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September 22-24, 2016 Phoenix, USA

## Reduction of feedback inhibition in homoserine kinase (ThrB) enhances the L-threonine biosynthesis

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-threonine is a limiting amino acid in livestock diets and its deficiency results in not only malnutrition of the animals Libut also releasing increased volumes of nitrogen compounds to the environment. Addition of L-threenine into livestock feed is therefore important for healthier animals as well as a cleaner environment. Moreover, L-threonine is utilized in both pharmaceutical and cosmetic industries. Currently, L-threonine is produced by E. coli, which makes the purification of L-threonine difficult because it produces endotoxins. Thus, we seek to over-produce L-threonine by using Corynebacterium glutamicum, a GRAS (generally regarded as safe) microorganism. C. glutamicum produces L-threonine from aspartate through an enzymatic pathway involving aspartate kinase (LysC), aspartate semialdehyde dehydrogenase (Asd), homoserine dehydrogenase (Hom), homoserine kinase (ThrB) and threonine synthase (ThrC). Among these, LysC, Hom and ThrB are feedback inhibited by the end-product, L-threonine. Releasing the feedback inhibition in LysC and Hom through mutating their allosteric site has successfully increased L-threonine biosynthesis. However, it has been unsuccessful to remove the feedback inhibition in ThrB because L-threonine inhibits the enzyme by competing with L-homoserine (substrate) for the same active site. To genetically separate the catalytic activity and the feedback inhibition in ThrB of C. glutamicum, we mutated a residue at the gate of the active site into various amino acids such as hydrophobic residues (Leu and Val) and hydrophilic residue (Ser and Gly). Enzymatic kinetics with the wild-type and mutant forms of ThrB showed that one specific mutation increased Ki for L-Threonine about 5-fold while it increased Km for homoserine only 2-fold. More importantly, when we introduced this mutation into C. glutamicum and E. coli, it increased approximately 20% of L-threonine production. Our approach can be applied to other metabolic enzymes that are similarly regulated by competitive feedback inhibition.

## Biography

Choong-Min Kang has completed his PhD in Microbiology from UC Davis and Postdoctoral studies from Children's Hospital Boston, Harvard Med School. He is currently an Associate Professor at California State University, USA. He has published more than 27 papers in reputed journals.

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