

ภาษาอังกฤษ

ส่วนที่ 1

Research Title “Cloning of a gene encoding keratinase from hyperthermophilic *Fervidobacterium* species isolated from hot springs in Thailand, for feather waste treatment from slaughterhouses”

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ส่วนที่ 2 Abstract (ไม่เกิน 250 คำ)

Fervidobacterium sp. FC2004 and *Fervidobacterium* sp. FA004 are extreme thermophilic bacteria belonging to order Thermotogales which can degrade feathers. This research aims to search a gene encoding keratinase from genomic DNA of a *Fervidobacterium* sp., and to clone the gene for future applications. In this study, a putative gene encoding a serine protease (522 amino acids) named “proA1” (named protein as ProA1) is identified in chromosome of *Fervidobacterium* sp. FC2004 using shotgun sequencing methods. BlastP analysis reveals that amino acid sequence of ProA1 is similar to large peptidase S8_subtilase superfamilies of serine proteases (size range of 682 – 710 amino acids) of *Fervidobacterium pennivorans* DSM 9078^T (WP_064012406.1; 52 %), *F. islandicum* AW-1 (WP_052107197.1; 48 %) and *F. nodosum* Rt17-B1T (WP_011994230.1; 48 %). 3-D model of ProA1 is similar to fervidolysin (a large S8_subtilase). ProA1 molecule is composed of a signal peptide (approx. 20 amino acids), PD (propeptide domain), CD (catalytic domain) and a domain similar to SD or SD-like (substrate binding domain), but the second substrate binding domain (SD2) was not detected. The CD region of ProA1 contains a catalytic triad of D169, H207

and S379, and a calcium binding site that is composed of E136, D178, D220, K222 and V224. The results suggest that ProA1 might be produced as ProA1 precursor (proProA, inactive enzyme) that requires cleavage off PD to become mature ProA1 (active enzyme). In this study, DNA segments encoding ProA1, CD and SD-like are amplified using primers confirming the existing of *proA1* gene sequence in the chromosome of *Fervidobacterium* sp. FC2004. Difficulty in cloning the gene into an expression vector might result from toxicity of the mature ProA1 and unknown intrinsic factors from the gene and host. Although, *Fervidobacterium* sp. FC2004 is able to degrade native feather and SD-like of ProA1 has similar 3D-structure to target specific binding domain of collagen binding protein and fibronectin binding protein, function of SD-like in binding to its keratin substrate is unclear.

Key words : keratinase, serine protease, thermostable, extreme thermophile, *Fervidobacterium*