CRYSTALLIZATION NOTES
Crystallization of Recombinant Chitinase from the Cloned \textit{chiA} Gene of \textit{Serratia marcescens}

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The \textit{chiA} gene encoding for the chitinase enzyme from \textit{Serratia marcescens} was efficiently overexpressed under the \textit{pL} promoter and the enzyme was secreted into the growth medium. The chitinase was purified to homogeneity using affinity chromatography on a Phenyl-Sepharose column and the protein was successfully crystallized. The crystals are presently in the form of small needles in space group \textit{C222}_1 and have unit cell dimensions \(a = 204(\pm 0.5)\) Å, \(b = 134(\pm 0.5)\) Å, \(c = 60(\pm 0.5)\) Å. The crystals diffract X-rays to about 3 Å resolution and are suitable for three-dimensional structural analysis.

\textit{Keywords:} chitinase; recombinant; crystallization; \textit{Serratia marcescens}; X-ray

\textit{Serratia marcescens} is a Gram-negative bacterium found to be an effective biocontrol agent of \textit{Sclerotium rolfsii} under greenhouse conditions (Ordentlich \textit{et al.}, 1988). A particular feature of this bacterium is its capability to secrete several chitinolytic enzymes. The major enzyme is encoded by the \textit{chiA} gene (Jones \textit{et al.}, 1986).

The \textit{chiA} gene has been isolated and cloned into the pBR322 plasmid under the control of the \textit{\lambda}\textit{OPL} promoter, producing the expression plasmid ppLCHIA (Shapira \textit{et al.}, 1989). The plasmid has been introduced into \textit{Escherichia coli} strain A2097, carrying the defective prophage \textit{\lambda}\textit{c1857ΔH1ΔBωH1}, to yield the A5745 clone. Chitinase expression is achieved by heating to 42°C for a short time (30 min), followed by continuous incubation at 40°C for several hours. Under these conditions, the cells continuously overexpress the recombinant chitinase, which is secreted into the growth medium as a major protein product (Oppenheim \textit{et al.}, 1990). The mechanism of chitinase secretion into the growth medium is not yet known. Presumably a leader sequence at the amino terminal end of the protein directs the synthesized protein to the periplasm where the leader sequence is removed by an enzymatic activity. Preliminary observations suggest that during the thermal induction, the outer bacterial membrane undergoes certain modifications that lead to secretion of the accumulated chitinase into the growth medium.

The chitinase was isolated from the growth medium by ammonium sulphate precipitation to 80\% saturation and the precipitated protein was recovered by centrifugation. The precipitated proteins were dissolved in buffer A (20 mm-Tris·HCl (pH 8.0), 1 mm-EDTA, 0.1 mm-phenylmethyl sulphonylflouride, 1 mm-ammonium sulphate) and directly applied to a Phenyl-Sepharose CL-6B column (Pharmacia) equilibrated in the same buffer. The bound chitinase was eluted at the very end of a descending ammonium sulphate gradient from 10 to 0.1 M. The chitinase was about 75\% pure as judged by SDS-PAGE. The final purification was achieved using a Mono-Q FPLC column at pH 8.0, where the protein was found in the flowthrough to be nearly homogeneous. Figure 1(a) shows a 12.5\% SDS-PAGE protein profile of purified chitinase (lane 1). The purified protein was further concentrated using a Centricon, dialysed against buffer B.

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(10 mm-sodium phosphate (pH 7.0), 1 mm-EDTA, 100 mm-NaCl) and used for crystallization experiments.

The crystallization of chitinase was screened using the “50 different conditions system” at 4°C and 16°C (Jancarik & Kim, 1991). The protein was at a concentration of 8 to 14 mg/ml in buffer B. Using hanging drop vapour diffusion 2:5 to 5 µl of protein solution were mixed with an equal volume of precipitating solution and equilibrated against 0.75 to 1.0 ml precipitating solution (McPherson, 1982). Useful crystals were obtained in the pH range 7.4 to 8.0 with 1.8 to 1.9 M-ammonium sulphate in the presence of 0.75 to 1% isopropanol. The crystals appeared in an interesting manner. After mixing the drop with isopropanol, a precipitate appeared after 24 hours. The crystals grow from the precipitate in the following 24 hours.

The crystals were mounted in a glass capillary and diffraction data measured using the EMBL X31 beam line in HASYLAB on the DORIS storage ring. Data were collected using a MAR research imaging plate scanner with a crystal to plate distance of 420 mm and radiation of wavelength 1.099 Å (1 Å = 0.1 nm).

The unit cell dimensions, \( a = 204(\pm 0.5) \) Å, \( b = 134(\pm 0.5) \) Å, \( c = 60(\pm 0.5) \) Å, were determined from the diffraction images. The molecular mass of recombinant chitinase, 60,800 Da, and the unit cell volume, \( 1.6 \times 10^6 \) Å\(^3\), give a \( V_m = 34 \) Å\(^3\)/Da. This value suggests that there are one or two chitinase protein molecules in the asymmetric unit. Although the crystals were quite small, the diffraction extended to about 3 Å. We are currently working on improving the crystallization procedure.

In summary, the overexpression of recombinant chitinase in E. coli and the simple and fast purification scheme made crystallization trials possible. In a relatively short time we obtained crystals that diffract to medium resolution. Preliminary X-ray diffraction data are reported for the chitinase crystals and we will start to use the diffraction data for the structural determination of the protein.

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**References**


