

Motifs in the C-terminal region of the *Penicillium chrysogenum* ACV synthetase are essential for valine epimerization and processivity of tripeptide formation

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The first step in the penicillin biosynthetic pathway is the non-ribosomal condensation of L- α -aminoadipic acid, L-cysteine and L-valine into the tripeptide γ -(L- α -aminoadipyl)-L-cysteinyl-D-valine (ACV). This reaction is catalysed by the multienzyme ACV synthetase (ACVS), which is encoded in the filamentous fungus *Penicillium chrysogenum* by the *pcbAB* gene. This enzyme contains at least ten catalytic domains. The precise role of the C-terminal domain of this multidomain NRPS still remains obscure. The C-terminal region of ACVS bears the epimerase and the thioesterase domains and may be involved in the epimerization of LLL-ACV to LLD-ACV and in the hydrolysis of the thioester bond. In this work, the conserved motifs 3371EGHGRE 3376 (located in the putative epimerase domain) and 3629GWSFG 3633 (located in the thioesterase domain) were changed by site-directed-mutagenesis to LGFGLL and GWAFG, respectively. In addition, the whole thioesterase domain (230 amino acids) and the different part