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Datasheet for the decision of 6 July 2022

Case Number: T 1600/19 - 3.3.09

Application Number: 10723329.8

Publication Number: 2421963

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Language of the proceedings: EN

Title of invention:
METHOD OF PRODUCING A LIPOLYTIC ENZYME

Patent Proprietor:
DuPont Nutrition Biosciences ApS

Opponent:
NOVOZYMES A/S

Headword:
Lipolytic Enzyme/NOVOZYMES

Relevant legal provisions:
EPC Art. 54(2), 56, 83, 84, 123(2)
RPBA Art. 12(4)

Keyword:
Main Request: Admission - (yes); Added Matter - (no); Clarity - (yes); Sufficiency of Disclosure - (yes); Novelty - (yes); Inventive Step - (Yes)

Decisions cited:
T 1074/00, T 0029/05, T 0137/01

Catchword:

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Case Number: T 1600/19 - 3.3.09

D E C I S I O N
of Technical Board of Appeal 3.3.09
of 6 July 2022

Appellant: (Opponent)	NOVOZYMES A/S Krogshoejvej 36 2880 Bagsvaerd (DK)
Representative:	Potter Clarkson The Belgrave Centre Talbot Street Nottingham NG1 5GG (GB)
Respondent: (Patent Proprietor)	DuPont Nutrition Biosciences ApS Langebrogade 1 1411 Copenhagen K (DK)
Representative:	D Young & Co LLP 120 Holborn London EC1N 2DY (GB)
Decision under appeal:	**Interlocutory decision of the Opposition Division of the European Patent Office posted on 8 April 2019 concerning maintenance of the European Patent No. 2421963 in amended form.**

Composition of the Board:

Chairman A. Haderlein
Members: A. Veronese
D. Rogers

Summary of Facts and Submissions

I. The appeal was filed by the opponent (appellant) against the decision of the opposition division finding that the European patent as amended according to the then auxiliary request 1 met the requirements of the EPC.

II. With its notice of opposition, the opponent had requested revocation of the patent in its entirety on the grounds under Articles 100(a) (lack of novelty and lack of inventive step), 100(b) and 100(c) EPC.

III. The documents submitted during the opposition proceedings included:

D1: Bradner et al., Curr. Genet., vol. 44(4), 2003, 224-30
D2: Zhang et al., Appl. Biochem. Biotechnol., vol. 176, 2015, 1722-35
D3: Kontkanen et al., Biotech. Bioeng., vol. 94, 2006, 407-15
D5: WO 2008/007510
D9: WO 98/45453
D10: US 4,797,361

IV. Claims 1 and 11 of auxiliary request 1 (current main request) read:

"*1. A method of producing a lipolytic enzyme comprising the steps of:*

(i) providing a transformed or transfected Trichoderma reesei cell comprising

a) at least one heterologous nucleotide sequence encoding a lipolytic enzyme comprising an amino acid sequence shown as SEQ ID NO: 1 or SEQ ID NO: 2 or an amino acid sequence which has at least 40% sequence identity to SEQ ID NO: 1 or 2; and/or

b) at least one heterologous nucleotide sequence encoding a lipolytic enzyme wherein the nucleotide sequence comprises the nucleotide sequence shown as SEQ ID NO: 3 or SEQ ID NO: 4 or a nucleotide sequence which has at least 40% sequence identity to SEQ ID NO: 3 or SEQ ID NO: 4; and/or

c) at least one heterologous nucleotide sequence encoding a lipolytic enzyme wherein the nucleotide sequence comprises the nucleotide sequence which hybridizes to SEQ ID NO: 3 or SEQ ID NO: 4 or a nucleotide sequence which is at least 40% sequence identity to SEQ ID NO: 3 or SEQ ID NO: 4 or the complement of any thereof under stringent conditions; and

(ii) culturing the cell under conditions to allow for expression of said heterologous nucleotide sequence(s) encoding said lipolytic enzyme; and

(iii) raising the pH at the end of the fermentation to a pH above the pH of the culture conditions in step (ii)."

"*11. A method of producing a lipolytic enzyme comprising the steps of:*

(i) providing a transformed or transfected Trichoderma reesei cell comprising at least one heterologous nucleotide sequence encoding a lipolytic enzyme;

(ii) culturing the cell at about pH 4.5 under conditions to allow for expression of said heterologous nucleotide sequence(s) encoding said lipolytic enzyme;

(iii) isolating, purifying or concentrating the enzyme in a medium at about pH 6,

wherein the at least one heterologous nucleotide sequence encoding a lipolytic enzyme is:

a) at least one heterologous nucleotide sequence encoding a lipolytic enzyme comprising an amino acid sequence shown as SEQ ID NO: 1 or SEQ ID NO: 2 or an amino acid sequence which has at least 40% sequence identity to SEQ ID NO: 1 or 2; and/or

b) at least one heterologous nucleotide sequence encoding a lipolytic enzyme wherein the nucleotide sequence comprises the nucleotide sequence shown as SEQ ID NO: 3 or SEQ ID NO: 4 or a nucleotide sequence which has at least 40% sequence identity to SEQ ID NO: 3 or SEQ ID NO: 4; and/or

c) at least one heterologous nucleotide sequence encoding a lipolytic enzyme wherein the nucleotide sequence comprises the nucleotide sequence which hybridizes to SEQ ID NO: 3 or SEQ ID NO: 4 or a nucleotide sequence which is at least 40% sequence identity to SEQ ID NO: 3 or SEQ ID NO: 4 or the complement of any thereof under stringent conditions."

V. In its decision, the opposition division found that auxiliary request 1 was to be admitted and that the claimed subject-matter met the requirements of the EPC. The opposition division found that:

- the definitions "about pH 4.5" and "about pH 6" and the expression "stringent conditions" were clear

- feature (iii) in claim 1 was based on page 9, lines 4 to 9 and page 21, lines 32 to 33 as filed

- the patent provided sufficient information to carry out the invention; reaching high yields was not a feature of claims 1 and 11, thus it was not relevant for assessing sufficiency of disclosure; the patent disclosed methods for achieving the yield mentioned in claim 3

- the claimed process was novel over that disclosed in D1, which did not include the final step (iii) of raising the pH

- the claimed process involved an inventive step; it differed from that of D9, the closest prior art, in that *Trichoderma reesei* was used as the host to produce the lipase and in that the pH was raised after fermentation; the effects of these differences were a higher yield and a lipase having a more favourable glycosylation pattern; the prior art did not provide any prompt towards the claimed solution

VI. With its statement setting out the grounds of appeal, the appellant filed, *inter alia*:

D22: First declaration of Kim Borch

D23: Second declaration of Kim Borch

VII. With its reply to the statement setting out the grounds of appeal, the proprietor (respondent) filed, *inter alia*:

D24: Treichel et al., Food and bioprocess technology, vol.3(2), 2010, 182-96
D29: Declaration of Robert Pratt

VIII. The **appellant** argued essentially that:

- the main request was not to be admitted
- the expressions "stringent conditions", "about pH 4.5" and "about pH 6" in claim 11 were unclear
- the insertion of step (iii) in claim 1 added subject-matter
- the claimed invention was not sufficiently disclosed; claims 1 and 11 encompassed pH values which could not be used to carry out the invention and yields which were impossible to achieve
- the claimed subject-matter was not novel over D1 and did not involve an inventive step starting from D9 as closest prior art

The **respondent** argued essentially that:

- the main request was to be admitted in the appeal proceedings
- the wording of claim 1 was clear

- basis for the subject-matter of claim 1 could be found on page 9, lines 4 to 6 and 19 to 21 and on page 21, lines 31 to 33 as filed

- the invention was sufficiently disclosed: achieving the yields mentioned in claim 3 was not a requirement of claim 1; there was no evidence that it could not be carried out at certain pH values or that the yields of claim 3 could not be achieved

- the claimed subject-matter was novel over D1, which did not disclose at least step (iii) of the method

- the claimed subject-matter involved an inventive step over D9, the closest prior art

IX. In a communication issued in preparation for the oral proceedings, the board expressed the preliminary opinion that the appeal was to be dismissed. In reaction to this communication, the appellant withdrew its request for oral proceedings. The oral proceedings were then cancelled.

The requests

X. The appellant requested that the decision under appeal be set aside and that the patent be revoked.

XI. The respondent requested that the appeal be dismissed or, alternatively, that the patent be maintained on the basis of one of auxiliary requests 1 to 72, filed with the reply to the statement setting out the grounds of appeal.

Reasons for the Decision

Main request

1. *Admissibility*

1.1 The appellant requested that the main request not be admitted into the appeal proceedings.

1.2 The main request was filed as auxiliary request 1 during the opposition proceedings after expiry of the time limit set by the opposition division under Rule 116 EPC. It was based on a previously filed main request and differed from it only in the definition of the pH in claim 11. The opposition division decided to admit this request, considering it to be filed in direct response to objections raised by the opponent during the opposition proceedings (see point 2.2 and 2.3 of the decision). The request was examined and considered to fulfil the requirements of the EPC.

1.3 There is no reason to consider that the opposition division exercised its discretion in an unreasonable manner when admitting this request. Thus, there is also no reason to overrule its decision and disregard the main request in this appeal by applying the provisions of Article 12(4) RPBA 2007.

2. *Clarity*

2.1 The appellant considered unclear the following wordings used in claim 11:

- "stringent conditions"

- "about pH 4.5" and "about pH 6"

2.2 It is established case law that the expression "hybridising under stringent conditions" is sufficiently clear for the purposes of Article 84 EPC given the nature of the subject-matter claimed (see Case Law of the Board of appeal, 9th edition, section II.A.3.4 and the cited decisions T 1074/00 and T 29/05). In these decisions, although different experimental protocols might be applied for assessing the hybridisation of nucleotides under "stringent conditions", it was considered that this does not mean that these protocols led to different results as far as the detected nucleotide sequence was concerned. Moreover, it was considered that the claimed subject-matter was also defined by a functional feature relating to the biological activity of the relevant nucleotides. This applies to the current case since claim 11 relates to nucleotides coding for a lipolytic enzyme, i.e. an enzyme having lipolytic activity.

2.3 The opposition division held that the term "about" made technical sense and was clear within the meaning of the EPO Guidelines for Examination in the context of pH values in the method called for in claim 11.

2.4 This finding is correct. As argued by the respondent, the term "about" used in claim 11 has to be interpreted as being as accurate as the method used to measure it. The skilled person would know that numerical values relating to measurements are subject to measurement errors which place limits on their accuracy. The general convention in the scientific and technical literature is that the last decimal place of a numerical value indicates its degree of accuracy.

Reference is made to point 2.1.3 of decision T 137/01. In this decision, albeit in the context of added matter, the board gave an analogous interpretation and considered the "about" wording to be clear. This approach was followed by the opposition division in its decision (see point 4.4).

2.5 The appellant argued that the first value in claim 11 has a decimal number, whereas the second value does not. In its opinion, this renders the degree of tolerance around the given values unclear. This argument is not convincing. The skilled person would in fact understand that the first of the given values, showing a decimal number, reflects the accuracy of the method and that this value is to be taken into account when determining the meaning of the word "about" in claim 11.

2.6 Therefore, in the context of claim 11, the use of the aforementioned expressions does not render the claimed subject-matter unclear (Article 84 EPC).

3. *Amendments*

3.1 According to the appellant, claim 1 contravened Article 123(2) EPC. The appellant considered that step (iii) of raising the pH at the end of the culturing step (ii) to above the pH used during the culturing step added subject-matter.

3.2 However, as decided by the opposition division and endorsed by the respondent, the passages on:

- page 9, lines 4 to 6 and 19 to 21, which refer to raising the pH of the medium after fermentation, and

- page 21, lines 31 to 33, stating that preferably, "the pH is adjusted to a pH above the pH of the fermentation broth"

read in the context of the disclosure of the application as filed, as a whole, provide the basis for claim 1. The omission of the additional steps of isolation, purification and concentration mentioned on page 9, lines 4 to 6 does not create new subject-matter. This is confirmed by the wording used on page 9, lines 19 to 21 "...the pH of the medium for culturing is about 4.5 and then the pH of the medium is raised, such that the pH of the medium for isolating and/or purifying and/or concentrating the enzyme is about pH 6". This wording discloses the possibility of raising the pH at the end of the fermentation, with no provision concerning either isolation, purification or concentration steps. This interpretation is confirmed by the aforementioned last sentence of page 21 reading "Preferably the pH is adjusted to a pH above the pH of the fermentation broth".

3.3 Thus, claim 1 does not contain added subject-matter (Article 123(2) EPC).

4. *Sufficiency of disclosure*

4.1 The appellant considered that the claimed invention was not sufficiently disclosed for the following reasons:

- Claims 1 and 11 encompassed "non-functional" pH values which did not induce the re-solubilisation of precipitated enzyme. Since this effect was not achieved, the invention could not be carried out.

- The claimed process was not suitable for achieving the purported product yields and the enzyme concentration of 20 g/l mentioned in claim 3. D2 raised reasonable doubts that such yields could be achieved.

4.2 These reasons are not convincing.

4.3 The process defined in claims 1 and 11 does not require the lipase to be precipitated and then redissolved. These steps are not required for the invention to be carried out. Thus, the argument that the claimed invention is not sufficiently disclosed because it encompasses "embodiments in which the pH is not functional" is not persuasive.

4.4 Claims 1 and 11 do not require any particular yield to be achieved either. Thus, as observed by the opposition division in its decision (points 5.16 and 5.17), whether the process defined in these claims achieves a specific yield is irrelevant for the issue of sufficiency of disclosure.

4.5 The appellant drew attention to claim 3, which defines a process affording an enzyme concentration of 20 g/l in the culture supernatant. However, this represents an embodiment of the invention defined in claim 1. Thus, it is not necessary that all embodiments encompassed by claim 1 achieve this concentration for the requirement of sufficiency of disclosure to be fulfilled.

4.6 Furthermore, the patent provides detailed instructions and examples showing how to produce the claimed lipolytic enzymes. It describes examples of suitable nucleotide sequences, the host cell for expressing it and how to carry out the fermentation process.

Examples 3 and 8 describe processes in which more than 20 g/l of lipase 3 were produced by carrying out the process. The fact that Figures 8 and 14, mentioned in the examples, do not indicate the yield data does not undermine the credibility of the information given in these examples. Furthermore, the concentration observed by the appellant when carrying out its own experiments was close to these values: 16 g/l (see D23). As submitted by the respondent, it is conceivable that higher concentrations could have been achieved had the fermentation time been extended. For these reasons, even if, as noted by the appellant, D2 describes a process affording yields lower than those specified in claim 3, there is no reason to assume that the claimed invention cannot be carried out (Article 83 EPC).

5. *Novelty over D1*

5.1 According to the appellant, the method of claim 1 lacked novelty over D1. In its opinion, the production process described in D1 included, implicitly, a step as defined in point (iii) of claim 1, i.e. a step in which, at the end of the fermentation step, the pH was raised above that for culturing used during step (ii).

5.2 The board does not agree. D1 is an article describing the isolation of a lipase gene from a *Penicillium*, its integration into a *Trichoderma reesei (T. reesei)* host, and the expression and excretion of the lipase into the growth medium. The section "Culture conditions for lipase production" on page 227 of D1 describes the conditions applied during the expression, stating that the culture medium was maintained at 28 °C. The pH is, however, not indicated. No step in which the pH of the culture medium is increased above that used during fermentation is mentioned either.

5.3 Relying on D3 to D5 and D10, the appellant submitted that the pH of the medium for culturing *T. reesei* was 5 to 6 and that it was known from D2 that the pH increased during the fermentation. For this reason, a step as defined in point (iii) of claim 1 was inherently disclosed.

5.4 This argument is not persuasive. Novelty can only be denied if the claimed subject-matter is directly and unambiguously disclosed. D1 does not qualify as such disclosure.

5.5 First, as mentioned above, D1 does not indicate the pH during fermentation. Furthermore, paragraph [0258] of the patent teaches that fermentation with *T. reesei* can be carried out with a pH of up to 7, a value significantly above that suggested by the appellant. Moreover, an alleged "inevitable increase" in pH during fermentation would not be a step, as defined in claim 1, in which the pH is raised after the end of the fermentation.

5.6 The section "Lipase activity assay and characterisation of the enzyme" on page 227 of D1 discloses tests in which the enzyme and its activity were characterised. Enzyme activity was assayed at a pH ranging from 4 to 10. However, the skilled person would not consider the pH adjustments at this stage to be part of the method for producing the lipolytic enzyme as defined in claim 1. Since the assays are described in the section relating to the characterisation of the enzyme, rather than its production, there is no room for other interpretations.

5.7 For these reasons, as decided by the opposition division, the claimed subject-matter is novel over D1 (Article 54(2) EPC).

6. *Inventive step*

6.1 The claimed invention relates to a method for producing a lipolytic enzyme which has the amino acid sequences or which is coded by the nucleotide sequences defined in the claims. According to the patent, the lipase produced by implementing the processes described in the prior art, and in particular in WO98/45453 (D9 in the current decision), was overglycosylated. This could result in a loss of enzymatic activity. It was thus desirable to produce a lipase in high yields, avoiding the problems induced by overglycosylation (paragraphs [0002] to [0008]).

The closest prior art

6.2 The opposition division decided that D9 represents the closest prior art. D9 describes the cloning and expression of lipolytic enzymes from *Aspergillus tubigensis (A. tubigensis*). A preferred enzyme is "lipase 3" or "lip3". This is the enzyme defined in the claims of the opposed patent by the amino acid sequences SEQ ID NO:1 and NO:2, relating to the full-length and mature forms of the enzyme, respectively, and by the nucleotide sequences of SEQ ID NO:3 and NO:4 (see the claims and paragraph [0294] of the patent).

6.3 Example 1 of D9 discloses the production, isolation, purification and characterisation of lipase 3 in *A. tubigensis* cells. Example 6 shows that a lipase mutant obtained from *A. tubigensis 6M 179* is overglycosylated and has low enzymatic activity.

However, examples 7 and 8 teach that lipase 3 mutants having less glycosylation sites and higher enzyme activity could be obtained using other *A. tubigensis* strains. Thus, D9 relates to the production of lipase 3 and addresses the problem of overglycosylation of this enzyme.

6.4 The whole thrust of D9 is toward the production of lipase 3 in *A. tubigensis,* and all the tests disclosed in this document relate to this micro-organism. Other micro-organisms are briefly mentioned, but their use is not investigated. For these reasons, the methods of producing lipase in *A. tubigensis* and in particular the methods of examples 7 and 8 are considered the closest prior art.

6.5 The appellant drew attention to page 12, lines 7 to 17 of D9, which mentions *T. reesei* among cells capable of expressing lipase and page 12 (lines 24 to 27), which mentions a method for preparing the polypeptides of the invention "in appropriate transformed host cells". In its opinion, a method of producing lipase in *T. reesei* was the most suitable starting point disclosed in D9. This method differed from the claimed one only in the absence of step (iii), involving an increase of the pH.

6.6 This argument is not convincing. As mentioned above, the whole thrust of D9 is toward the production of lipase in *A. tubigensis*. The problems associated with overglycosylation are only addressed in connection with *A. tubigensis*. *T. reesei* is only mentioned in passing in D9, in a passage mentioning cells "capable of expressing the polypeptide". However, this passage is very generic and does not even mention the production of the enzyme. A further passage mentioned by the appellant, starting on page 12, line 24, refers

generically to methods of producing polypeptides of the invention but does not mention *T. reesei*. Here mention is made of fungi of the genus Aspergillum, *A. tubigensis* in particular and yeast cells, such as *Saccharomyces cerevisiae* and *Pichia pastoris*.

The difference

6.7 For these reasons, as decided by the opposition division, the claimed method differs from that of the closest prior art in that:

- a different host, *T. reesei*, is used to express the lipase

- the method includes the step (iii) of raising the pH at the end of the fermentation to a pH above the pH of the culture during step (ii)

The technical effect

6.8 Concerning the first difference. The patent shows that high amounts, exceeding 20 g/l, of lipase 3 and lipase from *T. lanuginosus*, having 50% amino acid identity with lipase 3 are produced in *T. reesei* (see examples 3 and 8 and Figures 8 and 14). Furthermore, example 3 shows that the amount of lipase produced in *T. reesei* exceeds by far the amounts produced by other microbial species and in particular by *A. tubigensis* (example 3 and Figure 8). The appellant noted that the yield of lipase obtained using *T. reesei* in the process described in D2 was actually lower. However, as countered by the respondent, the results observed in D2 could relate to an isolated, non-optimised case not representing an optimised production process. Even

lower yields could have been obtained had *A. tubigensis* been used applying the experimental setting of D2.

6.9 In addition, example 9 and table 3 of the patent show that the glycosylation pattern of the lipase 3 produced in *T. reesei* differs considerably from that observed in lipase 3 produced in *A. tubigensis* and other expression hosts, such as *Pichia pastoris* and *Hansenula Polymorpha RB11.* This different glycosylation pattern is also shown to preserve enzymatic activity.

6.10 Referring to the declaration from a technical expert (D22) and an annexed experimental report, the appellant submitted that these effects could not be expected using all lipase types encompassed by claim 1. It noted that the activity of a lipase from *T. lanuginosus* produced in *A. oryzae* was lower than that produced in *T. reesei*.

6.11 This argument is not convincing. As noted by the respondent, the results in D22 do not relate to the glycosylation state of the lipase (see also the technical opinion D29). Furthermore, the lipase produced in *T. reesei* was not compared to that produced in *A. tubigensis*, which is the closest prior art, but to that produced in *A. oryzae*.

6.12 The appellant also argued that glycosylation is not necessarily detrimental to enzyme activity. For this reason, in its opinion, the results shown in the patent could not be generalised. This argument is not persuasive either. It was for the opponent to provide evidence that glycosylation is not detrimental to lipase activity. Such evidence has not been provided.

6.13 As stated by the respondent, it is credible that the claimed lipase types share common structural and functional features. Thus, it is reasonable to assume that the observed increase in yield and the favourable glycosylation pattern can be obtained across the entire scope of the claims.

6.14 Concerning the second difference. The respondent contended that step (iii) of the claimed method was associated with a new effect: the re-solubilisation of lipase precipitated during culturing. This effect was unexpected and relevant for assessing inventive step. This argument is not convincing. As shown in the tests annexed to D23, a lipase having a sequence as in claim 1 does not precipitate at a concentration of 16 g/l. This means that step (iii) does not induce the purported effect when this non-precipitating lipase is produced. Thus, the relevance of step (iii) does not extend across the entire scope claimed.

The underlying technical problem

6.15 Taking into account the results discussed above, starting from D9, the underlying technical problem is the provision of an improved method for producing a lipolytic enzyme as defined in claim 1 which has a higher yield and induces a more favourable glycosylation pattern compared to that observed in *A. tubigensis*.

Non-obviousness of the claimed solution

6.16 As a solution to the underlying problem, the patent proposes the production of the claimed lipase in *T. reesei*. Neither D9 nor any of the other cited prior-art documents suggests to the skilled person confronted

with the aforementioned problem selecting *T. reesei* as the host for culturing.

6.17 D9 mentions, in passing, *T. reesei* as a useful cell capable of expressing the polypeptides described in that document. However, D9 does not provide any prompt to select this micro-organism to solve the underlying problem. When mentioning methods for producing lipases, D9 refers to, as an alternative to *Aspergillum* (the preferred being *A. tubigensis*), *Saccharomyces cerevisiae* and *Pichia pastoris* (page 13, lines 14 to 36). No reference is made to *T. reesei* in this context. The other documents mentioning *T. reesei* as the host (e.g. D1) do not address the underlying problem either. Thus, the skilled person would not have had any reasonable expectation of solving the problem when selecting *T. reesei*.

6.18 Furthermore, D24, a review paper published a few months before the priority date which mentions a considerable number of micro-organisms used to produce lipases, does not even mention *T. reesei*.

6.19 For these reasons, it is concluded that the subject-matter of independent claims 1 and 11, as well as that of the dependent claims, which are more limited in scope, involves an inventive step.

Order

For these reasons it is decided that:

The appeal is dismissed.

The Registrar: The Chairman:

A. Nielsen-Hannerup A. Haderlein

Decision electronically authenticated