OPIC
Office de la propriété intellectuelle du Canada

CIPO
Canadian Intellectual Property Office

(12) (19) (CA) **Demande-Application**

(21) (A1) **2,276,093**
(86) 1997/12/29
(87) 1998/07/09

(72) PHILIPPSEN, PETER, CH
(72) POMPEJUS, MARKUS, DE
(72) SEULBERGER, HARALD, DE
(71) BASF AKTIENGESELLSCHAFT, DE

(51) Int.Cl.[6] C12N 15/53, C12N 15/54, C12P 25/00
(30) 1996/12/31 (0016/97) CH

(54) **SEQUENCES PARTIELLES DE GENES DE BIOSYNTHESE DE PURINE DE L'ASHBYA GOSSYPII ET LEUR UTILISATION DANS LA SYNTHESE DE RIBOFLAVINE PAR DES MICROBES**

(54) **PARTIAL SEQUENCES OF PURINE BIOSYNTHESIS GENES FROM ASHBYA GOSSYPII AND THEIR USE IN THE MICROBIAL RIBOFLAVIN SYNTHESIS**

(57) L'invention concerne des séquences partielles des gènes de biosynthèse de purine de l'Ashbya gossypii, leur production et leur utilisation.

(57) Partial sequences of purine biosynthesis genes from Ashbya gossypii are disclosed, as well as their production and use.

PCT

WELTORGANISATION FÜR GEISTIGES EIGENTUM
Internationales Büro

INTERNATIONALE ANMELDUNG VERÖFFENTLICHT NACH DEM VERTRAG ÜBER DIE INTERNATIONALE ZUSAMMENARBEIT AUF DEM GEBIET DES PATENTWESENS (PCT)

(51) Internationale Patentklassifikation [6] :
C12N 15/53, 15/54, C12P 25/00

A3

(11) Internationale Veröffentlichungsnummer: **WO 98/29539**

(43) Internationales Veröffentlichungsdatum: 9. Juli 1998 (09.07.98)

(21) Internationales Aktenzeichen: PCT/EP97/07312

(22) Internationales Anmeldedatum: 29. Dezember 1997 (29.12.97)

(30) Prioritätsdaten:
0016/97 31. Dezember 1996 (31.12.96) CH

(71) Anmelder *(für alle Bestimmungsstaaten ausser US)*: BASF AKTIENGESELLSCHAFT [DE/DE]; D–67056 Ludwigshafen (DE).

(72) Erfinder; und
(75) Erfinder/Anmelder *(nur für US)*: PHILIPPSEN, Peter [DE/CH]; Mühlestiegstrasse 28, CH–4125 Riehen (CH). POMPEJUS, Markus [DE/DE]; Lerchenstrasse 72, D–67165 Waldsee (DE). SEULBERGER, Harald [DE/DE]; Speyerer Wingert 25, D–67141 Neuhofen (DE).

(74) Gemeinsamer Vertreter: BASF AKTIENGESELLSCHAFT; D–67056 Ludwigshafen (DE).

(81) Bestimmungsstaaten: AL, AU, BG, BR, BY, CA, CN, CZ, GE, HU, ID, IL, JP, KR, KZ, LT, LV, MX, NO, NZ, PL, RO, RU, SG, SI, SK, TR, UA, US, eurasisches Patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), europäisches Patent (AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE).

Veröffentlicht
Mit internationalem Recherchenbericht.
Vor Ablauf der für Änderungen der Ansprüche zugelassenen Frist. Veröffentlichung wird wiederholt falls Änderungen eintreffen.

(88) Veröffentlichungsdatum des internationalen Recherchenberichts: 12. November 1998 (12.11.98)

(54) Title: PARTIAL SEQUENCES OF PURINE BIOSYNTHESIS GENES FROM *ASHBYA GOSSYPII* AND THEIR USE IN THE MICROBIAL RIBOFLAVIN SYNTHESIS

(54) Bezeichnung: TEILSEQUENZEN VON GENEN DER PURINBIOSYNTHESE AUS *ASHBYA GOSSYPII* UND DEREN VERWENDUNG IN DER MIKROBIELLEN RIBOFLAVINSYNTHESE

(57) Abstract

Partial sequences of purine biosynthesis genes from *Ashbya gossypii* are disclosed, as well as their production and use.

(57) Zusammenfassung

Teilsequenzen von Genen der Purinbiosynthese aus *Ashbya gossypii*, deren Herstellung und Verwendung.

PARTIAL SEQUENCES OF PURINE BIOSYNTHESIS GENES FROM *ASHBYA GOSSYPII* AND THEIR USE IN THE MICROBIAL RIBOFLAVIN SYNTHESIS

Partial sequences of genes of purine biosynthesis from Ashbya gossypii and the use thereof in microbial riboflavin synthesis

The present invention relates to partial sequences of genes of purine biosynthesis from Ashbya gossypii and to the use thereof in riboflavin synthesis.

Vitamin B2, also called riboflavin, is essential for humans and animals. Vitamin B2 deficiencies are associated with inflammations of the oral and pharyngeal mucous membranes, itching and inflammation in the skin folds and similar skin damage, conjunctival inflammations, reduced visual acuity and clouding of the cornea. In infants and children there may be cessation of growth and loss of weight. Vitamin B2 therefore has economic importance, in particular as vitamin supplement for vitamin deficiencies and as animal feed supplement. It is also employed for coloring foodstuffs, for example in mayonnaise, ice-cream, blancmange, etc.

Vitamin B2 is prepared either chemically or microbially (see, for example, Kurth et al. (1996) Riboflavin, in: Ullmann's Encyclopedia of industrial chemistry, VCH Weinheim). In the chemical preparation processes, riboflavin is usually obtained as a pure final product in multistage processes, it being necessary to employ relatively costly starting materials such as D-ribose. An alternative to the chemical synthesis of riboflavin is the preparation of this substance by microorganisms. The starting materials used in this case are renewable raw materials such as sugars or vegetable oils. The preparation of riboflavin by fermentation of fungi such as Eremothecium ashbyii or Ashbya gossypii is known (The Merck Index, Windholz et al., eds. Merck & Co., page 1183, 1983), but yeasts such as Candida, Pichia and Saccharomyces, or bacteria such as Bacillus, Clostridia or Corynebacteria, are also described as riboflavin producers.

EP 405370 describes riboflavin-overproducing bacterial strains obtained by transformation of the riboflavin biosynthesis genes from Bacillus subtilis. These genes described therein and other prokaryotic genes involved in vitamin B2 biosynthesis are unsuitable for a recombinant riboflavin preparation process using eukaryotes such as Saccharomyces cerevisiae or Ashbya gossypii.

DE4420785 describes six riboflavin biosynthesis genes from Ashbya gossypii, and microorganisms transformed with these genes, and the use of such microorganisms for riboflavin synthesis.

It is possible with these processes to generate producer strains for microbial riboflavin synthesis. However, these producer strains often have metabolic limitations which cannot be eliminated by the inserted biosynthesis genes or are sometimes introduced thereby. It is therefore desirable to enhance other areas of metabolic pathways, to eliminate metabolic bottlenecks in this way and to optimize further in this way the riboflavin synthesis capability of the microorganisms employed for microbial riboflavin synthesis. Optimizations of this type can be carried out by genetic modification of microorganisms. Genetically modified microorganisms mean those where the activity of genes or the gene copy number shows differences from the natural genetic situation.

The invention relates to novel partial sequences of novel genes of purine biosynthesis and to the use thereof for microbial riboflavin synthesis.

Purine metabolism (for a review, see, for example, Voet, D. and Voet, J.G., 1994, Biochemie, VCH Weinheim, pages 743-771; Zalkin, H. and Dixon, J.E., 1992, De novo purine nucleotide biosynthesis, in: Progress in nucleic acid research and molecular biology, Vol. 42, pages 259-287, Academic Press) is a part of the metabolism which is essential for all life forms.

The invention relates firstly to a gene fragment which comprises the nucleotide sequence depicted in SEQ ID NO:1 or a nucleotide sequence obtainable from SEQ ID NO:1 by substitution, insertion or deletion of up to 20% of the nucleotides.

The invention further relates to a gene fragment which comprises the nucleotide sequence depicted in SEQ ID NO:2 or a nucleotide sequence obtainable from SEQ ID NO:2 by substitution, insertion or deletion of up to 20% of the nucleotides.

The invention further relates to a gene fragment which comprises the nucleotide sequence depicted in SEQ ID NO:3 or a nucleotide sequence obtainable from SEQ ID NO:3 by substitution, insertion or deletion of up to 15% of the nucleotides.

The invention further relates to a gene fragment which comprises the nucleotide sequence depicted in SEQ ID NO:4 or a nucleotide sequence obtainable from SEQ ID NO:4 by substitution, insertion or deletion of up to 20% of the nucleotides.

The invention further relates to the use of the nucleic acid sequence depicted in SEQ ID NO:1 or SEQ ID NO:2 or SEQ ID NO:3 or SEQ ID NO:4 for constructing genetically modified microorganisms.

These microorganisms can be any suitable bacteria, but preferably bacteria of the genera Bacillus and Corynebacterium. These microorganisms can be any suitable eukaryotic microorganisms, preferably fungi of the genera Ashbya or Eremothecium, in particular Ashbya gossypii, or else yeasts, but preferably yeasts of the genera Candida, Pichia and Saccharomyces.

The invention further relates to the use of such genetically modified microorganisms for producing riboflavin.

The following examples describe the preparation of the nucleic acids according to the invention and the use thereof for producing microorganisms with increased riboflavin synthesis.

Example 1

Preparation of a genomic gene bank from Ashbya gossypii ATCC10895

Genomic DNA from Ashbya gossypii ATCC10895 can be prepared by conventional methods, for example as described in WO9703208. The genomic gene bank can be constructed starting from this DNA by conventional methods (e.g. Sambrook, J. et al. (1989) Molecular cloning: a laboratory manual, Cold Spring Harbor Laboratory Press or Ausubel, F.M. et al. (1994) Current protocols in molecular biology, John Wiley and sons) in any suitable plasmids such as in pRS316 and other plasmids of the pRS series (Sikorski and Hieter (1989) Genetics, 19-27). The Ashbya gossypii genomic DNA fragment used can have any suitable size, but Sau3A fragments between 2 and 9 kb in length, which can be cloned into a BamHI cleavage site, are preferred.

Example 2

Analysis of nucleic acid sequences of the genomic gene bank

It is possible to select single clones from E.coli clones harboring the gene bank described in Example 1. The cells can be cultivated by conventional methods in suitable media (e.g. LB with 100 mg/l ampicillin), and plasmid DNA can be isolated from these cells. After cloning of the fragments of the genomic DNA from Ashbya gossypii (inserts) into a cleavage site of the polylinker of pRS plasmids (described in Example 1) it is possible to determine, with suitable oligonucleotides and

standard methods, the nucleotide sequence of partial regions of the inserts. Oligonucleotides particularly suitable for this purpose are 5'-GTAAAACGACGGCCAGT-3' and 5'-GGAAACAGCTATGACCATG-3'

Example 3

Computer-assisted analysis of nucleotide sequences found

Comparisons of newly identified sequences with existing DNA and protein databanks can be carried out, for example, with BLAST algorithms (Altschul et al. (1990) J. Mol. Biol. 215, 403-410) or the Clustal algorithm using the PAM250 weighting table or the Wilbur-Lipman DNA alignment algorithm (as implemented, for example, in the MegAlign 3.06 program package from DNAstar). It is possible in this way to discover similarities between the newly discovered sequences and previously known sequences and to describe the function of the novel partial sequences of novel genes.

Example 4

Identification of E. coli clones which harbor fragments of the gene for glutamine phosphoribosylpyrophosphate amidotransferase from Ashbya gossypii (AgADE4).

Examination of the clones as described in Example 2 and analysis of the resulting sequences as in Example 3 reveals sequences as described in SEQ ID NO:1 and SEQ ID NO:2 and SEQ ID NO:3. On use of the BLASTN algorithm (see Example 3), these sequences show the following similarities with partial regions of ADE4 genes, in particular with partial regions of the ADE4 gene from Saccharomyces kluyveri (Genbank entry SKU32992) and with partial regions of the ADE4 gene from Saccharomyces cerevisiae (Genbank entry YSCADE4): nucleotide sequence in SEQ ID NO:1: 74% and 69%, respectively, similarity, nucleotide sequence in SEQ ID NO:2 : 78% and 73%, respectively, similarity and nucleotide sequence in SEQ ID NO:3: 81% and 75%, respectively, similarity.

Example 5

Identification of E. coli clones which harbor fragments of the gene for inosine-5'-monophosphate dehydrogenase from Ashbya gossypii (AgGUA1).

Examination of clones as described in Example 2 and analysis of the resulting sequences as in Example 3 reveals a sequence as described in SEQ ID NO:4. On use of the BLASTN algorithm (see

Example 3), this sequence shows 67% similarity with partial regions of the sequence of chromosome XII and 65% similarity with partial regions of the sequence of chromosome VIII from Saccharomyces cerevisiae (Genbank entries YSCL9753 and YSCH9177). Similarities with various genes for inosine-5'-monophosphate dehydrogenases were found, in particular 67% similarity with partial regions of the sequence of a human inosine-5'-monophosphate dehydrogenase (Genbank entry HUMIMPH).

Example 6

Cloning of the complete genes for glutamine phosphoribosyl-pyrophosphate amidotransferase (AgADE4) and inosine-5'-monophosphate dehydrogenase (AgGUA1) from Ashbya gossypii

The nucleic acid sequences depicted in SEQ ID NO:1 or SEQ ID NO:2 or SEQ ID NO:3 can be used as probes for cloning the complete gene for glutamine phosphoribosylpyrophosphate amidotransferase (AgADE4) from Ashbya gossypii. The nucleic acid sequence depicted SEQ ID NO:4 can be used as probe for cloning the complete gene for inosine-5'-monophosphate dehydrogenase (AgGUA1) from Ashbya gossypii. It is possible to use for this purpose, for example, a genomic gene bank from Ashbya gossypii as described in Example 1. Using conventional methods (see, for example, Sambrook, J. et al. (1989) Molecular cloning: a laboratory manual, Cold Spring Harbor Laboratory Press) of Southern blot analysis and of colony hybridization it is possible to identify E. coli clones harboring the complete AgADE4 and AgGUA1 genes.

Example 8

Use of the genes for glutamine phosphoribosylpyrophosphate amidotransferase (AgADE4) and inosine-5'-monophosphate dehydrogenase (AgGUA1) from Ashbya gossypii for enhancing riboflavin production

The genes for glutamine phosphoribosylpyrophosphate amidotransferase (AgADE4) and inosine-5'-monophosphate dehydrogenase (AgGUA1) from Ashbya gossypii can be introduced into Ashbya gossypii or other microorganisms such as Saccharomyces cerevisiae or other yeasts, as described in WO9703208 or DE4420785. It is possible in this way to construct genetically modified microorganisms in which the activity of genes or the gene copy number shows differences from the natural situation. The microorganisms constructed in this way can be used for the biosynthesis of riboflavin.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT:
(A) NAME: BASF Aktiengesellschaft
(B) STREET: Carl-Bosch-Strasse 38
(C) CITY: Ludwigshafen
(E) COUNTRY: Federal Republic of Germany
(F) POSTAL CODE: D-67056
(G) TELEPHONE: 0621/6048526
(H) TELEFAX: 0621/6043123
(I) TELEX: 1762175170

(ii) TITLE OF APPLICATION: Partial sequences of genes of purine biosynthesis from Ashbya gossypii and the use thereof in microbial riboflavin synthesis

(iii) NUMBER OF SEQUENCES: 4

(iv) COMPUTER-READABLE FORM:
(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: PatentIn Release #1.0, Version #1.25 (EPO)

(2) INFORMATION FOR SEQ ID NO: 1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 747 base pairs
(B) TYPE: Nucleic acid
(C) STRANDEDNESS: Double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTISENSE: NO

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Ashbya gossypii

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 1:

GATCAGGCCG CTATTGTTTG GTGAGCGCGT CAACGATGAC GGCACCATGG GACTACATGC 60

TAGCGTCCGA AAGTGTCGTT CTTAAGGCCC ACCGCTTCCA AAACATACGT GATATTCTTC 120

CCGGCCAAGC CGTCATTATC CCTAAAACGT GCGGCTCCAG TCCACCAGAG TTCCGGCAGG 180

TAGTGCCAAT TGAGGCCTAC AAACCGGACT TGTTTGAGTA CGTGTATTTC GCTCGTGCTG 240

ACAGCGTTCT GGACGGTATT TCCGTTTACC ATACACGCCT GTTGATGGGT ATCAAACTTG 300

CCGAGAACAT CAAAAAACAG ATCGATCTGG ACGAAATTGA CGTTGTTGTA TCTGTTCCTG 360

ACACTGCACG TACCTGTGCA TTGGAGTGTG CCAACCATTT AAACAAACCT TATCGCGAAG 420

GATTTGTCAA GAACAGATAT GTTGGAAGAA CATTTATCAT GCCAAACCAA AAAGAGCGAG 480

TATCTTCTGT GCGCCGCAAG TTGAACCCAA TGAACTCAGA ATTTAAAGAC AAGCGCGTGC 540

TGATTGTCGA TGATTCCATT GTGCGAGGTA CCACTTCCAA AGAGATTGTT AACATGGCGA 600

AGGAATCCGG TGCTGCCAAG GTCTACTTTG CCTCTGCAGC GCCAGCAATT CGTTTCAATC 660

ACATCTACGG GATTGACCTA GCAGATACTA AGCAGCTTGT CGCCTACAAC AGAACTGTTG 720

AAGAAATCAC TGCGGAGCTG GGCTGTG 747

(2) INFORMATION FOR SEQ ID NO: 2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 488 base pairs
(B) TYPE: Nucleic acid
(C) STRANDEDNESS: Double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTISENSE: NO

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Ashbya gossypii

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 2:

GATCTGGACG AAATTGACGT TGTTGTATCT GTTCCTGACA CTGCACGTAC CTGTGCATTG 60

GAGTGTGCCA ACCATTTAAA CAAACCTTAT CGCGAAGGAT TTGTCAAGAA CAGATATGTT 120

GGAAGAACAT TTATCATGCC AAACCAAAAA GAGCGAGTAT CTTCTGTGCG CCGCAAGTTG 180

AACCCAATGA ACTCAGAATT TAAAGACAAG CGCGTGCTGA TTGTCGATGA TTCCATTGTG 240

CGAGGTACCA CTTCCAAAGA GATTGTTAAC ATGGCGAAGG AATCCGGTGC TGCCAAGGTC 300

TACTTTGCCT CTGCAGCGCC AGCAATTCGT TTCAATCACA TCTACGGGAT TGACCTAGCA 360

GATACTAAGC AGCTTGTCGC CTACAACAGA ACTGTTGAAG AAATCACTGC GGAGCTGGGC 420

TGTGACCGCG TCATCTATCA ATCTTTGGAT GACCTCATCG ACTGTTGCAA GACAGACATC 480

ATCTCAGA 488

(2) INFORMATION FOR SEQ ID NO: 3:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 447 base pairs
(B) TYPE: Nucleic acid
(C) STRANDEDNESS: Double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTISENSE: NO

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Ashbya gossypii

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 3:

GATCTGGACG AAATTGACGT TGTTGTATCT GTTCCTGACA CTGCACGTAC CTGTGCATTG 60

GAGTGTGCCA ACCATTTAAA CAAACCTTAT CGCGAAGGAT TTGTCAAGAA CAGATATGTT 120

GGAAGAACAT TTATCATGCC AAACCAAAAA GAGCGAGTAT CTTCTGTGCG CCGCAAGTTG 180

AACCCAATGA ACTCAGAATT TAAAGACAAG CGCGTGCTGA TTGTCGATGA TTCCATTGTG 240

CGAGGTACCA CTTCCAAAGA GATTGTTAAC ATGGCGAAGG AATCCGGTGC TGCCAAGGTC 300

TACTTTGCCT CTGCAGCGCC AGCAATTCGT TTCAATCACA TCTACGGGAT TGACCCTAGC 360

AGGATACTTA AGCAGCTTGT CGCCCTACAA CAGAACTGTT GAAGAAATCA CTGCTGCAGC 420

TGGGCTGTGA CCCGCGTCAT CTATCAA 447

(2) INFORMATION FOR SEQ ID NO: 4:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 656 base pairs
(B) TYPE: Nucleic acid
(C) STRANDEDNESS: Double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTISENSE: NO

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Ashbya gossypii

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 4:

GATCTCAGAC ACAAGCAGGG TCTCGTCCTC GACAAACTGG ATGTCACGGG ACGTGATGAT 60

CCCCTGCAGC TTCCCGGTCG GCTTGCCATC ATCTGCTGAT GTGTTAGTAC ATGTTCCTGC 120

CAAAAAGGCG CTGCACGTCT GCGGTCAGAG TAAAAGGTGT CTACACGATC GTTTGATCGC 180

AGTCAGTAGA ACGAAAGTTA TTGATTTATG ATCATCATCC CAGCGTGCAG CCCCGCGTGC 240

CACTCTAACA TACCTGTCAC AGGAAATCCT GCAAACCCAA ACTCGTTCTT CATCCGGCGC 300

ACGTCCGCCA CCGTCGCGTC CGGCCCCACG ACCACGGGGG CGTTGATGAA CCCGTTTTCG 360

TACTTCTTGA CCCGGCGCAC CATCTCCGCC TGCTCCTCCG CAGTGCAGTT GTGGTGGATG 420

ATCCCGATGC CGCCCAGGAG CGCCATGTGG ATCGCCATGT CGGCCTCCGT CACCGTGTCC 480

ATCGGCGACG ACACAAACGG CGCGTTCAAG GTGATCTTCT TGGTCAGGCG CGACGACAGC 540

ACCACCTCCG ACGATGGGAA GTCGATCTTG CCCGGCAAGA ACARGAAGTC GTTGTACGTC 600

AACCCGCCCC GCGTCTTGGA GTTCATCAAC TGCTCCACGG ACAGCCCGTC CTTCTC 656

We claim:

1. A gene fragment which comprises the nucleotide sequence depicted in SEQ ID NO:1 or a nucleotide sequence obtainable from SEQ ID NO:1 by substitution, insertion or deletion of up to 20%, preferably up to 15%, particularly preferably up to 10%, especially preferably up to 5%, of the nucleotides.

2. A gene fragment which comprises the nucleotide sequence depicted in SEQ ID NO:2 or a nucleotide sequence obtainable from SEQ ID NO:2 by substitution, insertion or deletion of up to 20%, preferably up to 15%, particularly preferably up to 10%, especially preferably up to 5%, of the nucleotides.

3. A gene fragment which comprises the nucleotide sequence depicted in SEQ ID NO:3 or a nucleotide sequence obtainable from SEQ ID NO:3 by substitution, insertion or deletion of up to 15%, preferably up to 10%, particularly preferably up to 5%, of the nucleotides.

4. A gene fragment which comprises the nucleotide sequence depicted in SEQ ID NO:4 or a nucleotide sequence obtainable from SEQ ID NO:4 by substitution, insertion or deletion of up to 20%, preferably up to 15%, particularly preferably up to 10%, especially preferably up to 5%, of the nucleotides.

5. The use of one or more of the nucleic acid sequences as claimed in claims 1 to 4 for the construction by genetic manipulation of microorganisms able to produce riboflavin.

6. A process for producing riboflavin by cultivating microorganisms which have been genetically modified in such a way that at least one gene which comprises nucleotide sequences as claimed in claim 1 to 4 has been modified.

7. A process as claimed in claim 6, wherein the microorganism is a bacterium.

8. A process as claimed in claim 6, wherein the microorganism is a bacterium of the genus Bacillus or Corynebacterium.

9. A process as claimed in claim 6, wherein the microorganism is a eukaryotic microorganism.

10. A process as claimed in claim 6, wherein the microorganism is a filamentous fungus.

11. A process as claimed in claim 6, wherein the microorganism is a filamentous fungus of the genera Ashbya or Eremothecium.

12. A process as claimed in claim 6, wherein the microorganism is Ashbya gossypii.

13. A process as claimed in claim 6, wherein the microorganism is a yeast.

14. A process as claimed in claim 6, wherein the microorganism is a yeast of the genus Candida, Pichia or Saccharomyces.

15. The use of one or more of the nucleic acid sequences as claimed in claim 1-3 for isolating the complete gene for glutamine phosphoribosylpyrophosphate amidotransferase (ADE4).

16. The use of a nucleic acid sequence as claimed in claim 4 for isolating the complete gene for inosine-5'-monophosphate dehydrogenase (GUA1).