BESCHWERDEKAMMERN DES EUROPÄISCHEN PATENTAMTS

BOARDS OF APPEAL OF THE EUROPEAN PATENT OFFICE

CHAMBRES DE RECOURS DE L'OFFICE EUROPEEN DES BREVETS

A		B		C	X

File No.: T 0816/90 - 3.3.2

Application No.: 85 902 041.4

Publication No.: 0 214 971

Classification: C12N 15/00

Title of invention: Yeast strains producing cellulolytic enyzmes and methods and means for constructing them

D E C I S I O N
of 7 September 1993

Applicant: OY ALKO AB

Proprietor of the patent:

Opponent:

Headword: CBH II/ALKO

EPC: Art. 56, 83, 84

Keyword: "Inventive step (yes)" "non-obvious modification of analogous process" Main and first auxiliary requests: "Reproducibility of specific plasmids (no)" - "Clarity (no)- arbitrary designation of plasmids"

Headnote
Catchwords

Europäisches Patentamt | European Patent Office | Office européen des brevets

Beschwerdekammern | Boards of Appeal | Chambres de recours

Case Number: T 0816/90 - 3.3.2

D E C I S I O N
of the Technical Board of Appeal 3.3.2
of 7 September 1993

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Decision under appeal: **Decision of the Examining Division of the European Patent Office dated 25 April 1990 refusing European patent application No. 85 902 041.4 pursuant to Article 97(1) EPC.**

Composition of the Board:

Chairman: P.A.M Lançon
Members: L. Galligani
E.M.C. Holtz

Summary of Facts and Submissions

I. European patent application No. 85 902 041.4 published as an International application under No. WO 85/04672 (European publication No. 0 214 971) was refused by the Examining Division.

The decision was taken on the basis of Claims 1 to 17 as filed by letter dated 16 June 1989.

Claim 1 was directed to "a DNA sequence which codes for the cellulase enzyme cellobiohydrolase II from *Trichoderma reesei* which is capable, when correctly combined with an expression vector, of expressing a protein having the cellulolytic activity of the said enzyme upon transformation of a host organism by the vector", said DNA sequence being one that codes for the specific amino acid sequence recited in the claim or "for a substantially identical amino acid sequence showing the same enzymatic activity".

Claim 2 was directed to "a DNA sequence according to Claim 1 which codes for the cellulase enzyme cellobiohydrolase II" which has the specific nucleotide sequence recited in the claim or "a substantially identical nucleotide sequence coding for an amino acid sequence showing the same enzymatic activity".

II. The Examining Division refused the application under Article 97(1) EPC on the grounds that the subject-matter of Claim 1 did not involve an inventive step within the meaning of Article 56 EPC, having regard to the following documents:

(1) BIO/TECHNOLOGY, October 1983, pages 691 to 695;
(2) BIO/TECHNOLOGY, October 1983, pages 696 to 699;
(3) FEBS Letters, September 1980, Vol.119 (1), pages 974 to 100.

The main reasons given for the decision are as follows:

(a) the isolation and characterisation of cellobiohydrolase II (CBH II) was known from document (3);

(b) the molecular cloning and characterization of the gene encoding cellobiohydrolase I (CBH I) was known both from document (1) and (2) wherein essentially the same techniques are used;

(c) in (1) it is stated that the method used for the isolation of CBH I should be generally applicable to other cellulolytic genes;

(d) Claim 1 is not limited to a specific DNA sequence or to a specific construct suitable to be expressed in yeast, but relates broadly to genes encoding CBH II;

(e) a skilled person by following the procedures described in document (1) or (2) would inevitably have arrived at the isolation of a DNA sequence encoding

CBH II from *Trichoderma reesei (T.reesei)*. With respect to the known procedures this merely required the additional step of the preparation of an antiserum against CBH II which is a routine matter. The isolation of the CBH II gene in the present application has indeed exactly followed the said procedures.

III. The Appellant has lodged an appeal against this decision and has paid the appeal fee.

IV. In filing the Statement of Grounds the Appellant, as an auxiliary request, offered to combine Claims 1 and 2 and to renumber the remaining claims and their appendancies (auxiliary request I).

V. In reply to a communication of the Board pursuant to Article 110 (2) EPC, the Appellant filed by letter dated 2 June 1993 (received on 5 June 1993) further observations together with two additional auxiliary requests (II and III) in which the claims quoting specific plasmids (see Claims 6 to 7, 11 to 12, 14 of main request) were deleted . Said auxiliary requests II and III correspond to the main request (rejected claims) and to auxiliary request I, respectively.

VI. The Appellant's main argument is essentially that, in spite of the teachings of documents (1) and (2) (isolation of DNA encoding CBH I) and of the information on the amino acid sequence of CBH II available from document (3), the successful cloning and expression of CBH II required **more than** the routine adaptation of the known procedures. This is mainly because of the difficulties in obtaining mRNA of a quality good enough for use in a differential hybridisation method. Said difficulties, which were caused by the relatively smaller amount of mRNA for CBH II produced by *T.reesei*, were overcome by the Appellant by adopting the expedient

of grinding the frozen mycelia into fine powder under liquid nitrogen, i.e. at -170°C (see description page 7, third paragraph). This critical alteration of the known procedure wherein **frozen cells** were ground at -20°C could not be considered as a routine adaptation. Neither the information from the prior art nor the awareness of the skilled person of the differences between CBH I and CBH II and of the necessity of isolating best quality mRNA suggested the said modification.

VII. The Appellant requests the setting aside of the appealed decision and the allowance either of the main request or of one of the auxiliary requests.

Reasons for the Decision

1. The appeal is admissible.

2. *Amendments (Article 123 (2) EPC)*

The Board endorses the finding of the Examining Division that the claims of the main request comply with Article 123(2) EPC. There are no objections to the proposed amendments in any of the auxiliary requests as the combination of Claims 1 and 2 (auxiliary requests I and III) and/or the deletion of the claims which quote specific plasmids (auxiliary requests II and III) do not result in an extension of the application beyond the contents of the application as filed.

3. *Novelty (Article 54 EPC)*

Novelty was not contested by the Examining Division with respect to the main request and there is no reason for the Board to further examine this question of its own

motion. In view of the nature of the amendments in the auxiliary requests, there can be no novelty objection also with respect to these latter.

4. *Main request and auxiliary request I*

4.1. Clarity (Article 84 EPC) and sufficiency of disclosure (Article 83 EPC)

These issues are examined by the Board of its own motion under Article 114(1) EPC as no objections under Articles 83 and 84 EPC were raised by the Examining Division in the appealed decision.

Claims 6 to 7, 11 to 12, 14 of the main request (Claims 5 to 6, 10 to 11, 13 in auxiliary request I) quote specific plasmids, namely pMP91 and pMA29 as an intermediate and a final product, respectively. As observed in the official communication of the Board to the Appellant:

i) said plasmids are defined by use of a designation which has no technical meaning *per se*. Moreover, their structure is defined by reference to a figure. Such a definition of a plasmid cannot be accepted because it is contrary to the requirements of Article 84 EPC (see also decision T 269/87 of 24 January 1989, not published in the OJ EPO);

ii) the Appellant did not directly deposit the said plasmids and relied on the written description. Thus, the repeatibility of the said plasmids is dependent upon the possibility for the skilled person to **identically reproduce** them by following the instructions provided by the description. This latter, however, does not appear to contain sufficient details to ensure said identical reproducibility (see

description page 12, lines 25 to 27 and page 13, item b). The Appellant has not proven the contrary. Thus, the Board considers that the said plasmids and, consequently, the subject-matter of the quoted claims cannot be reproduced. This is contrary to the provisions of Article 83 EPC.

In reply to the said communication of the Board, the Appellant declared that he was prepared to withdraw the quoted claims. This has in fact been done in auxiliary requests II and III (see section V above).

For the above reasons, the main request as well as auxiliary request I, of which the quoted claims are part, are rejected under Articles 83 and 84 EPC.

5. *Auxiliary request II*

5.1 Clarity (Article 84 EPC) and sufficiency of disclosure (Article 83 EPC)

This request differs from the main request merely in that the claims quoting plasmids pMP91 and pMA29 have been deleted. The remaining claims and their appendancies have been renumbered. Thus, the objections under Article 84 and Article 83 EPC raised in section 4.1 above do not longer apply.

On the other hand, the non-identical reproducibility of the quoted plasmids does not prejudice the reproducibility of the application as a whole because the skilled person, in the light of the disclosure of the complete nucleotide and amino acid sequences for CBH II, can easily put the claimed invention into practice (see also decision T 281/86, OJ EPO, 1989, 202).

5.2 *Inventive step (Article 56 EPC)*

5.2.1 The closest prior art is represented in the present case by Document (3). This document discloses the isolation in homogeneous form of CBH II from *T.reesei* and its partial physico-chemical characterisation . The sequence of the first 20 amino acids of CBH II at the N-terminal end as well as a comparison with that of CBH I (see Figure 4) are reported. The said comparison shows that there is no obvious sequence homology within the first 20 residues between CBH I and CBH II. Document (3) points to the need for a highly purified preparation of CBH II in order to further elucidate the structure and the properties of the enzyme (see page 100, first paragraph).

5.2.2 In the light of (3), the problem to be solved by the present application can be seen in **the provision of an improved method for the production of CBH II in larger amounts** so as to allow, for instance, the elucidation of its structure and properties.

5.2.3 The solution provided by the present application is a method for the production of CBH II of *T.reesei* in a recombinant system. The necessary tools to be used therefor, such as DNA sequences encoding CBH II, the corresponding plasmids and yeast host cells are also provided (see e.g. Claims 1 to 5, 8 to 10, 13, 17).

The Board is satisfied that the underlying technical problem has indeed been solved in view of the results reported in the application (see in particular page 16, item 3).

5.2.4 In the search for a solution to the underlying technical problem as defined above, the skilled person, besides considering prior art documents which deal with CBH

II, would also direct his/her attention to documents which deal with the closely related enzyme CBH I. He/she would, therefore, be familiar with the contents of documents (1) and (2) - referred to by the Examining Division in its decision - which describe the molecular cloning of CBH I from *Trichoderma reesei*. Document (1) discloses the genomic map of CBH I and the complete nucleotide and amino acid sequences of the enzyme. Document (2) discloses the restriction map of the CBH I gene and some sequence data. In both documents the differential hydridisation technique is used as a step in the experimental protocol for the isolation of CBH I clones. The experimental protocols of (1) and (2) are essentially the same.

The relevant question to be asked in assessing inventive step is whether the skilled person, starting from document (3), would have arrived, in the light of the teachings of documents (1) and (2), at a method for the production of CBH II by recombinant DNA technology in a rather straightforward way. This amounts to the question whether the cloning and expression of CBH II would have been obtained by way of the simple adaptation of the differential hybridisation approach used in (1) and (2) for the cloning of CBH I or by way of any other straightforward approach.

5.2.5 With respect to the above question the Board observes the following:

i) the teaching of documents (1) and (2) is limited to the **cloning** of DNA encoding CBH I. The **expression** of CBH I in a host cell is not disclosed therein;

ii) although the cloning of CBH II from *T.reesei* has been achieved by the Appellant by way of a procedure which is largely similar to that disclosed in

documents (1) and (2), nevertheless a modification has been introduced in the step of isolation of cellular RNA, namely "the grinding of frozen mycelia under liquid nitrogen". This modification is not derivable from the cited documents. In fact, document (1) refers to " a modification of the method of Chirwing et al." and merely states that "frozen cells were ground in a mortar and pestle and homogenized.....". Document (2) refers in vague terms to the "GuGCl-LiCl method of Ohi and Short, modified by Salovuori et al...", but does not give any detailed information (N.B. the reference to Salovuori et al. is "submitted for publication", thus it is not retrievable).

Thus, it cannot be said that the Appellant followed **exactly** the procedures known from documents (1) and (2) because at least one modification has been introduced therein. Moreover, the experimental work in the present case went few steps further since **not only** the cloning of the CBH II was achieved, **but also** its expression in yeast cells.

5.2.6 During the appeal proceedings, the Appellant strongly emphasized the critical importance of the said modification in the RNA isolation procedure. In his submissions the optimization of the RNA isolation method thereby achieved made possible to obtain high quality RNA and thus assured success of the cloning and expression efforts. The necessity to optimize the RNA isolation method in order to obtain high quality RNA from filamentous fungi is confirmed in a later publication by one of the present inventors (see T.T.Teeri "The cellulolytic enzyme system of ***Trichoderma reesei***", May 1987, Technical Research Center of Finland, Publication 38 - hereinafter document (4) -, in particular item 3.2.1 on pages 24 to 26).

5.2.7 The Board is aware of the fact that, even when it is possible to theoretically conceive a straightforward approach to solve a specific technical problem, the skilled person might be confronted with unexpected difficulties when trying to put the conceived strategy into practice. Sometimes these difficulties can be overcome by introducing modifications in the known protocols. Said modifications can sometimes prove to be decisive for the successful conclusion of the research effort.

In the present case, the available prior art offered undeniably good indications on how the underlying technical problem could be solved. However, the experimental approach successfully used by the Appellant comprised a modification of the known procedure. It is true that the existence of a cause/effect relationship between the modification in the RNA isolation step and the successful cloning and expression of CBH II has not been proven. However, at the present stage, neither concrete evidence nor well-founded reasons are available to state that the said modification was **unessential** and that the skilled person would have achieved the cloning of CBH II merely by using the experimental approach of (1) and (2) in an analogous manner. Moreover, no prior document is available which discloses the grinding of cells under liquid nitrogen for the purpose of isolating cellular RNA. Although the skilled person is generally aware of the fact that appropriate refrigeration is essential to prevent RNA degradation during its isolation, nevertheless the use of **ultra low temperatures** appears at present to be a non-obvious measure.

Under these circumstances, the Board is bound to concede that the successful cloning and expression of CBH II was

not merely a matter of routine adaptation of the known procedures.

5.2.8 As to the question whether the skilled person would have achieved the same result by a different experimental approach, e.g. through the use of an oligonucleotide probe constructed on the basis of the amino acid sequence information in document (3), the Board considers that at this stage this is a purely academic question. Firstly, what appeared to be the most probable approach (analogous use of the differential hybridisation techniques described in documents (1) and (2)), has already been considered on the whole as non-obvious (see item 5.2.7 above). Secondly, no prior art document is at present available which shows that, starting from the information in document (3), the skilled person would have easily arrived at the claimed subject-matter.

In conclusion, at the present stage nothing allows the Board to conclude that the skilled person would have arrived at a method for the production of CBH II of *T. reesei* in a recombinant system in a straightforward way from the known art. Consequently, auxiliary request II is patentable.

6. *Auxiliary request III*

In view of the allowability of the auxiliary request II, a discussion of the auxiliary request III, in which the main claim is more limited in scope, is not necessary.

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 grant a patent on the basis of the claims of the auxiliary request II and of a description to be appropriately amended.

The Registrar: The Chairman:

P. Martorana P.A.M. Lançon