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Fermentative Production of L-Lysine: Bacterial Fermentation-I

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Lysine is an essential, economically important amino acid used as food and feed supplement. It has also some pharmaceutical applications in the formulation of diets with balanced amino acid composition and in amino acid infusions. Chemical, enzymatic and fermentation processes have been used to synthesize lysine. This review outlines the efforts of various researchers, which provide useful information regarding the fermentative production of lysine by bacteria. It also discusses different methods, including the development of new auxotrophic mutants and optimization of culture conditions used in order to improve the total yield and quality of lysine.

Key words: L-Lysine, fermentation, new auxotrophs, culture conditions, yield

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Introduction

Lysine is one of the essential amino acids not synthesized biologically in the body. Children and growing animals have a high requirement of lysine, since it is needed for bone formation. Lysine is generally recognized as the most deficient amino acid in the food supply of both man and domestic meat producing animals. Since animal feed, such as grain and defatted oil seeds contain only small quantities of lysine, poultry, cattle and other live stocks are unable to synthesize this amino acid. So it must be added to these feed stuff to provide adequate diet (Tosaka *et al.*, 1983).

Dagley and Mangioli (1950) described the excretion of small amount of alanine, glutamic acid, aspartic acid and histidine in a culture of *E. coli*. They also found that addition of ammonium salt in excess of that required for growth resulted in increased amino acid production. The principles of the fermentative method quickly gained acceptance, and systematic work soon began on the production of other amino acids. This marked the birth of the amino acid fermentation industry (Aida, 1972).

Research on the possible utilization of wild strain revealed that many microorganisms, such as bacteria, yeast, filamentous fungi and actinomycetes, accumulated amino acids in culture containing a supplementary source of nitrogen. Many efforts have recently been devoted to elucidating the mechanisms of microbial production of amino acids. The most outstanding results concern metabolic regulation and amino acid transport. The biosynthetic pathways of most amino acids are now well documented, and the focus of attention has therefore, moved to metabolic control and its break down, including the genus and species specificity of the phenomenon (Aida, 1972).

Protein production by microorganisms rich in essential amino acids has been studied in many laboratories, both as a food supplement and as a source of amino acid. Fifteen amino acids were found in cell hydrolyzate, of which arginine (1.14 g L^{-1}) and L-lysine 0.4g L^{-1} were the most abundant (Nakayama, 1972).

Most natural strains cannot produce industrially significant amounts of L-lysine in the culture broth due to various metabolic regulation mechanisms. However, alteration of these mechanism can lead to L-lysine accumulation (Nakayama, 1972). Two distinct biosynthetic pathways are known for L-lysine production. In certain actinomycetes fungi and algae the carbon skeleton of L-lysine arises from acetate and α-ketoglutarate by biosynthetic sequences that include α-amino adipic acid. The other pathway has been found in bacteria, higher plants, blue green algae and certain fungi (some phycomycetes) and protozoa. The L-lysine carbon chain is synthesized from pyruvate and aspartate and α-ϵ-diaminopimelic acid is a key intermediate.

Production of L-lysine by bacteria: Kinoshita *et al.* (1958) for the first time developed homoserine auxotroph of *Micrococcus glutamicus* by UV radiation and found it able to accumulate large amounts of L-lysine in the culture broth. The chief ingredients in the medium were 2.5% glucose, 0.5% NH_4Cl, 0.2% NZ amine and mineral salts. Many other microorganisms with similar mutational block have been isolated (Nakayama *et al.*, 1961). White's (1972) medium was found to be the best one. With optimum glucose, ammonium nitrate and biotin, the strain AEC V1 yielded 36 g L-lysine per liter in flask culture (Samanta and Bhattacharyya, 1991).

Sen and Chatterjee (1983), isolated *Arthrobacter globiformis* from Burdwan (India) soil and found it able to accumulate 3.4 g L^{-1}, L-lysine in the growth medium. The strain grew and accumulated L-lysine in purely synthetic medium. Among the different hydrocarbon and nitrogen source tested straight run (SR) gas oil at 4% and ammonium sulphate at 0.4%, respectively, were found most to be suitable. Different vitamins and antibiotics stimulated growth and L-lysine yield; inoculum of 7% (v/v) of the medium was found to be optimal. The yield of L-lysine under optimal condition was 3.4 g per liter medium. Sen *et al.* (1983) isolated *Micrococcus varians* from Assam (India) soil and was able to accumulate 2.6 g L^{-1} L-lysine in a purely synthetic medium under optimal conditions. The supplementation of the synthetic medium with casamino acids significantly improved the yield. Sen (1985) tested 263 hydrocarbon utilizer strains for their L-lysine production. He observed that only 24 isolates (9%) produced L-lysine, but most of them as mixture with other amino acids. Two isolates (8.5%) were found to produce L-lysine alone. The isolates were identified as *Arthrobactor globiformis* and *Micrococcus varians*, respectively. Sen and Chatterjee (1985) found that at optimum pH, carbon and nitrogen sources, a hydrocarbon utilizing strain of *Arthrobactor globiformis* yielded 3.4 g L^{-1} L-lysine in the medium and addition of antibiotic and micro nutrients to that optimal media stimulated cell growth and enhanced L-lysine yield. Samanta *et al.* (1988) isolated a number of methionine plus threonine double auxotrophs from a glutamate producing *Arthrobactor globiformis* by mutagenesis with N-methyl-N-nitro-N-nitroso-guanidine. Sen and Chatterjee (1989) further studied the effect of B-Vitamins and trace element on L-lysine production by *Micrococcus varians* 2 Fa, which produced 2.6 g L^{-1} L-lysine. Addition of B-vitamins and trace elements to the optimal media has been found to stimulate growth and enhance L-lysine yield.

Bhattacharyya and Samanta (1992) isolated a few microorganisms, among them an L-lysine excreter (2 g L^{-1}) was identified as *Arthrobacter globiformis* TR 9 and was selected for further improvement. Sano and Shiio (1970) developed AEC resistance mutant of *Bacillus subtilis*, *Brevibacterium flavum* and *E. coli*. Among them, *B. flavum* mutant resistant to the growth inhibition of AEC plus threonine was the best one, producing 32 g L-lysine for 100g glucose. One of the mutants HBR-2 (Thialysine^r, Leucine$^-$, Homoserine$^-$) produced L-lysine at a concentration of 30 mg ml^{-1} in a molasses medium containing 10% reducing sugar. (Hagino *et al.*, 1981).

Kalcheva *et al.* (1991) observed that a low concentration of dimethyl sufoxide had a stimulatory effect on L-lysine production by the methionine sensitive mutant of *Bacillus subtilis*. Crociani *et al.* (1991) isolated auxotrophic regulatory mutant of *Bacillus stearothermophilus* that is a mutant which was resistant to S-(2-aminoethyl-L-cystine) and homoserine super (negative), produced L-lysine at the concentration of 7.5 g L^{-1}.

In immobilized cell preparations, growth of cells outside the immobilization matrix, as free cells, is normally undesirable due to the appearance of cells in the product stream and clogging of such systems. Antibiotics could be used to arrest such free cell growth, while allowing the synthesis and excretion of the product into the medium. Chloramphenicol at 200 μg ml^{-1} effectively arrests free cell growth and hence the L-lysine being produced can be entirely attributed to the immobilized cells. Novobiocine, on the other hand, at concentration of 100 μg ml^{-1}, stopped free cell growth, but also prevented the production of L-lysine. Productivity and yields of L-lysine were adversely affected by chloramphenicol and novobiocin probably due to a great decrease in cell viability (Israilides *et al.*, 1989). A new route for large-scale production of L-lysine is from methanol (CH_3OH) using auxotrophic mutant of the thermotolerent *Bacillus methianolicus*. Schendel *et al.* (1990) isolated a gram positive, endospore-forming methylotrophs that grew rapidly on methanol at 60 °C and did not sporulate readily at temperature above 50 °C, developed homoserine auxotrophs and AEC resistance and were capable of secreting nearly 20 g L^{-1} L-lysine in feed batch fermentation.

Recently, a simulation was developed based on the experimental data obtained in 14 L reactor to predict the growth and L-lysine accumulation. Grace *et al.* (1996) reported the development of a useful model that can be applied to choose the most beneficial volume control strategy for the optimization of L-lysine accumulation by mutant of *Bacillus methanolicus*. Based on data obtained in a 14-L-system, a three-phase approach was used to predict the rate of change of culture volume based on CO_2 production and methanol consumption. The model was used for the evaluation of volume control strategies to optimize L-lysine productivity at constant volume reactor process, with variable feeding and continuous removal of broth and cell resulted in higher L-lysine productivity, than a feed batch process with out

volume control. 0.141 g of L-lysine was produced per gram of methanol. Shah *et al.* (2002) improved the microbial production of L-lysine by developing a new auxotrophic mutant strain of *Cortnebacterium glutamicum*.

Kubota *et al.* (1970) produced auxotrophic mutant strains of *B. lactofermentum* No. 2256-213, which required threonine, isoleucine and valine for their growth by ultraviolet radiation and cultured them on an otherwise conventional medium using glucose. The amount of L-lysine produced was as highly as 5.2 gram dL^{-1}. Nakayama *et al.* (1973) also obtained a mutant strain of *B. flavum* LT-1 ATCC 21258. This was used as the seed strain. Culturing was carried out under the same conditions as described, except that 200 $\mu g\ L^{-1}$ of threonine was added to the seed culture medium and the fermentation medium. After the completion of culturing, 25 mg ml^{-1} of L-lysine was found to be accumulated in the culture liquor). When the threonine-requiring strain *B. flavum* ATCCC 2129 was used and was cultured in a similar manner as a control, only 17 mg ml^{-1} of L-lysine was formed in the culture liquor. The condition of L-lysine continuous biosynthesis was studied by Pilat and Paleckora (1982) using the suppresser mutants *Brevibacterium* sp. CCM AO 6 / 79. The highest yields were achieved using the two stage continuous culture with the semicontinuous regime in the second stage. When compared with batch culture the production of L-lysine was increased roughly by 70%. Beker (1982) developed threonine, methionine double auxotrophic mutant of *Brevibacterium flavum*, which required biotin. He found that at low concentration of biotin biosynthesis of glutamic acid takes place; and intensive synthesis of L-lysine can be observed at the beginning of the stationary phase of growth. Certain concentrations of threonine and L-lysine act as enzyme inhibitors. Glucose, the basic carbon source, at concentration higher than 5% has a repressive effect.

Young and Chipley (1983) studied microbial production of L-lysine and threonine from whey permeate by using *Brevibacterium lactofermentum* ATTCC 21086 and *E. coli* ATCC 21151. The highest amount of L-lysine 3.3 g L^{-1} was produced from a mixture of acid hydrolyzed whey permeate and yeast extract (0.2%). Tosaka *et al.* (1979 a, b) investigated the effect of biotin levels on L-lysine formation in *B. lactofermentum*. They reported that accumulation of L-lysine was stimulated considerably by increasing the biotin level. Young and Chipley (1984) investigated the role of biotin in L-lysine production by *B. lactofermentum* ATCC 21086 in acid-hydrolyzed whey permeate medium with and without biotin. Biotin stimulated L-lysine production and growth of *B. lactofermentum* and 5 μg / biotin per 100 ml was the optimum level. Zaki *et al.* (1987) worked on the effect of non-ionic detergents and vitamin on the production of amino acids by *B. ammoniagenes*. They found that the presence of 20$\mu g\ L^{-1}$ biotin induced the production of about 166 mg % L-lysine and 105 mg % arginine. In the presence of 100-400 mg % sodium oleate, 100-169 mg % L-lysine was produced but less amount of L-lysine was produced in the presence of Tween 20 and 80.

According to Nomura *et al.* (1987) *B. flavum* QL-5, aspartate kinase was sensitive to feed back inhibition in the simultaneous presence of L-lysine and L-threonine. The simultaneous addition of these two amino acids (1mM each) produced about 60% inhibition. The inhibition was reduced to about 40% by dialysis. Similarly, in L-lysine production by resting cells, the simultaneous addition of these two amino acids (1mM each) produced 35% inhibition and dialysis reduced the inhibition to 12%. In dialysis cultures the lag-phase was shortened and cell mass increased, as compared to non dialysis culture. Moreover, in dialysis cultures, L-lysine was produced earlier and the maximum productivity of L-lysine (1.50 g L^{-1}) was obtained in 6 through 10 hrs cultivation. Yokota and Shiio (1988), studied the effect of reduced citrate synthetase activity and feedback resistant phosphoenol pyruvate carboxylase on L-lysine productivities. Aspartokinase and S-2 aminoethyl cystein (AEC) resistant mutant plus threonine auxotroph of *B. flavum* was found to produced more than 40 gL^{-1} of L-lysine as its HCl salt in the medium containing 10 % glucose. In particular, strain No. 664-7 with normally active and completely feed back resistant. AK produced 45g L^{-1} of L-lysine, HCl. A homoserine dehydrogenase-defective mutant (HD), H-3-4, with low level citrate synthetase and phosphoenol pyruvate carboxylase character also showed higher L-lysine productivity, 41 g / L, than the HD mutant, H1013, which was derived directly from the wild strain. Thus it was concluded that the low level citrate synthetase and phosphoenol pyruvate carboxylase character were effective for the enhancement of the L-lysine productivities of both aspartokinase resistant and HD type producers. Smekal *et al.* (1988) studied the control of L-lysine biosynthesis with chromogene mutants of *Brevibacterium* Species M-27. They found 43 to 49 g L-lysine per liter in 96 hours with conversion of 45 to 49%.

Effect of exogenous betaine on the growth of an L-lysine producing mutant of *B. lactofermentum* was examined by Kawashara *et al.* (1990a) in a medium containing different carbon sources such as glucose, fructose or sucrose. The growth rate decreased significantly with a rise in temperature when sucrose was the carbon source. Both the specific sucrose consumption rate and the invertase activity of the mutant decreased with the culture period when the cultivation temperature was 35 °C. The addition of betaine restored both growth and invertase activity on medium containing sucrose as the carbon source at 35 °C. Betaine protected the invertase activity against the inactivity effects of high temp *in vitro*. Furthermore, the addition of exogenous invertase into the production medium at 35 °C restored the growth rate to that at 35 °C. Kawashara *et al.* (1990b) studied the effect of glycine betaine on growth of *B. lactofermentum* during L-lysine production and found that it stimulated growth rate in minimal medium, especially in culture medium of inhibitory osmotic stress. Wang *et al.* (1991) examined the culture conditions for production of L-lysine by *Brevibacterium* sp. P1-13. The optimal concentration of initial sugar for molasses and raw sugar media were 9 and 16%, respectively. For obtaining high yield of L-lysine, it was necessary to maintain 2% $(NH_4)_2SO_4$ in medium throughout fermentation and it was also very effective to increase oxygen supply during fermentation. Under optimal condition, 7.35% of L-lysine HCl was accumulated from molasses medium with product yield of 36% in 96-hour fermentation.

The effect of synthetic carbohydrate (SC) on L-lysine biosynthesis by *B. flavum* 22L cells was studied by Sukharevich *et al.* (1992). The said strains were grown in a medium containing molasses, protein-vitamin hydrolyzate and mineral salt supplemented with SC. An 11-17% increase in L-lysine concentration was obtained in a medium supplemented with 0.05% SC and found that the enhancement of L-lysine biosynthesis by SC was not associated with any effect on the TCA cycle. Enzymes cultivation of *B. flavum* 22L in media containing SC and glycosidase resulted in an 18 and 16% increase in L-lysine yield, respectively. *Brevibacterium* sp. 221, which is resistant to ketobutric acid, are capable of producing L-lysine. (Anonymous, 1992 a, b). Shiio *et al.* (1993) isolated α-ketobutyrate (α-KB) resistant mutant of *B. flavum* with aspartate kinase desensitized to feed back inhibition by nitrosoguanidine treatment observed to produce 29.4 to 41.9g L^{-1} L-lysine.

Trifonova *et al.* (1993) studied the possible application of various types of fruit and vegetable raw materials to microbial synthesis of L-lysine. They used *Brevibacterium* sp. and found that substrates high in carbohydrates gave higher L-lysine level than low carbohydrate substrates The highest L-lysine yield on single substrate was obtained with sugar beet (8.4 g carbohydrate 100 ml^{-1}) and gave 25.9 g L^{-1} L-lysine. Arutyunyan *et al.* (1993) added mixture of phases to the seed material before culturing the L-lysine producing *Brevibacterium* in final concentration of the phase in the fermentation was 200 particles ml^{-1}. They found that the phases prevented L-lysine losses if the nutrient media was infected with *Proteus* sp.

Liu and Wu (1994) used a recirculation loop to investigate the fermentation of L-lysine production by regulatory mutant of

B. flavum with the recirculation. Overchenko *et al.* (1996) noted effective biosynthesis of L-lysine during culture of the auxotrophic strain of *Brevibacterium* sp. E531 in fruit and vegetable media, using Chinese cabbage juice.

Nakayama *et al.* (1973), obtained a mutant strain of *Corynebacterium glutamicum*. Only 34.5mg ml^{-1} of L-lysine was formed in the fermentation liquor when a homoserine and leucine requiring strain of *C. glutamicum* (ATCC21253) was used. When culturing was carried out in a fermentation medium into which 500 μg ml^{-1} of L-threonine has been added, 32.5 mg ml^{-1} of L-lysine was formed with *C. glutamicum* BL-25 ATCC 21526. Nakayama *et al.* (1973) obtained *C. glutamicum* RL-9 ATCC 21543, T-135 ATCC 21527 and LY-32-6 ATCC 21544. The amounts of L-lysine formed were, 39.4, 38.2 and 38.1 mg ml^{-1}, respectively.

Production of L-lysine was followed in two L-lysine accumulating mutants of *C. glutamicum* ATCC 13287 in media containing sucrose, ethanol, acetic acid, or a mixture of acetic acid and ammonium or sodium acetate. Pelechova *et al.* (1981) found that acetate was the best substitution for sucrose. Zaki *et al.* (1982) reported the effect of tetracycline and erythromycin on the fermentation production of L-lysine. They found that some 22 to 24 g L^{-1} L-lysine could be produced by *Micrococcus glutamicum,* when tetracycline and etheryromycin were added to the fermentation culture. Smekel *et al.* (1982), tested the effect of several types of polar and nonpolar tensides on the biosynthesis of L-lysine using the strain *C. glutamicum.* Only the definite concentrations of liquid Tween 60 and 80 have a stimulating effect on a production of L-lysine and using the liquid Tween the yield was increased about 10 to 30%.

Production strains of *C. glutamicum* and *Brevibacterium* sp. are able to grow and synthesize L-lysine in the fermentation medium with the paper hydrolyzate as the source of monosaccharides. The production of 20-24 g of L-lysine L^{-1} was achieved in media where hydrolyzate was supplemented with saccharose that permitted the sufficient growth with the simultaneous initiation of the production of L-lysine (Pelechova *et al.*, 1983). The production of L-lysine with the strain of *B. flavum* and *C. glutamicum,* using saccharose technology and non standard nitrogen sources such as hydrolysates of extracted rap, flax and cotton plant crush and hydrolysates of fodder yeast, was studied by Smekel *et al.* (1982). Using these N-sources the production in a range from 36 to 45 g L-lysine per liter was achieved. Smekal (1983) studied the same strain of *C. glutamicum* 10-20/60, which needed homoserine for growth. The accumulation with standard carbon sources (acetic acid, hydrolysates of cereal starch, mixture of molasses-acetate and enzyme hydrolysis of paper) produced 36-44g L^{-1} lysine.

The influence of temperature on the growth and L-lysine formation in *C. glutamicum* 9366 was studied by Hilliger *et al.* (1984). The optimum temperature for both, the biomass yield and product formation was found to be 29 °C. At the temperature above 29°C biomass yield and L-lysine excretion decreased. Smekel *et al.* (1984) studied the biosynthesis of L-lysine in *C. glutamicum* and *B. flavum* using media with a hydrolyzate of phosphocarpus flour, with a yield of 44 and 30 g L^{-1}, respectively. Smekal *et al.* (1985) achieved 36 g of L-lysine per liter of the medium containing saccharose and non-standard nitrogen sources such as hydrolyzate of rap, flax and cotton. Plachys and Ulbert (1985), isolated chlorolysine resistant mutant of *C. glutamicum,* which produced 45 g L^{-1} L-lysine after 4 days cultivation in 20 L fermenter.

The effect of threonine and methionine on the culture growth and L-lysine production was studied by Zaitseva and Konovalova (1986), using four homoserine dependent mutants viz., *C. glutamicum* 95 and *Brevibacterium* sp. 22I (sensitive) and *C. glutamicum* 1020-60 and 410-6 (resistant). The L-lysine accumulation was proportional to the threonine content. The high L-lysine producing strains were not particularly good consumers of glucose. Tosaka *et al.* (1983), however, demonstrated that a high L-lysine producer, high-glucose-consumer could be produced from fusion of a high-L-lysine, low glucose strain with a low L-lysine, highly glucose strain.

Hadj-Sassi *et al.* (1988), reported the influence of initial concentration of glucose from 60 to 233 g L^{-1} on the production of L-lysine by *Corynebacterium* sp. in batch and feed batch culture. The maximum conversion rate into L-lysine was obtained at 165 g L^{-1} and the best specific production rate of L-lysine was observed at 65 g L^{-1} of glucose. Sobotkova *et al.* (1989) isolated multiple auxotrophic, regulatory and penicillin resistant mutants from a β- galactosidase-hyper producing strain of *E. coli* K_{12}. These mutants exhibited, for the most part, a high reversion rate. Wam *et al.* (1991) isolated DAPA gene (L-2, 3-dihydrodipicolinate synthetase DHDP synthetase) of *C. glutamicum* JS231, a L-lysine over producer. The DHDP synthetase activity of *E. coli* TFI, carrying PSHDP5812, showed high resistance towards inhibition by L-lysine.

Pham *et al.* (1989) studied the microbial production of L-lysine using sugar cane juice, enriched with coconut water (an industrial waste product) by a homoserine auxotroph 9NG7, derived from *C. glutamicum* ATTCC 13032. The L-lysine yield increased 1.5 fold to 16.9 g L^{-1} when sugarcane juice enriched with coconut water was used.

Hilliger and Prauser (1989) screened a number of bacteria not reported to produce L-lysine. Twenty-five *Coryneform* and *Nocardioform* bacteria were selected with this property. Of these many *Oerskonia* strains were secreting L-lysine. The AEC resistant mutants accumulated upto 10 g L^{-1} under the same condition. Mankel *et al.* (1989), studied the utilization of fumarate by recombinant strain of *C. glutamicum.* They reported that upon addition of fumarate to a strain with a feedback resistant aspartate kinase, the L-lysine yield increased from 20 to 30 mM. Hadj-Sassi *et al.* (1990), grew the mutant strain of *C. glutamicum* in a medium containing 17.5% glucose, 5.5% ammonium sulphate and 2% yeast extract. Under laboratory conditions, it produced high amounts of L-lysine in this optimized medium. A new *C. glutamicum* strain CS-755 has been claimed, capable of producing L-lysine (Anonymous, 1990).

Hirao *et al.* (1990) studied L-lysine production in continuous culture with a single stage cultivation process using the L-lysine hyper producing mutant B-6 of *C. glutamicum.* Strain B-6 showed stable L-lysine production for over 500 hours. The maximum values of L-lysine concentration and volumetric productivity were 105 g L^{-1} and 5.6 g L^{-1} hr^{-1}, respectively. Coello *et al.* (1992) worked on the physiological aspect of L-lysine production. They observed that in case *C. glutamicum,* phosphate limited cultures at low growth rates were favourable to L-lysine production. Konicek *et al.* (1991) studied the effect of Tween 80 and dimethyl sulfoxide on biosynthesis of L-lysine in regulatory mutants of *C. glutamicum.* They observed that by using dimethyl sulfoxide or Tween 80 the production of L-lysine was increased by 20-28% and 23-25%, respectively. The stimulation observed is supposed to be caused by influencing cellular surface structure.

A thermophilic mutant strain of *C. thermoaminogenes* produced 3.2 g L^{-1} L-lysine using conventional C, N and mineral sources (Anonymous, 1992e). Moszezyensky *et al.* (1991) studied immobilized *C. glutamicum* for L-lysine production and observed 10 fold less L-lysine yield than that of free cells. This was probably due to oxygen transfer limitations. Ferreira and Duarte (1991) isolated fluoro-acetate sensitive mutant of *C. glutamicum* following mutagenesis with nitrosoguanidine having a maximum yield of 1.3 g L^{-1}.

Zhou *et al.* (1991) obtained the fusion strain 24413, which used sugar beet molasses with high output of above 6.5% L-lysine through the protoplast fusion between *B. subtilis* BR151 and *C. perkinense* 1134 derivatives. Mutant strain of *Corynebacterium* and *Brevibacterium,* which are resistant against reverse coupling inhibition by 2-azido-epsilon caprolactum, produced L-lysine in higher yields than those produced by microorganisms obtained by selection with previously used compound such as flouro or chloro-caprolactum. The efficiency in selecting the mutants is higher with 2-azido-epsilon caprolactum (Anonymous, 1992c). Selenalysine resistant mutants of *C. glutamicum, B. lactofermentum* and *C.acetoacidophilum* have been developed, accumulating 2.21 g L^{-1} L-lysine in the culture broth (Anonymous, 1992d).

L-lysine production by AEC resistant mutant of *C. glutamicum* using ethanol as the principal carbon source was 4.6 g L^{-1} (Anonymous, 1993b). A method for improving L-lysine secretion

by a *Coryneform* bacterium, involving induction of L-aspartic acid-β-methyl ester (AME) resistance has been patented (Anonymous, 1993a). The strain was used for fermentative production of L-lysine produced 29.9 g L^{-1} L-lysine. Sander *et al.* (1994) expressed *E. coli* Lys-C gene (AK -111-M4) and the dap-A gene from *C. glutamicum*. They encoding L-lysine insensitive forms of AK and DHDPS, respectively, alone and in combination with the seed rape (*Brassica napus*) and observed large increase (as much as 100 times) in the level of free L-lysine. Kim (1994) developed 4-Azaleucine and rifampicin resistant mutants of *C.glutamicum* from a homoserine deficient mutant and observed L-lysine production by feed batch fermentation.

Falco *et al.* (1995) observed that the L-lysine content in the seed of rap (*Brassica napus*) wester and soybean plants was increased by circumventing the normal feed back regulation of enzymes of the biosynthetic pathway; aspartate kinase and DHDPS. Expression of *Corynebacterium* DHDPS resulted in more than 100-fold increase in free L-lysine accumulation in rap seeds; Total seed L-lysine content approximately doubled. Hadj-Sassi *et al.* (1996) studied the effect of O_2, CO_2 and redox potential on L-lysine production biomass formation and substrate consumption for *C. glutamicum* ATTCC 21513 and found that O_2 limitation caused a decrease in substrate consumption rate and conversion efficiency of substrate to L-lysine. The maximum conversion rate into L-lysine was obtained at 30-35% dissolved O_2 saturation without CO_2 addition and a redox potential of 440 mv. Sambanthamurthi *et al.* (1984) developed a homoserine auxotrophic mutant of *Pseudomonas aeroginosa* PAC35. They examine that in minimal salt medium, with growth limiting concentration of homoserine, excreted L-lysine into the medium and this did not occur when oxygenous homoserine, or threonine, was in excess of requirements.

Kikuchi *et al.* (1996) claimed a new L-lysine decorboxylase of *E. coli* W 3110 and the DNA sequences, leading to high expression of L-lysine. Sergeichuk *et al.* (1995) examined that *Staphylococcus* sp. and Micrococcal strains completely consumed L-lysine in the culture medium. Thus affected the level of L-lysine accumulation in the culture medium. Smirnov *et al.* (1994) isolated *Azomonas* sp. from culture broth at different stages of fermentation during L-lysine production and found that this bacteria were not fastidious for culture conditions, grew rapidly, consumed a broad range of carbohydrate and actively produced extracellular slim protein-poly saccharide. They actively consumed the surrounding amino acids thus were dangerous contaminants of biotechnological process.

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