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**Datasheet for the decision  
of 4 February 2011**

**Case Number:** T 1451/09 - 3.3.08

**Application Number:** 01960723.3

**Publication Number:** 1317547

**IPC:** C12N 15/54

**Language of the proceedings:** EN

**Title of invention:**

Isolation and sequencing of the pknB gene of *C. glutamicum*

**Applicant:**

Evonik Degussa GmbH

**Headword:**

Corynebacteria lysin production/EVONIK

**Relevant legal provisions:**

EPC Art. 54, 56

**Relevant legal provisions (EPC 1973):**

-

**Keyword:**

"Main request - novelty (yes); inventive step (yes)"

**Decisions cited:**

T 0038/84

**Catchword:**

-



Case Number: T 1451/09 - 3.3.08

**D E C I S I O N**  
of the Technical Board of Appeal 3.3.08  
of 4 February 2011

**Appellant:** Evonik Degussa GmbH  
Rellinghauser Straße 1-11  
D-45128 Essen (DE)

**Representative:** -

**Decision under appeal:** Decision of the Examining Division of the  
European Patent Office posted 13 February 2009  
refusing European patent application  
No. 01960723.3 pursuant to Article 97(2) EPC.

**Composition of the Board:**

**Chairman:** M. Wieser  
**Members:** P. Julià  
C. Heath

## Summary of Facts and Submissions

- I. The applicant (appellant) lodged an appeal against the decision of the examining division dated 13 February 2009, whereby the European patent application No. 01 960 723.3 published as WO 02/22828 (hereinafter referred to as "the application as filed") was refused on the basis of Article 97(2) EPC.
- II. Basis for the refusal was the set of claims then on file, corresponding to claims 1 to 15 and claim 16 (partially) filed with letter dated 5 November 2008 and claim 16 (partially) and claims 17 and 18 filed with letter dated 13 November 2008, which was considered by the examining division not to fulfil the requirements of Articles 56, 57, 83 and 84 EPC (cf. point X *infra*).
- III. With the statement setting out the grounds of appeal, the appellant filed a main request and three auxiliary requests, the main request being identical to the set of claims refused by the examining division. Oral proceedings were requested as a precautionary measure.
- IV. The examining division did not rectify its decision and referred the appeal to the Board of Appeal (Article 109(2) EPC).
- V. In a communication pursuant to Article 15(1) RPBA annexed to the summons to oral proceedings, the board informed the appellant of the main issues to be discussed at the upcoming oral proceedings, in particular those concerning Articles 84, 56 and 83 EPC. The board also introduced document D4 into the appeal proceedings (cf. point IX *infra*).

VI. On 3 February 2011 (one day in advance of the oral proceedings), the appellant filed a further amended set of claims 1 to 13 as its fourth auxiliary request.

VII. Oral proceedings took place on 4 February 2011. In these proceedings, the appellant filed a new main and sole request to replace all its previous requests then on file. The appellant also filed a description adapted to this new main and sole request.

VIII. The appellant's main and sole request consisted of claims 1 to 12, wherein claim 1 read as follows:

"1. An isolated polynucleotide from corynebacteria selected from the group comprising:

- a) a polynucleotide containing the nucleotide sequence of SEQ ID NO: 1;
- b) a polynucleotide which is complementary to the polynucleotide of a)."

Claims 2 and 3 related to particular embodiments of claim 1. Claims 4 to 6 were directed to corynebacteria in which the *pknB* gene - defined as a polynucleotide containing the nucleotide sequence of SEQ ID NO: 1 - was overexpressed. Claim 7 was directed to a vector comprising a polynucleotide according to claims 1 to 3. Claims 8 to 12 were directed to a fermentation process for the preparation of L-lysine using the transformed corynebacteria of claims 4 to 6.

IX. The following documents are cited in the present decision:

- D1: Database SWALL EBI; 1 October 1996, "Mycobacterium leprae pknB", Accession number P54744;
- D2: EP-A-1 108 790 (filing date: 18 December 2000, publication date: 20 June 2001);
- D3: EP-A-1 029 919 (filing date: 8 February 2000, publication date: 23 August 2000);
- D4: Fiuza M. et al. 2008, J. Biol. Chem., Vol. 283 No. 26, pages 18099 to 18112;

"Example 3" filed during examination proceedings with the appellant's letter dated 13 January 2009.

- X. The reasoning which led the examining division to the refusal was briefly the following:

Document D3, which was considered to be the closest prior art, disclosed a mutant strain of a *Corynebacterium glutamicum* that overexpressed an enzyme involved in the metabolism of L-amino acids. Starting from that prior art, the technical problem to be solved was formulated as the provision of further *C. glutamicum* mutants that overexpressed enzymes involved in L-amino acid metabolism. The solution was seen in the provision of a polynucleotide containing the nucleotide sequence of SEQ ID NO: 1 and encoding the amino acid sequence of SEQ ID NO: 2. This solution was considered not to be inventive because there was no technical data in the application to support the alleged function of the encoded protein of SEQ ID NO: 2 as a protein kinase. This deficiency was considered not to be overcome in the light of the sequence alignment

of SEQ ID NO: 2 and the *pknB* sequence from *Mycobacterium leprae* disclosed in document D1 or in view of the results shown in "Example 3" filed by the applicant. Whereas the low sequence similarity - less than 40% identity - did not allow to conclude with certainty that the protein of sequence SEQ ID NO: 2 was a protein kinase, the results of "Example 3" showed that the difference in the production of L-lysine - between a strain transformed with a control plasmid and a strain transformed with the *pknB* disclosed in the application - was rather insignificant and not sufficient to support a possible role of the disclosed *pknB* in the metabolism of lysine. Thus, the provision of the sequences SEQ ID NO: 1 and 2 did not solve the technical problem or, indeed, any problem at all. The lack of a confirmed function was also detrimental for industrial applicability.

XI. The appellant's arguments, as far as relevant for the present decision, may be summarized as follows:

The *pknB* protein disclosed in the application had been detected in the course of general screening methods and unexpectedly shown to have an effect on the production of amino acids. Indeed, as supported by "Example 3", overexpression of the *pknB* gene resulted in an increase production of L-lysine. There were no criteria set out in the EPC or in the established case law of the Boards of Appeal for defining when a quantitative improvement was significant or not. According to decision T 38/84 (OJ EPO, 1984, page 368) even "*(t)he achievement of a numerically small improvement of a process commercially used on a large scale (...) represents a worthwhile technical problem which must not be disregarded in*

*assessing the inventive step of its solution as claimed*". The production of L-lysin involved a large-scale commercial process for which small improvements provided significant benefit. Whereas in decision T 38/84 (*supra*), a 0.5% improvement in a one-step process had been considered significant, in the present case an improvement of almost 5% was achieved in a multi-step process. This was all the more surprising because it was not to be expected that an improvement in only one step of a multi-step process would still be evident at the end of that process. Although the yield improvement shown in "Example 3" was below that obtained with the method disclosed in document D3, there was nevertheless a significant improvement in relation to non-modified bacteria for which there was absolutely no hint in the prior art.

Contrary to the detrimental and toxic effects found in document D4 when overexpressing a *pknB* gene in *C. glutamicum*, "Example 3" showed that the optical density of non-transfected *C. glutamicum* strains was the same as that of strains transfected with the *pknB* gene disclosed in the application, i.e. the same number of cells was present in both cases. This result spoke against the presence of eventual toxic effects on cell viability when overexpressing the *pknB* gene. Indeed, the growth curves shown in Figure 7B of document D4 confirmed that the non-transfected, control strain had already stopped growing while the transfected strain was still growing.

Although the nucleotide sequence used in the experiments reported in "Example 3" did not correspond to the whole sequence of SEQ ID NO: 1 but only to that

encoding a full-length, complete pknB protein (which had a 19 amino acid residues longer N-terminus compared to that of the sequence SEQ ID NO: 2 disclosed in the application), there was no evidence on file - nor was the appellant aware of any - showing that the use of the whole sequence SEQ ID NO: 1 could have any impact on the results obtained in these experiments.

XII. The appellant requested that the decision under appeal be set aside and a patent be granted on the following basis:

- Claims 1 to 12 according to the main (and sole) request as filed in oral proceedings, and a description as follows:
- description pages 1-6, 8-10, 12-16, 18 and 21 as filed in oral proceedings;
- description pages 7, 11, 17 and 19-20 as published;
- sequence listing pages 1-12 as published.

## **Reasons for the Decision**

### *Admissibility of the appellant's main and sole request*

1. The main and sole request filed at the oral proceedings is based on a request filed on the day before the oral proceedings which took into account the objections raised by the board in its communication under Article 15(1) RPBA by limiting the claimed subject-matter. The amendments and corrections carried out directly address issues and objections raised by the board. They do not introduce unexpected subject-matter but instead consist of deletion of subject-matter present in previous requests on file. As



such, the board in exercising its discretion according to Article 13(1) RPBA decides to admit the appellant's main and sole request into the appeal proceedings.

*Appellant's main and sole request*

*Articles 123(2) and 84 EPC*

2. There were no objections raised under Article 123(2) EPC by the examining division in the decision under appeal nor by the board in its communication pursuant to Article 15(1) RPBA (*supra*). The board sees no reason to deviate from this position and considers that the claimed subject-matter fulfils the requirements of Article 123(2) EPC.
  
3. The subject-matter of the appellant's main and sole request relates only and exclusively to an isolated polynucleotide containing the nucleotide sequence of SEQ ID NO: 1. It is noted that the sequence SEQ ID NO: 1 encodes the N-terminal truncated pknB protein of sequence SEQ ID NO: 2 (starting ATG codon at position 594-596 of SEQ ID NO: 1) disclosed in the application as well as the full-length, complete sequence of the pknB protein (alternative starting GTG codon at position 537-539 of SEQ ID NO: 1) disclosed in document D2 (SEQ ID NO: 46 and 3546 of document D2) and in Figure 1 of post-published document D4 (cited as an expert opinion). Whereas in document D2 - as in the present application - a kinase activity is postulated based only on sequence comparison (cf. point 5 *infra*), a kinase activity is measured and reported in both "Example 3" (cf. point 11.3 *infra*) and document D4 (cf. page 18102, left-hand column, last paragraph and page 18103, right-hand column, last full paragraph of

document D4). Since none of the claims of the appellant's main and sole request is directed to the specific N-terminal truncated pknB protein of SEQ ID NO: 2 disclosed in the application, the board does not see any reason to raise an objection under Article 84 EPC in this respect. Nor does the board see any other reason to raise an objection under this Article for the claimed subject-matter, which is thus considered to fulfil the requirements of Article 84 EPC.

*Article 54 EPC*

4. Document D2 - with a filing date of 18 December 2000 - is part of the prior art pursuant to Article 54(3) EPC and, since the present application is entitled to the first claimed priority (DE 100 44 912.3 of 12 September 2000), it is thus only relevant for the assessment of novelty.
  
5. Document D2 discloses the complete genome of the *Corynebacterium glutamicum* ATCC 13032 strain, with a size of 3.309.400 bp, obtained by constructing shotgun and cosmid libraries, and then sequencing and assembling the library clones (cf. SEQ ID NO: 1 and page 9, paragraph [0018] to page 13, paragraph [0088]). Identification of open reading frames (ORFs) and the putative function of the encoded proteins is established by sequence homology with known protein and amino acid sequences (cf. page 35, paragraphs [0367] to [0373] and pages 36 to 223, Table 1). In Table 1, SEQ ID NO: 46 is identified - among 3501 listed nucleotide sequences - as coding for a serine/threonine protein kinase with amino acid sequence SEQ ID NO: 3546 which

is homologous - 40.6% identity and 68.7% homology - to the known *pknB* from *M. leprae* (cf. page 36).

6. The polynucleotide of sequence SEQ ID NO: 46 has a length of 1938 nucleotides corresponding only to the coding region of the *pknB* gene and is, therefore, shorter than the sequence of SEQ ID NO: 1 disclosed in the present application (2875 nucleotides). Thus, the specific sequence SEQ ID NO: 46 of document D2 does not contain the claimed nucleotide sequence SEQ ID NO: 1 and, as such, it does not fall within the scope of the claimed subject-matter. Moreover, it is not directly derivable from the information given in the examples of document D2 whether a polynucleotide containing the polynucleotide sequence of SEQ ID NO: 46 was found within any of the isolated clones used to determine the whole genome sequence of *C. glutamicum* ATCC 13032 or else if it was derived from several isolated clones having overlapping nucleotide sequences.
  
7. Although the whole genomic sequence of *C. glutamicum* ATCC 13032 disclosed in document D2 appears to include the complementary sequence of the nucleotide sequence SEQ ID NO: 1 of the present application (cf. nucleotides 43049 to 40175 of SEQ ID NO: 1 of document D2), this sequence, however, is not disclosed as an "isolated polynucleotide". Indeed, the polynucleotide sequence of SEQ ID NO: 1 disclosed in document D2 was assembled - in its whole - using a computer and the sequence information derived from several, partially overlapping nucleotide sequences from different clones (cf. in particular paragraphs [0360] and [0361]). It is also noted that there is no reference to SEQ ID NO: 46 or SEQ ID NO: 3546 in Examples 2 and 3 of document D2

concerned with L-lysine producing *C. glutamicum* strains nor in Example 4, which describes the production of a DNA array and uses thereof, or in any of the other examples of that document which include the search and identification of expressed proteins effective in lysine production (cf. pages 224 to 235 of document D2).

8. Document D2 is considered not to anticipate the claimed subject-matter which is thus acknowledged to fulfil the requirements of Article 54 EPC.

*Article 56 EPC*

*Determination of the closest prior art and the technical problem to be solved*

9. According to the established case law of the Boards of Appeal, the closest prior art is normally a prior art document disclosing subject-matter conceived for the same purpose or aiming at the same objective as the claimed invention (cf. "Case Law of the Boards of Appeal of the EPO", 6th edition 2010, I.D.3.1, page 163). Document D3, which, like the present application, is directed to the production of L-amino acids (L-lysine) using *Corynebacterium glutamicum*, is considered to be the closest prior art. This document discloses the transformation of a specific *C. glutamicum* ATCC 13032 strain - the same strain as that used in the present application - to overexpress a glutamate-dehydrogenase gene in order to increase the production of L-amino acids such as L-lysine (cf. page 5, line 30 to page 6, line 40, Examples 1 and 2, and page 7, line 34 to page 8, line 30, Example 5). The specific reference strain used in Examples 1, 2 and 5 of that document is *C. glutamicum* DSM5715, a L-lysine

producing strain derived from the *C. glutamicum* MH20-22B strain (cf. point 11.3 *infra*) which has been transformed with genes encoding enzymes involved in the biosynthesis of L-lysine (cf. page 5, line 24 in paragraph [0030] of document D3 with reference to EP 0 435 132).

10. Starting from this closest prior art, the technical problem to be solved is formulated as the provision of a further (alternative) method for improving the yield of L-lysine production in *C. glutamicum* when compared to the yield obtained in a non-genetically modified or non-transformed *C. glutamicum* strain. The solution proposed by the application is a *C. glutamicum* strain transformed with - and overexpressing - the *pknB* gene of sequence SEQ ID NO: 1.

*Does the solution proposed in the application solve the technical problem?*

11. Contrary to the examining division (cf. point X *supra*), the board considers that the proposed solution solves the above technical problem and that the application provides enough data to allow the skilled person to conclude that the protein encoded by SEQ ID NO: 1 is a protein kinase which has an effect on the production of L-lysine.
  - 11.1 First, the degree of homology within different protein members of a protein family is not always high. More significant than the overall homology is the presence of relevant highly conserved domains and/or motifs which are known to be of importance for the common function(s) and/or structure of the members of that

protein family, such as in the serine/threonine kinases. In the present case, although not explicitly mentioned in the application, the amino acid sequence of SEQ ID NO: 2 contains domains and motifs which allow the skilled person to identify the protein as a member of the serine/threonine kinase family. This is confirmed by document D2 which, based also only on homology comparison, acknowledges this amino acid sequence - albeit a full-length, complete pknB sequence (SEQ ID NO: 46 and 3546) - to be homologous to the pknB sequence known from document D1 (cf. page 38 of document D2). In post-published document D4 (cited as an expert opinion), the presence of those relevant conserved domains and motifs within the (full-length, complete pknB) amino acid sequence is explicitly shown in Figure 1 (cf. page 18104, Figure 1 of document D4). In the light of all this evidence, the board does not see any reason to doubt that the identification in the application of the amino acid sequence of SEQ ID NO: 2 as a pknB protein and of its suggested function as a kinase is correct (cf. *inter alia* page 6, last two lines of the present application).

- 11.2 Second and notwithstanding the above observations, the board considers that whether the protein encoded by the sequence of SEQ ID NO:1 is a kinase (and for that matter a pknB kinase) or not, is of little weight for assessing whether the claimed subject-matter actually solves the technical problem. Independently of its putative function as a kinase, the critical issue to be settled by the board is whether its overexpression in *C. glutamicum* does provide the effect of improving the production of L-lysine as alleged in the application. Indeed, the disclosure of the application explicitly

refers to an improvement of amino acid production by overexpression of the protein kinase gene disclosed in the application (cf. page 10, third paragraph and page 12, second paragraph of the application). There is, however, no technical evidence in the application to support these statements. Technical evidence was filed by the appellant during the examination proceedings in a document entitled "Example 3" (cf. point IX *supra*). According to the case law of the Boards of Appeal, post-published and/or late experimental evidence may in the proper circumstances be taken into consideration, namely when it is used to support information or to back up results which are already derivable from the original application (cf. "Case Law", *supra*, I.D.4.6, page 175). The board is convinced that, in the current circumstances, the experiments reported in "Example 3" must be taken into consideration.

- 11.3 Third, the experiments reported in "Example 3" show that the transformation of a reference MH20-22B strain - an aminoethyl-cysteine-resistant, L-leucine auxotrophic, L-lysin producing *C. glutamicum* strain obtained by random mutagenesis which has a feedback-resistant aspartate kinase and is devoid of isopropylmalate dehydratase (cf. page 3, last paragraph in "Example 3" with reference to EP 0 318 663) - with the *pknB* gene disclosed in the application results in increased L-lysin production after 72 hours of culture when compared to production of the non-transformed MH20-22B strain (cf. page 6, Table 1 of "Example 3"). Although this increase is far from that achieved in document D3 (more than 30% versus less than 10%), the board, contrary to the examining division (cf. point X *supra*), considers this increase to be significant and

sufficient to support the statements found in the application, namely that the overexpression of the disclosed *pknB* gene has a positive effect, although admittedly small, on the production of L-lysine by *C. glutamicum*.

12. The board notes that the polynucleotide of sequence SEQ ID NO: 1 is not used in the experiments reported in "Example 3" but only a shorter sequence which essentially includes only the coding region of a full-length, complete *pknB* kinase as disclosed in documents D2 and D4. Indeed, the primers of sequences SEQ ID NO: 5 and 6 in "Example 3" correspond to regions close, respectively, to the starting GTG codon and to the C-terminus encoding codon of SEQ ID NO: 1 (cf. page 1, example 3.1 of "Example 3"). As a result thereof, the cDNA cloned in "Example 3" is about 800 nucleotides (about 500 nucleotides at the 5' upstream region and about 300 nucleotides at the 3' downstream region) shorter than the polynucleotide of sequence SEQ ID NO: 1. However, in the absence of any evidence to the contrary, the board accepts the appellant's allegations that the presence of these additional 800 nucleotides has no effect on the expression of the encoded *pknB* kinase or on the results obtained in the production of L-lysine.
  
13. As regards the disclosure of post-published document D4 (cited as an expert opinion) which reports the presence of important problems when a *pknB* gene is overexpressed in *C. glutamicum*, such as delayed growth rate, diminished viability and presence of aberrant cells (lacking DNA) (cf. page 18110, left-hand column, last paragraph to page 18111, left-hand column, first



paragraph and page 18110, Figure 7B of document D4), the appellant has drawn the attention of the board to Table 1 of "Example 3" which, in the column under the heading "OD" (optical density of cell cultures which corresponds to the cell density), does not show any impaired growth of the transformed host bacteria MH20-22B/pEC-XK99EpknB when compared to that of the non-transformed reference MH20-22B strain (cf. page 6, Table 1 of "Example 3"). Moreover, document D4 concerns a *C. glutamicum* ATCC 13869 strain (cf. *inter alia* page 18101, left-hand column, first paragraph of document D4) which is different from the *C. glutamicum* ATCC 13032 strain used in the present application. Figure 7B of document D4 also shows different growth patterns for non-transformed and transformed strains, the former reaching a plateau earlier than the latter but all reaching similar levels after enough culture time. The appellant has also referred to in-house industrial and experimental results for which none of the drawbacks outlined in document D4 were found.

14. It follows from the considerations above and the evidence on file, that the solution proposed in the application, i.e. the claimed subject-matter, solves the technical problem as formulated in point 10 above.

*Is the solution proposed in the application obvious?*

15. There is no indication in the prior art on file suggesting that the pknB protein kinase from *C. glutamicum* - or any of its homologues known from the prior art - may be involved or have a possible function in the biosynthesis of L-amino acids and, in particular, of L-lysine, let alone that an overexpression of the

pknB gene in Corynebacteria could result in increased production of L-lysin. In the absence of such an indication, the board is convinced that it would not have been obvious for a skilled person to arrive at the claimed solution which is thus considered to be unexpected and surprising.

*Conclusion on Article 56 EPC*

16. In the light of the foregoing considerations, the board comes to the conclusion that the subject-matter of the appellant's main and sole request fulfils the requirements of Article 56 EPC.

*Articles 83 and 57 EPC*

17. In the decision under appeal, the examining division considered that the set of claims then on file contravened Articles 83 and 57 EPC because, in its opinion, no conclusions could be drawn from the experimental results filed by the appellant nor could a function be assigned to the polypeptide disclosed in the application (cf. points II and X *supra*). The board, however, cannot agree with the reasoning of the examining division in this respect.
18. As regards Article 83 EPC, the board considers the application to provide an enabling disclosure of the polynucleotide sequence of SEQ ID NO: 1 and a clear indication on how to use that sequence for achieving the claimed embodiments. The results provided in "Example 3" support only statements already found in an explicit manner in the application and, although only a relatively small effect is shown in "Example 3", this

effect is considered to be significant (cf. points 11.1 to 11.3 *supra*), in particular, in the absence of any evidence to the contrary (cf. points 12 and 13 *supra*).

19. As regards Article 57 EPC, the importance of the industrial production of L-lysine is well acknowledged in the art and, in view of the effect shown when overexpressing the disclosed *pknB* gene, the board is convinced that industrial applicability is given for the claimed subject-matter.

*Adaptation of the description*

20. The granted description has been amended to bring it into line with the invention as claimed in the appellant's main and sole request and it does not give rise to any objection under the EPC.

**Order**

**For these reasons it is decided that:**

1. The decision under appeal is set aside.
2. The case is remitted to the first instance with the order to grant a patent based on the set of claims filed in the oral proceedings, and description pages 1-6, 8-10, 12-16, 18 and 21 filed during oral proceedings, description pages 7, 11, 17, 19 and 20 as published, and sequence listing pages 1-12 as published.

The Registrar:

The Chairman:

A. Wolinski

M. Wieser

**Internal distribution code:**

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**Datasheet for the decision  
of 4 February 2011**

**Case Number:** T 1451/09 - 3.3.08

**Application Number:** 01960723.3

**Publication Number:** 1317547

**IPC:** C12N 15/54

**Language of the proceedings:** EN

**Title of invention:**

Isolation and sequencing of the pknB gene of *C. glutamicum*

**Applicant:**

Evonik Degussa GmbH

**Headword:**

Corynebacteria lysin production/EVONIK

**Relevant legal provisions:**

EPC R. 140

**Relevant legal provisions (EPC 1973):**

-

**Keyword:**

"Correction of an error"

**Decisions cited:**

-

**Catchword:**

-



Case Number: T 1451/09 - 3.3.08

**D E C I S I O N**  
of  
correcting an error in the decision of  
the Technical Board of Appeal 3.3.08  
of 4 February 2011

**Appellant:** Evonik Degussa GmbH  
Rellinghauser Straße 1-11  
D-45128 Essen (DE)

**Representative:** -

**Decision under appeal:** Decision of the Examining Division of the  
European Patent Office posted 13 February 2009  
refusing European patent application  
No. 01960723.3 pursuant to Article 97(2) EPC.

**Composition of the Board:**

**Chairman:** M. Wieser  
**Members:** P. Julià  
C. Heath

In application of Rule 140 EPC, the decision of the Technical Board of Appeal dated 4 February 2011 is hereby corrected as follows:

On page 2, line 5 of the adapted description remitted to the department of first instance the wording "This object is achieved according to claims 1 to 13" is replaced by:

"This object is achieved according to claims 1 to 12."

The Registrar:

The Chairman:

A. Wolinski

M. Wieser