SOLUBLE EXPRESSION OF GENES FOR ENZYMES IN ESCHERICHIA COLI

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During the studies to exploit enzymes from plants and animals, we found that the gene for hydroxynitrile lyase from cassava (*Manihot esculenta*) is expressed insolubly in *E. coli*, but genes for variants in which His103Leu or three Lys positions are replaced with Pro are expressed soluble as active enzymes. To cope with the problem of insoluble expression in genetic engineering and to prove the generality of such mutations, we proposed "alpha-helix rule" and "INTMSAlign-HiSol method" to predict soluble mutation hotspots for producing enzymes as active entities, which has been a problem in genetic recombination technology. The INTMSAlign software, which is the core of this technique, targets enzyme and protein primary sequence databases and uses the multiple sequence alignment (MSA) method to rapidly estimate the conservation of amino acid residues[1]. By using this software, the location of hot spots and substituted amino acids can be predicted in a short time not only for soluble expression of enzymes in *E. coli*, but also for changing the properties of the enzymes.

Some successful examples for enzyme application in combination with other digital information would be introduced in this presentation.

1. Developed by Prof. Shogo Nakano of University of Shizuoka.