

Metabolic engineering of corynebacteria to create a producer of L-valine

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L-valine, along with L-leucine and L-isoleucine (valine, leucine, isoleucine), is a proteinogenic branched-chain amino acid (BCAA). As an essential amino acid, valine should be present in the diet of humans and animals: not only is it a building material for proteins, but it also regulates protein and energy metabolism, participates in neurotransmission, and is a donor of an amino group for glutamate biosynthesis.

Valine is used in food industry, medicine and cosmetics, but its main use is as a feed additive. Adding valine to fodder, alone or mixed with other BCAAs, allows for the use of feeds with less crude protein, increases the quality and quantity of meat in pigs and broiler chickens, as well as improves the reproductive functions of farm animals.

The main method of industrial production of valine is biotechnological, the key element of which is strains-producers. Despite the fact that the market for valine is constantly growing, this amino acid is not yet produced in our country. Given the circumstances, the task of organizing local valine production and, consequently, the task of creating industrial producers, are of paramount importance.

One of the most common basic microorganisms for the creation of amino acid producers is the soil bacterium *Corynebacterium glutamicum*. It is well studied from the genetic, biochemical and metabolic points of view, it is safe and easy to grow. Nowadays, producer strains based on *C. glutamicum* are constructed using metabolic engineering, a method based on the analysis of metabolic pathways that allows selection of the most suitable target genes among genes with known functions.

In the present study, the features of corynebacterial BCAA metabolism will be reviewed and strategies for modifying the *C. glutamicum* genome to increase valine production will be proposed. Using several valine-producing strains as examples, we will demonstrate how the level of valine production is affected by modification of the genes responsible for its biosynthesis (*ilvBNCDE*), for the availability of the process precursor (pyruvate) and cofactor (NADPH), for BCAA import and export (*brnQ* and *brnFE* respectively), for the formation of by-products (leucine, isoleucine, D-pantothenate and others).

In addition, the results of the analysis of the complete genomes of valine-producing strains of *C. glutamicum* obtained by nondirectional mutagenesis will be presented. Such an analysis not only makes it possible to identify previously unknown mutations in known genes related to valine production, but also to expand the range of possible target

genes. The results of experimental testing of the effect of new modifications on valine production by *C. glutamicum* strains will also be presented.

The approaches we have tested are of interest not only in terms of creating a corynebacterial valine producer, but may also be applied in the development of producers of other BCAA and related compounds.

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