METABOLIC INTERLOCK BETWEEN BRANCHED CHAIN AMINO ACID AND PROLINE BIOSYNTHESIS IN *STREPTOMYCES* GENUS: THE ROLE OF ENZYME PROMISCUITY

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Introduction. Catalytic promiscuity plays a role in the divergent evolution of enzymes by providing a head start in activity and a possible selective advantage to a duplicated gene (1,2). In *Streptomyces coelicolor* mutation of *proC* gene (pyrroline-5-carboxylate reductase, P5CR, proline biosynthesis) yields a prototrophic strain (3). Since no obvious *proC* homologue was found in *S. coelicolor*, we propose that ketol-acid reductoisomerase (KARI, *ilvC*, branched-chain amino acid biosynthesis), with a paralogue in this genome, is a promiscuous enzyme that carries out the P5CR activity. Therefore, the aim of this work is to determine the metabolic and evolutionary connection between proline and branched-chain amino acid biosynthesis and prove through an in vivo and in vitro approach that this connection occurs via enzyme promiscuity.

Methods. Search for remote homologues enzymes of P5CR were made by structural homology and sequence profiles using Dali server and PSI-BLAST respectively. Once was established KARI as a candidate enzyme, to demonstrate the connection between proline and branched-chain amino acid biosynthesis we selected a set of KARI homologues from a phylogenetic analysis of the Actinobacteria group. Eleven KARI homologues were selected and functionally characterized. For in vitro analysis, the enzymatic assays were made using native substrates of KARI reaction, likewise, the substrate for P5CR activity (see results). All substrates were chemically synthesized. For in vivo demonstration, a *S. coelicolor* triple mutant was constructed and the activity complementation assays with the set of KARI homologues were made.

Results. *Search of P5CR remote homologues:* From the structural (Dali server) and PSI-BLAST search of P5CR homologues we obtained a set of reductases and dehydrogenases enzymes, all of them belonging to the superfamily of 6-phosphogluconate dehydrogenase, that itself belongs to the large group of NAD(P)-binding Rossmann-like dehydrogenases. We selected KARI (encoded by *ilvC* gene), as a candidate enzyme since both (P5CR and KARI) are involved in amino acid biosynthesis. *Selection of KARI homologues*: Phylogenetic reconstruction and analysis of the genomic context allowed us to select eleven *ilvC* homologues with a probable evolutionary relationship and showed us that KARI has a paralogue with high sequence identity in a few *Streptomyces* species.

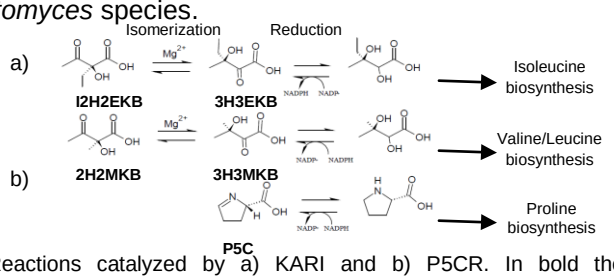

Fig.1.Reactions catalyzed by a) KARI and b) P5CR. In bold the intermediaries of the reaction that were chemically synthesized; 2-hydroxy-2-ethyl-ketobutyrate (2H2EKB), 3-hydroxy-3-ethyl-ketobutyrate (3H3EKB) (isoleucine biosynthesis). 2-hydroxy-2-methyl-ketobutyrate (2H2MKB), 3-hydroxy-3-methyl-ketobutyrate (3H3EKB) intermediaries for valine and leucine biosynthesis, and pirrolyne-5-carboxylate (P5CR) for proline biosynthesis.

In vivo and in vitro demonstration of metabolic interlock: construction of a triple *S. coelicolor* mutant (*ΔilvC1, ΔilvC2, ΔproC*) was carried out. Given the characteristics of mutagenesis method we found counterselection to mutate the three genes in the same strain. Proof of this is that the triple mutant besides auxotrophy for branched-chain amino acids and proline had an unexpected arginine auxotrophy, for this reason we propose that genome sequencing of this mutant is important to understand what kind of chromosomal rearrangements resulted when these metabolic pathways are connected. Moreover, enzymatic assays demonstrate in vitro P5CR activity for all KARI homologues. Kinetic parameters for native KARI substrates suggest a subfunctionalization event since the paralog of the gene has a reduced catalytic efficiency.

Conclusions. In this study, we showed the interaction mediated by enzyme promiscuity between amino acid biosynthesis in *Streptomyces*. Our results shown that the paralogous relationship supporting the functional overlap of three enzymes can be unveiled by bioinformatic analyses and confirmed with a biochemical and genetic approach that help to understand the importance of the this enzyme property in the fitness of the organisms.

References.

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