powdery mildew; hyperparasite)

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SUMMARY

Degenerate oligodeoxynucleotide primers, designed on the basis of conserved regions of the chitin synthase gene family, were used to amplify a fragment of the *Ampelomyces quisqualis* (*Aq*) *chsA* gene. Subsequently, the PCR product was used as a probe in order to identify and isolate genomic clones harboring the entire *chsA* gene. *Aq chsA* is 2786-nt long, has one intron and encodes a 910-amino-acid polypeptide belonging to the class-I chitin synthases. Low-stringency Southern hybridizations to *Aq* genomic DNA provided evidence for the presence of additional DNA fragments resembling *chsA* in the fungal genome, suggesting the presence of a multigene family of chitin synthases in *Aq*.

INTRODUCTION

Ampelomyces quisqualis (*Aq*) Ces. (Coelomycetes (Sphaerosidales)), a hyperparasite on Erysiphales, has been reported as a possible control agent against powdery mildews (Szejnberg et al., 1989; 1990). The potential of this biocontrol agent in integrated pest management has brought us to initiate the investigation of the growth, development and hyperparasitism of *Aq* at the molecular level.

Chitin, a polymer of β (1,4)-linked *N*-acetylglucosamine (GlcNAc) is an integral component of the cell wall of many fungi. Initial studies have shown that the chitin content of the *Aq* cell wall changes markedly during vari-

ous phases of fungal differentiation (N.W., A.S. and O.Y., unpublished), thus warranting the analysis of the chitin synthase (*chs*) gene family of this fungus. In *Saccharomyces cerevisiae* (*Sc*), chitin is essential for viability; three Chs, each with different functions, account for the total cellular chitin (Buliawa, 1993; Choi et al., 1994). Analyses of DNA fragments from taxonomically diverse fungal species have shown that most fungi have three to six *chs* (Bowen et al., 1992; Mehmman et al., 1994; Mellado et al., 1995). The *chs* gene fragments which have significant homology to the *CHS1* and *CHS2* genes of *Sc* were grouped into three classes (I, II, III) by Bowen et al. (1992).

EXPERIMENTAL AND DISCUSSION

(a) Cloning of the *chsA* gene

For isolating *chsA*, we amplified a fragment of the gene with the aid of degenerate oligo primers, which are a slight modification (in restriction sites and degeneracy levels used) of those designed on the basis of conserved regions of the class-I, -II and -III Chs found in various fungal species (Bowen et al., 1992). Low-stringency PCR

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Abbreviations: aa, amino acid(s); *Aq*, *Ampelomyces quisqualis*; *Af*, *Aspergillus fumigatus*; *An*, *Aspergillus nidulans* bp, base pair(s); Chs, chitin synthase(s); *chsA*, gene encoding ChsA; kb, kilobase(s) or 1000 bp; nt, nucleotide(s); oligo, oligodeoxynucleotide(s); PCR, polymerase chain reaction; *Sc*, *Saccharomyces cerevisiae*; SDS, sodium dodecyl sulfate; SSC, 0.15 M NaCl/0.015 M Na₂ citrate pH 7.6.

upstream sequences. Using a strategy identical to that described above, pNW27, a 4.3-kb *Hind*III fragment present in pNW25, yet with additional upstream DNA was gel-isolated and cloned. The full nt sequence (determined by sequencing both strands of the relevant regions of pNW25 and pNW27) and predicted aa sequence of *chsA* is shown in Fig. 1.

The *chsA* gene of *Aq* carries some of the common structural hallmarks of genes isolated from other fungal species. The presumed *chsA* start codon segment is CCGCTATGGC, which is highly similar to the GCCRMTGG translation initiation consensus (where M=A or C; R=A or G) found in other fungi as well as in higher eukaryotes (Kozak, 1987; Bruchez et al., 1993a). The nt sequences resembling putative CAAT and TATA boxes were identified 266 and 181 nt upstream from the tentative translation start site, respectively. In addition, *chsA* has 1 putative intron (Fig. 1) which was identified on the basis of aa sequence alignment with other *chs* gene products and the presence of intron boundary sequences found in other fungi (Bruchez et al., 1993b). The closest match to a polyadenylation signal in eukaryotes (AATAAA; Proudfoot and Brownlee, 1976) was a AATTA segment at nt 3434–3438 (Fig. 1).

Based on comparison of the predicted aa sequence of ChsA to those identified in other filamentous fungi (Yarden and Yanofsky, 1991; Bowen et al., 1992; Motoyama et al., 1994a,b; Yanai et al., 1994), it is evident that the *chsA* gene product is highly similar to the products of the *chs* gene family in other fungi and it most resembles (62% identity) the *Aspergillus nidulans* (*An*) ChsC polypeptide, which has been designated as belonging to class I. This class-I *chs* of *An* has been shown to be non-essential (Motoyama et al., 1994b). Once transformation and gene disruption procedures are established for *Aq*, elucidation of the role of *chsA* may prove to be possible in this organism. The single putative *chsA* intron is not located at the same position as any of the four introns identified in the *An chsC* gene (Motoyama et al., 1994b). Interestingly, however, the *chsA* intron is identical in its positioning to that of class-II *Rhizopus oligosporus chsI* intron-3 (Motoyama et al., 1994a) and almost identical in its positioning to the third intron in the *An* class-III *chsB* gene (Yanai et al., 1994).

(c) *chsA* is a member of a multi-gene family

Recently, Mellado et al. (1995) have shown that a multiple *chs* gene family is present in the human pathogen *Aspergillus fumigatus* (*Af*). In order to obtain an indication if a similar situation is present in *Aq*, we used an 585-bp gel-purified *Eco*RI fragment of the *chsA* gene, isolated from pNW25, in order to probe *Aq* DNA at low stringency.

Based on previous standard stringency Southern analysis (data not shown), the *Eco*RI fragment should have hybridized to 0.9-, 1.2- and 4.3-kb fragments of *Aq* genomic DNA digested with *Eco*RI, *Bam*HI and *Hind*III, respectively. In the low-stringency Southern blot, the

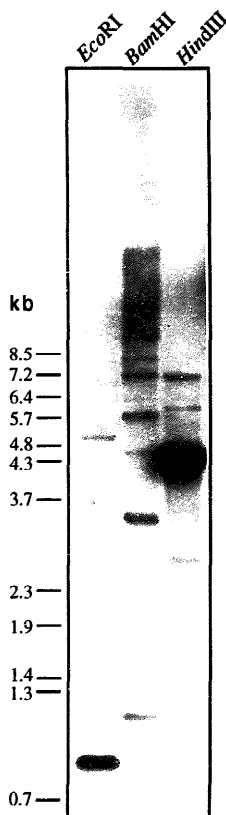


Fig. 2. Low-stringency Southern hybridization of an 885-bp *Eco*RI hexamer-labelled *Aq chsA* gene fragment with digested *Aq* genomic DNA resolved on a 1% agarose gel and blotted on a nylon membrane. Prehybridization and hybridization were performed at 42°C in the presence of 35% formamide/6 × SSC/5 × Denhardt's solution/0.5% SDS/100 µg/ml denatured Salmon sperm DNA. The final membrane washes were at 42°C in the presence of 1 × SSC/0.1% SDS.

labelled probe hybridized to the expected size fragments, yet clear evidence of additional bands cross-hybridizing to the *chsA* probe was obtained (Fig. 2). Thus, our results indicate that similar to *Af*, as well as other filamentous fungi, it is most likely that *Aq* has a *chs* gene family, probably comprised of 4–6 *chs*-related genes. Cloning and subsequent sequencing of the other genomic DNA fragments cross hybridizing with *chsA* is required for the verification of the number and determining the structural relationship between the members of the *Aq chs* gene family.

(d) Conclusions

(1) The *chsA* gene of *Aq* was cloned and structurally analyzed.

(2) *chsA* encodes a class-I Chs, most resembling the *An chsC* gene product.

(3) *chsA* is a member of a multiple *chs* gene family.

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REFERENCES

- Beth Din, A. and Yarden, O.: The *Neurospora crassa chs-2* gene encodes a non-essential chitin synthase. *Microbiology* 140(1994) 2189–2197.
- Bowen, A.R., Chen-Wu, J.L., Momany, M., Young, R., Szanislo, P.J. and Robbins, P.W.: Classification of fungal chitin synthases. *Proc. Natl. Acad. Sci. USA* 89(1992) 519–523.
- Bruchez, J.J.P., Eberle, J. and Russo, V.E.A.: Regulatory sequences involved in the translation of *Neurospora crassa* mRNA: Kozak sequences and stop codons. *Fungal Genet. Newslett.* 40 (1993a) 85–88.
- Bruchez, J.J.P., Eberle, J. and Russo, V.E.A.: Regulatory sequences in the transcription of *Neurospora crassa* genes: CAAT box, TATA box, introns, poly(A) tail formation sequences. *Fungal Genet. Newslett.* 40 (1993b) 89–96.
- Bulawa, C.: Genetics and molecular biology of chitin synthesis in fungi. *Annu. Rev. Microbiol.* 47 (1993) 505–534.
- Choi, W.J., Santos, B., Duran, A. and Cabib, E.: Are yeast chitin synthases regulated at the transcriptional or the post-translational level? *Mol. Cell. Biol.* 14 (1994) 7685–7694.
- Kozak, M.: An analysis of 5'-noncoding sequences from 699 vertebrate messenger RNAs. *Nucleic Acids Res.* 15 (1987) 8125–8148.
- Mehmann, B., Brunner, I. and Braus, G.H.: Nucleotide sequence variation of chitin synthase genes among ectomycorrhizal fungi and its potential use in taxonomy. *Appl. Environ. Microbiol.* 60 (1994) 3105–3111.
- Mellado, E., Auaufvre-Brown, A., Specht, C.A., Robbins, P.W. and Holden, D.W.: A multigene family related to chitin synthase genes of yeast in the opportunistic pathogen *Aspergillus fumigatus*. *Mol. Gen. Genet.* 246 (1995) 353–359.
- Motoyama, T., Kojima, N., Horiuchi, H., Ohta, A. and Takagi, M.: Isolation of a chitin synthase gene (*chsC*) of *Aspergillus nidulans*. *Biosci. Biotech. Biochem.* 58 (1994a) 2254–2257.
- Motoyama, T., Sudoh, M., Horiuchi, H., Ohta, A. and Takagi, M.: Isolation and characterization of two chitin synthase genes of *Rhizopus oligosporus*. *Biosci. Biotech. Biochem.* 58 (1994b) 1685–1693.
- Proudfoot, N.J. and Brownlee, G.G.: 3' non-coding region sequences in eucaryotic mRNA processing intermediates. *Nature* 263 (1976) 211–214.
- Sambrook, J., Fritsch, E.F. and Maniatis, T.: *Molecular Cloning. A Laboratory Manual*, 2nd ed. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, 1989.
- Sanger, F., Nicklen, S. and Coulson, A.R.: DNA sequencing with chain-terminating inhibitors. *Proc. Natl. Acad. Sci. USA* 74 (1977) 5463–5467.
- Sztejnberg, A., Galper, S. and Lisker, N.: Conditions for pycnidial production and spore formation by *Ampelomyces quisqualis*. *Can. J. Microbiol.* 36 (1990) 193–198.
- Sztejnberg, A., Galper, S., Mazar, S. and Lisker, N.: *Ampelomyces quisqualis* for biological and integrated control of powdery mildews in Israel. *J. Phytopathol.* 124 (1989) 285–295.
- Yanai, K., Kojima, N., Takaya, N., Horiuchi, H., Ohta, A. and Takagi, M.: Isolation and characterization of two chitin synthase genes from *Aspergillus nidulans*. *Biosci. Biotech. Biochem.* 58 (1994) 1828–1835.
- Yarden, O. and Yanofsky, C.: Chitin synthase I plays a major role in cell wall biogenesis of *Neurospora crassa*. *Genes Dev.* 5 (1991) 2420–2430.