

Site-Directed Mutagenesis of Proline-285 to Leucine in *Cephalosporium acremonium* Isopenicillin N – Synthase Affects Catalysis and Increases Soluble Expression at Higher Temperatures

Paxton Loke and Tiow-Suan Sim*

Department of Microbiology, Faculty of Medicine, National University of Singapore, MD4/4A, 5 Science Drive 2, Singapore 117597. Fax: 65-7766872. Email: micsimts@nus.edu.sg

* Author for correspondence and reprint requests

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The conversion of δ -(L- α -amino adipyl)-L-cysteinyl-D-valine (ACV) to isopenicillin N is dependant on the catalytic action of isopenicillin N – synthase (IPNS), an important enzyme in the penicillin and cephalosporin biosynthetic pathway. One of the amino acid residues suggested by the *Aspergillus nidulans* IPNS crystal structure for interaction with the valine isopropyl group of ACV is proline-283. Site-directed mutagenesis of the corresponding proline-285 to leucine in *Cephalosporium acremonium* IPNS resulted in non-measurable activity but an increased soluble expression at higher temperatures in a heterologous *E. coli* host.