COMMONWEALTH of AUSTRALIA

PATENTS ACT 1952

APPLICATION FOR A STANDARD PATENT

I/We BOEHRINGER MANNHEIM GmbH

of Sandhofer Strasse 112-132
D-6800 Mannheim-Waldhof
Federal Republic of Germany

LODGED AT SUB-OFFICE
27 APR 1988
Melbourne

hereby apply for the grant of a Standard Patent for an invention entitled:

"STABLE CREATINE AMIDINOHYDROLASE MUTANTS"

which is described in the accompanying ~~provisional~~ complete specification.

Details of basic application(s):—

Number	Convention Country	Date
P 37 15 841.4	Federal Republic of Germany	12th May, 1987
P 38 03 175.2	Federal Republic of Germany	3rd February, 1988

APPLICATION ACCEPTED AND AMENDMENTS ALLOWED 23.11.89

The address for service is care of DAVIES & COLLISON, Patent Attorneys, of 1 Little Collins Street, Melbourne, in the State of Victoria, Commonwealth of Australia.

Dated this 27th day of April 19 88

To: THE COMMISSIONER OF PATENTS

H. M. Pimington
(a member of the firm of DAVIES & COLLISON for and on behalf of the Applicant).

Davies & Collison, Melbourne and Canberra.

FORM 36

COMMONWEALTH OF AUSTRALIA

DECLARATION CONCERNING THE DEPOSIT, IN RELATION TO A PATENT APPLICATION, OF A MICRO-ORGANISM WITH AN INTERNATIONAL DEPOSITORY AUTHORITY

In the matter of the application made by
BOEHRINGER MANNHEIM GmbH

for a patent for an invention entitled

"Stable Creatine Amidinohydrolase Mutants"

I, JOHN MICHAEL SLATTERY,

of 1 Little Collins Street, Melbourne 3000, Victoria, Australia

declare as follows:

~~*1. I am the applicant for the patent for the invention referred to above.~~

*1. I am authorised by Boehringer Mannheim GmbH the applicant for the patent for the invention referred to above, to make this declaration on its behalf.

* See attached sheet.

2. ~~A micro-organism was deposited with~~

~~on~~ ~~and accorded the file, accession or registration number~~

3. The deposit referred to in paragraph 2 was an original deposit within the meaning of Rule 7.3 of the Budapest Treaty Regulations.

4. Samples of that micro-organism are obtainable from that international depository authority as provided by:

(a) such provisions of the Budapest Treaty on the International Recognition of the Deposit of Micro Organisms for the purposes of Patent Procedures, and the Regulations annexed to that Treaty as affected by any amendments made under Article 12 of that Treaty, as are applicable; and

(b) such provisions of the Patents Regulations as are applicable.

(continued overleaf)

*5. ~~*I am~~/*the applicant for the patent for the invention referred to above is/the depositor, within the meaning of Article 2 of the Budapest Treaty, of the deposit referred to in paragraph 2.

*5. The depositor, within the meaning of Article 2 of the Budapest Treaty, of the deposit referred to in paragraph 2 is

*6. *I rely/*I have the consent of the depositor referred to above to rely/on that deposit for the purposes of section 40 of the Patents Act 1952 in relation to the application for the patent for the invention referred to above

*6 *The applicant for the patent for the invention referred to above relies/~~*The applicant for the patent for the invention referred to above has the consent of the depositor referred to above to rely/on~~ that deposit for the purposes of section 40 of the Patents Act 1952 in relation to the application for the patent for the invention referred to above.

Declared at Melbourne
this 5th day of July, 19 88.

.....
(Signature of declarant)

(*Omit whichever is inapplicable)

To: The Commissioner of Patents

Lodged by: DAVIES & COLLISON, 1 Little Collins Street, Melbourne, 3000, Victoria; for and on behalf of the Applicant.

2. The micro-organisms were deposited with Deutsche Sammlung von Mikroorganismen, Grisebachstrasse 8, Gottingen, West Germany on October 1, 1981; October 1, 1981; December 13, 1984; April 29, 1987 and April 29, 1987 and accorded the file, accession or registration numbers DSM 2102; DSM2106; DSM 3143; DSM 4105 and DSM 4106, respectively.

(12) PATENT ABRIDGMENT (11) Document No. AU-B-15186/88
(19) AUSTRALIAN PATENT OFFICE (10) Acceptance No. 593438

(54) Title
STABLE CREATINE AMIDINOHYDROLASE MUTANTS

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DAVIES & COLLISON, MELBOURNE

(57) The present invention is concerned with mutants of creatine amidinohydrolase which are more stable than the wild type enzyme and are, therefore, more suitable for the enzymatic determination of the creatinine content of serum, plasma and urine.

CLAIM

1. Creatine amidinohydrolase synthesized in Escherichia coli or Pseudomonas putida, which catalyses the reaction:

creatine + H20 → sacarosine + urea

wherein, in position 109 of an amino acid sequence of the wild type enzyme, alanine is replaced by valine.

2. Creatine amidinohydrolase according to claim 1, wherein at least one further amino acid of the wild type enzyme is additionally mutated.

8. Process for the production of the enzyme according to claim 1, wherein, according to known gene-technological methods, into an appropriate expression system there is introduced a recombinant DNA for expression which contains

a creatine amidinohydrolase gene which, in position 326 of the natural gene, contains the desoxyribonucleotide T instead of C.

COMMONWEALTH OF AUSTRALIA

PATENT ACT 1952

COMPLETE SPECIFICATION

(ORIGINAL)

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NAME OF APPLICANT: BOEHRINGER MANNHEIM GmbH

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Peter BUCKEL

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1 Little Collins Street, Melbourne, 3000.

COMPLETE SPECIFICATION FOR THE INVENTION ENTITLED:

"STABLE CREATINE AMIDINOHYDROLASE MUTANTS"

The following statement is a full description of this invention, including the best method of performing it known to us :-

The present invention is concerned with mutants of creatine amidinohydrolase which are more stable than the wild type enzyme and are, therefore, more suitable for the enzymatic determination of the creatinine content of serum, plasma and urine.

The enzyme creatine amidinohydrolase (EC 3.5.3.3) is used industrially for the determination of creatinine. It is used, inter alia, in clinical analysis for the diagnosis of kidney diseases in which creatinine contents occur in the serum and urine which differ from those of the healthy organism. Micro-organisms are known, for example types of Pseudomonas, which, with induction by creatine, are able to produce creatine amidinohydrolase in an amount making working up worthwhile but the achievable yields and the costs of isolation of the enzyme are still limiting factors for the industrial use of the enzyme.

As is described in Federal Republic of Germany Patent Specification No. 35 00 184, it has been possible to isolate from Pseudomonas putida the gene coding creatine amidohydrolase and to introduce it, for example, into the plasmid pßR 322. After transformation of a micro-organism of the genus Escherichia coli or Pseudomonas putida, it is possible to obtain creatine amidinohydrolase constitutively from these. This gene-technological production of creatine amidinohydrolase is more effective and easier to carry out than the

isolation from an induced micro-organism which does not contain a plasmid. However, this process has the disadvantage that the wild type enzyme obtained therefrom has only a limited detergent and thermal stability and thus is not optimally suitable for use in clinical test processes.

It is an object of the present invention to provide creatine amidinohydrolase mutants which do not display the described disadvantages of the prior art or only to a slight extent.

According to the present invention, this object is achieved by creatine amidinohydrolase mutants which catalyse the reaction:

$$\text{creatine} + H_2O \longrightarrow \text{sarcosine} + \text{urea}$$

corresponding to the wild type enzyme, wherein, in comparison with the wild type enzyme, at least one amino acid exchange has taken place in position 109. This exchange concerns the amino acid alanine which has been replaced by valine. The so mutated enzyme has a considerably better stability than the wild type enzyme.

Apart from this amino acid exchange, without impairment of the enzymatic activity, other mutations on the amino acid plane can also be present and a still higher stability can then be achieved. Apart from the amino acid exchange in position 109, a preferred enzyme contains a further amino acid exchange.

The present invention also provides the plasmids

pBT 109 and pBT 119. These contain the creatine amidinohydrolase genes of the wild type but, in both plasmids, on the DNA plane there is not only the amino acid exchange at position 109 and in the case of pBT 119 also in a further position, namely, position 355 in the form of an exchange of Val by Meth by corresponding exchange of the triplet coding these amino acids in the wild type gene. By sequencing of pBT 119, we have ascertained that at position 1063 of the sequence in the coding strand, a G is replaced by A (G $\longrightarrow$ A) so that the triplet GTG of the wild type is changed into ATG. A preferred microorganism of the genus Escherichia coli according to the present invention, Escherichia coli, DSM 4105, contains the plasmid pBT 109 and a further preferred microorganism Escherichia coli, DSM 4106, contains, according to the present invention, the plasmid pBT 119. According to the present invention, further preferred microorganisms are those of the genus Escherichia coli or Pseudomonas putida which constitutively express a creatine amidinohydrolase which contains the said mutations.

The preparation of the mutated enzymes according to the present invention takes place in that, according to known gene-technological methods, a recombinant DNA is brought to expression in an appropriate expression system which contains a creatine amidinohydrolase gene which essentially has the sequence of the wild type gene

but contains, at least in position 326 of the natural gene, a T instead of the desoxyribonucleotide C. A gene is preferably expressed in which, furthermore, a base is exchanged in a further position. Especially preferably, in position 1063, A is present instead of G.

As recombinant DNA, there is preferably expressed one of the plasmids pBT 109, DSM 4108P or pBT 119, DSM 4107P. However, as recombinant DNA, there can also be used any recombinant DNA which contains the DNA sequence for the wild type enzyme described in Federal Republic of Germany Patent Specification No. 35 00 184 or an equivalent thereof coding according to the genetic code for the same amino acid sequence in which, however, at position 326 of the natural gene, the desoxyribonucleotide T is present instead of C. In such a recombinant DNA, in addition there can be contained a further base exchange which leads to a further amino acid mutation in the enzyme. In position 1063, G is thereby preferably exchanged for A.

As expression system, there is preferred a micro-organism of the genus Escherichia coli or Psuedomonas putida. Especially preferably, there is used the micro-organism Escherichia coli ED 8654, DSM 2102, or Pseudomonas putida, DSM 2106. Further processes of production according to the present invention involve culturing the preferred micro-organisms Escherichia coli, DSM 4105 and Escherichia coli, DSM 4106.

The present invention is also concerned with the use of the mutated creatine amidinohydrolases according to the present invention, which are more stable than the wild type enzyme, for the determination of the creatinine content in serum, plasma and urine.

Investigations of the cloned creatine amidinohydrolase described in Federal Republic of Germany Patent Specification No. 35 00 184 have shown that the enzyme catalyses the rate-determining step in the reaction sequence:

$$\text{creatine} + H_2O \xrightarrow{\text{creatine amidinohydrolase}} \text{sarcosine} + \text{urea}$$

An increase of the rate of the substrate reaction cannot be achieved under the optimum working conditions by increasing the amount of enzyme. Under test conditions for the determination of the creatinine content of serum, plasma or urine at 37°C., the enzyme displays a limited stability, which leads to a reduction of the substrate reaction at the above-mentioned temperature. The creatine amidinohydrolase according to the present invention, which contains a mutation of the amino acid 109 of the wild type enzyme from alanine into valine, has, in comparison, under various test conditions (addition of detergent), in the case of approximately the same initial enzyme activity, already a strongly

increased detergent stability, as is shown by the comparative experimental results set out in the following Table I. The temperature behaviour under optimum conditions is shown in the following Table II.

TABLE I

Enzyme determination at 37°C.

	time (minutes)	creatinine amidinohydrolase wild type (DE 35 00 184 A1) (DSM 3143) comparison	creatine amidinohydrolase mutant (amino acid 109 = Val) (DSM 4105) according to the invention	test conditions
I	0	3.3 U/ml. (activity = 100%)	3.0 U/ml. (activity = 100%)	Testmix 1 from test
	15	1.9 U/ml. (residual activity = 59%)	3.0 U/ml. (residual activity = 100%)	combination
	30	0.6 U/ml. (residual activity = 18%)	2.7 U/ml. (residual activity = 91%)	"Creatinin-PAP"
	60	0.2 U/ml. (residual activity = 6%)	2.5 U/ml. (residual activity = 83%)	(BM No.839 434)
II	0	3.3 U/ml. (activity = 100%)	3.0 U/ml. (activity = 100%)	in 125 mmole/litre
	15	0.7 U/ml. (residual activity = 21%)	2.7 U/ml. (residual activity = 91%)	phosphate buffer
	30	0.0 U/ml. (residual activity = 0%)	2.7 U/ml. (residual activity = 91%)	+ detergent
	60	0.0 U/ml. (residual activity = 0%)	2.1 U/ml. (residual activity = 71%)	

The enzymes were incubated under the conditions given in the Table and subsequently an enzyme determination was carried out with the use of the test combination "urea" (BM Order No. 124770).

Table II

Enzyme determination under optimum conditions at different temperatures

Test conditions: 20 mMole/litre phosphate buffer (pH 8.0).

1 = creatine amidinohydrolase wild type: 1.05 mg. protein/ml. (8.0 U/mg.)

2 = creatine amidinohydrolase mutant: 1.10 mg. protein/ml. (8.7 U/mg.) (amino acid 109 = Val)

temp.	creatinase residual activity in % (incubation in minutes					
	1. (DSM 3147)			2. (DSM 4105)		
	30 m.	60 m.	120 m.	30 m.	60 m.	120 m.
37°C.	100	86	80	100	96	90
42°C.	57	36	13	84	73	63
47°C.	1	0.4	0	42	2	0
52°C.	0	-	-	3	-	-

The enzymes were incubated under the given test conditions and subsequently an enzyme determination was carried out with the use of the test combination "urea" (BM Order No. 124 770).

The creatine amidinohydrolase enzyme according to the present invention which, in addition to this mutation, carries a further amino acid mutation, also possesses, in the case of almost the same initial enzyme activity, a

still greater stability in comparison with the wild type enzyme (see Example 1, Table III).

With the creatine amidinohydrolase mutants according to the present invention, it is, therefore, possible to avoid an enzyme activity loss in the case of the creatinine determination due to the detergent and thermal lability of the wild type enzyme and also to carry out such determinations dependably over longer periods of time than was hitherto possible. Because of the improved properties, a reduction of the amount of enzyme in the creatinine test is also possible.

The following Examples are given for the purpose of illustrating the present invention:

Example 1.

Cells of Escherichia coli DSM 4105, Escherichia coli DSM 4106 and, for comparison, Escherichia coli DSM 3143 (Federal Republic of Germany Patent Specification No. 35 00 184), were cultured overnight in 11 fermenters.

Medium

complete medium (yeast/peptone extract)

0.4% glucose

100 mMole/litre phosphate buffer.

After centrifuging for 15 minutes at 800 r.p.m., the cells were broken down in a French press and the creatine amidinohydrolase purified by fractionation over a molecular sieve (Sephacryl S 200).

The enzymes obtained were incubated under the conditions given in the following Table III and subsequently an enzyme determination was carried out with the use of the test combination "urea" (BM Order No. 124 770).

The following Table III gives the stability behaviour of the creatine amidinohydrolase mutants according to the present invention in comparison with the wild type enzyme (Federal Republic of Germany Patent Specification No. 35 00 184 - Escherichia coli DSM 3143).

Table III

Stability at 37°C.

Test conditions: Testmix 1 from the test combination "creatinine-PAP" (BM Order No. 839 434)

Initial activity: 12 U/ml. (= 100%)

creatine amidinohydrolase from Escherichia coli (coding plasmid)	residual activity as % of the initial activity after			
	15 m.	30 m.	45 m.	60 m.
DSM 3143 (pBT 2a-1, DSM 3148P)	39	51	39	27
DSM 4105 (pBT 109, DSM 4108P)	96	90	84	84
DSM 4106 (pBT 119, DSM 4107P)	102	102	102	102

Comparison of the Michaelis constants (Km) at 37°C
test conditions: 0.1 mole/litre Tris-HCl (pH 8.0)
(optimum test conditions)

creatine amidinohydrolase from Escherichia coli (coding plasmid)	Km, mMole/Litre
DSM 3143 (pBT 2a-1, DSM 3148P)	~~24~~ 25
DSM 4105 (pBT 109, DSM 4108P)	20
DSM 4106 (pBT 119, DSM 4107P)	16

Example 2.

The stable creatine amidinohydrolase mutant from the micro-organism Escherichia coli, DSM 4105, which contains the plasmid pBT 109, was isolated from a 100 litre fermentation in the manner described in Federal Republic of Germany Patent Specification No. 35 00 184. 121 g. of protein were obtained with a specific activity of 10.4 U/mg. This corresponds to a yield of 63%.

Example 3.

Plasmid pBT 119 carries a mutation at positions 109 and 355 of the gene encoding creatine amidinohydrolase and the enzyme thus encoded has improved stability as shown in the Table below:

	residual activity after 0'	15'	30' in %
wild type enzyme	100	41	40
pBT 109 enzyme	100	80	80
pBT 119 enzyme	100	102	100
mutant enzyme 119 without mutation of plasmid 109	100	38	25

The results of the above Table show that the advantage achieved through the mutation at position 109 is maintained even if additional mutations take place at other positions.

Deposits:

Depository	Date	Accession No.
Deutsche Sammlung von Mikroorganismen*	October 1, 1981	DSM 2102
"	October 1, 1981	DSM 2106
"	December 13, 1984	DSM 3143
"	April 29, 1987	DSM 4105
"	April 29, 1987	DSM 4106

* Address - Grisebachstrasse 8,
D-3400 Gottingen

THE CLAIMS DEFINING THE INVENTION ARE AS FOLLOWS:

1. Creatine amidinohydrolase synthesized in Escherichia coli or Pseudomonas putida, which catalyses the reaction:

creatine + H20 → ~~sacarosine~~ sarcosine + urea

wherein, in position 109 of an amino acid sequence of the wild type enzyme, alanine is replaced by valine.

2. Creatine amidinohydrolase according to claim 1, and being a functional equivalent thereof, wherein at least one further amino acid of the wild type enzyme is additionally mutated.

3. Plasmid pBT 109, DSM 4108P, as hereinbefore described wherein it contains a gene encoding creatine amidinohydrolase according to claim 1.

4. Plasmid pBT 119, DSM 4107P, as hereinbefore described wherein it contains a gene encoding a creatine amidinohydrolase according to claim 2.

5. Micro-organism Escherichia coli, DSM 4105, as hereinbefore described wherein it contains the plasmid pBT 109.

6. Micro-organism Escherichia coli, DSM 4106, as hereinbefore described wherein it contains the plasmid pBT 119.

7. Micro-organism of the genus Escherichia coli or Pseudomonas putida, wherein it constitutively forms a creatine amidinohydrolase according to claim 1 or 2.

8. Process for the production of the enzyme according to claim 1, wherein, according to known gene-technological methods, into an appropriate expression system there is introduced a recombinant DNA for expression which contains a creatine amidinohydrolase gene which, in position 326 of the natural gene, contains the

desoxyribonucleotide T instead of C.

9. Process according to claim 8, wherein as recombinant DNA there is expressed the plasmid pBT 109, as hereinbefore described DSM 4108P.

10. Process according to claim 8, wherein a recombinant DNA is used which contains the sequence illustrated in Fig. 1 of the accompanying drawings or an equivalent thereof coding according to the genetic code for the same amino acid sequence.

11. Process for the production of the enzyme according to claim 2, wherein, according to known gene technological methods, in an appropriate expression system, a recombinant DNA is brought to expression which contains a creatine amidinohydrolase gene which, in addition to the mutation of the base C into T on position 326 of the natural gene, contains a further mutation which brings about a further amino acid exchange in the enzyme.

12. Process according to claim 11, wherein a recombinant creatine amidinohydrolase gene is expressed which contains a mutation of the base G into A in position 1063.

13. Process according to claim 12, wherein the plasmid pBT 119, DSM 4107P, as hereinbefore described is expressed as recombinant DNA.

14. Process according to any of claims 8 to 13, wherein, as expression system, there is used a microorganism of the genus Escherichia coli or Pseudomonas putida.

15. Process according to claim 14, wherein there is used Escherichia coli ED 8654, DSM 2102 as hereinbefore described.

16. Process according to claim 14, wherein there is used Pseudomonas putida.

17. Process according to claim 8 or 9, wherein Escherichia coli, DSM 4105, as hereinbefore described is cultured.

18. Process according to any of claims 11 to 13, wherein Escherichia coli, DSM 4106, as hereinbefore described is cultured.

19. Process according to any of claims 8 to 18, substantially as hereinbefore described and exemplified.

20. Enzymes, whenever produced by the process according to any of claims 8 to 19.

21. Use of an enzyme according to claim 1 or 2 for the determination of the ~~creatine~~ creatinine content in serum, plasma or urine.

DATED this 14th day of June, 1989
DAVIES & COLLISON
Patent Attorneys for
BOEHRINGER MANNHEIM GmbH

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MetGlnMetProLysThrLeuArgIleArgAsnGlyAspLysValArgSerThrPheSer

        70                  90                  110
GCCCAGGAATACGCCAATCGCCAAGCCAGGCTGCGCGCCCACCTGGCGGCGGAGAACATC
AlaGlnGluTyrAlaAsnArgGlnAlaArgLeuArgAlaHisLeuAlaAlaGluAsnIle

       130                 150                 170
GACGCCGCGATCTTCACCTCGTACCACAACATCAACTACTACTCCGACTTCCTCTACTGC
AspAlaAlaIlePheThrSerTyrHisAsnIleAsnTyrTyrSerAspPheLeuTyrCys

       190                 210                 230
TCCTTCGGCCGCCCCTACGCGTTGGTGGTGACCCAGGACGACGTCATCAGCATCAGCGCC
SerPheGlyArgProTyrAlaLeuValValThrGlnAspAspValIleSerIleSerAla

       250                 270                 290
AACATCGACGGCGGCCAGCCGTGGCGCCGCACCGTCGGCACCGACAACATCGTCTACACC
AsnIleAspGlyGlyGlnProTrpArgArgThrValGlyThrAspAsnIleValTyrThr

       310                 330                 350
GACTGGCAGCGCGATAACTACTTCGTCGCCATCCAGCAGGCGTTGCCGAAGGCCCGCCGC
AspTrpGlnArgAspAsnTyrPheValAlaIleGlnGlnAlaLeuProLysAlaArgArg

       370                 390                 410
ATCGGCATCGAACATGACCACCTGAACCTGCAGAACCGCGACAAGCTGGCCGCGCGCTAT
IleGlyIleGluHisAspHisLeuAsnLeuGlnAsnArgAspLysLeuAlaAlaArgTyr

       430                 450                 470
CCGGACGCCGAGCTGGTGGACGTGGCCGCCGCCTGCATGCGTATGCGCATGATCAAATCC
ProAspAlaGluLeuValAspValAlaAlaAlaCysMetArgMetArgMetIleLysSer

       490                 510                 530
GCCGAAGAGCACGTGATGATCCGCCACGGCGCGCGCATCGCCGACATCGGTGGTGCGGCG
AlaGluGluHisValMetIleArgHisGlyAlaArgIleAlaAspIleGlyGlyAlaAla

       550                 570                 590
GTGGTCGAAGCCCTGGGCGACCAGGTACCGGAATACGAAGTGGCGCTGCATGCCACCCAG
ValValGluAlaLeuGlyAspGlnValProGluTyrGluValAlaLeuHisAlaThrGln

       610                 630                 650
GCCATGGTCCGCGCCATTGCCGATACCTTCGAGGACGTGGAGCTGATGGATACCTGGACC
AlaMetValArgAlaIleAlaAspThrPheGluAspValGluLeuMetAspThrTrpThr

       670                 690                 710
TGGTTCCAGTCCGGCATCAACACCGACGGCGCGCACAACCCGGTGACCACCCGCAAGGTG
TrpPheGlnSerGlyIleAsnThrAspGlyAlaHisAsnProValThrThrArgLysVal

       730                 750                 770
AACAAGGGCGACATCCTCAGCCTCAACTGCTTCCCGATGATCGCCGGCTACTACACCGCG
AsnLysGlyAspIleLeuSerLeuAsnCysPheProMetIleAlaGlyTyrTyrThrAla

       790                 810                 830
TTGGAGCGCACCCTGTTCCTCGACCACTGCTCGGACGACCACCTGCGTCTGTGGCAGGTC
LeuGluArgThrLeuPheLeuAspHisCysSerAspAspHisLeuArgLeuTrpGlnVal
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FIG. 1 CONT'D.

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       850                 870                 890
AACGTCGAGGTGCATGAAGCCGGCCTGAAGCTGATCAAGCCCGGTGCGCGTTGCAGCGAT
AsnValGluValHisGluAlaGlyLeuLysLeuIleLysProGlyAlaArgCysSerAsp

       910                 930                 950
ATCGCCCGCGAGCTGAACGAGATCTTCCTCAAGCACGACGTGCTGCAGTACCGCACCTTC
IleAlaArgGluLeuAsnGluIlePheLeuLysHisAspValLeuGlnTyrArgThrPhe

       970                 990                1010
GGCTACGGCCACTCCTTCGGCACGCTCAGCCACTACTACGGCCGCGAGGCCGGCTTGGAA
GlyTyrGlyHisSerPheGlyThrLeuSerHisTyrTyrGlyArgGluAlaGlyLeuGlu

      1030                1050                1070
CTGCGCGAGGACATCGACACCGTGCTGGAGCCGGGCATGGTGGTGTCGATGGAGCCGATG
LeuArgGluAspIleAspThrValLeuGluProGlyMetValValSerMetGluProMet

      1090                1110                1130
ATCATGCTGCCGGAAGGCCTGCCGGGCGCCGGTGGCTATCGCGAGCACGACATCCTGATC
IleMetLeuProGluGlyLeuProGlyAlaGlyGlyTyrArgGluHisAspIleLeuIle

      1150                1170                1190
GTCAACGAGAACGGTGCCGAGAACATCACCAAGTTCCCCTACGGCCCGGAGAAAAACATC
ValAsnGluAsnGlyAlaGluAsnIleThrLysPheProTyrGlyProGluLysAsnIle

      1210
ATCCGCAAATGA
IleArgLysEnd
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