Direct Fermentative Production of Lysine (Review)

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(Received 16th July 2007, revised 27th August 2007)

With the tremendous utilization of lysine in animal fields its economic importance increases continuously as well. This focuses the attention of over production of this essential amino acid. This review concentrates on the developed auxotrophic and regulatory mutants and its potency for lysine production under optimal condition.

The 1950s were the early days of synthesis of amino acids from microbial origin. Two-step methods of production were developed for at least two amino acids-glutamic acid and lysine. In the case of glutamic acid, one specie; of microbe was used to produce α-ketoglutarate, the immediate precursor of L-glutamic acid, from glucose. Another specie was used to convert α-ketoglutarate to glutamic acid. Similarly, a two-step method for lysine production was developed where diaminopimelic acid was accumulated by a strain of *E. coli*. The diaminopimelic acid was then converted to lysine by diaminopimelic acid decarboxylase from the bacteria *Aerobacter aerogenes*. These two-step methods did not readily lend themselves to industrial production.

In 1956, a major breaks through occurred with the isolation of a bacteria that was capable of accumulating large quantities of L-glutamic acid. By this, two-step amino acid production gave way to "direct" fermentation. The principles of the fermentation method quickly gained acceptance, and systemic screening of microbes soon began on the production of other amino acids. This was the beginning of the amino acid fermentation industry. L-lysine production by "direct" fermentation soon followed in 1958, and the process was industrialized to a technical scale.

Since amino acids are essential components of microbial cells, their biosynthesis is generally regulated at an optimum level by the microbe. Therefore, amino acids are normally synthesized in limited quantities. Some special means must be found to overcome such regulation in order to accumulate large quantities of amino acids. To overcome this regulation of amino acid, a great variety of microorganisms have been reported to overproduce L-lysine, including auxotrophic mutants and regulatory mutants. In addition to the conventional mutation techniques used to obtain these strains, protoplast fusion and recombinant DNA technologies have recently been introduced into the breeding of lysine producers. Microbial process using molasses or starch hydrolysates as raw material have become firmly established as the major methods for producing L-lysine, having replaced methods based on the hydrolysis of proteins. The development of better strains of microbe to produce lysine has had a major impact on the economics of the fermentation process and it is on this aspect of lysine production that author focus.

L-lysine was the first amino acid to be produced on an industrial scale with the aid of auxotrophs when homoserine-requring auxotrophic mutants of *Corynebacterium glutamicum* were derived as L-lysine producers [1-3]. It was found that, in the genra *Corynebacterium and Brevibacterium*, aspartokinase catalyses the first step in the syntheis of L-lysine and L-threonine from aspartate, and that this reaction is subject to a concerted feedback inhibition by L-lysine and L-threonine. Hence, a homoserine auxotroph and a threonine-methionine double auxotroph were isolated from glutamate producing strains of *Corynebacterium glutamicum, Brevibacterium flavum* and *Brevibacterium lactofermention* [4]. The intracellular pool of L-threonine in the these organisms is considerably reduced and they are capable of accumulating 13-34 g of L-lysine per litre [4].

Another effective technique for the overproduction of amino acids is the use of regulatory mutants. The key to this method is to obtain mutants, which are insensitive of feedback inhibition of

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repression. Such organisms have been isolated as mutants, which are resistant to amino acid analogs, or as revertants derived from the auxotroph deficient in regulatory enzymes. A typical example is an L-lysine producer derived from *Brevibacterium flavum* [5-6]

The regulation of L-lysine biosynthesis in *Brevibacterium flavum* is less complicated than that in *E. coli* only one species of aspartokinase is subject to a concerted feedback inhibition b L-lysine and L-threonine in *Brevibacterium flavum*. growth of this organism is inhibited by an analog of lysine, S-(2-aminoethyl) L-cysteine (AEC). This inhibition is markedly enhanced by L-threonine, but reversed by L-lysine. This implies that AEC behaves as a false feedback inhibitor of aspartokinase. Regulatory mutants in which aspartokinase is insensitive of the concerted feedback inhibition were expected to be found among mutants resistant to AEC. One such mutant, FAI-30, produces 31-33 g per litre of L-lysine; its aspartokinase is approximately 150-fold less sensitive to feedback inhibition by L-lysine plus L-threonine than the parent strain.

Better producers of L-lysine were obtained by repeated mutation. The lysine productivity was improved stepwise by successive mutations, which endowed the bacteria with resistance to AEC, a requirement for alanine, resistance to a-chloro-caprolactam (CCL) and y-methyl-L-lysine (ML) and sensitivity to fluoropyruvate (FP) [5-6].

Kinoshita *et al.*, [1] for the first time developed homoserine auxotroph of *Micrococcus glutamicus* by ultrviolet (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 % ammonium chloride, 0.2 % amino acids mixture and mineral salts.

Ever since Kinoshita *et al.*, [1] discovered that mutants of *Micrococcus glutamicus* auxotrophic for homoserine (or requiring methionine plus threonine) excreted large amount of L-lysine, many other microorganisms with similar mutational block have been isolated [2]. Using mutagenesis with N-methyl-N-Nitro-N-nitrosoguanidine, a number of homoserine auxotroph have been isolated from a glutamate producing *Athrobactor globiformis* excreting L-lysine in good amounts, for further improvement, mutants resistant to the L-lysine analogue (AEC) have been isolated from homoserine auxotrophs for the three potent mutant tested. Whites (1972) [7] medium was found to be the best glucose, ammonium nitrate and biotin were found to be optimum at 280 m mol / L, 40 m mol / L and 22 m mol / L, respectively. With optimum glucose and ammonium nitrate and biotin, the strain AEC V1 yielded 36 g L-lysine per liter in flask culture [8].

Samanta *et al.*, [9] 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, biotin, ammonium chloride and glucose was found to be optimum at 5 μg / L, 40 mM and 4 % level, respectively. With such optimal C and N source, the strain MT 35 yielded 28.0 g L-lysine per liter of medium in shake flask culture.

Sano and Shiio [5] developed AEC resistance mutant of *Bacillus-subtilis*, *Brevibacterium flavum* and *E. coli*. Among *Brevibacterium flavum* mutant resistant to the growth inhibition of AEC plus threonine was the best one producing 32 g L-lysine for 100 g glucose. AEC an analogue of L-lysine strongly inhibited the growth of *Bacillus subtilis* and *E. coli* in minimal medium. In case of *Brevibacterium flavum*, the inhibition of AEC alone was weak L-threonine was found to be one of the strongest stimulators. This phenomenon is reasonable considering the fact that aspartokinase of this bacteria is inhibited by L-lysine plus threonine. The fact that methionine as well as L-lysine restored the growth for inhibition by AEC plus threonine suggests that the primary inhibition target of this agent may be aspartokinase.

Bacillus licheniformis able to grow at 46 °C was isolated for soil. L-lysine producing mutants were derived from the bacterium by the introduction of resistance to S-(2 amino) ethyl. L-cystein (thialysine) and auxotrophy. One of the mutants HBR-2 (Thialysine^r, Leucine, Homo-serine) produced L-lysine at a concentration of 30 mg / ml in a molasses medium containing 10% reducing sugar [10].

Kalcheva *et al.*, [11] 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.*, [12] 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 per liter in shaken flask in minimal medium containing 5 % glucose at 60-65 °C.

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. Chloroamphenicol (200 μg / ml) and / or novobiocine (10 μg / ml), when added during the growth of *Bacillus subtilis,* allow the production and excretion of L-lysine into the medium. Chloroamphenicol at 200 μg / ml 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, stopped free cell growth, but also prevented the production of L-lysine. Productivity and yields of L-lysine were adversely affected by chloroamphenicol and novobiocin probably due to a great decrease in cell viability 66.6 mg L-lysine / liter was produced [13].

A new route for the large scale production of L-lysine is from methanol using auxotrophic mutant of the thermotolerant *Bacillus methianolicus.* Schendel *et al.*, [14] isolated a gram positive, endosporeforming 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 / Liter L-lysine in feed batch fermentation.

Recently, a simulation was developed based on experimental data obtained in 14 L reactor to predict the growth and L-lysine accumulation. Homoserine auxotrophs of *Bacillus methanolicus* MGA3 are unique methylotrophs because of their ability to secret L-lysine during aerobic growth and threonine starvation at 50 °C.

Kubota *et al.*, [15] produced auxotrophic mutant strains of *Brevibacterium lactofermentum* No. 2256-213, which required threonine, isoleucine and valine for their growth by UV radiation and cultured them on an otherwise conventional medium using glucose. The amount of L-lysine (as hydrochloride) produced was as highly as 5.2 gram per deci-liter.

Nakayama *et al.*, [16] also obtained a mutant strain of *Brevibacterium flavum* LT-1 ATCC 21258, a strain having a requirement for threonine and a resistance to the L-lysine analogue S-(β-amino ethyl)-cysteine and the threonine analogue α-amino-β-hydroxy valeric acid. This was used as the seed strain; culturing was carried out under the same conditions as described, except that 200 μg / L of threonine was added to the seed culture medium and the fermentation medium. After the completion of culturing, 25 mg / ml of L-lysine (as the hydrochloride was found to be accumulated in the culture liquor). When the threonine-requiring strain *Brevibacterium flavum* ATCCC 2129 was used and was cultured in a similar manner as a control, only 17 mg / ml of L-lysine was formed in the culture liquor.

Yokota and Shiio [17], studied the effect of reduced citrate synthetase activity and feedback resistant phosphoenol pyruvate carboxylase on L-lysine productivities. Aspartokinase and AEC resistant mutant plus threonine auxotroph of *Brevibacterium flavum* was found to produced more than 40 g / L 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 of L-lysine, HCl, whereas an AK^R L-lysine producer, FAI-30, derived directly from wild strain No. 2247 produced only 20 g/ L. A homoserine dehydrogenase-defective mutant (HD), H-3-4, with low level citrate synthatase 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, and produced 35 g / L of L-lysine. Thus it was concluded that the low level citrate synthetase and phosphoenol pyruvate carboxylase character were effective for enhancement of the L-lysine productivities of both aspartokinase resistant and HD type producers.

Shiio *et al.*, [18] isolated alpha-ketobutyrate (α-KB) resistant mutant of *Brevibacterium flavum* with aspartate kinase desensitized to feed back inhibition by nitrosoguanidine treatment observed to produced 29.4 to 41.9 g / L L-lysine.

Overchenko *et al.*, [19] 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.*, [16], obtained a mutant strain of *Corynebacterium glutamicum*, a strain having a requirement for homoserine and leucine and in resistance to L-lysine analogue, S-(β-amino ethyl) cysteine, was used as the seed strain. Culturing in molasses medium at 30 °C for 4 days at pH 7.5 with aerobic shaking of the culture, resulting in the fermentation 30.5 mg / ml of L-lysine (as the hydrochloride) in the resultant culture liquor. Only 34.5 mg / ml of L-lysine was formed in the fermentation liquor when a homoserine and leucine requiring strain of *Corynebacterium glutamicum* (ATCC21253) is used and is cultured in similar manner. When culturing was carried out in a fermentation medium into which 500 μg / ml of L-Threonine has been added, 32.5 mg / ml of L-lysine was formed with *Corynebacterium glutamicum* BL-25 ATCC 21526. Nakayama *et al.*, [16] obtained RL-9 ATCC 21543, a strain having a requirement for homoserine and leucine and a resistance to the L-lysine analogue S-(β-amino ethyl) cysteine. *Corynebacterium glutamicum* T-135 ATCC 21527, having a requirement for homoserine and leucine and a resistance 2-α-amino, β-hydroxy-valeric acid and *Corynebacterium glutamicum* LY-32-6 ATCC 21544 having a requirement for homoserine and leucine and a resistance to the isoleucine analogue, 2-amino-3-methylthio-butyric acid, were used as seed strains and were cultured in the same manner. The amount of L-lysine formed 39.4 mg / ml, 38.2 mg / ml and 38.1 mg / ml, respectively.

Smekel [20] studied the same strain of *Corynebacterium glutamicum* 10-20/60 which needed homoserine for growth and resistant S(2-amino ethyl)-L-cystein. The accumulation with standard carbon sources (acetic acid, hydrolysates of cereal starch, mixture of molasses-acetate and enzyme hydrolysis of paper) produced 36-44 g L-lysine per liter.

Plachy and Ulbert [21], isolated chlorolysine resistant mutant of *Corynebacterium glutamicum* which produced 45 g / L L-lysine after 4 days cultivation in 20 L fermentor.

The effect of threonine and methionine, on the culture growth and L-lysine production was studied by Zaitseva and Konovalova [22], using four homoserine dependent mutants, sensitive (*Corynebacterium glutamicum* 95 and *Brevibacterium Sp* 221) and resistant (*Corynebacterium glutamicum* 1020-60 and 410-6) to the analogue of L-lysine, S-(2-aminoethyl-L-cysteine). All the mutants studied utilized almost equal amounts of L-threonine, but different amounts of L-methionine. All the mutant used threonine only in L-form, while methionine in both L and D-forms. The L-lysine accumulation was proportional to the threonine content. The maximal specific productivity of the mutants was observed at a different threonine-methionine ratio in the medium. The optimal Thr / Met ratio was less than 4 for AEC sensitive strain while for AEC resistant mutants was more than 7.5. The high-L-lysine-producing strains were not particularly good consumers of glucose. Tosaka *et al.*, [23], 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.

Sobotkova *et al.*, [24], isolated from a beta galactosidase-hyper producing strain of *E. coli* K_{12} multiple auxotrophic, regulatory and penici-llin resistant mutants. These mutants exhibited, for the most part, a high reversion rate, but some of them produced about 2 mg / ml L-lysine in an enriched fermentation medium. Wam *et al.*, (1991) [25], isolated DAPA gene (L-2, 3-dihydrodipicolinate synthetase DHDP synthetase) of *Corynebacterium glutamicum* JS231, a L-lysine over producer. The cloned and sub cloned in *E. coli* / *Corynebacterium glutamicum* shuttle vector PECCG 117 was used to transform *E. coli* threonine producer and threonine and L-lysine co-producer. The plasmid PSHPS812 carrying gen. of *Coryne-bacterium glutamicum* led to an increase in L-lysine production in these *E. coli* strains. Threo-nine and L-lysine co-producer *E. coli* TFI with DHDP 5812 produced L-lysine with small amount of threonine. The DHDP synthetase activity of *E. coli* TFI, carrying PSHDP5812, showed highly resistance towards inhibition by L-lysine.

Pham *et al.*, [26] 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 *Corynebacterium glutamicum* ATTCC 13032, by chemical mutagenesis in batch culture in stirred tank fermentor. The effect of feed back inhibition by L-lysine in the fermentation broth containing glucose AR grade or sugar cane juice was laggend when the homoserine auxotroph 9NG7 was used instead of the Parent strain. The L-lysine yield increased 1.5 fold to

16.9 g / L when sugarcane juice enriched with coconut water.

Hilleger and Prauser [27] 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. *Oerskonia turbata* was used for further treatment. Threonine, methionine and threonine-methionine auxotrophs and S-2-aminoethyl-L-cystien (AEC) resistant mutants were selected after pulse mutagenesis with highly concentrations of nitrosoguanidine. The auxotroph produced low amount of L-lysine (3-4 g / L) after 72 hours cultivation in shake flasks. The AEC resistant mutants accumulated upto 10 g / L under the same condition.

A new *Corynebacterium glutamicum* strain CS-755 has been claimed, capable of producing L-lysine, possesses resistance to alpha-amino-beta-hydroxy valeric acid, AEC, methyl L-lysine and arginine analogues. The new strain was found to produce increased yield of L-lysine [28]. L-lysine production was also studied by a thermophilic mutant strain of *Corynebacterium thermoaminogenes* capable of growth at 40 °C and resistant to AEC. This strain produced 3.2 g / L L-lysine using conventional C-source and N-source and mineral [29].

Ferreira and Duarte [30] isolated fluoro-acetate sensitive mutant of *Corynebacterium glutamicum* following mutagenesis with nitroso-guanidine. The maximum specific rate of L-lysine production for the mutant was 1.3 g/ L. 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. [31].

Selenalysine resistant mutants of *Corynebacterium glutamicum, Brevibacterium lactofermentum* and *Corynebacterium acetoacido-philum* have been developed accumulated 2.21 g / L L-lysine in the culture broth [32].

L-lysine production by AEC resistant mutant of *Corynebacterium glutamicum* have been examined using ethenol as the principal carbon source. Composition of the fermentation of vol / vol % ethenol, mineral salt and 2 g / L ammonium sulphate accumulated 4.6 g / L L-lysine [33]. A method for improving L-lysine secretion from a *Coryneform* bacterium involves induction of L. aspartic acid beta methyl ester (AME) resistance has been patent [34]. The strain was used for fermentative production of L-lysine produced 29.9 g / L L-lysine.

Kim (1994) [35] developed 4-Azaleucine and rifampicin resistant mutant of *Corynebacterium glutamicum* from homoserine deficient mutant and observed L-lysine production by feed batch fermentation.

Sambanthamurthi *et al.* [36], 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 oxygennous homoserine, or threonine, was in excess of requirements.

Although these successive mutations did increase L-lysine production, the over producing strain often showed lower rate of glucose consumption and growth. Tosaka *et al.*, [23], demonstrated the advantage of protoplast fusion in *Brevibacterium - lactofermentation* to improve the glucose consumption rate of these L-lysine producer. One of the two strains fused was a glutamic acid producer, resistant to decoyinine (DC) and ketomelonat (KM), which was able to consume glucose rapidly. The other was the L-lysine producer A, 11214 with a lower glucose consumption rate. Resistant to AEC, DC and KM was used as the selection marker. One strain among fusants consumed 130 g of initial sugar per liter in 30 hours as compared with 100 hours for the parent L-lysine producer and it produced 3 times as much L-lysine.

A major aim of recombinant DNA technology in the amino acid fermentation industry is to increase the number of genes which encode the key enzyme (s) for the biosynthesis of an amino acid. Such an increase in the number of genes. (carried on plasmids) would lead to the over production of an amino acid, if the synthesis of the relevant enzyme (s) increase with the gene copy number and, if the reaction it catalyzes is one of the limiting steps in the synthesis of this amino acid. The principle of gene

amplification has already been developed for L-lysine fermentation as well as for L-threonine fermentation.

Several genes of the L-lysine biosynthetic pathway have been cloned on the highly copy number plasmid PBR322. These hybrids were used to transform and *E. coli* strain, TOCR21, that over produces L-lysine because of a mutation in its aspartokinase. TOCR21 and the plasmide-labouring strain accumulated 4 and 6.5 g of L-lysine per liter, respectively. Only plasmids, which carried the DAPS gene (encoding dihydrodipicolinate synthetase) caused an increase in L-lysine production. This clearly indicates that the limiting step in the synthesis of L-lysine in *E. coli,* is the condensation step catalyzed by this enzyme. Further improvement has been attempted for this strain by amplifying the lysc gene (aspartokinase-III) and the lys A gene (DAP decarboxylase) and trying to reduce the activity of inducible L-lysine decarboxylase. However, the production of L-lysine by these strains of *E. coli* was still much lower than of the analog-resistant mutants derived from *Brevibacterium* and *Corynebacterium* [23].

To reproduce the results of laboratory-scale fermentation in large commercial operations a number of biochemical engineering problems must be solved: pure cultures must be maintained; the fermentation broth must be aerated and agitated; and pH, temperature and foaming must be controlled.

Molasses or starch hydrolysate are now generally used as carbon sources. The optimum fermentation temperature is 32 °C and the pH is maintained near neutrality during the fermentation by feeding ammonia. The supply of oxygen is particularly important in L-lysine fermentations.

The biosynthesis of L-lysine is an aerobic process and Akashi *et al.*, [37] elucidated the relationship between oxygen supply and product formation in lysine fermentation's. The maximum accumulation of L-lysine was observed when the cells' requirements for oxygen respiration are satisfied; a limited oxygen supply results in the accumulation of lactic acid.

Hilliger *et al.*, [38] also reported that oxygen depletion irreversibly inhibited the production of L-lysine by a homoserine auxotroph of *Corneybacterium glutamicum*. This inhibition was accompanied by an increase in the intracellular pools of L-lysine and phospholipid. This suggests that the reduction of L-lysine accumulation under these conditions was due to an alteration of the cell membrane which decreases the permeability of L-lysine.

Shah *et al.*, [39] develop a new auxotrophic mutant from a regulatory mutant and to examine its potency for lysine production. Glutamate-homoserine-alanine triple auxotrophic mutant MRLH-GHA_{10} was derived from thiosine resistant mutant of *Corneybacterium glutamicum*. Its potency was examined in various media containing different carbon sources. The most potent auxotrophic mutant developed in this investigation produced 38, 33 and 28.5 g L^{-1} lysine, in the media containing glucose, molasses and starch hydrolysate, respectively as carbon sources in a stirred tank fermenter, at 30 °C and at 500 rpm agitation.

Shah *et al.*, [40] optimized the culture conditions for lysine fermentaiton. In this regard the role of various physical and nutritional parameters was examined. The culture was incubated in a 500 ml Erlenmeyer flask in a rotatory shaking incubator at 200 rpm. The appropriate conditions were obtained only when 50 ml medium was charged for fermentation at 30 °C, pH 7.5 and at 10 % inoculum size. The finally selected medium per 100 ml distilled water formulated was 10 g glucose, 2.5 g ammonium sulfate, 2.0 g calcium carbonate, 0.5 g bactocas-amino acid, 20 microgram thiamine hydrochlorid, 5 microgram D-biotin, 0.1 g potassium dihydrogen phosphate, 0.05 g magnesium sulfate heptahydrate, 0.2 mg ferrous sulfate heptahydrate and 0.2 mg managanese chloride tetrahydrate. The optimzed culture conditions resulted into elevated amount of L-lysine.

Conclusions

The fermentation method has important advantages of yielding the optically active L-form of L-lysine directly. The demand for L-lysine in food, animal feeds and pharmaceuticals is still increasing. To meet this demand the amino acid industry is attempting to improve the production technology by reducing costs and utilizing un-usual resources. Promising approaches for improving the productivity of L-lysine fermentation in future include.

Breeding better L-lysine producer with the aid of genetic engineering and protoplast fusion. The

high L-lysine producing strains were not particularly good consumers of glucose. Hence, their growth rate were not optimal. High L-lysine producers and high-glucose consumers could be produced from the fusion of high L-lysine, low-glucose strain with a low L-lysine, high-glucose strain.

The L-lysine feed back insensitive bacterial aspartokinase encoded by *Corynebacterium* could be expressed in plant like *Brassica napus* to increase free L-lysine accumulation in rap seed. Success in these endeavours will ensure that the L-lysine industry continues to grow in economic importance.

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