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54	TITLE OF INVENTION
	Method for producing L-threonine using recombinant enterobacteriaceae with increased enolase activity

57	ABSTRACT (NOT MORE THAN 150 WORDS)

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The sheet(s) containing the abstract is/are attached.

If no classification is furnished, Form P.9 should accompany this form.
The figure of the drawing to which the abstract refers is attached.

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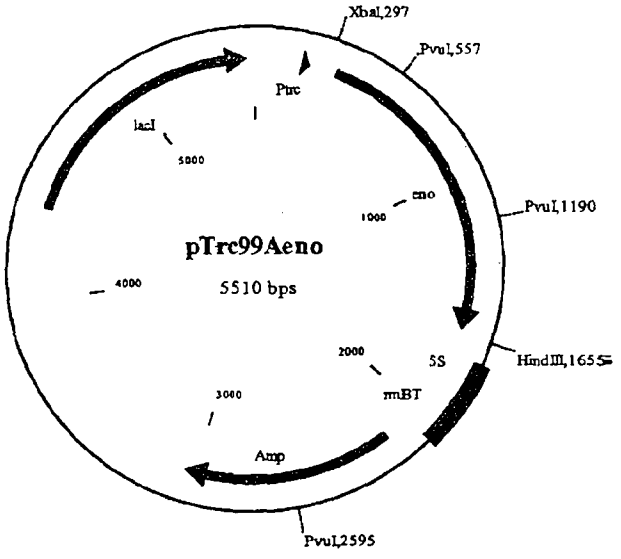
(54) Title: METHOD FOR PRODUCING L-THREONINE USING RECOMBINANT ENTEROBACTERIACEAE WITH INCREASED ENOLASE ACTIVITY



(57) Abstract: The invention relates to a process for the preparation of L-amino acids, in particular L-threonine, in which the following steps are carried out: a) fermentation of the microorganisms of the Enterobacteriaceae family which produce the desired L-amino acid and in which the eno gene or nucleotide sequences or alleles which code for the gene product thereof is/are enhanced, b) concentration of the desired L-amino acid in the medium or in the cells of the microorganisms, and c) isolation of the desired L-amino acid.

WO 2005/071094 A3

Process for the Preparation of L-Amino Acids using Strains of the Enterobacteriaceae Family

Field of the Invention

This invention relates to a process for the preparation of L-amino acids, in particular L-threonine, using recombinant microorganisms of the Enterobacteriaceae family in which the eno gene is enhanced, in particular over-expressed, and to these microorganisms.

Background of the Invention

L-Amino acids, in particular L-threonine, are used in human medicine and in the pharmaceuticals industry, in the foodstuffs industry and very particularly in animal nutrition.

It is known that L-amino acids are prepared by fermentation of strains of Enterobacteriaceae, in particular Escherichia coli (E. coli) and Serratia marcescens. Because of their great importance, work is constantly being undertaken to improve the preparation processes. Improvements to the process can relate to fermentation measures, such as e.g. stirring and supply of oxygen, or the composition of the nutrient media, such as e.g. the sugar concentration during the fermentation, or the working up to the product form, by e.g. ion exchange chromatography, or the intrinsic output properties of the microorganism itself.

Methods of mutagenesis, selection and mutant selection are used to improve the output properties of these microorganisms. Strains which are resistant to antimetabolites, such as e.g. the threonine analogue α-amino-β-hydroxyvaleric acid (AHV), or are auxotrophic for metabolites of regulatory importance and produce L-amino acid, such as e.g. L-threonine, are obtained in this manner.

Methods of the recombinant DNA technique have also been employed for some years for improving the strain of strains of the Enterobacteriaceae family which produce L-amino acids, by amplifying individual amino acid biosynthesis genes and investigating the effect on the production. Summarizing information on the cell and molecular biology of Escherichia coli and Salmonella are to be found in Neidhardt (ed): Escherichia coli and Salmonella, Cellular and Molecular Biology, 2nd Edition, ASM Press, Washington, D.C., USA (1996).

Object of the Invention

The object of the invention is to provide new measures for improved fermentative preparation of L-amino acids, in particular L-threonine.

Summary of the Invention

The invention provides recombinant microorganisms of the Enterobacteriaceae family which contain an enhanced or over-expressed eno gene, which codes for enolase, or alleles thereof, and which produce L-amino acids, in particular L-threonine, in an improved manner.

The starting point for the comparison are in each case the microorganisms which are not recombinant for the eno gene and contain no enhanced eno gene.

These microorganisms include, in particular, microorganisms of the Enterobacteriaceae family in which a polynucleotide which codes for a polypeptide of which the amino acid sequence is identical to the extent of at least 90%, in particular to the extent of at least 95%, preferably to the extent of at least 98% or at least 99%, particularly preferably to the extent of 99.7% and very particularly preferably to the extent of 100% to an amino acid sequence chosen from the group consisting of SEQ ID No. 4, SEQ ID No . 6, and SEQ ID No. 8 is enhanced.

Amino acid sequences which are identical to those from SEQ ID No. 4, SEQ ID No. 6 or SEQ ID No. 8 are preferred.

The microorganisms mentioned contain enhanced or over-expressed polynucleotides chosen from the group consisting of:

a) polynucleotide with the nucleotide sequence SEQ ID No. 3, SEQ ID No. 5 or SEQ ID No. 7;

b) polynucleotide with a nucleotide sequence which corresponds to SEQ ID No. 3, SEQ ID No. 5 or SEQ ID No. 7 within the context of degeneration of the genetic code;

c) polynucleotide sequence with a sequence which hybridizes under stringent conditions with the sequence complementary to SEQ ID No. 3, SEQ ID No. 5 or SEQ ID No. 7;

d) polynucleotide with a sequence SEQ ID No. 3, SEQ ID No. 5 or SEQ ID No. 7 which contains neutral-function sense mutants,

where the polynucleotides code for enolase.

The invention also provides a process for the fermentative preparation of L-amino acids, in particular L-threonine, using recombinant microorganisms of the Enterobacteriaceae family which, in particular, already produce L-amino acids and in which at least the eno gene or nucleotide sequences which code for the gene product thereof is or are enhanced.

Detailed Description of the Invention

The microorganisms described are preferably employed.

Where L-amino acids or amino acids are mentioned in the following, this means one or more amino acids, including their salts, chosen from the group consisting of

L-asparagine, L-threonine, L-serine, L-glutamate, L-glycine, L-alanine, L-cysteine, L-valine, L-methionine, L-proline, L-isoleucine, L-leucine, L-tyrosine, L-phenylalanine, L-histidine, L-lysine, L-tryptophan, L-arginine and L-homoserine. L-Threonine is particularly preferred.

The term "enhancement" in this connection describes the increase in the intracellular activity or concentration of one or more enzymes or proteins in a microorganism which are coded by the corresponding DNA, for example by increasing the number of copies of the gene or genes by at least one (1) copy, linking a potent promoter functionally to the gene or using a gene or allele which codes for a corresponding enzyme or protein with a high activity, and optionally combining these measures.

Alleles are in general understood as meaning alternative forms of a given gene. The forms are distinguished by differences in the nucleotide sequence.

The protein coded by a nucleotide sequence, i.e. an ORF, a gene or an allele, or the ribonucleic acid coded is in general called the gene product.

By enhancement measures the activity or concentration of the corresponding protein is in general increased by at least 10%, 25%, 50%, 75%, 100%, 150%, 200%, 300%, 400% or 500%, up to a maximum of 1,000% or 2,000%, based on that of the wild-type protein or on the activity or concentration of the protein in the microorganism or parent strain which is not recombinant for the corresponding enzyme or protein. Microorganism or parent strain which is not recombinant is understood as meaning the microorganism on which the inventive measures are carried out.

The invention also provides a process for the preparation of L-amino acids by fermentation of recombinant

microorganisms of the Enterobacteriaceae family, characterized in that

a) the microorganisms of the Enterobacteriaceae family which produce the desired L-amino acid and in which the eno gene or alleles thereof is/are enhanced, in particular over-expressed, are cultured in a medium under conditions suitable for the formation of the eno gene product,

b) the desired L-amino acid is concentrated in the medium or in the cells of the microorganisms, and

c) the desired L-amino acid is isolated, constituents of the fermentation broth and/or the biomass in its entirety or portions (≥ 0 to 100%) thereof optionally remaining in the product which has been isolated or being completely removed.

The microorganisms, in particular recombinant microorganisms, with an enhanced eno gene which the present invention also provides can produce L-amino acids from glucose, sucrose, lactose, fructose, maltose, molasses, optionally starch, optionally cellulose or from glycerol and ethanol. They are representatives of the Enterobacteriaceae family chosen from the genera Escherichia, Erwinia, Providencia and Serratia. The genera Escherichia and Serratia are preferred. Of the genus Escherichia in particular the species Escherichia coli and of the genus Serratia in particular the species Serratia marcescens are to be mentioned.

Recombinant microorganisms are in general produced by transformation, transduction or conjugation, or a combination of these methods, with a vector which contains the desired gene, an allele of this gene or parts thereof and/or a promoter which enhances the expression of the gene. This promoter can be the promoter originating by enhancing mutation from the endogenous regulatory sequence

upstream of the gene, or an efficient promoter that has been fused with the gene.

Suitable strains, which produce L-threonine in particular, of the genus Escherichia, in particular of the species Escherichia coli, are, for example

- Escherichia coli H4581 (EP 0 301 572)
- Escherichia coli KY10935 (Bioscience Biotechnology and Biochemistry 61(11):1877-1882 (1997)
- Escherichia coli VNIIgenetika MG442 (US-A-4278,765)
- Escherichia coli VNIIgenetika M1 (US-A-4,321,325)
- Escherichia coli VNIIgenetika 472T23 (US-A- 5,631,157)
- Escherichia coli BKIIM B-3996 (US-A- 5,175,107)
- Escherichia coli kat 13 (WO 98/04715)
- Escherichia coli KCCM-10132 (WO 00/09660).

Suitable L-threonine-producing strains of the genus Serratia, in particular of the species Serratia marcescens, are, for example

- Serratia marcescens HNr21 (Applied and Environmental Microbiology 38(6): 1045-1051 (1979))
- Serratia marcescens TLr156 (Gene 57(2-3): 151-158 (1987))
- Serratia marcescens T-2000 (Applied Biochemistry and Biotechnology 37(3): 255-265 (1992)).

Strains from the Enterobacteriaceae family which produce L- threonine preferably have, inter alia, one or more genetic or phenotypic features chosen from the group consisting of: resistance to α-amino-β-hydroxyvaleric acid, resistance to thialysine, resistance to ethionine,

resistance to α-methylserine, resistance to diaminosuccinic acid, resistance to α-aminobutyric acid, resistance to borrelidin, resistance to cyclopentane-carboxylic acid, resistance to rifampicin, resistance to valine analogues, such as, for example, valine hydroxamate, resistance to purine analogues, such as, for example, 6-dimethylaminopurine, a need for L-methionine, optionally a partial and compensatable need for L-isoleucine, a need for meso-diaminopimelic acid, auxotrophy in respect of threonine-containing dipeptides, resistance to L-threonine, resistance to threonine raffinate, resistance to L-homoserine, resistance to L-lysine, resistance to L-methionine, resistance to L-glutamic acid, resistance to L-aspartate, resistance to L-leucine, resistance to L-phenylalanine, resistance to L-serine, resistance to L-cysteine, resistance to L-valine, sensitivity to fluoropyruvate, defective threonine dehydrogenase, optionally an ability for sucrose utilization, enhancement of the threonine operon, enhancement of homoserine dehydrogenase I-aspartate kinase I, preferably of the feed back resistant form, enhancement of homoserine kinase, enhancement of threonine synthase, enhancement of aspartate kinase, optionally of the feed back resistant form, enhancement of aspartate semialdehyde dehydrogenase, enhancement of phosphoenol pyruvate carboxylase, optionally of the feed back resistant form, enhancement of phosphoenol pyruvate synthase, enhancement of transhydrogenase, enhancement of the RhtB gene product, enhancement of the RhtC gene product, enhancement of the YfiK gene product, enhancement of a pyruvate carboxylase, and attenuation of acetic acid formation.

It has been found that microorganisms of the Enterobacteriaceae family produce L-amino acids, in particular L-threonine, in an improved manner after enhancement, in particular over-expression, of the eno gene or alleles thereof.

The nucleotide sequences of the genes of Escherichia coli belong to the prior art (see the following text references) and can also be found in the genome sequence of Escherichia coli published by Blattner et al. (Science 277: 1453-1462 (1997)). It is known that enzymes endogenous in the host (methionine aminopeptidase) can split off the N-terminal amino acid methionine.

The nucleotide sequences for the eno gene are likewise known from Shigella flexneri and Salmonella typhimurium, which also belong to the Enterobacteriaceae family.

The eno gene is described, inter alia, by the following data:

Description:	enolase, phosphopyruvate hydratase
Function:	enolization (splitting off of water): 2-phosphoglycerate to phosphoenol pyruvate in glycolysis
EC No.:	EC 4.2.1.11
Reference:	Spring TG. and Wold F.; The Journal of Biological Chemistry 246(22) : 6797-6802 (1971)
	Klein et al.; DNA Sequence 6 (6): 351-355 (1996)
	Gulick et al.; Biochemistry 40(51): 15716 - 15724 (2001)
	Kaga et al.; Bioscience, Biotechnology and Biochemistry 66(10): 2216-2220 (2002)
Accession No.:	AE000361

The eno gene from Salmonella typhimurium is described, inter alia, in the following reference: Garrido-Pertierra A.; Revista Espanola de Fisiologia 36(1): 33-39 (1980).

The nucleic acid sequences can be found in the databanks of the National Center for Biotechnology Information (NCBI) of the National Library of Medicine (Bethesda, MD, USA), the nucleotide sequence databank of the European Molecular

Biologies Laboratories (EMBL, Heidelberg, Germany or Cambridge, UK) or the DNA databank of Japan (DDBJ, Mishima, Japan).

For better clarity, the known nucleotide sequence of the eno gene from Escherichia coli is to be found in SEQ ID No. 3 and the known sequences for the eno gene from Shigella flexneri (AE015293) and Salmonella typhimurium (AE008835) are to be found under SEQ ID No. 5 or SEQ ID No. 7. The amino acid sequences of the proteins coded by these reading frames are shown as SEQ ID No. 4, SEQ ID No. 6 or SEQ ID No. 8.

The genes described in the text references mentioned can be used according to the invention. Alleles of the genes which result from the degeneracy of the genetic code or due to "sense mutations" of neutral function can furthermore be used. The use of endogenous genes is preferred.

"Endogenous genes" or "endogenous nucleotide sequences" are understood as meaning the genes or alleles or nucleotide sequences present in the population of a species.

Suitable alleles of the eno gene which contain neutral-function sense mutations include, inter alia, those which lead to at most 50 or to at most 40 or to at most 30 or to at most 20, preferably to at most 10 or to at most 5, very particularly preferably to at most 3 or to at most 2 or to at least one (1) conservative amino acid exchange in the protein coded by them.

In the case of aromatic amino acids, conservative exchanges are referred to when phenylalanine, tryptophan and tyrosine are exchanged for one another. In the case of hydrophobic amino acids, conservative exchanges are referred to when leucine, isoleucine and valine are exchanged for one another. In the case of polar amino acids, conservative exchanges are referred to when glutamine and asparagine are exchanged for one another. In the case of basic amino

acids, conservative exchanges are referred to when arginine, lysine and histidine are exchanged for one another. In the case of acidic amino acids, conservative exchanges are referred to when aspartic acid and glutamic acid are exchanged for one another. In the case of amino acids containing hydroxyl groups, conservative exchanges are referred to when serine and threonine are exchanged for one another. All other amino acid exchanges are called non-conservative amino acid exchanges.

In the same way, those nucleotide sequences which code for variants of the proteins mentioned which additionally contain a lengthening or shortening by at least one (1) amino acid on the N or C terminus can also be used. This lengthening or shortening is not more than 50, 40, 30, 20, 10, 5, 3 or 2 amino acids or amino acid radicals.

Suitable alleles also include those which code for proteins in which at least one (1) amino acid is inserted (insertion) or removed (deletion). The maximum number of such changes, called indels, can relate to 2, 3, 5, 10, 20 but in no case more than 30 amino acids.

Suitable alleles furthermore include those which are obtainable by hybridization, in particular under stringent conditions, using SEQ ID No. 3, SEQ ID No. 5 or SEQ ID No. 7 or parts thereof, in particular the coding regions or the sequences complementary thereto.

Instructions for identifying DNA sequences by means of hybridization can be found by the expert, inter alia, in the handbook "The DIG System Users Guide for Filter Hybridization" from Boehringer Mannheim GmbH (Mannheim, Germany, 1993) and in Liebl et al. (International Journal of Systematic Bacteriology 41: 255-260 (1991)). The hybridization takes place under stringent conditions, that is to say only hybrids in which the probe and target sequence, i.e. the polynucleotides treated with the probe,

are at least 80% identical are formed. It is known that the stringency of the hybridization, including the washing steps, is influenced or determined by varying the buffer composition, the temperature and the salt concentration. The hybridization reaction is in general carried out under a relatively low stringency compared with the washing steps (Hybaid Hybridisation Guide, Hybaid Limited, Teddington, UK, 1996).

A buffer corresponding to 5x SSC buffer at a temperature of approx. 50°C - 68°C, for example, can be employed for the hybridization reaction. Probes can also hybridize here with polynucleotides which are less than 80% identical to the sequence of the probe. Such hybrids are less stable and are removed by washing under stringent conditions. This can be achieved, for example, by lowering the salt concentration to 2x SSC and optionally subsequently 0 .5x SSC (The DIG System User's Guide for Filter Hybridisation, Boehringer Mannheim, Mannheim, Germany, 1995) a temperature of approx. 50°C - 68°C, approx. 52°C - 68°C, approx. 54°C - 68°C, approx. 56°C - 68°C, approx. 58°C - 68°C, approx. 60°C - 68°C, approx. 62 - 68°C, approx. 64°C - 68°C, approx. 66°C — 68°C being established. Temperature ranges of approx. 64°C - 68°C or approx. 66°C - 68°C are preferred. It is optionally possible to lower the salt concentration to a concentration corresponding to 0.2x SSC or 0.1x SSC. Polynucleotide fragments which are, for example, at least 80% or at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98% or at least 99% identical to the sequence of the probe employed or to the nucleotide sequences shown in SEQ ID No. 3, SEQ ID No. 5 or SEQ ID No. 7 can be isolated by increasing the hybridization temperature stepwise from 50°C to 68°C in steps of approx. 1 - 2°C. Further instructions on hybridization are obtainable on the market in the form of so-called kits (e.g. DIG Easy Hyb from Roche Diagnostics GmbH, Mannheim, Germany, Catalogue

No. 1603558). The nucleotide sequences obtained in this way code for polypeptides which are at least 90% identical, in particular identical to the extent of at least 95%, preferably to the extent of at least 98% or at least 99%, very particularly preferably to the extent of 99.7% to the amino acid sequences shown in SEQ ID No . 4, SEQ ID No. 6 or SEQ ID No. 8.

To achieve an enhancement, for example, expression of the genes or the catalytic properties of the enzyme proteins can be increased. The two measures can optionally be combined.

Thus, for example, the number of copies of the corresponding genes can be increased, or the promoter and regulation region or the ribosome binding site upstream of the structural gene can be mutated. Expression cassettes which are incorporated upstream of the structural gene act in the same way. By inducible promoters, it is additionally possible to increase the expression in the course of fermentative L-threonine production, and it may furthermore be advantageous also to use promoters for the gene expression which allow another gene expression with respect to time. The expression is likewise improved by measures to prolong the life of the m-RNA. Furthermore, the enzyme activity is also increased by preventing the degradation of the enzyme protein. The genes or gene constructs can either be present in plasmids with a varying number of copies, or can be integrated and amplified in the chromosome. Alternatively, an over-expression of the genes in question can furthermore be achieved by changing the composition of the media and the culture procedure.

Methods of overexpression are adequately described in the prior art - for example by Makrides et al. (Microbiological Reviews 60 (3), 512-538 (1996)). By using vectors, the number of copies is increased by at least one (1) copy. Vectors which can be used are plasmids such as are

described, for example, in US 5,538,873. Vectors which can also be used are phages, for example the phage Mu, as described in EP 0332448, or the phage lambda (λ). An increase in the number of copies can also be achieved by incorporating a further copy into a further site of the chromosome - for example into the att site of the phage λ (Yu and Court, Gene 223, 77-81 (1998)). US 5,939,307 discloses that by incorporation of expression cassettes or promoters, such as, for example, the tac promoter, trp promoter, lpp promoter or P_L promoter and P_R-promoter of the phage λ for example upstream of the chromosomal threonine operon it was possible to achieve an increase in the expression. The promoters of the phage T7, the gear-box promoters or the nar promoter can be used in the same way. Such expression cassettes or promoters can also be used, as described in EP 0 593 792, to overexpress plasmid-bound genes. By using the $lacI^Q$ allele, the expression of plasmid-bound genes can in turn be controlled (Glascock and Weickert, Gene 223, 221-231 (1998)). It is furthermore possible that the activity of the promoters is increased by modification of their sequence by means of one or more nucleotide exchanges, by insertion(s) and/or deletion(s). Another gene expression with respect to time can be achieved, for example, as described by Walker et al. (Journal of Bacteriology 181: 1269-80 (1999)) by using the growth phase-dependent fis promoter.

The expert can find general instructions in this respect, inter alia, in Chang and Cohen (Journal of Bacteriology 134: 1141-1156 (1978)), in Hartley and Gregori (Gene 13: 347-353 (1981)), in Amann and Brosius (Gene 40: 183-190 (1985)), in de Broer et al. (Proceedings of the National Academy of Sciences of the United States of America 80: 21-25 (1983)), in LaVallie et al. (BIO/TECHNOLOGY 11: 187-193 (1993)), in PCT/US97/13359, in Llosa et al. (Plasmid 26: 222-224 (1991)), in Quandt and Klipp (Gene 80: 161-169 (1989)), in Hamilton et al. (Journal of Bacteriology 171:

4617-4622 (1989)), in Jensen and Hammer (Biotechnology and Bioengineering 58: 191-195 (1998)) and in known textbooks of genetics and molecular biology.

Plasmid vectors which can replicate in Enterobacteriaceae, such as e.g. cloning vectors derived from pACYC184 (Bartolomé et al.; Gene 102: 75-78 (1991)), pTrc99A (Amann et al.; Gene 69: 301-315 (1988)) or pSC101 derivatives (Vocke and Bastia; Proceedings of the National Academy of Sciences USA 80(21): 6557-6561 (1983)) can be used. A strain transformed with a plasmid vector, where the plasmid vector carries at least the eno gene or a nucleotide sequence which codes for the gene product thereof or an allele, can be employed in a process according to the invention.

The term transformation is understood as meaning the uptake of an isolated nucleic acid by a host (microorganism).

It is also possible to transfer mutations which affect the expression of the particular genes into various strains by sequence exchange (Hamilton et al.; (Journal of Bacteriology 171: 4617-4622 (1989)), conjugation or transduction.

More detailed explanations of the terms in genetics and molecular biology are found in known textbooks of genetics and molecular biology, such as, for example, the textbook by Birge (Bacterial and Bacteriophage Genetics, 4th ed., Springer Verlag, New York (USA), 2000) or the textbook by Berg, Tymoczko and Stryer (Biochemistry, 5th ed., Freeman and Company, New York (USA), 2002) or the handbook by Sambrook et al. (Molecular Cloning, A Laboratory Manual, (3 volume set), Cold Spring Harbor Laboratory Press, Cold Spring Harbor (USA), 2001).

It may furthermore be advantageous for the production of L-amino acids, in particular L-threonine, with strains of the Enterobacteriaceae family to enhance one or more

enzymes of the known threonine biosynthesis pathway or enzymes of anaplerotic metabolism or enzymes for the production of reduced nicotinamide adenine dinucleotide phosphate or enzymes of glycolysis or PTS enzymes or enzymes of sulfur metabolism, in addition to the enhancement of the eno gene. The use of endogenous genes is in general preferred.

Thus, for example, at the same time one or more of the genes chosen from the group consisting of

- at least one gene of the thrABC operon which codes for aspartate kinase, homoserine dehydrogenase, homoserine kinase and threonine synthase (US-A-4,278,765),
- the pyc gene of Corynebacterium glutamicum which codes for pyruvate carboxylase (WO 99/18228),
- the pps gene which codes for phosphoenol pyruvate synthase (Molecular and General Genetics 231(2): 332-336 (1992)),
- the ppc gene which codes for phosphoenol pyruvate carboxylase (WO 02/064808),
- the pntA and pntB genes which code for subunits of pyridine transhydrogenase (European Journal of Biochemistry 158: 647-653 (1986)),
- the rhtB gene which codes for the protein which imparts homoserine resistance (EP-A-0 994 190),
- the rhtC gene which codes for the protein which imparts threonine resistance (EP-A-1 013 765),
- the thrE gene of Corynebacterium glutamicum which codes for the threonine export carrier protein (WO 01/92545),

- the gdhA gene which codes for glutamate dehydrogenase (Nucleic Acids Research 11: 5257-5266 (1983); Gene 23: 199-209 (1983)),
- the pgm gene which codes for phosphoglucomutase (WO 03/004598),
- the fba gene which codes for fructose biphosphate aldolase (WO 03/004664),
- the ptsH gene of the ptsHIcrr operon which codes for the phosphohistidine protein hexose phosphotransferase of the phosphotransferase system PTS (WO 03/004674),
- the ptsI gene of the ptsHIcrr operon which codes for enzyme I of the phosphotransferase system PTS (WO 03/004674),
- the crr gene of the ptsHIcrr operon which codes for the glucose-specific IIA component of the phosphotransferase system PTS (WO 03/004674),
- the ptsG gene which codes for the glucose-specific IIBC component (WO 03/004670),
- the lrp gene which codes for the regulator of the leucine regulon (WO 03/004665),
- the fadR gene which codes for the regulator of the fad regulon (WO 03/038106),
- the iclR gene which codes for the regulator of central intermediate metabolism (WO 03/038106),
- the ahpC gene of the ahpCF operon which codes for the small sub-unit of alkyl hydroperoxide reductase (WO 03/004663),

- the ahpF gene of the ahpCF operon which codes for the large sub-unit of alkyl hydroperoxide reductase (WO 03/004663),
- the cysK gene which codes for cysteine synthase A (WO 03/006666),
- the cysB gene which codes for the regulator of the cys regulon (WO 03/006666),
- the cysJ gene of the cysJIH operon which codes for the flavoprotein of NADPH sulfite reductase (WO 03/006666),
- the cysI gene of the cysJIH operon which codes for the haemoprotein of NADPH sulfite reductase (WO 03/006666),
- the cysH gene of the cysJIH operon which codes for adenylyl sulfate reductase (WO 03/006666),
- the rseA gene of the rseABC operon which codes for a membrane protein with anti-sigmaE activity (WO 03/008612),
- the rseC gene of the rseABC operon which codes for a global regulator of the sigmaE factor (WO 03/008612),
- the sucA gene of the sucABCD operon which codes for the decarboxylase sub-unit of 2-ketoglutarate dehydrogenase (WO 03/008614),
- the sucB gene of the sucABCD operon which codes for the dihydrolipoyltranssuccinase E2 sub-unit of 2-ketoglutarate dehydrogenase (WO 03/008614),
- the sucC gene of the sucABCD operon which codes for the β-sub-unit of succinyl-CoA synthetase (WO 03/008615),
- the sucD gene of the sucABCD operon which codes for the α-sub-unit of succinyl-CoA synthetase (WO 03/008615),

- the aceE gene which codes for the E1 component of the pyruvate dehydrogenase complex (WO 03/076 635),
- the aceF gene which codes for the E2 component of the pyruvate dehydrogenase complex (WO 03/076635), and
- the rseB gene which codes for the regulator of sigmaE factor activity (Molecular Microbiology 24(2): 355-371 (1997)),
- the gene product of the open reading frame (ORF) yodA of Escherichia coli (Accession Number AE000288 of the National Center for Biotechnology Information (NCBI, Bethesda, MD, USA), DE10361192.4),
- the gene product of the open reading frame (ORF) yaaU of Escherichia coli (Accession Number AE005 181 of the National Center for Biotechnology Information (NCBI, Bethesda, MD, USA), DE10361268.8) and
- the malT gene which codes for the positive transcriptional activator of the maltose regulon (Gene 42: 201-208 (1986)

can be enhanced.

It may furthermore be advantageous for the production of L-amino acids, in particular L-threonine, in addition to the enhancement of the eno gene, for one or more of the genes chosen from the group consisting of

- the tdh gene which codes for threonine dehydrogenase (Journal of Bacteriology 169: 4716-4721 (1987)),
- the mdh gene which codes for malate dehydrogenase (E.C. 1.1.1.37) (Archives in Microbiology 149 : 36-42 (1987)),
- the gene product of the open reading frame (orf) yjfA of Escherichia coli (Accession Number AAC77180 of the National Center for Biotechnology Information (NCBI,

Bethesda, MD, USA),
WO 02/29080)),

- the gene product of the open reading frame (orf) ytfP of Escherichia coli (Accession Number AAC77179 of the National Center for Biotechnology Information (NCBI, Bethesda, MD, USA),
WO 02/29080)),

- the pckA gene which codes for the enzyme phosphoenol pyruvate carboxykinase (WO 02/29080),

- the poxB gene which codes for pyruvate oxidase (WO 02/36797),

- the dgsA gene which codes for the DgsA regulator of the phosphotransferase system (WO 02/081721) and is also known under the name of the mlc gene,

- the fruR gene which codes for the fructose repressor (WO 02/081698) and is also known under the name of the cra gene,

- the rpoS gene which codes for the sigma^{38} factor (WO 01/05939) and is also known under the name of the katF gene, and

- the aspA gene which codes for aspartate ammonium lyase (WO 03/008603)

to be attenuated, in particular eliminated or for the expression thereof to be reduced.

The term "attenuation" in this connection describes the reduction or elimination of the intracellular activity or concentration of one or more enzymes or proteins in a microorganism which are coded by the corresponding DNA, for example by using a weaker promoter than in the microorganism or parent strain which is not recombinant for the corresponding enzyme or protein or a gene or allele

which codes for a corresponding enzyme or protein with a low activity or inactivating the corresponding enzyme (protein) or the open reading frame or the gene and optionally combining these measures.

By attenuation measures, the activity or concentration of the corresponding protein is in general reduced to 0 to 75%, 0 to 50%, 0 to 25%, 0 to 10% or 0 to 5% of the activity or concentration of the wild-type protein or of the activity or concentration of the protein in the microorganism or parent strain which is not recombinant for the corresponding enzyme or protein. Microorganism or parent strain which is not recombinant is understood as meaning the microorganism on which the inventive measures are carried out.

To achieve an attenuation, for example, expression of the genes or open reading frames or the catalytic properties of the enzyme proteins can be reduced or eliminated. The two measures can optionally be combined.

The reduction in gene expression can take place by suitable culturing, by genetic modification (mutation) of the signal structures of gene expression or also by the antisense-RNA technique. Signal structures of gene expression are, for example, repressor genes, activator genes , operators, promoters, attenuators, ribosome binding sites, the start codon and terminators. The expert can find information in this respect, inter alia, for example, in Jensen and Hammer (Biotechnology and Bioengineering 58: 191 -195 (1998)), in Carrier und Keasling (Biotechnology Progress 15: 58-64 (1999)), Franch and Gerdes (Current Opinion in Microbiology 3: 159-164 (2000)) and in known textbooks of genetics and molecular biology, such as, for example, the textbook by Knippers ("Molekulare Genetik [Molecular Genetics]", 6th edition, Georg Thieme Verlag, Stuttgart, Germany, 1995) or that by Winnacker ("Gene und Klone [Genes and Clones]", VCH Verlagsgesellschaft, Weinheim, Germany, 1 990).

Mutations which lead to a change or reduction in the catalytic properties of enzyme proteins are known from the prior art. Examples which may be mentioned are the works of Qiu and Goodman (Journal of Biological Chemistry 272: 8611-8617 (1997)), Yano et al. (Proceedings of the National Academy of Sciences of the United States of America 95: 5511-5515 (1998)), Wente and Schachmann (Journal of Biological Chemistry 266: 20833-20839 (1991)). Summarizing descriptions can be found in known textbooks of genetics and molecular biology, such as e.g. that by Hagemann ("Allgemeine Genetik [General Genetics]", Gustav Fischer Verlag, Stuttgart, 1986).

Possible mutations are transitions, transversions, insertions and deletions of at least one (1) base pair or nucleotide. Depending on the effect of the amino acid exchange caused by the mutation on the enzyme activity, "missense mutations" or "nonsense mutations" are referred to. Missense mutation leads to an exchange of a given amino acid in a protein for another, this being, in particular, a non-conservative amino acid exchange. The functional capacity or activity of the protein is impaired by this means and reduced to a value of 0 to 75%, 0 to 50%, 0 to 25%, 0 to 10% or 0 to 5%. Nonsense mutation leads to a stop codon in the coding region of the gene and therefore to a premature interruption in the translation. Insertions or deletions of at least one base pair in a gene lead to frame shift mutations, which lead to incorrect amino acids being incorporated or translation being interrupted prematurely. If a stop codon is formed in the coding region as a consequence of the mutation, this also leads to a premature termination of the translation. Deletions of at least one (1) or more codons typically also lead to a complete loss of the enzyme activity.

Instructions on generation of such mutations are prior art and can be found in known textbooks of genetics and molecular biology, such as e.g. the textbook by Knippers

("Molekulare Genetik [Molecular Genetics]", 6th edition, Georg Thieme Verlag, Stuttgart, Germany, 1995), that by Winnacker ("Gene und Klone [Genes and Clones]", VCH Verlagsgesellschaft, Weinheim, Germany, 1990) or that by Hagemann ("Allgemeine Genetik [General Genetics]", Gustav Fischer Verlag, Stuttgart, 1986).

Suitable mutations in the genes can be incorporated into suitable strains by gene or allele replacement.

A common method is the method, described by Hamilton et al. (Journal of Bacteriology 171: 4617-4622 (1989)), of gene replacement with the aid of a conditionally replicating pSC101 derivative pMAK705. Other methods described in the prior art, such as, for example, that of Martinez-Morales et al. (Journal of Bacteriology 181 : 7143-7148 (1999)) or that of Boyd et al. (Journal of Bacteriology 182: 842-847 (2000)), can likewise be used.

It is also possible to transfer mutations in the particular genes or mutations which affect expression of the particular genes or open reading frames into various strains by conjugation or transduction.

In addition to enhancement of the eno gene it may furthermore be advantageous for the production of L-amino acids, in particular L-threonine, to eliminate undesirable side reactions (Nakayama: "Breeding of Amino Acid Producing Microorganisms", in: Overproduction of Microbial Products, Krumphanzl, Sikyta, Vanek (eds.), Academic Press, London, UK, 1982).

The microorganisms produced according to the invention can be cultured in the batch process (batch culture), in the fed batch (feed process), in the repeated fed batch process (repetitive feed process) or in a continuous process (DE102004028859.3 or US5,763,230). A summary of known culture methods is described in the textbook by Chmiel (Bioprozesstechnik 1. Einführung in die

Bioverfahrenstechnik (Gustav Fischer Verlag, Stuttgart, 1991)) or in the textbook by Storhas (Bioreaktoren und periphere Einrichtungen (Vieweg Verlag, Braunschweig/Wiesbaden, 1994)).

The culture medium to be used must meet the requirements of the particular strains in a suitable manner. Descriptions of culture media for various microorganisms are contained in the handbook "Manual of Methods for General Bacteriology" of the American Society for Bacteriology (Washington D.C., USA, 1981).

Sugars and carbohydrates, such as e.g. glucose, sucrose, lactose, fructose, maltose, molasses, starch and optionally cellulose, oils and fats, such as e.g. soya oil, sunflower oil, groundnut oil and coconut fat, fatty acids, such as e.g. palmitic acid, stearic acid and linoleic acid, alcohols, such as e.g. glycerol and ethanol, and organic acids, such as e.g. acetic acid, can be used as the source of carbon. These substances can be used individually or as a mixture.

Organic nitrogen-containing compounds, such as peptones, yeast extract, meat extract, malt extract, corn steep liquor, soya bean flour and urea, or inorganic compounds, such as ammonium sulfate, ammonium chloride, ammonium phosphate, ammonium carbonate and ammonium nitrate, can be used as the source of nitrogen. The sources of nitrogen can be used individually or as a mixture.

Phosphoric acid, potassium dihydrogen phosphate or dipotassium hydrogen phosphate or the corresponding sodium-containing salts can be used as the source of phosphorus. The culture medium must furthermore comprise salts of metals, such as e.g. magnesium sulfate or iron sulfate, which are necessary for growth. Finally, essential growth substances, such as amino acids and vitamins, can be employed in addition to the abovementioned substances.

Suitable precursors can moreover be added to the culture medium. The starting substances mentioned can be added to the culture in the form of a single batch, or can be fed in during the culture in a suitable manner.

The fermentation is in general carried out at a pH of 5.5 to 9.0, in particular 6.0 to 8.0. Basic compounds, such as sodium hydroxide, potassium hydroxide, ammonia or aqueous ammonia, or acid compounds, such as phosphoric acid or sulfuric acid, can be employed in a suitable manner to control the pH of the culture. Antifoams, such as e.g. fatty acid polyglycol esters, can be employed to control the development of foam. Suitable substances having a selective action, e.g. antibiotics, can be added to the medium to maintain the stability of plasmids. To maintain aerobic conditions, oxygen or oxygen-containing gas mixtures, such as e.g. air, are introduced into the culture. The temperature of the culture is usually 25°C to 45°C, and preferably 30°C to 40°C. Culturing is continued until a maximum of L-amino acids or L-threonine has formed. This target is usually reached within 10 hours to 160 hours.

The analysis of L-amino acids can be carried out by anion exchange chromatography with subsequent ninhydrin derivatization, as described by Spackman et al. (Analytical Chemistry, 30: 1190-1206 (1958)) or it can be carried out by reversed phase HPLC, as described by Lindroth et al. (Analytical Chemistry 51: 1167-1174 (1979)).

The process according to the invention is used for the fermentative preparation of L-amino acids, such as, for example, L-threonine, L-isoleucine, L-valine, L-methionine, L-homoserine, L-tryptophan and L-lysine, in particular L-threonine.

The following microorganism has been deposited at the Deutsche Sammlung für Mikroorganismen und Zellkulturen

(DSMZ = German Collection of Microorganisms and Cell Cultures, Braunschweig, Germany) in accordance with the Budapest Treaty:

- Escherichia coli strain E. coli MG442 as DSM 16574.

The present invention is explained in more detail in the following with the aid of embodiment examples.

The minimal (M9) and complete media (LB) for Escherichia coli used are described by J.H. Miller (A Short Course in Bacterial Genetics (1992), Cold Spring Harbor Laboratory Press). The isolation of plasmid DNA from Escherichia coli and all techniques of restriction, ligation, Klenow and alkaline phosphatase treatment are carried out by the method of Sambrook et al. (Molecular Cloning - A Laboratory Manual (1989) Cold Spring Harbor Laboratory Press). Unless described otherwise, the transformation of Escherichia coli is carried out by the method of Chung et al. (Proceedings of the National Academy of Sciences of the United States of America 86: 2172-2175 (1989)).

The incubation temperature for the preparation of strains and transformants is 37°C.

Example 1

Construction of the expression plasmid pTrc99Aeno

The eno gene from E. coli K12 is amplified using the polymerase chain reaction (PCR) and synthetic oligonucleotides. Starting from the nucleotide sequence of the eno gene in E. coli K12 MG1655 (Accession Number AE000361, Blattner et al. (Science 277: 1453-1474 (1997)), PCR primers are synthesized (MWG Biotech, Ebersberg, Germany). The primers contain sequences for restriction enzymes which are marked by underlining in the nucleotide sequence shown below. The primer eno1 contains the

restriction cleavage site for XabI, the primer eno2 contains that for HindIII.

eno1:
5' - GTTTGTCTAGAGTTTCAGTTTAACTAGTGAC - 3' (SEQ ID No. 1)

eno2:
5' - CCGGAGGCTGGCAAGCTTAAATCAG - 3' (SEQ ID No. 2)

The chromosomal E. coli K12 MG1655 DNA employed for the PCR is isolated according to the manufacturers instructions with "Qiagen Genomic-tips 100/G" (QIAGEN, Hilden, Germany). A DNA fragment approx. 1,381 bp in size can be amplified with the specific primers under standard PCR conditions (Innis et al. (1990) PCR Protocols. A Guide to Methods and Applications, Academic Press) with Vent-DNA polymerase (New England BioLabs, Frankfurt, Germany) (SEQ ID No. 3).

The amplified eno fragment is restricted with the restriction enzymes HindIII and XbaI and after purification (Purification Kit, QIAGEN, Hilden, Germany) checked in a 0.8% agarose gel. The vector pTrc99A (Pharmacia Biotech, Uppsala, Sweden) is cleaved with the enzymes HindIII and XbaI and ligation is carried out with the restricted eno fragment. The E. coli strain TOP10 One Shot (TOPO TA Cloning Kit, Invitrogen, Groningen, The Netherlands) is transformed with the ligation batch and plasmid-carrying cells are selected on LB agar, to which 50 µg/ml ampicillin is added. Successful cloning can be demonstrated after plasmid DNA isolation by control cleavage with the enzymes HindIII/XbaI and PvuI. The plasmid is called pTrc99Aeno (figure 1).

Example 2

Preparation of L-threonine with the strain MG442/pTrc99Aeno

The L-threonine-producing E. coli strain MG442 is described in the patent specification US-A-4,278,765 and deposited as

CMIM B-1628 at the Russian National Collection for Industrial Microorganisms (VKPM, Moscow, Russia) and as DSM 16 574 at the Deutsche Sammlung für Mikroorganismen und Zellkulturen (DSMZ = German Collection of Microorganisms and Cell Cultures, Braunschweig, Germany) in accordance with the Budapest Treaty.

The strain MG442 is transformed with the expression plasmid pTrc99Aeno described in example 1 and with the vector pTrc99A and plasmid-carrying cells are selected on LB agar with 50 µg/ml ampicillin. Successful transformations can be demonstrated after plasmid DNA isolation by control cleavages with the enzymes HindIII/XbaI. The strains MG442/pTrc99Aeno and MG442/pTrc99A are formed in this manner. Selected individual colonies are then multiplied further on minimal medium with the following composition: 3.5 g/l $Na_2HPO_4*2H_2O$, 1.5 g/l KH_2PO_4, 1 g/l NH_4Cl, 0.1 g/l $MgSO_4*7H_2O$, 2 g/l glucose, 20 g/l agar, 50 mg/l ampicillin. The formation of L-threonine is checked in batch cultures of 10 ml contained in 100 ml conical flasks. For this, 10 ml of preculture medium of the following composition: 2 g/l yeast extract, 10 g/l $(NH_4)_2SO_4$, 1 g/l KH_2PO_4, 0.5 g/l $MgSO_4*7H_2O$, 15 g/l $CaCO_3$, 20 g/l glucose, 50 mg/l ampicillin are inoculated and the batch is incubated for 16 hours at 37 ºC and 180 rpm on an ESR incubator from Kühner AG (Birsfelden, Switzerland).

250 µl portions of this preculture are transinoculated into 10 ml of production medium (25 g/l $(NH_4)_2SO_4$, 2 g/l KH_2PO_4, 1 g/l $MgSO_4*7H_2O$, 0.03 g/l $FeSO_4*7H_2O$, 0.018 g/l $MnSO_4*1H_2O$, 30 g/l $CaCO_3$, 20 g/l glucose, 50 mg/l ampicillin) and the batch is incubated for 48 hours at 37ºC. For complete induction of the expression of the eno gene, 100 mg/l isopropyl β-D-thiogalactopyranoside (IPTG) are added in parallel batches. The formation of L-threonine by the starting strain MG442 is investigated in the same manner, but no addition of ampicillin to the medium takes place. After the incubation the optical density (OD) of the

culture suspension is determined with an LP2W photometer from Dr. Lange (Düsseldorf, Germany) at a measurement wavelength of 660 nm.

The concentration of L-threonine formed is then determined in the sterile-filtered culture supernatant with an amino acid analyzer from Eppendorf-BioTronik (Hamburg, Germany) by ion exchange chromatography and post-column reaction with ninhydrin detection.

The result of the experiment is shown in Table 1.

Table 1

Strain	Additives	OD (660 nm)	L-Threonine g/l
MG442	-	5.6	1.4
MG442/pTrc99A	-	3.8	1.3
MG442/pTrc99Aeno	-	2.6	1.7
MG442/pTrc99Aeno	IPTG	5.1	2.6

Brief description of the Figure:

Figure 1: Map of the plasmid pTrc99Aeno containing the eno gene

The length data are to be understood as approx. data. The abbreviations and designations used have the following meaning:

- Amp: ampicillin resistance gene
- lacI: gene for the repressor protein of the trc promoter

- Ptrc: trc promoter region, IPTG-inducible
- eno: coding region of the eno gene
- 5S: 5S rRNA region
- rrnBT: rRNA terminator region

The abbreviations for the restriction enzymes have the following meaning

- HindIII: restriction endonuclease from Haemophilus influenzae R_c
- PvuI: restriction endonuclease from Proteus vulgaris
- XbaI: restriction endonuclease from Xanthomonas campestris

What is claimed is:

1. Recombinant microorganisms of the Enterobacteriaceae family which contain an enhanced or over-expressed eno gene, which codes for enolase, and which produce L-amino acids in an improved manner.

2. Microorganisms according to claim 1 in which a polynucleotide which codes for a polypeptide of which the amino acid sequence is identical to the extent of at least 90% to an amino acid sequence chosen from the group consisting of SEQ ID No. 4, SEQ ID No. 6, and SEQ ID No. 8 is enhanced.

3. Microorganisms according to claim 2, wherein they contain an over-expressed or enhanced polynucleotide corresponding to the eno gene chosen from the group consisting of:

   a) polynucleotide with the nucleotide sequence from SEQ ID No. 3, SEQ ID No. 5 or SEQ ID No. 7;

   b) polynucleotide with a nucleotide sequence which corresponds to SEQ ID No. 3, SEQ ID No. 5 or SEQ ID No. 7 within the context of degeneration of the genetic code;

   c) polynucleotide sequence with a sequence which hybridizes under stringent conditions with the sequence complementary to SEQ ID No. 3, SEQ ID No. 5 or SEQ ID No. 7;

   d) polynucleotide with a sequence SEQ ID No. 3, SEQ ID No. 5 or SEQ ID No. 7 which contains neutral-function sense mutants.

4. Microorganisms according to claim 2, wherein the polypeptide has an amino acid sequence which is identical to the extent of at least 95% to one of the

sequences chosen from the group consisting of SEQ ID No. 4, SEQ ID No. 6 and SEQ ID No. 8.

5. Microorganisms according to claim 2, wherein the polypeptide has the amino acid sequence which is 100% identical to that from SEQ ID No. 4, SEQ ID No. 6 or SEQ ID No. 8.

6. Microorganisms according to claims 1 to 5, wherein they are produced by transformation, transduction or conjugation, or a combination of these methods, with a vector which contains the eno gene, an allele of this gene or parts thereof and/or a promoter.

7. Microorganisms according to claims 1 or 6, in which the number of copies of the eno gene or of the alleles is increased by at least 1.

8. Microorganisms according to claim 7, wherein the increase in the number of copies of the eno gene by at least 1 is effected by integration of the ORF or the alleles into the chromosome of the microorganisms.

9. Microorganisms according to claim 7, wherein the increase in the number of copies of the eno gene by at least 1 is achieved by a vector which replicates extrachromosomally.

10. Microorganisms according to claims 1 or 2, wherein, to achieve the enhancement,

a) the promoter and regulation region or the ribosome-binding site upstream of the eno gene is mutated, or

b) expression cassettes or promoters are incorporated upstream of the eno gene.

11. Microorganisms according to claims 1 or 2, wherein the eno gene is under the control of a promoter which enhances expression of the gene.

12. Microorganisms according to claims 1 to 11, wherein the concentration or activity of the eno gene product (protein) is increased by at least 10%, based on the activity or concentration of the gene product in the microorganism or parent strain which is not recombinant for the eno gene, by the enhancement of the eno gene.

13. Microorganisms according to claims 1 to 12, wherein the microorganisms are chosen from the genera Escherichia, Erwinia, Providencia and Serratia.

14. Microorganisms according to claim 13, wherein further genes of the biosynthesis pathway of the desired L-amino acids are additionally enhanced, in particular over-expressed.

15. Microorganisms according to claims 1 to 14, wherein they produce L-threonine.

16. Process for the preparation of L- amino acids by fermentation of recombinant micro-organisms of the Enterobacteriaceae family, wherein

a) the microorganisms which produce the desired L-amino acid and in which the eno gene or nucleotide sequences or alleles which code for the gene product thereof are enhanced, in particular over-expressed, are cultured in a medium under conditions under which the desired L-amino acid is concentrated in the medium or in the cells, and

b) the desired L-amino acid is isolated, constituents of the fermentation broth and/or the biomass in its entirety or portions ($\geq$ 0 to 100%) thereof

remaining in the product which has been isolated or being completely removed.

17. Process according to claim 16, wherein the microorganisms according to claims 1 to 15 are employed.

18. Process according to claim 16 or 17, wherein, for the preparation of L-threonine, microorganisms of the Enterobacteriaceae family in which additionally at the same time one or more of the genes chosen from the group consisting of:

18.1 at least one gene of the thrABC operon which codes for aspartate kinase, homoserine dehydrogenase, homoserine kinase and threonine synthase,

18.2 the pyc gene of Corynebacterium glutamicum which codes for pyruvate carboxylase,

18.3 the pps gene which codes for phosphoenol pyruvate synthase,

18.4 the ppc gene which codes for phosphoenol pyruvate carboxylase,

18.5 the pntA and pntB genes which code for subunits of pyridine transhydrogenase,

18.6 the rhtB gene which codes for the protein which imparts homoserine resistance,

18.7 the rhtC gene which codes for the protein which imparts threonine resistance,

18.8 the thrE gene of Corynebacterium which codes for the threonine export carrier protein,

18.9 the gdhA gene which codes for glutamate dehydrogenase,

18.10 the pgm gene which codes for phosphoglucomutase,

18.11 the fba gene which codes for fructose biphosphate aldolase,

18.12 the ptsH gene which codes for the phosphohistidine protein hexose phosphotransferase,

18.13 the ptsI gene which codes for enzyme I of the phosphotransferase system,

18.14 the crr gene which codes for the glucose-specific IIA component,

18.15 the ptsG gene which codes for the glucose-specific IIBC component,

18.16 the lrp gene which codes for the regulator of the leucine regulon,

18.17 the fadR which codes for the regulator of the fad regulon,

18.18 the iclR gene which codes for the regulator of central intermediate metabolism,

18.19 the ahpC gene which codes for the small subunit of alkyl hydroperoxide reductase,

18.20 the ahpF gene which codes for the large subunit of alkyl hydroperoxide reductase,

18.21 the cysK gene which codes for cysteine synthase A,

18.22 the cysB gene which codes for the regulator of the cys regulon,

18.23 the cysJ gene which codes for the flavoprotein of NADPH sulfite reductase,

18.24 the cysI gene which codes for the haemoprotein of NADPH sulfite reductase,

18.25 the cysH gene which codes for adenylyl sulfate reductase,

18.26 the rseA gene which codes for a membrane protein with anti-sigmaE activity,

18.27 the rseC gene which codes for a global regulator of the sigmaE factor,

18.28 the sucA gene which codes for the decarboxylase sub-unit of 2-ketoglutarate dehydrogenase,

18.29 the sucB gene which codes for the dihydrolipoyltranssuccinase E2 sub-unit of 2-ketoglutarate dehydrogenase,

18.30 the sucC gene which codes for the β-sub-unit of succinyl-CoA synthetase,

18.31 the sucD gene which codes for the α-sub-unit of succinyl-CoA synthetase,

18.32 the aceE gene which codes for the E1 component of the pyruvate dehydrogenase complex,

18.33 the aceF gene which codes for the E2 component of the pyruvate dehydrogenase complex,

18.34 the rseB gene which codes for the regulator of sigmaE factor activity,

18.35 the gene product of the open reading frame (ORF) yodA of Escherichia coli, and

18.36 the gene product of the open reading frame (ORF) yaaU of Escherichia coli

is or are enhanced, in particular over-expressed, are fermented.

19. Process according to claims 16 to 18, characterized in that microorganisms in which the metabolic pathways which reduce the formation of the desired L-amino acid are at least partly attenuated are employed.

20. Process according to claim 19, wherein, for the preparation of L-threonine, microorganisms of the Enterobacteriaceae family in which additionally at the same time one or more of the genes chosen from the group consisting of:

20.1 the tdh gene which codes for threonine dehydrogenase,

20.2 the mdh gene which codes for malate dehydrogenase,

20.3 the gene product of the open reading frame (ORF) yjfA of Escherichia coli,

20.4 the gene product of the open reading frame (ORF) ytfP of Escherichia coli,

20.5 the pckA gene which codes for phosphoenol pyruvate carboxykinase,

20.6 the poxB gene which codes for pyruvate oxidase,

20.7 the dgsA gene which codes for the DgsA regulator of the phosphotransferase system,

20.8 the fruR gene which codes for the fructose repressor,

20.9 the rpoS gene which codes for the sigma^{38} factor, and

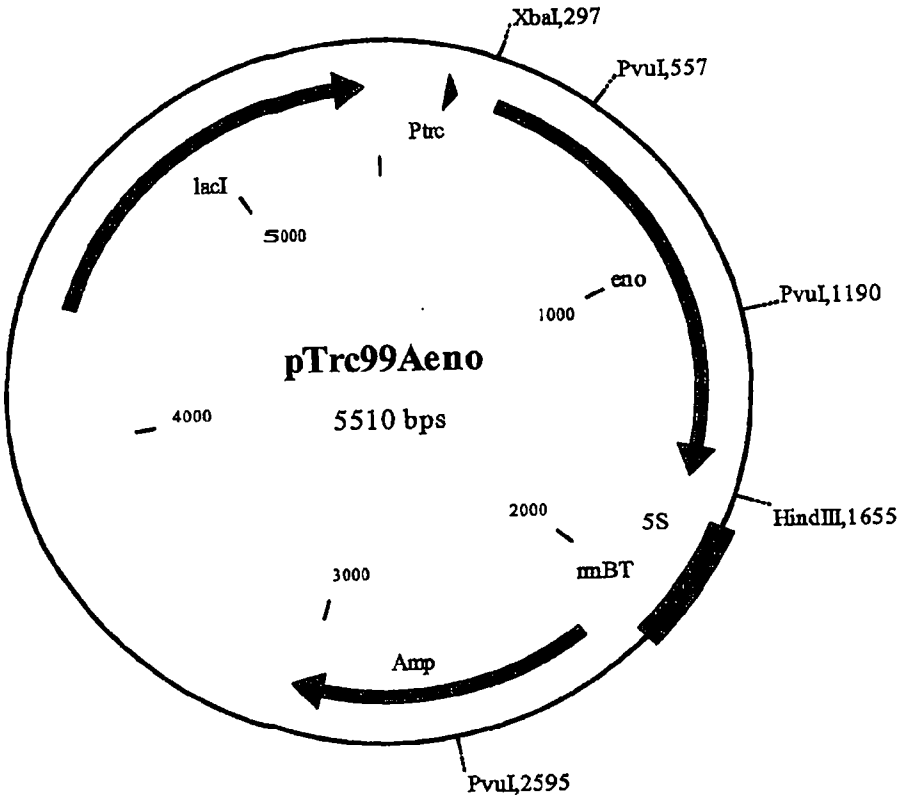
20.10 the aspA gene which codes for aspartate ammonium lyase

is or are attenuated, in particular eliminated or reduced in expression, are fermented.

21. Process according to claims 16 to 20, wherein L-amino acids chosen from the group consisting of L-asparagine, L-serine, L-glutamate, L-glycine, L-alanine, L-cysteine, L-valine, L-methionine, L-proline, L-isoleucine, L-leucine, L-tyrosine, L-phenylalanine, L-histidine, L-lysine, L-tryptophan, L-arginine and L-homoserine are prepared.

22. Process according to claim 21, wherein L-amino acids chosen from the group consisting of L-isoleucine, L-valine, L-methionine, L-homoserine, L-tryptophan and L-lysine are prepared.

Figure 1: Map of the plasmid pTrc99Aeno



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Ala Pro Ser Gly Ala Ser Thr Gly Ser Arg Glu Ala Leu Glu Leu Arg
            40                  45                  50

gat ggc gac aaa tcc cgt ttc ctg ggt aaa ggc gta acc aaa gct gtt      249
Asp Gly Asp Lys Ser Arg Phe Leu Gly Lys Gly Val Thr Lys Ala Val
        55                  60                  65

gct gcg gta aac ggc ccg atc gct cag gcg ctg att ggc aaa gat gct      297
Ala Ala Val Asn Gly Pro Ile Ala Gln Ala Leu Ile Gly Lys Asp Ala
    70                  75                  80

aaa gat cag gct ggc att gac aag atc atg atc gac ctg gac ggc acc      345
Lys Asp Gln Ala Gly Ile Asp Lys Ile Met Ile Asp Leu Asp Gly Thr
85                  90                  95                  100

gaa aac aaa tcc aaa ttc ggc gcg aac gca atc ctg gct gta tct ctg      393
Glu Asn Lys Ser Lys Phe Gly Ala Asn Ala Ile Leu Ala Val Ser Leu
                105                 110                 115

gct aac gcc aaa gct gct gca gct gct aaa ggt atg ccg ctg tac gag      441
Ala Asn Ala Lys Ala Ala Ala Ala Ala Lys Gly Met Pro Leu Tyr Glu
            120                 125                 130

cac atc gct gaa ctg aac ggt act ccg ggc aaa tac tct atg ccg gtt      489
His Ile Ala Glu Leu Asn Gly Thr Pro Gly Lys Tyr Ser Met Pro Val
        135                 140                 145

ccg atg atg aac atc atc aac ggt ggt gag cac gct gac aac aac gtt      537
Pro Met Met Asn Ile Ile Asn Gly Gly Glu His Ala Asp Asn Asn Val
    150                 155                 160

gat atc cag gaa ttc atg att cag ccg gtt ggc gcg aaa act gtg aaa      585
Asp Ile Gln Glu Phe Met Ile Gln Pro Val Gly Ala Lys Thr Val Lys
165                 170                 175                 180

gaa gcc atc cgc atg ggt tct gaa gtt ttc cat cac ctg gca aaa gtt      633
Glu Ala Ile Arg Met Gly Ser Glu Val Phe His His Leu Ala Lys Val
                185                 190                 195

ctg aaa gcg aaa ggc atg aac act gct gtt ggt gac gaa ggt ggc tat      681
Leu Lys Ala Lys Gly Met Asn Thr Ala Val Gly Asp Glu Gly Gly Tyr
            200                 205                 210

gcg ccg aac ctg ggt tcc aac gct gaa gct ctg gct gtt atc gct gaa      729
Ala Pro Asn Leu Gly Ser Asn Ala Glu Ala Leu Ala Val Ile Ala Glu
        215                 220                 225

gct gtt aaa gct gct ggt tat gaa ctg ggc aaa gac atc act ttg gcg      777
Ala Val Lys Ala Ala Gly Tyr Glu Leu Gly Lys Asp Ile Thr Leu Ala
    230                 235                 240

atg gac tgc gca gct tct gaa ttc tac aaa gat ggt aaa tac gtt ctg      825
Met Asp Cys Ala Ala Ser Glu Phe Tyr Lys Asp Gly Lys Tyr Val Leu
245                 250                 255                 260
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gct ggc gaa ggc aac aaa gcg ttc acc tct gaa gaa ttc act cac ttc      873
Ala Gly Glu Gly Asn Lys Ala Phe Thr Ser Glu Glu Phe Thr His Phe
                265                 270                 275

ctg gaa gaa ctg acc aaa cag tac ccg atc gtt tct atc gaa gac ggt      921
Leu Glu Glu Leu Thr Lys Gln Tyr Pro Ile Val Ser Ile Glu Asp Gly
            280                 285                 290

ctg gac gaa tct gac tgg gac ggt ttc gca tac cag acc aaa gtt ctg      969
Leu Asp Glu Ser Asp Trp Asp Gly Phe Ala Tyr Gln Thr Lys Val Leu
        295                 300                 305

ggc gac aaa atc cag ctg gtt ggt gac gac ctg ttc gta acc aac acc     1017
Gly Asp Lys Ile Gln Leu Val Gly Asp Asp Leu Phe Val Thr Asn Thr
    310                 315                 320

aag atc ctg aaa gaa ggt atc gaa aaa ggt atc gct aac tcc atc ctg     1065
Lys Ile Leu Lys Glu Gly Ile Glu Lys Gly Ile Ala Asn Ser Ile Leu
325                 330                 335                 340

atc aaa ttc aac cag atc ggt tct ctg acc gaa act ctg gct gca atc     1113
Ile Lys Phe Asn Gln Ile Gly Ser Leu Thr Glu Thr Leu Ala Ala Ile
                345                 350                 355

aag atg gcg aaa gat gct ggc tac act gca gtt atc tct cac cgt tct     1161
Lys Met Ala Lys Asp Ala Gly Tyr Thr Ala Val Ile Ser His Arg Ser
            360                 365                 370

ggc gaa act gaa gac gct acc atc gct gac ctg gct gtt ggt act gct     1209
Gly Glu Thr Glu Asp Ala Thr Ile Ala Asp Leu Ala Val Gly Thr Ala
        375                 380                 385

gca ggc cag atc aaa act ggt tct atg agc cgt tct gac cgt gtt gct     1257
Ala Gly Gln Ile Lys Thr Gly Ser Met Ser Arg Ser Asp Arg Val Ala
    390                 395                 400

aaa tac aac cag ctg att cgt atc gaa gaa gct ctg ggc gaa aaa gca     1305
Lys Tyr Asn Gln Leu Ile Arg Ile Glu Glu Ala Leu Gly Glu Lys Ala
405                 410                 415                 420

ccg tac aac ggt cgt aaa gag atc aaa ggc cag gca taa gactgacttt      1354
Pro Tyr Asn Gly Arg Lys Glu Ile Lys Gly Gln Ala
                425                 430

atctgattta agcttgccag cctccgg                                      1381
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<210> 4
<211> 432
<212> PRT
<213> Escherichia coli

<400> 4

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Met Ser Lys Ile Val Lys Ile Ile Gly Arg Glu Ile Ile Asp Ser Arg
1               5                  10                  15

Gly Asn Pro Thr Val Glu Ala Glu Val His Leu Glu Gly Gly Phe Val
            20                  25                  30

Gly Met Ala Ala Ala Pro Ser Gly Ala Ser Thr Gly Ser Arg Glu Ala
        35                  40                  45

Leu Glu Leu Arg Asp Gly Asp Lys Ser Arg Phe Leu Gly Lys Gly Val
    50                  55                  60
```

Thr Lys Ala Val Ala Ala Val Asn Gly Pro Ile Ala Gln Ala Leu Ile
65 70 75 80

Gly Lys Asp Ala Lys Asp Gln Ala Gly Ile Asp Lys Ile Met Ile Asp
85 90 95

Leu Asp Gly Thr Glu Asn Lys Ser Lys Phe Gly Ala Asn Ala Ile Leu
100 105 110

Ala Val Ser Leu Ala Asn Ala Lys Ala Ala Ala Ala Ala Lys Gly Met
115 120 125

Pro Leu Tyr Glu His Ile Ala Glu Leu Asn Gly Thr Pro Gly Lys Tyr
130 135 140

Ser Met Pro Val Pro Met Met Asn Ile Ile Asn Gly Gly Glu His Ala
145 150 155 160

Asp Asn Asn Val Asp Ile Gln Glu Phe Met Ile Gln Pro Val Gly Ala
165 170 175

Lys Thr Val Lys Glu Ala Ile Arg Met Gly Ser Glu Val Phe His His
180 185 190

Leu Ala Lys Val Leu Lys Ala Lys Gly Met Asn Thr Ala Val Gly Asp
195 200 205

Glu Gly Gly Tyr Ala Pro Asn Leu Gly Ser Asn Ala Glu Ala Leu Ala
210 215 220

Val Ile Ala Glu Ala Val Lys Ala Ala Gly Tyr Glu Leu Gly Lys Asp
225 230 235 240

Ile Thr Leu Ala Met Asp Cys Ala Ala Ser Glu Phe Tyr Lys Asp Gly
245 250 255

Lys Tyr Val Leu Ala Gly Glu Gly Asn Lys Ala Phe Thr Ser Glu Glu
260 265 270

Phe Thr His Phe Leu Glu Glu Leu Thr Lys Gln Tyr Pro Ile Val Ser
275 280 285

Ile Glu Asp Gly Leu Asp Glu Ser Asp Trp Asp Gly Phe Ala Tyr Gln
290 295 300

Thr Lys Val Leu Gly Asp Lys Ile Gln Leu Val Gly Asp Asp Leu Phe
305 310 315 320

Val Thr Asn Thr Lys Ile Leu Lys Glu Gly Ile Glu Lys Gly Ile Ala
325 330 335

Asn Ser Ile Leu Ile Lys Phe Asn Gln Ile Gly Ser Leu Thr Glu Thr
340 345 350

Leu Ala Ala Ile Lys Met Ala Lys Asp Ala Gly Tyr Thr Ala Val Ile
355 360 365

Ser His Arg Ser Gly Glu Thr Glu Asp Ala Thr Ile Ala Asp Leu Ala
370 375 380

Val Gly Thr Ala Ala Gly Gln Ile Lys Thr Gly Ser Met Ser Arg Ser
385 390 395 400

Asp Arg Val Ala Lys Tyr Asn Gln Leu Ile Arg Ile Glu Glu Ala Leu
405 410 415

Gly Glu Lys Ala Pro Tyr Asn Gly Arg Lys Glu Ile Lys Gly Gln Ala
420 425 430

<210> 5
<211> 1299
<212> DNA
<213> Shigella flexneri

<220>
<221> CDS
<222> (1)..(1299)
<223> eno coding region

<400> 5
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Met Ser Lys Ile Val Lys Ile Ile Gly Arg Glu Ile Ile Asp Ser Arg
1 5 10 15

ggt aac ccg act gtt gaa gcc gaa gta cat ctg gag ggt ggt ttc gtc 96
Gly Asn Pro Thr Val Glu Ala Glu Val His Leu Glu Gly Gly Phe Val
20 25 30

ggt atg gca gct gct ccg tca ggt gct tct act ggt tcc cgt gaa gct 144
Gly Met Ala Ala Ala Pro Ser Gly Ala Ser Thr Gly Ser Arg Glu Ala
35 40 45

ctg gaa ctg cgc gat ggc gac aaa tcc cgt ttc ctg ggt aaa ggc gta 192
Leu Glu Leu Arg Asp Gly Asp Lys Ser Arg Phe Leu Gly Lys Gly Val
50 55 60

acc aaa gct gtt gct gcg gta aac ggc ccg atc gct cag gcg ctg att 240
Thr Lys Ala Val Ala Ala Val Asn Gly Pro Ile Ala Gln Ala Leu Ile
65 70 75 80

ggc aaa gat gct aaa gat cag gct ggc att gac aag atc atg atc gac 288
Gly Lys Asp Ala Lys Asp Gln Ala Gly Ile Asp Lys Ile Met Ile Asp
85 90 95

ctg gac ggc acc gaa aac aaa tcc aaa ttc ggc gcg aac gca atc ctg 336
Leu Asp Gly Thr Glu Asn Lys Ser Lys Phe Gly Ala Asn Ala Ile Leu
100 105 110

gct gta tct ctg gct aac gcc aaa gct gct gca gct gct aaa ggt atg 384
Ala Val Ser Leu Ala Asn Ala Lys Ala Ala Ala Ala Ala Lys Gly Met
115 120 125

ccg ctg tac gag cac atc gct gaa ctg aac ggt act ccg ggc aaa tac 432
Pro Leu Tyr Glu His Ile Ala Glu Leu Asn Gly Thr Pro Gly Lys Tyr
130 135 140

tcc atg ccg gtt ccg atg atg aac atc atc aac ggt ggt gag cac gct 480
Ser Met Pro Val Pro Met Met Asn Ile Ile Asn Gly Gly Glu His Ala
145 150 155 160

gac aac aac gtt gat atc cag gaa ttc atg att cag ccg gtt ggc gcg 528
Asp Asn Asn Val Asp Ile Gln Glu Phe Met Ile Gln Pro Val Gly Ala
165 170 175

aaa act gta aaa gaa gcc atc cgc atg ggt tct gaa gtt ttc cat cac 576
Lys Thr Val Lys Glu Ala Ile Arg Met Gly Ser Glu Val Phe His His
180 185 190

ctg gca aaa gtt ctg aaa gcg aaa ggc atg aac act gct gtt ggt gac 624

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Leu Ala Lys Val Leu Lys Ala Lys Gly Met Asn Thr Ala Val Gly Asp
        195                 200                 205

gaa ggt ggc tat gcg ccg aac ctg ggt tcc aac gct gaa gct ctg gct      672
Glu Gly Gly Tyr Ala Pro Asn Leu Gly Ser Asn Ala Glu Ala Leu Ala
    210                 215                 220

gtt atc gct gaa gct gtt aaa gct gct ggt tat gaa ctg ggc aaa gac      720
Val Ile Ala Glu Ala Val Lys Ala Ala Gly Tyr Glu Leu Gly Lys Asp
225                 230                 235                 240

atc act ctg gcg atg gac tgc gca gct tct gaa ttc tac aaa gat ggt      768
Ile Thr Leu Ala Met Asp Cys Ala Ala Ser Glu Phe Tyr Lys Asp Gly
                245                 250                 255

aaa tac gtt ctg gct ggc gaa ggc aac aaa gcg ttc acc tct gaa gaa      816
Lys Tyr Val Leu Ala Gly Glu Gly Asn Lys Ala Phe Thr Ser Glu Glu
            260                 265                 270

ttc act cac ttc ctg gaa gaa ctg acc aaa cag tac ccg atc gtt tct      864
Phe Thr His Phe Leu Glu Glu Leu Thr Lys Gln Tyr Pro Ile Val Ser
        275                 280                 285

atc gaa gac ggt ctg gac gaa tct gac tgg gac ggt ttc gca tac cag      912
Ile Glu Asp Gly Leu Asp Glu Ser Asp Trp Asp Gly Phe Ala Tyr Gln
    290                 295                 300

acc aaa gtt ctg ggc gac aaa atc cag ctg gtt ggt gac gac ctg ttc      960
Thr Lys Val Leu Gly Asp Lys Ile Gln Leu Val Gly Asp Asp Leu Phe
305                 310                 315                 320

gta acc aac acc aag atc ctg aaa gaa ggt atc gaa aaa ggt atc gct     1008
Val Thr Asn Thr Lys Ile Leu Lys Glu Gly Ile Glu Lys Gly Ile Ala
                325                 330                 335

aac tcc atc ctg atc aaa ttc aac cag atc ggt tct ctg acc gaa act     1056
Asn Ser Ile Leu Ile Lys Phe Asn Gln Ile Gly Ser Leu Thr Glu Thr
            340                 345                 350

ctg gct gca atc aag atg gcg aaa gat gct ggc tac act gca gtt atc     1104
Leu Ala Ala Ile Lys Met Ala Lys Asp Ala Gly Tyr Thr Ala Val Ile
        355                 360                 365

tct cac cgt tct ggt gaa act gaa gat gct acc atc gct gat ctg gct     1152
Ser His Arg Ser Gly Glu Thr Glu Asp Ala Thr Ile Ala Asp Leu Ala
    370                 375                 380

gtt ggt act gct gca ggc cag atc aaa act ggt tct atg agc cgt tct     1200
Val Gly Thr Ala Ala Gly Gln Ile Lys Thr Gly Ser Met Ser Arg Ser
385                 390                 395                 400

gac cgt gtt gct aaa tac aac cag ctg att cgt atc gaa gaa gct ctg     1248
Asp Arg Val Ala Lys Tyr Asn Gln Leu Ile Arg Ile Glu Glu Ala Leu
                405                 410                 415

ggc gaa aaa gca ccg tac aac ggt cgt aaa gag atc aaa ggc cag gca     1296
Gly Glu Lys Ala Pro Tyr Asn Gly Arg Lys Glu Ile Lys Gly Gln Ala
            420                 425                 430

taa                                                                1299

<210> 6
<211> 432
<212> PRT
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<213> Shigella flexneri

<400> 6

Met Ser Lys Ile Val Lys Ile Ile Gly Arg Glu Ile Ile Asp Ser Arg
1 5 10 15

Gly Asn Pro Thr Val Glu Ala Glu Val His Leu Glu Gly Gly Phe Val
20 25 30

Gly Met Ala Ala Ala Pro Ser Gly Ala Ser Thr Gly Ser Arg Glu Ala
35 40 45

Leu Glu Leu Arg Asp Gly Asp Lys Ser Arg Phe Leu Gly Lys Gly Val
50 55 60

Thr Lys Ala Val Ala Ala Val Asn Gly Pro Ile Ala Gln Ala Leu Ile
65 70 75 80

Gly Lys Asp Ala Lys Asp Gln Ala Gly Ile Asp Lys Ile Met Ile Asp
85 90 95

Leu Asp Gly Thr Glu Asn Lys Ser Lys Phe Gly Ala Asn Ala Ile Leu
100 105 110

Ala Val Ser Leu Ala Asn Ala Lys Ala Ala Ala Ala Ala Lys Gly Met
115 120 125

Pro Leu Tyr Glu His Ile Ala Glu Leu Asn Gly Thr Pro Gly Lys Tyr
130 135 140

Ser Met Pro Val Pro Met Met Asn Ile Ile Asn Gly Gly Glu His Ala
145 150 155 160

Asp Asn Asn Val Asp Ile Gln Glu Phe Met Ile Gln Pro Val Gly Ala
165 170 175

Lys Thr Val Lys Glu Ala Ile Arg Met Gly Ser Glu Val Phe His His
180 185 190

Leu Ala Lys Val Leu Lys Ala Lys Gly Met Asn Thr Ala Val Gly Asp
195 200 205

Glu Gly Gly Tyr Ala Pro Asn Leu Gly Ser Asn Ala Glu Ala Leu Ala
210 215 220

Val Ile Ala Glu Ala Val Lys Ala Ala Gly Tyr Glu Leu Gly Lys Asp
225 230 235 240

Ile Thr Leu Ala Met Asp Cys Ala Ala Ser Glu Phe Tyr Lys Asp Gly
245 250 255

Lys Tyr Val Leu Ala Gly Glu Gly Asn Lys Ala Phe Thr Ser Glu Glu
260 265 270

Phe Thr His Phe Leu Glu Glu Leu Thr Lys Gln Tyr Pro Ile Val Ser
275 280 285

Ile Glu Asp Gly Leu Asp Glu Ser Asp Trp Asp Gly Phe Ala Tyr Gln
290 295 300

Thr Lys Val Leu Gly Asp Lys Ile Gln Leu Val Gly Asp Asp Leu Phe
305 310 315 320

Val Thr Asn Thr Lys Ile Leu Lys Glu Gly Ile Glu Lys Gly Ile Ala

325 330 335

Asn Ser Ile Leu Ile Lys Phe Asn Gln Ile Gly Ser Leu Thr Glu Thr
340 345 350

Leu Ala Ala Ile Lys Met Ala Lys Asp Ala Gly Tyr Thr Ala Val Ile
355 360 365

Ser His Arg Ser Gly Glu Thr Glu Asp Ala Thr Ile Ala Asp Leu Ala
370 375 380

Val Gly Thr Ala Ala Gly Gln Ile Lys Thr Gly Ser Met Ser Arg Ser
385 390 395 400

Asp Arg Val Ala Lys Tyr Asn Gln Leu Ile Arg Ile Glu Glu Ala Leu
405 410 415

Gly Glu Lys Ala Pro Tyr Asn Gly Arg Lys Glu Ile Lys Gly Gln Ala
420 425 430

<210> 7
<211> 1299
<212> DNA
<213> Salmonella typhimurium

<220>
<221> CDS
<222> (1)..(1299)
<223> eno coding region

<400> 7

atg tcc aaa atc gtt aaa gtc atc ggt cgt gaa atc atc gac tcc cgt 48
Met Ser Lys Ile Val Lys Val Ile Gly Arg Glu Ile Ile Asp Ser Arg
1 5 10 15

ggt aac ccg act gtt gaa gct gaa gta cac ctg gaa ggt ggt ttc gta 96
Gly Asn Pro Thr Val Glu Ala Glu Val His Leu Glu Gly Gly Phe Val
20 25 30

ggt atg gcg gcg gct ccg tca ggt gct tct act ggt tcc cgc gaa gcg 144
Gly Met Ala Ala Ala Pro Ser Gly Ala Ser Thr Gly Ser Arg Glu Ala
35 40 45

ctg gaa ctg cgc gat ggc gac aaa tcc cgt ttc ctg ggt aaa ggc gta 192
Leu Glu Leu Arg Asp Gly Asp Lys Ser Arg Phe Leu Gly Lys Gly Val
50 55 60

acc aaa gct gtt ggc gcg gtt aac ggc ccg atc gct cag gct att ctt 240
Thr Lys Ala Val Gly Ala Val Asn Gly Pro Ile Ala Gln Ala Ile Leu
65 70 75 80

ggc aaa gac gct aaa gac cag gct ggc atc gac aaa atc atg atc gac 288
Gly Lys Asp Ala Lys Asp Gln Ala Gly Ile Asp Lys Ile Met Ile Asp
85 90 95

ctg gac ggt act gaa aac aaa tct aac ttc ggt gca aac gcc att ctg 336
Leu Asp Gly Thr Glu Asn Lys Ser Asn Phe Gly Ala Asn Ala Ile Leu
100 105 110

gct gtc tct ctg gct aac gcc aaa gct gct gct gcc gct aaa ggt atg 384
Ala Val Ser Leu Ala Asn Ala Lys Ala Ala Ala Ala Ala Lys Gly Met
115 120 125

ccg ctg tac gag cac att gct gaa ctg aat ggc acg ccg ggc aaa tac 432
Pro Leu Tyr Glu His Ile Ala Glu Leu Asn Gly Thr Pro Gly Lys Tyr

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      130                 135                 140
tcc atg ccg gtt ccg atg atg aac atc atc aac ggc ggc gag cac gct      480
Ser Met Pro Val Pro Met Met Asn Ile Ile Asn Gly Gly Glu His Ala
145                 150                 155                 160

gac aac aac gtc gac atc cag gaa ttc atg atc cag ccg gtt ggc gcg      528
Asp Asn Asn Val Asp Ile Gln Glu Phe Met Ile Gln Pro Val Gly Ala
                165                 170                 175

aaa acg gtt aaa gaa gcc atc cgt atg ggt tct gaa gtt ttc cat cac      576
Lys Thr Val Lys Glu Ala Ile Arg Met Gly Ser Glu Val Phe His His
            180                 185                 190

ctg gca aaa gtg ctg aaa ggc aaa ggc atg aac acc gct gtg ggt gac      624
Leu Ala Lys Val Leu Lys Gly Lys Gly Met Asn Thr Ala Val Gly Asp
        195                 200                 205

gaa ggc ggc tat gcg ccg aac ctg ggt tcc aac gct gaa gcg ctg gct      672
Glu Gly Gly Tyr Ala Pro Asn Leu Gly Ser Asn Ala Glu Ala Leu Ala
    210                 215                 220

gtt atc gct gaa gcg gtt aaa gcg gct ggt tac gag ctg ggt aaa gac      720
Val Ile Ala Glu Ala Val Lys Ala Ala Gly Tyr Glu Leu Gly Lys Asp
225                 230                 235                 240

atc acc ctg gcg atg gac tgc gca gca tct gaa ttc tac aaa gac ggt      768
Ile Thr Leu Ala Met Asp Cys Ala Ala Ser Glu Phe Tyr Lys Asp Gly
                245                 250                 255

aaa tac gtt ctg gct ggc gaa ggc aac aaa gcg ttc acc tcc gaa gaa      816
Lys Tyr Val Leu Ala Gly Glu Gly Asn Lys Ala Phe Thr Ser Glu Glu
            260                 265                 270

ttc acc cac ttc ctg gaa gaa ctg acc aaa cag tac ccg atc gtt tcc      864
Phe Thr His Phe Leu Glu Glu Leu Thr Lys Gln Tyr Pro Ile Val Ser
        275                 280                 285

atc gaa gat ggt ctg gac gag tct gac tgg gac ggt ttt gca tac cag      912
Ile Glu Asp Gly Leu Asp Glu Ser Asp Trp Asp Gly Phe Ala Tyr Gln
    290                 295                 300

acc aaa gta ctg ggc gac aaa atc cag ctg gtt ggt gac gac ctg ttc      960
Thr Lys Val Leu Gly Asp Lys Ile Gln Leu Val Gly Asp Asp Leu Phe
305                 310                 315                 320

gta acc aac acc aaa atc ctg aaa gaa ggc atc gag aaa ggc atc gct     1008
Val Thr Asn Thr Lys Ile Leu Lys Glu Gly Ile Glu Lys Gly Ile Ala
                325                 330                 335

aac tcc atc ctg atc aaa ttc aac cag atc ggt tct ctg acc gaa act     1056
Asn Ser Ile Leu Ile Lys Phe Asn Gln Ile Gly Ser Leu Thr Glu Thr
            340                 345                 350

ctg gct gca atc aag atg gcg aaa gac gct ggc tat act gct gtc atc     1104
Leu Ala Ala Ile Lys Met Ala Lys Asp Ala Gly Tyr Thr Ala Val Ile
        355                 360                 365

tct cac cgt tct ggc gaa act gaa gac gct acc atc gct gac ctg gct     1152
Ser His Arg Ser Gly Glu Thr Glu Asp Ala Thr Ile Ala Asp Leu Ala
    370                 375                 380

gtt ggt acc gct gca ggc cag atc aaa acc ggt tct atg agc cgt tct     1200
Val Gly Thr Ala Ala Gly Gln Ile Lys Thr Gly Ser Met Ser Arg Ser
385                 390                 395                 400
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gac cgt gtt gct aaa tac aac cag ctg att cgt atc gaa gaa gct ctg 1248
Asp Arg Val Ala Lys Tyr Asn Gln Leu Ile Arg Ile Glu Glu Ala Leu
405 410 415

ggc gaa aaa gca ccg tat aac ggt cgt aaa gag atc aaa ggc cag gcg 1296
Gly Glu Lys Ala Pro Tyr Asn Gly Arg Lys Glu Ile Lys Gly Gln Ala
420 425 430

taa 1299

<210> 8
<211> 432
<212> PRT
<213> Salmonella typhimurium

<400> 8
Met Ser Lys Ile Val Lys Val Ile Gly Arg Glu Ile Ile Asp Ser Arg
1 5 10 15

Gly Asn Pro Thr Val Glu Ala Glu Val His Leu Glu Gly Gly Phe Val
20 25 30

Gly Met Ala Ala Ala Pro Ser Gly Ala Ser Thr Gly Ser Arg Glu Ala
35 40 45

Leu Glu Leu Arg Asp Gly Asp Lys Ser Arg Phe Leu Gly Lys Gly Val
50 55 60

Thr Lys Ala Val Gly Ala Val Asn Gly Pro Ile Ala Gln Ala Ile Leu
65 70 75 80

Gly Lys Asp Ala Lys Asp Gln Ala Gly Ile Asp Lys Ile Met Ile Asp
85 90 95

Leu Asp Gly Thr Glu Asn Lys Ser Asn Phe Gly Ala Asn Ala Ile Leu
100 105 110

Ala Val Ser Leu Ala Asn Ala Lys Ala Ala Ala Ala Ala Lys Gly Met
115 120 125

Pro Leu Tyr Glu His Ile Ala Glu Leu Asn Gly Thr Pro Gly Lys Tyr
130 135 140

Ser Met Pro Val Pro Met Met Asn Ile Ile Asn Gly Gly Glu His Ala
145 150 155 160

Asp Asn Asn Val Asp Ile Gln Glu Phe Met Ile Gln Pro Val Gly Ala
165 170 175

Lys Thr Val Lys Glu Ala Ile Arg Met Gly Ser Glu Val Phe His His
180 185 190

Leu Ala Lys Val Leu Lys Gly Lys Gly Met Asn Thr Ala Val Gly Asp
195 200 205

Glu Gly Gly Tyr Ala Pro Asn Leu Gly Ser Asn Ala Glu Ala Leu Ala
210 215 220

Val Ile Ala Glu Ala Val Lys Ala Ala Gly Tyr Glu Leu Gly Lys Asp
225 230 235 240

Ile Thr Leu Ala Met Asp Cys Ala Ala Ser Glu Phe Tyr Lys Asp Gly
245 250 255

```
Lys Tyr Val Leu Ala Gly Glu Gly Asn Lys Ala Phe Thr Ser Glu Glu
            260                 265                 270

Phe Thr His Phe Leu Glu Glu Leu Thr Lys Gln Tyr Pro Ile Val Ser
        275                 280                 285

Ile Glu Asp Gly Leu Asp Glu Ser Asp Trp Asp Gly Phe Ala Tyr Gln
    290                 295                 300

Thr Lys Val Leu Gly Asp Lys Ile Gln Leu Val Gly Asp Asp Leu Phe
305                 310                 315                 320

Val Thr Asn Thr Lys Ile Leu Lys Glu Gly Ile Glu Lys Gly Ile Ala
                325                 330                 335

Asn Ser Ile Leu Ile Lys Phe Asn Gln Ile Gly Ser Leu Thr Glu Thr
            340                 345                 350

Leu Ala Ala Ile Lys Met Ala Lys Asp Ala Gly Tyr Thr Ala Val Ile
        355                 360                 365

Ser His Arg Ser Gly Glu Thr Glu Asp Ala Thr Ile Ala Asp Leu Ala
    370                 375                 380

Val Gly Thr Ala Ala Gly Gln Ile Lys Thr Gly Ser Met Ser Arg Ser
385                 390                 395                 400

Asp Arg Val Ala Lys Tyr Asn Gln Leu Ile Arg Ile Glu Glu Ala Leu
                405                 410                 415

Gly Glu Lys Ala Pro Tyr Asn Gly Arg Lys Glu Ile Lys Gly Gln Ala
            420                 425                 430
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