Cloning and Primary Structure of the chiA Gene from *Aeromonas caviae*

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Received 21 January 1995/Accepted 8 May 1995

The chiA gene from *Aeromonas caviae* encodes an extracellular chitinase, 865 amino acids long, that shows a high degree of similarity to chitinase A of *Serratia marcescens*. Expression in *Escherichia coli* yielded an enzymatically active protein from which a leader sequence was removed, presumably during transport of the enzyme across the cell membrane.

Hydrolysis of chitin to disaccharides and larger oligomeric saccharides usually takes place extracellularly by the action of chitinases. These enzymes have been shown to play an important role in biological control of soil plant pathogens (1, 6, 8). The number of chitinases isolated from a variety of organisms is rapidly expanding. Recently we compared the primary sequences of 56 known chitinases and demonstrated that many of these proteins have a central region containing several highly conserved domains (10). We have previously found processing of the secreted chitinase A (Chia_Serma) of *Serratia marcescens* in *Escherichia coli* (4, 11). Inbar and Chet isolated from soil a strain of *Aeromonas caviae* which showed a high level of chitinolytic activity (3). This bacterial strain was found to secrete chitinase into the growth medium when grown on chitin as the sole carbon source (3). In this report, we describe the cloning and sequencing of the complete chiA gene from *A. caviae* and comparison of the protein to closely related chitinase proteins.

The chiA gene of *A. caviae* was subcloned as a 4.5-kb HindIII segment in pBluescriptII KS. The strategy used is described in the legend to Fig. 1. To characterize the chitinase activity, we purified the chitinase enzyme from *E. coli*, which does not express chitin-binding activities. Strain XL1-Blue (Stratagene, La Jolla, Calif.), carrying the cloned chiA gene, was grown at 37°C in Luria-Bertani medium in the presence of ampicillin (100 mg/ml), and the chitinase protein was purified by standard procedures. In brief, the cells were collected, sonicated, and spun to remove debris. Colloidal chitin (0.02% [wt/vol]) was added and stirred to allow chitinase binding. The bound chitinase was collected by centrifugation, and the enzyme was dissociated from the chitin by incubation at 4°C in 10 mM HCl for 20 min. Following centrifugation, the free enzyme was dialyzed against 0.2 M sodium acetate buffer at pH 6.8. In preliminary studies, we found that in *E. coli*, part of the enzymatic activity is secreted into the medium while most of the enzymatic activity is cell associated (data not shown). We have previously found that for Chia_Serma, the enzyme accumulates in the periplasmic space unless the expression levels are very high. The enzymatic activity of the protein was assayed by the ability to degrade *p*-nitrophenyl-β-d-N,N'-diacetylchitobiose (described by Kobyl et al. [5]). Optimal enzymatic activity was found over a pH range of 5 to 7 in 0.1 M phosphate buffer. The highest activity was obtained at a temperature of 50°C. The purified enzyme was boiled in the presence of sodium dodecyl sulfate and β-mercaptoethanol and separated by electrophoresis on a sodium dodecyl sulfate-polyacrylamide gel. Staining with Coomassie brilliant blue revealed a single protein band with an estimated molecular mass of about 94 kDa.

The amino acid sequence at the N terminus of the protein band was determined by Edman degradation and found to be NH₂-Ala-Ala-Pro-Ala-Lys-Pro-Thr-Ile-Gly-Ser-Gly-Pro-Thr-Lys. The complete sequence of the chiA gene was obtained, and the deduced amino acid sequence of the chitinase protein (Chia_Aerca) yielded a large open reading frame, 2,595 nucleotides long, coding for 865 amino acids (Fig. 1). A putative ribosome-binding site (UAAGGAG) was found seven nucleotides upstream of the AUG initiation codon. However, the N terminus of the purified protein is identical to the amino acid sequence, starting at the Ala located at position 24 of the deduced amino acid sequence. This finding suggests the presence of a 23-amino-acid leader sequence, which is cleaved away, probably in the process of protein transport to the periplasmic space.

Comparison of the amino acid sequences shows that the chitinase from *A. caviae* (Chia_Aerca) is similar to chitinase A of *S. marcescens* and the chitinase of *Alteromonas* sp. strain O-7 (Fig. 1) (12). The sequence of Chia_Serma was corrected in accordance with the results reported by Perrakis et al. (9). The similarity of the chitinase of *Alteromonas* sp. strain O-7 (Chia_Altso) to chitinases A and B of *S. marcescens* has been previously noted (12). Both Chia_Aerca and Chia_Altso are larger than chitinase A of *S. marcescens* by 301 and 258 residues, respectively. This extension is restricted to the C terminus (Fig. 1).

The three-dimensional structure of the chitinase A protein of *S. marcescens* is composed of three major domains (9). The N-terminal domain (with the exception of the signal peptide) is a 134-amino-acid all-β domain similar to the fibronectin III motif. The major catalytic domain is 331 amino acids long and has an α/β barrel fold. This domain is interrupted by a 74-amino-acid domain with an α + β fold. Because of the high
DNA of *A. caviae* nases. The complete nucleotide sequence of the chitinases from the package, the coding region of the gene was analyzed and the sequence was translated to the single-amino-acid code. The amino acid sequence alignment of the Chia_Aerca subdomain (residues 766 to 804) is 51% identical to the terminal region of Chia_Altso. This subdomain of Chia_Aerca shows significant similarity (36% identity) to the same region of the cellulase gene product (CELB1). This domain also aligns with the last 40 residues of two more *Bacillus* cellulase gene products (CELA [residues 447 to 482] and CELB [residues 368 to 403]) (2). These three domains, CELA, CELB, and CELB1, are almost identical (89% identity) (Fig. 2b). These observations suggest that the C-terminal regions of the *A. caviae* chitinase and the *Bacillus* sp. strain N-4 cellulases are functionally related and may be involved in the ability of these enzymes to degrade highly hydrophobic substrates. The Trp and Tyr residues that are highly conserved between the two C termini of the *A. caviae* chitinase subdomains and the *Bacillus* cellulases possibly provide a hydrophobic protein environment, facilitating binding to chitin.

**Nucleotide sequence accession number.** The sequence described here has been submitted to the GenBank under accession no. U09139.

We thank Tova Treibish and Tevi Melman for assistance. This work was conducted in part at the Irene and Davide Sala Laboratory for Molecular Genetics.

**References**


5. Koby, S., H. Schickler, I. Chet, and A. B. Oppenheim. 1994. The chitinase encoding Tn7-based chiA gene endows *Pseudomonas* fluorescens with the level of similarity of Chia_Aerca and Chia_Altso to Chia_Serma, one can assume that the three enzymes have very similar three-dimensional structures, with few differences reflected by their primary structures. Asp-391 and Glu-315 of Chi-Aerca, which have been suggested to be involved in the acid-base catalysis of chitin, are conserved in Chia_Aerca (9, 13, 14).

The C-terminal extension found in the chitinase of *A. caviae* is similar to that of the chitinase of *Aeromonas* sp. strain O-7 but is longer by 50 amino acid residues (Fig. 1). This part of the protein is very likely organized as a separate domain whose function is unknown. A computer search of the entire protein and of its C terminus suggested that the C-terminal domain contains two small related sequences, about 40 amino acids long. The strong similarity between these amino acid sequences suggests that they perform similar functions and arose by gene duplication (Fig. 2a). Similarly, computer searches of the available data banks revealed that the first C-terminal subdomain of Chia_Aerca (residues 766 to 804) is 51% identical to the terminal region of Chia_Altso. This subdomain shows strong similarity (48% identity) to the last 39 amino acids of the cellulase gene product (CELB1) (Fig. 2b) (2). The second C-terminal subdomain (residues 819 to 856) of Chia_Aerca is involved in the biological control of soil-borne plant pathogens by this bacterium. Soil Biol. Biochem. 23:973–978.