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D E C I S I O N
of 12 July 2006

Case Number: T 0814/04 - 3.3.08

Application Number: 96918619.6

Publication Number: 0833898

IPC: C12N 9/76

Language of the proceedings: EN

Title of invention:
A process for producing trypsin (trypsinogen)

Applicant:
Novozymes A/S

Headword:
Trypsin/NOVOZYMES

Relevant legal provisions:
EPC Art. 54, 56

Keyword:
"Main request - Novelty (no)"
"First auxiliary request - Inventive step (no)"
"Second auxiliary request - Novelty (no)"

Decisions cited:
G 0010/93

Catchword:
-

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Case Number: T 0814/04 - 3.3.08

D E C I S I O N
of the Technical Board of Appeal 3.3.08
of 12 July 2006

Appellant: Novozymes A/S
Kroghoejvej 36
DK-2880 Bagsvaerd (DK)

Representative: -

Decision under appeal: **Decision of the Examining Division of the European Patent Office posted 26 January 2004 refusing European application No. 96918619.6 pursuant to Article 97(1) EPC.**

Composition of the Board:

Chairman: P. Julià
Members: T. J. H. Mennessier
B. Günzel

Summary of Facts and Submissions

I. The applicant (appellant) lodged an appeal against the decision of the examining division of 26 January 2004 refusing the European patent application No. 96 918 619.6 with publication number 0 833 898. The application, entitled "*A process for producing trypsin (trypsinogen)*", originated from an International patent application published as WO 97/00316, to be referred to in the present decision as "the application as published".

II. Basis for the refusal was the only request then on file, namely claims 1 to 7 filed with the letter dated 5 April 2002.

III. The application was refused by reason of non-compliance with the requirements of Article 56 EPC.

IV. The appellant filed a statement setting out the grounds of appeal which was accompanied by a main and two auxiliary requests. The main request was identical with the request on which the decision under appeal was based.

V. The examining division did not rectify its decision and referred the appeal to the Board of Appeal (Article 109 EPC).

VI. A communication under Article 11(1) of the Rules of Procedure of the Boards of Appeal (RPBA) (OJ EPO 2003, 89) presenting some preliminary and non-binding views of the Board was sent to the appellant. In that communication, the Board introduced document D3 in

respect of the meaning to be attributed to the wording "trypsin-like protease" and expressed particular concerns as to whether the main and the second auxiliary requests met the requirements of Article 54 EPC over document D1 and as to whether the first auxiliary request met in particular the requirements of Article 56 EPC.

VII. The appellant made no substantive reply to the Board's communication and with letter dated 29 June 2006 informed the Board of its intention not to attend oral proceedings.

VIII. Oral proceedings took place as scheduled on 12 July 2006 in the absence of the appellant.

IX. Claim 1 of the main request read:

"1. A process for the production of trypsin in a filamentous fungus of an *Aspergillus sp.*, the process comprising

(a) transforming an *Aspergillus sp.* host cell with a recombinant DNA vector which comprises a DNA sequence encoding trypsinogen (protrypsin) N-terminally fused to a DNA sequence encoding a signal peptide,

(b) culturing the transformed *Aspergillus sp.* host cell in a suitable culture medium under conditions conducive to the expression of protrypsin and secretion thereof to the medium, and

(c) recovering the protrypsin or trypsin from the medium."

X. Claim 1 of the first auxiliary request differed from that of the main request only in that it specified in the preamble that the trypsin was a mammalian trypsin.

XI. Claim 1 of the second auxiliary request differed from that of the main request only in that it was limited to a process for the production of trypsin in two definite *Aspergillus* species, namely *Aspergillus niger* and *Aspergillus oryzae*.

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

(D1) WO-A-94/25583 (published on 10 November 1994);

(D2) EP-A-0 597 681 (published on 18 May 1994);

(D3) W.R. Rypniewski et al., Protein Engineering, Vol. 6, No. 4, 1993, Pages 341 to 348.

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

Novelty

No arguments were provided in reply to the Board's objection raised under Article 54 EPC (see Section VII *supra*).

Inventive step (first auxiliary request)

The technical problem solved by the invention was the provision of technical means for expressing high levels of trypsin.

Neither document D1 nor document D2 disclosed or suggested expression of a trypsin in host cells of *Aspergillus sp.*, e.g. *Aspergillus oryzae*. Indeed, document D1 disclosed expression in *Aspergillus sp.* host cells of a trypsin-like protease while document D2 was concerned with the expression of bovine trypsin and trypsinogen in *E. coli* strains.

However, if the skilled person were to apply the teaching of document D1 he would have been directed towards selecting the host cell among a list of different bacteria, yeast and filamentous fungi including *Aspergillus*, *Bacillus* and *Saccharomyces*. Document D1 did not discriminate between the different host cells and there was no teaching in that document which would have directed the skilled person towards selecting an *Aspergillus sp.* host cell as the preferred host.

The evidence provided with the letter of 27 August 2001 indicated that expression levels of a mammalian trypsin in *Aspergillus oryzae* cells were several fold increased when compared to expression levels in *Bacillus subtilis* and *Saccharomyces cerevisiae*.

The skilled person would not have reasonably expected that expression of trypsin/trypsinogen in an

Aspergillus sp. host cell would have resulted in yield of secreted trypsin/trypsinogen as high as 0.5 g/l.

The inventors had unexpectedly found that *Aspergillus sp.* were particularly efficient producers of trypsin relative to other microbial hosts.

XIV. The appellant requested in writing that the decision under appeal be set aside and that a patent be granted on the basis of, in order of preference, the main request or the first auxiliary request or the second auxiliary request, all filed with the statement setting out the grounds of appeal.

Reasons for the Decision

Procedural matters

1. The present application had not been refused in the decision under appeal by reason of lack of novelty. Nevertheless, exercising its discretionary power, as expressed in decision G 10/93 (OJ EPO 1995, 172; see the order which reads "*In an appeal from a decision of an examining division in which a European patent application was refused, the board of appeal has the power to examine whether the application or the invention to which it relates meets the requirements of the EPC. The same is true for requirements which the examining division did not take into consideration in the examination proceedings or which it regarded as having been met. If there is reason to believe that such a requirement has not been met, the board shall include this ground in the proceedings.*"), the Board,

having preliminary informed the appellant of its intention to do so in its communication pursuant to Article 11(1) RPBA (see Section VI *supra*), has decided to include this ground in the proceedings.

Main request

Article 54 EPC

2. Claim 1 of the main request is directed to a process for the production of a **trypsin** in a filamentous fungus of an *Aspergillus sp.*. According to page 3 (see the third full paragraph) of the description in the application as published, the trypsin may be **of any origin**.

3. Document D1 describes a "trypsin-like protease" which was isolated from a strain of *Fusarium oxysporum* a culture of which had been deposited at the DSM under the accession number DSM 2672. The protease is characterised by its amino acid sequence consisting of 224 amino acids which is represented in the sequence listing by the sequence identified as SEQ ID NO:2 (see pages 33 and 34 in the application as published).

4. The very same protease is acknowledged to be a **trypsin** in document D3, a document to which contributed the inventors of D1, in which the sequence (see Fig. 2 on page 342) and the refined crystal structure of the protease are reported. The classification of the protease as a trypsin is evident from the whole document (see in particular the first sentence of the abstract which reads: "*The trypsin from Fusarium oxysporum is equally homologous to trypsins from*

Streptomyces griseus, Streptomyces erythraeus and to bovine trypsin."; see further the second sentence of the introduction on page 341, the first paragraph of the Section entitled "Results and discussion" on page 345 and the last paragraph of the document on page 347). Indeed, the protease of *Fusarium oxysporum* has the catalytic activity of a trypsin (see the introduction on page 342) and exhibits a marked structural homology with other trypsins (see the sixth sentence on page 341 together with Fig. 6 on page 344 and the last paragraph of the document on page 347).

5. Thus, the fact that the protease in document D1 is referred to as a "trypsin-like protease" is irrelevant, that wording being apparently used therein to differentiate that particular trypsin from bovine trypsin (see page 4, lines 14 to 19).

6. In document D1, the gene encoding the trypsinogen corresponding to that trypsin from *Fusarium oxysporum* with a signal peptide (see page 8, lines 14 to 21 and page 26, lines 19 to 24) is expressed using a procedure which, as detailed on page 27, is identical to the preferred procedure of the present invention. In particular, the same fungal expression vector p777 is used to prepare an expression vector that is co-transformed into the particular strain IFO 4177 of *Aspergillus oryzae* together with plasmid pToC90 in document D1 (see page 27) or with plasmid pToC186 in the present application (see page 10 as published), both plasmids carrying the *amdS* gene from *Aspergillus nidulans*.

6.1 Thus, it has to be concluded that the process of claim 1 of the main request lacks novelty over document D1. Therefore, the main request contravenes Article 54 EPC.

First auxiliary request

Article 56 EPC

7. Claim 1 of the first auxiliary request is directed to a process for the production in a recombinant form of **a mammalian trypsin,** i.e. in view of the teaching of document D3, of a protease (possibly referred to as a trypsin-like protease) found in mammalian cells which is capable of specifically cleaving the peptide bond on the C-terminal side of lysine or arginine and shares some structural identity, in particular in the area of the active and binding sites and in the core of the protein (see page 347, left-hand column, of document D3), with the bovine **trypsin**.

8. The skilled person reading the sentence bridging pages 4 and 5 of document D1, which contemplates *inter alia* such trypsins of mammalian origin, *would* have realised that document D1 is in fact concerned with the production in a recombinant form not only of the trypsin from *Fusarium oxysporum* but also of trypsins from mammals.

9. Thus, the skilled person would have regarded it as straightforward to carry out the process detailed on page 27 of document D1, which relies on the use of a particular strain of *Aspergillus orizae*, replacing the cDNA sequence identified as SEQ ID NO:1 in the listing

sequence of document D1 by a corresponding cDNA sequence encoding a mammalian trypsinogen (such as the sequence encoding the bovine trypsinogen identified as SEQ ID NO:24 in the sequence listing of document D2) and, thereby, producing a mammalian trypsin according to a process as featured in claim 1.

10. The argument made by the appellant that the skilled person would have had to select *Aspergillus sp*. among a number of other organisms is not tenable as indeed the only expression system actually exemplified in document D1 is the one claimed in the present request and, thus, is the immediate choice to be made from document D1. Since the use of that expression system is obvious, then the presence of higher levels of expression of a mammalian trypsin in *Aspergillus oryzae* compared with levels of expression in *Bacillus subtilis* or *Saccharomyces cerevisiae* is considered to be only a bonus effect (see Case Law of the Boards of Appeal of the EPO, I.D. 7.7.1, 138 to 140).

11. Therefore, the subject-matter of claim 1 does not involve an inventive step and the first auxiliary request contravenes Article 56 EPC.

Second auxiliary request

Article 54 EPC

12. Claim 1 of the second auxiliary request is directed to a process for the production of **trypsin** in *Aspergillus niger* **or** *Aspergillus oryzae*. There is no restriction whatsoever on the origin of the trypsin. Therefore, exactly for the same reasons as explained with respect

to the main request, the subject-matter of claim 1 also lacks novelty and the second auxiliary request contravenes Article 54 EPC.

Conclusion

13. As none of the requests on file meets the requirements of the EPC, there is no request on the basis of which a patent could be granted.

Order

For these reasons it is decided that:

The appeal is dismissed.

The Registrar: The Chairman:

A. Wolinski P. Julià