ABSTRACT

Chitinase gene (*chi*) from *Aeromonas caviae* WS7b, which was cloned previously in pUC19 based plasmid vector, has been sequenced. Its completed nuceleotide sequence was determined. The structural gene consists of 2.937 bp with an ORF encoding 865 amino acids. DNA sequence analysis indicated that the gene was cloned without its indigenous promoter. Comparison to other chitinase genes in database shows that the deduced amino acid sequence was nearly identical (97%) to *chiA* from *A. caviae* isolated from Israel. In this study, *chi* was cloned either under a strong constitutive promoter, i.e. PKm^R, as a transcriptional fusion, or under an inducible strong promoter, P*tac*. To introduce the gene fusion/s into *Pseudomonas fluorescens*(Pf), the constructs were cloned into broad host range plasmid vectors such as pRK415, pBBR1MCS2, and pVSP61. Chitinolytic expression of PKm^R-*chi* fusion on chitin agar plate as clear zones could only be demonstrated in a recombinant plasmid based on pBBR1MCS2 replicon, designated as pAM340. pBBR1MCS2, which has higher copy number than the either pRK415 or pVSP61, was used as a vector for the construction of P*tac-chi*, which was designated as pAM630. A PKm^R-*chi* fusion in a suicide vector, pUTmini-Tn5 Sp/Sm, has been constructed as well, and was designated as pAM520. Construction of pAM520 was performed to obtain the *chi* fusion clone that integrated into Pf chromosome, and that will be stably maintained as a single copy number. From the three transcriptional fusion recombinants, only *Escherichia coli* harboring pAM520 recombinant could not demonstrated chitinolytic activity. To mobilize the *chi* fusion recombinant into Pf, gene tranfer was performed employing bacterial conjugation. Based on the time of incubation required to display transparent zone on chitin agar plate, the *chi* gene expression in Pf demonstrated much higher activity than when the same construct was present in *E. coli*. Chitinase activities were determined spectrophotometrically using colloidal chitin azure as a specific substrate. The results showed that the expression of *chi* fusion in *E. coli* was significantly

influenced by plasmid copy number as shown in *E. coli* (pWS506), which expressed intracellular chitinase activity (4.17 U/mg protein) several folds higher than that of medium copy plasmid in *E. coli* (pAM630) (1.05 U/mg protein). Pf5100 (pAM630) overnight culture accumulated chitinase in the intracellular fraction which is three folds higher (2.53 U/mg protein) than the control strain. There was no significant chitinase activity in the extracellular fraction of Pf5100 (pAM630). From these results, it could be concluded that the expression of *chi* was influenced by gene copy number of constructed gene, as well as the nature of bacterial host used in this study.