International Journal of Sciences: Basic and Applied Research (IJSBAR)

ISSN 2307-4531
(Print & Online)

http://gssrr.org/index.php?journal=JournalOfBasicAndApplied

Increase of Lysine Production in *Corynebacterium glutamicum* by using Gamma Irradiation

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Abstract

Corynebacterium glutamicum has been developed mostly by repeated random mutation and selection. In order to study the molecular mode of action on increase of lysine production in *Corynebacterium glutamicum* exposed to gamma rays at doses of 0,100, 200 and 300 Gy, mutant strain was selected first by bacteria counts and lysine production level, then it was used in molecular experiments. Wild and mutant strains of these bacteria were cultured in liquid media and proteins were extracted. Protein concentration was measured according to Bradford assay and sufficient amount of protein was loaded in Polyacrylamide gel electrophoresis. Electrophoresis of proteins was performed on 13% acrylamide running gel (1.0mm×110mm×140mm) with 3.75% acrylamide stacking gel. Based on the results of this study, gamma irradiation at dose of 200 Gy could induce mutation in *Corynebacterium glutamicum* and increased lysine production by 67 % in mutant strain. Electrophoretic techniques showed changes in key enzymes of lysine biosynthesis pathway (aspartate kinase). Changes in the protein pattern are indicative of a change in the direction of lysine production; this change can be explanation for the increase in lysine producing mutant strain.

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Keywords: *Corynebacterium glutamicum*; Gamma irradiation; Lysine; Polyacrylamide gel electrophoresis.

1. Introduction

Corynebacterium glutamicum is a Gram-positive soil bacterium used for the industrial production of amino acids [8,9]. In the wild type due to different mechanisms of metabolic regulation, lysine is synthesized in small amounts and can't be used for industrial production, but production can be increased with the manipulation of metabolism [17]. Mutation is a successful method to obtain high producing strains. By the use of different mutant strains more than 1 000 000 tons of L-glutamate and 450 000 tons of L-lysine are produced per year beside smaller amounts of the industrially less important amino acids L-alanine, L-isoleucine, and L-proline. While *C. glutamicum* is generally recognized as nonhazardous organism, which is safe to handle, phylogenetically it is closely related to pathogenic species, *e.g. Corynebacterium diphtheriae* or *Mycobacterium leprae* [9,15]. Commrcially potent amino acids producrs of *C. glutamicum* have been developed mostly by repeated random mutation and slection [8,9,19]. Mutation of the successful methods is to obtain high-producing strains. 10 enzyme, lysine biosynthetic pathway that control the activity of this pathway in wild type aspartate kinase is strongly inhibited in the presence of lysine and threonine In the mutant strains, Feedback Control of aspartate kinase and aspartate kinase resistant to feedback is removed, resulting in increased amounts of lysine [9]. The purpose of this study was to use gamma ray as a mutagen, to create high-producing bacterial strains. In order to investigate the changes in the pattern of protein was used to the technique polyacrylamide gel electrophoresis.

2. Materials and Methods

2.1 Bacterial preparations order to radiation

C. glutamicum wild-type strain ATCC 13032 was grown aerobically on a rotary shaker 30˚C in TSB medium. Samples in 20 replicate gamma irradiated with doses of 0, 100, 200 and 300 Gy using a cobalt-60 gamma cell (AEOI, Tehran Irradiation Application Center, Iran) at a dose rate of 300 Gy per min.

2.2 Bacteria selected suitable

After 24 hours of fermentation in bacterial isolates from the irradiated samples, bacterial population was obtained using standard log CFU/ml against optical density. Using an index calculated by dividing the amount of lysine production (mg/mL) by log CFU/ml (lysine production divided by population), samples with index values above control levels (at 200 Gy) were selected and used for inducing mutations for molecular tests.

2.3 sample preparation

In order to extract the bacterial protein; *C. glutamicum* ATCC 13032 was grown on a rotary shaker at 30˚C, the cells were harvested by centrifugation , in a centrifuge during each supernatant was discarded and the pellet was collected. The resulting pellet was washed in phosphate buffered saline. The cell lysate using a variety of physical and chemical methods, including the use of lysis buffer, sonicator, glass beads and freeze- dfreeze was

used. Then the proteins were extracted from the mutant and wild strains of bacteria were lyophilized. Optimal protein concentration for polyacrylamide gel electrophoresis was determined according to the Bradford method [1] and patterns of bacterial protein were analyzed using the technique polyacrylamide gel electrophoresis by Lammeli method [9].

3. Results

Due to the low amount of lysine production in *Corynebacterium glutamicum* wild type, mutant bacterial strains were created using various doses of gamma ray and density of bacteria and the production of lysine in bacteria and compared to other bacteria in the mutant. Wild type had the lowest population density and the lowest amount of lysine. Irradiation results showed changes in bacterial density and lysine production in the mutant strain. Bacterial population increased in irradiated samples. Also, lysine production was higher in mutant bacteria than in the wild strain and the trend was linear up to dosage 200 Gy and decreased at dose level 300 Gy. The mutant strains had the highest population densities of strain were irradiated with 100 Gy and the maximum amount of lysine was observed in strain were irradiated with 200 Gy.The findings showed that the highest amount of lysine production was 16/38 mg/mL, which was observed in bacteria irradiated with gamma radiation at dose level 200 Gy (Figure1).

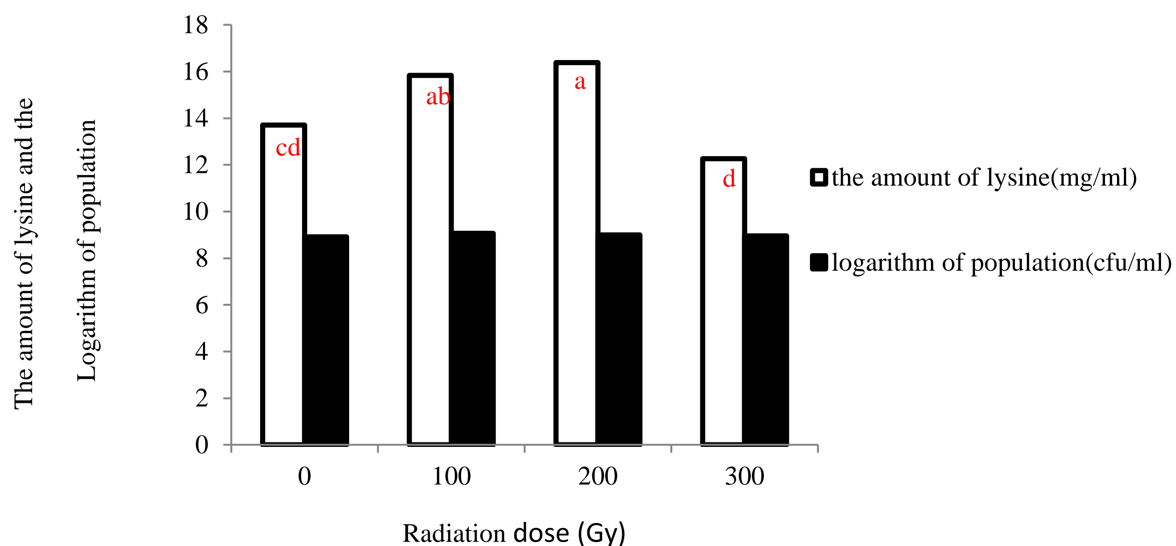

Figure1: lysine production rate and the logarithm of population of bacteria before and after irradiation

The pattern of protein subunits in the wild strain of *Corynebacterium glutamicum* was studied. Protein electrophoresis showed changes in key enzyme of lysine biosynthesis (aspartate kinase). Protein subunit structure of wild and mutant strains of *C. glutamicum* indicate that the small subunit of aspartate kinase undergoes some changes in the mutant strain (Figure2). In this study, three major protein types were observed in *C. glutamicum*, pertaining to enzymes aspartate kinase (58 and 18 kDa), homoserine dehydrogenase (46 kDa), and homoserine kinase (28 kDa). Densitometry findings suggest that three main proteins of *C. glutamicum*, i.e. aspartate kinase (28%), homoserine dehydrogenase (16.8%) and homoserine kinase (18%), constitute about

62.8% of the total proteins of *C. glutamicum*. The comparison of key Enzyme in wild and mutant strains indicated a 38 percent decrease of Density of Aspartate kinase large subunit in mutant strain compared to the wild strain, and 60 percent increase of Density of the same enzyme's small subunit in mutant strains.

Change in the small subunit of aspartate kinase could be due to an increase of the rate of production of lysine in these strains. So that in this mutant strain is capable of producing 38 g L-lysine for 3 days, respectively, whereas the wild type produced in the same period, 13 g L-lysine. Protein subunit structure of wild and mutant strains of *C. glutamicum* indicate that the small subunit of aspartate kinase undergoes some changes in the mutant strain (Figure2).

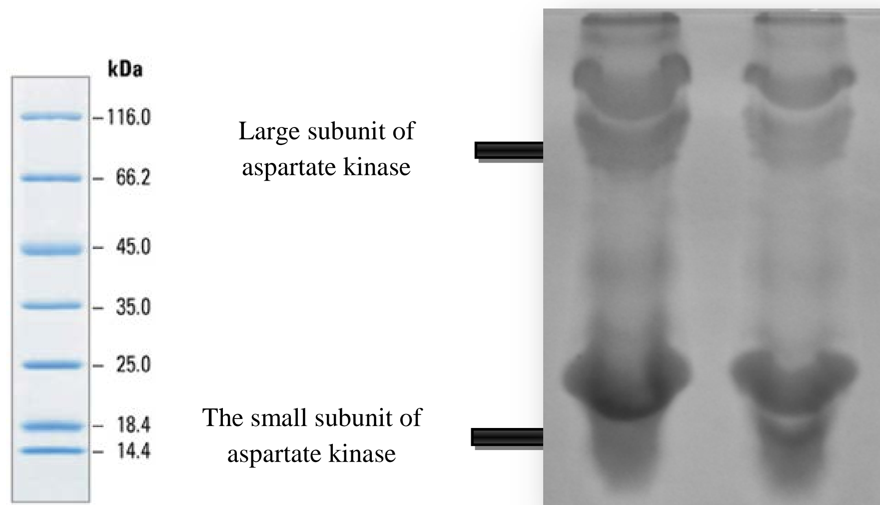

Figure2: pattern protein subunits of wild type (left) and mutant (right) *Corynebacterium glutamicum*

4. Conclusions

In the last few years the biochemistry, physiology, and molecular biology of amino acid biosynthesis in *C.glutamicum* have been intensively studied; and especially by development and application of genetic techniques, a lot of information has emerged [2].

The purpose of this study was to use gamma ray as a mutagen, to create high-producing bacterial strains. In order to investigate the changes in the pattern of protein was used to the technique polyacrylamide gel electrophoresis. The molecular weight calculated in the current study for these subunits is near to the figures reported by [12]. The small differences in molecular weights observed between the current study and the investigation of [12] can be attributed to various factors, including heterogeneity, sample electrophoresis conditions, gel concentration, and the molecular weight of protein markers.

Furthermore, they reported that aspartate kinase subunits are connected to each other by disulfide bridges, producing a protein with molecular weight of 280 kDa. The β-mercaptoethanol present in the extraction buffer results in the reduction of disulfide bonds and separation of aspartate kinase subunits. Protein subunit structure of wild and mutant strains of *C. glutamicum* indicate that the small subunit of aspartate kinase undergoes some changes in the mutant strain. These changes could be an expression of feedback regulation of allosteric changes. Given the importance of lysine production by *Corynebacterium glutamicum* and widely lysine, we suggest proteome analyzed using electrophoresis 2-D page to Base on gel electrophoresis of proteins appear to alter the expression of specific proteins and enzymes aspartate check that the addition of proteins which are involved in the production of lysine.

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