MOLECULAR GENETIC STUDIES ON SOME AMINO ACIDS PRODUCTION FROM BACTERIA

BY

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B.Sc. Agric. Sc. (Genetics), Ain Shams University, 2000

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ABSTRACT

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In this study three different strains of *Corynebacterium glutamicum i.*e., C*. glutamicum xerioss, C. glutamicum* (ATCC 21513) and *C. glutamicum* (ATCC 13032) were obtained. The production of amino acids lysine and methionine by these strains was determined. *C. glutamicum* (ATCC 13032) gave the highest level of the two amino acids, 0.083 g/l lysine and 0.45 g/l methionine. This strain was subjected to N-methyl-N-nitro-N-nitrosoguanidine mutagenesis. Several mutants with different abilities to produce the desired amino acids were obtained. Of these mutants were isolated and selected based on resistance to lysine analogue (s-aminoethyl-cysteine) and the other were isolated and selected based on resistance to methionine analogue (selenomethionine). The amount of amino acids produced by the first step mutagenesis comparing with original strain was reached to more than 86.7 times in the case of lysine and three times in the case of methionine, (7.2 g/l and 1.4 g/l for lysine and methionine, respectively). In the second step mutagenesis, the amount of the amino acids produced was highly increased, it was 34.84 g/l and 29.14 g/l for lysine and methionine, respectively, i.e. the production capacity was increased 419.6 times in the case of lysine and 63.6 times in the case of methionine comparing with the original strain. To confirm the genetic changes and find molecular markers for high production, molecular and biochemical fingerprints including REP-PCR and SDS-PAGE were carried out on the resultant mutants.

Key words: *Corynebacterium glutamicum*, mutagenesis, NTG, lysine**,** Methionine, SDS-PAGE, REP-PCR, BOX-PCR.

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Amino acids are the building blocks of protein, and are utilized by every cell in the body for a variety of crucial functions. Normally, the amino acids necessary for animals and humans are obtained from food sources, particularly those high in protein content. The body breaks these proteins down into their constituent parts of amino acids; and then our cells use these amino acids to build specific types of protein that needs. There are two types of amino acids, essential and nonessential. Essential ones are defined as those that the body cannot synthesize them and must obtain them from food sources (or supplements). Nonessential ones, on the other hand, can be synthesized by bodies, but can also be consumed as supplements **(Jalkanen, *et al.*, 2004).**

Amino acids are used in a variety of ways, most of them associated with food. Amino acids are also used as food and feed additives. As well known, the farm animals cannot synthesize lysine, methionine, tryptophan, leucine, valine, phenylalanine, threonine, and arginine as essential amino acids. They must obtain them from the proteins in their food. However, the less expensive, more abundant sources of food proteins, the seed of crop plants, are rather deficient in some of the essential amino acids, particularly lysine, methionine, and tryptophan **(Soda, *et al.*, 1983).** For example, the corn proteins contain only 0.2% lysine, whereas the animal meat proteins contain 2.6% lysine. The nutritional value of plant seed proteins can be increased significantly if the seed proteins can be fortified with the deficient amino acids. Another important use of amino acids is as a starting material for the production of other compounds. One of the most famous is the sweetener aspartame, which produced from L-phenylalanie and L-aspartic. Finally, some amino acids have medical uses, whether for specific therapeutic effects or as components of intravenous infusions given to patients who have difficulty taking in