PRIMARY METABOLIC PATHWAYS PROVIDING BUILDING BLOCKS IN FK506/520 BIOSYNTHESIS

Hrvoje Petković[1,2], Gregor Kosec[1], Dušan Goranovič[1], Štefan Fujs[1], Marko Blažič[1,3], Jaka Horvat[1], Enej Kuščer[1]

[1] Acies Bio d.o.o.,Tehnoloski Park 21, SI-1000 Ljubljana, Slovenia
[2] Instituto de Biomedicina y Biotecnología de Cantabria (IBBTEC)
Universidad de Cantabria-CSIC-SODERCAN, Avda. Cardenal Herrera Oria, s/n.
39011 Santander, Cantabria, Spain
[3] University of Ljubljana, Biotechnical Faculty, Department of Food Science and Technology, Jamnikarjeva 101, SI-1000 Ljubljana, Slovenia

Hrvoje Petkovic: hrvoje.petkovic@aciesbio.com

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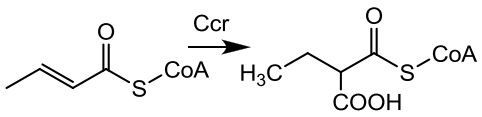
Introduction. The medically important immunosuppressant FK506 and its biogenetically related natural product FK520 and rapamycin are synthesized by combined polyketide synthase (PKS) / non-ribosomal peptide synthetase (NRPS) systems in several species of *Streptomyces*. In addition to the high complexity of this combined PKS/NRPS system, the diversity of building blocks used for the biosynthesis of these complex metabolites is also exceptionally diverse. In addition to the usual malonyl and methylmalonyl-CoA, more unusual building blocks, such as shikimate-derived starter unit, amino acid derived pipecolic acid, methoxymalonyl-CoA, ethylmalonyl-CoA and unusual 5-carbon allylmalonyl-CoA are incorporated into the FK506 structure. Thus, primary metabolic pathways involved in the provision of these building blocks play a key role in the yield of FK506 and biosynthesis in undesired structurally related metabolites. We were particularly interested in the primary biosynthetic pathways involved in the provision of the ethylmalonyl-SCoA and the unusual extender unit allylmalonyl-SCoA.

Methods. The entire genome of the *Streptomyces tsukubaensis* NRRL18488 was sequenced using the 454 pyrosequencing method (Macrogen Inc., S. Korea). We have identified primary metabolic pathways putatively involved in the provision of key primary metabolites (FK506 building blocks) using standard bioinformatics tools, such as BLAST program package and KEGG database. Gene inactivation and *in-trans* gene complementation and over-expression experiments were carried out to evaluate putative functions of the selected genes or gene pathways.

Results. Among others, we identified a group of genes encoding biosynthesis of the extender unit, which forms the allyl group at the carbon 21 of FK506. Based on our results, we proposed a biosynthetic pathway for the provision of this unusual five-carbon extender unit, which is carried out by a novel diketide synthase complex, small but rather complex biosynthetic machinery (Goranovic et al., 2010). This machinery shows significant promiscuity, resulting in the simultaneous production of 5 carbon allylmalonyl-CoA as well as ethylmalonyl-CoA (Kosec et al., 2012)



crotonyl-CoA ethylmalonyl-CoA

Fig.1: A key reaction in the biosynthesis of ethylmalonyl-CoA is catalysed by crotonyl-CoA carboxylase/reductase

We have also identified in the genome of *S. tsukubaensis* an operon of five genes, encoding the relatively recently discovered “ethylmalonyl-CoA pathway” (Erb et al., 2007), involved in acetate assimilation in many *Streptomyces* species. Interestingly, this operon contains a crotonyl-CoA carboxylase/reductase gene, a homologue of which is also involved in the provision of allylmalonyl-CoA for FK506 biosynthesis. Our results show that the primary metabolic ethylmalonyl-CoA pathway genes can also be involved in the provision of ethylmalonyl-CoA, which is incorporated by the FK506 PKS, resulting in FK520.

Conclusions. The biosynthesis of ethylmalonyl-CoA, the building block for the biosynthesis of the FK506-related metabolite FK520 in *S. tsukubaensis* is rather complex and is shared between primary metabolic pathways, and enzymes encoded by genes of the FK506 gene cluster.

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