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Datasheet for the decision of 27 May 2010

Case Number: T 1539/08 - 3.3.08

Application Number: 91200003.1

Publication Number: 0440273

IPC: C12N 9/92

Language of the proceedings: EN

Title of invention:
A method for obtaining glucose isomerases having altered substrate specificity

Patentees:
GENENCOR INTERNATIONAL, INC., et al

Opponent:
NOVOZYMES A/S

Headword:
Glucose isomerases/GENENCOR

Relevant legal provisions:
EPC Art. 56, 83

Relevant legal provisions (EPC 1973):
-

Keyword:
"Main request: sufficiency of disclosure (yes)"
"Inventive step (yes)"

Decisions cited:
-

Catchword:
-

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Case Number: T 1539/08 - 3.3.08

D E C I S I O N
of the Technical Board of Appeal 3.3.08
of 27 May 2010

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Decision under appeal: **Decision of the Opposition Division of the European Patent Office posted 2 June 2008 revoking European patent No. 0440273 pursuant to Article 101(2)(3)b EPC.**

Composition of the Board:

Chairman: L. Galligani
Members: T. J. H. Mennessier
R. Moufang

Summary of Facts and Submissions

I. The patentees (appellants) lodged an appeal against the decision of the opposition division dated 2 June 2008, whereby European patent 0 440 273 was revoked. The patent had been granted on European patent application No. 91 200 003.1 entitled "*Novel glucose isomerases having altered substrate specificity".* The application which was filed on 2 January 1991 claimed the priority date of 4 January 1990.

II. The patent had been opposed by one opponent. The grounds for opposition relied on were lack of novelty (Article 100(a) EPC), 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. The decision was based on the main and auxiliary requests filed on 10 August 2007 as auxiliary requests 2 and 4, respectively, and renamed at the oral proceedings held on 11 October 2007. The main request was considered to be sufficiently disclosed (Article 83 EPC) but, as the auxiliary request, was refused for reasons of lack of inventive step (Article 56 EPC).

IV. The notice of appeal was filed on 6 August 2008. It was accompanied by a main request which corresponded to the main request on which the decision was based.

The main request consisted of 8 claims of which claim 1 read as follows:

"1. A method for obtaining a glucose isomerase enzyme with an altered substrate specificity by changing an amino acid which is selected according to a method comprising the following criteria:

(a) select all residues and crystallographically assigned water molecules which have at least one atom within a sphere of 4 angstroms surrounding the atoms of the substrate or of a substrate analogue or of an inhibitor bond [sic] in the active site;

(b) select all the residues which are in Van der Waals contact with the residues and water molecules obtained by the selection according to criterion (a);

(c) discard from the selected list of residues identified in steps (a) and (b) those that are implied in catalysis, cofactor binding (such as metal ions and nucleotides) and essential intersubunit interactions in the case of oligomeric enzymes;

(d) discard from the selected list of residues identified in steps (a) and (b) those residues that interfere with the structural role of the selected residues."

Claim 1 differed from claim 1 as granted only in that its preamble had been limited to a method for obtaining a particular enzyme, namely a glucose isomerase.

Claims 2 to 8 were dependent on claim 1 and directed to particular embodiments thereof.

V. The statement of grounds of appeal was filed on 13 October 2008. It was accompanied by three auxiliary requests. Auxiliary request 1 corresponded to the auxiliary request considered by the opposition division.

VI. The opponent (respondent) replied on 4 February 2009, arguing that (i) all the requests lacked an inventive step (Article 56 EPC), (ii) the patent failed to disclose the claimed method (whatever the requests) sufficiently clearly and completely to allow it to be repeated (Article 83 EPC) and (iii) auxiliary requests 2 and 3 had formal problems under Article 123(2) EPC and, in the case of auxiliary request 3, also Article 84 EPC.

VII. The board issued on 3 December 2009 a communication pursuant to Article 15(1) of the Rules of Procedure of the Boards of Appeal in which provisional and non-binding opinions on the issues of inventive step and sufficiency of disclosure were expressed.

VIII. In a letter dated 4 January 2010, the respondent withdrew its request for oral proceedings on condition that the appellants did not file a set of claims that was any broader in respect of the definition of the enzyme than the sets of claims on file.

IX. In reaction to the respondent's letter, the appellants informed the board on 12 January 2010 that they withdrew their request for oral proceedings in the event that the board would be willing to maintain the patent in the form of the main request or any of the auxiliary requests filed with their letter of 13 October 2008.

X. With a letter dated 30 April 2010, the appellants withdrew their request for oral proceedings.

XI. On 4 May 2010, the board informed the parties that it had decided to cancel oral proceedings and to continue the proceedings in writing.

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

(D2) A. R. Fersht, "Overview: Kinetic Aspects of Purposely Modified Proteins". In: D.L. Oxender and C.F. Fox (Editors), 1987, Protein Engineering, Alan R. Liss Inc,, New York, pages 221 to 224

(D3) EP 0 351 029 A1 (published on 17 January 1990)

(D10) K. Henrick et al., J. Mol. Biol., Vol. 208, 1989, pages 129 to 157

(D11) A.-M. Lambeir et al., Biochemistry, Vol. 31, 1992, pages 5459 to 5466

(D12) J. A. Wells et al., Proc. Natl. Acad. Sci. USA, Vol. 84, August 1987, pages 5167 to 5171

(D13) J. A. Wells and D. A. Estell, TIBS, Vol. 13, August 1988, pages 291 to 297

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

Main request

Sufficiency of disclosure (Article 83 EPC)

No comments were made.

Inventive step (Article 56 EPC)

Document D3 described the generation of mutant glucose isomerases derived from *Actinoplanes missouriensis*. These mutants were designed to have "improved properties" which were defined (see page 4, lines 48 to 51) as being higher conversion performance and/or improved stability, especially heat stability, relative to the wild type enzyme. "Improved stability" also included increased stability of the enzyme at different pHs optionally in combination with enhanced thermostability. Thus, the purpose of document D3 was not to produce a glucose isomerase with an altered specificity, as specified in claim 1 of the main request. Nevertheless, some of the mutants described in document D3 did happen to have altered substrate specificity for glucose versus xylose (see Table 3, on page 16), as assessed by a change in V_{max}(gl/xy) or K_m(xy/gl) compared to the wild type enzyme.

Therefore, the objective technical problem to be solved could be formulated as the provision of an alternative method for producing a glucose isomerase with altered substrate specificity.
The present invention solved this problem by providing rational stepwise criteria for selecting residues for mutation, the selection being based solely on the crystal structure of the enzyme.

On the other hand, the residues to be mutated in document D3 were selected according to very different criteria and with a different purpose in mind. These criteria were primarily aimed at enhancing the interactions between glucose isomerase subunits, in order to stabilise its tetrameric

structure (see page 4, line 57 to page 5, line 4), which was thought to be required for enzymatic activity.

Thus, document D3 was concerned with selection and modification of residues participating in the interfaces between subunits. These criteria were also concerned with selecting and replacing residues that were implied in essential intersubunit interactions with the tetrameric glucose isomerase. Such residues were specifically rejected in step (c) of the method of claim 1. Therefore, document D3 taught away from the claimed invention.

Therefore, document D3 did not provide a clear and unambiguous disclosure of any part of step (c) of claim 1. Nor was there any suggestion therein of the selection criteria listed in steps (a) and (b) of claim 1. Specifically, nowhere in document D3 did the authors disclose that residues and crystallographically assigned water molecules which had at least one atom within a sphere of 4 angstroms surrounding the atoms of the substrate or of a substrate analogue or of an inhibitor bound in the active site, or residues that were in van der Waals contact with these residues and/or water molecules should be selected for mutation. Instead, document D3 was solely concerned with identifying and replacing residues that were directly involved in electrostatic interactions, in order to increase the stability of the enzyme.

A combination of documents D12 and D13 with document D3 would not have been made by the skilled person for the reasons set out below.

Firstly, documents D12 and D13 were both concerned with mutating residues in subtilisin, which was a single domain protein whereas glucose isomerase was a tetramer. Given that document D3 was primarily concerned with stabilising the interaction between the subunits of glucose isomerase, it was highly unlikely that the skilled person having read document D3 and seeking to alter the substrate specificity would have turned to the disclosure of document D12 or document D13.

Secondly, documents D12 and D13 were at least partially concerned with altering the substrate specificity of subtilisin, whereas document D3 was concerned with improving the stability of glucose isomerase. The skilled person would have understood that very different considerations applied to each of these purposes and would not have considered the teaching of documents D12 and D13 applicable to that of document D3.

Finally, document D3 (like the patent-in-suit) was concerned with modification of residues based solely on knowledge of the structure of the enzyme to be modified. The approach in document D3 was used specifically because a naturally-occurring glucose isomerase with the desired properties was

not available. By contrast, the approach detailed in documents D12 and D13 was designed to recruit desirable properties from one type of subtilisin enzyme to another. Thus, they involved comparing sequences and structures of two enzymes with known properties and attempting to determine which of the sequence and structural differences were responsible for the observed differences in activity and specificity. Therefore, it would not have been possible for the skilled person to select residues for mutation in glucose isomerase by applying the methods described in documents D12 and D13.

Moreover, even if document D12 and/or document D13 were combinable with document D3, this combination of documents did not disclose or suggest all the selection criteria specified in claim 1. In particular, none of these documents suggested selecting for mutation those residues that were in van der Waals contact with residues or crystallographically assigned water molecules having at least one atom within a sphere of 4 angstroms surrounding the atoms of the substrate or of a substrate analogue or of an inhibitor bound in the active site.

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

Main request

Sufficiency of disclosure (Article 83 EPC)

The patent failed to describe the method according to claim 1 sufficiently clearly and completely to allow the discarding steps (c) and (d) to be performed. One of the requirements of step (c) was that the skilled person had to perform the mental act of "discarding" residues that were implied in "essential intersubunit interactions". Before these residues could be discarded, they had to be identified and, therefore, the patent could only fulfil the requirements of Article 83 EPC if it were possible for the skilled person to identify residues implied in essential intersubunit interactions without undue burden.

There was no disclosure in the patent about how to identify essential intersubunit interactions. In particular, the patent did not teach the skilled person how to distinguish between essential and non-essential subunit interactions. Nor was it possible for the skilled person to identify these essential intersubunit interactions using knowledge outside the teaching of the patent. In this respect, since a residue (Arg140) that was identified in the prior art document D10 as being essential for intersubunit interaction in a closely

related glucose isomerase (according to the patent), was not discarded when it apparently should have been, this made it impossible for the skilled person to know how to carry out step (c) of the method of claim 1. Conversely, a residue (Phe26), which was identified in the post-published document D11 as providing hydrophobic binding surface in the *Actinoplanes missouriensis* glucose isomerase for the substrate backbone, was one of the residues that was shown in the description (see page 5, lines 32 to 33 in the patent specification) to be discarded by applying the criterion of step (c) of the method of claim 1.

Inventive step (Article 56 EPC)

Document D3 was the closest state of the art. D3 disclosed mutant glucose isomerase enzymes having altered substrate specificity and how they could be produced by rational modification using information derived from the 3D structure of the enzyme. The basis of the method described in document D3 was enhancement of subunit interactions which was achieved by replacement of amino acid residues that were not involved in catalysis and cofactor binding, while retaining intersubunit interaction.

Taking document D3 as the closest state of the art, the technical problem to be solved was seen as the provision of an alternative method for making mutant glucose isomerases having altered substrate specificity.

The solution to that problem was the method according to claim 1 which consisted in selecting for modification amino acid residues in or near to the binding site rather than those residues involved in subunit interactions.

This solution was obvious from common general knowledge, as evidenced by document D2 or from any of documents D12 and D13.

Claim 1 involved nothing more than the application of the skilled person's general knowledge about enzyme structure as derivable from the prior art document D2. Said document showed that the skilled person was aware of the possibility of engineering enzymes based on their 3D structures and stated that amino acid side chains that were not so obviously involved in catalysis but just appeared to be involved in binding the substrates might be modified to give enzymes of slightly changed activities. Thus, it was clear that the underlying concept of the invention, namely that of modifying enzyme specificity by changing amino acids in or near the binding site, was part of the common general knowledge of the skilled person.

Documents D12 and D13 which described the protein engineering of subtilisin proteases to alter substrate specificity were not relevant only for those proteases. The basic principles that they described were applicable to

enzymes in general and were not specific to subtilisin proteases.

Document D12 disclosed that substrate specificity could be altered by modification of residues that were in direct contact with the substrate as well as those that were slightly further away from the substrate. The effect of altered substrate specificity by changing amino acids involved in binding was expected by the authors of document D12. They observed an effect when a residue was substituted just outside of direct contact distance and taught that the modification of these residues altered substrate specificity.

Document D13 which referred to document D12 concluded that predictable changes in substrate specificity could be produced by alteration in the binding site.

Documents D12 and D13 each disclosed that substrate specificity could be altered by substitution of amino acid residues that bound the substrate or that neighboured these binding residues. This set of residues was the set identified by selection criteria (a) and (b) in claim 1.

XV. The appellants (patentees) requested that the decision be set aside and the patent maintained on the basis of the main request filed with the notice of appeal on 6 August 2008, or in the alternative, of one of the auxiliary requests 1 to 3 filed with the statement of grounds on 13 October 2008.

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

Reasons for the Decision

Main request

Compliance with the requirements of Article 54, 84 and 123(2) and (3) EPC

1. Neither the opposition division nor the respondent raised objections under Articles 54, 84 and 123(2) and (3) EPC. The board is also satisfied that the main request complies with the requirements of those articles.

Compliance with the requirements of Article 83 EPC

2. The respondent argues that the patent fails to disclose how residues which are implied in essential intersubunit interactions as referred to in step (c) of claim 1 can be identified. Its reasoning is based on analysis of documents D10 and D11.

3. Document D10 reports how the structures of D-xylose isomerase (glucose isomerase) from *Arthrobacter* strain B3728 containing the polyol inhibitors xylitol and D-sorbitol have

been solved at 2.5 and 2.3 angstrom resolution respectively. In the paragraph bridging pages 148 and 149, well-defined salt-bridges linking five pairs of amino acids that take part in the formation of dimers are referred to. The salt-bridge linking Asp23 and Arg139 is believed by the authors to play a key role in stabilising the geometry of the active site.

4. The respondent deduces therefrom that said salt-bridge is an essential intersubunit interaction and concludes that the corresponding residues Asp24 and Arg140 in the *Actinoplanes missouriensis* glucose isomerase which is analysed in detail in the patent, being implied in an essential intersubunit interaction, should be discarded from the residues selected for substitution when performing the method of claim 1 in order to prepare a mutant enzyme with altered substrate specificity. As neither Asp24 nor Arg140 have been discarded in the experiment reported on page 5 of the patent specification (see paragraph 0035), the respondent contends that the method of claim 1 is not sufficiently disclosed.

5. The board notes that the amino acid sequence of the enzyme from Arthrobacter B3728 given in Table 3 on page 134 of document D10 corresponds to the sequence of the Arthrobacter strain represented in Figure 2 of the patent specification. A comparison of that sequence (denoted "Art") with the sequence of the glucose isomerase from Actinoplanes missouriensis (denoted "Ami") shows that their primary structures are related with around 64% of identity. This may reflect that both microorganisms have had a common ancestor but does not necessarily imply that a conserved amino acid present in both sequences (such as the aspartic acid residue at position 23 in the *Art* sequence and position 24 in the *Ami* sequence or the arginine residue at position 139 in the *Art* sequence and position 140 in the *Ami* sequence) plays for both enzymes the same role in the structural integrity of the molecule. The board sees no reason to question the observation reported in document D10 that Asp23 and Arg139 in the enzyme from Arthrobacter B3728 play a key role in stabilising the active dimer. Conversely, the board sees no reason not to admit the analysis of the inventors who, while being certainly aware of the pre-published document D10, did not allocate such a role to Asp24 and Arg140 in the enzyme from Actinoplanes missouriensis and, therefore, did not discard those two amino acid residues from the ones to be substituted according to the method of claim 1. Thus, the respondent's objection based on document D10 is not tenable.

6. Post-published document D11 reports on the properties of a series of active site mutant xylose (glucose) isomerases from *Actinoplanes missouriensis*. While residue Phe26 is identified therein as providing a hydrophobic binding surface for the substrate backbone (see page 5463, right-hand column, fifth full paragraph, third to fifth lines), there is no indication that this particular residue is implied in any essential intersubunit interaction.

Nevertheless, in the experiment reported in the patent specification (see page 5, lines 22 to 37), this particular residue has been discarded according to the criterion (c) of claim 1.

7. The board notes that it was not the object of the authors of document D11 to assign to each and every residue a role in the essential intersubunit interactions which contribute to maintain the stability of the enzyme. According to the authors, the role of residue Phe26 seems to be purely structural (see last sentence of the abstract on page 5459). This remark is an indication that said residue may be involved in an essential intersubunit interaction. Thus, the respondent's objection based on document D11 is not tenable.

8. It is noted that, since the patent identifies in the glucose isomerase from *Actinoplanes missouriensis* those residues which are implied in essential intersubunit interactions (see paragraph 0034 on page 5 of the patent specification), the skilled person is provided with relevant guidance for the identification of such residues in other glucose isomerases.

9. The board concludes that, since the respondent has not provided any convincing fact and evidence in support of its objections, the claimed invention has to be considered as being sufficiently disclosed. Therefore, the main request complies with Article 83 EPC.

Compliance with the requirements of Article 56 EPC

10. Document D3 has been considered by the opposition division to represent the closest state of the art. The respondent agrees. The appellants do not contest. Notice is taken that document D3, which was published after the priority date claimed for the patent at issue but before the filing date of the application on which the same was granted, belongs to the state of the art by virtue of the invalidity of the said priority as argued in the notice of opposition and as implicitly acknowledged in the appealed decision. This is because the method according to claim 1 as granted was not described in the priority document, whatever the enzyme to be obtained, the same reason applying to claim 1 of the main request which differs from claim 1 as granted only in that it is limited to the preparation of a glucose isomerase (see Section IV *supra*).

11. Document D3 describes methods for enhancing the interactions between glucose isomerase subunits which contribute to stabilise the tetrameric structure of the enzyme. The methods generate mutant glucose isomerases having improved properties, namely a higher conversion performance and/or improved stability, relative to the corresponding wild-type enzymes or an increased stability at different pHs as such

or in combination with enhanced thermostability (see page 4, line 42 to page 5, line 2).

12. The methods of document D3 involve introduction of ionic bridges or special mutations (see page 5, lines 3 to 57). For the introduction of ionic bridges, residues are selected which participate in the interfaces (see page 6, lines 45 to 50). Preferred mutations involve substituting arginine residues for specific lysine residues, in particular those lysine residues which occur within interfaces between subunits (see page 7, lines 19 to 32). Example 3 reports the identification of an appropriate lysine residue (Lys294) in the subunit interfaces of the glucose isomerase of *Actinoplanes missouriensis* to produce the K294R mutant (see pages 14 to 16), which is shown to display an enzymatic activity that is 85% of the wild-type's with xylose as a substrate (see Table 3 on page 16).

13. Thus, the purpose of document D3 is not to produce a glucose isomerase with an altered substrate specificity. In fact, it is concerned with selection and modification of residues participating in the interfaces between subunits.

14. The K294R mutant is also one of the mutants with an improved specificity for glucose the preparation of which is described in the patent at issue according to the method of claim 1 (see Example 1, pages 7 and 8 and Example 6, pages 10 in the patent specification). Accordingly, unawares document D3 describes a method which results in a glucose isomerase with an altered substrate specificity.

15. Taking document D3 as the closest state of the art, the objective technical problem to be solved can be seen in the provision of an alternative method for producing a glucose isomerase with an altered specificity.

16. As for inventive step, the question to be answered is whether the state of the art would have led the skilled person facing the above technical problem to depart from the method of document D3 and arrive without undue burden to the method of claim 1.

17. Documents D12 and D13 have been relied on by the opposition division in the decision under appeal and by the respondent in its reply to the statement of grounds. The respondent also argues that document D2 is of relevance.

18. Document D2 is part of a textbook published in 1987 (at the onset of protein engineering) and its content may be considered to belong to the common general knowledge of the skilled person at the filing date. It provides no more than a theoretical thought about the identification of the requirements of systems for studying the rules of folding of proteins and the basis of enzyme catalysis by preparing mutant enzymes of different thermodynamic and kinetic

properties of folding. For that purpose making small changes in proteins of known three-dimensional structure is recommended. In the last paragraph of page 222, it is mentioned that it would be desirable to alter properties of existing enzymes, *inter alia* their specificity by small modifications of the parent structure, it being added that some of these goals could be achieved just by simple substitution of amino acid residues. Nevertheless, this general assumption does not allow to identify which residues should be substituted for obtaining an enzyme with altered substrate specificity.

19. The respondent's argument that the remark made in document D2 (see page 222, right-hand column, lines 28 to 32) that "*amino acid side chains that are not so obviously involved in catalysis but just appear to be involved in binding the substrates may be modified to give enzymes of slightly changed activities*" is the basis for the selecting steps (a) and (b) of claim 1 is not convincing. This mere statement does not indeed amount to a description whatsoever of the definite technical features of those steps.

20. Document D12 reports an investigation aiming to assess whether specificity properties of two distantly related and functionally divergent subtilisins can be exchanged by limited amino acid replacements.

20.1 The two wild-type subtilisins respectively produced by *Bacillus licheniformis* and *Bacillus amyloliquefaciens* were known to differ dramatically in catalytic efficiency against a given substrate, the *B. licheniformis* subtilisin differing for example by more than sixty times in catalytic efficiency toward substrates containing a glutamate residue in the P_1 position (C-terminal residue).

20.2 Residues within 4 angstroms, i.e. within van der Waals contact distance, of a model substrate bound to subtilisin were identified for each of the two subtilisins. There were 19 of them (see Table 1 on page 5168), of which only two, namely residues 156 and 217, were different in the two subtilisins (see Table 1 and right-hand column on page 5168).

20.3 To evaluate the extent to which those two residues and a third residue (Ala169) within 7 angstroms from a model substrate can account for the specificity differences between the subtilisins, the three *B. licheniformis* substitutions (Ser156/Ala169/Leu217) were introduced into the *B. amyloliquefaciens* subtilisin by site-directed mutagenesis.

20.4 The substrate specificity of the triple mutant was found to approach that of *B. licheniformis* when assayed with seven different substrates which vary in charge, size, and hydrophobicity.

20.5 Thus, it may be concluded that document D12 describes a method for deriving from a given enzyme (to be referred to *infra* as the receiver enzyme) an enzyme with an improved specificity toward a model substrate, the method comprising the steps of:
(a) selecting a distantly related and functionally divergent enzyme having a better catalytic efficiency against said substrate (to be referred to *infra* as the donor enzyme),
(b) identifying the residues of the binding sites of both enzymes which are within van der Waals contact distance of the model substrate,
(c) selecting among the residues identified at step (b) those which differ in the two enzymes,
(d) selecting a further residue located just outside of direct contact distance (just as Ala-169 in *B. licheniformis* subtilisin which has been shown to have substantial effects on substrate binding), and
(e) replacing by site-directed mutagenesis in the receiver enzyme the residues selected at steps (c) and (d) by the corresponding residues of the donor enzyme.

20.6 Therefore, the method of document D12 differs fundamentally from the method of claim 1, in that it involves comparative crystallographic studies of the enzyme to be mutated and of an enzyme taken as a reference. Moreover, the residues selected for substitution should include a residue which is just outside of direct contact distance with the substrate.

20.7 The board concludes that the skilled person would not have found in document D12 the necessary information to design a method for obtaining a glucose isomerase enzyme with an altered substrate specificity with the technical features of the method of claim 1.

21. Document D13 is a review contemplating subtilisin as an enzyme designed to be engineered. Two strategies for engineering substrate specificity of subtilisins are discussed (see the Sections entitled "*Engineering substrate specificity*", pages 293 to 295), the one developed in document D12 which is citation 19 in document D13 (see point 12 *supra*) and an alternative strategy in which substrates are distinguished primarily by their ability to participate directly in the catalytic mechanism.

21.1 Therefore, whereas document D13 describes some principles useful for the skilled person aiming to prepare mutants of a subtilisin exhibiting an altered specificity, those principles are far away from those on which the method of claim 1 relies. In particular, there is no description or suggestion of how to select for substitution amino acid residues according to claim 1. In this respect, the board cannot see any pointer to the selection steps (a) and (b) of claim 1 in the mere statement found on page 293, right-hand column, as referred to on page 10 of the decision under appeal, that "*Although these data* [derivable from document

D12] *show that changes in direct contact residues largely account for the specificity differences, other substitutions further removed from the substrate binding site must account for the remaining discrepancy between the triple mutant and the B. licheniformis sutilisin*".

21.2 The argument relied on in the decision under appeal that document D13 teaches that the catalytic site should not be mutated to avoid the catalytic function to be severely impaired is contradicted by the comments made in the paragraph bridging pages 294 and 295 of the document in connection with the "*substrate-assisted catalysis*" strategy to engineering substrate specificity. As reported in said paragraph, the His64 residue in the catalytic site of *Bacillus amyloliquefaciens* subtilisin was indeed replaced by an alanine by means of site-directed mutagenesis. Therefore, the reasoning in the decision under appeal based on the further argument that the teaching in document D12 is similar to the one in the patent (see point 20.6 *supra*) is erroneous. In the board's judgement, contrary to the view of the opposition division, the method of claim 1 is not obviously derivable from document D3 taken in combination with both documents D12 and D13.

22. In view of the remarks made at points 10 to 21, the conclusion is reached that the method of claim 1 involves an inventive step. The same conclusion applies to claims 2 to 8, which are dependent on claim 1. Therefore, the main request complies with the requirements of Article 56 EPC.

Conclusion

23. Since neither the opposition division nor the respondent have raised further objections against the main request, the board concludes that the main request meets the requirements of the EPC and forms a basis for the maintenance of the patent in an amended form.

Order

For these reasons it is decided that:

1. The decision under appeal is set aside.

2. The case is remitted to the first instance with the order to maintain the patent on the basis of the main request filed with the notice of appeal on 6 August 2008 and a description to be adapted thereto.

The Registrar The Chairman

V. Commare L. Galligani