

Internal distribution code:

- (A) [-] Publication in OJ
(B) [-] To Chairmen and Members
(C) [-] To Chairmen
(D) [X] No distribution

**Datasheet for the decision
of 11 July 2019**

Case Number: T 0451/14 - 3.3.08

Application Number: 05018139.5

Publication Number: 1612271

IPC: C12N15/57, C12N9/54, C11D3/386,
A23K1/165

Language of the proceedings: EN

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

Patent Proprietor:
Danisco US Inc.

Opponents:
Novozymes A/S
Henkel AG & Co. KGaA

Headword:
Subtilisin/DANISCO

Relevant legal provisions:
EPC Art. 83, 113(1)
RPBA Art. 15(1), 15(3)

Keyword:

Main request and auxiliary request - sufficiency of disclosure
(no)

Decisions cited:

T 0190/99

Catchword:



Beschwerdekammern
Boards of Appeal
Chambres de recours

Boards of Appeal of the
European Patent Office
Richard-Reitzner-Allee 8
85540 Haar
GERMANY
Tel. +49 (0)89 2399-0
Fax +49 (0)89 2399-4465

Case Number: T 0451/14 - 3.3.08

D E C I S I O N
of Technical Board of Appeal 3.3.08
of 11 July 2019

Appellant: Novozymes A/S
(Opponent 1) Krogshøjvej 36
2880 Bagsværd (DK)

Representative: Grünecker Patent- und Rechtsanwälte
PartG mbB
Leopoldstraße 4
80802 München (DE)

Respondent: Danisco US Inc.
(Patent Proprietor) 925 Page Mill Road
Palo Alto, CA 94304 (US)

Representative: Mewburn Ellis LLP
City Tower
40 Basinghall Street
London EC2V 5DE (GB)

Party as of right: Henkel AG & Co. KGaA
(Opponent 2) Henkelstrasse 67
40589 Düsseldorf (DE)

Representative: Henkel AG & Co. KGaA
CLI Patents
Z01
40191 Düsseldorf (DE)

Decision under appeal: **Decision of the Opposition Division of the
European Patent Office posted on 20 December
2013 rejecting the opposition filed against
European patent No. 1612271 pursuant to Article
101(2) EPC.**

Composition of the Board:

Chairman	B. Stolz
Members:	P. Julià
	J. Geschwind

Summary of Facts and Submissions

- I. European patent no. 1 612 271 is based on European patent application no. 05 018 139.5 (hereinafter "the patent application"), a divisional application of the earlier European patent application no. 98 955 111.4, published under the PCT as International patent application WO 99/20771. The patent was granted with four claims. Two oppositions were filed on the grounds as set forth in Articles 100(a), (b) and (c) EPC. The opposition division considered that none of the grounds of opposition prejudiced the maintenance of the patent as granted and, accordingly, rejected the opposition.
- II. An appeal was lodged by opponent 01 (appellant). In the statement setting out the grounds of appeal, the appellant maintained the objections raised on the grounds of Articles 100(a), (b) and (c) EPC. In reply to the statement of grounds of appeal, the patent proprietor (respondent) filed an auxiliary request. Both parties requested oral proceedings as an auxiliary measure.
- III. The appellant made further substantive submissions and the respondent replied to them.
- IV. No submissions were filed by opponent 02 (party as of right).
- V. The board summoned the parties to oral proceedings. In a communication pursuant to Article 15(1) of the Rules of Procedure of the Boards of Appeal (RPBA), the parties were informed of the board's provisional, non-binding opinion on some of the issues of the case. The board stated *inter alia* that the main request and

the auxiliary request did not sufficiently disclose the claimed subject matter.

- VI. In reply thereto and without making any substantive submissions, the appellant and the respondent, under cover of letters dated 2 July 2019 and 8 July 2019, respectively, informed the board of their intention not to attend the oral proceedings.
- VII. Oral proceedings were held on 11 July 2019 in the absence of all parties.
- VIII. Claim 1 of the main request (claims as granted) reads as follows:

"1. A method of improving the performance of a subtilisin in a low, medium and high detergent concentration system having less than about 800 ppm, between about 800 ppm and about 2000 ppm, and above about 2000 ppm detergent components present in the wash water respectively:

a) substituting an amino acid at one or more residue positions in a precursor subtilisin to produce a subtilisin variant wherein the substitution alters the charge at that position to make the charge more positive or less negative compared to the precursor;

b) substituting an amino acid at one or more residue 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;

c) testing the variant to determine its effectiveness compared to the precursor subtilisin in a low, medium

and high detergent concentration system having less than about 800 ppm, between about 800 ppm and about 2000 ppm, and above about 2000 ppm detergent components present in the wash water respectively; and

d) repeating steps a) - c) as necessary to produce a subtilisin variant that is more effective in a low, medium and high detergent concentration system than a precursor protease."

IX. Claim 1 of the auxiliary request reads as claim 1 of the main request, except for step d) which reads as follows:

"1. [as in claim 1 of the main request] ...,

d) repeating steps a) - c) at least once to produce a subtilisin variant that is more effective in a low, medium and high detergent concentration system than the precursor subtilisin." (underlined by the board)

X. The following document is cited in this decision:

(C11): Tables 1 and 2 in W.R. Taylor, "The classification of amino acid conservation", Journal of Theoretical Biology, Vol. 119, 1986, pages 205 to 218.

XI. Appellant's submissions, insofar as relevant to this decision, may be summarised as follows:

Article 83 EPC; Main request and auxiliary request

Claim 1 was directed to a method of improving the washing performance/effectiveness of a subtilisin, however without defining the reference subtilisin.

Claim 1 required to test a subtilisin variant and a subtilisin precursor under three different washing conditions so as to determine their washing performance/effectiveness. However, claim 1 did not define whether the subtilisin precursor used to make the substitutions in steps (a) and (b) (thereby producing a subtilisin variant) was the same as the subtilisin precursor used in step (d) for testing/ comparing the washing performance/effectiveness. Even if it was assumed that there was only one reference subtilisin, serving as the starting point for carrying out the substitutions and for comparing the washing performance/effectiveness, it could still be any subtilisin. In the absence of a defined subtilisin reference in claim 1, there was no single trend which could be ascribed to the substitutions made before testing for wash performance/effectiveness; two changes of charges could lead to arbitrary results or to contradictory results with regard to the washing performance/effectiveness, depending on what type of subtilisin reference was used.

An iterative method required that, upon conducting a sequence of steps (steps (a) to (c) of claim 1), an iteration took place leading to an approximation towards an improved effect (washing performance/ effectiveness). Such iteration required starting from the result of the last step of a first round of steps, i.e. the subtilisin variant of a first round of substitutions, and further improving this subtilisin in a second iterative round. However, neither step (a) nor step (b) of claim 1 referred back to a subtilisin which was the result of step (c) and/or step (d) of claim 1, a prerequisite for an iteration. Claim 1 did not even define which subtilisin was used in steps (a) or (b), it only required the repetition of steps (a) to (c) but

not an iteration. Claim 1 merely defined a simple trial and error repetition with an undefined starting point. Although there was no difficulty in performing steps (a) to (c) of claim 1, which were only routine steps, they did not allow for any type of improvement of the washing performance/effectiveness. It was impossible to rework the combination of steps (a) to (d) as required by claim 1 without undue burden because important information was missing how these steps were linked with one another. The skilled person had to perform a research program.

The result shown in Table 14 of Example 2 of the patent application was not a technical teaching because it was merely a matter of the sorting algorithm applied to show that all subtilisin variants were better than the specific (worst) subtilisin reference chosen/selected by this algorithm. The results were entirely different when using the same information shown in Table 14 but a different sorting algorithm (selecting thereby a different subtilisin reference). Example 2 did not demonstrate that introducing at least one positive (less negative) charge and at least one negative (less positive) charge in an arbitrary subtilisin reference improved the washing performance/effectiveness. It was totally unpredictable whether a change of two or more charges (at least one positive/less negative and at least one negative/less positive) led to an improvement in the subtilisin performance under low, medium and/or high washing conditions.

Moreover, neither the effectiveness of the wash performance nor the washing conditions were defined in claim 1. However, the amount and nature of the washing powder, the amount of subtilisin, the temperature and pH, all were known to influence the washing

performance/effectiveness of the compositions used. The lack of definition of proper washing conditions in claim 1 led to an insufficiency of disclosure in the sense of Article 83 EPC. The more so, because there was an ambiguous functional feature in claim 1, namely the term "effectiveness", which covered a plurality of different possible meanings (proteolytic activity, substrate specificity, pH activity profile, etc.).

XII. Respondent's submissions, insofar as relevant to this decision, may be summarised as follows:

Article 83 EPC ; Main request and auxiliary request

Claim 1 provided clearly defined instructions for achieving the desired result. The amino acid substitutions indicated in claim 1 were not uncharacterised; they were clearly and precisely defined, requiring to change the charges of at least two residues of a subtilisin; one more positive, the other more negative. Sufficient examples were provided by the patent application to demonstrate the success of this approach, namely to make more-positive and more-negative amino acid substitutions for improving the washing performance/effectiveness of a subtilisin. The skilled person was capable of mutating a subtilisin precursor to alter its charge in accordance with steps (a) and (b) of claim 1 (paragraph [0033] of the patent application), and to test/compare the washing performance/effectiveness of the subtilisin variant with that of the subtilisin precursor in low, medium and high detergent concentration systems (e.g. paragraphs [0020] to [0022] and Examples 1 and 2 of the patent application). Importantly, the method of claim 1 relied on a relative test rather than an absolute one; the washing performance/effectiveness of the subtilisin

variant was tested under the same conditions as the subtilisin precursor, and the results were normalised to those of the subtilisin precursor (paragraph [0073] of the patent application). Claim 1 provided: i) a structurally-defined starting composition (subtilisin precursor), ii) clearly defined instructions for adapting the starting composition (steps (a) and (b)), and iii) clearly defined selection rules (steps (c) and (d)). The skilled person was able to routinely perform each step of claim 1 and to achieve the desired result (improved washing performance/effectiveness) without undue burden or having to resort to undue trial and error. Indeed, the opposition division acknowledged that a skilled person could carry out the method of claim 1 without undue burden and to achieve a success rate of about 30%, which was considered to be sufficient.

The testing and repetition defined in steps (c) and (d) of claim 1 showed the claimed method to be an iterative method, in which subtilisin variants were produced that balanced the changes to improve the washing performance/effectiveness in all three detergent concentration systems. The skilled person with a mind willing to understand (cf. T 190/99 of 6 March 2001) would interpret the subtilisin precursor of step (d) to correspond to the subtilisin precursor of steps (a) to (c) of claim 1. Claim 1 required steps (a) to (c) to be performed iteratively, as often as necessary, so as to produce a more-effective subtilisin variant. It was illogical to read the term "precursor subtilisin" in steps (a) and (b) of claim 1 as referring to two different subtilisin precursors, not least because the reference to the subtilisin precursor in step (c) showed that there was only a single subtilisin precursor against which the subtilisin variant was

compared. The skilled person, when considering the claim and ruling out illogical interpretations, would have had no doubt about the source of the subtilisin variant of step (c) of claim 1 and that the subtilisin variants produced according to steps (a) to (c) could be again substituted and tested as necessary, as defined in step (d) of claim 1.

In Table 14 of Example 2, the subtilisin with the fewest substitutions with respect to the wild-type subtilisin from *B. amyloliquefaciens* (N76D-S103A-V104I, designated S1) was the reference subtilisin. It was therefore entirely reasonable to take the subtilisin with the fewest substitution as the subtilisin precursor from which further subtilisin variants were made. Table 14 showed that the changes made in accordance with steps (a) and (b) of claim 1 did not compete or cancel each other. On the contrary, they were balanced such that (washing performance/ effectiveness) improvement was seen across the range of detergent concentrations used. Steps (c) and (d) of claim 1 set out the testing and repetition steps that "wed out" those changes that moved washing performance/ effectiveness in the wrong direction in the detergent systems. Although steps (a) and (b) of claim 1 could be theoretically used to undo or reverse the improvement-conferring charge changes described in Table 14 (as done in appellant's submissions), such a reversal did not fall within the scope of claim 1 because the claim clearly defined, and was explicitly directed to, a method of improving the washing performance/ effectiveness of a subtilisin variant. This reversal approach required a forced misinterpretation of claim 1.

The patent application provided suitable testing conditions (Examples) and required only to assess the relative washing performance/effectiveness (i.e. normalised to the subtilisin precursor). The detergent concentrations were defined in claim 1 and there was no evidence that other parameters could affect some subtilisin variants more than other variants and/or than the subtilisin precursor. Nor was evidence on file that a reasonable variation in temperature or subtilisin concentration could change the washing performance/effectiveness of the subtilisin variants in an unpredictable way, much less that the relative performance/effectiveness of the subtilisin precursor and the subtilisin variant could be reversed by such conditions. All terms in claim 1 had a clear meaning for a person skilled in the art who could perform each (routine) step without undue burden.

- XIII. The appellant (opponent 01) requested that the decision under appeal be set aside and the patent revoked.
- XIV. The respondent (patent proprietor) requested that, as its main request, the appeal be dismissed or, in the alternative, the decision under appeal be set aside and the patent maintained in amended form on the basis of the auxiliary request filed on 8 September 2014.
- XV. There were no requests on file from the party as of right (opponent 02).

Reasons for the Decision

Article 113(1)EPC

1. By their decision not to attend the oral proceedings and not to file substantive arguments in reply to the issues raised in the board's communication pursuant to Article 15(1) RPBA, all parties have chosen not to make use of the opportunity to comment on the board's provisional, non-binding opinion, either in writing or at the oral proceedings, in particular not the respondent to whom this opinion was unfavourable. According to Article 15(3) RPBA, the board is not obliged to delay any step in the proceedings, including its decision, by reason only of the absence at the oral proceedings of any party duly summoned who may then be treated as relying on its written case.

2. In the light thereof, the present decision is based on the same grounds, arguments and evidence on which the provisional, non-binding opinion of the board was based.

Main request (Claims as granted)

Article 100(b) EPC

3. The board considers the teaching of the patent application to be summarised in paragraph [0017], which describes:

(i) a method for producing "a protease variant that is more efficacious in a low detergent concentration system" corresponding to step (b) of claim 1 (see also paragraph [0057] of the patent application), and (ii) a method for producing "a protease variant that is more efficacious in a high detergent concentration system"

corresponding to step (a) of claim 1 (see also paragraph [0058] of the patent application).

It is also indicated in paragraph [0017] that "many of the protease variants useful in the low detergent concentration system and/or the high detergent concentration system also are effective in a medium detergent concentration system" (underlined by the board). However, there is no information in the patent application disclosing the actual proportion or amount of protease variants effective in a medium detergent, neither for variants useful in the low or the high detergent concentration systems nor for variants effective in low and high detergent systems. There are no results reported in Examples 1 and 2 for a medium detergent concentration system and there is no information given on the performance or effectiveness in such a system for any of the subtilisin variants shown in these Examples. The meaning of the term "many" is not defined in the patent application and remains fully open to interpretation. In the board's view, it cannot be equated to any percentage such as, for instance, the 30% referred to by the respondent.

In the same paragraph [0017], it is further stated that "[b]y balancing these changes, it is possible to produce a protease variant that works well in low detergent concentration systems, low and medium detergent concentration systems, medium and high detergent concentration systems, high detergent concentration systems, or all three detergent concentration systems" (underlined by the board). There is however no indication given to the skilled person on how to perform this balance and thereby, produce protease variants that might work well in all three detergent concentration systems.

4. Paragraphs [0018] and [0019] of the patent application are also highly relevant. Paragraph [0018] indicates that the "electrostatic charge ... in aqueous solution is a function of the pH" and that the dissociation ranges for amino acid side chains indicated therein "are average values for many proteins but they are known to be affected by unusual structural configurations in some proteins". In line therewith, paragraph [0019] states that a "protein will lose or gain charge when the pH is shifted or when an amino acid with an ionizable side chain residue is added or removed" and therefore, all references to a (net) increase/decrease of the positive/negative charge are explicitly indicated to be "at a given pH", "at the given pH" and "at the pH of observation" (see document (C11) for the properties of the amino acid residues).

In this context, it is worth noting the indication in paragraph [0063] that the "proteases of the invention can be formulated into ... detergents having pH between 6.5 and 12.0" and, in paragraph [0064], that "any ... pH suitable for the detergent is also suitable for the present compositions as long as the pH is within the above range". According thereto, it is appropriate to assume that the detergent systems used in Examples 1 and 2 of the patent application (Column B in Example 1 and column A in Example 2: "Ariel Ultra", a low concentration detergent system; Column C in Example 1 and column B in Example 2: "Ariel Futur", a high concentration detergent system) have a pH within the pH range indicated above. Therefore, it is also appropriate to assume that the charge difference of the subtilisin variants given in column A of Example 1 is also measured at the pH of these detergent systems.

In view of this information, the board considers that, at the alkaline pH range of the detergent systems used in Examples 1 and 2, the substitution/introduction of acidic residues (Glu/E; Asp/D) "at one or more positions in a precursor subtilisin" may not always and necessarily result in an alteration of "the charge at that position". This is also the case for the substitution/introduction of the basic residues (His/H; Lys/K; Arg/R) when using high-alkaline detergent systems. In the board's view, the skilled person would read and interpret the charge differences given in column A of Example 1 in the light of this information and taking into account the whole disclosure of the patent application.

5. The detergent composition and washing conditions, i.e. the complete detergent system, used for testing and comparing the "performance" (preamble of claim 1) or the "effectiveness" (steps (c) and (d) of claim 1) of the precursor subtilisin and the subtilisin variants, not only influence the results of said testing and comparison, as argued by the appellant, but the charge difference of the substitutions carried out in steps (a) and (b) of this method as well. However, neither the detergent composition nor the washing conditions are defined in claim 1. Likewise, although there is a reference in claim 1 to the detergent concentration in the wash water, there is no indication whatsoever as regards the cleaning application of these detergent which is thus not limited to "the treatment of a textile", such as "silk or wool" (cf. paragraph [0068] of the patent application), but may include *inter alia* any of the applications referred to in paragraph [0062] of the patent application, such as dishcare formulations, contact lens cleaning solutions

or products, etc. In line with the established case law (cf. "Case Law of the Boards of Appeal of the EPO", 8th edition 2016, I.C.4.8, 110), there is no reason to interpret and limit the scope of claim 1 to a particular group of detergents, washing conditions and/or cleaning applications.

6. It is also in line with this case law that the terms "subtilisin" and "precursor subtilisin" are understood in the broadest possible way. All the more so in view of the definition given to the term "subtilisin" in the patent application, namely "a naturally-occurring subtilisin or a recombinant subtilisin" (cf. paragraph [0030] of the patent application); a definition which is also in line with that given to "precursor proteases" (cf. paragraph [0033] of the patent application). According to paragraph [0031], the definition of "recombinant subtilisin" and "recombinant protease" includes the "substitution, deletion or insertion of one or more amino acids in the naturally-occurring amino acid sequence". There is no limitation as regards the nature and number of these substitutions, deletions or insertions or their position within the naturally-occurring subtilisin sequence. Indeed, all the precursor subtilisins used in Example 1 for testing the performance of the subtilisin variants are "recombinant subtilisins" which, as shown in Tables 1 to 13, have several substitutions within the naturally-occurring subtilisin sequence. The number of substitutions is as low as four (Tables 1, 3 and 4) and as high as ten residues (Table 13), and include substitutions that may result in negative (N62D, N76D, G159D, N248D) as well as in positive (Q12R, Q109R, Q236H, Q245R, N252K) charge differences depending on the pH of the detergent composition and washing conditions used. It is worth noting here that, as

stated in point 4 above, the position of the substituted residue within the structural configuration of the subtilisin may also strongly influence the charge difference resulting from this substitution; amino acid residues at the surface of the subtilisin and in direct contact with the detergent and washing system may well have different (charge) properties than other residues buried inside the structure of the subtilisin and having no contact with the detergent and washing system.

In the light thereof, the board does not agree with respondent's assertion that "it is entirely reasonable to take the subtilisin with fewest substitutions as the precursor from which further subtilisin variants were made". Although the N76D-S103A-V104I subtilisin (designated S1) in Table 14 of the patent application has the fewest substitutions, there is no reason for considering this variant as the precursor subtilisin. According to the definition of "precursor subtilisin" given in the description, any subtilisin of Table 14 (designated S1 to S17) may well be considered as a precursor subtilisin, and neither step (a) nor step (b) exclude in any manner substitutions that may result in an amino acid sequence closer to that of a "naturally-occurring" subtilisin.

7. In fact, the substitutions defined in steps (a) and (b) of claim 1 lead to opposite effects. Whilst in step (a) the substitution(s) result in a "more positive or less negative" subtilisin, they result in a "more negative or less positive" variant in step (b). There is however no feature in claim 1 defining the net charge of the subtilisin variant resulting from carrying out steps (a) and (b) when compared to the precursor subtilisin. Nor is such a feature derivable from step (d) of

claim 1 which only requires to "repeat steps a)-c) as necessary". Neither the number of changes, nor their position within the amino acid sequence of the subtilisin, nor the net charge of the subtilisin variant is defined in any of these steps, and no guidance is provided in the claim - nor in the patent application itself - as to the criteria to follow for carrying out the iterative step (d).

In the board's view, the iteration of step (d) cannot be performed in a manner that directly leads to the balancing of the changes as stated in the patent application (see point 3 above); said iteration does not exclude or, in the respondent's words, "weed out those changes that move effectiveness in the wrong direction in the detergent systems". The subtilisin variant resulting from carrying out said iteration will not necessarily have an improved performance compared to the former variant tested in previous step (c) or, for that case, to the original precursor subtilisin used as the starting point before carrying out any iteration step. No matter how many iteration cycles are carried out, at the end of each iteration the result to be expected is the very same as that of the first iteration. Apart from the performance of the specific variant obtained in each iteration cycle, no other information can be derived from the iteration so as to guide the skilled person in the next iteration cycle. Step (d) is always performed in a manner that the desired improved performance or effectiveness is achieved, if at all, by trial and error, i.e. mere chance.

It is also worth noting that, from the wording of step (d) of claim 1, it is not clearly derivable whether the iteration referred to in said step requires

to use the subtilisin variant tested in step (c) as the new "precursor subtilisin" in steps (a) and (b) instead of the original precursor subtilisin used as the starting precursor subtilisin before carrying out any iteration step at all. Should this be the case, the question might arise as to which "precursor subtilisin" is actually meant when, as required in step (c), the effectiveness of the new subtilisin variant is tested.

8. In the light of the above considerations, the board concludes that the guidance provided by the patent application is so incomplete that the skilled person cannot perform the claimed invention without undue burden.
9. Thus, the subject-matter of the main request is not sufficiently disclosed (Article 100(b) EPC).

Auxiliary request

10. The auxiliary request filed by the respondent on 8 September 2014 is identical to the auxiliary request filed with letter dated 8 October 2012 in opposition proceedings. In view of the decision of the opposition division to reject the opposition and thus, to maintain the patent as granted, there was no need for the opposition division to examine the patentability of this auxiliary request. Accordingly, there is no reference thereto in the decision under appeal.
11. The auxiliary request differs from the main request by the replacement of the wording "repeating steps a)-c) as necessary to produce ..." in step (d) of claim 1 by the wording "repeating steps a)-c) at least once to produce ...". Additionally, the reference in step (d)

to "a precursor protease" has been replaced by "the precursor subtilisin" (cf. point IX *supra*).

Article 83 EPC

12. The amendments introduced into step (d) of claim 1 do not overcome any of the deficiencies referred to above with respect to the main request. The guidance provided by the patent application is still incomplete and an undue burden is still put on the skilled person trying to perform the claimed invention. Thus, the auxiliary request does not fulfil the requirements of Article 83 EPC.

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:



L. Malécot-Grob

B. Stolz

Decision electronically authenticated