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) [ ] To Chairmen
(D) [X] No distribution

Datasheet for the decision of 8 June 2010

Case Number: T 1904/08 - 3.3.08

Application Number: 98955111.4

Publication Number: 1025240

IPC: C12N 15/57

Language of the proceedings: EN

Title of invention:
Multiply-substituted protease variants with altered net charge for use in detergents

Patentee:
GENENCOR INTERNATIONAL, INC.

Opponent:
NOVOZYMES A/S

Headword:
Protease variants/GENENCOR

Relevant legal provisions:
EPC Art. 56, 83, 114(2)

Relevant legal provisions (EPC 1973):
-

Keyword:
"Documents cited on appeal - admitted (no)"
"Main request: inventive step (no)"
"Auxiliary request: inventive step (no)"

Decisions cited:
T 0019/90

Catchword:
-

Europäisches Patentamt | European Patent Office | Office européen des brevets

Beschwerdekammern | Boards of Appeal | Chambres de recours

Case Number: T 1904/08 - 3.3.08

D E C I S I O N
of the Technical Board of Appeal 3.3.08
of 8 June 2010

Appellant: (Opponent)
NOVOZYMES A/S
Krogshoejvej 36
DK-2880 Bagsvaerd (DK)

Representative:
Jensen, Bo Hammer
Novozymes A/S
Patents
Krogshöjvej 36
DK-2880 Bagsvaerd (DK)

Respondent: (Patent Proprietor)
GENENCOR INTERNATIONAL, INC.
925 Page Mill Road
Palo Alto, California 94304 (US)

Representative:
Casley, Christopher Stuart
Mewburn Ellis LLP
33 Gutter Lane
London EC2V 8AS (GB)

Decision under appeal:
Interlocutory decision of the Opposition Division of the European Patent Office posted 21 July 2008 concerning maintenance of European patent No. 1025240 in amended form, pursuant to Article 101(3)(a) EPC.

Composition of the Board:

Chairman: L. Galligani
Members: T. J. H. Mennessier
C. Heath

Summary of Facts and Submissions

I. Both the opponent (appellant) and the patentee (now respondent) lodged an appeal against the interlocutory decision of the opposition division dated 21 July 2008, whereby European patent No. 1 025 240, which had been granted on European application No. 98 955 111.4 published under the international publication No. WO 99/20771, was maintained in an amended form on the basis of the first auxiliary request (claims 1 to 4) filed on 24 April 2008. The main request had been refused for lack of novelty.

II. The patent had been opposed on the grounds as set forth in (i) Article 100(a) EPC that the invention was neither new nor inventive (cf. Articles 54 and 56 EPC), (ii) Article 100(b) EPC that it was not sufficiently disclosed (cf. Article 83 EPC) and (iii) Article 100(c) EPC that the patent contained subject-matter which extends beyond the content of the application as filed (cf. Article 123(2) EPC). The objection of lack of novelty was based on four documents, including D1 and D5 (see Section X, *infra*).

III. The patentee withdrew its appeal on 28 November 2008. Consequently, it is now only respondent to the appeal of the appellant.

IV. In its statement of grounds of appeal the appellant (opponent) made reference to three new documents, including documents D10 and D12 (see section X, *infra*).

V. On 8 April 2009, the respondent replied to the statement of grounds of the appellant and submitted as

main request the request on the basis of which the patent had been maintained (claims 1 to 4). An auxiliary request (noted "1" with four claims) was also filed.

The **main request** consisted of 4 claims, claim 1 reading:

"1. A method of improving the performance of a subtilisin in a low detergent concentration system having less than 800 ppm detergent components present in the wash water, comprising:
a) substituting an amino acid residue at one or more positions in a precursor subtilisin to produce a subtilisin variant wherein the substitution alters the charge at that position to make the charge more negative or less positive compared to the precursor; and
b) testing the variant in a low detergent system having less than 800 ppm detergent components present in the wash water by comparing the ability of the precursor and the variant to remove a stain."

Claim 1 of the **auxiliary request** differed from claim 1 of the main request only in that in step a) it was specified that the substitution altered the net charge of the precursor subtilisin to make the net charge more negative or less positive compared to the precursor.

VI. On 26 February 2010, the board issued a communication under Article 15(1) of the Rules of Procedure of the Boards of Appeal (RPBA) expressing a provisional, non-binding opinion on some of the pending issues.

VII. On 7 May 2010, the respondent replied to the board's communication by stating not to have any further written submissions.

VIII. The appellant replied to the board's communication with a letter dated 10 May 2010 in which four new documents were enclosed. The appellant submitted that the main request was not new over document D10 or document D12, and pointed out that, in relation to its objection of lack of novelty, it was no longer pursuing its arguments in respect of any of the previous documents filed with its notice of opposition. As regards inventive step, it maintained that document D1 was to be considered as the closest prior art and indicated that it would like to use documents D10 and D12 in support of its objection of lack inventive step. Furthermore, it withdrew its arguments on added matter and maintained its position that, in view of the very broad definition of subtilisin in the patent at issue, the exemplification in the description was insufficient to demonstrate that the invention worked with all subtilisins.

IX. Oral proceedings took place on 8 June 2010.

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

(D1) WO 91/00345 (published on 10 January 1991)

(D5) EP A1 0 328 229 (published on 16 August 1989)

(D8) EP A1 0 670 367 (published on 6 September 1995)

(D9) EP A2 0 496 361 (published on 29 July 1992)

(D10) EP A1 1 029 920 (published as WO 99/18218 on 15 April 1999; with a priority date of 7 October 1997)

(D12) Document entitled "Novo Nordisk Enzyme Symposium 1998 - New Challenges and Discoveries in Enzyme Research", cover page "Program and Abstracts" and pages 56 to 59 with the title "Development of a low-temperature protease for the detergent industry"

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

Admissibility of documents D10 and D12

Documents D10 and D12 which were identified only at the onset of the appeal proceedings could not have been submitted earlier. Document D10 was of interest for the assessment of novelty and inventive step, although admittedly the mutagenesis step of claim 1 was not described therein. In contrast, document D12 which was available to the public at the filing date of the patent at issue was highly relevant. The document reported that a new detergent protease called Kannase® had been developed and tested at typical Japanese conditions (see the last sentence on page 59), it being specified that such a protease could be developed partly by random mutagenesis and selection. Admittedly, there was no *expressis verbis* teaching about specifically changing the charge of a protease.

Main request

Novelty (Article 54 EPC)

Both documents D10 and D12 described subtilisin variants which fell under the scope of the claims and thus affected their novelty. However, if documents D10 and D12 were not introduced into the proceedings, lack of novelty would no longer be an issue to be discussed.

Sufficiency of disclosure (Article 83 EPC)

The patent provided a very broad definition of the precursor subtilisins to be substituted, as according to paragraph [0025] bridging pages 4 and 5 of the patent specification, they were "non-human subtilisins" obtainable from prokaryotic and eukaryotic organisms. In contrast, only one particular precursor subtilisin was referred to in the patent, namely the one obtainable from Bacillus lentus GG36 (see paragraph [0063] on page 8 of the patent specification).

Claim 1 was broadly drafted in that any substitution of an amino acid residue at any one or more positions was appropriate, provided that it altered the charge at the given position to make the charge more negative or more positive compared to the precursor. There was no proviso that the net charge of the molecule should be changed.

The patent provided only a rudimentary experimentation which could not be repeated by the skilled person.

Inventive step (Article 56 EPC)

Making substitutions according to step a) of claim 1 was well-known. Furthermore, as derivable from document D9 (see page 15, lines 1 to 43), it was known to test detergent compositions comprising an enzyme at a low concentration detergent such as 660 ppm.

The patent provided an obvious proposal which consisted in substituting any amino acid residue at any one or more positions of the precursor subtilisin and testing it according to the Asian market detergent conditions. Inevitably, an improvement would be obtained with a broad majority of the variants.

In view of document D1 taken as the closest prior art, the technical problem to be solved was to be seen in the generation of subtilisin variants that provided improved wash performance for the Asian market.

Document D5 (see page 2, lines 40 to 44) would have incited the skilled person to test appropriate variants prepared according to the teaching of document D1 under the Asian market detergent conditions.

No prejudice was derivable from document D1, in which experiments were performed at a low pH, i.e. at pH conditions used in Asia (see documents D8, page 10, lines 3 to 4 and D9, page 25, Example 7), that would have prevented the skilled person from carrying out such a test. Therefore, the method according to claim 1 was not inventive.

First auxiliary request

Inventive step (Article 56 EPC)

The reasoning made with respect to the main request also applied to the first auxiliary request, the only difference being that the latter request was limited to substitutions which changed the net charge of the molecule. Examples of such substitutions were described in document D1 (see page 28).

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

Admissibility of documents D10 and D12

Documents D10 and D12 should not be introduced into the proceedings for the reason that they were filed by the appellant only together with its statement of grounds and were *prima facie* no more relevant than the documents already on file. There was no clear-cut disclosure of a mutagenesis step in either of the two documents. Moreover, the three particular enzymes disclosed in document D10 were not subtilisins within the meaning of the present invention. Furthermore, there was no evidence on file that document D12 had been made publicly available at the relevant filing date.

Main request

Sufficiency of disclosure (Article 83 EPC)

The appellant had not substantiated its objections by verifiable facts and evidence. There was no difficulty in finding amino acid residue positions appropriate for substitution, in particular in view of the fact that no change of the net electrostatic charge of the molecule was required (see Table 14 on pages 13 and 14 of the patent specification).

Novelty (Article 54 EPC)

An objection of lack of novelty based on the late filed documents D10 and D12 amounted to a fresh case.

Inventive step (Article 56 EPC)

The choice of the substitutions to be made according to claim 1 was not an obvious one. The skilled person had to make a selection among a variety of known mutations, which included not only substitutions of many kinds but also deletions or insertions of amino acid residues, to finally find out the particularly appropriate type of substitutions as referred to in claim 1. There was no motivation in the prior art for him/her to test those substitutions in a low detergent concentration system having, as referred to in claim 1, less than 800 ppm detergent components present in the wash water, i.e. under conditions in use in Asia, more particularly in Japan. He/she would not have performed step a) of claim 1 and then step b) thereof. Performing such two steps was to be regarded as an inventive selection

which contributed to the art in allowing the number of failures when looking for efficient enzyme detergent compositions to be reduced. Moreover, no correlation had been established in the prior art, including document D5, between that category of substitutions which change the charge of an amino acid residue as referred to in claim 1 and the concentration of the detergent components. Therefore, the method according to claim 1 was inventive.

First auxiliary request

Inventive step (Article 56 EPC)

The above reasoning made with respect to the main request also applied to the first auxiliary request, the only difference being that in the latter request a reduced number of substitutions were appropriate, namely only those which changed the net charge of the molecule.

XIII. The appellant (opponent) requested that the decision under appeal be set aside and the patent be revoked.

XIV. The respondent (patentee) requested that the appeal be dismissed, in the alternative that the patent be maintained on the basis of the auxiliary request as filed with letter of 8 April 2009.

Reasons for the decision

Admissibility of documents D10 and D12

1. Documents D10 and D12 were filed by the appellant together with its statement of grounds in support of its objections of lack of novelty. In its letter of 10 May 2010, the appellant, without giving any further details, expressed its intention to subsequently use the same documents in support of its arguments against inventive step. The respondent argued that an attack on novelty and/or inventive step based on documents D10 and D12 constituted a fresh case with respect to the decision under appeal, and thus requested that the documents in question not be admitted into the proceedings. It was also argued that it had not been established whether document D12 had been made available to the public before the relevant filing date.

Document D10

2. Document D10 is a European patent application which belongs to the state of the art pursuant to Article 54(3) EPC. Its disclosure is centred in particular on three alkaline proteases, denoted "KP-9860" (see page 15, paragraph [0084] and sequence SEQ ID NO:3 on pages 32 to 42), KP-43 (see page 15, paragraph [0085] and sequence SEQ ID NO:4 on pages 37 to 42) and KP-1790 (see page 15, paragraph [0085] and sequence SEQ ID NO:5 on pages 42 to 47), respectively. These proteins differ from the subtilisins referred to in the patent at issue. The only reference to subtilisin is found on page 15, paragraph [80] where the three amino acids of the active centre of alkaline

proteases (Asp, His, Ser) are given without specifying their order in the amino acid sequence. The document admittedly does not disclose a method in which an amino acid is substituted to alter the charge. The only reference to amino acid substitution in claim 3 is of a general nature. Thus, document D10 is manifestly no more relevant than the documents already on file for the assessment of novelty and inventive step of the claimed invention.

Document D12

3. As for document D12, it consists of a cover page and a short technical paper. The cover page refers to "Program and Abstracts" of the 'Novo Nordisk Enzyme Symposium', held on 25 September 1998 in Kyoto, Japan. The link with the attached paper entitled "Development of a low temperature protease for the detergent industry" is unclear in that it is not known whether the latter is an abstract which was distributed together with the programme, or a later article. In any case, the said paper relates to the development of a new detergent protease called Kannase® with high stain removal efficiency at very low washing temperature of 10 to 20°C , which is an example of a protein that can be developed partly by random mutagenesis and screening. The paper also reports a general discussion about random mutagenesis with a brief overview of some experimental tools (oligonucleotides for PCR, shuttle vector, mutant libraries, etc ..) used at Novo Nordisk, and a short description of an automated high throughput screening which was developed to screen the mutants. The paper further describes how the Kannase® protease was purified from the medium in which it had been

secreted by the Bacillus cells and how wash performance of the Kannase® protease was evaluated using "typical Japanese conditions" (see the last sentence on page 59) which are not specified. A figure is given which illustrates the superiority of Kannase® compared to Savinase® in the evaluation test.

4. Admittedly, the document does not contain the teaching of specifically changing the charge of a protease and thus fails to describe the paramount technical feature of claim 1 of the main request.

5. In view of the uncertainty about the availability of the document and in consideration of its contents, document D12 is no more relevant than the documents already on file for the assessment of novelty and inventive step of the claimed invention.

Conclusion

6. In view of the above, in exercise of the discretionary power conferred upon it by Article 114(2) EPC, the board decides not to admit documents D10 and D12 into the proceedings.

Main request

Admissibility of the amendments (Article 123(2) EPC)

7. The appellant did no longer pursue its objections under Article 123(2) EPC against the main request, this being the former auxiliary request on the basis of which the patent was maintained by the opposition division. The

board is satisfied that the main request complies with the requirements of Article 123(2) EPC.

Sufficiency of the disclosure (Article 83 EPC)

8. Two objections were raised by the appellant under this heading: i) the expression "precursor subtilisin" as used in claim 1 was considered to encompass a very large number of molecules, whereas only one precursor subtilisin had actually been exemplified in the experimental part of the description; ii) the substitutions covered by claim 1 were seen as insufficiently specified to allow the skilled person to determine without undue burden what they should be.

9. As regards i), it is noted that the subtilisins to be used as a precursor according to claim 1 are defined in the description of the patent at issue (cf paragraphs [0003] and [0022] to [0025]). These are serine proteases with a molecular weight of approximately 27.500 daltons having their catalytic triad made of histidine, aspartic acid and serine in the order, reading form the amino to the carboxy terminus, "Asp-His-Ser". Furthermore, they are secreted from a wide variety of Bacillus species and other microorganisms, including prokaryotic organisms such as gram-negative or gram-positive bacteria and eukaryotic organisms such as yeasts and fungi (see paragraph [0025] bridging pages 4 and 5 of the patent specification). A preferred subtilisin is "subtilisin 309" (see paragraph [0022] on page 4). In the experimental part of the description one precursor subtilisin is referred to, namely the one produced by the Bacillus lentus GG36 strain (see paragraph [0063] on page 8).

10. In the board's judgment, the description provides sufficient information to enable a skilled person to choose without undue burden an appropriate precursor subtilisin.

11. As regards ii), it is noted that, according to claim 1, any amino acid substitution is convenient, whatever the nature and the position of the amino acid residue provided it alters the charge at that position in the variant to make the charge more negative or less positive compared to the precursor. There is no requirement that the net charge of the whole subtilisin be altered as clearly illustrated in Table 14 (see pages 13 and 14 of the patent specification).

12. In the board's judgment, choosing a convenient substitution is a rather easy task for the skilled person which can be performed without undue burden.

13. It is observed that the appellant has acknowledged not having attempted to reproduce the experiments of the patent and has not substantiated its objections of insufficiency of disclosure by the provision of concrete evidence or verifiable facts (see decision 19/90, OJ EPO 1990, 476).

14. The board's conclusion is thus that the invention according to claim 1 is sufficiently disclosed for it to be put into practice by the skilled person and claim 1 and the main request as a whole meet the requirements of Article 83 EPC.

Novelty (Article 54 EPC)

15. The appellant has abandoned its novelty objections based on documents filed together with its notice of opposition. Moreover, as documents D10 and D12 have not been admitted into the proceedings, the objections based thereon cannot be considered. Thus, there is no issue of novelty to be examined.

Inventive step (Article 56 EPC)

16. The method according to claim 1 comprises two steps: a) a substitution step aiming at altering at one or more positions of a precursor subtilisin the charge by making it more negative or less positive, and b) a test step for assessing whether the variant thereby obtained performs better than the precursor subtilisin in a low detergent concentration system having less than 800 ppm detergent components present in the wash water (Japanese wash conditions; see paragraph [0017] on page 4 of the patent specification).

17. Document D1, which represents the closest prior art and deals with the problem of generally improving the wash performance of a subtilisin, proposes substitutions by mutation in a precursor subtilisin at one or more amino acid positions which result in a change in the surface charge of the molecule. A list of preferred substitutions to be performed on subtilisin 309 (the preferred subtilisin also in the patent-in-suit) is provided on pages 28 and 29. This includes substitutions in the sense of step a) of claim 1, i.e. making the charge more negative or less positive. What document D1 does not disclose is the testing of the

resulting variants under "Asian market detergent conditions", i.e. step b) of claim 1. In fact, the tests are carried out at high detergent concentrations (see page 53).

18. The respondent maintains that the skilled person would not have found any incentive in the state of the art to combine the particular kind of substitutions indicated in step a) with test conditions specific for the Asian market. In its view, the inventive contribution lies in establishing a correlation between detergent concentration, wash performance and enzyme charge.

19. The board cannot concur with this view for the reasons outlined hereinafter.

20. In addressing the general problem of improving the wash performance of subtilisin by introducing mutations of one or more amino acids, document D1 proposed a broad catalogue of possibilities, including mutations resulting in a negative charge. In view of said prior art knowledge, the underlying technical problem is defined as finding in the large number of putative variants thereby obtained those suitable for a particular market segment (e.g. the European, the American or the Asian).

21. The solution proposed in claim 1 of the main request is a method comprising testing the class of variants in which the charge of one or more amino acid residues has been rendered more negative (or less positive) under the conditions known to be typical of the Asian market, i.e. at low detergent concentrations. It is noted that claim 1 is not limited to any particular variants, but

proposes a broad catalogue of possibilities within the framework of charge change. In fact, any amino acid residue at one or more positions in the precursor subtilisin which is around 270 amino acid residues long (see Figure 3 in the patent specification) may be substituted.

22. The relevant question in relation to inventive step is whether the skilled person would have taken into consideration testing such a broad range of variants under the conditions typical for household application in that geographical area, given that document D1 indicates for testing conditions which are typical of other market segments.

23. In the board's judgement, the skilled person is well aware of the fact that the performance of proteolytic enzymes such as subtilisin may well depend on the washing conditions and that there is no such a thing as universal washing conditions (cf e.g. document D5). When wishing to penetrate in a given market segment with a further variant(s), the skilled person would not hesitate to test it (them) under the conditions typical for that market. This is seen by the board as belonging to the normal set of mind of the skilled person in real life. Thus, proposing a test under Asian conditions for selecting suitable candidates among the broad range of variants with a more negative charge cannot be seen as requiring inventive talent, but rather as a standard control to be carried out to ascertain the reality of the invention.

24. Moreover, there was no prejudice in the state of the art which would have prevented the skilled person from

performing such a control under Japanese wash conditions. On the contrary, methods therefor had been disclosed in the state of the art (see, for example, document D9, page 15, lines 14 to 30 in which tests are described for an alkaline protease in a 0,417% detergent solution, i.e. a wash liquor with about 400 ppm detergent components).

25. Thus, no inventive contribution is seen in the method of claim 1 as a whole, and consequently the main request is not allowed under Article 56 EPC.

Auxiliary request

26. Claim 1 differs from claim 1 of the main request only in that the substitutions to be performed should precisely make the net charge of the variant more negative or less positive compared to the precursor. The reasoning made above with respect to the main request also applies to this request which likewise is not allowed under Article 56 EPC.

Conclusion

27. Neither of the main request and the auxiliary request can form a basis for maintenance of the patent in amended form. Therefore, in the absence of any other claim request, the patent should be revoked.

Order

For these reasons it is decided that:

1. The decision under appeal is set aside.

2. The patent is revoked.

The Registrar The Chairman

A. Wolinski L. Galligani