Volume 71 (2015)

Supporting information for article:

Expression, purification, crystallization and X-ray diffraction analysis of ChiL, a chitinase from *Chitiniphilus shinanonenesis*

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Figure S1 (A) Multi-cloning site of pENTR-TEV-ccdB, a modified Gateway entry vector with a tobacco etch virus (TEV) protease cleavage site (ENLYFQ|G), derived from pENTR1A (Invitrogen, Thermo Fisher Scientific). (B) Map of the protein expression plasmid vector, pCold-ChiL(41-406), which was constructed using LR reaction of Gateway technology (Invitrogen) with pENTR-TEV-L-ChiL(41-406) and pCold I-DEST, a modified pCold I DNA (Takara Bio) expression vector bearing a cold shock protein cspA promoter, an N-terminal His6-tag, and a Gateway reading frame cassette (Invitrogen). The vector map was created using ApE (A plasmid Editor by M. Wayne Davis) (http://biologylabs.utah.edu/jorgensen/wayned/ape/).
**Figure S2** SDS–PAGE analysis of ChiL(41-406) purification by immobilized-metal affinity chromatography (IMAC). Lanes M, molecular mass standards (protein molecular weight marker (broad), Takara Bio); lane L, lysate of cells; lane P, pellet of cell extract, lanes 1–9, eluted fractions of ChiL(41-406) by IMAC. Proteins were detected by staining with Coomassie Brilliant Blue (CBB).
**Figure S3** SDS–PAGE analysis of ChiL(41-406) purification process: cleavage of a His$_6$ tag by TEV protease. Lanes M, molecular mass standards; lanes 1 and 3, the sample before TEV protease treatment; lane 2, the sample after TEV protease treatment; lane 4, the TEV protease-treated sample after removal of the His$_6$ tag by IMAC. Proteins were detected by staining with CBB.
Figure S4 ChiL(41-406) purification by anion exchange chromatography (AEC).

(A) Chromatogram of ChiL(41-406) purification by AEC using an AKTAexplorer HPLC system with a RESOURCE Q (6 ml) column (GE healthcare). (B) SDS–PAGE analysis of ChiL(41-406) purification by AEC. Lanes M, molecular mass standards; lane 1, ChiL(41-406) protein sample before AEC; lanes 2–11, eluted peak fractions of ChiL(41-406) by AEC. Proteins were detected by staining with CBB.
**Figure S5** Chromatogram of ChiL(41-406) purification by size exclusion chromatography (SEC) using an AKTAexplorer 10S HPLC system with a HiLoad 16/600 Superdex 75 pg column (GE Healthcare). Proteins were detected by staining with CBB.
Figure S6 Crystallizability prediction results of the full-length ChIL(1-410) by XtalPred web server (Slabinski et al., 2007).
**Figure S7** Crystallizability prediction results of the truncated Chl(L41-406) (i.e. the catalytic domain) by **XtalPred** web server (Slabinski *et al.*, 2007).
Figure S8 Crystals obtained by the sitting drop vapor diffusion method with 96-well protein crystallization plates using crystallization screens, Index HT (Hampton Research), PEGRx HT (Hampton Research), and Wizard Classic 1 and 2 block (Rigaku Reagents).