(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
15 September 2011 (15.09.2011)

PCT

(10) International Publication Number
WO 2011/111073 A2

(51) International Patent Classification:
C12P 13/08 (2006.01)

(21) International Application Number:
PCT/IN2011/000162

(22) International Filing Date:
10 March 2011 (10.03.2011)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
644/MUM/2010 11 March 2010 (11.03.2010) IN
668/MUM/2011 10 March 2011 (10.03.2011) IN

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(81) Designated States *(unless otherwise indicated, for every kind of national protection available)*: AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IS, JP, KE, KG, KM, KN, KP, KR, KZ, LA, LC, LK, LR, LS, LT, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PE, PG, PH, PL, PT, RO, RS, RU, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

(84) Designated States *(unless otherwise indicated, for every kind of regional protection available)*: ARIPO (BW, GH, GM, KE, LR, LS, MW, MZ, NA, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

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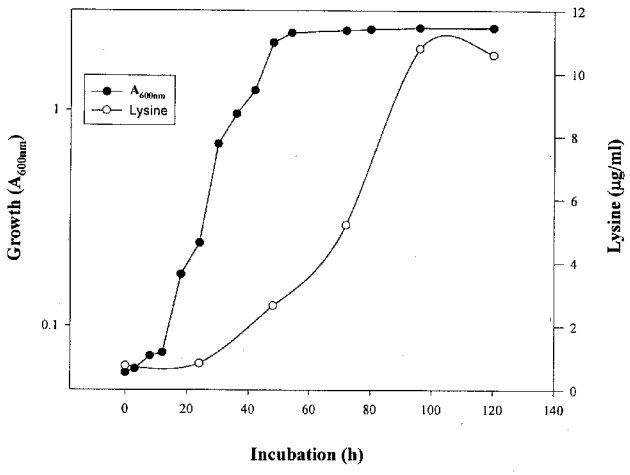
— *of inventorship (Rule 4.17(iv))*

[Continued on next page]

(54) Title: PROCESS FOR BIOGENESIS OF L-LYSINE FROM ε-CAPROLACTAM OR ε-CAPROLACTAM DEGRADATION OR RELATED INTERMEDIATES

Fig # 1

Demonstration of formation of L-Lysine during microbial degradation of ε-Caprolactam



(57) Abstract: A process of biogenesis of L-Lysine from ε-caprolactam or ε-caprolactam degradation or related intermediates selected from ε-Amino Caproic Acid [ACA], Amino Adipic Acid, Adipic Acid, NorLeucine or any combinations thereof, by microorganisms or their enzymes, and more particularly microorganisms belonging to groups Alcaligenes spp., *Arthrobacter* spp., *Bacillus* spp., *Pseudomonas* spp. and *Rhodococcus* spp.; in a reaction medium. The biotransformation process is performed leading to biogenesis of L-Lysine from ε-caprolactam or ε-caprolactam degradation or related intermediates selected from ε-Amino Caproic Acid [ACA], Amino Adipic Acid, Adipic Acid, NorLeucine or combination thereof, in presence of any or combination of modulators/ inhibitors, and then isolating the L-Lysine from the reaction mixture.

WO 2011/111073 A2

Published:

— *without international search report and to be republished upon receipt of that report (Rule 48.2(g))*

PROCESS FOR BIOGENESIS OF L-LYSINE FROM ε-CAPROLACTAM OR ε-CAPROLACTAM DEGRADATION OR RELATED INTERMEDIATES

Field of Invention:

The present invention relates to L-Lysine formation by biogenesis from ε-caprolactam or ε-caprolactam degradation or related intermediates selected from ε-Amino Caproic Acid [ACA], Amino Adipic Acid, Adipic Acid, NorLeucine or any combinations thereof.

The present invention relates to biogenesis of L-Lysine by the microbial isolates belonging to groups selected from Groups *Alcaligenes* spp., *Arthrobacter* spp., *Bacillus* spp., *Pseudomonas* spp. & *Rhodococcus* spp. during degradation of ε-caprolactam.

BACKGROUND OF THE INVENTION

L-Lysine is an essential amino acid and is widely used as additive to human and animal food. It is also employed in medicine as component of infusion solutions.

Lysine is one of the eight amino acids identified as essential for animals and human beings. Thomas Burr Osborne and his associates at the Connecticut Agricultural Experiment Station found in the experiments with rats that a diet containing corn as the sole source protein (known to be deficient in lysine and tryptophan) produced obvious signs of malnutrition. Strikingly, in the children in the countries where corn is the principal food, there existed a high frequency of the protein-deficiency syndrome – Kwashiorkor [Harpstead, D. D. (1971) *Sci.*

Am. **225(2):** 34-42]. Earlier, Rose *et al,* while reporting the findings of Albanese *et al* that human subjects receiving diets deficient in lysine not only complain of nausea, dizziness and hypersensitivity to metallic sounds, but also manifest a sharp rise in the urinary output of non-ketonic organic acids; concluded that lysine is the one of the most variable of the entire group of essential amino acids [Rose *et al,* (1955) *J. Biol. Chem.*, **214**: 579-587]. Rose *et al,* while establishing L-Lysine as an essential amino acid; found that young men experienced a loss of appetite, an increase in nervous irritability, and a sensation of fatigue and pronounced negative nitrogen balance, which is relieved by return of lysine to the diet [Rose *et al,* (1954) *J. Biol. Chem.*, **206**: 421-430].

Large-scale production of amino acids, hence, is felt necessary, to be used as food supplement to combat the widespread malnutrition. Besides their use as dietetic supplements, amino acids today find application in various ways, such as flavouring agents, for improvement of taste of food; as therapeutic agents and as laboratory reagent [Chatterjee and Chatterjee (1997) *Hind. Antibiot. Bull.*, **39**: 20-49].

Production of L-Lysine on a large scale started in 1958, with two main processes: Two-stage Fermentation and Single-Stage Fermentation. Kyowa Hakko Kogyo Company established a Two-stage fermentation process using auxotrophic mutants of microorganisms belonging to Groups – *Brevibacterium, Arthrobacter, Aerobacter, Micrococcus-22, E. coli, etc. While* Ajinomoto Company established a Single-stage Fermentation process using auxotrophs belonging to Group *Corynebacterium* (especially, *glutamicum*). The productivity was later increased by manipulations of export carrier genes and regulatory mutants.

It is known that L-Lysine is produced by hydrolysis of α-amino-ε-caprolactam (Helv. Chim. Acta, XLI, P 186 [1958]). However, the advent of biotransformation changed the market dynamics to a great extent.

Seto *et al* in U.S. Pat. No. 3,056,729 discloses conversion of DL-α-Amino-ε-Caprolactam to L-Lysine. But the rate of conversion of DL-α-Amino-ε-Caprolactam to L-Lysine in the process is at the most 30 percent.

Takashi *et al* in U.S. Pat. No. 3,770,585 describes α-amino-ε-caprolactam (ACL) biotransformation process for production of L-Lysine by an enzymatic route. The hydrolysing activity of microorganism belonging to *Cryptococcus, Candida, Trichosporon and enzyme produced by said microorganisms brings about the conversion of* α-amino-ε-caprolactam (ACL) to L-Lysine via a selective hydrolytic step.

The enzymatic production of lysine amounts to the tune of only about 10% of the total global production (Nakayama, 1985). In this enzymatic process, DL-α-Aminocaprolactam is incubated with a mixture of acetone dried cells of *Cryptococcus laurentii* and *Achromobacter obae* nov. sp., L-Lysine is obtained. *C. laurentii* produces L-aminocaprolactam hydrolase inductively in the medium containing DL-α-aminocaprolactam, while *A. obae* produces aminocaprolactam racemase using both D- and L-α-aminocaprolactam as inducer. A similar optimal pH value of both enzymes allows the efficient conversion in what appears to be a single step.

This Biotransformation based method offers low recovery cost and there is no problem of effluent disposal. However, amino caprolactam need to be specially synthesized chemically from cyclohexane adding to the total cost.

In contrast, to the existing methods, this invention describes a method never tried so far, based on biotransformation (and hence, at an advantage over fermentative methods) from a substrate much cheaper (and hence, of economic interests) and of hazardous & polluting value (and hence, role in bioremediation).

SUMMARY OF THE INVENTION

In one aspect, the present invention provides, biogenesis of L-Lysine from ε-caprolactam or ε-caprolactam degradation or related intermediates selected from the group of ε-Amino Caproic Acid [ACA], Amino Adipic Acid, Adipic Acid, NorLeucine or any combinations thereof; by the biomass and more particularly selected from the groups like *Alcaligenes* spp., *Arthrobacter* spp., *Bacillus* spp. *Pseudomonas* spp. and *Rhodococcus* spp.

According to the present invention, biogenesis of Lysine by degradation of ε-caprolactam or ε-caprolactam degradation or related intermediates selected from the group of ε-Amino Caproic Acid [ACA], Amino Adipic Acid, Adipic Acid, NorLeucine or any combinations thereof; comprises, contacting biomass and more particularly selected from the groups like *Alcaligenes* spp., *Arthrobacter* spp., *Bacillus* spp., *Pseudomonas* spp. and *Rhodococcus* spp. with any of the substrates like ε-Caprolactam, or ε-caprolactam degradation or related intermediates selected from the group of ε-Amino Caproic Acid [ACA], Amino Adipic Acid, Adipic Acid, NorLeucine or any combinations thereof; in presence of biochemical reaction modulators particularly selected from the group like ammonium chloride, azide, o-phenanthroline, 8-hydroxyquinoline, pyridoxal phosphate, malonic acid and like.

Thus, in various embodiments, the present invention provides a method of degradation ε- caprolactam or ε-caprolactam degradation intermediates selected from ε-Amino Caproic Acid [ACA], Amino Adipic Acid, Adipic Acid, NorLeucine or combination thereof; to L-Lysine by processes which comprise the steps of:

a) Generating biomass for degradation of substrate(s) by utilizing microorganisms selected from the genera *Alcaligenes*, *Arthrobacter*, *Bacillus*, *Pseudomonas*, *Rhodococcus*, and like, in media – rich or defined;

b) Optionally washing the culture with saline, with or without starvation, with or without adaptation in caprolactam or ε-caprolactam degradation intermediates selected from ε-Amino Caproic Acid [ACA], Amino Adipic Acid, Adipic Acid, NorLeucine or combination thereof, with or without the conventional chilled acetone treatment

c) contacting the washed biomass with any of the substrates like ε-caprolactam or ε-caprolactam degradation intermediates selected from ε-Amino Caproic Acid [ACA], Amino Adipic Acid, Adipic Acid, NorLeucine or combination thereof; in presence or absence of any one or combination of biochemical reaction modulators like ammonium chloride, azide, o-phenanthroline, 8-hydroxyquinoline, pyridoxal phosphate, malonic acid;

d) isolating the formed L-Lysine from the biotransformation medium by methods known earlier.

BRIEF DESCRIPTION OF THE DRAWINGS

The present invention will become fully understood from the detailed description and the accompanying drawings, wherein:

FIG. 1 Formation of Lysine

FIG. 2 Formation of Lysine by cultures R, B, F & Rh

FIG. 3 Cap degradation pathway describes the bacterial degradation of ε-caprolactam.

It should be noted that these figures are intended to exemplify the general characteristics of the invention for the purpose of the description of such embodiments herein. These figures may not precisely reflect the characteristics of any given embodiment and is not necessarily intended to define or limit specific embodiments within the scope of this invention.

DETAIL DESCRIPTION OF THE INVENTION:

The ability to degrade ε-caprolactam is a rare feature in the microbial world [Shama and Wase, (1981) *International Biodeterioration Bulletin* **17**: 1-9]. Kato and Fukumura (1962) reported the bacterial breakdown of ε-caprolactam. Tosa and Chibata (1965) [*J. Bact.* 89 (3): 919-920] were first to report formation of ω-amino acids from cyclic amides suggesting 'cyclic amide hydrolase' activity in microorganisms. T. Fukumura (1966) [*Plant & Cell Physiol.*, 7: 93-104] isolated 11 different types of bacteria growing on ε-caprolactam as sole source of carbon and nitrogen in presence of small amount of yeast extract. Of these, one strain was found to be capable of utilizing cyclic and linear oligomers of 6-Amino caproic acid [ACA].

Though some workers have reported microbial degradation of ε-caprolactam [Shama and Wase, 1981; Esikova *et al*, (1992) (*Mikrobiologiya.* 59(4): 547-552); Kulkarni and Kanekar, (1998) (*Process Control Qual.*, 9: 31-37)], but none have reported the formation of L-Lysine during microbial degradation of ε-caprolactam.

Thus, there is no information regarding biogenesis of L-lysine during ε-caprolactam or ε-caprolactam degradation intermediates selected from ε-Amino Caproic Acid [ACA], Amino Adipic Acid, Adipic Acid, NorLeucine or combination thereof, utilization by microorganisms or their enzymes. In contrast to the other workers working on ε-caprolactam degradation, which have never previously reported formation of lysine from ε-caprolactam, in present invention; inventor could conclusively detect formation of lysine [Fig. # 1] during ε-caprolactam degradation by biomass and more particularly belonging to group *Alcaligenes* spp., *Arthrobacter* spp., *Bacillus* spp., *Pseudomonas* spp. and *Rhodococcus* spp.

As evident from the Figure # 1, the Lysine formation profile matched the log phase of the growth profile of the growth of cultures. Indeed, the essential amino acid is a product of primary metabolism.

Biotransformation medium consists of ε-caprolactam or ε-caprolactam degradation intermediates or any combinations thereof along with salts consisting of Mg^{+2} and PO_4^{-3}.

The Salts of Mg^{+2} are selected from group of Magnesium hydroxide, chloride, oxide, gluconate, malate, orotate, citrate, sulfate (inclusive of Epsom salts), borate or salicylate and Salts of PO_4^{-3} are selected from group of Potassium dihydro phosphate, Dipotassium hydrogen phosphate, Sodium phosphate, Magnesium phosphate, and likes and their mixtures thereof.

The medium employed for enrichment and cultivation of caprolactam utilizing micro-organisms, contained caprolactam as sole carbon and nitrogen source with the following composition (g l^{-1}): $MgSO_4.7H_2O$ 0.70, KH_2PO_4 1.00,

Caprolactam 10.00 (pH 7.6) [CM]. The minimal medium without Caprolactam is also referred to as Basal Medium, elsewhere. Ammonium chloride [0.6 g l^{-1}] was supplemented depending upon the need. In the case of solid media, 2.5 g% of agar-agar was used. In the above medium, caprolactam was replaced with ε-caprolactam degradation intermediates selected from ε-Amino Caproic Acid [ACA], Amino Adipic Acid, Adipic Acid, NorLeucine or combination thereof, other substrates like Sucrose (10g l^{-1}), or ε-Amino Caproic Acid [ACA] (10g l^{-1}) for alternate media.

Biomass was generated in rich medium like Luria Bertani medium, with 2% v/v inoculums, incubation on shaker at 180 rpm, and 30 ± 2 °C. Cells were grown to an °A600 nm = 0.6, washed thoroughly in saline twice and resuspended in one tenth of the original volume of Caprolactam medium [CM]. The cells were thus, adapted to caprolactam for 7 hours on rotary shaker at 180 rpm. The cells were then harvested by centrifugation in a bench-top medium-speed centrifuge at 10,000 x g for 10 minutes. Acetone treatment was given with chilled extrapure acetone twice for 15 minutes each at –20 °C. Every time, the cells were thoroughly washed washed with extrapure acetone and centrifuged at 10, 000 x g for 6 minutes. Such acetone-dried biomass was carefully collected weighed and small aliquots of the same stored at -20 °C till further use.

In the case of biomass generated from defined medium, the biomass generated from Caprolactam medium or Sucrose (+NH_4Cl) medium was harvested by centrifugation in a bench-top medium-speed centrifuge at 10,000 x g for 10 minutes and washed with one-tenth volume of normal saline. Acetone treatment was given with chilled extrapure acetone twice for 15 minutes each at –20 °C. Every time, the cells were thoroughly washed washed with extrapure acetone and centrifuged at 10,000 x g (Remi centrifuge) for 6 minutes. Such

acetone-dried biomass was carefully collected weighed and small aliquots of the same stored at -20 °C till further use.

Substrates with different concentrations added to the Minimal/Basal medium, filter sterilized using 0.1 µm Nitrocellulose filters were incubated with acetone-dried biomass at 30 ± 2 °C on rotary shaker at 180 rpm for 16 hours. The incubation/reaction was stopped by centrifuging the system at 10,000 x g for 6 min and the supernatant was separated and used for various analyses. Cell free supernatant was used for paper chromatography and Lysine bioassay.

After initial qualitative assessment of lysine by Paper Chromatography, the lysine was quantified by microbiological assay using the organism *Pediococcus acidilactici* (previously called as *Leuconostoc mesenteroides*) ATCC 8042 - a lysine auxotroph, following a method, essentially given by Meites (1963) [Difco Manual of dehydrated culture media and reagents; Difco Laboratories, Inc., Detroit, p. 233].

Contacting of biomass with any of the substrates like ε-caprolactam or ε-caprolactam degradation intermediates selected from ε-Amino Caproic Acid [ACA], Amino Adipic Acid, Adipic Acid, NorLeucine or combination thereof; in presence or absence of any one or combination of biochemical reaction modulators like ammonium chloride, azide, o-phenanthroline, 8-hydroxyquinoline, pyridoxal phosphate, malonic acid

According to the present invention, the microorganisms belonging to Groups *Alcaligenes spp., Arthrobacter spp., Bacillus spp. Pseudomonas* spp., *Rhodococcus spp., and the likes;* and enzymes produced by said microorganisms or their enzymes brings about the conversion of ε-caprolactam or ε-caprolactam degradation intermediates selected from ε-Amino Caproic Acid

[ACA], Amino Adipic Acid, Adipic Acid, NorLeucine or combination thereof to L-Lysine via contacting step, whereby the desired product is readily obtained. In other words, in accordance with the process of this invention, ε-caprolactam or ε-caprolactam degradation intermediates selected from ε-Amino Caproic Acid [ACA], Amino Adipic Acid, Adipic Acid, NorLeucine or combination thereof gives pure L-Lysine by biogenesis.

Wherein the microorganisms belonging to group *Alcaligenes* spp. belonged to *Alcaligenes faecalis* [designated as R_2 or R, herein further]; microorganisms belonging to group *Arthrobacter* spp. belonged to *Arthrobacter citreus* [designated as F or fgc, herein further]; microorganisms belonging to group *Bacillus* spp. belonged to *Bacillus sphaericus* [designated B, herein further]; and microorganisms belonging to group *Rhodococcus* spp. belonged to *Rhodococcus erythropolis* [designated as Rh, herein further] [Fig. # 2].

Legend to Fig. # 2

Plates	Isolates
A	R (*Alcaligenes faecalis*)
B	B (*Bacillus sphaericus*)
C	F (*Arthrobacter citreus*)
D	Rh (*Rhodococcus erythropolis*)

Lanes	Spots
1	'0' h sample
2	Lane 1 + co-chromatography
3	Standard Lysine
4	Standard ACL (in the case of Plate C, Standard ACA)
5	Lane 6 + co-chromatography
6	'21' h sample

Loading	Sample:	always 30 µl of the sample
	Standard:	As in Standards as well as in Cochromatography
		10 µg of the standard in 5 µl of [i)DL-ACL, ii) ACA, iii) Lysine]
Solvent System	Propan-1-ol: Ethyl Acetate: Ammonia: Water[PEAW] =	
	6 : 1 : 1 : 3	

In an embodiment of the present invention method for formation of L-Lysine which comprises the steps of:

a) Generating biomass for degradation of caprolactam by utilizing microorganisms selected from the genera *Alcaligenes*, *Arthrobacter*, *Bacillus*, *Pseudomonas*, *Rhodococcus*, and like, in media – rich or defined;

b) Optionally washing the culture with saline, with or without starvation, with or without adaptation in caprolactam or ε-caprolactam degradation or related intermediates selected from the group of ε-Amino Caproic Acid [ACA], Amino Adipic Acid, Adipic Acid, NorLeucine or any combinations thereof, with or without the conventional chilled acetone treatment

c) contacting the washed biomass with any of the substrates like ε-caprolactam or ε-caprolactam degradation intermediates selected from ε-Amino Caproic Acid [ACA], Amino Adipic Acid, Adipic Acid, NorLeucine or combination thereof; in presence or absence of any one or combination of biochemical reaction modulators like ammonium chloride, azide, o-phenanthroline, 8-hydroxyquinoline, pyridoxal phosphate, malonic acid;

d) isolating the formed L-Lysine from the biotransformation medium by methods known earlier.

Example I

Bacillus sphaericus, from the slant of Caprolactam Medium [CM] [in (g l^{-1}): $MgSO_4.7H_2O$ 0.70, KH_2PO_4 1.00, Caprolactam 10.00 (pH 7.6)], was grown in CM that was previously sterilized at 15 pounds/sq. inch for 15 mins.

Samples were withdrawn from different flasks at periodic intervals to find $°A_{600nm}$ and also assayed for lysine by method essentially given by Meites (1963) [Difco Manual of dehydrated culture media and reagents; Difco Laboratories, Inc., Detroit, p. 233]. After about 105 hours of incubation, 11. 2 µg/ml of L-Lysine was formed matching during the log phase of the growth profile of the bacterial culture [Fig. # 1]. Indeed, the essential amino acid is a product of primary metabolism.

Example II

Microbial cultures viz. *Alcaligenes faecalis* [designated as R_2 or R, herefurther], *Arthrobacter citreus* [designated as F or fgc herefurther], *Bacillus sphaericus* [designated as B, herefurther], and *Rhodococcus erythropolis* [designated as Rh, herefurther]grown in Luria-Bertani medium, washed in normal saline and resuspended in Caprolactam Medium [CM] [of Example I]. Equal volume of cells [of $°A_{600nm}$ = 0.6] were washed with normal saline and resuspended in one tenth volume of Biotransformation Medium - 5X Caprolactam Medium [with 50 g l^{-1} of Caprolactam in the CM of Example I], were allowed to incubate in shaking conditions at 180 rpm at 30 °C ± 2 °C for 21 hours. The reaction is terminated by separating the cells from the medium by centrifugation. The L-Lysine formed was in the range of 35 to 48 µg/ml [Fig. # 2].

Example III

In the Example II, the biomass (for Microbial cultures *Alcaligenes faecalis*, *Arthrobacter citreus*, and *Bacillus sphaericus*) was generated in the Caprolactam Medium of Example I upto $°A_{600nm}$ = 0.6 and then is treated with chilled

extrapure acetone twice for 15 minutes each at -20 °C. Equal quantity of acetone treated biomass in powder form was then resuspended in the Biotransformation Medium (of Example II). The L-Lysine formed was in the range of 30 to 40 µg/10 mg of acetone treated biomass/ml.

Example IV

In the Example III, the biomass is generated in the Luria-Bertani Medium, the biomass is washed with Normal Saline, adapted in Caprolactam Medium of Example I for 7 hours in shaking conditions at 180 rpm at 30 °C ± 2 °C and then acetone treatment & rest of the processing and the reaction as in Example III. The L-Lysine formed was in the range of 500 to 1500 µg/10 mg of acetone treated biomass/ml.

Example V

In the Example III, the biomass is generated in Defined Media viz. the Sucrose (+ Ammonium Chloride) Medium (Same as Caprolactam medium, with caprolactam being replaced by Sucrose 10 g l^{-1}; NH_4Cl 0.6 g l^{-1}), AND the Caprolactam medium [CM] and then acetone treatment & rest of the processing and the reaction as in Example III. The L-Lysine formed was in the range of 500 to 1500 µg/10 mg of acetone treated biomass/ml.

Since many different embodiments of the invention may be made without departing from the spirit and scope thereof, it is to be understood that the invention is not limited by the specific illustrations except to the extent defined in the following claims.

We claim,

1. A method for formation of L-Lysine which comprises degradation of ε-caprolactam by microorganisms or their enzymes.

2. L-Lysine formation which comprises degradation of ε-caprolactam or ε-caprolactam degradation or related intermediates, by microorganisms or their enzymes.

3. A method for formation of L-Lysine which comprises of:
   (a) Generating biomass of ε-caprolactam or ε-caprolactam degradation or related intermediates selected from ε-Amino Caproic Acid [ACA], Amino Adipic Acid, Adipic Acid, NorLeucine or combination thereof utilizing microorganisms or their enzymes in media;
   (b) Optionally on adaptation in ε-caprolactam or ε-caprolactam degradation or related intermediates selected from ε-Amino Caproic Acid [ACA], Amino Adipic Acid, Adipic Acid, NorLeucine or their combinations thereof;
   (c) Optionally with Acetone treatment to the culture,
   (d) On contacting the biomass with the biotransformation medium in presence or absence of biochemical reaction modulators;
   (e) Isolating the formed L-Lysine from the biotransformation medium by methods known earlier.

4. L-Lysine formation, according to preceding claims wherein the likes of ε-caprolactam or ε-caprolactam degradation or related intermediates selected from ε-Amino Caproic Acid [ACA], Amino Adipic Acid, Adipic Acid, NorLeucine or combination thereof.

5. A method for producing L-Lysine, according to preceding claims wherein the microorganisms are selected from the genera *Alcaligenes, Arthrobacter, Bacillus, Pseudomonas, Rhodococcus or any combinations, thereof*.

6. A method for producing L-Lysine, according to Claim 3 step [a] wherein the biomass generating medium is rich or defined.

7. A method for producing L-Lysine, according to Claim 3 Step [d] wherein the biotransformation medium consists of ε-caprolactam or ε-caprolactam degradation/related intermediates or any combinations thereof; optionally along with salts consisting of Mg^{+2} and PO_4^{-3}.

8. A method for producing L-Lysine, according to Claim 7 wherein the Salts of Mg^{+2} are selected from group of Magnesium hydroxide, chloride, oxide, gluconate, malate, orotate, citrate, sulfate (inclusive of Epsom salts), borate or salicylate and Salts of PO_4^{-3} are selected from group of Potassium dihydro phosphate, Dipotassium hydrogen phosphate, Sodium phosphate, Magnesium phosphate, and likes and their mixtures thereof.

9. A method for producing L-Lysine, according to Claim 3 Step (d) wherein the biochemical reaction modulators are selected from ammonium chloride, azide, o-phenanthroline, 8-hydroxyquinoline, pyridoxal phosphate, malonic acid, and any combinations thereof.

10. A method for producing L-Lysine by biotransformation, which comprises of culturing microorganisms or extracting enzymes thereof, with or without acetone treatment; when contacted with a biotransformation

medium, and recovering the L-Lysine which accumulates in the culture medium.

Fig # 1

Demonstration of formation of L-Lysine during microbial degradation of ε-Caprolactam

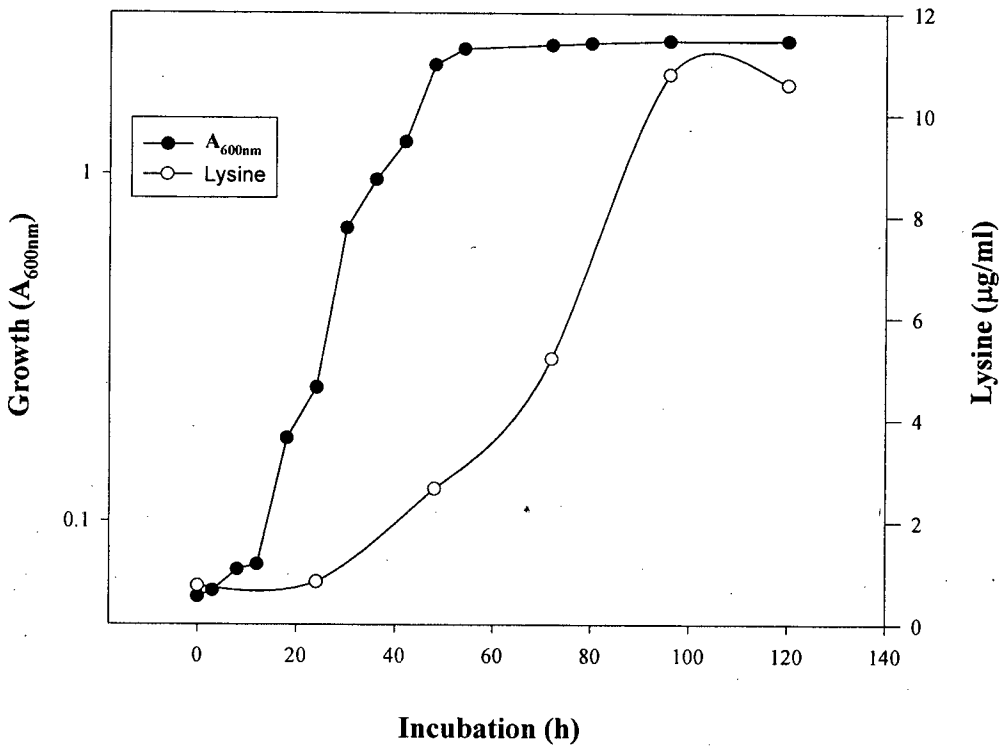
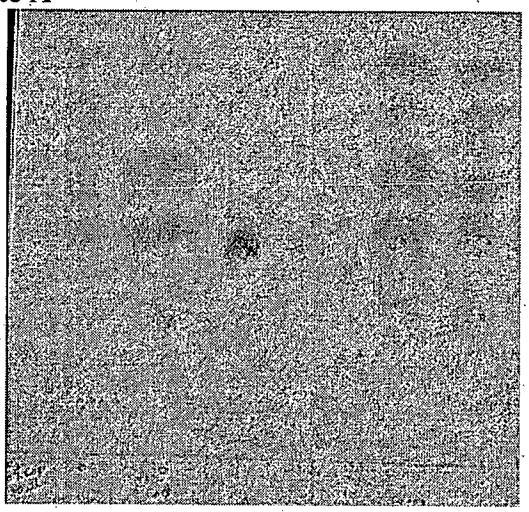


Fig. # 2

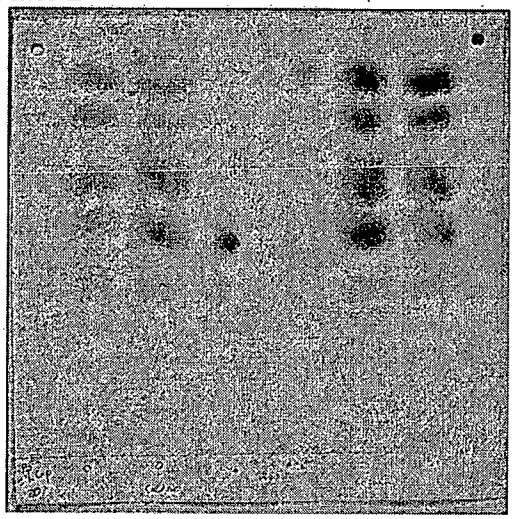
Paper Chromatographic analysis
Demonstrating Lysine formation by taxonomically different bacterial isolates

Plate A



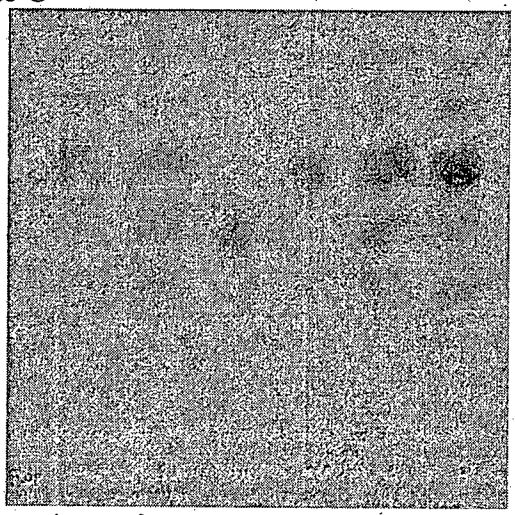
1 2 3 4 5 6

Plate B



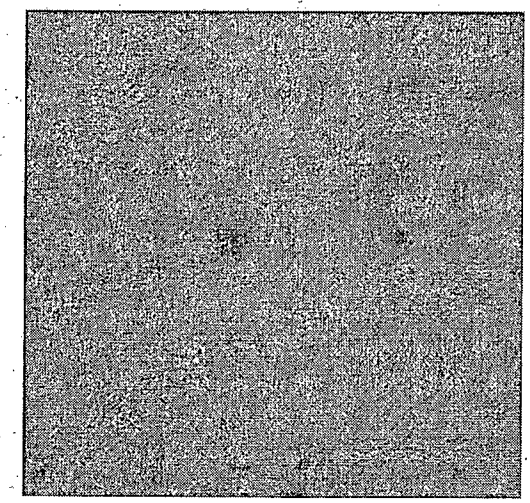
1 2 3 4 5 6

Plate C



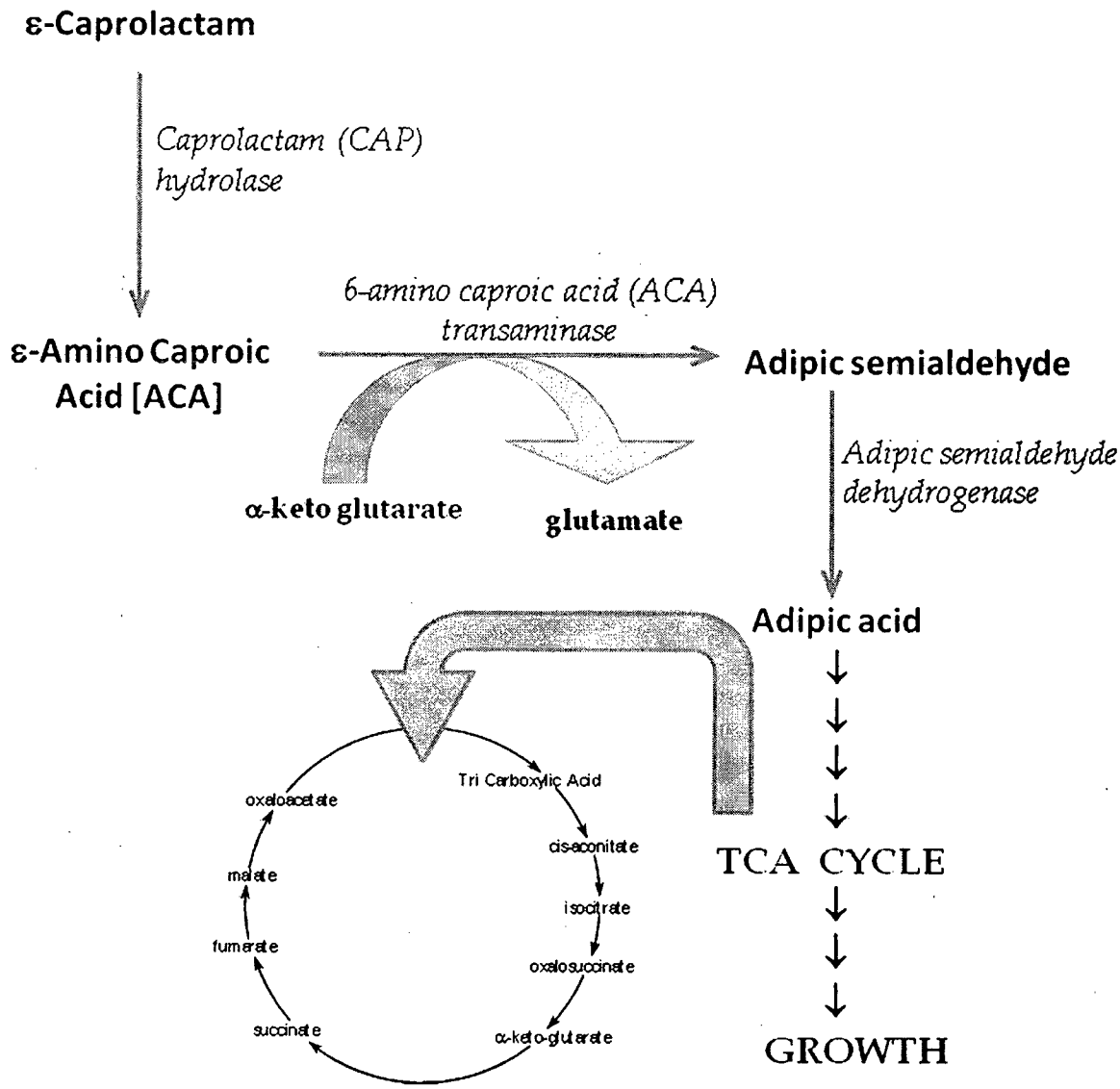
1 2 3 4 5 6

Plate D



1 2 3 4 5 6

Fig. # 3


ε-Caprolactam
Caprolactam (CAP) hydrolase
ε-Amino Caproic Acid [ACA]
6-amino caproic acid (ACA) transaminase
α-keto glutarate
glutamate
Adipic semialdehyde
Adipic semialdehyde dehydrogenase
Adipic acid
TCA CYCLE
GROWTH
Tri Carboxylic Acid
oxeloacetate
cis-aconitate
isocitrate
oxalosuccinate
α-keto-glutarate
succinate
fumarate
malate