“Characterization of the Product of ORF12, a Hypothetical Gene Essential to the Clavulanic Acid Biosynthesis”

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Streptomyces clavuligerus synthesizes diverse secondary metabolites, such as cephamycin-C, and clavulanic acid (CA), among many others. CA is a *ß*-lactam compound of clinical and industrial importance exhibiting a remarkable *ß*-lactamase inhibitory activity. *?*-lactamases are enzymes responsible for the resistance that some bacteria show against common *ß*-lactamic antibiotics, like penicillins and cephalosporins. Because of this *ß*-lactamase inhibitory activity, CA has been successfully used in combination with common *ß*-lactamic antibiotics in the treatment of infections caused by otherwise antibiotic-resistant bacteria. Thus, it is of industrial productivity interest the knowledge of the biochemical regulation of CA biosynthesis. However, its biosynthesis is not yet completely known, still missing undescribed enzymatic steps. This work presents the cloning, heterologous expression, purification and initial characterization of the protein encoded by ORF12, a putative gene that has been proven essential for the CA biosynthesis pathway in *S. clavuligerus*. Activity assays for the ORF12 recombinant product confirmed a sequence predicted *ß*-lactamase activity and also showed a possible bacterial proliferation triggering activity. Circular dichroism measurements indicated a typical *a+ß* or *a/ß* spectrum. Crystallization conditions were obtained and one dataset was collected up to 2.3Å resolution. Even using several molecular replacement procedures it was not possible to solve the structure by MR, due to the lack of significant sequence identity to any other *ß*-lactamase in the PDB, leading to the belief that it might show a somewhat distinct structure. Efforts are now being conducted to obtain better suited crystals to perform SAD experiments and to further define its activity.