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D E C I S I O N
of 11 June 2003

Case Number: T 0003/00 - 3.3.8

Application Number: 91309737.4

Publication Number: 0482879

IPC: C12N 15/57

Language of the proceedings: EN

Title of invention:
Protease from Bacillus licheniformis

Patentee:
SHIONOGI SEIYAKU KABUSHIKI KAISHA trading under the name of SHIONOGI & CO. LTD.

Opponent:
NOVOZYMES A/S

Headword:
Protease/SHIONOGI

Relevant legal provisions:
EPC Art. 56, 123(2)

Keyword:
"Main request: added matter (yes)"
"Auxiliary request: inventive step (yes)"

Decisions cited:
-

Catchword:
-

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Case Number: T 0003/00 - 3.3.8

D E C I S I O N
of the Technical Board of Appeal 3.3.8
of 11 June 2003

Appellant: (Opponent)	NOVOZYMES A/S Krogshoejvej 36 DK-2880 Bagsvaerd (DK)
Representative:	Bublak, wolfgang, Dr Patent- und Rechtsanwälte Bardehle, Pagenberg, Dost, Altenburg, Geissler Galileiplatz 1 D-81679 München (DE)
Respondent: (Proprietor of the patent)	SHIONOGI SEIYAKU KABUSHIKI KAISHA trading under the name of SHIONOGI & CO. LTD. 1-8, Doshomachi 3-chome Chuo-ku Osaka 541 (JP)
Representative:	Benedum, Ulrich Max, Dr Haseltine Lake Partners Rosenheimerstrasse 30 D-81669 München (DE)
Decision under appeal:	**Decision of the Opposition Division of the European Patent Office posted 12 October 1999 concerning maintenance of European patent No. 0482879.in amended form.**

Composition of the Board:

Chairman: L. Galligani
Members: T. J. H. Mennessier
S. C. Perryman

Summary of Facts and Submissions

I. The opponent (appellant) lodged an appeal against the interlocutory decision of the opposition division dated 12 October 1999, whereby the European patent No. 0 482 879 was maintained on the basis of the fourth auxiliary request as taken into consideration at the oral proceedings on 10 February 1998.

II. The patent had been opposed under Article 100(a) EPC on the grounds that the invention was not new and did not involve an inventive step. The opposition division had refused the other requests then on file on the grounds that they contravened, respectively, Article 123(3) EPC (main request), Article 123(2) EPC (second auxiliary request) and Article 54 EPC (first and third auxiliary requests).

III. In reply to the statement of grounds of appeal, the respondent (patentee) filed observations with a letter dated 2 August 2000.

IV. On 11 March 2003, the board issued a communication under Article 11(2) of the Rules of Procedure of the Boards of Appeal indicating some preliminary and non-binding views of the board on the matters of the case.

V. Oral proceedings took place on 11 June 2003. They were attended by both parties. In addition to the claims as maintained by the opposition division, which it regarded as its **main request**, the respondent filed an **auxiliary request.**

VI. Claim 1 of the **main request** (claims 1 to 31) read:

"1. A DNA sequence encoding a protease molecule which contains an amino acid sequence from serine in the +1 position to glutamine in the +222 position of SEQ ID NO: 1, and cleaves the peptide bonds at the carboxyl termini of glutamic acid residues in polypeptides, which DNA sequence hybridizes to a base sequence complementary to a base sequence from the thymine residue in the 605 position to the adenine residue in the 1270 position of SEQ ID NO:1 with at least 80% homology."

VII. The **auxiliary request** consisted of 15 claims.

Claim 1, 4, 6, 8 and 9 read:

"1. A DNA sequence encoding a protease molecule which contains an amino acid sequence from serine in the +1 position to glutamine in the +222 position of SEQ ID NO: 1, and cleaves the peptide bonds at the carboxyl termini of glutamic acid residues in polypeptides, which DNA sequence contains a base sequence from the thymine residue in the 605 position to the adenine residue in the 1270 position of SEQ ID NO:1."

"4. An expression vector containing a DNA sequence of claim 1, 2, or 3."

"6. A transformant obtainable by introducing the expression vector of claim 4 or 5 into a host."

"8. A method for producing a protease comprising the steps of cultivating a transformant of claim 6 or 7 in a culture medium and recovering the produced protease from the culture medium."

"9. A method for producing a protease which contains an amino acid sequence from serine in the +1 position to glutamine in the +222 position of SEQ ID NO:1, which method comprises:
(i) isolating, from Bacillus licheniformis strain ATCC 14580, a DNA sequence encoding said protease;
(ii) constructing an expression vector containing said DNA sequence;
(iii) transforming a host with said expression vector to produce a transformant; and
(iv) cultivating the transformant in a culture medium and recovering the produced protease from the medium."

The remaining claims were dependent claims.

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

(D5) US-A-4 266 031;

(D6) Takuro Niidome et al., J. Biochem., Vol. 108, No. 6, December 1990, Pages 965 to 970;

(D7) Norio Yoshida et al., J. Biochem., Vol. 104, 1988, Pages 451 to 456;

(D10) EP-A2-0 369 817;

(D12) Comparative data (BLase DNA sequence versus RP-II DNA sequence) filed by the appellant;

(D14) Alan Sloma et al., J. Bacteriol., Vol. 172, No. 2, February 1990, Pages 1024 to 1029;

(D16) Declaration of Prof. Dr Dieter H. Wolf dated 16 February 2000, with exhibits A to F;

(D17) Gerald A. Rufo et al., J. Bacteriol., Vol. 172, No. 2, February 1990, Pages 1019 to 1023.

IX. The appellant's submissions in writing and during the oral proceedings, insofar as they are relevant to the present decision, can be summarized as follows:

Main request (added matter; sufficiency of disclosure)

Claim 1 included the additional feature related to the degree of homology of the DNA sequence, thereby defining a range of homology with a lower limit of 80% and an upper limit of 100%. There was no explicit disclosure for that range in the application as filed. The half-sentence from page 17, lines 14 and 15 of the published application (A2 publication) referred to by the respondent as providing a support for that feature related to a "DNA fragment which has about 80% homology with the DNA sequence of BLase", "BLase" being the protease which was isolated from Bacillus licheniformis strain ATCC 14580. Moreover, that half-sentence had been taken out of context, by ignoring the precisely defined experimental conditions which had allowed to obtain a DNA fragment with about 80% homology. The feature had been added arbitrarily and was not clearly and

unambiguously derivable from the technical information contained in the application as filed, with the result that the requirements of Article 123(2) EPC were contravened. Moreover, there was no disclosure provided by the description with respect to the specific conditions required for obtaining a DNA sequence having at least 80% homology to that of BLase, except a sequence which had about 80% homology. Thus, the requirements of Article 83 EPC were not met.

Auxiliary request (inventive step)

Although there were no objections under Articles 123, 84, 83 and 54 EPC, the subject-matter of claim 1 lacked an inventive step. The underlying technical problem, in the light of the protein RP-II described in the closest prior art document (D10) and of the DNA encoding that protein as referred to therein, was regarded as the provision of a DNA sequence encoding a further protein with similar proteolytic activity. The solution proposed in claim 1 was obvious in view of document (D10) in combination with documents (D14) and (D17), both relating to a protein Mpr which was the same protein as RP-II, as well as, subsidiarily, document (D5), the component C of which was the BLase protein referred to in the patent. The declaration of Prof. Wolf (D16) further confirmed this view.

X. The respondent's submissions in writing and during the oral proceedings, insofar as they are relevant to the decision, can be summarized as follows:

Main request (added matter, sufficiency of disclosure)

The additional feature in relation to the degree of homology of the DNA sequence in claim 1 found a support in a passage of the application as filed corresponding to the passage from line 44 on page 16 to line 15 on page 17 in the published application. It was inherent therefrom that not only a DNA having 80% homology but also DNAs having from 80 to 100% homology were described. Thus, the requirements of both Articles 123(2) and 83 EPC were met.

Auxiliary request (inventive step)

Document (D10) did not represent the closest prior art. The starting point for evaluating inventive step was the knowledge about the glutamic acid-specific serine protease derived from the V8 pathogenic strain of Staphyloccus aureus, as referred to in the patent specification (cf page 3, lines 15 to 19). The technical problem was the provision of a DNA encoding an alternative glutamic acid specific serine protease which is less toxic and dangerous than the V8 serine protease. The solution to that problem as proposed in claim 1 involved an inventive step.

XI. The appellant requested that the decision under appeal be set aside and that the patent be revoked.

XII. The respondent requested that the appeal be dismissed or as auxiliary request that the decision be set aside and the patent be maintained on the basis of:

Claims:
1 to 15 as submitted at the oral proceedings on
11 June 2003;

Description:
Pages 3, 3a and 4 to 23 as submitted at oral proceedings on 11 June 2003;

Figures:
As granted.

Reasons for the Decision

Claims as maintained by the opposition division (main request)

Article 123(2) EPC

1. Claim 1 is directed to a DNA sequence which is characterised in that: (a) it contains an amino acid sequence from serine in the +1 position to glutamine in the +222 position of SEQ ID NO: 1, (b) cleaves the peptide bond at the carboxy termini of glutamic acid residues in polypeptides, and (c) hybridizes to a base sequence complementary to a base sequence from the thymine residue in the 605 position to the adenine residue in the 1270 position of SEQ ID NO:1 **with at least 80% homology**.

2. The appellant argues that, due to the expression "with at least 80% homology" introduced into the claim during the opposition proceedings, feature (c) has no basis in the application as filed.

3. The respondent submits that feature (c) has support in the passage of the description starting at line 44 on page 16 and finishing at line 15 on page 17 in the A2-publication (which corresponds in its wording to the application as filed). That passage discusses how to prepare a DNA sequence which hybridizes to the DNA sequence of "BLase", "BLase" being the protease which is produced by Bacillus licheniformis ATCC No. 14580 (see page 3, lines 42 to 44 in the A2-publication). The procedure referred to therein basically relies on a screening process of DNA fragments derived from various organisms using as a probe the whole or a part of the DNA sequence of BLase as represented in SEQ ID NO: 1. An example is given: after the hybridization has been carried out at 65°C overnight, employing the Southern hybridization technique and using a well-defined hybridization buffer, a filter, to which the probe has been hybridized, is washed. When the filter is washed once for 20 minutes at 50°C, **a DNA fragment which has about 80% homology with the DNA sequence of BLase** can be obtained.

4. There is no evidence whatsoever that the so obtained DNA sequence, which was not tested, actually encodes the protease molecule of 222 amino acid residues of SEQ ID NO: 1, as required in claim 1.

5. There is, therefore, no unambiguous implicit or explicit support, in the only passage of the application as filed referred to by the respondent, for a DNA sequence which hybridizes to a base sequence from the thymine residue in the 605 position to the adenine residue in the 1270 position of SEQ ID NO: 1 with about 80% homology, **and** which encodes the protease molecule

of 222 amino acid residues of SEQ ID NO: 1, let alone for such a DNA sequence which has "at least 80% homology". Consequently, the introduction into claim 1 of the contested expression has resulted in the patent being amended in such a way that it contains subject-matter which extends beyond the content of the application as filed. Therefore, the main request does not meet the requirements of Article 123(2) EPC and, thus, is not allowable.

Auxiliary request

Formal requirements of Articles 84 and 123 EPC, sufficiency of disclosure (Article 83 EPC), novelty (Articles 54 EPC)

6. The appellant has no objections as regards the compliance of the amended claims with the requirements of Articles 123, 84, 83 and 54 EPC. Also in the board's judgment these requirements are met, as the subject-matter of all claims is within the ambit of protection of the claims as granted, is supported by the application as filed, is clearly and unambiguously defined, is sufficiently disclosed and is novel. Moreover, the amendments proposed arise from the appeal and there is no case of "*reformatio in peius*".

Inventive step (Article 56 EPC)

The invention (claim 1)

7. Claim 1 is directed to a DNA sequence which encodes a protease having 222 amino acid residues as represented in SEQ ID NO: 1. Said protease was isolated from **Bacillus licheniformis** ATCC No. 14580 (see page 4,

lines 38 to 40, in the patent specification). It **is a glutamic acid specific endopeptidase** and is **a typical serine protease** (see page 7, lines 6 to 8 and 35 to 43, respectively, in the patent specification).

The state of the art as cited by the parties in the opposition proceedings

8. For the assessment of inventive step the parties rely on the prior art documents (D17), (D14), (D10) and (D5). These documents are hereinafter briefly analysed in order to establish their suitability to represent the closest prior art.

9. Document (D17) describes the isolation and characterisation of **Mpr**, a protease which is secreted by **Bacillus subtilis**. To categorize Mpr with regard to it being (1) a serine protease, (2) an acid or aspartic protease, (3) a cysteine or sulfhydryl protease, or (4) a metalloprotease type, several compounds were tested as potential inhibitors of Mpr activity. Based on the knowledge that a serine protease is inhibited by hydroxyl-reactive organofluorides such as diisopropylfluorophosphate (also abbreviated "DFP") and phenylmethylsulfonyl fluoride (also abbreviated "PMSF"), Mpr is not classified as a serine protease, because PMSF, up to concentrations as high as 10 mM, fails to inhibit its esterase or protease activity (see on page 1022, the sixth to ninth lines of the right-hand column and on page 1021, the sentence bridging the two columns, as well as the abstract). It is rather suggested that **Mpr is a metalloprotease** (see page 1021, first full paragraph of the right-hand column). The enzyme's esterase activity is measured by using a N-

tert-butoxy-carbonyl-L-glutamic acid-"-phenyl esterase assay.

10. Document (D14), which makes reference *inter alia* to document (D17), discloses the cloning of the gene encoding **Mpr**. The nucleotide and deduced amino acid sequences of the *mpr* gene are shown in Figure 4 (see page 1026). In the concluding paragraph of page 1029, the authors state that "[I]t seems likely that there should exist protease genes in other gram-positive organisms that are similar to *mpr*.".

11. Document (D10), to which some of the authors of document (D14) and the four authors of document (D17) contributed, deals basically with the creation of a Bacillus strain which is substantially devoid of proteolytic activity (see page 4, lines 1 and 2). The strategy followed includes the identification of novel proteolytic activities, and thereby the isolation and characterisation of the RP-II gene and protein (see page 11, lines 1 to 19), in view of making deletions in the gene. **The RP-II protein is regarded as not being a serine protease** (see page 14, line 28). It is characterised as possessing esterase activity, as demonstrated by its ability to hydrolyse phenylalanine methyl ester and N-tert-butoxy-carbonyl-L-glutamic acid-"-phenyl ester. Figure 14 reports the DNA sequence encoding RP-II and the deduced amino acid sequence of the protein. This figure is identical to Figure 4 of document (D14) which reports the nucleotide and deduced amino acid sequence of the mpr gene.

12. Document (D5) describes a washing composition which is prepared using strains of **Bacillus licheniformis** that have been mutated to block the synthesis of the minor protease component of the commercially available enzyme preparation known as "Alcalase" in such a way that the washing composition is free of that component which is undesirable as being allergenic. The minor protease component, referred to in the document as component C, is poorly characterised in terms of its structural features (in particular, no amino acid or DNA sequence is provided). From inhibition studies (see Section entitled "Inhibition studies" in column 7), it is concluded that component C **is not a serine protease** (for the reason that it is not inhibited by either DFP or PMSF).

13. The arguments that the Mpr/RP-II protease is a glutamic acid-specific serine protease, put forward in document (D16) by the appellant with the view of establishing that document (D10) represents the closest prior art, cannot be accepted, the reasons therefor being as follows:

13.1 Although it is reported in document (D17) that the protein was capable of cleaving the N-tert-butoxy-carbonyl-L-glutamic acid-"-phenyl substrate, it has not been proved therein that Mpr **specifically** cleaves peptide bonds at the carboxy termini of glutamic acid residues, this specificity meaning that the other amino acid residues, inclusive of the aspartic amino acid residues, are not cleaved.

13.2 The authors of document (D17) duly took into account the correlation existing between the DFP inhibitor effect of a protein and classification of the same as a serine protease (see the first sentence of the first full paragraph of the right-hand column on page 1022) but considered that the PMSF inhibitory effect, as characterised in their observations, was sufficient for them to conclude that Mpr was not a serine protease. Their conclusion is reinforced by document (D5) in which DFP and PMSF are each reported to have no inhibitor effect on the component C (see lines 15 to 20 in column 7).

14. The further argument that the assertion on page 1029 of document (D14) (see point 10, *supra*) that protease genes similar to *mpr* might exist in other gram-positive organisms would have prompted the person skilled in the art to investigate strains of Bacillus licheniformis for a DNA encoding a protein which, such as BLase, had the amino acid sequence of SEQ ID NO: 1 and was a **glutamic acid specific serine protease** is not tenable. Indeed, the person skilled in the art would at best have expected to identify in Bacillus licheniformis a gene encoding a protein **similar** to Mpr, ie, as indicated in the art (see document (D17), not a serine protease, such as BLase, but a metalloprotease. Further, in view of the poor homology between the DNA sequences encoding respectively Mpr and BLase (see document (D12), it is highly doubtful whether the skilled person would have been in a position to derive from the Mpr DNA sequence probes appropriate for the retrieval of any BLase encoding gene in the genome of a Bacillus licheniformis strain. There is certainly no evidence showing that such a probe could be made.

Assessment of inventive step

15. In the board's jugdment, none of the documents (D17), (D14), (D10) and (D5) referred to above is suitable to represent the closest prior art as none of them deals with **a serine protease** which **specifically cleaves a peptide bond at the carboxy termini of glutamic acid residues**. Rather, the background art cited in the patent specification (see page 3, lines 15 to 19) constitutes a more appropriate starting point for the evaluation of inventive step.

16. In this respect, reference is made in the patent specification to **the protease derived from the pathogenic V8 strain of Staphlylococcus aureus** which specifically cleaves the peptide bond at the carboxyl terminal of glutamic acid residues and is classified as a serine protease. The DNA sequence encoding this enzyme is also stated to have been cloned. Reference is also made to the serine protease of document (D7) which is derived from Streptomyces griseus. This protease is strictly speaking not glutamic acid specific, but rather acidic amino acid specific as it cleaves peptide bonds at the carboxyl terminal side of either glutamic or aspartic acid (see the abstract on page 451 of document (D7)). The patent specification further refers to an endoprotease which is specific for glutamic acid residue derived from Bacillus subtilis, citing both document (D6), which, having been published after the undisputedly valid priority date, is not prior art, and "Abstracts of 62nd General Conference of the Japan Biochemical Society", for which no other details are available.

17. In the board's judgement, the knowledge derivable from said documents, whether taken alone or taken in combination with each other or with any of the other documents cited by the parties, would **not** have given the skilled person, who was faced with the problem of finding a further glutamic acid specific serine protease, any clue or incentive in respect of isolating from a Bacillus licheniformis strain, in particular from the strain ATCC 14580, a DNA sequence encoding a serine protease having such specificity and containing the amino acid sequence as recited in claim 1.

18. Therefore, the board reaches the conclusion that the subject-matter of claim 1 involves an inventive step. As all the remaining claims contain an explicit or implicit reference to a DNA sequence as defined in claim 1, the same conclusion applies to the whole claimed subject-matter. Thus, the auxiliary request meets the requirements of Article 56 EPC.

Amendments of the description

19. The respondent requests that the description on file be replaced by an amended description, consisting of pages 3, 3a, and 4 to 23.

20. The requested amendments result in an appropriate adaptation of the description to the claims of the auxiliary request and are in compliance with the requirements of Article 123(2) 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 maintain the patent on the basis of:

   Claims:
   1 to 15 as submitted at the oral proceedings on 11 June 2003;

   Description:
   Pages 3, 3a, and 4 to 23 as submitted at oral proceedings on 11 June 2003; and

   Figures:
   As granted.

The Registrar: The Chairman:

A. Wolinski L. Galligani