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D E C I S I O N
of 14 November 2001

Case Number: T 0749/98 - 3.3.4

Application Number: 91909971.3

Publication Number: 0531372

IPC: C12N 9/42

Language of the proceedings: EN

Title of invention:

A cellulase preparation comprising an endoglucanase enzyme

Patentee:

Novozymes A/S

Opponents:

GENENCOR INTERNATIONAL INC.
Unilever N.V., Rotterdam Patent Division

Headword:

Cellulase/NOVOZYMES A/S

Relevant legal provisions:

EPC Art. 83, 56

Keyword:

"Sufficiency of disclosure - main request (no)"
"Inventive step - auxiliary request (yes)"

Decisions cited:

T 0225/93

Catchword:

-



Case Number: T 0749/98 - 3.3.4

D E C I S I O N
of the Technical Board of Appeal 3.3.4
of 14 November 2001

Appellant I: Novozymes A/S
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Decision under appeal: Interlocutory decision of the Opposition Division
of the European Patent Office posted 26 May 1998
concerning maintenance of European patent
No. 0 531 372 in amended form.

Composition of the Board:

Chairwoman: U. M. Kinkeldey
Members: F. L. Davison-Brunel

S. C. Perryman

Summary of Facts and Submissions

- I. The appeals lie from the decision of the Opposition Division issued on 26 Mai 1998 whereby the European patent No EP-A-0 531 372 with the title "A cellulase preparation comprising an endoglucanase enzyme" was maintained in amended form pursuant to Article 102(2) EPC.

Granted claim 1 read as follows:

"1. A cellulase preparation consisting essentially of a homogenous endoglucanase component which is active between pH 6.0 and 10.0, and which is immunoreactive with an antibody raised against a highly purified ~43kD endoglucanase derived from **Humicola insolens**, DSM 1800, or which is a derivative of said ~43kD endoglucanase."

- II. At oral proceedings on 14 November 2001, Appellants I (Patentees) submitted an auxiliary request.

Claim 1 of this request read as follows:

"1. A cellulase preparation consisting essentially of a homogeneous enzyme exhibiting endoglucanase activity, which enzyme has the amino acid sequence shown in the appended Sequence Listing ID#2 or ID#4 or is a derivative of either."

Claims 2 and 3 were directed to further features of the cellulase preparation. Claims 4 to 8 were directed to DNA constructs/expression vector/transformed cells comprising the DNA sequences encoding the enzyme as claimed in any of claims 1 to 3. Claim 9 was addressed to a process for producing the claimed endoglucanase.

Claims 10 to 14 and 15 to 20 were addressed to detergent additives and detergent compositions, respectively. Claims 21 to 25 were directed to various methods making use of the cellulase preparations of claims 1 to 3.

III. The following documents are mentioned in this decision:

(1): WO 89/09259

(9): Schülein, M. et al., Proceedings of the second TRICEL symposium on *TRICHODERMA REESEI* CELLULASES AND OTHER HYDROLASES, Espoo 1993, ed. by P. Suominen & T. Reinikainen. Foundation for Biotechnical and Industrial Fermentation Research 8, pages 109 to 116, (1993).

IV. The arguments by Appellants I in writing and during oral proceedings insofar as they are relevant to the present decision can be summarized as follows:

Main request

Article 83 EPC in relation to the subject-matter of claim 1

The patent specification (page 4, lines 12 to 15) provided the information that the immunochemical characterization of the 43kD endoglucanase from *Humicola insolens* could be carried out as described in document (1) (page 15) ie by immunoprecipitation involving a polyclonal antiserum (Ouchterlony method, Mancini test). It also described on page 8, lines 31 to 35 how to raise a polyclonal antiserum against the

43kD protein. Following these teachings, the skilled person would consider that immunoprecipitation was the method to use to show the presence of an endoglucanase such as claimed in claim 1 (capable of **immunoreacting with an antibody** against the purified 43kD endoglucanase) in a cellulase preparation.

It had been shown that the 43kD endoglucanase from *Humicola insolens* could be immunoprecipitated from an impure enzyme preparation by the anti-43kD polyclonal antiserum and also that not all cellulase mixtures were immunoprecipitated under the same conditions. Furthermore, evidence had been provided that immunoprecipitation could successfully be carried out between the 43kD endoglucanase and the polyclonal antiserum over a wide range of concentrations. All these data constituted strong evidence that immunoprecipitation could be used as a means to identify an endoglucanase such as claimed and even to monitor its purification.

The patent also taught how to obtain the claimed endoglucanases by recombinant means. It provided sets of primer pairs which enabled the cloning of the corresponding genes from *Fusarium* and from many other species by the PCR method. It was possible that all the primer pairs did not work for all species and the experimental conditions to use would not be exactly the same as those described in the patent, yet no evidence had been provided that an endoglucanase gene could not be isolated using some of the primer pairs and the experimental conditions described.

The arguments by Appellants II (Opponents I) that other methods for determining immunoreactivity may have been

chosen by the skilled person with the risk of getting a false negative/positive result did not hold in view of the reference to document (1) in the specification of the patent in suit. In relation to this point, it was admitted that the earlier statement that the term "**immunoreactive with an antibody**" (claim 1) also comprised the recognition of the endoglucanase by a monoclonal antibody other than those specific for the cellulose binding domain was wrong.

Appellants II' objection that immunoprecipitation would not be an adequate method to obtain the claimed endoglucanases was not backed up by any experimental evidence although it would have been possible to produce such evidence starting from the *Humicola insolens* cellulase preparation which contained the 43 kD endoglucanase in small quantities.

Auxiliary request

Article 56 EPC; claim 1

The closest prior art was document (1) which disclosed a cellulase preparation from *Humicola insolens* with high endoglucanase activity containing two proteins of 65kD and 50kD.

The problem to be solved could be defined as retrieving from the cellulase preparation the protein responsible for the observed endoglucanase activity.

The solution provided was a 43kD endoglucanase.

Document (1) identified the 65kD protein as the endoglucanase. In a first step, one had to recognize

that this protein was not the endoglucanase looked for and, then, the skilled person would have to begin a research programm to find out which protein was the desired endoglucanase. Once the 50kD protein was identified as having endoglucanase activity (which it had, document (9)), there was no reason to look for a further endoglucanase. And besides, it was not obvious to isolate the 43kD enzyme which was present in the preparation in very small quantities. The immunoprecipitation route which had been used was not a routine method of protein purification.

- V. The arguments by Appellants II in writing and during oral proceedings as well as by the Respondents (Opponents 2), insofar as they are relevant to the present decision, can be summarized as follows:

Main request

Article 83 EPC in relation to the subject-matter of claim 1

The patent specification did not tell the skilled reader which method to use to show that an endoglucanase was **immunoreactive with an antibody** against the 43kD endoglucanase in accordance with the claim. In 1990, there were many techniques for showing immunoreactivity such as Western blot assays, radioimmunoassays, ELISAs, immunodiffusion or immunoprecipitation, which would not necessarily give the same results.

Although Appellants I now contended that it was readily apparent from the patent specification that immunoprecipitation was the method of choice, they

themselves in an earlier statement (see submissions of 18 February 1998) considered that a possible method for identifying an endoglucanase falling within the scope of the claim was to show that it interacted with an anti-43kD monoclonal antibody (Mab) (other than an Mab specific of the cellulose binding site).

It would be extremely difficult to obtain the claimed endoglucanase by immunoprecipitation starting from a preparation which would not be known to contain it and being unaware of the level of homology which may exist between said endoglucanase and the 43kD endoglucanase. The experiments done by Appellants I to back up their argument that this would be possible were beside the point. Indeed, while it would be fully expected that an enzyme mixture containing the 43kD endoglucanase (even in small quantities) could be shown to react with the anti-43kD polyclonal antiserum, this would not necessarily apply for another endoglucanase which may not sufficiently cross-react with the polyclonal antiserum for the cross-reaction to be observed. (false negative). In the same manner, Appellants I' experiment showing that a given anti-43kD polyclonal antiserum did not react with some cellulases from other species did not mean that another anti-43kD polyclonal antiserum would not react with these cellulases as each polyclonal antiserum was different from the next.

Appellants II were not in a position to prove that the invention could not be put into practice because the patent specification did not teach which method to use to show whether or not it had been put into practice.

Appellants I themselves did not go the "immunoreactivity route" to try and isolate the claimed

endoglucanase. They chose instead to use the PCR method to isolate a gene from another organism which would have a certain degree of homology to the 43kD endoglucanase gene. The PCR method as described in the patent in suit was not of general applicability. And, besides, it had not been shown that the cloned *Fusarium* gene so obtained encoded an endoglucanase, let alone that this endoglucanase would be immunoreactive with an antibody against the 43kD endoglucanase, whether it be by immunoprecipitation or by any other method.

Auxiliary claim request

Article 56 EPC; claim 1

The closest prior art to the *Humicola insolens* enzyme (Sequence Listing #2) was document (1) which described a cellulase preparation with endoglucanase activity and containing two proteins of 65kD and 50kD respectively.

Starting from document (1), the problem to be solved was to find out which component of the preparation was responsible for the high endoglucanase effect which was observed.

The solution was the isolation from the cellulase preparation of a 43kD protein with endoglucanase activity.

Once the skilled person had found out that the 65kD protein initially thought to be the endoglucanase had in fact no endoglucanase activity, it was inevitable that he/she would continue the purification procedure in a routine manner and they, thus, would necessarily get to the 43kD protein in addition to the 50kD enzyme.

Nothing in the purification process per se was inventive as it only involved the raising of antibodies and their use to test the cellulase preparation in a routine manner.

- VI. Appellants I requested that the decision under appeal be set aside and that the patent be maintained, as main request as granted or as auxiliary request on the basis of claims 1 to 25 submitted as New Auxiliary Request 1 at the oral proceedings on 14 November 2001.

Appellants II and the Respondents requested that the decision under appeal be set aside and that the patent be revoked.

Reasons for the Decision

Main request

Article 83 EPC in relation to the subject-matter of claim 1

1. The cellulase preparation (endoglucanase) of claim 1 is characterised in particular by the functional feature that it must be **immunoreactive with an antibody** raised against a *Humicola insolens* 43kD endoglucanase. This feature implies that the claim not only covers the latter endoglucanase isolated according to the patent in suit but also all endoglucanases capable of the above mentioned immunoreaction. While the Board accepts that the *Humicola insolens* 43kD endoglucanase can be isolated in a repeatable manner and, thus, that the requirements of Article 83 EPC are fulfilled so far, it must also be decided in the context of sufficiency of

disclosure whether the patent specification provides sufficient technical advice to be able to obtain any other endoglucanases comprised within the claim.

2. In the patent specification, page 4, lines 12 to 15, it is taught that the 43kD endoglucanase may be immunologically characterized as described in WO 89/00069 (in fact, WO89/ 09259 with the international application number PCT/DK89/00069), that is, by immunoprecipitation with a polyclonal antiserum, the production of which is described on page 8, lines 31 to 35. No general teachings or specific examples are provided for the immunological identification of an endoglucanase other than the 43kD endoglucanase itself.
3. Appellants I argue that the skilled person would have understood from the reference to WO 89/00069 that immunoprecipitation was the method to use to ensure that the claimed feature was being met. The Board is not convinced that this is the case. The skilled person would, of course, assume that the 43kD endoglucanase will react with a polyclonal antiserum raised against itself so that optimal conditions exist for identifying the enzyme by immunoprecipitation with the polyclonal antiserum, even if it is only present in small quantities in the sample to be tested. Yet, he/she would not necessarily expect that an endoglucanase from another source would react with the anti-43kD polyclonal antiserum with suitable efficiency to be observed in immunoprecipitation because this other endoglucanase may share some of the 43kD endoglucanase epitopes but will not possess them all. Thus, he/she would have no compelling reasons to choose immunoprecipitation rather than any other methods for testing the feature "**immunoreactivity to an antibody**".

4. In 1990, there were several techniques which could have been used to determine immunoreactivity: Western blot assays, radioimmunoassays, ELISAs, immunoprecipitation... Western blots assays are not suited to show the presence of conformational epitopes and, as above stated, immunoprecipitation may not be sensitive enough to identify proteins with low levels of homology to the 43kD protein. Interaction with an anti-43Kd monoclonal antibody (ELISA) was also originally envisaged by Appellants I as one of the suitable techniques and, thus, it is reasonable to expect that it would come to the skilled person's mind to detect the endoglucanase in that way. By this method, only endoglucanases sharing the same epitope as the one recognized by the anti-43kD monoclonal antibody would be found.

5. Because these techniques for determining immunoreactivity give different answers and no information is given in the patent in suit as to which of them to use, the skilled person choosing anyone of them would not know when getting a positive or a negative result, whether he/she had succeeded or failed to reproduce the invention as claimed. Accordingly, the conclusion is reached that the patent in suit fails to give sufficient technical advice to isolate the claimed endoglucanase because it fails to identify the means by which to assess its claimed property.

6. The situation is very much alike to that encountered in case T 225/93 (of 13 Mai 1997). There, the claimed subject-matter made use of a calcium carbonate defined by the size of its particles which was an essential feature of the invention. No technical advice was given in the patent specification as to how to measure the

size of the particles and different methods of measurement existed in the art which did not all give the same result. The then competent Board decided that the skilled person would not know which method to choose to arrive at the claimed calcium carbonate and, therefore, could not reproduce the claimed invention.

7. Appellants I' other argument in favour of sufficiency of disclosure went to show that the patent in suit gave adequate information for the person skilled in the art to be able to isolate endoglucanase genes from other species on the basis of their expected homology to the 43kD endoglucanase gene. Whether or not this is enabled by the patent in suit need not be decided here insofar as even if an endoglucanase gene and the corresponding protein are obtainable in the described way, there is no technical teaching in the patent in suit for identifying the endoglucanase as exhibiting the claimed feature (see points 1 to 5 above).
8. Finally, Appellants I also argued that the onus was on Appellants II and on the Respondents to show that the claimed endoglucanase could not be obtained and identified on the basis of the teachings in the patent in suit. Whereas this approach to sufficiency of disclosure is in general valid, it cannot be applied in the present case because the opposing parties could not have carried out the relevant experiments in the absence of knowledge of what these might be.
9. The main request is rejected for lack of sufficiency of disclosure.

Auxiliary request

10. No objections were raised against this request under Articles 123(2)(3), 84, 83 or 54 EPC. The Board agrees that the sole issue to be decided is that of inventive step.
11. The closest prior art to the *Humicola insolens* enzyme of claim 1 (sequence Listing ID#2) is document (1) which discloses a partially purified cellulase preparation (F1P1C2) from *Humicola insolens* exhibiting endoglucanase activity (page 13) and comprising a main 65kD protein and a minor amount of a protein with a molecular weight of 50kD (page 14). The endoglucanase activity of F1P1C2 is attributed to the presence of the 65kD protein in the mixture.
12. Starting from this closest prior art, the problem to be solved can be defined as further investigating the partially purified F1P1C2 preparation in terms of its endoglucanase activity.
13. The solution provided is that this activity is attributable to a protein with a molecular weight of 43kD.
14. The skilled person taught by document (1) that the endoglucanase activity was due to the 65kD protein would in the first instance have attempted to purify this protein and, thus, would probably have discarded the protein fractions containing the 43kD protein. The fact that no endoglucanase activity could be associated with the 65kD protein would have come as a surprise. The Board agrees, however, with the position of Appellants II and the Respondents that once it had turned out that it was not the 65kD protein which was responsible for the endoglucanase activity, the skilled

person would have investigated F1P1C2 further. It would, then, have been a matter of routine to test the only other protein known to be present in the preparation, ie the 50kD protein, for being an endoglucanase. The skilled person investigating the enzymic property of this protein would have come to the result that it was an endoglucanase. In fact, post-published document (9) (to be taken as an expert document) discloses that *Humicola insolens* produces two endoglucanases with the same 50kD molecular weight (EGI and EG2) in addition to the 43kD endoglucanase.

15. In the Board's judgment, the skilled person having found a protein having endoglucanase activity in F1P1C2 had no reason to look further for another such enzyme. The isolation of the 43kD endoglucanase is, thus, fully unexpected.

16. For these reasons, inventive step is acknowledged to the subject-matter of claim 1 being a cellulase preparation consisting essentially of a homogeneous enzyme exhibiting endoglucanase activity, which enzyme has the amino acid sequence shown in the appended Sequence Listing ID#2 and derivatives thereof.

17. Claim 1 is also directed to a second cellulase preparation containing an enzyme from *Fusarium* (sequence listing ID#4). This enzyme, which is "derived" from the inventive 43kD endoglucanase protein in the sense that the DNA encoding it was isolated on the basis of the *Humicola insolens* 43Kd endoglucanase DNA sequence must also be inventive, as well as its derivatives if, as the Board has found, the skilled person would not have arrived at the 43Kd endoglucanase protein, he or she would not have arrived at this

Fusarium enzyme by any obvious route either.

18. The requirements of Article 56 EPC are fulfilled by claim 1 and claims 2 to 25 dependent thereon.

Order

For these reasons it is decided that:

1. The decision under appeal is set aside.
2. The matter is remitted to the first instance with the order to maintain the patent on the basis of claims 1 to 25 submitted as New Auxiliary Request 1 at the oral proceedings on 14 November 2001 and a description adapted thereto.

The Registrar:

The Chairwoman:

P. Cremona

U. Kinkeldey