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Datasheet for the decision of 15 April 2010

Case Number: T 0938/08 - 3.3.08

Application Number: 99102452.2

Publication Number: 0945502

IPC: C12N 9/54

Language of the proceedings: EN

Title of invention:
A mutated subtilisin protease

Patentee:
Novozymes A/S

Opponent:
Genencor International, Inc.

Headword:
Subtilisin/NOVOZYMES

Relevant legal provisions:
EPC Art. 56, 83, 123(2)

Relevant legal provisions (EPC 1973):
-

Keyword:
"Main request: inventive step (no)"
"Auxiliary request 1: added matter (no)"
"Sufficiency of disclosure (yes)"
"Inventive step (yes)"

Decisions cited:
T 1329/04

Catchword:
-

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Beschwerdekammern | Boards of Appeal | Chambres de recours

Case Number: T 0938/08 - 3.3.08

D E C I S I O N
of the Technical Board of Appeal 3.3.08
of 15 April 2010

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Decision under appeal:
Decision of the Opposition Division of the European Patent Office posted 14 March 2008 revoking European patent No. 0945502 pursuant to Article 101(2)(3)(b) EPC.

Composition of the Board:

Chairman: C. Heath
Members: T. J. H. Mennessier
M. R. Vega Laso

Summary of Facts and Submissions

I. The patentee (appellant) lodged an appeal against the decision of the opposition division dated 14 March 2008, whereby European patent 0 945 502 was revoked. The patent had been granted on European patent application No. 99 102 452.2 entitled "*A mutated subtilisin protease*" which was filed as a divisional application to the parent application 90 910 604.9 published as the international publication WO 91/00345.

II. The patent had been opposed by one opponent. The grounds for opposition relied on were lack of inventive step (Article 100(a) EPC), insufficiency of disclosure (Article 100(b) EPC) and presence of added matter (Article 100(c) EPC).

III. In the decision under appeal, the opposition division found that the main request (claims as granted) and the sole auxiliary request then on file (filed as auxiliary request 2 on 12 December 2007) lacked an inventive step (Article 56 EPC).

IV. The statement of grounds of appeal was filed on 22 July 2008. It was accompanied by two auxiliary requests (1 and 2), of which auxiliary request 2 corresponded to the auxiliary request refused by the opposition division. 16 documents were attached thereto.

V. The opponent (respondent) replied thereto on 16 December 2008. It argued that the three requests on file, i.e. the main request (claims as granted) and the two auxiliary requests filed with letter of 22 July 2008, did not comply with the requirements of Articles

56 and 83 EPC. Three further new documents were attached thereto.

VI. On 21 September 2009, the Board issued a communication pursuant to Article 15(1) of the Rules of Procedure of the Boards of Appeal, in which a provisional and non-binding opinion on issues to be discussed at the oral proceedings was expressed.

VII. In reply to the board's communication, the appellant with a letter dated 15 March 2010 filed further submissions together with five additional sets of claims as auxiliary requests 1 and 4 to 7, and renumbered the previous auxiliary requests 1 and 2 of 22 July 2008 as its auxiliary requests 2 and 3, respectively. 14 new documents were enclosed with the letter.

VIII. Then, on 23 March 2010, the appellant filed a further document to confirm the exact date of publication of one of the documents filed on 15 March 2010.

IX. Oral proceedings took place on 15 April 2010, at which the appellant withdrew its auxiliary requests 1 to 3 and 5 to 7, and adopted as its auxiliary request 1 the set of claims according to its previous auxiliary request 4.

X. Claim 1 of the main request (claims as granted) read:

"1. A method of preparing a mutated subtilisin protease for use in a detergent composition, which comprises changing the net electrostatic charge of the protease in comparison to the parent protease at the same pH,

such that, in said protease there are, relative to said parent protease, fewer or more positively-charged amino acid residue(s) and/or more or fewer negatively-charged amino acid residue(s), wherein the parent protease is selected from subtilisin BPN', subtilisin amylosacchariticus, subtilisin 168, subtilisin mesentericopeptidase, subtilisin Carlsberg, subtilisin DY, subtilisin 309, subtilisin 147, thermitase, aqualysin, Bacillus PB92 protease, proteinase K, Protease TW7, and Protease TW3 **characterised in that** one mutation is effected at a position corresponding to position 252 in subtilisin BPN' by substitution, whereby said mutated subtilisin protease has an isoelectric point (pI_o) lower or higher than that of said parent protease and the pI_o of the mutated subtilisin protease is closer to the pH of the wash liquor formed by the detergent composition than is the pI_o of the parent protease, such that the mutated subtilisin protease exhibits improved wash performance relative to the parent protease in said wash liquor."

(underlining added by the board)

XI. The set of claims according to the auxiliary request 1 consisted of 6 claims which differed from the claims as granted only in that the expression "or higher" in claim 1 (see the underlined expression in claim 1 as granted in section X *supra*) had been deleted.

Claims 2 to 6 were dependent on claim 1 and directed to particular embodiments thereof.

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

(D1) A. J. Russell and A. R. Fersht, Nature, Vol. 328, 6 August 1987, pages 496 to 500

(D13) Report submitted by the appellant during the examination proceedings together with its letter of 10 October 2003

(D43) Alessandra Bossi et al., Electrophoresis, Vol. 15, 1994, pages 1535 to 1540

(D44) B. Bjellqvist et al., Electrophoresis, Vol. 15, 1994, pages 529 to 539

(D46) A.W. Kenchington and A.G. Ward, Biochem. J., Vol. 58, No. 2, 1954, pages 202 to 207

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

Main request

Inventive step (Article 56 EPC)

The data provided in the application as filed showed that, in almost all cases, mutations to parent subtilisin enzymes that shifted the pI_o resulted in enhanced wash performance in a detergent solution where the shifted pI_o was closer to the pH of the detergent solution, and the converse where the shifted pI_o was further away from the pH of the detergent solution. The patentee had found that the relationship between pI_o and

the operational pH was an important contributing factor to optimising wash performance.

Based on the data in Table III alone, 21 different mutants having mutations at one or more of eleven different positions, that caused the pI_o of the protein to be shifted compared to the wild-type protein, had all been demonstrated to follow the trend underlying the present invention by showing an associated shift in performance.

It was difficult to draw any clear conclusions from the data in Table XI, due to the small sample size. Nevertheless, the results in Table XI were almost consistent with the general principle underlying the invention, particularly when taking the pH drift towards acidity into account for the lower pH values (where the initial pH was close to the pI_o of the tested enzymes and thus the pH drift became influential), whereas results at the higher pH values of 11.5 and 12.0 (more distant from the tested pI_o values, and thus less influenced by the effect of the pH drift towards acidity) showed that there was a clear trend of increasing wash performance as pI_o approached the operational pH.

In view of the fact that mutations could alter a protein's wash performance by both pI_o-dependent and pI_o-independent mechanisms, it would not have been at all surprising if some mutations did not follow exactly the general trend of pI_o to pH wash relationship compared to other mutants. A small amount of non-conforming data did not detract for the vast majority of data that clearly supported the trend.

The quality of the evidence in the application (which provided multiple proven solutions to the problem) was of a suitably high quality to make "at least plausible" that the effect demonstrated for other positions could also be achieved at the claimed position 252. The application as filed provided multiple examples of making mutations at other positions (including adjacent position 251) in order to solve the problem, and there were no data to suggest that the other positions that were listed, but not tested in the application as filed would be unsuitable alternatives to those positions proven to provide the effect. Accordingly, it was proper to admit the post-filed evidence D13 that merely confirmed the successful effect of a previously untested, but structurally-related solution that was explicitly disclosed in the application as filed.

Starting from document D1, the technical problem was seen as the provision of a method of generating subtilisin mutants with improved wash performance at a given pH. The solution provided by claim 1 was to modify the subtilisin by mutation at position 252, so that the pI_o of the mutant was closer to the pH of the wash liquor than the pI_o of the parent enzyme. The question to be answered, therefore, was whether there was anything in the art that would have motivated the skilled person to modify a subtilisin enzyme in order to move the pI_o of the enzyme closer to the wash pH, in particular by making a mutation at position 252, in the expectation of achieving some benefit.

There was nothing in the prior art that would have motivated the skilled person to modify the teaching of

document D1 in such a way as to arrive at a method having all of the features of claim 1.

The experimental report D13 fully supported a technical effect associated with the claimed subject-matter when modifying position 252. Mutants N252D and N252E did, indeed, perform better than the wild-type parent subtilisin because their pI_o was closer to the pH of the wash liquor, whereas N252K and N252R did, indeed, perform worse than the wild-type parent subtilisin because their pI_o was further away from the pH of the wash liquor.

The rationale for mutating the 66 amino acid residues listed in original claim 1, which included position 252, in the context of the present invention was that they were located on or close to the surface of the resulting enzyme and, consequently, modifications to the charge of these amino acids could have an impact on the pI_o of the protein. Those amino acids did not represent an arbitrary selection from the totality of amino acids in subtilisin. The skilled person would have immediately and unambiguously understood that position 252 was a position that could be modified in the practice of the invention.

It followed that it was permissible to take into account the data in document D13 as evidence when assessing inventive step, since it merely confirmed that the technical effect taught in the application as filed to be associated with modifying position 252 was, indeed, correct.

There was nothing in decision T 1329/04 of 28 June 2005, as relied on by the opponent, to support its position that document D13 should not be taken into account. There was indeed nothing in the application (or elsewhere in the art) that would have led the skilled person to the conclusion that it was implausible that the observed effects might not be obtained by making modifications at residue 252.

The data in document D13 confirmed that the claimed invention did, indeed, solve the problem that was taught to be solved by the application as filed, and so supported a finding in favour of inventive step.

Auxiliary request 1

Allowability of amendments (Article 123(2) EPC)

Support for claim 1, in particular with regard to the feature '*the pI_o of the mutated subtilisin protease is closer to the pH of the wash liquor formed by the detergent composition than is the pI_o of the parent protease*', could be found in various passages of the application as filed, namely on page 74, lines 15 to 19; page 75, lines 36 to 38; page 77, lines 17 to 19 and, more particularly, on page 22, lines 20 to 28.

The term "low" as used on page 22, line 24 to qualify the wash liquor pH was a relative term for which no particular definition had been given in the application as filed. As such, it could be ignored.

Sufficiency of disclosure (Article 83 EPC)

It was only required that the invention was disclosed in a manner that was sufficiently clear and complete for it to be 'performable' by the skilled person. Thus, for the assessment of sufficiency, the issue was whether the application provided adequate guidance to allow the skilled person to perform the claimed invention without undue burden.

In this respect, the skilled person was readily able to determine whether the isoelectric point of the mutant was lower or higher compared to the parent protein, using entirely ordinary methodologies that were well known at the filing date. Paragraph [0138] of the patent made it clear that calculated pI_o values were, at the very least, useful to indicate in what direction the pI_o for a given mutant enzyme will move in comparison to the pI_o of the parent enzyme. A calculation of the exact modified pI_o value was not necessarily required to perform the invention. It could be enough, for the purposes of practicing the present invention, to know that the pI_o had moved in a particular direction, i.e. either towards or away from the operational pH value of interest, and this could be readily achieved using calculated pI_o values. In any case, methods of accurately calculating the pI_o of proteins were known in the art at the filing date (see document D44) and if an accurate pI_o value was required, the skilled person would have been able to determine such value by well known experimental protocols. As discussed at paragraph [0140] of the patent, pI_o values could also be measured (i.e. "observed") if an accurate value was deemed necessary.

All steps of the claimed method were readily performable, without undue burden, by the skilled person, and so the claims fully complied with Article 83 EPC.

Inventive step (Article 56 EPC)

The test data for mutants that had a lower pI_o than their parents clearly demonstrated that they reliably possessed improved wash activity compared to their parents in wash liquors that had a pH value lower than the pI_o of the parent protein. It was clear that the data in the application as filed made it at least "plausible" that the same effect could be achieved by making similar modifications at position 252. Accordingly, a proper assessment of inventive step of the claimed invention should take into account the post-filing data provided by document D13. That document confirmed that those modifications at position 252, which reduced the pI_o of the protein and thereby moved the pI_o closer to the pH of the wash liquor than was the pI_o of the parent protein, did lead to improved performance. In the application as filed, such a technical effect was ascribed to this modification.

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

Main request

Inventive step (Article 56 EPC)

The application proposed that the wash performance of a subtilisin protease at a particular pH could be improved by altering its isolelectric point to lie closer to that pH. This hypothesis required that a shift in the pI_o would result in enhanced wash performance in a detergent solution where the shifted pI_o was closer to the pH of the detergent solution and the converse where the shifted pI_o was further away from the pH of the detergent solution. The hypothesis predicted that the peak of activity occurred where pH was equal to pI_o and that activity should fall away on either side of this value. If the patent's hypothesis were correct, then it should always be possible to improve wash performance by moving the pI_o of an enzyme towards the pH of the wash liquor. It followed that the optimum performance should be found when the pI_o and the pH were equal. This, however, was not the case, as could be clearly illustrated by the data in Table VI (see page 37 of the patent specification). Thus, the main request did not involve an inventive step.

The post-filed data provided in document D13 certainly did show that technical benefits could be achieved, under some circumstances, by substitution at position 252. The question to be addressed was whether the application provided the kind of disclosure in respect of position 252 which was suitable for support by those post-filed data. Because the link between pI_o, pH and wash performance was flawed, the application did not teach (in the sense of providing a real substantial

technical teaching) that mutation at position 252 could improve wash performance. It simply proposed 252 as a candidate position for mutation, which could, or could not lead to an improvement of the wash performance. Thus, the improvements found by mutation at position 252 were a "new effect" not disclosed in the application as filed because they were position- and substituent-specific and had nothing to do with pI_o. While the application as filed merely put forward a problem to be solved, it was only the data in document D13 which provided the basis to establish that the claimed subject-matter solved indeed the problem it purported to solve. Thus, in line with decision T 1329/04 (see above), document D13 could not be used to support an inventive step.

Auxiliary request 1

Allowability of amendments (Article 123(2) EPC)

The passage on page 22 lines 20 to 28 of the application as filed and the equivalent passage in the parent application as filed (see WO 91/00345, page 22, lines 18 to 26) referred to a wash liquor of low pH and therefore provided support only for a specific embodiment of the method according to claim 1.

Sufficiency of disclosure (Article 83 EPC)

The method of calculating the pI_o referred to in the patent-in-suit relied primarily on the assignment of a pK value to each potentially charged amino acid residue, while the pK of a given amino acid residue would depend on its neighbours. As regards the Tyr residues, only

three pK values were given, whereas the protein contained seven such residues. As the patent did not specify which Tyr residue corresponded to which pH value, the skilled person would not have known how the pI_o would be affected by mutation of any particular Tyr residue. Therefore, no valid method of calculating the pI_o was provided by the patent. Nor was the skilled person looking for an alternative method taught how to experimentally measure the pI_o. In this respect, document D46 was of no relevance, as the titration technique it described was for gelatine, a non-enzymatic protein, which moreover was tested in a denatured form. Post-published document D43, which described attempts to measure the pI_o of subtilisins by isoelectric focusing, illustrated the lack of reliability of such a determination, as the measured value (11.15) was more than a whole pH unit away from the predicted calculated value as given in the patent (10.06 or 10.02), and substantially different from the "observed value" of 9.7 quoted in the patent.

The patentee attempted to explain some discrepancies in its results by invoking "pH drift", apparently caused by environmental CO_2 and the grass juice released from the cloth during cleaning. Notably, the patentee provided no evidence that such drift actually occurred during their wash tests. If the patentee was correct about pH drift, then the skilled person did not know what pH he/she was designing the enzyme for.

Inventive step (Article 56 EPC)

The respondent did not provide additional comments on this issue.

XV. The appellant (patentee) requested that the decision under appeal be set aside and the patent be maintained as granted (main request) or on the basis of the auxiliary request 1 filed as auxiliary request 4 with letter dated 15 March 2010.

XVI. The respondent (opponent) requested that the appeal be dismissed.

Reasons for the Decision

Main request

Inventive step (Article 56 EPC)

1. As a preliminary remark, the board notes that claim 1 was derived from claim 22 as originally filed, which was directed to mutated proteases prepared from a parent enzyme chosen among a limited list of 13 wild-type subtilisin proteases. The parent proteases as originally claimed were mutated at any one or more of 66 positions in their amino acid sequence, including position 252 (see claim 1 as originally filed). In the course of the examination proceedings, the claimed subject-matter was limited to a method of preparing subtilisin proteases mutated at least at that latter position. This limitation is contained in claim 1 of the main request. However, in the experiments reported in the description, no subtilisin proteases mutated at least at position 252 were tested. Results concerning such mutated subtilisin proteases were provided for the first time in an experimental report (document D13)

filed by the appellant during the examination proceedings with letter dated 10 October 2003.

2. Another point which is of importance for the assessment of inventive step is the fact that, as implied by the wording of claim 1, the wash performance of a given mutant, measured either as an improvement factor (see Tables III and VII) or a differential remission (denoted "delta R" in Tables VIII to XI), should not be compared to the wash performance of any other given mutant, but to the performance of the corresponding wild-type parent subtilisin protease.

3. Inventive step must be ascertained by an analysis of the experimental data contained in the application. In fact, the general concept which - purportedly - underlies the method of claim 1 must be supported by this data. For this analysis, the board has paid particular attention to Table XI (see page 40 of the patent specification which, due to printing errors, is to be read in the light of the same table on page 77 of the international application WO 91/00345). Table XI contains the results of a significant number of wash assays performed with the parent subtilisin 309 (S000) and five mutants thereof, namely S027, S028, S031, S032 and S033, each having been tested at five different wash liquor pH values.

4. The analysis of the results contained in Table XI shows that, when the pI_o of the mutant is **higher** than that of the parent subtilisin and closer to the pH of the wash liquor than is the pI_o of the parent subtilisin protease, an improvement is not achieved in a number of cases; rather, a decrease of the wash performance is observed.

This is the case for (i) mutant S027 with a wash liquor pH of 10.25, 10.50, 10.75, 11.0, and 12.0, (ii) mutant S031 with a wash liquor pH of 10.25, 10.50, 10.75 and 11.0, (iii) mutant S032 with a wash liquor pH of 11,0 and (iv) mutant S033 with a wash liquor pH of 10.25, 10.50, 10.75 and 11.0. In the board's judgment, the inconsistencies with the general concept, which affect the five mutants tested, in particular three of them for at least four of the pH values tested, must be interpreted as indicating the absence of an established correlation between (i) a pI_o of the mutant **higher** than that of the parent subtilisin protease and closer to the pH of the wash liquor than is the pI_o of the parent subtilisin protease, and (ii) an improvement of the wash performance.

5. The board thus concludes that the general concept which - purportedly - underlies the method of claim 1 is not supported for most cases of mutants having a pI_o **higher** than that of the parent subtilisin protease. As a result, the subject-matter of claim 1 does not involve an inventive step, as the purported teaching does not solve the problem it is meant to solve. Therefore, the main request does not comply with the requirements of Article 56 EPC and cannot form a basis for the maintenance of the patent.

Auxiliary request 1

Allowability of amendments (Article 123(2) EPC

6. During the oral proceedings, the question arose whether, in view of the feature contained in claim 1 of auxiliary request 1 that '*the pI_o of the mutated subtilisin protein is closer to the pH of the wash liquor formed by the detergent composition than is the pI_o of the parent protease*' introduced in the course of the examination proceedings, the subject-matter of claim 1 extends beyond the content of both the application as filed on which the patent-in-suit was granted, and the parent application as filed.

7. Of particular relevance for this assessment is the passage on page 22, lines 20 to 28 of the application as filed, which reads "*Stated differently, it was found that changing the isoelectric point, pI_o, of the enzyme in a direction to approach a lower pH, also shifted the pH of optimum wash performance of the enzyme to a lower value, meaning that in order to design an enzyme to a wash liquor of low pH, in which the enzyme is to be active, improvement in the wash performance of a known subtilisin enzyme may be obtained by mutating the gene of the known subtilisin enzyme to obtain a mutant enzyme having a lower pI_o*" (underlining added by the board). This passage exposes the concept on which claim 1 relies, i.e. the preparation of a mutated subtilisin protease having a pI_o which is lower than that of the parent subtilisin protease and closer to the pH of the wash liquor than is the pI_o of the parent subtilisin protease. The same relevant passage is found

in the parent application as filed (see page 22, lines 18 to 26 in the international application WO 91/00345).

8. The respondent argued that claim 1 of the auxiliary request failed to specify that the method was for a wash liquor of low pH and that, therefore, it was not supported by the afore-mentioned passage. This argument is not convincing. The board notes that "low" is a relative term for the interpretation of which no general and/or detailed guidance - apart from the mere indication that in the context of the experiments the results of which are reported in Table III (see page 49 of the application as filed) a wash liquor pH of 8.3 was considered to be low - has been given.

9. It is concluded that claim 1 does not contain matter which extends beyond the content of either the parent application as filed, or the present application as filed and that, therefore, the requirements of Articles 76(1) and 123(2) EPC are complied with.

Sufficiency of disclosure (Article 83 EPC)

10. The respondent's objections which are directed against the method of claim 1 are twofold. One of the objections is based on the argument that the patent does not provide a valid method for determining the pI_o values. The other objection is associated with the concept of 'pH drift' which was introduced by the appellant in its statement of grounds.

11. Calculation of the isoelectric point of the parent subtilisin 309 wild type enzyme (S000) is disclosed in the section entitled "*Computation of isoelectric point*

(pI$_o$)" of the description (see paragraphs [0133] to [0138] on pages 23 to 24 of the patent specification, including Table II). The calculation procedure was as follows: pK values were assigned to each potentially charged amino acid residue (Tyr, Asp, Glu, C-terminal Arg, Cys, Arg, His, Lys, N-terminal Ala) and Ca_{2+}, account being taken of the environment of each of the residues. The ratio of the occurrence of an amino acid residue at a given pH in charged or uncharged form was calculated for both negative and positive charges. Subsequently, the relative charge or charge contribution allocated to each charged residue was calculated. The isoelectric point, i.e. the pH value where the sum of all the charge contributions from the charged residues is equal to zero, was found by iteration. In Table II, the calculated isoelectric point for S000 was found to be 10.06. In the board's judgment, this calculation procedure amounts to a valid method for calculating pI$_o$.

12. As indicated at paragraph [0138] of the patent specification (see page 24), the pK value assigned to a particular amino acid residue will vary depending on its environment, which in itself will be influenced by the experimental conditions. This explains why different pI$_o$ calculated values are indicated in the experimental part of the description for a given subtilisin: in this respect, the calculated pI$_o$ of S000 has been found to be 10.02 (see Tables III on page 24 and VI on page 37) or 10.06 (see Table II on page 24 as well as Tables VIII on page 38, IX on page 39 and X on page 39).

13. In the board's judgment, in order to carry out the method of claim 1, the skilled person, who is someone making evaluations which are realistic and technically sound, will apply exactly the same criteria to calculate each of the respective isoelectric points of the mutated subtilisin protease and the parent subtilisin protease. He/she will therefore be in a position to determine whether the mutated subtilisin protease has a pI_o lower than that of the parent subtilisin protease. Applying the same criteria, variations in the calculation resulting from the variability of the environment of each residue under the influence of the experimental conditions, if any, will be of the same order for both pI_o, and it will always be possible to determine with certainty whether the pI_o of the mutated subtilisin is lower than that of the parent subtilisin protease, and whether the pI_o of the mutated subtilisin protease is closer to the pH of the wash liquor formed by the detergent composition than is the pI_o of the parent subtilisin protease.

14. Furthermore, the respondent's argument that, because in the exemplified calculation procedure, as derivable from Table II, it has not been indicated for each of the seven Tyr residues which precise pK had been assigned, the method for calculating the pI_o was not valid, is not tenable. In the board's judgment, this information is not necessary. What is required is that a pK value be assigned by the skilled person to each of the Tyr residues of the subtilisin protease taking into account the same criteria, whether it is the mutant or the parent, and that he/she computes all the values in the way indicated in paragraphs [0135] to [0137] of the patent specification. Thus, relying on the information

contained in the patent, the skilled person is in a position to determine the pI_0 values. There is no need for the board to assess whether a method of measurement such as the one described in either of documents D43 and D46 may be applied to a subtilisin protease.

15. Thus, it is concluded that the method according to claim 1 is sufficiently disclosed in the patent and that, therefore, auxiliary request 1 complies with the requirements of Article 83 EPC.

Inventive step (Article 56 EPC)

16. Document D1, which was considered by the opposition division to represent the closest state of the art, shows that, by changing the surface charge of subtilisin BPN' from Bacillus amyloliquefaciens by site-directed mutagenesis, mutated subtilisins with significantly shifted pH-activity profiles, higher catalytic activities and altered specificities are obtained. Single and double mutants were constructed by mutating Asp99 and Glu156 residues. None of the mutants were tested for their wash performance in a detergent composition.

17. Taking document D1 as the closest state of the art, the technical problem to be solved is seen in the provision of a method of generating a mutated subtilisin protease exhibiting improved behaviour in detergent solutions at a given pH compared to a wild-type parent subtilisin protease. The solution to this problem is a method according to claim 1. This method is characterised in that one mutation is effected in the parent subtilisin protease (to be chosen from a limited list of 13

enzymes) at a position corresponding to position 252 in subtilisin BPN', by substitution and in such a way that the mutated subtilisin has an isoelectric point (pI_o) which is **lower** than that of said parent subtilisin protease and closer to the pH of the wash liquor than the pI_o of the parent subtilisin protease.

18. An analysis of all the results presented in the patent (see Tables III, VI, VII, IX and X on pages 24, 25 and 37 to 39 of the patent specification) with respect to a mutated subtilisin protease having an isoelectric point (pI_o) which is **lower** than that of said parent subtilisin and closer to the pH of the wash liquor than the pI_o of the parent subtilisin protease, shows that mutated subtilisin proteases which fulfil these requirements always exhibit an improved wash performance relative to the parent subtilisin (see mutants S001, S003, S004, S005, S012, S019, S020, S021, S022, S023, S024, S025, S035, S201, S202 and S203 with a wash liquor pH of 8.3 in Table III; mutants S001, S003, S004, S005, S012 and S019 with a wash liquor pH of 8.0 and 9.0 in Table VI; mutant S020 with a wash liquor pH of 8.0 in Table VI; mutant S021 with a wash liquor pH of 9.85 in Table VII; mutants S003, S004 and S006 with a wash liquor pH of 9.1 in Table VIII; mutants S015, S017, S021, S023 and S025 with a wash liquor pH of 7.0, 8.0 and 9.0 in Table X; and mutant S024 with a wash liquor pH of 7.0 and 8.0 in Table X).

19. The board regards these results as an unequivocal evidence that confirms the general concept on which the method of claim 1 relies, i.e. the concept that a mutated subtilisin protease having an isoelectric point (pI_o) which is **lower** than that of said parent subtilisin

and closer to the pH of the wash liquor than is the pI_o of the parent subtilisin, exhibits an improved wash performance. Whilst in the experiments shown in the application as filed none of the tested mutants differs from the parent wild-type subtilisin in that it has been mutated at position 252, document D13 shows that the two mutants tested with a mutation at position 252 (N252D and N252E), both having a pI_o (9.9) lower than the pI_o (10.2) of the parent subtilisin (the savinase wild-type subtilisin 309) and closer to the pH (7.7-7.9) of the wash liquor than is the pI_o of the parent subtilisin, exhibit an improved performance relative to the parent subtilisin.

20. Contrary to the position taken by the opposition division, document D13 can be taken into account because it provides only a confirmation that the general concept underlying the method of the invention is sound. In this appreciation, the board does not deviate from decision T 1329/04 (see *supra*). In this decision, the then competent board ruled that those post-published documents which are the first disclosure going beyond speculation should not be taken into consideration for the assessment of inventive step (see point 12 of the decision). Since, in the present case, the experimental data provided in the application as filed made plausible the general concept underlying the invention, document D13 cannot be regarded as the first disclosure going beyond speculation, but rather as the confirmation that the general concept works also for the specific mutation at position 252.

21. In view of the above remarks, there is no reason to doubt that the technical problem is solved over the whole ambit of claim 1.

22. None of the available prior art document other than document D1 relate to methods for preparing mutant subtilisins, let alone mutants exhibiting wash performance of interest. As none of those documents suggests that a correlation exists between a mutation at position 252 and an improvement in wash performance, in the board's judgement, the skilled person would not have realised that a subtilisin mutated at position 252 could be associated with an improvement in wash performance. Thus, he/she would not have been in a position to arrive at the method of claim 1. It is therefore concluded that the subject-matter of claim 1 involves an inventive step. Since the remaining claims are dependent on claim 1, auxiliary request 1 as a whole meets the requirements of Article 56 EPC.

23. As auxiliary request 1 complies with the requirements of the EPC, it can form the basis for the maintenance of the patent.

Order

For these reasons it is decided that:

1. The decision under appeal is set aside.

2. The case is remitted to the opposition division with the order to maintain the patent on the basis of the auxiliary request 1 filed as auxiliary request 4 with letter dated 15 March 2010 and a description to be adapted thereto.

The Registrar | The Chairman

A. Wolinski | C. Heath