BESCHWERDEKAMMERN DES EUROPÄISCHEN PATENTAMTS

BOARDS OF APPEAL OF THE EUROPEAN PATENT OFFICE

CHAMBRES DE RECOURS DE L'OFFICE EUROPEEN DES BREVETS

Internal distribution code:
(A) [ ] Publication in OJ
(B) [ ] To Chairmen and Members
(C) [X] To Chairmen
(D) [ ] No distribution

Datasheet for the decision of 10 January 2012

Case Number: T 0817/09 - 3.3.08

Application Number: 95938648.3

Publication Number: 796912

IPC: C12N 15/60, C12N 9/88, C12P 13/08

Language of the proceedings: EN

Title of invention:
Novel lysine decarboxylase gene and process for producing L-lysine

Patentee:
Ajinomoto Co., Inc.

Opponents:
Changchun Dacheng Bio-chem Engineering Development Co.
Helm AG

Headword:
Lysine decarboxylase/AJINOMOTO

Relevant legal provisions:
EPC Art. 54, 56, 83, 84, 114(2), 123(2)
RPBA Art. 13(1)

Keyword:
"Admissibility of a scientific report filed one month before the oral proceedings (no)"
"Main request - added subject-matter (yes)"
"First auxiliary request - requirements of the EPC met (yes)"

Decision cited:
T 0019/90

Catchword:
-

Case Number: T 0817/09 - 3.3.08

D E C I S I O N
of the Technical Board of Appeal 3.3.08
of 10 January 2012

Appellant I: (Patent Proprietor)
Ajinomoto Co., Inc.
No. 15-1, Kyobashi 1-chome
Chuo-ku
Tokyo 104 (JP)

Representative:
Bijvank, Koen Mattijs Lodewiek
Vereenigde
Johan de Wittlaan 7
NL-2517 JR Den Haag (NL)

Appellant II: (Opponent 01)
Changchun Dacheng Bio-chem Engineering
Development Co.
886 Xihuangcheng Road
Processing Corn Dist
Changchun Economic and Techn. Development Zone
Changchun City, Jilin Province (CN)

Representative:
Wittop Koning, Tom Hugo
Exter Polak & Charlouis B.V.
P.O. Box 3241
NL-2288 GE Rijswijk (NL)

Party as of right: (Opponent 02)
Helm AG
Nordkanalstrasse 28
D-20097 Hamburg (DE)

Representative:
Muth, Heinz-Peter
Uexküll & Stolberg
Patentanwälte
Beselerstrasse 4
D-22607 Hamburg (DE)

Decision under appeal:
Interlocutory decision of the Opposition Division of the European Patent Office posted 30 January 2009 concerning maintenance of European patent No. 796912 in amended form.

Composition of the Board:

Chairman: M. Wieser
Members: T. J. H. Mennessier
J. Geschwind

Summary of Facts and Submissions

I. The patent proprietor (appellant I) and opponent 01 (appellant II) each lodged an appeal against the interlocutory decision of the opposition division dated 30 January 2009, whereby European patent No. 0 796 912, which had been granted on European application No. 95 938 648.3 (published - in Japanese language - under the international publication No. WO 96/17930), was maintained in an amended form on the basis of the first auxiliary request (claims 1 to 9) filed at the oral proceedings held on 18 September 2008.

II. The main request (claims 1 to 9 as granted) had been refused for reasons of non-compliance with the requirements of Article 123(2) EPC.

III. The patent had been opposed by opponent 01 and opponent 02, who is party to the appeal proceedings as of right, on the grounds as set forth in Article 100(a) EPC that the invention was neither new nor inventive, Article 100(b) EPC and Article 100(c) EPC.

IV. Claim 1 as granted read as follows:

"1. A gene which codes for lysine decarboxylase having an amino acid sequence defined in the following (A) or (B):
(A) an amino acid sequence shown in SEQ ID NO:4,
(B) an amino acid sequence having substitution, deletion or insertion of 3 amino acid residues or less in the amino acid sequence shown in SEQ ID NO:4 and having lysine decarboxylase activity."

V. The first auxiliary request of 18 September 2008 consisted of 9 claims of which claims 1, 4, 5, 8 and 9 read as follows:

"1. A gene which codes for lysine decarboxylase having an amino acid sequence defined in the following (A) or (B):
(A) an amino acid sequence shown in SEQ ID NO:4,
(B) an amino acid sequence having substitution, deletion or insertion of 3 amino acid residues or less in the amino acid sequence shown in SEQ ID NO:4 **without any substantial deterioration of** lysine decarboxylase activity."

(emphasis added by the Board)

"4. A method for decreasing or disappearing the activity of the lysine decarboxylase encoded by the gene according to claim 1 or 2, wherein the gene according to claim 1 or 2 is modified by substitution, deletion, insertion, addition or inversion of one or a plurality of nucleotides in a nucleotide sequence in the gene."

"5. A microorganism belonging to the genus Escherichia, wherein the gene according to claim 1 or 2, a promoter sequence in the gene or a region between an SD sequence and an initiation codon of the gene is modified by substitution, deletion, insertion, addition or inversion of one or a plurality of nucleotides in the nucleotide sequence of the gene, the promoter sequence or the region between an SD sequence and an initiation codon, whereby the activity of a lysine decarboxylase

encoded by the gene is decreased or disappeared in cells."

"8. The microorganism according to any of claims 5, 6 and 7, which belongs to the genus Escherichia and has L-Lysine productivity."

"9. A method of producing L-lysine comprising the step of cultivating a microorganism according to claim 8 in a liquid medium."

Claim 2 was dependent on claim 1. Claim 3 was directed to a DNA fragment containing a gene according to claim 1 or 2. Claims 6 to 7 were dependent on claim 5.

VI. Both appellants filed their respective statements of appeal.

VII. On 2 November 2009, each appellant replied to the other appellant's statement of grounds. In addition to the claims as granted and the first auxiliary request, appellant I filed a second auxiliary request (claims 1 to 8). Two additional documents were also attached to its letter.

VIII. On 27 September 2011, the Board sent a communication pursuant to Article 15(1) of the Rules of Procedure of the Boards of Appeal (RPBA) in which provisional and non-binding opinions were expressed.

IX. On 9 December 2011, appellant II filed further submissions in reply to the Board's communication. Three additional documents, referred to in the decision as documents D37A, D37B and D56 (see Section X, *infra*)

as a well as a clean copy of document D16 were attached to the letter.

X. The following documents are referred to in the present decision:

(D3) S-Y. Meng and G. N. Bennett, Journal of Bacteriology, Vol. 174, No. 8, April 1992, pages 2659 to 2669

(D11) J. M. Wright and S. M. Boyle, Journal of Bacteriology, Vol. 159, No. 3, September 1984, pages 1074 to 1076

(D12) Y. Kikuchi, et al., Journal of Bacteriology, Vol. 179, No. 14, July 1997, pages 4486 to 4492

(D16) L.F. Fecker et al., Molecular and General Genetics, Vol. 203, 1986, Pages 177 to 184

(D20) J. Alvarez-Jacobs et al., Biotechnology Letters, Vol. 12, No. 6, 1990, pages 425 to 430

(D37) Bergey's Manual of Determinative Bacteriology, Ninth Edition, Williams & Wilkins publishers, Baltimore, 1 September 1993, pages 179, 180, 233 and 234

(D37A) Bergey's Manual of Determinative Bacteriology, Ninth Edition, Williams & Wilkins publishers, Baltimore, 1 September 1993, double-sided cover page and page 222

(D37B) E-mail from the publishing company Wolters Kluwer dated 2 December 2011

(D39) "Molecular cloning: a laboratory manual", T. Maniatis et al, 1982, Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y. pages 382 to 389

(D56) Scientific report dated 2 December 2011 filed on behalf of appellant II

XI. The submissions made by appellant I, insofar as they are relevant to the present decision, can be summarised as follows:

Admissibility of the clean copy of document D16 and of documents D37A, D37B and D56 all submitted on 9 December 2011

The clean copy of document D16, and documents D37A, D37B and D56 had been submitted long after the date of the decision under appeal. These pieces of evidence could have been submitted earlier and should not be admitted in the appeal proceedings.

Main request (claims as granted)

The skilled person would have understood that the term "*equivalent*", found on page 7, line 17 of the application as filed, was used to distinguish the proteins of the invention from the known lysine decarboxylase encoded by the *cadA* gene. Since this decarboxylase differed from the sequence SEQ ID NO:4 by at least 200 amino acid residues, the use of the term

"*equivalent*" in embodiment B of claim 1 would have been superfluous as this embodiment was limited to modifications of 3 amino acid residues or less. Therefore, claim 1 did not contain added matter.

First auxiliary request

Article 123(2) EPC

Claims 1 and 3 as filed provided the language of claim 1 of the first auxiliary request with the only exception that the term "*plurality*" has been limited to "*3 amino acid residues or less*". This limitation had a basis on page 7, line 20, which, when read together with page 6 as filed, described the genes for which protection was sought in claim 1(B). Those genes encoded an enzyme which, as the known CadA enzyme, was a lysine decarboxylase. This explained the use of the phrase "*the genes which code for proteins having equivalent lysine decarboxylase activity*" on page 7, lines 16 to 17, wherein the term "equivalent" was employed to distinguish the lysine decarboxylase encoded by a gene according to claim 1 from the CadA lysine decarboxylase.

Article 84 EPC

The use of relative terms such as "*substantial*" did not always lead to ambiguity. In the light of the disclosure on pages 6 and 7 of the application as filed (see paragraphs [0018] and [0019] of the patent specification), the meaning of the term "*substantial*" was clear: the activity should not have been lost.

Article 83 EPC

Appellant II did not explain why the skilled person would not be able to repeat the experiment of Example 2 and arrive at the data presented in Figure 3. A method to measure cadaverine, a decomposition product of L-lysine, was described in detail in the prior art document D3. No experimental evidence was provided that different results would have been obtained using this method.

Article 54 EPC

The gene encoding the second lysine decarboxylase (Ldc) could be present but was either only weakly expressed under the given growth conditions or was not expressed at all in the *Escherichia coli* HB101 strain described in document D16 (see page 180, bottom of the right-hand column). The same applied to the bacteria shown in document D37 to be negative in reactions for lysine decarboxylase.

Article 56 EPC

The authors of document D3 reported on page 2666 (see the first paragraph of the left-hand column), that they had carried out molecular research to identify a gene encoding a second lysine decarboxylase in addition to the gene encoding the CadA lysine decarboxylase. They admitted that they had not been successful, as expressed in the passage reading "*The locus of a second lysine decarboxylase gene has not been mapped, and the purified protein has not been reported. Our preliminary Southern hybridisation experiments using cadA to probe*

E. coli chromosomal DNA failed to identify a second region homologous to cadA under the conditions used.".

Document D3 did not at all specify the conditions under which the Southern experiments were carried out. Furthermore, at the priority date, it was not established that a "second" lysine decarboxylase gene existed in addition to the *cadA* gene decarboxylase. Therefore, it was no surprise for the skilled person that the attempt of the authors of document D3 to identify the hypothetical "second" gene failed.

Since the gene encoding the second lysine decarboxylase was not known at the priority date, it was not possible to determine its homology with the *cadA* gene. The skilled person would have derived from the statement reporting the failure of the authors of document D3 to identify the second gene, which they had assumed to have homology with the *cadA* gene, that *a contrario* this second gene was not sufficiently homologous to the *cadA* gene to permit its identification with Southern experiments.

Therefore, the skilled person would not have found any incentive in other prior art documents to repeat the Southern experiments of document D3 using different conditions but rather would have considered that there was no expectation of success. Claim 1, therefore, involved an inventive step.

Document D12 did not belong to the state of the art. Any reasoning derived therefrom was based on hindsight.

The argument presented by appellant II at the oral proceedings against claim 9 had not been raised before. At the time document D20 was published, the gene of claim 1 was unknown. The skilled person would have had no incentive to block its expression. It was the identification of the gene encoding the second lysine decarboxylase by the inventors, which allowed to prepare microorganisms capable of overproducing L-lysine by blocking its expression. Therefore, also claim 9 involved an inventive step.

XII. The submissions made by appellant II, insofar as they are relevant to the present decision, can be summarised as follows:

Admissibility of the clean copy of document D16 and of documents D37A, D37B and D56 all submitted on 9 December 2011

Document D16 had been submitted with the appellant I's notice of opposition. As it appeared in the meantime that the quality of the copy distributed was not satisfactory, a clean copy was submitted on 9 December 2011 in preparation for the oral proceedings.

Document D37A was a selected set of additional pages from the Bergey's manual, of which pages 179, 180, 233 and 234 had been submitted earlier as document D37. The additional pages were the double-sided cover page of the manual, to provide a full identification of the manual, and page 222 which explained the meaning of symbols used throughout the manual, more particularly in the tables of pages 233 and 234.

Document D37B was an e-mail sent by the present publisher of the Bergey's manual which confirmed that the said manual was available to the public on 1 September 1993.

Document D56 was filed with the letter of 9 December 2009 to complete appellant II's argument regarding lack of novelty of claim 5 of the first auxiliary request and was not accepted by the opposition division.

The clean copy of document D16 as well as documents D37A, D37B and D56 should be admitted in the appeal proceedings.

Main request (claims as granted)

The term "*plurality*", as used in claim 3 as filed, could not be changed into "*3 amino acid residues or less*" as used in claim 1 as granted. The phrase "*3 amino acid residues or less*" was not found in the application as filed.

A difference in 2 or 3 amino acids was only referred to in the particular context of a lysine decarboxylase having an enzymatic activity equivalent to that of the known CadA lysine decarboxylase (see page 7, lines 16 to 20 as filed). This essential feature, based on the unclear notion of equivalency, was not contained in claim 1.

First auxiliary request

Article 123(2) EPC

Claim 1 differed from claim 1 of the main request in that the modification of 3 amino acid residues or less was associated with the condition that it did not result in any substantial deterioration of the lysine decarboxylase activity.

This amendment resulted from an improper combination of two passages of the application as filed. The first passage read: "*The lysine decarboxylase encoded by the gene of the present invention may have substitution, deletion, or insertion of one or a plurality of amino acid residues without substantial deterioration of the lysine decarboxylase activity, in the amino acid sequence described above*." (see page 6, lines 10 to 15; the sequence referred to was SEQ ID NO:4). The second passage read: "... *the genes which code for the proteins having equivalent lysine decarboxylase activity are included in the present invention even if they are different from one having the amino acid sequence shown in SEQ ID NO:3 with respect to two or three amino acid residues*" (see page 7, lines 16 to 20).

The limitation in the second passage to two or three amino acid residues was made in the context of a protein having equivalent lysine decarboxylase compared to the CadA lysine decarboxylase, whereas in the first passage the reference to two or three amino acid residues (included in the phrase "plurality of amino acid residues") was made in the context of a protein without substantial deterioration of the lysine

decarboxylase activity. Indeed, the concept of a modification of the amino acid sequence resulting in an equivalent lysine decarboxylase activity was different from the concept of a modification of the amino acid sequence resulting in no substantial deterioration of the lysine decarboxylase activity.

Article 84 EPC

The wording "*without any substantial deterioration of the lysine decarboxylase activity*" as used in claim 1 was not clear due to the relative term "*substantial*". There was no indication in the patent at issue concerning the exact meaning of the phrase "*substantial deterioration*". According to claim 1 up to three changes in the amino acid sequence were possible, as long as the encoded protein had a certain degree of activity, whereas, according to claim 4, even a single nucleotide change could effect a decrease or the disappearance of the enzyme activity. It was not clear whether in this context, a decrease as referred to in claim 4 could also be interpreted as a small but not substantial deterioration of the enzymatic activity.

Article 83 EPC

In Example 2, the amount of L-lysine was said to be determined by using a given analyser without any further details (see paragraph [0048] on page 9 of the patent specification). Therefore, the data of Figure 3 could not be reproduced. Furthermore, the method to determine lysine decarboxylase activity described in document D3 clearly and significantly differed from what was described in Example 2. No routine technique

to measure said activity was described in the patent specification. Furthermore, claims directed to a microorganism of the genus *Escherichia* were too broad as only strains of *Escherichia coli* were tested in the examples of the patent. Said claims could not be reworked if strains of the *Escherichia hermannii* species which did not have lysine decarboxylase activity were used, as indeed in these strains the lysine decarboxylase activity could not be decreased. Moreover, carrying out the claimed multi-step process represented an immense workload.

Article 54 EPC

Document D16 described that the *Escherichia coli* strain HB101 did not have Ldc lysine decarboxylase activity under standard growth conditions. This was in line with document D37, which pointed to the existence of a biogroup of *Escherichia coli* and bacteria of the *Escherichia hermannii* species which were negative in reactions for lysine decarboxylase (see page 180, left-hand column and Table 5.17 on page 233). This was a proof of the existence of bacteria belonging to the genus *Escherichia* in which this enzyme activity was decreased or has been disappeared. Therefore, claim 5 which included microorganisms wherein the gene of claim 1 or 2 had been completely deleted and claims dependent thereon lacked novelty.

In *Escherichia hermannii*, the Ldc lysine decarboxylase activity seemed to have been lost by evolutionary mechanisms involving a modification as referred to in claim 5. A skilled person would have realised that the loss of lysine decarboxylase activity was not due to

the absence of the complete gene(s), but merely to a genetic event affecting one or more nucleotides and leading to the inactivation of the gene(s).

Article 56 EPC

Document D3 was the closest prior art. It described experiments in which Southern blots were performed to show the presence of disrupted *cadA* genes in a series of mutant constructs, as illustrated in Figure 1. These experiments were made on purpose to map the gene encoding the CadA lysine decarboxylase.

As indicated on page 2666 in the third full sentence of the left-hand column, the existence of a "second" lysine decarboxylase in Escherichia coli was known.

As derivable from the sentence reading "*Our preliminary Southern hybridisation experiment using cadA to probe E. coli chromosomal DNA failed to identify a second region homologous to cadA under the conditions used*", the authors of document D3 noticed that in the course of the Southern hybridisation mapping experiments, which they made with the only intention to identify the *cadA* gene, they did not identify the gene encoding the "second" lysine decarboxylase. They concluded that Southern experiments with other conditions were needed.

The said sentence contained the indication that the gene encoding the "second" lysine decarboxylase shared at least some homology with the *cadA* gene.

Document D39, the basic laboratory manual of Maniatis et al. for molecular cloning, was reference 36 of

document D3. It was cited on page 2659 of document D3 (see the bottom of the right-hand column) which read: "*All cloning experiments were conducted according to standard procedures*." This was a clear indication that standard stringency conditions had been used in the Southern experiments of document D3. Document D12 later on confirmed that the authors of document D3 had used standard conditions (see page 4488, the sentence beginning with "*Meng and Bennett*" in the left-hand column). The reference to document D39 in document D3 provided the skilled practitioner performing Southern hybridisations also with the advice that "*If the homology between the probe and the DNA bound to the filter is inexact, the washing should be carried out under less stringent conditions*" (see the *Note* of paragraph 11 on page 388 of document D39).

The skilled person would also have been aware of document D11 describing Southern experiments using low stringency conditions, as illustrated in the legend of Figure 1 on page 1075, in a similar situation. Indeed, the purpose of the experiments of document D11 was to identify in *E. coli* a new gene - encoding a biodegradative ornithine decarboxylase - having homology with the known *speC* gene encoding the biosynthetic ornithine decarboxylase. It was evident that the skilled person would have been prompted by document D11 to repeat the Southern experiments of document D3 by using the *cadA* gene as a probe in Southern experiments under less stringent conditions, as the inventors of the patent at issue and, later on, the authors of document D12 have done (see D12, page 4488, the phrase beginning with "*in this study*" in the left-hand column). By doing so the skilled person

would have identified the gene encoding the "second" lysine decarboxylase without the exercise of inventive skills. Therefore, claim 1 lacked inventive step.

The skilled person would have derived from document D20, which described lysine and methionine overproduction by an *Escherichia coli* strain, that a microorganism belonging to the genus *Escherichia coli* was the obvious choice for the production of L-lysine. Therefore, claim 9 lacked inventive step.

XIII. Oral proceedings took place on 10 January 2012 in the presence of the two appellants. As announced with letter of 15 December 2011, the party as of right did not attend.

XIV. Appellant I (patentee) requested that the decision under appeal be set aside and that the patent be maintained as granted, or in the alternative that the appeal of appellant II be dismissed.

XV. Appellant II (opponent 01) requested that the decision under appeal be set aside and the patent be revoked.

Reasons for the decision

Admissibility of the clean copy of document D16 and of documents D37A, D37B and D56 all submitted on 9 December 2011

1. Document D16 was submitted by appellant II together with its notice of opposition. The copy submitted was incomplete as it lacked the upper portion of all pages. In particular, the two first lines of the right-hand

column of page 179 were missing. This was the reason why appellant II has submitted a further "clean" copy of the document with its letter of 9 December 2011 in preparation for the oral proceedings. Document D16 had correctly been identified when first filed and its whole content had become available to the other parties. The newly filed "second" copy is not a new piece of evidence. Its submission has served the only purpose to correct a material defect. Therefore, the Board admits it in the appeal proceedings.

2. Document D37A consists of a copy of the double-sided cover page and page 222 from the ninth edition of the Bergey's manual, a textbook known to all those working in the field of microbiology. Page 222 explains the meaning of the symbols used in the tables of pages 233 (see the footnote of Table 5.17) and 234 (see the footnote of Table 5.18) of the same document submitted together with pages 179 and 180 as document D37 by appellant II during the opposition proceedings. The information contained in page 222 does not constitute new evidence. Therefore, the Board admits document D37A in the appeal proceedings.

3. Document D37B is a letter from the present publisher of the Bergey's manual. It conveys the information that the pages of Bergey's manual which were presented in document D37 originate from the ninth edition thereof (this had not been indicated earlier; see the appellant II's letter of 18 July 2008) and that said edition was published on 1 September 1993, i.e. before the priority date. As neither the origin of the pages filed as document D37 nor their availability to the public before the priority date have been disputed,

either during the opposition proceedings or at the appeal stage, the Board admits document D37B into the appeal proceedings.

4. Document D56 is a scientific report which describes experimental procedures and their results. Appellant II was not able to give a reason why this evidence was submitted only one month before the oral proceedings. It is the Board's opinion that such evidence could and should have been presented earlier in order to give appellant I a fair opportunity to examine it. Therefore, using the discretional power conferred to it by Article 114(2) EPC and Article 13(1) of the Rules of Procedure of the Boards of Appeal (RPBA), the Board does not admit document D56 in the appeal proceedings.

Main request

5. According to its embodiment (B), claim 1 as granted is directed to a gene which codes for a lysine decarboxylase having an amino acid sequence which has been modified by substitution, deletion or insertion **of 3 amino acid residues or less** in the amino acid sequence shown in SEQ ID NO:4 **and has lysine decarboxylase activity**. Appellant II has argued that this gene was not described in the application as filed (see the English translation filed on 5 June 1997 when entering the regional phase before the EPO). Appellant I contends that a reading of claim 3 as filed together with the description on pages 6 and 7 as filed constitutes a basis for this embodiment.

6. Claim 3 as filed is directed to a gene which codes for lysine decarboxylase having an amino acid sequence

which has been modified by substitution, deletion or insertion **of one or a plurality** (a term which includes two or three) **of amino acid residues** in the amino acid sequence shown in SEQ ID NO:4 **without any substantial deterioration of lysine decarboxylase activity**. From the passage on page 6, lines 10 to 15, it can be derived that the genes described in the application as filed are those which encode a lysine decarboxylase having either the sequence shown in SEQ ID NO:4 or a sequence differing therefrom by the substitution, deletion or insertion of one or a plurality of amino acid residues, provided that this **does not result in any substantial deterioration of the lysine decarboxylase activity**.

7. The passage on page 7, lines 2 to 20, provides additional information about the genes more generally referred to on page 6, lines 10 to 15, by stating that they code for a lysine decarboxylase which has been modified by substitution, deletion, insertion of two or three amino acid residues with regard to the amino acid sequence shown in SEQ ID NO:3, but whose activity has not been deteriorated.

8. Thus, due to the use of the term "*having lysine decarboxylase activity*" instead of the original term "***without any substantial deterioration of the lysine decarboxylase activity*****",** claim 1 is directed to genes which are not described in the application as filed. As, therefore, claim 1 contains subject-matter which extends beyond the content of application as filed, the main request does not meet the requirements of Article 123(2) EPC.

First auxiliary request

Requirements of Article 123(2) EPC

9. Claim 1, which differs from claim 1 of the main request in that the phrase "***and having lysine decarboxylase activity***" has been replaced by the phrase "***without any substantial deterioration of the lysine decarboxylase activity***", is directed to those genes which are generally described on page 6 as filed (see lines 10 to 15), with the further limitation that no more than three amino acids are modified. Genes encoding a lysine decarboxylase with two or three modified amino acid residues are described as a particular embodiment on page 7 as filed (see line 20).

10. As explained at point 7, *supra*, the Board is of the view that the particular genes referred to on page 7, lines 2 to 20, are specific examples of the genes generally described on page 6 which code for a lysine decarboxylase with an amino acid sequence having substitution, deletion or insertion of one or a plurality of amino acid residues in the amino acid sequence shown in SEQ ID NO:4 **without any substantial deterioration of the lysine decarboxylase activity.** Therefore, appellant II's argument that claim 1 results from the combination of two different embodiments, one being "*without any substantial deterioration of the lysine decarboxylase activity*" and the other "*having equivalent lysine decarboxylase activity*" is not tenable. No such combination is evident as claim 1 relies on the general description given on page 6, lines 10 to 15, complemented with the optional feature

described on page 7 that the *"plurality"* of modified amino acid residues is limited to two or three residues.

11. Therefore, the genes according to claim 1 are disclosed in the application as filed. As claims 2 to 9 have the same wording as granted claims 2 to 9 which were not objected to under Article 123(2) EPC by either the opposition division in its decision or by appellant II in its submissions at the appeal stage, the auxiliary request as a whole meets the requirements of Article 123(2) EPC.

Requirements of Article 84 EPC

12. Appellant II's objection is based on the presence of the term "*substantial*" in claim 1. The term is part of the phrase "*without substantial deterioration of the lysine decarboxylase activity*" which is present on page 6, lines 13 to 15, of the application as filed. Although, "*substantial*" without doubt is a relative term, the Board considers that its meaning in the context of the patent in suit is clear to a skilled person reading the description with a mind willing to understand. It is emphasised on pages 6 and 7 of the application as filed that the modified enzymes encoded by the claimed genes must retain a lysine decarboxylase activity close to the activity value of the unmodified enzyme. Therefore, the term "*substantial*" does not render ambiguous the wording of claim 1.

13. The Board reaches the conclusion that claim 1 is clear and that the requirements of Article 84 EPC are met.

Requirements of Article 83 EPC

14. The objections raised by appellant II are as follows:

a) The data of Figure 3 of the patent cannot be reproduced because Example 2 fails to describe how the amount of L-lysine was measured and because no routine technique to measure the lysine carboxylase activity is referred to in the patent.

b) Starting from a bacterium of the genus *Escherichia,* which does not have lysine decarboxylase activity, such as strains of the species *E. hermannii*, the invention according to claims 4 to 8 cannot be carried out.

c) There is a multitude of steps to be carried out in order to perform the claimed invention, which may take days and which represents an immense workload.

d) There is no indication in the patent of how to keep the enzymatic activity without substantial deterioration.

15. With its first objection regarding reproducibility of the experiment of Example 2, appellant II has argued that the statement in paragraph [0048] on page 9 of the patent specification that "*The amount of L-lysine was quantitatively determined by using Biotech Analyzer AS-210 (produced by Asahi Chemical Industry)*" provides an insufficient disclosure as regards the means to be used for measuring the amount of L-lysine. This argument cannot as such be regarded as a proof that there are serious doubts substantiated by verifiable doubts (see decision T 19/90, OJ EPO 1990, 476) that at

the relevant filing date a skilled person would not have been in a position to quantitatively determine the remaining L-lysine amounts in culture liquids referred to in Example 2. In this respect, the Board notes that appellant II has not denied that at the relevant filing date the said analyser was available to the skilled person. Nor has it contested that the biochemistry of L-lysine was well-established at the said date. Therefore, the objection is not tenable.

16. In Example 2, lysine decarboxylase activity is determined by measuring the amount of cadaverine, a decomposition product of L-lysine. The skilled person is taught that the amount of cadaverine was quantitatively measured by using high performance liquid chromatography (see paragraph [0050] on page 9 of the patent specification). The precise procedure which was used in this respect by the inventors is not detailed. Nevertheless, the Board is convinced that a skilled person at the relevant filing date would have known how to proceed. Moreover, he would have found in document D3, which is cited in paragraph [0002] on page 3 of the patent specification, a detailed description of an alternative method to measure cadaverine (see the second part of the right-hand column on page 2660 of the document). The argument that the method of document D3 is not the method referred to in Example 2, and that consequently the results thereof could not be reobtained, is simply meaningless. Therefore, the objection that Example 2 is not reproducible because the skilled person would not have been in a position to measure lysine decarboxylase activity is not tenable.

17. The Board holds that a skilled person aiming at preparing a microorganism according to any of claims 5 to 8 would obviously have chosen to mutate a bacterium in which the presence of a gene according to claims 1 or 2 had been ascertained. It would have been meaningless for him to choose a bacterium, such as a strain of *Escherichia hermannii*, which was known to exhibit no lysine decarboxylase activity. Therefore, also the objection that the disclosure of the invention in the patent at issue was insufficient as regards the preparation of the claimed mutated microorganisms is not tenable.

18. The objection that the skilled person would have had to perform many steps to carry out the claimed invention which is time consuming and represents a lot of work is meaningless as such considerations have nothing to do with the question of sufficiency of disclosure that has to be answered positively if a complete and sufficiently clear information to carry out the invention is provided in the application under consideration.

19. Claim 1 refers to a gene coding for an enzyme, which is defined by its amino acid sequence and its activity. The claim does not contain a feature referring to the maintenance of the enzyme activity over time, so that appellant II's objection in this respect is meaningless.

20. In view of the above remarks, the conclusion is reached that the first auxiliary request meets the requirements of Article 83 EPC.

Requirements of Article 54 EPC

21. Claim 5 has been objected to for reasons of lack of novelty in view of either of documents D16 and D37.

22. Document D16 describes the cloning and characterisation of a lysine decarboxylase gene from the enterobacterium *Hafnia alvei* in the *Escherichia coli* strain HB101. It has been submitted by appellant II because it reports that no lysine decarboxylase activity has been detected after electroblotting of the proteins contained in a lysate of the HB101 strain from SDS-polyacrylamide gels onto nitrocellulose, as illustrated in Figure 4 (see page 180).

23. Document D37 is a compilation of four pages from the ninth edition of the Bergey's manual. On pages 179 (see the right-hand column) and 180 (see the left-hand column), the genus *Escherichia* is generally described. On page 180, following the remark that "*Another biogroup of E. coli is negative in reactions for lysine carboxylase, arginine dihydrolase, and ornithine decarboxylase, which make them similar to Enterobacter (Pantoea) agglomerans and other species that are negative in these tests*", reference is made to Table 5.17. This table on page 233 of document D37 shows, as interpreted on page 222, that no lysine decarboxylase activity has been found in 90% or more of the strains of *Escherichia hermanii*.

24. Consequently, each of documents D16 and D37 shows that bacteria belonging to the genus *Escherichia* exist which, at least under certain conditions, either do not produce any lysine decarboxylase (see document D16) or

do not exhibit any lysine decarboxylase activity (see document D37).

25. A microorganism falling within the scope of claim 5 must contain a gene which can be recognised as a modified version of the chromosomal *ldc* gene contained in the W3110 strain of *Escherichia coli* K-12 (see paragraph [0009] and paragraph [0038] of the patent specification) having the sequence shown in SEQ ID NO:4 as referred to in claim 1.

26. Neither document D16 nor document D17 refer to a gene encoding a lysine decarboxylase originating from *Escherichia coli*, let alone to a gene having the sequence shown in SEQ ID NO:4 or a mutated version thereof. Indeed, both documents only refer to strains belonging to the genus *Escherichia* which do not exhibit lysine decarboxylase activity.

27. Appellant II's argument that the said bacterial strains of documents D16 and D37 may have derived from an ancestor having a gene of the sequence shown in SEQ ID NO:4 is to be regarded as a mere assumption and, therefore, has to be disregarded.

28. Thus, the Board reaches the conclusion that claim 5 is new over either of documents D16 and D37. The conclusion extends to dependent claims 6 to 8. As no other claim has been objected to for reasons of lack of novelty, the first auxiliary request meets the requirements of Article 54 EPC.

Requirements of Article 56 EPC

29. Both claims 1 and 9 have been objected to by appellant II for reasons of lack of inventive step. They will be successively assessed using the problem-solution approach.

30. Document D3 has been considered by the opposition division and the appellants to represent the closest state of the art as regards claim 1. The Board sees no reason to depart from this choice.

31. Document D3 reports the complete sequencing of the *Escherichia coli cad* operon, including *cadA*, the gene encoding the inducible lysine decarboxylase.

32. Document D3 mentions that, in addition to the CadA inducible lysine decarboxylase, the existence of a "second" lysine decarboxylase had been previously observed in *E. coli* (see page 2659, left-hand column, last sentence of the first paragraph, and page 2666, left-hand column, top paragraph).

33. Therefore, the technical problem underlying the patent in suit in the light of the disclosure in document D3 is defined as the provision of this "second" lysine decarboxylase. As a solution to said problem the patent provides a gene according to claim 1 coding for the non-inducible lysine decarboxylase. The technical problem is credibly solved.

34. The remaining question to be answered is whether a skilled person would have found any incentive in the prior art documents on file that would have allowed him

to arrive at the claimed subject-matter in an obvious way.

35. Appellant II has argued as follows:

35.1 The skilled person would have regarded it as highly probable that the *cadA* gene and the gene encoding the constitutive lysine decarboxylase were **significantly homologous**.

35.2 The skilled person would have realised that the failure reported by the authors of document D3 in the sentence reading "*Our preliminary Southern hybridization experiment using cadA to probe E. coli chromosomal DNA failed to identify a second region homologous to cadA **under the conditions used***" (see page 2666, left-hand column, top paragraph; emphasis added by the Board) was due to the use of **standard** stringency conditions for the performance of the Southern blots.

35.3 The skilled person would have derived from document D39 (see on page 388, the note reading "*If the homology between the probe and the DNA bound to the filter is inexact, the washing should be carried out under less stringent conditions*"), which was citation 36 of document D3, that, in view of the homology between the two lysine decarboxylase encoding genes, **less stringent conditions** than those assumed to be standard were appropriate.

35.4 A skilled person being convinced that the known *cadA* gene and the "second" gene he was looking for had significant homology and that the latter gene could be identified in Southern blots using less stringent

conditions, would have been prompted to apply the conditions used in document D11 for the identification of a "second ornithine decarboxylase" in *E. coli.* He would have performed a Southern blot using the *cadA* gene as a probe under the low stringency conditions referred to in the legend of Figure 1 on page 1075 of document D11.

36. The Board cannot adhere to the appellant II's argument for the following reasons:

36.1 Document D3 is silent as regards the stringency conditions which have been used for the Southern hybridisations referred to on page 2659. The same applies to the conditions which have been used for the Southern hybridisation in the experiments carried out to map the *cad* operon. The mere reference to the Maniatis manual (D39) in the sentence reading "*All cloning experiments were conducted according to standard procedures*" (see page 2659, bottom of the right-hand column) does not allow to conclude exactly which stringency conditions were used in any of the experiments of document D3, including the "*preliminary Southern hybridization experiment*" as referred to in the sentence of page 2666 (see point 35.2, *supra*).

36.2 The Maniatis manual (D39) was only generally referred to in document D3 as citation (36) (see the first and the last sentences of the paragraph entitled "*Recombinant DNA techniques*" bridging pages 2659 and 2660 of document D3). No reference was made to a particular passage of the manual, let alone to the note of paragraph 11 on page 388, on which appellant II has relied.

37. Furthermore, the Board sees no convincing basis in the prior art to support appellant II's contention that the skilled person at the relevant date was convinced that the *cadA* gene, encoding the known inducible CadA lysine decarboxylase, and the unknown second gene, encoding the other (non-inducible) lysine decarboxylases of *E. coli,* shared significant homology. Also this is a mere assumption.

38. Rather the failure to identify this second gene by performing an experiment based in the hypothesis of such a homology, as reported in document D3 (see page 2666 and point 35 *supra*), would have left the skilled person with the assumption that this hypothesis was wrong.

39. Moreover, it is the Board's view that document D11 is not relevant for the present assessment. This document reports the use as a probe of a plasmid (pODC-1) which bears the *speC* gene encoding the biosynthetic ornithine decarboxylase in *Escherichia coli* in hybridisation assays. Hybridisation of the pODC-1 probe to DNA of *E. coli* UW44 revealed a different pattern of radioactive bands from those detected in the DNA of *E. coli* C600. As *E. coli* UW44 was known to possess both the biosynthetic and biodegradative ornithine decarboxylases, whereas *E. coli* C600 was known to possess only the biosynthetic ornithine decarboxylase, the authors of document D11 have concluded that "*the additional radioactive bands detected in endonuclease digests of E. coli UW44 relative to E. coli C600 may represent portions of the biodegradative ornithine decarboxylase gene which are partially homologous to*

speC" (see page 1075, left-hand column). The authors did not prove that they had actually identified a second ornithine decarboxylase gene. It cannot be concluded that the biosynthetic and biodegradative ornithine decarboxylases of *E. coli* share some significant structural homology. Thus, the skilled person would not have derived any useful teaching from document D11 which he would have considered helpful in order to solve the technical problem underlying the patent in suit.

40. Appellant II also referred to document D12, which however has been published in 1997, i.e. long after the relevant filing date of the patent at issue, and which does not belong to the state of the art. Therefore, the sentence "*Meng and Bennett* [i.e. document D3] *previously reported that preliminary Southern hybridization using cadA to probe E. coli chromosomal DNA failed to identify a second region homologous to cadA under standard conditions*" referred to by appellant II is not relevant for the assessment of inventive step in the present case.

41. In view of the above comments, the Board arrives at the conclusion that a skilled person facing the technical problem as defined at point 33, *supra*, would not have been prompted to use the *cadA* gene in a Southern hybridisation experiment under the stringency conditions referred in the legend of Figure 1 of document D11. Therefore, the Board concludes that claim 1 involves an inventive step.

42. Appellant II has argued separately in respect of the inventive step of the subject-matter of claim 9. It has

contended that the skilled person would have derived from document D20 that *Escherichia coli* was an obvious choice for the production of L-lysine. Appellant II has concluded that claim 9 does not therefore involve an inventive step.

43. The Board cannot adhere to this argument. The method of claim 9 uses a microorganism according to claim 8 which itself is dependent of claim 5, which requires that microorganism contains a modified version of the gene of claim 1 or 2. Insofar as the gene of claim 1 is acknowledged to be inventive, any activity based on the knowledge of said gene is inventive. It is the identification of the gene according to claim 1 which permits to produce L-Lysine in a more efficient way. Therefore, also claim 9 involves an inventive step. Thus, it is concluded that the first auxiliary request meets the requirements of Article 56 EPC.

Order

For these reasons it is decided that:

The appeals are dismissed.

The Registrar | The Chairman

A. Wolinski | M. Wieser