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

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

Nava Weiss, Abraham Sztejnberg and Oded Yarden

Department of Plant Pathology and Microbiology, The Faculty of Agriculture, The Hebrew University of Jerusalem, Rehovot 76100, Israel

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SUMMARY

Degenerate oligodeoxyribonucleotide 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 (Sphaeropsidales)), a hyperparasite on Erysiphales, has been reported as a possible control agent against powdery mildews (Sztejnberg et al., 1989; 1990). The potential of this biocontrol agent in integrated pest managment has brought us to initiate the investigation of the growth, developement 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content of the *Aq* cell wall changes markedly during various phases of fungal differentiation (N.W., A.S. and O.Y., unpublished), thus warranting the analysis of the chitin synthase (*chs*) gene family of this fungus.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 (Bulawa, 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; Mehmann 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).

Correspondence to: Dr. O. Yarden, Department of Plant Pathology and Microbiology, Faculty of Agriculture, The Hebrew University of Jerusalem, Rehovot 76100, Israel. Tel. (972-8) 481-298; Fax (972-8) 466-794; e-mail: yardeno@indycc1.agri.huji.ac.il

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, oligodeoxyribonucleotide(s); PCR, polymerase chain reaction; *Sc*, *Saccharomyces cerevisiae*; SDS, sodium dodecyl sulfate; SSC, 0.15 M NaCl/0.015 M Na_3-citrate pH 7.6.

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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conditions (1.5 min at 92°C, followed by 35 cycles of 2 min at 50°C, 5 min at 60°C, and 1 min at 92°C) were employed to allow for possible mismatches between the primers and the template. The obtained amplified product mixture was cloned, and among the clones analyzed by sequencing, one (pNW2), bearing high sequence similarity to the class-I Chs, was identified and used for further analyses. In order to isolate the entire genomic *chsA* fragment, we used hexamer-labelled pNW2 to probe Southern blots of agarose gels on which various endonuclease restriction digests of *Aq* genomic DNA (prepared from mycelia and pycnidia of *Aq* grown on potato dextrose broth for four days at 25°C) had been resolved. Genomic DNA was prepared from lyophilized mycelia as described by Beth Din and Yarden (1994). Based on the hybridization signals, an *Aq* mini genomic DNA library was prepared by cloning gel-purified 3.2–3.5-kb *Pst*I-*Hin*dIII fragments corresponding to the hybridization signal obtained with pNW2. The fragments were cloned into a multicopy plasmid vector (Bluescript SK-; Stratagene) and transformed into *E. coli* (DH5α). Colony hybridization procedures were carried out (Sambrook et al., 1989), and a colony harboring a *chsA* genomic fragment was isolated and designated pNW25.

(b) Structural analysis of *chsA*

Subcloning and use of custom oligo primers was used in order to perform dideoxy chain-termination sequencing (Sanger et al., 1977). The *chsA* coding region was located by comparison of the predicted aa sequence with that of other *chs* gene products (Beth Din and Yarden, 1994). In order to find the presumed translation start site, it was necessary to isolate an additional, overlapping clone, containing the 5′ coding and untranslated

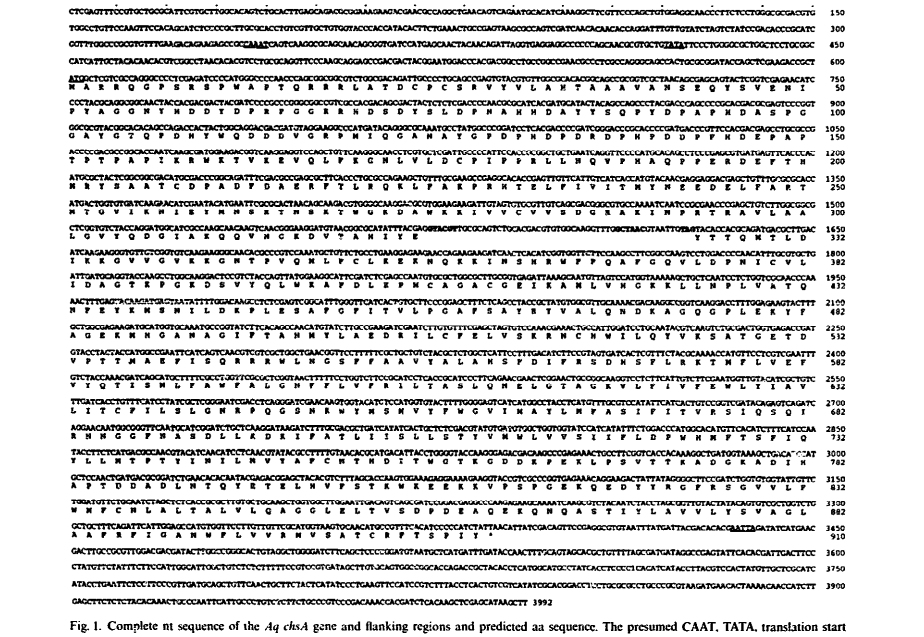

Fig. 1. Complete nt sequence of the *Aq chsA* gene and flanking regions and predicted aa sequence. The presumed CAAT, TATA, translation start and polyadenylation signal sequences are underlined. Conserved intron boundaries and presumed lariat sequences are shown in boldface. The nt sequence reported has GenBank accession No. X86802. Oligo mixtures used for amplification of the initial *chsA* fragment were 5′-GGAA-TTCTNACNATGTAYAAYGARGAT and 5′-CGGGATCCYTTRTATTCRAARTTYTG (where N = A or C or G or T; R = A or G; Y = C or T).

upstream sequences. Using a stratagy identical to that described above, pNW27, a 4.3-kb *Hin*dIII 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 segement 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 boundry 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 chs1* 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 885-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 *Hin*dIII, respectively. In the low-stringency Southern blot, the

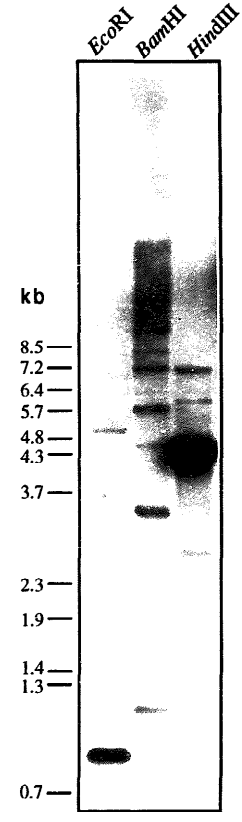

Fig. 2. Low-stringency Southern hybridization of an 885-bp *Eco*RI hexamer-labelled *Aq chsA* gene fragment with digested *Aq* genomic DNA resolved on a 1% agarose gel and blotted on a nylon membrane. Prehybridization and hybridization were performed at 42°C in the presence of 35% formamide/6 × SSC/5 × 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 hybridyzed to the expected size fragments, yet clear evidence of additional bands cross-hybridyzing 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 hybridyzing with *chs*A 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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