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D E C I S I O N
of 6 September 2001

Case Number: T 0479/97 - 3.3.4

Application Number: 89810545.7

Publication Number: 0353188

IPC: C12N 15/60

Language of the proceedings: EN

Title of invention:
Novel expression system

Patentee:
Novartis AG

Opponent:
DSM Gist Holding B.V.

Headword:
Expression system/NOVARTIS AG

Relevant legal provisions:
EPC Art. 54, 56, 87 to 89

Keyword:
"Right to priority - main request - part of claim 1 and claim 5 - (no) - auxiliary request - (yes)"
"Novelty - main and auxiliary requests - (yes)"
"Inventive step - main request (no) - auxiliary request (yes)"

Decisions cited:
T 0002/83, T 0081/87, T 0301/87, T 0500/91, T 0277/95

Catchword:
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Beschwerdekammern | Boards of Appeal | Chambres de recours

Case Number: T 0479/97 - 3.3.4

D E C I S I O N
of the Technical Board of Appeal 3.3.4
of 6 September 2001

Appellant: (Opponent)
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Decision under appeal: **Interlocutory decision of the Opposition Division of the European Patent Office posted 10 April 1997 concerning maintenance of European patent No. 0 353 188 in amended form.**

Composition of the Board:

Chairman: U. M. Kinkeldey
Members: F. L. Davison-Brunel
W. Moser

Summary of Facts and Submissions

I. European patent No. 0 353 188 relating to "Novel expression system" and claiming priorities from GB 8818046 of 28 July 1988 (PI) and GB 8914666 of 26 June 1989 (PII) was granted on the basis of the European application No. 89 810 545.7 with forty claims for all designated Contracting States except for ES and with 38 claims for ES.

II. A notice of opposition was filed whereby the revocation of the European patent was requested on the grounds of Article 100(a) to (c) EPC.

III. The decision of the Opposition Division was to maintain the patent in amended form on the basis of a main request filed at oral proceedings together with an amended description. Claims 1, 5 and 40 of this request for all designated Contracting States except ES read as follows:

"1. A recombinant DNA molecule comprising a DNA sequence coding for pectin lyase pelA having the amino acid sequence set forth in fig.10; pectin lyase pelB having the amino acid sequence set forth in fig.11; pectin lyase pelC having the amino acid sequence set forth in fig.12; pelE obtainable by expression of pelE contained in pGW880 shown in fig.5, deposited under accession no. DSM 4392 or pelF obtainable by expression of pelF contained in pGW860 shown in fig.6, deposited under accession no. DSM 4391 or a derivative thereof, wherein a derivative designates a larger derivative including flanking sequences or DNA sequences which are degenerated in accordance with the genetic code."

"5. A recombinant DNA molecule according to claim 1, comprising a DNA sequence of the formula III shown in figure 12 or a derivative thereof as defined in claim 1."

"40. Pectin lyases PLA, PLB, PLC, PLE or PLF in pure form, obtainable by transforming a host which is not capable of expressing any pectin lyase with a recombinant DNA molecule according to claim 1 and isolating said pectin lyases."

Dependent claims 2 to 4, 6 to 22 related to further embodiments of the recombinant molecule of claim 1. Claim 23 was directed to a process for the preparation of a DNA molecule according to claim 1. Claim 24 and claims 25 to 33 dependent thereof related to various embodiments of a transformed host containing a recombinant molecule according to claim 1 and claims 34 and 35 related to a method for preparing such transformed host. Claims 36 to 39 were directed to a method for producing the pectin lyase polypeptides.

The corresponding claims were filed for ES.

IV. The Appellants (Opponents) lodged an appeal, paid the appeal fee and submitted a statement of grounds of appeal.

V. The Respondents (Patentees) filed an answer to the grounds of appeal together with an auxiliary request for all designated Contracting States, except ES and a test report.

This auxiliary request differed from the main claim request for all designated Contracting States, except

ES accepted by the Opposition Division in that claim 5 was deleted and all other claims were renumbered accordingly; in addition, claim 1 was amended as follows:

"1. A recombinant DNA molecule comprising a DNA sequence coding for pectin lyase pelA having the amino acid sequence set forth in fig.10; pectin lyase pelB having the amino acid sequence set forth in fig.11; pectin lyase pelC **obtainable by expression of pelC contained in pGW850 shown in fig.3, deposited under accession no. DSM 4390**; pelE obtainable by expression of pelE contained in pGW880 shown in fig.5, deposited under accession no. DSM 4392 or pelF obtainable by expression of pelF contained in pGW860 shown in fig.6, deposited under accession no. DSM 4391 or a derivative thereof, wherein a derivative designates a larger derivative including flanking sequences or DNA sequences which are degenerated in accordance with the genetic code." (amendments emphasized by the Board)

VI. The Board issued a communication pursuant to Article 11(2) of the Rules of Procedure of the Boards of Appeal, summoning oral proceedings and setting out its provisional, non-binding opinion.

VII. On 21 February 2000 and 2 March 2000 respectively, the parties announced their intention not to attend oral proceedings and requested that a decision be made based on the state of the file.

VIII. The Board sent a communication indicating its intention to grant a patent on the basis of the auxiliary request for all designated Contracting States, except ES and requested from the Respondents that they filed the

corresponding set of claims for ES.

IX. The Appellants were set a term to provide their comments on the auxiliary request for ES. No answer was received within the imparted time limit.

X. The documents cited in the present decision are the following:

(1): EP-A-0 278 355

(3): Maniatis et al., Molecular Cloning, A Laboratory Manual, Cold Spring Harbor Laboratory, 1982,

(4): Van Houdenhoven, F.E.A., Studies on pectin lyases, Ph.D. thesis, Agricultural University of Wageningen, Editors H.Veenman and Zonen B.V., October 1975,

(5): Edman, P. and Begg,G., European J.Biochem., Vol. 1 pages 80 to 91, 1967,

(6): Kusters-van Someren, M., Characterization of an Aspergillus niger pectin lyase gene family, Ph.D. thesis, Rijksuniversiteit, Utrecht, January 1991,

(10): Kusters-van Someren, M.A. et al., Curr.Genet., Vol. 20, pages 293 to 299, 1991.

XI. The arguments in writing by the Appellants insofar as they are relevant to the proceedings can be summarized as follows:

Main request for all designated Contracting States except ES:

- Claim 40 lacked novelty over document (4) under Article 54(1)(2) EPC and over document (1) under Article 54(3)(4) EPC as both these documents disclosed the pectin lyase PLII which was the same protein as the claimed PLA by all tested criteria.

- The patent in suit did not enjoy any priority rights for two reasons:

- The two priority applications, filed on 28 July 1988 and 26 June 1989, respectively, disclosed members of the pectin lyase family of Aspergillus niger (A.niger). If there was an invention in finding such a family, this invention was disclosed when the first member of the family was described. The first member of the pectin lyase family of A.niger was described in the patent application GB 8702475 filed on 1 February 1988 which served as priority application to the patent EP-A-0 278 355 (document (1) on file). Consequently, neither of the priority applications of the patent in suit were the first application to have been filed in respect of the pectin lyase family. In accordance with Article 87(4) EPC, they could not serve as a basis on which to establish priority.

- Claim 1 was directed, in particular, to the DNAs encoding pelF, pelE and pelC and claim 5 was directed to the DNA encoding pelC, which latter DNA was identified in both claims by reference to Figure 12 ie by its sequence. In contrast, the sequence was not disclosed in the priority applications which provided the recombinant

plasmid encoding pelC. In accordance with the case law (T 301/87, OJ EPO 1990, 335) that a deposit of a DNA molecule does not give a right of priority for its sequence, none of these priority applications could serve as a basis on which to acknowledge priority rights to claims 1 and 5. The priority date in relation to these claims was the filing date of the patent in suit.

- In view of the findings with regard to priority, document (1) published on 17 August 1988 belonged to the prior art.

Document (4) disclosed that there were two members in the pectin lyase family. Document (1) described the cloning of one of them. The skilled person would use the information given in document (1) to screen routinely for the other pectin lyases and would, thus, necessarily arrive at the claimed pectin lyases. No inventive step was involved.

Auxiliary request for all designated Contracting States except ES:

Document (4) taught a method for the complete purification of PLI and/or PLII. This allowed routine sequencing of short stretches of either of the proteins according to document (5). On the basis of these short sequences, probes could be prepared according to document (3). These probes could then be used in a routine manner to recover the claimed pectin lyase genes. The requirement for inventive step was not fulfilled.

XII. The Respondents answered essentially as follows:

Main request for all designated Contracting States except ES:

- Claim 40 directed to the PLA protein in pure form was novel over document (4) which disclosed a mixture of pectin lyases. It was also novel over document (1) as the pectin lyase PLII was different from PLA.

- The pelA, pelB, pelC, pelE and pelF genes and proteins were disclosed for the first time in the first priority application of the patent in suit, PI. It, thus, could not be said that the priority application GB 8702475 for document (1) was a first filing of the invention comprising said genes and proteins. Therefore, it was allowable to claim priority from PI in accordance with Article 87(1) EPC.

  In PI, the pelA and pelB genes and proteins were described in terms of their DNA and amino-acid sequences. The other pectin lyases and the genes encoding them were characterized through the deposition and the structural features of the relevant plasmids. Thus, PI disclosed the same invention as the patent in suit and all claims enjoyed priority rights from 28 July 1988.

- In view of these findings with regard to priority, document (1) did not belong to the state of the art. Starting from document (4) as closest prior art, the problem to be solved could be defined as the provision in pure form of distinct pectin lyases. Since neither document (4) nor any other documents of the state of the art suggested the

existence of the five pectin lyases, it was not obvious to search for the genes encoding them. Inventive step must be acknowledged.

Auxiliary request for all designated Contracting States except ES:

Claim 5 of the main request was deleted and claim 1 was redrafted in such a way that pelC was characterized by features which were disclosed expressis verbis in PI. The claim request as a whole enjoyed priority rights from 28 July 1988. Thus, the same reasoning applied with regard to inventive step of claim 1 (comprising the pelC gene) over document (4) as was presented in relation to claim 1 of the main request.

XIII. The Appellants requested that the decision under appeal be set aside and that the patent be revoked.

The Respondents requested that the appeal be dismissed or, as an auxiliary request, that the decision under appeal be set aside and the patent be maintained on the basis of claims 1 to 39 for all designated Contracting States except ES filed on 3 March 1998 and claims 1 to 39 for ES filed on 18 May 2001.

Reasons for the Decision

Main request for all designated Contracting States, except ES
Articles 87 to 89 EPC; right of priority

1. The Appellants argued that the patent application GB 8702475 which serves as priority application for

document (1) was the first application to disclose the pectin lyase family of A. niger and, therefore, that PI could not serve as a basis under the provision of Article 87(4) EPC for claiming a right of priority for the pelA, pelB, pelC, pelE and pelF genes and proteins which belonged to this family.

2. The patent application GB 8702475 is not one of the documents on file. There is, thus, no evidence that its content differs from that of document (1). This latter document mentions the common general knowledge that many pectin lyases are synthesized in A.niger, including PLI and PLII, and it also describes the cloning and the expression of the PLI gene (also known as pelD). It is, however, wholly silent on the number and characteristics of further pectin lyase genes and proteins. In contrast, claim 1 of the main request is directed to five genes other than pelD, which encode pectin lyases of A.niger. It is, thus, concluded that GB 8702475 does not disclose the same invention as claim 1 because the disclosure of one protein with a given function (here, pelD) does not make available proteins with the same function. GB 8702475 cannot be considered as a first application under Article 87(4) EPC for the recombinant DNA molecules of claim 1 carrying the DNA sequences coding for the pelA, pelB, pelC, pelE and pelF proteins. Consequently, PI may serve as a basis for claiming a right of priority.

3. According to Article 88(3) and (4) EPC, the right of priority shall cover those elements of the application which are specifically disclosed as a whole in the application whose priority is claimed. In decision T 81/87 (OJ EPO 1990, 250), it was made clear that the disclosure of the essential features must be either

express, or be directly and unambiguously implied by the text, and that missing elements which are to be recognized as essential only later on are thus not part of the disclosure.

4. In claim 1, the pelA, pelB, pelE anf pelF genes are characterized by features which were already described in PI (Figures 9 and 10, page 62). Claim 1, thus, enjoys priority rights from 28 July 1998 in respect of these genes.

5. The DNA encoding pelC is characterized in claims 1 and 5 by reference to Figure 12. This figure shows a 3168 base pair (bp) long sequence. The pelC open-reading frame (ORF) starts at position 1368, it is 1373 bp long and comprises three introns. In the first priority document, the pelC gene is characterized as being on a 5Kb DNA fragment which is carried by the deposited plasmid pGW850. The issue to be decided is thus, whether the provision of pGW850 can be considered as a direct and unambiguous disclosure of the pelC gene.

6. To characterize the pelC gene in the 5 Kb insert, it is necessary to sequence this insert, to identify in the sequence the elements, promoters, terminators, open-reading frames, introns which it may contain and, finally, to determine which combination of these elements constitute the pelC gene. It may be accepted that the necessity of sequencing the 5 Kb DNA fragment does not deter from the conclusion that the provision of said fragment amounts to an implicit disclosure of its sequence, taking into consideration that, in 1988, the DNA sequencing of fragments of such length would routinely be achieved. Yet, this sequence being known does not necessarily imply that the provision of the

DNA fragment makes available in a direct and unambiguous manner a specific gene which it contains, if only because the 5Kb fragment which is bigger than the gene of interest may carry more than one gene. Thus, priority rights for the pelC gene as claimed in claim 1 cannot be derived from PI.

7. The facts of the present case shows some similarity to those dealt with in decision T 277/95 of 16 April 1999. There, a claim to a method of producing in CHO cells recombinant human erythropoietin (rhEPO) characterized by the presence of a specific glycosilation pattern was found not to enjoy priority from a priority application which failed to disclose any glycosilation pattern for the rhEPO produced by the CHO deposited cell line. The then competent Board came to the conclusion that, in spite of the availablity of the cell line, the skilled person in the absence of any information about the presence of a specific glycosilation pattern could not derive from the priority document the specific features which characterized the claimed method.

Article 56 EPC; inventive step of claims 1 and 5 comprising the recombinant DNA encoding the pelC pectin lyase.

8. Novelty was not challenged in relation to these two claims. The issue of inventive step is to be decided, taking into account that, in view of the findings under point 6 above, document (1) published on 17 August 1988 belongs to the state of the art as far as pelC is concerned. Document (1) is considered to be the closest prior art. It discloses a recombinant DNA comprising the A.niger pelD gene encoding the PLD pectin lyase known in the art as PLI.

9. Starting from document (1), the objective problem to be solved is to isolate a recombinant DNA encoding a further pectin lyase of A.niger.

10. The solution provided by the patent in suit is to screen a gene bank of A.niger with the pelD DNA as a probe and to recover the DNAs hybridising to said probe. The Board is satisfied that the above defined problem is solved in this manner as a recombinant DNA is isolated, the expression of which leads to the protein PLC with pectin lyase activity (patent in suit, passage bridging pages 8 and 9).

11. Document (4) (page 17) summarizes the common general knowledge as early as 1975 with regard to the ability of fungi to produce extracellular enzymes: *" ..fungi often excrete more than one enzyme which catalyzes the same reaction...This means that...the presence of only one enzyme activity does not exclude the possibility that more than one enzyme is present...Many pectic enzymes...have very similar molecular weights and charges, with the result that they are very difficult to separate by conventional techniques of protein fractionation."* Document (4) also provides experimental evidence confirming the common general knowledge on specific pectin lyases: two pectin lyases are purified from a preparation of Asp.niger: PLI and PLII which have nearly identical amino-acid composition and the same C-terminal amino-acids (page 46).

12. In the Board's judgment, the knowledge that it is difficult to separate pectin lyases by conventional fractionation techniques would lead the skilled person to try and obtain them in some other way. Document (1) already applied recombinant DNA technique for the

isolation of the gene coding for pelD and in 1988, the recombinant DNA route to the production of a protein in pure form, including the screening of the gene encoding said protein with a homologous DNA probe, had become a matter of general common knowledge (document (3)). Thus, aware of the fact that pectin lyases are very similar in their amino-acid compositions, ie that the DNAs encoding them share substantial homology, the skilled person would be able to isolate said DNAs in a routine manner by hybridisation of a Asp.niger bank to the pelD DNA probe available from document (1). Otherwise stated, it did not require inventive skills to obtain the recombinant DNA carrying the pelC gene.

13. Therefore, it is concluded that the subject-matter of claims 1 and 5, when directed to the pelC gene, does not involve an inventive step. If one alternative of a claim covering several alternative inventions does not fulfil the requirements of Article 56 EPC, then the whole claim fails. The main request is thus refused for failing to fulfill the requirements of Article 56 EPC.

Auxiliary request, claims for all designated Contracting States except ES

Article 123(2)(3) EPC; Article 84 EPC

14. This set of claims differs from that of the main request in that claim 5 has been deleted and, in claim 1, the pelC recombinant DNA is characterized by reference to the name and the deposit number of the recombinant plasmid carrying it. Support for this feature may be found on page 72, line 5 of the application as filed. The requirements of Article 123(2) EPC are fulfilled.

15. The scope of claim 1 comprising the pelC recombinant DNA was not enlarged by making reference to the specific deposited recombinant plasmid carrying the pelC coding sequence rather than to the sequence per se. The requirements of Article 123(3) EPC are also fulfilled.

16. The claim is clear (Article 84 EPC)

Article 54 EPC; novelty of claim 39 (former claim 40 of the main request)

17. The novelty of claim 39 was challenged (insofar as the PLA protein is claimed) on the basis of document (4) which discloses a purified preparation of a pectin lyase named PLII. Document (4) describes the isolation of PLII in a specific manner from a specific origin:the commercial enzyme preparation Ultrazym^{R}. A comparison between the properties of PLA and those of an enzyme considered to be PLII is found in example 6.4 in the patent in suit as well as in a test report filed by the Respondents in the course of the appeal and in the post-published documents (6) and (10) (to be taken as expert documents). Each of these documents will be considered in turn to assess whether or not PLA as claimed in claim 39 and PLII as isolated from Ultrazym^{R} are the same enzymes.

18. In Example 6.4 of the patent in suit, PLA isolated from the wild-type strain A.niger N400 or from a transformed A.niger host according to the purification procedure given in the patent in suit is compared with a pectin lyase named PLII. This pectin lyase is obtained by the purification procedure used in document (4), but starting from the A.niger strain N400 rather than from

the Ultrazym^{R} preparation. This example does not, thus, provide a comparison which is relevant to assessing whether PLA and the PLII enzyme present in Ultrazym^{R} and isolated according to the state of the art (document (4)) are the same proteins.

19. In the test report filed by the Respondents on appeal, the properties of PLA are said to be compared with that of "purified PLII isolated from a commercial pectinase preparation (Ultrazyme^{R}, Van Houdenhoven, 1975)". The purification method, however, is not specified. It is, thus, impossible to assess whether or not the additional polygalacturonase activity observed in the PLII enzyme preparation would have been present in the PLII enzyme preparation as obtained by the method of document (4) ie whether PLA and the PLII enzyme isolated from Ultrazyme^{R} by the method of document(4) would differ by this feature.

20. The post-published document (6) presents an exhaustive study of, in particular, PLA and a comparison is carried out between this enzyme and PLII purified from Ultrazym^{R} as described in document (4). In the passage bridging pages 11 and 12, it is stated: *" PLA...was...shown to be the same enzyme as PLII"* whereas on page 17, the opinion is given that: *" ...we provide strong evidence that the pelA gene product PLA is the same as the enzyme PLII"* and on page 47:*"...we have now clearly demonstrated that Asp.niger N400 PLA has the same properties as PLII from Ultrazym^{R}"*. Yet, on page 41, the molecular weight of mature PLA is said to be "*slightly higher than that of PLII",* and the isoelectric points of both enzymes are given as being 3.5 and 3.7, respectively; these differences being attributed to the fact that **both** enzymes are produced

in A.niger strains of different origins. On page 42, it is stated: *"it is clear that PLII and PLA are closely related if not identical enzymes"*.

21. In document (10) (summary) published in 1991, the identity of PLA and PLII is once more suggested but not affirmed: *"... it was shown that the pelA encoded PLA is **probably** the same enzyme as PLII from Ultrazym..."* (emphasis added by the Board).

22. The Board understands the informations given in documents (6) and (10) as implying that PLA and PLII have the same functional properties, but also that, even a long time after the filing date of the patent in suit, the skilled person was not sure whether or not they represented the same molecule.

23. In the absence of a firm and unambiguous disclosure of the identity between PLA and PLII, the Board concludes that the subject-matter of claim 39 including PLA is novel over the disclosure of PLII in document (4).

24. It was also argued that document (1) was detrimental to the novelty under Article 54(3)(4) EPC of claim 1 comprising the PLA protein. PLII is mentioned once on page 3 of this document as one of the pectin lyases, the purification of which was described in document (4). The disclosure, thus, does not add to the disclosure of PLII in document (4): the reasoning presented under points 17 to 21 which led the Board to conclude that the subject-matter of claim 39 including PLA is novel over the disclosure of PLII in document (4), applies.

Articles 87 to 88 EPC; priority rights

25. All of the recombinant DNAs of claim 1 including the one encoding pelC are characterized in the same manner as in PI (Figures 9 and 10, page 62). Thus, claim 1 enjoys priority from 28 July 1988.

Article 56 EPC, inventive step

26. In view of the findings under point 25 above, document (1), published on 17 August 1988, does not belong to the state of the art. The closest prior art is document (4), the content of which is summarized under point 11 above.

27. Starting from this prior art, the objective problem to be solved is to provide further pectin lyases in pure form.

28. The proposed solution is to clone and express separately each of the genes encoding said pectin lyases. The Board is satisfied that the above defined problem is solved in this manner as five genes are isolated, the expression of which leads to proteins with pectin lyase activity (patent in suit, passage bridging pages 8 and 9).

29. Differently from document (7) (see point 12 above) document (4) does not suggest to take the DNA recombinant route to the purification of the pectin lyase genes. Neither does any of the other documents belonging to the state of the art. as said in point 12 above, it could be accepted that in 1988, on the basis of the then prevailing general common knowledge, this route was an obvious one to take for the skilled person, as recombinant DNA techniques were major tools for the separation of proteins (document (3)). Yet, it

remains that on the basis of the disclosure of document (4), which does not provide a clear teaching of a single pectin lyase, let alone an amino-acid sequence which could be a basis for preparing a probe, the cloning of these genes becomes a research programm including the isolation of said probe. In this respect, the argument by the Appellants (see section XI above) that all techniques necessary to isolate the probe were known from documents (3) and (5), thus making said isolation obvious, is not convincing: indeed, according to the case law of the Boards of Appeal (see, for example, T 2/83, OJ EPO 1984, 265), it is not a question of whether a skilled person could have carried out the invention but whether he would have done so. Taking into account that in accordance with the case law (cf. T 500/91 of 21 October 1992), the average skilled person in the field of biotechnology would not be expected to solve technical problems through scientific research, it is concluded that the subject-matter of claim 1 and of all of the other claims which are dependent thereof was obtained by exercising inventive skills. Accordingly, it is decided that the requirements of Article 56 EPC are fulfilled.

30. For these reasons, it is concluded that the auxiliary request comprising claims 1 to 39 for all designated Contracting States except ES and the request concerning claims 1 to 39 for ES, which correspond to claims 1 to 39 of the auxiliary request for all other designated Contracting States fulfill the requirements for patentability.

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 following documents:

   (a) claims 1 to 39 for all designated Contracting States except ES filed on 3 March 1998 as auxiliary request; and

   (b) claims 1 to 39 for the contracting State ES filed on 18 May 2001; and

   (c) description pages 2,3,5 to 20, 22 to 33 and page 34, lines 1 to 41 as granted and pages 4 and 21, filed on 5 December 1996; and

   (d) drawings: Figures 1 to 15 as granted.

The Registrar: The Chairwoman:

U. Bultmann U. Kinkeldey