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**Datasheet for the decision
of 18 October 2012**

Case Number: T 2352/09 - 3.3.08
Application Number: 04028877.1
Publication Number: 1526181
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C12P 13/24, C12N 1/21,
C12N 15/31, C07K 14/245,
C12R 1/185

Language of the proceedings: EN

Title of invention:

Method for producing l-amino acid using bacteria belonging to the genus escherichia

Patentee:

Ajinomoto Co., Inc.

Opponent:

Evonik Degussa GmbH

Headword:

Amino acid production/AJINOMOTO

Relevant legal provisions:

EPC Art. 83, 54, 56

Keyword:

"Claims as granted - sufficiency of disclosure (yes), novelty (yes), inventive step (yes)"
"Appeal dismissed"

Decisions cited:

G 0002/88, T 0018/09

Catchword:

-



Case Number: T 2352/09 - 3.3.08

D E C I S I O N
of the Technical Board of Appeal 3.3.08
of 18 October 2012

Appellant:
(Opponent)

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Respondent:
(Patent Proprietor)

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Decision under appeal:

**Decision of the Opposition Division of the
European Patent Office posted on 8 October 2009
rejecting the opposition filed against European
patent No. 1526181 pursuant to Article 101(2)
EPC.**

Composition of the Board:

Chairman: M. Wieser
Members: P. Julià
J. Geschwind

Summary of Facts and Submissions

- I. European patent no. 1 526 181, based on European patent application no. 04 028 877.1, is a divisional application of the earlier European patent application no. 02 003 335.3, published as EP 1 239 041 (Article 76 EPC). An opposition was filed against the patent on the grounds of Articles 100(a) (lack of novelty and of inventive step; Articles 54 and 56 EPC) and 100(b) EPC (insufficiency of disclosure; Article 83 EPC). The opposition division decided that the patent fulfilled the requirements of the EPC and rejected the opposition.
- II. The opponent (appellant) lodged an appeal against this decision and, in the statement setting out its grounds of appeal, maintained all grounds of opposition.
- III. The patentee (respondent) replied thereto and requested the board to dismiss the appeal and maintain the patent as granted.
- IV. A summons to oral proceedings was issued by the board and, in a communication pursuant to Article 15(1) of the Rules of Procedure of the Boards of Appeal (RPBA) annexed thereto, the parties were informed of the board's preliminary, non-binding opinion on substantive issues of the appeal.
- V. In reply to the board's communication, the appellant filed further submissions.
- VI. Oral proceedings took place on 18 October 2012. Following a proposition of the board made in its communication pursuant to Article 15(1) RPBA, the

parties agreed to deal the present appeal case together with the appeal case number T 2354/09, as was also done by the first instance at the opposition proceedings.

VII. Claims 1 and 3 as granted read as follows:

"1. An L-amino acid producing bacterium belonging to the genus *Escherichia*, wherein the bacterium has been modified so that the L-amino acid production by said bacterium is enhanced by enhancing activities of proteins as defined in the following (G) or (H) in a cell of said bacterium:

(G) a protein which comprises the amino acid sequence shown in SEQ ID NO:15 in Sequence listing;

(H) a protein which comprises an amino acid sequence including deletion, substitution, insertion or addition of 1-20 amino acids in the amino acid sequence shown in SEQ ID NO:15 in Sequence listing, and which has an activity of making bacterium having enhanced resistance to L-amino acids and/or its analogs, such as DL- α -methylserine, 6-diazo-5-oxo-L-norleucine and DL- β -hydroxy-norvaline, and having enhanced sensitivity to S-(2-aminoethyl)cysteine;

the activities of proteins being enhanced by transformation of said bacterium with DNA coding for protein as defined in (G) or (H), or by alteration of promoter sequence of said DNA on the chromosome of the bacterium."

"3. A method for producing L-amino acid, which comprises cultivating the bacterium according to any of claim 1 or 2 in a culture medium and collecting from

the culture medium L-amino acid to be produced and accumulated."

Claim 2 was an embodiment of claim 1. Claims 4 and 6 were embodiments of claim 3 and defined the L-amino acid produced as L-arginine and L-proline, respectively. Claims 5 and 7 were embodiments of claims 4 and 6, respectively, requiring the bacterium to be modified so as to have an enhanced expression of the arginine regulon (claim 5) or of genes for proline biosynthesis (claim 7).

VIII. The following documents are cited in this decision:

D1: F.R. Blattner et al., *Science*, 5 September 1997, Vol. 277, pages 1453 to 1462;

D2: S. Lisser and H. Margalit, *Nucleic Acid Research*, 1993, Vol. 21, No. 7, pages 1507 to 1516;

D4: EP 1 033 407 (Ajinomoto Co., Inc.; publication date 06 September 2000).

IX. The arguments of the appellant may be summarized as follows:

Article 100(b) EPC; Article 83 EPC

Article 83 EPC required the disclosure of the patent to allow a skilled person to carry out the invention over the whole scope of the claims. This was not the case for the patent-in-suit due to the following reasons:

1) the proteins defined in parts (G) and (H) of claim 1 were identified as putative membrane exporters. Their actual activities were not disclosed in the patent and thus, a skilled person was not in a position to measure them and assess whether they were enhanced as required by claim 1. An increase in the amount of these proteins could not be equated to an enhanced activity.

2) Table 1 of the patent showed that an *E. coli* strain transformed with a nucleic acid encoding a protein as defined in part (G) of claim 1 had an enhanced resistance to L-amino acid analogs unrelated to the L-amino acids produced by this strain (L-arginine, L-proline), the first to which resistance was to be expected. No correlation was found between the L-amino acid produced by the transformed strain and the L-amino acid to which this strain showed enhanced resistance. Thus, claim 1 comprised *E. coli* strains transformed with a nucleic acid encoding a protein as defined in part (H) of claim 1 which made these strains to have an enhanced sensitivity to S-(2-aminoethyl)cysteine and an enhanced resistance to any possible L-amino acid and/or analogs thereof and not necessarily to the specific L-amino acid (L-arginine, L-proline) produced by these strains. Since there was no definition in the patent of the expression "*L-amino acid and/or its analogs*", it could be broadly interpreted and the number of potential substances to which an *E. coli* strain transformed with a nucleic acid encoding a protein as defined in part (H) of claim 1 could have an enhanced resistance was almost unlimited. Moreover, a protein as defined in part (H) of claim 1 could have an almost unlimited number of possible modifications (deletions, insertions or substitutions of 1-20 amino acids and

combinations thereof). For each of them, the skilled person had to assess whether the transformed *E. coli* strain had an enhanced resistance to any possible L-amino acid and/or analogs thereof and, if it was so, whether this strain had also an enhanced production of whatever L-amino acid. The scope of claim 1 was comparable to that of a reach-through claim, it was nothing more than an invitation to perform a research programme. This was not allowable according to the established case law.

Claim 1 comprised an embodiment, wherein an *E. coli* strain transformed with a nucleic acid encoding a protein as defined in part (H) of claim 1 made this strain to have an enhanced resistance to all L-amino acids and/or analogs thereof and simultaneously an enhanced sensitivity to S-(2-aminoethyl)cysteine. The results of Example 2 of the patent showed the presence of an enhanced resistance only to a very limited number of specific L-amino acids and analogs thereof. There was no teaching in the patent to enable a skilled person to achieve that embodiment without undue burden or inventive skill.

3) Examples 10 and 11 of the patent showed an enhanced production of L-arginine and L-proline. In view of the divergent biosynthetic pathways for the production of different L-amino acids, it was highly improbable and not credible that the production of other L-amino acids could be enhanced by increasing only the activity of the non-specific putative membrane (exporters) proteins as defined in parts (G) and (H) of claim 1 as required in claim 3.

4) Claim 1 comprised an embodiment, wherein the activity of the proteins as defined in parts (G) and (H) of claim 1 was enhanced by alteration of the endogenous DNA promoter sequence on the chromosome of the *E. coli* bacterium. However, the endogenous promoter of the b3434 gene was neither disclosed in the patent nor in the prior art document D2 which disclosed a compilation of *E. coli* promoter sequences. Document D2 referred to the presence of several problems that could be encountered when identifying promoter sequences and it showed that the endogenous promoter of the b3434 gene could not be identified by standard methods. The identification and modification of the endogenous promoter of the b3434 gene for enhancing the activity of the proteins as defined in parts (G) and (H) of claim 1 amounted to an undue burden for a skilled person.

Article 100(a) EPC; Article 54 EPC

The production of L-amino acids was an inherent property of *E. coli* bacteria. Document D1 disclosed the complete *E. coli* K-12 genome which included the b3434 gene (SEQ ID NO:15) encoding a protein as defined in part (G) of claim 1. Document D1 referred to the high redundancy of the sequencing strategy and to subclones with genome fragments of a size (15 to 20 kb; 250 kb) greater than that of the b3434 gene. Thus, following this sequencing strategy, it was inevitable to obtain subclones containing the complete b3434 gene. When cloning the *E. coli* K-12 genome, the number of copies of the b3434 gene as well as those of other genes, was increased and thus, the *E. coli* bacterium of claim 1 was inherently obtained. Moreover, according to

standard practice, transformed *E. coli* bacteria were always cultured in order to have enough material for sequencing and thus, the method of claim 3 was also inherently disclosed. Therefore, in line with the criteria set out in the decision G 2/88 (OJ EPO 1990, page 93) as regards the disclosure of inherent features, document D1 anticipated the claimed subject-matter.

Article 100(a) EPC; Article 56 EPC

It was known in the prior art that an increased L-amino acid secretion enhanced the productivity of a strain producing this L-amino acid and that the transformation of *E. coli* strains with genes encoding putative *E. coli* membrane proteins could enhance the production of L-amino acids (cf. paragraph [0004] of the patent and the prior art cited therein). This body of prior art documents represented the closest prior art. Starting therefrom, the technical problem to be solved was the provision of alternative putative *E. coli* membrane proteins that enhanced the production of L-amino acids in *E. coli*. This problem was solved by the claimed subject-matter which however was obvious to a skilled person. Document D1 disclosed the complete sequence of the *E. coli* genome and standard methods were known for the analysis of this genome, the identification of putative membrane proteins and the screening for those involved in enhanced production of L-amino acids. As shown in paragraph [0006] of the patent-in-suit, the skilled person would have inevitably arrived at the b3434 gene encoding a protein as defined in part (G) of claim 1. According to the case law, the same standard had to be used when assessing Articles 83 and 56 EPC. Thus, if no problems existed to identify the endogenous

b3434 promoter sequence, there was no reason to expect them when identifying the b3434 gene.

According to the case law, an advantageous effect could only substantiate an inventive step, if it was credibly shown to exist over the whole scope of the claims. In the present case, it was not credible that the technical problem was solved over the whole scope of the claims. A large number of modifications could be contemplated for obtaining a protein as defined in part (H) of claim 1 and it was not credible that all achieved the advantageous effect disclosed in the patent-in-suit and thereby solved the technical problem. Likewise, it was not credible that all alterations of the b3434 promoter sequence provided this technical effect and thereby solved the technical problem. Moreover, based on the limited disclosure of the patent, it was not credible that an enhanced production of L-amino acids other than those exemplified in the patent-in-suit (L-arginine and L-proline) could be achieved.

- X. The arguments of the respondent may be summarized as follows:

Article 100(b) EPC; Article 83 EPC

The patent-in-suit provided examples of *E. coli* bacteria transformed with a nucleic acid sequence encoding a protein as defined in part (G) of claim 1 (SEQ ID NO:15) and having increased production of L-arginine and L-proline. A skilled person would have understood that an enhanced expression by transformation with nucleic acid sequences encoding the

proteins as defined in parts (G) or (H) of claim 1, or promoter alteration or replacement by a stronger promoter, would lead to enhanced activities of these proteins in transformed *E. coli* bacteria.

No technical difficulties were encountered by a skilled person when obtaining a protein as defined in part (H) of claim 1, since it required only to carry out a limited number of modifications to SEQ ID NO:15 encoding a protein as defined in part (G) of claim 1 (1-20 amino acid substitutions, insertions or deletions). The proteins defined in parts (G) and (H) of claim 1 were defined not only structurally but also functionally. Standard methods were known for screening those variants which made the transformed bacteria to have enhanced resistance and increased production of L-amino acids. The experimental results disclosed in the patent-in-suit had never been contested and they showed that the L-amino acid to which resistance was developed and the L-amino acid produced were not necessarily the same. It was expected but not necessarily required that the proteins as defined in part (H) of claim 1 had similar functional properties as those of the non-modified protein defined in part (G) of claim 1. Important was that these proteins had all properties required in claim 1 and that methods were known in the prior art for identifying them.

Claim 1 required the *E. coli* bacteria transformed with a nucleic acid sequence encoding a protein as defined in part (H) to have an enhanced resistance to at least two amino acids but not to all L-amino acids and/or analogs thereof. Likewise, claim 1 required the transformed *E. coli* bacteria to have an enhanced

production of one L-amino acid, there was no requirement to have an enhanced production for all L-amino acids. The expression "*L-amino acids and/or analogs thereof*" was usual in the field and its meaning well-known to a skilled person. Appellant's objection concerning the interpretation of this expression related to Article 84 EPC and not to Article 83 EPC.

The promoter of SEQ ID NO:15 could be identified in the upstream region of this sequence in the publicly available *E. coli* genome sequence by using known methods. Likewise, conventional techniques, such as these cited in document D4, were known to a skilled person for altering or replacing a promoter sequence and for screening for promoters having an enhanced gene expression. The patent-in-suit also referred to the use of a strong promoter for expressing the nucleic acid sequence SEQ ID NO:15 (promoter replacement). Document D2 contained a general disclosure of *E. coli* promoter sequences but not a complete compilation. It could not be used as evidence for the presence of technical difficulties in the determination of the promoter sequence of the b3434 gene.

Article 100(a) EPC; Article 54 EPC

In line with the principles laid down in the decision G 2/88 (*supra*), the critical question was not whether the sequence SEQ ID NO:15 was contained in the *E. coli* K-12 genome but whether document D1 made publicly available this sequence. Document D1 did neither identify it as such, let alone variants thereof, nor did it disclose *E. coli* strains transformed with this

sequence and having an increased production of L-amino acids.

Article 100(a) EPC; Article 56 EPC

Starting from the body of prior art documents cited in paragraph [0004] of the patent-in-suit, the technical problem to be solved was the provision of further *E. coli* genes which, when over-expressed in transformed *E. coli* strains, increased the production of L-amino acids, i.e. the provision of transformed *E. coli* bacteria having increased L-amino acid production. Examples 10 and 11 of the patent showed that the over-expression of the b3434 gene encoding a protein as defined in part (G) of claim 1 in a transformed *E. coli* strain resulted in increased production of L-arginine and L-proline. Thus, the technical problem was solved by the claimed subject-matter, which comprised only those sequences encoding the proteins as defined in part (H) of claim 1 having the functional properties recited in this claim.

Although the relevance of *E. coli* membrane proteins in the production of L-amino acids was known in the prior art, a large number of sequences encoding putative membrane proteins could be identified in the *E. coli* genome. However, these sequences encoded only putative membrane proteins whose actual cellular location and function was not known and not directly derivable from this prior art. Moreover, not all *E. coli* membrane proteins were involved in L-amino acid production or had any effect on this production. These putative membrane proteins could have many possible functions unrelated to L-amino acid production. There was no hint

in the prior art, including document D1, that could have led a skilled person to sequence SEQ ID NO:15 encoding a protein as defined in part (G) of claim 1 in an obvious manner, let alone to variants thereof, such as the proteins defined in part (H) of claim 1.

XI. The appellant (opponent) requested to set aside the decision under appeal and to revoke the patent-in-suit.

XII. The respondent (patentee) requested to dismiss the appeal.

Reasons for the Decision

Articles 123(2) and 84 EPC

1. The objections raised under Articles 123(2) and 84 EPC were not admitted into the opposition proceedings for reasons given by the opposition division on page 2, points 9 and 10 of the decision under appeal. This decision has not been contested in appeal and thus, it is not part of the present appeal proceedings.

Article 100(b) EPC; Article 83 EPC

2. Claim 1 as granted is directed to an L-amino acid producing *Escherichia* bacterium, which has been modified so that its L-amino acid production is increased by enhancing the activities of the proteins as defined in parts (G) or (H) of claim 1. The enhancement is obtained by: i) transformation with a nucleic acid sequence encoding the amino acid sequence shown in SEQ ID NO:15 (part (G) of claim 1), ii)

- transformation with a nucleic acid sequence encoding the amino acid sequence shown in SEQ ID NO:15 which has been mutated as defined in part (H) of claim 1 and has the properties indicated therein (enhanced resistance to L-amino acids and/or its analogs and enhanced sensitivity to S-(2-maminoethyl)cysteine), or iii) alteration of the promoter sequence of the said DNA sequence on the chromosome of the bacterium (cf. Section VII *supra*).
3. According to the patent-in-suit, the b3434 gene was selected by screening the complete *Escherichia coli* genome for genes encoding proteins having 4 or more putative transmembrane segments. Thus, the encoded b3434 protein was identified as a putative membrane exporter protein. The nucleic acid sequence of the b3434 gene, also known as *yhgN*, and the encoded amino acid sequence were shown in SEQ ID NO:15 (cf. page 2, paragraph [0006] and page 4, paragraph [0022] of the patent-in-suit). Transformation of *E. coli* with plasmid pYHGN, carrying the b3434 gene under the control of a strong lactose promoter (P_{lac} UV5) and prepared as indicated in Example 1 (cf. page 6, paragraph [0042] of the patent-in-suit), resulted in an enhanced production of the L-amino acids Arg and Pro (cf. page 15, Example 10 and page 16, Example 11 of the patent-in-suit). This disclosure has not been contested in appeal proceedings or in the opposition proceedings.
 4. Thus, the subject-matter of claim 1 is exemplified by increasing the gene copy number and over-expression of the b3434 gene in order to obtain an *E. coli* bacterium with an increased production of L-amino acids. No technical problems or difficulties have been

encountered when carrying out this embodiment (cf. pages 4 and 5, paragraphs [0026] and [0027] of patent-in-suit).

5. Appellant's first objection is based on the absence of a disclosure in the patent of the actual activity of the encoded b3434 protein (cf. Section IX *supra*). Claim 1 states that the activity of the protein as defined in part (G) is enhanced by transforming the bacterium with a DNA sequence encoding this protein, i.e. by increasing the expression of a DNA sequence encoding this protein and eventually increasing the amount of this protein in the transformed *E. coli* bacterium. There is no requirement in claim 1 to enhance the (biological or cellular) activity of the protein as defined in part (G) of this claim other than by increasing its amount. Appellant's objection relates to the clarity of the claim and thus, to Article 84 EPC, rather than to the sufficiency of the disclosure of the patent-in-suit. However, Article 84 EPC is not a ground of opposition.

6. In part (H) of claim 1, the protein is structurally defined as comprising "... *an amino acid sequence including deletion, substitution, insertion or addition of 1-20 amino acids in the amino acid sequence shown in SEQ ID NO:15 in Sequence listing ...*" (cf. Section VII *supra*). Since the amino acid sequence shown in SEQ ID NO:15 has 197 residues, the modification of 1-20 amino acids represents a change of no more than about 10% of the whole sequence. Thus, the proteins defined in part (H) of claim 1 must have at least 90% identity to the amino acid sequence shown in SEQ ID NO:15. The production of proteins in accordance with this

definition, either by random or site-directed mutagenesis, does not pose any technical problem to a skilled person. This structural definition constitutes a generalization of the specific amino acid sequence SEQ ID NO:15 which is in line with the constant practice, and of general acceptance, in the field.

7. All the more so, since these proteins are further defined by functional terms in part (H) of claim 1 by requiring them to have "*... an activity of making bacterium having enhanced resistance to L-amino acids and/or its analogs ... and having enhanced sensitivity to S-(2-aminoethyl)cysteine*" (cf. Section VII *supra*). These proteins which, although fulfilling the structural requirements defined in claim 1 do not have similar functional properties as the specific protein having the amino acid sequence SEQ ID NO:15 shown in Table 1 of the patent-in-suit, do not fall under the scope of the claim.

8. Appellant's objection regarding the proteins defined in part (H) of claim 1 is based on two different arguments. As a first argument, it is argued that since the expression "*L-amino acids and/or its analogs*" is not defined in the patent-in-suit and no correlation is shown in the examples of the patent between the amino acids produced by a transformed bacterium and those to which this bacterium shows enhanced resistance, there is an unlimited number of possible L-amino acids and analogs thereof to which a bacterium transformed with the proteins defined in part (H) could have an enhanced resistance. In the appellant's view, no reliable method would be available to a skilled person for functionally screening these proteins (cf. Section IX *supra*).

9. The board cannot follow this argument. The results shown in Table 1 of the patent-in-suit have not been contested and, although they do not show the correlation referred to by the appellant, they inform a skilled person of the L-amino acid analogs to which an enhanced resistance may be first expected, namely to those to which an *E. coli* transformed with the original amino acid sequence SEQ ID NO:15 shows an enhanced resistance. Indeed, this can be expected for a large number of proteins defined in part (H) of claim 1 having only a small number of arbitrary modifications. Even if this expectation is not fulfilled, the patent-in-suit and, in particular Table 1, shows that a large number of L-amino acids and analogs thereof were available to a skilled person for functionally screening the proteins modified in accordance with part (H) of claim 1 (cf. also pages 3 and 4, paragraphs [0016] to [0019] of the patent-in-suit). Again, appellant's objection relates to the clarity of the claim and, thus to Article 84 EPC, rather than to the sufficiency of the disclosure.

10. In the board's view, this also applies for appellant's second argument which presupposes a reading of part (H) of claim 1 so that its subject-matter comprises an embodiment in which the modified protein makes the transformed bacterium to have an enhanced resistance to all L-amino acids and/or analogs thereof (cf. Section IX *supra*). Whereas the wording of claim 1 may be open to interpretation and thus objectionable under Article 84 EPC, the board does not find any support for appellant's interpretation which, in the light of the whole disclosure of the patent-in-suit and the prior

- art cited therein as well as that on file, is considered not to be technically sensible.
11. Likewise, the board cannot follow appellant's argument that the exemplified production of the L-amino acids Arg and Pro (Examples 10 and 11) does not allow for a generalization to a method of producing L-amino acids in general (cf. Section IX *supra*). In absence of any evidence to the contrary, in view of the structure and properties of these two amino acids, and the possible interconnection of their metabolic biosynthetic pathway with that of other (structurally) related amino acids, the board does not see any reason to deviate from the decision of the opposition division on this issue. As a putative membrane exporter, the b3434 protein could well be functionally appropriate for several amino acids other than Arg and Pro. Moreover, the proteins defined in part (H) of claim 1 (at least 90% identical to the amino acid sequence SEQ ID NO:15) may also allow for a certain degree of variation. The appellant's doubts have not been substantiated by verifiable facts as required by the established case law.

 12. Although the endogenous promoter sequence of the b3434 gene is not disclosed in the patent-in-suit, there is evidence on file, for instance in document D2, showing that methods for identifying consensus sequences of promoter function and specific recognition sequences of RNA polymerase were standard in the field. Even though this document discloses an extensive compilation of *E. coli* promoters, it does not disclose a complete list of all *E. coli* promoters. Document D2 was published in 1993, four years before the publication of the complete *E. coli* genome sequence in 1997 (document D1) and eight

years before the first priority date claimed by the patent-in-suit. The problems identified in document D2 and referred to by the appellant mainly concern deficiencies in the databanks available at that time, such as the presence of duplicated sequences and "*inaccuracies in the locations reported in the bank, in comparison to the experimental paper*", for which document D2 already indicates possible methods to overcome them (cf. page 1512, left-hand column, first two paragraphs of document D2). In the board's view, the absence of the b3434 promoter sequence in the list of document D2 cannot be taken as evidence that its identification involves real technical difficulties for a skilled person. Taking into account the submissions and evidence on file, the board considers that no undue burden would be required to identify and isolate the b3434 promoter sequence. There is also evidence on file showing that, using random mutagenesis and standard screening methods, altered promoters with the desired properties could also be obtained without undue burden (cf. *inter alia*, document D4).

13. It is further noted that, for carrying out the claimed invention, it is actually not even necessary to know the endogenous sequence of the b3434 promoter since a replacement of this sequence is also an "alteration" in the sense of claim 1. The patent-in-suit refers to the use of a strong promoter to express the b3434 or *yhgN* gene (cf. page 5, paragraph [0029] of the patent-in-suit). Indeed, Example 1 describes the construction of plasmid pYHGN carrying the b3434 gene under the control of the lactose promoter (P_{lac} UV5) and Examples 10 and 11 show the enhanced production of Arg and Pro by *E. coli* strains transformed with this

plasmid. As stated in point 3 *supra*, no technical problems arise from this type of promoter alteration (replacement) which is well-known in the art (cf. page 6, paragraph [0042] of the patent-in-suit).

14. Thus, the board considers the patent-in-suit according to the claims as granted to fulfil the requirements of Article 83 EPC.

Article 100(a) EPC; Article 54 EPC

15. Document D1, the sole document discussed in the decision under appeal in the context of novelty (cf. page 4, point 12 of the decision under appeal), discloses the complete genome of *E. coli* K-12. The appellant argues that, by determining this sequence, an *E. coli* bacterium as defined in the claims would have been "*inherently*" produced (cf. Section IX *supra*).
16. The genome of *E. coli* K-12 disclosed in document D1 is obtained by a combination of three different approaches. The first 1.92 Mb (positions 2,686,777 to 4,639,221 in base pairs) are sequenced from an overlapping set of 15 to 20 kb MG1655 lambda clones, a second segment (2,475,719 to 2,690,160) is sequenced using non-overlapping DNA fragments by a popout plasmid approach, and the largest portion of the genome (22,551 to 2,497,976) is sequenced from M13 Janus shotguns with fragments of about 250 kb. The M13 Janus shotgun strategy involves an initial random sequencing at a four to fivefold redundancy and it is the most efficient strategy (cf. page 1453, last paragraph middle column and right-hand column of document D1). There is, however, no information either in document D1

- or in any other document on file on the precise location of the b3434 gene within the *E. coli* K-12 genome and thus, it cannot be excluded with certainty that the b3434 gene is located within the second segment of the *E. coli* genome in which non-overlapping fragments were used.
17. Similarly, with the evidence at hand, it cannot be said with certainty that the complete, full-length sequence of the b3434 gene is inevitably present in at least one of the clones or subclones resulting from the three cloning strategies used in document D1. There is no evidence on file of the presence of a clone containing and identifying the specific sequence of the b3434 gene (SEQ ID NO:15). There is also no evidence on file that any of the clones or subclones obtained by the strategies used in document D1 results in an *E. coli* bacterium having an enhanced production of L-amino acids.
18. Moreover, according to the established case law (cf. *inter alia* T 18/09 21 October 2009, points 10 to 15 of the Reasons), the presence of a cDNA sequence - without any further (indexed) information or identification - in a clone collection, a sequence databank or, as in the present case, in a complete genome sequence of about 4.64 Mb, does not "make available" this cDNA sequence directly and unambiguously to the skilled person. This is in line with the difference made in the case law between an "implicit" and an "inherent" disclosure as laid down in the decision G 2/88 (*supra*).
19. Thus, the board considers the claimed subject-matter to fulfil the requirements of Article 54 EPC.

Article 100(a) EPC; Article 56 EPC

20. In the decision under appeal, the document WO 97/23597 cited in paragraph [0004] of the patent-in-suit is identified as the closest prior art, even though this document was not formally introduced into the opposition proceedings (cf. page 5, point 13.1 and page 6, point 13.3 of the decision under appeal). In paragraph [0004] of the patent-in-suit, it is stated that "... *increased L-amino acid excretion can enhance the productivity of strain producing L-amino acid. Strain of bacterium belonging to the genus Corynebacterium having increased expression of L-lysine excretion gene (lysE gene) is disclosed (WO 9723597A2)*". In the same paragraph, reference is made to other documents stating that "... *several Escherichia coli genes coding for putative membrane proteins enhancing L-amino acid production are disclosed*", such as the *rhtB* gene (EP 0 994 190), the *rhtC* gene (EP 1 013 765), *yahN*, *yeaS*, *yfiK* and *yggA* genes (EP 1 016 710). Although none of these documents was introduced into the opposition proceedings, in view of the strains used (*E. coli*), the nature of the isolated genes and the function of the putative membrane proteins (enhancement of L-amino acid production), the board considers the disclosure in these documents to be a whole body of prior art that may represent the closest prior art for the patent-in-suit.
21. Starting from this prior art, the technical problem to be solved is defined as the provision of further *E. coli* genes encoding membrane proteins that enhance the production of L-amino acids in a transformed

- E. coli* bacterium. Contrary to the appellant's view, the board considers that this technical problem is solved by the claimed subject-matter over the whole scope of the claims without requiring undue burden or the exercise of inventive skills.
22. As in the discussion under Article 83 EPC, the appellant argues that it is not credible that all proteins defined in part (H) of claim 1 and all alterations of the b3434 promoter sequence result in an enhanced production of L-amino acids (cf. Section IX *supra*). However, claim 1 clearly requires, in an explicit manner, the altered promoters and the proteins defined in part (H) of claim 1 to enhance the L-amino acid production of the transformed *E. coli* bacterium. In other words, proteins and altered promoters that do not enhance the production of L-amino acids are simply excluded from the scope of the claims. Claim 1 covers solely subject-matter that actually solves the technical problem. The board considers that the achievement of a technical effect stated in a product claim, which effect is a functional feature of the claimed product, is a requirement of Article 83 EPC and not of Article 56 EPC. In view of the conclusion arrived at by the board on Article 83 EPC (cf. points 2 to 14 *supra*), the board considers the technical problem to be solved by the claimed subject-matter and, indeed, over the whole scope of the claims.
23. Moreover, in view of the prior art on file, the board also considers that the claimed subject-matter is not obvious. Whereas computer-assisted methods for the analysis of the complete *E. coli* genome and the identification of putative membrane (exporter) proteins

were available to the skilled person, the results obtained by these methods could vary significantly depending on the combination of computer programs, parameters and evaluation criteria used. Moreover, as stated in the patent-in-suit (cf. page 2, paragraph [0006], lines 42 and 43 of the patent-in-suit), a further screening was required in order to select those genes which enhanced the production of L-amino acids among all identified genes encoding putative membrane proteins.

24. Although these computer-assisted and screening methods are similar to those methods discussed in the context of Article 83 EPC for the identification of the endogenous b3434 promoter sequence and they were known in the prior art and did not involve any real technical difficulties for a skilled person (cf. point 12 *supra*), the two situations are not comparable. Whereas for the identification of the b3434 promoter sequence, a skilled person had a starting point in the specific sequence SEQ ID NO:15 disclosed in the patent-in-suit and thus, a clear hint where to look for the promoter sequence (within the 5' upstream region of the b3434 gene), the skilled person had no such starting point in the body of prior art documents identified above that could guide his/her efforts to achieve the specific sequence SEQ ID NO:15 among all other possible sequences in an obvious manner.

25. Thus, the board considers the claimed subject-matter to fulfil the requirements of Article 56 EPC.

Order

For these reasons it is decided that:

The appeal is dismissed

The Registrar:

The Chairman:

A. Wolinski

M. Wieser