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D E C I S I O N
of 19 October 2004

Case Number: T 0537/02 - 3.3.8

Application Number: 92900769.8

Publication Number: 0563103

IPC: C12N 9/56

Language of the proceedings: EN

Title of invention:
Enzymes and enzymatic detergent compositions

Patentees:
UNILEVER PLC, et al

Opponent:
GENENCOR INTERNATIONAL INC.

Headword:
Detergent composition/UNILEVER

Relevant legal provisions:
EPC Art. 54, 56, 83

Keyword:
"Main request: sufficiency of disclosure (yes)"
"Novelty (yes)"
"Inventive step (no)"
"First auxiliary request: inventive step (no)"
"Second auxiliary request: inventive step (no)"

Decisions cited:
-

Catchword:
-

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Case Number: T 0537/02 - 3.3.8

D E C I S I O N
of the Technical Board of Appeal 3.3.8
of 19 October 2004

Appellants I: (Proprietors of the patent)
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Decision under appeal: **Interlocutory decision of the Opposition Division of the European Patent Office posted 3 April 2002 concerning maintenance of European patent No. 0563103 in amended form.**

Composition of the Board:

Chairman: L. Galligani
Members: T. J. H. Mennessier
M. B. Günzel

Summary of Facts and Submissions

I. The patent proprietors (appellants I) as well as the opponents (appellants II) lodged an appeal against the interlocutory decision of the opposition division given at oral proceedings on 7 March 2002 with written reasons posted on 3 April 2002, whereby the European patent No. 0 563 103 was maintained on the basis of the second auxiliary request (claims 1 to 6) filed at said oral proceedings. The patent had been granted on European application No. 92 900 769.8. which originated from an international application published as WO 92/11348.

II. The patent had been opposed on the grounds as set forth in Article 100(a) EPC that the invention was not new and did not involve an inventive step (Article 56 EPC), and on the ground as set forth in Article 100(b) EPC that the invention was not sufficiently disclosed (Article 83 EPC).

III. Basis for the decision under appeal were, in addition to the aforementioned second auxiliary request, the main request (claims 1 to 7), and the first auxiliary request (claims 1 to 7) both filed also on 7 March 2002. The main request was not accepted by the opposition division for lack of novelty (claim 1). The first auxiliary request was not allowed for lack of inventive step.

IV. Appellants I and appellants II filed their statements of grounds of appeal. Appellants I indicated therein that their main claim request was the main request refused by the opposition division. In support of their

statement appellants II filed therewith thirteen additional documents D2 to D14. Appellants I filed a reply to the statement of grounds of appellants II and appellants II a reply to the statement of grounds of appellants I.

V. A communication under Article 11 of the Rules of Procedure of the Boards of Appeal presenting some preliminary and non-binding views of the board was then sent to the parties.

VI. In reply to the board's communication, appellants II filed observations with a letter dated 22 March 2004.

VII. Oral proceedings took place on 19 October 2004, at which appellants I filed a first auxiliary request (claims 1 to 7) and a second auxiliary request (claims 1 to 6).

VIII. The claims on file were as follows:

(a) **Main request**

(i) Claim 1 read:

"1. An enzymatic detergent composition comprising a mutant subtilisin 147 or 309 protease carrying at least one mutation of its amino acid sequence resulting in a lower degree of variation, compared with the parent protease, of the molecular charge of the protease over a pH range of at least 0.5 pH unit within the pH range of about 7 to about 11, said protease comprising at

least one of the following mutations: H17Q, H39S, H120N, Y167E, Y167F, Y171V, Y192E, Y192F, Y209F, Y214F, H226S, Y263F."

(ii) Claims 2 to 7 were dependent on claim 1 and defined further embodiments thereof.

(b) **First auxiliary request**

(i) Claim 1 differed from claim 1 of the main request only in that it did not contain the terms "147 or".

(ii) Claims 2 to 7 were dependent on claim 1 and defined further embodiments thereof.

(c) **Second auxiliary request**

(i) Claim 1 read:

"1. An enzymatic detergent composition comprising a mutant subtilisin 309 protease carrying at least one mutation of its amino acid sequence resulting in a lower degree of variation, compared with the parent protease, of the molecular charge of the protease over a pH range of at least 0.5 pH unit within the pH range of about 7 to about 11, said protease comprising at least one of the following sets of mutations:

b - H17Q+K27R+H39S;
e - E54D+Y91F+K94R+H120N;
f - Y167F+Y171V+Y192F+Y209F+Y214T;
g - K235L+K237R+K251E+Y263F;
h - K235L+K237R+K251N+Y263F;
i - H226S+K235L+K237R+K251N+Y263F;
k - H226S+K235L+K237R+K251E+Y263F;
g'- K235R+K237R+K251E+Y263F;
h'- K235R+K237R+K251N+Y263F;
i'- H226S+K235R+K237R+K251N+Y263F;
k'- H226S+K235R+K237R+K251E+Y263F."

(ii) Claims 2 to 6 were dependent on claim 1 and defined further embodiments thereof.

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

(D1) WO-A-89/06279

(D4) EP-A-0 405 901

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

Admissibility of documents (D2) to (D14) into the proceedings

There was no justification for introducing into the present appeal proceedings documents which were part of the evidence cited with respect to the co-pending appeal T 0660/02 concerning patent EP-B-0 563 169. This latter patent and the present patent were derived from

a common source. However they had been granted on the basis of different sets of claims. Thus, newly filed documents D2 to D14, with the exception of those which had been cited in the present patent, should not be admitted into the proceedings.

Main request

- Article 83 EPC

Any mutant subtilisin 147 or 309 protease differing from the parent protease by only one mutation selected among the twelve possible mutations listed in claim 1 intrinsically exhibited a lower degree of variation, compared to the parent protease, of the molecular charge of the protease over a pH range of at least 0.5 pH unit within the pH range of about 7 to about 11. This functional feature was relative. Titration curves should be calculated in the same way for the mutant and the parent proteases.

- Article 54 EPC

Subtilisin Carlsberg would not have been regarded by a skilled person as a mutant subtilisin 147 or 309 protease as defined in claim 1, for the reason that subtilisin Carlsberg had an amino acid sequence substantially different from that of either subtilisin 147 or subtilisin 309.

- Article 56 EPC

The technical problem solved by the invention was the provision of detergent compositions which were

relatively resistant to changes in pH of wash liquid which occurred during the wash process and which mutants therefore showed improved wash performance. The mutant subtilisin proteases tested in Example B showed such an improvement. Thus, detergent compositions containing a protease as defined in claim 1 were inventive.

First auxiliary request (Article 56 EPC)

Claim 1 was restricted only to those detergent compositions encompassed by claim 1 of the main request which contained a mutant subtilisin 309 protease. Therefore, the invention involved an inventive step for the same reasons as those given for the main request.

Second auxiliary request (Article 56 EPC)

Claim 1 was directed to a subgroup of the detergent compositions encompassed by claim 1 of the first auxiliary request, namely those compositions which contained a mutant subtilisin 309 protease comprising at least one of eleven distinct sets of mutations, each containing at least one of nine of the twelve mutations listed in claim 1 of the main request. Therefore, the invention involved an inventive step for the same reasons as those given for the main request.

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

Admissibility of documents (D2) to (D14) into the proceedings

Although the present patent and patent EP-B-563 169 had different proprietors, they clearly derived from a common source with same content and inventorship, and each proprietor was aware of the opposition and appeal proceedings relating to the patent of the other proprietor. It was desirable that substantially the same material be considered in the present appeal and in the co-pending appeal T 0660/02, in order that consistent decisions be issued by the boards of appeal, insofar as the subject-matters of the claims were related. Therefore, documents D2 to D11, D13 and D14 which were part of the evidence cited in appeal T 0660/02 should be admitted into the present proceedings. Document D12 which had not been cited in the statement of grounds of appeal of appellants II could be disregarded by the board.

Main request

- Article 83 EPC

Mutant subtilisin 147 or 309 proteases having at least one of the twelve mutations listed in claim 1 could have been prepared by a skilled person using genetic engineering without undue burden. Nevertheless, the skilled person was not provided with the necessary means to assess whether such mutant proteases would exhibit a lower degree of variation, compared with the parent protease, of their molecular charge over a pH range of at least 0.5 pH unit within the pH range of about 7 to about 11. In particular, a skilled person

would not have known how to choose the pKa value to be allocated to each ionisable residue in the molecule. In any event, the patent did not disclose that "standard" pK values could be used for the calculations.

- Article 54 EPC

Claim 1 of the main request lacked novelty on the basis of the mutation H17Q in view of the fact that subtilisin Carlsberg had a Q (glutamine) residue at position 17 and had been disclosed as suitable for detergent use.

- Article 56 EPC

As shown by the results of wash tests presented in Example B, there was no correlation between the flattening of the titration curve expressing a lower degree of variation of the molecular charge of the mutant proteases tested associated with a particular mutation and an improvement in the wash performance. There was no inventive concept fit for generalisation which could be derived from the exemplified mutant proteases.

First auxiliary request (Article 56 EPC)

The reasoning given with respect to claim 1 of the main request applied similarly to claim 1 of the first auxiliary request.

Second auxiliary request (Article 56 EPC)

It was not possible to predict whether altering mutant protease B by adding one mutation or replacing one or two mutations by one, two or three further mutations would have resulted in a mutant protease performing in the same way as protease B. Therefore, there was no inventive concept fit for generalisation based on protease B.

XII. Appellants I requested that the decision under appeal be set aside and that the patent be maintained on the basis of the main request filed on 7 March 2002. As first and second auxiliary requests appellants I requested that the patent be maintained on the basis of any of the first and second auxiliary requests filed during the oral proceedings.

XIII. Appellants II requested that the decision under appeal be set aside and the patent be revoked.

Reasons for the Decision

Admissibility of documents D2 to D14 into the appeal proceedings

1. Thirteen additional documents (D2 to D14) were filed together with the statement of grounds of appeal of appellants II. Appellants I objected to the admission into the proceedings of those documents which have not been cited in the patent, namely documents D7, D9 and D11 to D14, and this issue was discussed during the oral proceedings. As the board has come to the

conclusion that all the requests lack an inventive step when compared with document D1, the only document previously on file, as will be set out below, this issue is not relevant for the present decision.

Main request

- Article 83 EPC

2. Claim 1 relates to a detergent composition which comprises a mutant subtilisin having a lower variation of its molecular charge within an alkaline pH range in comparison with the parent subtilisin 147 or 309, said mutant comprising at least one of the twelve different specific mutations indicated in the claim.

3. Two questions have to be answered: (i) whether the mutant subtilisin can be made, and (ii) whether its property can be tested.

4. Appellants II do not deny that mutations such as those listed in claim 1 can be introduced into subtilisins 147 or 309 without undue burden by routine techniques of genetic engineering. The board notes that document D1 (see point 14, infra) illustrates how to proceed in detail with subtilisin 309. However, appellants II argue that the property of the mutant protease cannot be tested, and, thus, the skilled person is not in a position to establish whether the claimed functional feature is satisfied.

5. Therefore, the question which remains at issue is whether the application as filed contains all the information that would have been necessary for a

skilled person to obtain subtilisins which, while differing from either subtilisin 147 or subtilisin 309 by the presence of at least one of the twelve mutations listed in claim 1, exhibit a lower variation of their molecular charge as defined in the claim.

6. Appellants II argue that the calculation of the molecular charge of any protein over a pH range requires calculation of the titration curve and that a skilled person would not be in a position to calculate a titration curve for any mutant subtilisin because the patent provides no guidance as to the choice of the pKa value to be allocated to each ionisable residue in the molecule, and notices in this respect that the patent does not disclose that standard pKa values can be used for the calculations.

7. The board finds this not convincing. In its view, a skilled person would be in a position to calculate titration curves. It is true that the use of standard pKa values is not referred to in the patent, but such values had been calculated for each of the amino acids commonly found in proteins and were available at the priority date. There was no reason for a skilled person not to take them as reference values and derive therefrom realistic values, ie values which take account of the specific wash conditions to be applied in a given test, such as the ionic strength of the medium in which the protease is contained, the nature of any salt in the medium or the temperature. A skilled person would therefore be in a position to calculate titration curves for any given mutant protease and, thus, would calculate the molecular charge.

8. Therefore, in the board's judgment the invention of claim 1 is sufficiently disclosed and the requirements of Article 83 EPC are met by the main request.

- Article 54 EPC

9. Claim 1 is directed to a detergent composition comprising a subtilisin 147 or 309 bearing at least one of twelve given mutations. Among them is the mutation H17Q.

10. Appellants II consider that detergent compositions of the art which contain subtilisin Carlsberg are encompassed by claim 1, because subtilisin Carlsberg would be regarded by a skilled person as a variant of subtilisin 147 or 309 with the mutation H17Q.

11. Each of subtilisins 147 and 309 is substantially different from any other of the subtilisins known in the art, including subtilisin Carlsberg. In this respect, the amino acid sequence of subtilisin Carlsberg (274 amino acids) differs in 118 and 101 positions from respectively that of wild-type subtilisin 147 (268 amino acids) and that of wild-type subtilisin 309 (269 amino acids)(see Table I on pages 14 to 20 of document D4, where (f) stands for subtilisin Carlsberg, (h) for subtilisin 309 and (i) for subtilisin 147).

12. The fact that subtilisin Carlsberg in position 17 bears the same amino acid residue Q as the claimed mutant H17Q subtilisin 147 or 309, is not a good reason for raising a lack of novelty objection as the rest of its amino acid sequence differs substantially in terms of

the nature and the position of the amino acid residues. The skilled person would not have regarded subtilisin Carlsberg as being a variant of a mutant subtilisin 147 or 309 protease bearing the mutation H17Q.

13. Thus, the subject-matter of claim 1 is new, and, as claims 2 to 7 are dependent on claim 1, the main request as a whole meets the requirement of Article 54 EPC.

- Article 56 EPC

14. Subtilisins 147 and 309 and muteins thereof exhibiting physical properties advantageous to industrial application, in particular in the detergent industry, were known in the art. Document D1 is regarded in this respect as the closest state of the art. It is reported therein that subtilisin genes were cloned from the 147 and 309 variants of the bacterium Bacillus lentus, and that the clones genes were sequenced. By comparing the deduced amino acid sequences of subtilisin 147 and 309 with each other and then, respectively, with sequences of other known subtilisins, sites were identified which, upon mutation, might alter the physical properties of the parent enzyme. Site-directed mutagenesis was used to generate mutations at several of these sites in the subtilisin 309 gene. The resultant mutant enzymes were then expressed in a Bacillus strain and tested in respect of various physical and chemical parameters. Several of the mutants were shown to exhibit properties desirable in enzymes used in detergent compositions.

15. In view of this state of the art, the technical problem to be solved by the invention may be regarded as the

provision of further detergent compositions containing muteins of subtilisin 147 or 309 showing improved washing performance compared with a detergent composition comprising wild-type subtilisin 147 or 309.

16. As a solution, claim 1 proposes a detergent composition which comprises a subtilisin 147 or 309 that contains at least one of twelve mutations, said mutation resulting in a lower degree of variation, compared with the parent protease, of its molecular charge over the alkaline pH range of 7 to 11.

17. The question to be addressed is whether the proposed solution solves indeed the underlying technical problem, ie whether there is really a cause-effect relationship between the proposed mutation and the improved wash performance.

18. To answer this question, one has to take into consideration the wash tests which are reported in the patent.

19. Wash tests have been performed using subtilisin 309 and two muteins thereof, namely proteases B and a+g'. Each contains the mutation Y263F, ie one of the twelve mutations listed in claim 1 (see Example B on pages 14 to 16 in the patent), but also other mutations, namely, protease B: K235R + K237R + K251E; protease a+g': K27R + K235R + K237R + K251E.

20. Titration curves (assumed to have been calculated using parameters that have been chosen in such a way that the wash conditions of the reported tests have been duly taken into account) have been provided (see Figure 1)

with respect to protease B and subtilisin 309 but not with respect to protease a+g'. Therefore, the tests results presented for the protease a+g' cannot be interpreted. Consequently, the answer to the afore-mentioned question can rely only upon the interpretation of the test results presented for the mutant protease B.

21. As a measure of the wash performance, differential reflectance has been used and an improvement factor has been calculated from a dose-response curve which relates to the amount of enzyme needed for each of the two mutant proteases tested for obtaining a given differential reflectance in comparison with subtilisin 309. From the table bridging pages 15 and 16 in the patent in suit, it can be seen that an improvement of the wash performance was observed at the four pH values (8, 9, 10 and 11)at which protease B was tested. This result shows that **co-introduction** of the four mutations K235R, K237R, K251E and Y263F in subtilisin 309 has resulted in a mutant protease, namely protease B, performing better than the parent wild-type subtilisin 309 during wash processing at pH 8 as well as at pH 9, 10 and 11 at which the titration curve of protease B is flattening compared to that of subtilisin 309. However, the experiment does not allow to evaluate the individual impact of each of the four mutations on the wash performance. Nor does it permit to ascribe the improvement in wash performance to the specific mutation Y263F out of the four mutations. Thus, since it is not possible to establish a causal link between the mutation and the improvement in wash performance, it is impossible to state that the proposed structural

change constitutes a solution to the underlying technical problem.

22. Under these circumstances, inventive step cannot be acknowledged, as simply proposing a series of possible mutations without showing an effect is not considered to involve any inventive contribution over the prior art wherein a number of other mutations has already been proposed. Thus, the requirements of Article 56 EPC are not met by the main request which, consequently, has to be refused.

First auxiliary request (Article 56 EPC)

23. Claim 1 is directed only to detergent compositions containing a mutant subtilisin 309 protease.

24. For the same reasons given for the main request (see points 14 to 23, supra), the subject-matter of claim 1 does not meet the requirements of Article 56 EPC. Thus, the first auxiliary request has to be refused.

Second auxiliary request (Article 56 EPC)

25. Claim 1 is further limited to only those detergent compositions comprising a mutant subtilisin 309 protease which contains at least one of eleven selected sets of three, four or five mutations.

26. Set g' corresponds to the group of four mutations contained in **protease B**. In addition to set g', seven other sets (sets g, h, i, k, h', i' and k') also contain the mutation **Y263F** which, as a matter of fact, is the only mutation shared by these eight sets. These

sets have no mutations in common with the three other sets listed in claim 1 (sets b, e and f).

27. Since it is not possible to establish a causal link between the mutation **Y263F** and the improvement in wash performance shown for **protease B** (see point 21, supra), it is impossible to state that the introduction in subtilisin 309 of any of the eight sets of mutations containing the mutation **Y263F** (sets g, h, i, k, g', h', i' and k') constitutes a solution to the underlying technical problem (see point 15, supra). Nor is it possible, in the absence of any relevant experimental data, to arrive at a positive conclusion with respect to the introduction in subtilisin 309 of any of the other sets of mutations (sets b, e and f).

28. Therefore, inventive step cannot be acknowledged for the subject-matter of claim 1. Thus, the second auxiliary request has to be refused.

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