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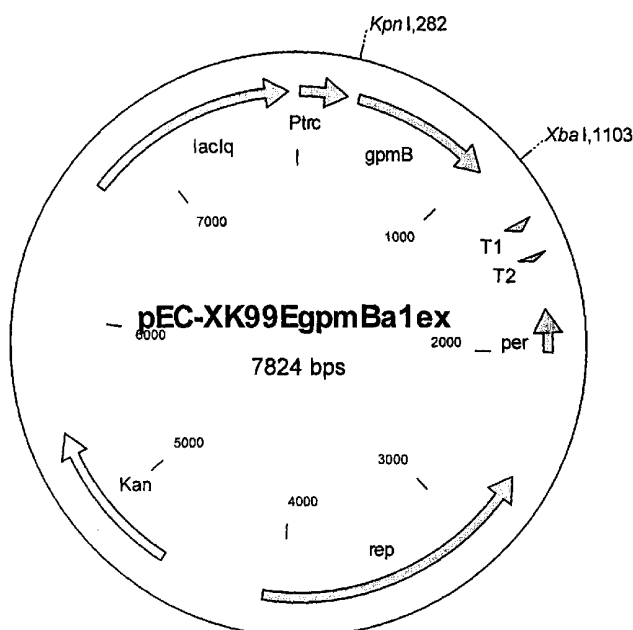
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(54) Title: NUCLEOTIDE SEQUENCES WHICH CODE FOR THE GPMB GENE

Map of the plasmid pEC-XK99EgpmBa1ex



(57) Abstract: The invention relates to an isolated polynucleotide comprising a polynucleotide sequence chosen from the group consisting of a) polynucleotide which is identical to the extent of at least 70% to a polynucleotide which codes for a polypeptide which comprises the amino acid sequence of SEQ ID No. 2, b) polynucleotide which codes for a polypeptide which comprises an amino acid sequence which is identical to the extent of at least 70% to the amino acid sequence of SEQ ID No. 2, c) polynucleotide which is complementary to the polynucleotides of a) or b), and d) polynucleotide comprising at least 15 successive nucleotides of the polynucleotide sequence of a), b) or c), and a process for the fermentative preparation of L-amino acids using coryneform bacteria in which at least the gpmB gene is present in enhanced form, and the use of polynucleotides which comprise the sequences according to the invention as hybridization probes.



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Nucleotide sequences which code for the gpmB gene

Field of the Invention

The invention provides nucleotide sequences from coryneform bacteria which code for the gpmB gene and a process for the fermentative preparation of amino acids using bacteria in which the gpmB gene is enhanced.

Prior Art

L-Amino acids are used in human medicine and in the pharmaceuticals industry, in the foodstuffs industry and especially in animal nutrition.

It is known that amino acids are prepared by fermentation from strains of coryneform bacteria, in particular *Corynebacterium glutamicum*. 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, for example, stirring and supply of oxygen, or the composition of the nutrient media, such as, for example, the sugar concentration during the fermentation, or the working up to the product form by, for example, 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 or are auxotrophic for metabolites of regulatory importance and produce amino acids are obtained in this manner.

Methods of the recombinant DNA technique have also been employed for some years for improving the strain of *Corynebacterium* strains which produce L-amino acid, by

amplifying individual amino acid biosynthesis genes and investigating the effect on the amino acid production.

Object of the Invention

The inventors had the object of providing new measures for
5 improved fermentative preparation of amino acids.

Summary of the Invention

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

15 The invention provides an isolated polynucleotide from coryneform bacteria, comprising a polynucleotide sequence which codes for the gpmB gene, chosen from the group consisting of

- a) polynucleotide which is identical to the extent of at
20 least 70% to a polynucleotide which codes for a polypeptide which comprises the amino acid sequence of SEQ ID No. 2,
- b) polynucleotide which codes for a polypeptide which comprises an amino acid sequence which is identical to
25 the extent of at least 70 % to the amino acid sequence of SEQ ID No. 2,
- c) polynucleotide which is complementary to the polynucleotides of a) or b), and
- d) polynucleotide comprising at least 15 successive
30 nucleotides of the polynucleotide sequence of a), b) or c),

the polypeptide preferably having the activity of phosphoglycerate mutase II.

The invention also provides the above-mentioned polynucleotide, this preferably being a DNA which is
5 capable of replication, comprising:

- (i) the nucleotide sequence shown in SEQ ID No. 1, or
- (ii) at least one sequence which corresponds to sequence (i) within the range of the degeneration of the genetic code, or
- 10 (iii) at least one sequence which hybridizes with the sequence complementary to sequence (i) or (ii), and optionally
- (iv) sense mutations of neutral function in (i).

The invention also provides

- 15 a polynucleotide, in particular DNA, which is capable of replication and comprises the nucleotide sequence as shown in SEQ ID No. 1;

- a polynucleotide which codes for a polypeptide which comprises the amino acid sequence as shown in SEQ ID
20 No. 2;

a vector containing the polynucleotide according to the invention, in particular a shuttle vector or plasmid vector, and

- 25 coryneform bacteria which contain the vector or in which the gpmB gene is enhanced.

The invention also provides polynucleotides which substantially comprise a polynucleotide sequence, which are obtainable by screening by means of hybridization of a corresponding gene library of a coryneform bacterium, which

comprises the complete gene or parts thereof, with a probe which comprises the sequence of the polynucleotide according to the invention according to SEQ ID No. 1 or a fragment thereof, and isolation of the polynucleotide sequence mentioned.

Detailed Description of the Invention

Polynucleotides which comprise the sequences according to the invention are suitable as hybridization probes for RNA, cDNA, and DNA, in order to isolate, in the full length, nucleic acids or polynucleotides or genes which code for phosphoglycerate mutase II or to isolate those nucleic acids or polynucleotides or genes which have a high similarity of sequence with that of the gpmB gene. They are also suitable for incorporation into so-called "arrays", "micro arrays" or "DNA chips", in order to detect and determine the corresponding polynucleotides.

Polynucleotides which comprise the sequences according to the invention are furthermore suitable as primers with the aid of which DNA of genes which code for phosphoglycerate mutase II can be prepared by the polymerase chain reaction (PCR).

Such oligonucleotides which serve as probes or primers comprise at least 25, 26, 27, 28, 29 or 30, preferably at least 20, 21, 22, 23 or 24, very particularly preferably at least 15, 16, 17, 18 or 19 successive nucleotides.

Oligonucleotides which have a length of at least 31, 32, 33, 34, 35, 36, 37, 38, 39 or 40, or at least 41, 42, 43, 44, 45, 46, 47, 48, 49 or 50 nucleotides are also suitable. Oligonucleotides with a length of at least 100, 150, 200, 250 or 300 nucleotides are optionally also suitable.

"Isolated" means separated out of its natural environment.

"Polynucleotide" in general relates to polyribonucleotides and polydeoxyribonucleotides, it being possible for these to be non-modified RNA and DNA or modified RNA and DNA.

5 The polynucleotides according to the invention include a polynucleotide according to SEQ ID No. 1 or a fragment prepared therefrom and also those which are at least 70 % to 80%, preferably at least 81% to 85%, particularly preferably at least 86% to 90%, and very particularly preferably at least 91%, 93%, 95%, 97% or 99% identical to
10 the polynucleotide according to SEQ ID No. 1 or a fragment prepared therefrom.

"Polypeptides" are understood as meaning peptides or proteins which comprise two or more amino acids bonded via peptide bonds.

15 The polypeptides according to the invention include a polypeptide according to SEQ ID No. 2, in particular those with the biological activity of phosphoglycerate mutase II and also those which are at least 70% to 80%, preferably at least 81% to 85%, particularly preferably at least 86% to
20 90%, and very particularly preferably at least 91%, 93%, 95%, 97% or 99% identical to the polypeptide according to SEQ ID No. 2 and have the activity mentioned.

The invention furthermore relates to a process for the fermentative preparation of amino acids chosen from the
25 group consisting of L-asparagine, L-threonine, L-serine, L-glutamate, L-glycine, L-alanine, L-cysteine, L-valine, L-methionine, L-isoleucine, L-leucine, L-tyrosine, L-phenylalanine, L-histidine, L-lysine, L-tryptophan and L-arginine using coryneform bacteria which in particular
30 already produce amino acids and in which the nucleotide sequences which code for the gpmB gene are enhanced, in particular over-expressed.

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

By enhancement measures, in particular over-expression, the
10 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 1000% or 2000%, based on that of the wild-type protein or the activity or concentration of the protein in the starting
15 microorganism.

The microorganisms which the present invention provides can produce L-amino acids from glucose, sucrose, lactose, fructose, maltose, molasses, starch, cellulose or from glycerol and ethanol. They can be representatives of
20 coryneform bacteria, in particular of the genus *Corynebacterium*. Of the genus *Corynebacterium*, there may be mentioned in particular the species *Corynebacterium glutamicum*, which is known among experts for its ability to produce L-amino acids.

25 Suitable strains of the genus *Corynebacterium*, in particular of the species *Corynebacterium glutamicum* (*C. glutamicum*), are in particular the known wild-type strains

Corynebacterium glutamicum ATCC13032
Corynebacterium acetoglutamicum ATCC15806
30 *Corynebacterium acetoacidophilum* ATCC 13870
Corynebacterium thermoaminogenes FERM BP-1539
Corynebacterium melassecola ATCC17965
Brevibacterium flavum ATCC14067

Brevibacterium lactofermentum ATCC13869 and
Brevibacterium devaricatum ATCC14020

and L-amino acid-producing mutants or strains prepared therefrom.

- 5 The new *gpmB* gene from *C. glutamicum* which codes for the enzyme phosphoglycerate mutase II (E.C. 5.4.2.1) has been isolated.

To isolate the *gpmB* gene or also other genes of *C. glutamicum*, a gene library of this microorganism is first
 10 set up in *Escherichia coli* (*E. coli*). The setting up of gene libraries is described in generally known textbooks and handbooks. The textbook by Winnacker: *Gene und Klone, Eine Einführung in die Gentechnologie* [Genes and Clones, An Introduction to Genetic Engineering] (Verlag Chemie,
 15 Weinheim, Germany, 1990), or the handbook by Sambrook et al.: *Molecular Cloning, A Laboratory Manual* (Cold Spring Harbor Laboratory Press, 1989) may be mentioned as an example. A well-known gene library is that of the *E. coli* K-12 strain W3110 set up in λ vectors by Kohara et al.
 20 (Cell 50, 495-508 (1987)). Bathe et al. (Molecular and General Genetics, 252:255-265, 1996) describe a gene library of *C. glutamicum* ATCC13032, which was set up with the aid of the cosmid vector SuperCos I (Wahl et al., 1987, Proceedings of the National Academy of Sciences USA,
 25 84:2160-2164) in the *E. coli* K-12 strain NM554 (Raleigh et al., 1988, Nucleic Acids Research 16:1563-1575).

Börmann et al. (Molecular Microbiology 6(3), 317-326) (1992) in turn describe a gene library of *C. glutamicum* ATCC13032 using the cosmid pH79 (Hohn and Collins, Gene
 30 11, 291-298 (1980)).

To prepare a gene library of *C. glutamicum* in *E. coli* it is also possible to use plasmids such as pBR 322 (Bolivar, Life Sciences, 25, 807-818 (1979)) or pUC9 (Vieira et al.,

1982, *Gene*, 19:259-268). Suitable hosts are, in particular, those *E. coli* strains which are restriction- and recombination-defective. An example of these is the strain DH5 α mcr, which has been described by Grant et al.

5 (Proceedings of the National Academy of Sciences USA, 87 (1990) 4645-4649). The long DNA fragments cloned with the aid of cosmids can in turn be subcloned in the usual vectors suitable for sequencing and then sequenced, as is described e.g. by Sanger et al. (Proceedings of the
10 National Academy of Sciences of the United States of America, 74:5463-5467, 1977).

The new DNA sequence of *C. glutamicum* which codes for the gpmB gene and which, as SEQ ID No. 1, is a constituent of the present invention has been found. The amino acid
15 sequence of the corresponding protein has furthermore been derived from the present DNA sequence by the methods described above. The resulting amino acid sequence of the gpmB gene product is shown in SEQ ID No. 2.

Coding DNA sequences which result from SEQ ID No. 1 by the degeneracy of the genetic code are also a constituent of
20 the invention. In the same way, DNA sequences which hybridize with SEQ ID No. 1 or parts of SEQ ID No. 1 are a constituent of the invention. Conservative amino acid exchanges, such as e.g. exchange of glycine for alanine or
25 of aspartic acid for glutamic acid in proteins, are furthermore known among experts as "sense mutations" which do not lead to a fundamental change in the activity of the protein. i.e. are of neutral function. It is furthermore known that changes on the N and/or C terminus of a protein
30 cannot substantially impair or can even stabilize the function thereof. Information in this context can be found by the expert, inter alia, in Ben-Bassat et al. (*Journal of Bacteriology* 169:751-757 (1987)), in O'Regan et al. (*Gene* 77:237-247 (1994)), in Sahin-Toth et al. (*Protein Sciences*
35 3:240-247 (1994)), in Hochuli et al. (*Bio/Technology*

6:1321-1325 (1988)) and in known textbooks of genetics and molecular biology. Amino acid sequences which result in a corresponding manner from SEQ ID No. 2 are also a constituent of the invention.

5 In the same way, DNA sequences which hybridize with SEQ ID No. 1 or parts of SEQ ID No. 1 are a constituent of the invention. Finally, DNA sequences which are prepared by the polymerase chain reaction (PCR) using primers which result from SEQ ID No. 1 are a constituent of the invention. Such
10 oligonucleotides typically have a length of at least 15 nucleotides.

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
15 Hybridization" from Boehringer Mannheim GmbH (Mannheim, Germany, 1993) and in Liebl et al. (International Journal of Systematic Bacteriology (1991) 41: 255-260). The hybridization takes place under stringent conditions, that is to say only hybrids in which the probe and target
20 sequence, i. e. the polynucleotides treated with the probe, are at least 70 % 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.
25 The hybridization reaction is preferably carried out under a relatively low stringency compared with the washing steps (Hybaid Hybridisation Guide, Hybaid Limited, Teddington, UK, 1996).

A 5 x SCC buffer at a temperature of approx. 50°C - 68°C,
30 for example, can be employed for the hybridization reaction. Probes can also hybridize here with polynucleotides which are less than 70% identical to the sequence of the probe. Such hybrids are less stable and are removed by washing under stringent conditions. This can be
35 achieved, for example, by lowering the salt concentration

to 2 x SSC and optionally subsequently 0.5 x SSC (The DIG System User's Guide for Filter Hybridisation, Boehringer Mannheim, Mannheim, Germany, 1995) a temperature of approx. 50°C - 68°C being established. It is optionally possible to
5 lower the salt concentration to 0.1 x SSC. Polynucleotide fragments which are, for example, at least 70 % or at least 80 % or at least 90 % to 95 % identical to the sequence of the probe employed can be isolated by increasing the hybridization temperature stepwise from 50°C to 68°C in
10 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).

Instructions for amplification of DNA sequences with the
15 aid of the polymerase chain reaction (PCR) can be found by the expert, inter alia, in the handbook by Gait: Oligonucleotide Synthesis: A Practical Approach (IRL Press, Oxford, UK, 1984) and in Newton and Graham: PCR (Spektrum Akademischer Verlag, Heidelberg, Germany, 1994).

20 It has been found that coryneform bacteria produce amino acids in an improved manner after over-expression of the gpmB gene.

To achieve an over-expression, the number of copies of the corresponding genes can be increased, or the promoter and
25 regulation region or the ribosome linking 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
30 fermentative amino acid production. The expression is likewise improved by measures to prolong the life of the mRNA. 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
35 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.

- 5 Instructions in this context can be found by the expert, inter alia, in Martin et al. (Bio/Technology 5, 137-146 (1987)), in Guerro et al. (Gene 138, 35-41 (1994)), Tsuchiya and Morinaga (Bio/Technology 6, 428-430 (1988)), in Eikmanns et al. (Gene 102, 93-98 (1991)), in EP 427,869,
 10 in US 4,601,893, in Schwarzer and Pühler (Bio/Technology 9, 84-87 (1991)), in Reinscheid et al. (Applied and Environmental Microbiology 60, 126-132 (1994)), in LaBarre et al. (Journal of Bacteriology 175, 1001-1007 (1993)), in WO 96/15246, in Malumbres et al. (Gene 134, 15-24 (1993)),
 15 in JP-A-10-229891, in Jensen and Hammer (Biotechnology and Bioengineering 58, 191-195 (1998)), in Makrides (Microbiological Reviews 60:512-538 (1996)) and in known textbooks of genetics and molecular biology.

- By way of example, for enhancement the gpmB gene according
 20 to the invention was over-expressed with the aid of episomal plasmids. Suitable plasmids are those which are replicated in coryneform bacteria. Numerous known plasmid vectors, such as e. g. pZ1 (Menkel et al., Applied and Environmental Microbiology (1989) 64:549-554), pEKEx1
 25 (Eikmanns et al., Gene 102:93-98 (1991)) or pHS2-1 (Sonnen et al., Gene 107:69-74 (1991)) are based on the cryptic plasmids pHM1519, pBL1 or pGA1. Other plasmid vectors, such as e.g. those based on pCG4 (US-A 4,489,160), or pNG2 (Serwold-Davis et al., FEMS Microbiology Letters 66, 119-
 30 124 (1990)), or pAG1 (US-A 5,158,891), can be used in the same manner.

- Plasmid vectors which are furthermore suitable are also those with the aid of which the process of gene amplification by integration into the chromosome can be
 35 used, as has been described, for example, by Reinscheid et

al. (Applied and Environmental Microbiology 60, 126-132 (1994)) for duplication or amplification of the hom-thrB operon. In this method, the complete gene is cloned in a plasmid vector which can replicate in a host (typically E. coli), but not in *C. glutamicum*. Possible vectors are, for example, pSUP301 (Simon et al., Bio/Technology 1, 784-791 (1983)), pK18mob or pK19mob (Schäfer et al., Gene 145, 69-73 (1994)), pGEM-T (Promega corporation, Madison, WI, USA), pCR2.1-TOPO (Shuman (1994), Journal of Biological Chemistry 269:2678-84; US-A 5,487,993), pCR®Blunt (Invitrogen, Groningen, The Netherlands; Bernard et al., Journal of Molecular Biology, 234:534-541 (1993)), pEM1 (Schrumpf et al., 1991, Journal of Bacteriology 173:4510-4516) or pBGS8 (Spratt et al., 1986, Gene 41:337-342). The plasmid vector which contains the gene to be amplified is then transferred into the desired strain of *C. glutamicum* by conjugation or transformation. The method of conjugation is described, for example, by Schäfer et al. (Applied and Environmental Microbiology 60, 756-759 (1994)). Methods for transformation are described, for example, by Thierbach et al. (Applied Microbiology and Biotechnology 29, 356-362 (1988)), Dunican and Shivnan (Bio/Technology 7, 1067-1070 (1989)) and Tauch et al. (FEMS Microbiological Letters 123, 343-347 (1994)). After homologous recombination by means of a "cross over" event, the resulting strain contains at least two copies of the gene in question.

In addition, it may be advantageous for the production of L-amino acids to enhance, in particular over-express one or more enzymes of the particular biosynthesis pathway, of glycolysis, of anaplerosis, of the citric acid cycle, of the pentose phosphate cycle, of amino acid export and optionally regulatory proteins, in addition to the gpmB gene.

Thus, for the preparation of L-amino acids, in addition to enhancement of the gpmB gene, one or more genes, according

to the biosynthesis pathway, chosen from the group consisting of

- the dapA gene which codes for dihydrodipicolinate synthase (EP-B 0 197 335),
- 5 • the gap gene which codes for glyceraldehyde 3-phosphate dehydrogenase (Eikmanns (1992), Journal of Bacteriology 174:6076-6086),
- the tpi gene which codes for triose phosphate isomerase (Eikmanns (1992), Journal of Bacteriology 174:6076-
10 6086),
- the pgk gene which codes for 3-phosphoglycerate kinase (Eikmanns (1992), Journal of Bacteriology 174:6076-6086),
- the zwf gene which codes for glucose 6-phosphate
15 dehydrogenase (JP-A-09224661),
- the pyc gene which codes for pyruvate carboxylase (DE-A 198 31 609),
- the mqo gene which codes for malate-quinone oxidoreductase (Molenaar et al., European Journal of
20 Biochemistry 254, 395-403 (1998)),
- the lysC gene which codes for a feed-back resistant aspartate kinase (Accession No.P26512; EP-B-0387527; EP-A-0699759),
- the lysE gene which codes for lysine export
25 (DE-A-195 48 222),
- the hom gene which codes for homoserine dehydrogenase (EP-A 0131171),
- the ilvA gene which codes for threonine dehydratase (Möckel et al., Journal of Bacteriology (1992) 8065-

8072)), or the *ilvA*(Fbr) allele which codes for a "feed back resistant" threonine dehydratase Möckel et al., (1994) Molecular Microbiology 13: 833-842),

- the *ilvBN* gene which codes for acetohydroxy-acid synthase (EP-B 0356739),
- the *ilvD* gene which codes for dihydroxy-acid dehydratase (Sahm and Eggeling (1999) Applied and Environmental Microbiology 65: 1973-1979),
- the *zwa1* gene which codes for the Zwa1 protein (DE: 19959328.0, DSM 13115)

can be enhanced, in particular over-expressed.

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

- the *pck* gene which codes for phosphoenol pyruvate carboxykinase (DE 199 50 409.1; DSM 13047),
- the *pgi* gene which codes for glucose 6-phosphate isomerase (US 09/396,478; DSM 12969),
- the *poxB* gene which codes for pyruvate oxidase (DE: 1995 1975.7; DSM 13114),
- the *zwa2* gene which codes for the Zwa2 protein (DE: 19959327.2, DSM 13113)

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

The term "attenuation" in this connection describes the reduction or elimination of the intracellular activity of one or more enzymes (proteins) in a microorganism which are coded by the corresponding DNA, for example by using a weak

promoter or using a gene or allele which codes for a corresponding enzyme with a low activity or inactivates the corresponding gene or enzyme (protein), and optionally combining these measures.

- 5 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
10 starting microorganism.

In addition to over-expression of it *gpmB* gene it may furthermore be advantageous for the production of amino acids to eliminate undesirable side reactions (Nakayama: "Breeding of Amino Acids Producing Micro-organisms", in:
15 Overproduction of Microbial Products, Krumphanzl, Sikyta, Vanek (eds.), Academic Press, London, UK, 1982).

The invention also provides the microorganisms prepared according to the invention, and these can be cultured continuously or discontinuously in the batch process (batch
20 culture) or in the fed batch (feed process) or repeated fed batch process (repetitive feed process) for the purpose of production of amino acids. A summary of known culture methods is described in the textbook by Chmiel (Bioprozesstechnik 1. Einführung in die
25 Bioverfahrenstechnik [Bioprocess Technology 1. Introduction to Bioprocess Technology (Gustav Fischer Verlag, Stuttgart, 1991)) or in the textbook by Storhas (Bioreaktoren und periphere Einrichtungen [Bioreactors and Peripheral Equipment] (Viehweg Verlag, Braunschweig/Wiesbaden,
30 (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 cellulose,
5 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.
10 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
15 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-
20 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
25 employed in addition to the above-mentioned 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.

30 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, such as 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 20°C to 45°C, and preferably 25°C to 40°C. Culturing is continued until a maximum of the desired product has formed. This target is usually reached within 10 hours to 160 hours.

Methods for the determination of L-amino acids are known from the prior art. The analysis can thus be carried out, for example, as described by Spackmann et al. (Analytical Chemistry, 30, (1958), 1190) by ion exchange chromatography with subsequent ninhydrin derivation, or it can be carried out by reversed phase HPLC, for example as described by Lindroth et al. (Analytical Chemistry (1979) 51: 1167-1174).

The process according to the invention is used for fermentative preparation of amino acids.

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

The following microorganism was deposited as a pure culture on 26th June 2001 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* DH5 α mcr/pEC-XK99EgpmBalex as DSM 14376.

The isolation of plasmid DNA from *Escherichia coli* and all techniques of restriction, Klenow and alkaline phosphatase treatment were carried out by the method of Sambrook et al. (Molecular Cloning. A Laboratory Manual (1989) Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, USA).

Methods for transformation of *Escherichia coli* are also described in this handbook.

The composition of the usual nutrient media, such as LB or TY medium, can also be found in the handbook by Sambrook et al.

Example 1

Preparation of a genomic cosmid gene library from *Corynebacterium glutamicum* ATCC 13032.

Chromosomal DNA from *Corynebacterium glutamicum* ATCC 13032 was isolated as described by Tauch et al. (1995, Plasmid 33:168-179) and partly cleaved with the restriction enzyme Sau3AI (Amersham Pharmacia, Freiburg, Germany, Product Description Sau3AI, Code no. 27-0913-02). The DNA fragments were dephosphorylated with shrimp alkaline phosphatase (Roche Diagnostics GmbH, Mannheim, Germany, Product Description SAP, Code no. 1758250). The DNA of the cosmid vector SuperCos (Wahl et al. (1987) Proceedings of the National Academy of Sciences USA 84:2160-2164), obtained from Stratagene (La Jolla, USA, Product Description SuperCos1 Cosmid Vector Kit, Code no. 251301) was cleaved with the restriction enzyme XbaI (Amersham Pharmacia, Freiburg, Germany, Product Description XbaI, Code no. 27-0948-02) and likewise dephosphorylated with shrimp alkaline phosphatase.

The cosmid DNA was then cleaved with the restriction enzyme BamHI (Amersham Pharmacia, Freiburg, Germany Product Description BamHI, Code no. 27-0868-04). The cosmid DNA treated in this manner was mixed with the treated ATCC13032 DNA and the batch was treated with T4 DNA ligase (Amersham Pharmacia, Freiburg, Germany, Product Description T4-DNA-Ligase, Code no. 27-0870-04). The ligation mixture was then packed in phages with the aid of Gigapack II XL Packing

Extract (Stratagene, La Jolla, USA, Product Description Gigapack II XL Packing Extract, Code no. 200217).

For infection of the E. coli strain NM554 (Raleigh et al. 1988, Nucleic Acid Research 16:1563-1575) the cells were
5 taken up in 10 mM MgSO₄ and mixed with an aliquot of the phage suspension. The infection and titering of the cosmid library were carried out as described by Sambrook et al. (1989, Molecular Cloning: A Laboratory Manual, Cold Spring Harbor), the cells being plated out on LB agar (Lennox,
10 1955, Virology, 1:190) with 100 mg/l ampicillin. After incubation overnight at 37°C, recombinant individual clones were selected.

Example 2

Isolation and sequencing of the gpmB gene.

15 The cosmid DNA of an individual colony was isolated with the Qiaprep Spin Miniprep Kit (Product No. 27106, Qiagen, Hilden, Germany) in accordance with the manufacturer's instructions and partly cleaved with the restriction enzyme Sau3AI (Amersham Pharmacia, Freiburg, Germany, Product
20 Description Sau3AI, Product No. 27-0913-02). The DNA fragments were dephosphorylated with shrimp alkaline phosphatase (Roche Diagnostics GmbH, Mannheim, Germany, Product Description SAP, Product No. 1758250). After separation by gel electrophoresis, the cosmid fragments in
25 the size range of 1500 to 2000 bp were isolated with the QiaExII Gel Extraction Kit (Product No. 20021, Qiagen, Hilden, Germany).

The DNA of the sequencing vector pZero-1, obtained from Invitrogen (Groningen, Holland, Product Description Zero
30 Background Cloning Kit, Product No. K2500-01), was cleaved with the restriction enzyme BamHI (Amersham Pharmacia, Freiburg, Germany, Product Description BamHI, Product No. 27-0868-04). The ligation of the cosmid fragments in the

sequencing vector pZero-1 was carried out as described by Sambrook et al. (1989, Molecular Cloning: A Laboratory Manual, Cold Spring Harbor), the DNA mixture being incubated overnight with T4 ligase (Pharmacia Biotech, Freiburg, Germany). This ligation mixture was then electroporated (Tauch et al. 1994, FEMS Microbiol Letters, 123:343-7) into the E. coli strain DH5 α MCR (Grant, 1990, Proceedings of the National Academy of Sciences U.S.A. 87:4645-4649) and plated out on LB agar (Lennox, 1955, Virology 1:190) with 50 mg/l zeocin.

The plasmid preparation of the recombinant clones was carried out with the Biorobot 9600 (Product No. 900200, Qiagen, Hilden Germany). The sequencing was carried out by the dideoxy chain-stopping method of Sanger et al. (1977, Proceedings of the National Academy of Sciences U.S.A., 74:5463-5467) with modification according to Zimmermann et al. (1990, Nucleic Acids Research, 18:1067). The "RR dRhodamin Terminator Cycle Sequencing Kit" from PE Applied Biosystems (Product No. 403044, Weiterstadt, Germany) was used. The separation by gel electrophoresis and analysis of the sequence reaction were carried out in a "Rotiphoresis NF Acrylamide/Bisacrylamide" Gel (29:1) (Product No. A124.1, Roth, Karlsruhe, Germany) with the "ABI Prism 377" sequencer from PE Applied Biosystems (Weiterstadt, Germany).

The raw sequence data obtained were then processed using the Staden program package (1986, Nucleic Acids Research, 14:217:231) version 97-0. The individual sequences of the pZero1 derivatives were assembled to a continuous contig. The computer-assisted coding region analysis was prepared with the XNIP program (Staden, 1986, Nucleic Acids Research, 14:217-231).

The resulting nucleotide sequence is shown in SEQ ID No. 1. Analysis of the nucleotide sequence showed an open reading

frame of 705 base pairs, which was called the gpmB gene. The gpmB gene codes for a protein of 235 amino acids.

Example 3

Preparation of the shuttle expression vector pEC-
5 XK99EgpmBalex for enhancement of the gpmB gene in *C.*
glutamicum

3.1 Cloning of the gpmB gene

From the strain ATCC 13032, chromosomal DNA was isolated by
the method of Eikmanns et al. (Microbiology 140: 1817-1828
10 (1994)). On the basis of the sequence of the gpmB gene
known for *C. glutamicum* from example 2, the following
oligonucleotides were chosen for the polymerase chain
reaction (see SEQ ID No. 3 and SEQ ID No. 4):

gpmBex1:

15 5' ca ggtacc tgg cta cga gga cga tta ag 3'

gpmBex2:

5' tg tctaga aag cat gcg gag gaa tca ac 3'

The primers shown were synthesized by MWG-Biotech AG
(Ebersberg, Germany) and the PCR reaction was carried out
20 by the standard PCR method of Innis et al. (PCR Protocols.
A Guide to Methods and Applications, 1990, Academic Press)
with Pwo-Polymerase from Roche Diagnostics GmbH (Mannheim,
Germany). With the aid of the polymerase chain reaction,
the primers allow amplification of a DNA fragment 827 bp in
25 size, which carries the gpmB gene. Furthermore, the primer
gpmBex1 contains the sequence for the cleavage site of the
restriction endonuclease Kpn1, and the primer gpmBex2 the
cleavage site of the restriction endonuclease XbaI, which
are marked by underlining in the nucleotide sequence shown
30 above.

The gpmB fragment 827 bp in size was cleaved with the
restriction endonucleases Kpn1 and XbaI and then isolated

from the agarose gel with the QiaExII Gel Extraction Kit (Product No. 20021, Qiagen, Hilden, Germany).

3.2 Construction of the shuttle vector pEC-XK99E

The *E. coli* - *C. glutamicum* shuttle vector pEC-XK99E was
5 constructed according to the prior art. The vector contains
the replication region rep of the plasmid pGA1 including
the replication effector per (US-A-5,175,108; Nesvera et
al., Journal of Bacteriology 179, 1525-1532 (1997)), the
kanamycin resistance gene aph(3')-IIa from *Escherichia coli*
10 (Beck et al. (1982), Gene 19: 327-336), the replication
origin of the trc promoter, the termination regions T1 and
T2, the lacI^q gene (repressor of the lac operon of *E. coli*)
and a multiple cloning site (mcs) (Norrander, J.M. et al.
Gene 26, 101-106 (1983)) of the plasmid pTRC99A (Amann et
15 al. (1988), Gene 69: 301-315).

The trc promoter can be induced by addition of the lactose
derivative IPTG (isopropyl β -D-thiogalactopyranoside).

The *E. coli* - *C. glutamicum* shuttle vector pEC-XK99E
constructed was transferred into *C. glutamicum* DSM5715 by
20 means of electroporation (Liebl et al., 1989, FEMS
Microbiology Letters, 53:299-303). Selection of the
transformants took place on LBHIS agar comprising 18.5 g/l
brain-heart infusion broth, 0.5 M sorbitol, 5 g/l Bacto-
tryptone, 2.5 g/l Bacto-yeast extract, 5 g/l NaCl and
25 18 g/l Bacto-agar, which had been supplemented with 25 mg/l
kanamycin. Incubation was carried out for 2 days at 33°C.

Plasmid DNA was isolated from a transformant by
conventional methods (Peters-Wendisch et al., 1998,
Microbiology, 144, 915 - 927), cleaved with the restriction
30 endonuclease HindIII, and the plasmid was checked by
subsequent agarose gel electrophoresis.

The plasmid construct obtained in this way was called pEC-
XK99E (figure 1). The strain obtained by electroporation of

the plasmid pEC-XK99E in the *C. glutamicum* strain DSM5715 was called DSM5715/pEC-XK99E and deposited as DSM13455 at the Deutsche Sammlung für Mikroorganismen und Zellkulturen (DSMZ = German Collection of Microorganisms and Cell
 5 Cultures, Braunschweig, Germany) in accordance with the Budapest Treaty.

3.3 Cloning of gpmB in the *E. coli*-*C. glutamicum* shuttle vector pEC-XK99E

The *E. coli* - *C. glutamicum* shuttle vector pEC-XK99E
 10 described in example 3.2 was used as the vector. DNA of this plasmid was cleaved completely with the restriction enzymes KpnI and XbaI and then dephosphorylated with shrimp alkaline phosphatase (Roche Diagnostics GmbH, Mannheim, Germany, Product Description SAP, Product No. 1758250).

15 The gpmB fragment approx. 817 bp in size described in example 3.1, obtained by means of PCR and cleaved with the restriction endonucleases KpnI and XbaI was mixed with the prepared vector pEC-XK99E and the batch was treated with T4 DNA ligase (Amersham Pharmacia, Freiburg, Germany, Product
 20 Description T4-DNA-Ligase, Code no.27-0870-04). The ligation batch was transformed in the *E. coli* strain DH5 α mc^r (Hanahan, In: DNA cloning. A practical approach. Vol. I. IRL-Press, Oxford, Washington DC, USA). Selection of plasmid-carrying cells was made by plating out the
 25 transformation batch on LB agar (Lennox, 1955, Virology, 1:190) with 50 mg/l kanamycin. After incubation overnight at 37°C, recombinant individual clones were selected. Plasmid DNA was isolated from a transformant with the Qiaprep Spin Miniprep Kit (Product No. 27106, Qiagen,
 30 Hilden, Germany) in accordance with the manufacturer's instructions and cleaved with the restriction enzymes XbaI and KpnI to check the plasmid by subsequent agarose gel electrophoresis. The resulting plasmid was called pEC-XK99EgpmBalex. It is shown in figure 2.

Example 4

Transformation of the strain DSM5715 with the plasmid pEC-XK99EgpmBalex

The strain DSM5715 was transformed with the plasmid pEC-
5 XK99EgpmBalex using the electroporation method described by
Liebl et al., (FEMS Microbiology Letters, 53:299-303
(1989)). Selection of the transformants took place on LBHIS
agar comprising 18.5 g/l brain-heart infusion broth, 0.5 M
sorbitol, 5 g/l Bacto-tryptone, 2.5 g/l Bacto-yeast
10 extract, 5 g/l NaCl and 18 g/l Bacto-agar, which had been
supplemented with 25 mg/l kanamycin. Incubation was carried
out for 2 days at 33°C.

Plasmid DNA was isolated from a transformant by
conventional methods (Peters-Wendisch et al., 1998,
15 Microbiology, 144, 915 - 927), cleaved with the restriction
endonucleases XbaI and KpnI, and the plasmid was checked by
subsequent agarose gel electrophoresis. The strain obtained
was called DSM5715/pEC-XK99EgpmBalex.

Example 5

20 Preparation of Lysine

The *C. glutamicum* strain DSM5715/pEC-XK99EgpmBalex obtained
in example 4 was cultured in a nutrient medium suitable for
the production of lysine and the lysine content in the
culture supernatant was determined.

25 For this, the strain was first incubated on an agar plate
with the corresponding antibiotic (brain-heart agar with
kanamycin (25 mg/l)) for 24 hours at 33°C. Starting from
this agar plate culture, a preculture was seeded (10 ml
medium in a 100 ml conical flask). The complete medium
30 CgIII was used as the medium for the preculture.

Medium Cg III

NaCl 2.5 g/l

Bacto-Peptone 10 g/l

Bacto-Yeast extract 10 g/l

Glucose (autoclaved separately) 2% (w/v)

The pH was brought to pH 7.4

Kanamycin (25 mg/l) was added to this. The preculture was incubated for 16 hours at 33°C at 240 rpm on a shaking machine. A main culture was seeded from this preculture
5 such that the initial OD (660nm) of the main culture was 0.1. Medium MM was used for the main culture.

Medium MM

CSL (corn steep liquor)	5 g/l
MOPS (morpholinopropanesulfonic acid)	20 g/l
Glucose (autoclaved separately)	50 g/l
 (NH ₄) ₂ SO ₄	 25 g/l
KH ₂ PO ₄	0.1 g/l
MgSO ₄ * 7 H ₂ O	1.0 g/l
CaCl ₂ * 2 H ₂ O	10 mg/l
FeSO ₄ * 7 H ₂ O	10 mg/l
MnSO ₄ * H ₂ O	5.0mg/l
Biotin (sterile-filtered)	0.3 mg/l
Thiamine * HCl (sterile-filtered)	0.2 mg/l
L-Leucine (sterile-filtered)	0.1 g/l
CaCO ₃	25 g/l

The CSL, MOPS and the salt solution were brought to pH 7 with aqueous ammonia and autoclaved. The sterile substrate and vitamin solutions were then added, as well as the CaCO₃ autoclaved in the dry state.

Culturing is carried out in a 10 ml volume in a 100 ml conical flask with baffles. Kanamycin (25 mg/l) and IPTG (1mM/l) was added. Culturing was carried out at 33°C and 80% atmospheric humidity.

After 48 hours, the OD was determined at a measurement wavelength of 660 nm with a Biomek 1000 (Beckmann

Instruments GmbH, Munich). The amount of lysine formed was determined with an amino acid analyzer from Eppendorf-BioTronik (Hamburg, Germany) by ion exchange chromatography and post-column derivation with ninhydrin detection.

- 5 The result of the experiment is shown in table 1.

Table 1

Strain	OD (660 nm)	Lysine HCl g/l
DSM5715	11.5	12.99
DSM5715/ pEC-XK99EgpmBalex	11.0	14.17

Brief Description of the Figures:

Figure 1: Map of the plasmid pEC-XK99E

- 10 Figure 2: Map of the plasmid pEC-XK99EgpmBalex

The abbreviations and designations used have the following meaning:

Kan:	Kanamycin resistance gene aph(3')-IIa from Escherichia coli
HindIII	Cleavage site of the restriction enzyme HindIII
XbaI	Cleavage site of the restriction enzyme XbaI
KpnI	Cleavage site of the restriction enzyme KpnI
P _{trc}	trc promoter
T1	Termination region T1
T2	Termination region T2
per	Replication effector per

rep	Replication region rep of the plasmid pGA1
lacIq	lacIq repressor of the lac operon of Escherichia coli
gpmB	Cloned gpmB gene

What is claimed is:

1. Isolated polynucleotide from coryneform bacteria,
comprising a polynucleotide sequence which codes for
the gpmB gene, chosen from the group consisting of
 - 5 a) polynucleotide which is identical to the extent of
at least 70% to a polynucleotide which codes for a
polypeptide which comprises the amino acid
sequence of SEQ ID No. 2,
 - b) polynucleotide which codes for a polypeptide which
10 comprises an amino acid sequence which is
identical to the extent of at least 70% to the
amino acid sequence of SEQ ID No. 2,
 - c) polynucleotide which is complementary to the
polynucleotides of a) or b), and
 - 15 d) polynucleotide comprising at least 15 successive
nucleotides of the polynucleotide sequence of a),
b) or c),

the polypeptide preferably having the activity of
phosphoglycerate mutase II.
- 20 2. Polynucleotide according to claim 1, wherein the
polynucleotide is a preferably recombinant DNA which
is capable of replication in coryneform bacteria.
3. Polynucleotide according to claim 1, wherein the
polynucleotide is an RNA.
- 25 4. Polynucleotide according to claim 2, comprising the
nucleic acid sequence as shown in SEQ ID No. 1.
5. DNA according to claim 2 which is capable of
replication, comprising

- (i) the nucleotide sequence shown in SEQ ID No. 1,
or
 - (ii) at least one sequence which corresponds to
sequence (i) within the range of the
5 degeneration of the genetic code, or
 - (iii) at least one sequence which hybridizes with the
sequence complementary to sequence (i) or (ii),
and optionally
 - (iv) sense mutations of neutral function in (i).
- 10 6. DNA according to claim 5 which is capable of
replication, wherein the hybridization of sequence
(iii) is carried out under a stringency corresponding
to at most 2x SSC.
- 15 7. Polynucleotide sequence according to claim 2, which
codes for a polypeptide which comprises the amino acid
sequence shown in SEQ ID No. 2.
8. Coryneform bacteria in which the gpmB gene is
enhanced, in particular over-expressed.
- 20 9. Escherichia coli strain DH5 α mc^r/pEC-XK99EgpmBalex as
DSM 14376 deposited at the Deutsche Sammlung für
Mikroorganismen und Zellkulturen [German Collection of
Microorganisms and Cell Cultures], DSMZ, Braunschweig,
Germany.
- 25 10. Process for the fermentative preparation of L-amino
acids, in particular L-lysine, wherein the following
steps are carried out:
- a) fermentation of the coryneform bacteria which
produce the desired L-amino acid and in which at
least the gpmB gene or nucleotide sequences which
30 code for it are enhanced, in particular over-
expressed,

b) concentration of the L-amino acid in the medium
or in the cells of the bacteria, and

c) isolation of the L-amino acid.

11. Process according to claim 10, wherein bacteria in
5 which further genes of the biosynthesis pathway of the
desired L-amino acid are additionally enhanced are
employed.

12. Process according to claim 10, wherein bacteria in
10 which the metabolic pathway which reduce the formation
of the desired L-amino acid are at least partly
eliminated are employed.

13. Process according to claim 10, wherein a strain
transformed with a plasmid vector is employed, and the
15 plasmid vector carries the nucleotide sequence which
codes for the gpmB gene.

14. Process according to claim 10, wherein the expression
of the polynucleotide(s) which code(s) for the gpmB
gene is enhanced, in particular over-expressed.

15. Process according to claim 10, wherein the catalytic
20 properties of the polypeptide (enzyme protein) for
which the polynucleotide gpmB codes are increased.

16. Process according to claim 10, wherein for the
preparation of L-amino acids, coryneform
25 microorganisms in which at the same time one or more
of the genes chosen from the group consisting of

16.1 the dapA gene which codes for
dihydrodipicolinate synthase,

16.2 the gap gene which codes for glyceraldehyde 3-
phosphate dehydrogenase,

- 16.3 the tpi gene which codes for triose phosphate isomerase,
- 16.4 the pgk gene which codes for 3-phosphoglycerate kinase,
- 5 16.5 the zwf gene which codes for glucose 6-phosphate dehydrogenase,
- 16.6 the pyc gene which codes for pyruvate carboxylase,
- 10 16.7 the mqo gene which codes for malate-quinone oxidoreductase,
- 16.8 the lysC gene which codes for a feed-back resistant aspartate kinase,
- 16.9 the lysE gene which codes for lysine export,
- 15 16.10 the hom gene which codes for homoserine dehydrogenase
- 16.11 the ilvA gene which codes for threonine dehydratase or the ilvA(Fbr) allele which codes for a feed back resistant threonine dehydratase,
- 20 16.12 the ilvBN gene which codes for acetohydroxy-acid synthase
- 16.13 the ilvD gene which codes for dihydroxy-acid dehydratase,
- 16.14 the zwal gene which codes for the Zwal protein,
- 25 is or are enhanced or over-expressed are fermented.
17. Process according to claim 10, wherein for the preparation of L-amino acids, coryneform

microorganisms in which at the same time one or more of the genes chosen from the group consisting of,

- 17.1 the pck gene which codes for phosphoenol pyruvate carboxykinase,
 - 5 17.2 the pgi gene which codes for glucose 6-phosphate isomerase,
 - 17.3 the poxB gene which codes for pyruvate oxidase,
 - 17.4 the zwa2 gene which codes for the Zwa2 protein,
- is or are attenuated are fermented.
- 10 18. Coryneform bacteria which contain a vector which carries a polynucleotide according to claim 1.
 - 19. Process according to one or more of claims 10-17, wherein microorganisms of the species *Corynebacterium glutamicum* are employed.
 - 15 20. Process according to claim 19, wherein the *Corynebacterium glutamicum* strain DH5 α mcr/pEC-XK99EgpmBalex is employed.
 - 21. Process for discovering RNA, cDNA and DNA in order to isolate nucleic acids, or polynucleotides or genes
 - 20 which code for phosphoglycerate mutase II or have a high similarity with the sequence of the gpmB gene, wherein the polynucleotide comprising the polynucleotide sequences according to claim 1,2,3 or 4 is employed as hybridization probes.
 - 25 22. Process according to claim 21, wherein arrays, micro arrays or DNA chips are employed.

Figure 1: Map of the plasmid pEC-XK99E

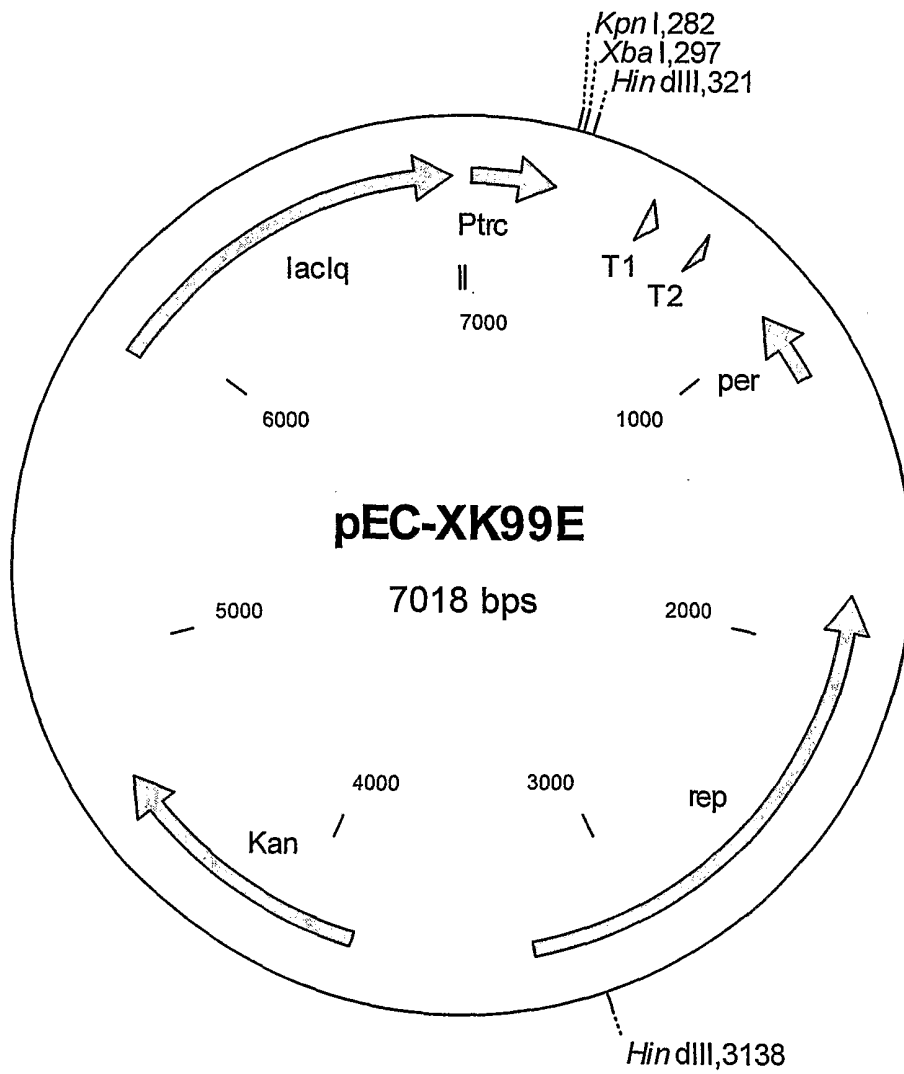
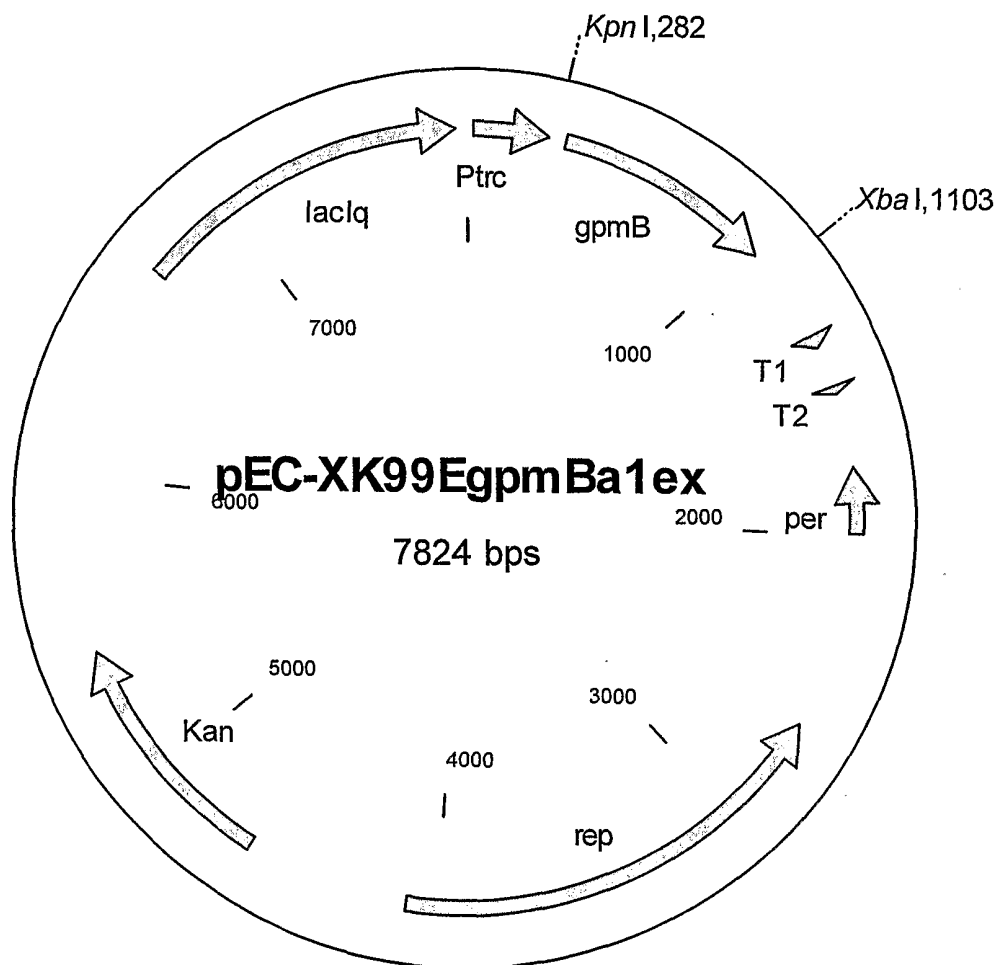


Figure 2: Map of the plasmid pEC-XK99EgpmBa1ex

5



SEQUENCE PROTOCOL

<110> Degussa AG

5 <120> Nucleotide sequences which code for the gpmB gene

<130> 000461 BT

<140>

10 <141>

<160> 4

<170> PatentIn Ver. 2.1

15

<210> 1

<211> 1800

<212> DNA

<213> Corynebacterium glutamicum

20

<220>

<221> CDS

<222> (500)..(1204)

<223> gpmB gene

25

<400> 1

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aagcagcgga tgaaaagaag gccgatgaca tcgctgtcat cgatgtctct gacatgatcg 120

30

caatcaccga ttgctttgtt gttgcatctg ctgacaatga gcgccagggtg ggcgccattg 180

ttgaggagat cgaagatgag atgaccaagg ctggtttoga gcctaagcgc cgtgaaggca 240

35

accgcgaaaa ccgttgggtt ctccttgact acggattggt tgttatccac gttcagcgac 300

aggcagagcg cgagttctac ggactggatc gtctgtaccg cgactgccca ctcatgaaa 360

ttgaaggact tgaaaccttc aagcgtgaat cctcctggtc tgatgaggcg gatatccgca 420

40

acatcgacag cattgatgaa ctcccacctt tgccagctga atacgagcct ggctacgagg 480

acgattaaga ggtagtcct gtg act cgt cgc ctg att ctg ctc cga cac ggg 532

45

Met Thr Arg Arg Leu Ile Leu Leu Arg His Gly
1 5 10

cag act gaa tac aac gcc acg tcc cga atg cag gga caa ttg gac aca 580

Gln Thr Glu Tyr Asn Ala Thr Ser Arg Met Gln Gly Gln Leu Asp Thr

50

15 20 25

gag ctg tct gac ctg ggc ttt caa cag gcg gcc agc gca gcc tca gtg 628

Glu Leu Ser Asp Leu Gly Phe Gln Gln Ala Ala Ser Ala Ala Ser Val

30 35 40

55

ctg gtt caa aaa aac atc acc cat gtg ttc agc tcg gat ctt tcc cgc 676

Leu Val Gln Lys Asn Ile Thr His Val Phe Ser Ser Asp Leu Ser Arg

45 50 55

	gcc ttc aac acc gca agc gcg gtt gcg gcg ctg att gac gcg gag gtg 724 Ala Phe Asn Thr Ala Ser Ala Val Ala Ala Leu Ile Asp Ala Glu Val 60 65 70 75
5	cgc gtc gat aag cgt ctt cgg gaa acg cat ttg ggt gag tgg cag gcc 772 Arg Val Asp Lys Arg Leu Arg Glu Thr His Leu Gly Glu Trp Gln Ala 80 85 90
10	aaa acc cac act gag gtg gat tcc gaa tat cca ggt gcg cgc gct caa 820 Lys Thr His Thr Glu Val Asp Ser Glu Tyr Pro Gly Ala Arg Ala Gln 95 100 105
15	tgg cgc cac gat ccg cag tgg gca cca ccc ggc ggc gaa tcg cgc gtg 868 Trp Arg His Asp Pro Gln Trp Ala Pro Pro Gly Gly Glu Ser Arg Val 110 115 120
20	gat gtt gcg cgc cgg gca cgc caa gtt gtc gac gag ttg atg gtg tcg 916 Asp Val Ala Arg Arg Ala Arg Gln Val Val Asp Glu Leu Met Val Ser 125 130 135
	ctt gat gat tgg gat gaa ggc acc gtg ctc atc gtg gct cac ggt ggc 964 Leu Asp Asp Trp Asp Glu Gly Thr Val Leu Ile Val Ala His Gly Gly 140 145 150 155
25	acg att aat gcg ctg acc tcg aat ctt ttg gac ctg gcg tat gat cag 1012 Thr Ile Asn Ala Leu Thr Ser Asn Leu Leu Asp Leu Ala Tyr Asp Gln 160 165 170
30	tac ccc atg ttc tct gga ctt gga aat acc tgt tgg gca caa ttg acc 1060 Tyr Pro Met Phe Ser Gly Leu Gly Asn Thr Cys Trp Ala Gln Leu Thr 175 180 185
35	gcc cga cct cgc tat tat gca ggt agt gag aac cca gaa gat gac ctc 1108 Ala Arg Pro Arg Tyr Tyr Ala Gly Ser Glu Asn Pro Glu Asp Asp Leu 190 195 200
40	aag att tct tcg gcg gtt tcc aac agc cct cat ttt gag ggc aac aat 1156 Lys Ile Ser Ser Ala Val Ser Asn Ser Pro His Phe Glu Gly Asn Asn 205 210 215
	gtg gaa aac gcc cag tgg tat ctt gac ggc tgg aac atg ggt gtt acg 1204 Val Glu Asn Ala Gln Trp Tyr Leu Asp Gly Trp Asn Met Gly Val Thr 220 225 230 235
45	cagtaaagaa gatggcaata aaaatgtgga ggagtaaagg cgatgccagt tcgggtaatt 1264 gttgattcct ccgcatgctt gccaacgcat gtggccgagg acctcgacat cacggtgatt 1324 aacttgcacg tgatgaataa cggatgaagaa cgcagtacat ccgggttgct gtcgttgga 1384
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 20 35 40 45
 Ile Thr His Val Phe Ser Ser Asp Leu Ser Arg Ala Phe Asn Thr Ala
 50 55 60
 25 Ser Ala Val Ala Ala Leu Ile Asp Ala Glu Val Arg Val Asp Lys Arg
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 Leu Arg Glu Thr His Leu Gly Glu Trp Gln Ala Lys Thr His Thr Glu
 85 90 95
 30 Val Asp Ser Glu Tyr Pro Gly Ala Arg Ala Gln Trp Arg His Asp Pro
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 Gln Trp Ala Pro Pro Gly Gly Glu Ser Arg Val Asp Val Ala Arg Arg
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 40 Glu Gly Thr Val Leu Ile Val Ala His Gly Gly Thr Ile Asn Ala Leu
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 Thr Ser Asn Leu Leu Asp Leu Ala Tyr Asp Gln Tyr Pro Met Phe Ser
 165 170 175
 45 Gly Leu Gly Asn Thr Cys Trp Ala Gln Leu Thr Ala Arg Pro Arg Tyr
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 Tyr Ala Gly Ser Glu Asn Pro Glu Asp Asp Leu Lys Ile Ser Ser Ala
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