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D E C I S I O N
of 13 March 2006

Case Number: T 0029/05 - 3.3.08

Application Number: 95120437.9

Publication Number: 0718402

IPC: C12N 15/55

Language of the proceedings: EN

Title of invention:
Penicillin V amidohydrolase gene from fusarium oxysporum

Applicant:
Bristol-Myers Squibb Company

Headword:
PVA/BRISTOL-MYERS

Relevant legal provisions:
EPC Art. 84, 54, 56

Keyword:
"Main request - added subject-matter (no)"
"Clarity (yes)"
"Novelty (yes)"
"Inventive step (yes)"

Decisions cited:
G 0001/03, T 0207/94, T 767/95, T 1074/00, T 1084/00, T 0090/03

Catchword:
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Beschwerdekammern | Boards of Appeal | Chambres de recours

Case Number: T 0029/05 - 3.3.08

D E C I S I O N
of the Technical Board of Appeal 3.3.08
of 13 March 2006

Appellant: Bristol-Myers Squibb Company
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Decision under appeal: **Decision of the Examining Division of the European Patent Office posted 25 June 2004 refusing European application No. 95120437.9 pursuant to Article 97(1) EPC.**

Composition of the Board:

Chairman: L. Galligani
Members: P. Julià
C. Rennie-Smith

Summary of Facts and Submissions

I. A request for grant of a European patent was filed on 22 December 1995. The application was given the European application number 95 120 437.9 and was published as EP-A2-0 718 402 on 26 June 1996 with the title "Penicillin V amidohydrolase gene from fusarium oxysporum". The application comprised 28 claims, wherein claim 1 read as follows:

"1. An isolated nucleic acid molecule having a sequence coding for the amino acid sequence of SEQ. ID. NOS.: 19 or 22; an isolated nucleic acid molecule having a sequence complementary to a nucleic acid sequence coding for said amino acid sequence; and an isolated nucleic acid molecule having a sequence capable of hybridizing to a nucleic acid having a sequence complementary to a nucleic acid sequence coding for said amino acid sequence."

II. The European Search Report was completed on 21 October 1997 and published as an A3 document on 17 December 1997.

III. In accordance with Article 94(2) EPC, the applicant requested by its letter dated 27 February 1998 the examination of the application and paid the official examination fee.

IV. The examining division issued a first official communication on 19 December 2000, wherein novelty was acknowledged but, on the basis of the first two documents cited in the European Search Report,

objections were raised against inventive step (cf. point XXI *infra*).

V. The applicant replied to these objections by its letter of 24 August 2001. Observations were made therein as to the relevance and deficiencies in the prior art cited by the examining division as well as to several technical problems which, allegedly, would have been encountered by the skilled person when attempting to follow the approach of the examining division. A document was filed supporting the applicant's arguments and new claims 22 to 29 were also filed to replace original claims 22 to 28.

VI. On 12 February 2002, the examining division issued a second official communication. Whereas the requirements of Article 123(2) EPC were considered to be met, the examining division raised several objections under Article 84 EPC and maintained the lack of inventive step objection with reference to the cited prior art and the common general knowledge of the skilled person.

VII. With its letter dated 6 December 2002, the applicant filed a new set of claims 1 to 29 and argued in favour of inventive step. Two documents cited in the European Search Report were put forward as further evidence in support of its arguments.

VIII. In an annex to the Summons to attend oral proceedings pursuant to Rule 71(1) EPC (issued on 13 March 2003), the examining division raised a clarity objection to the subject-matter of claim 1 (Article 84 EPC) and maintained the objection raised under Article 56 EPC in its second official communication.

IX. On 2 May 2003 and in preparation for the oral proceedings, the applicant filed auxiliary requests 1 and 2 and summarized its arguments in favour of inventive step. The applicant further noted that the examining division had provided no documentary evidence in rebuttal of the applicant's arguments in respect of inventive step.

X. In a communication dated 26 May 2003, the examining division acknowledged receipt of the auxiliary requests 1 and 2 and noted that the objections raised under Articles 84 and 56 EPC had not been overcome.

XI. At the oral proceedings before the examining division on 3 June 2003, the applicant filed an amended first and second auxiliary requests and provided further documents in support of inventive step. The examining division considered that the main request and the first auxiliary request did not fulfil the requirements of Article 56 EPC. The second auxiliary request (claims 1 to 10) was found to meet the requirements of the EPC and the applicant was informed that a patent could be granted on that basis.

XII. With its letter dated 2 July 2003, the applicant provided a clean copy of the second auxiliary request and a description adapted thereto.

XIII. A communication under Rule 54(1) EPC was issued on 20 October 2003 wherein the applicant was informed of the intention of the examining division to grant a patent on the basis of the documents indicated therein.

XIV. On 2 March 2004 the applicant indicated its disapproval of the documents intended for a grant and requested to grant a patent on the basis of the claims filed on 6 December 2002 (main request) or, alternatively, on the basis of the first auxiliary request filed with letter of 2 May 2003.

XV. A decision to refuse the application under Article 97(1) EPC was issued on 25 June 2004 by the examining division. The main request (claims 1 to 29) was held to lack both clarity and inventive step (Articles 84 and 56 EPC) and the first auxiliary request (claims 1 to 29) was held to lack inventive step (Article 56 EPC).

XVI. On 31 August 2004 the applicant (appellant) lodged an appeal against the decision of the examining division and paid the appeal fee. The statement of grounds of appeal was filed on 5 November 2004 with a main request and five auxiliary requests. The main request corresponded to the main request of the decision under appeal with minor corrections.

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

XVIII. On 21 December 2005, the board contacted appellant's representative by telephone and draw its attention to some editorial deficiencies in the main request.

XIX. The appellant filed a new main request on 2 March 2006 which took into account the board's comments. Claim 1 of this **main request** essentially corresponded to claim 1 of the main request refused by the examining

division in the decision under appeal and read as follows:

"1. An isolated nucleic acid molecule having a sequence coding for the amino acid sequence of SEQ. ID. NOS.: 19 or 22; or an
isolated nucleic acid molecule having a sequence complementary to a nucleic acid sequence coding for said amino acid sequence; or an isolated nucleic acid molecule of at least 20 nucleotides in length having a sequence capable of hybridizing under stringent conditions to a nucleic acid having a sequence complementary to a nucleic acid sequence coding for said amino acid sequence."

Claim 2 defined the nucleic acid molecule of claim 1 as a DNA molecule. Claim 3 was directed to an isolated DNA molecule having the nucleotide sequence of SEQ. ID. NOS.: 9, 10, 11, 14, 15, 16, 17, 18, 21, 23, 26, or 30, and claim 4 to an isolated polypeptide having the amino acid sequence of SEQ. ID. NOS.: 1, 2, 3, 4, 5, 6, 8, or 12. Claims 5 to 12 concerned expression vectors comprising a nucleic acid sequence coding for the amino acid sequence of SEQ. ID. NOS.: 19 or 22. Claims 13 to 18 concerned expression vectors comprising a promoter having the DNA sequence of SEQ. ID. NO.: 23. Claim 19 related to four specific expression vectors. Claims 20 to 23 were directed to host cells containing the expression vectors of claims 5 to 19 and claim 24 to a specific biologically pure culture of deposited Escherichia coli strains. Claims 25 to 28 related to a method for producing a polypeptide having the amino acid sequence of SEQ. ID. NOS.: 19 or 22 and claims 29 and 30 to a polypeptide having this amino acid sequence.

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

D1: D.A. Lowe et al., Biotechnology Letters, 1986, Vol. 8(3), pages 151 to 156;

D2: EP-A-0 302 473 (publication date: 8 February 1989).

XXI. The examining division's reasons on which the refusal of the application in suit was based may be summarized as follows:

Article 84 EPC

The requirement of "hybridizing under stringent conditions" had no functional restriction. Therefore, these conditions embraced subject-matter that had little, if anything, to do with the invention. As a consequence claim 1 was unclear and lacked support in the application.

Article 56 EPC

Document D1 was the closest prior art. This document disclosed the partial purification of the penicillin V amidohydrolase (PVA) from *F. oxysporum*. Starting from this prior art, the technical problem to be solved was seen in the isolation and sequencing of this PVA from *F. oxysporum*. Document D2 disclosed the use of pure PVA (isolated by the method of document D1) in a pharmaceutical context. Thus, the skilled person would try to optimise, if necessary at all, the method of document D1 using conventional techniques so as to

obtain the PVA protein and its amino acid sequence. The isolation and sequencing of the PVA protein was thus obvious. Likewise, the cloning of the PVA gene did not require any inventive faculty but merely the knowledge of cloning methods commonly used and known in the art.

The technical difficulties referred to by the applicant did not lower the expectation of success since well-known techniques and methods were at the disposal of the skilled person for overcoming them without requiring any inventive step. In particular, the (combination of) chromatographies used for purifying the PVA enzyme were mere routine methods which the skilled person could have easily derived without the need of inventive skill when confronted with the objective problem. As regards the possible problems encountered by the presence of a blocked N-terminus, reference was made to known N-terminal sequencing techniques as being widely used, well established and capable of overcoming that problem. As for the possible presence of heterogeneous termini (due to signal peptides), problematic amino acids at the N-terminus and O-glycosylation, the examining division considered that all these problems were only mere inconveniences that could be overcome in a variety of ways that were common practise and did not require any inventive skill.

XXII. The appellant's arguments in writing may be summarised as follows:

Article 84 EPC

Claim 1 specified the hybridizing conditions as well as the length of the hybridizing nucleotide sequences. By

defining a minimum length, there were no doubts that all partial sequences (which had to hybridize under stringent conditions to the complementary strand of the coding sequence defined in claim 1) were in fact related to the invention. As shown in the application, a probe of 20 nucleotides was successfully applied to the identification of PVA cDNA clones. This provided experimental evidence of the specificity of these short probes as well as of their relationship to the invention. The specific stringency conditions were not required to be identified in the claim since a skilled reader could deduce them from the application itself.

Article 56 EPC

Except for document D1, none of the other documents on file related to fungal PVA nor were any references therein to the production of recombinant fungal PVA. Document D1 merely disclosed a method for partial purification of the PVA from *F. oxysporum* which comprised a combination of precipitation steps followed by dialysis and lyophilization. This method was developed around processes that could be scaled-up for large scale PVA isolation. For analytical purposes only, document D1 referred to an exclusion chromatography. Starting from document D1 as the closest prior art, the problem to be solved was the provision of purified PVA, the corresponding sequence information and means for recombinant production. Evidence was provided (IEF gel electrophoresis) showing that the PVA preparation of document D1 was heterogeneous and that it did not have a purity sufficient for amino acid sequencing. This preparation was of about 50% purity, whereas a purity of at least 80% was required for obtaining reliable

sequence information by micro-sequencing. Document D2 referred to the PVA isolated from document D1 but nowhere in that document was it stated that the PVA had to be further purified to homogeneity for obtaining amino acid sequence information. Document D2 failed to provide any incentive to the skilled person.

None of the cited documents taught the skilled person how to perform an efficient separation of the complex protein mixture (with unknown proteins and activities) disclosed in document D1. A specific combination of chromatography steps (ion exchange DE-52 cellulose and size exclusion Biogel P-100) was required for resolving this complex protein mixture and for providing pure PVA of sufficient homogeneity. In fact, neither D1 nor D2 pointed the skilled reader to the complex heterogeneity of the protein mixture obtained in document D1. The skilled reader had no reasonable expectation of success when trying to purify this PVA preparation.

Moreover, none of the cited documents dealt with the recombinant production of fungal PVA. In fact, there was an extremely low level of knowledge as regards fungal PVA gene and protein regulation. This knowledge, however, was necessary for assisting a skilled person to arrive at the invention with a reasonable expectation of success. Even if the amino acid sequence of the PVA enzyme had been available, the nucleotide sequences encoding PVA or pre-PVA were not automatically achievable. Evidence was on file showing that a full-length DNA clone encoding mature PVA was not accessible from a cDNA library, and even when made accessible it did not express active PVA in E. coli or in yeast host cells. The provision of suitable

expression vectors, in particular of an expression vector useful for the expression of fungal PVA in a fungal host, was regarded as inventive too.

In summary, document D1 only provided a protein mixture with complex (micro)heterogeneity as shown by IEF electrophoresis. No purification strategy for separating this complex protein mixture was directly and unambiguously derivable from the prior art and no amino acid sequence was available for fungal PVA. Full-length cDNA was not obtainable as shown in Example 2 of the application and no expression of functional PVA was observed in *E. coli*, yeast or homologous fungi. Moreover, the N-terminal amino acid sequence of PVA was unsuitable for generating probes and there was no indication, let alone evidence, in the art that an additional 25 amino acid signal peptide was required for the correct expression of functional PVA.

XXIII. The appellant requested that the decision under appeal be set aside and that a patent be granted on the basis of the main request filed on 2 March 2006 or, alternatively, of one of the auxiliary requests filed with the grounds of appeal.

Reasons for the Decision

Article 123(2) EPC

1. No objections have been raised by the examining division under this article in the decision under appeal and the board sees no reason to differ. The claimed subject-matter has a formal basis in the claims

as filed as well as in the original description, where reference is made to the definition of "stringent conditions" and to nucleotide sequences of at least about 20 nucleotides as preferred sequences (cf. page 2, lines 55 to 58 of the application as published). Thus, the requirements of Article 123(2) EPC are fulfilled.

Article 84 EPC

2. According to the established case law of the Boards of Appeal, the requirement of clarity is fulfilled in a claim to a product if the characteristics of the product are specified by parameters related to the physical structure of the product, provided that those parameters are clearly and reliably determined by objective procedures which are usual in the art ("Case Law of the Boards of Appeal of the EPO", 4th edition 2001, II.A.6.1 and II.B.1.1, pages 154 and 157).

3. Claim 1 refers to "*a sequence capable of hybridizing under stringent conditions*" to a certain nucleic acid sequence (cf. point XIX *supra*). In relation to these conditions, the description refers to "stringent conditions" as meaning conditions no less stringent than the ones described in the "Detailed Examples of Preferred Embodiments" section (cf. page 2, lines 55 to 56 of the application as published). In point 5 of that section ("DNA Dot-Blot Hybridization"), hybridization conditions are disclosed when using a GT membrane or a nitrocellulose membrane (cf. page 11, lines 17 to 33 of the application as published). At the very beginning of this section, reference is made to a general handbook of molecular cloning (J. Sambrook et al., 1988, 2nd edition, Cold Spring Harbor Laboratory, CSH,

New York), which comprises some sections dealing with the effects of length and degeneracy of oligonucleotides on the specificity of hybridization and with the effects of modifying various physical and chemical parameters on the hybridization of oligonucleotide probes. Typical temperature ranges and salt concentrations are defined therein for stringent hybridization of probes of different length and compositions. Thus, although different experimental protocols may be applied for assessing hybridization under these conditions, they are usual in the art (cf. T 1084/00 of 11 April 2003, point 9.2 of the Reasons).

4. In fact, due to the existence of these different experimental protocols and to the possible variation in the length and composition of the nucleic acid sequences to be hybridized, it is normal patent practice to combine this (hybridization) feature with further functional features relating to the biological activity or with other structural features so as to exclude short nucleic acid sequences unrelated to the invention (cf. T 1074/00 of 13 May 2004, point 9 of the Reasons). There is no functional feature in claim 1 of the present request. However, the minimum length of the sequence to be hybridized is explicitly defined in claim 1, namely "*at least 20 nucleotides in length*", i.e. encoding at least about 6-7 amino acids which is about the length of a linear antigenic determinant (epitope) and which, according to the handbook on molecular cloning cited in the application, corresponds to the length of the short probes (17-20 nucleotides) normally used in hybridization studies. This is also the length of the specific fragments referred to in

claims 3 and 4 of the present request for isolated DNA molecules and isolated polypeptides, respectively.

5. In spite of this additional structural limitation, the decision under appeal refers to claim 1 as embracing "*subject-matter that has little if anything to do with the invention*". The existence of this unrelated subject-matter, however, is merely hypothetical and no evidence has been provided by the examining division to support its possible presence in the prior art, such as for instance the presence of domains in the PVA enzyme with a homology which is significant or high compared to other unrelated proteins, or the presence of domains required for certain - functional or structural - features or properties of the PVA enzyme (cellular location, (co)substrate binding, conformational structure, etc.) that might also be shared by other unrelated proteins, etc. In the absence of such evidence, each and every nucleic acid molecule - of at least 20 nucleotides in length - hybridizing under stringent conditions to a nucleic acid having a sequence complementary to a nucleic acid coding for the amino acid sequence of SEQ. ID. NOS.: 19 or 22 must be assumed to be related to the subject-matter disclosed in the application. The fact that a large number of possible nucleic acid sequences might fall within the scope of claim 1 is not a reason for raising a lack of clarity objection (cf. "Case Law", *supra*, II.B.1.1.3 and II.B.1.2.2, pages 159 and 162, respectively).

6. It follows from the foregoing considerations, and in accordance with the established case law of the Boards of Appeal which requires a balance to be struck between the interest of the applicant in obtaining adequate

protection and the interest of the public in determining the scope of protection with reasonable effort (cf. G 1/03, OJ EPO 2004, 413, point 3 of the Reasons), that the claimed subject-matter fulfils the requirements of Article 84 EPC.

Article 54 EPC

7. The provision of a purified homogeneous protein preparation, i.e. in a degree of purity that allows the determination of its amino acid sequence, is considered by the Boards of Appeal to justify the novelty of the pure protein preparation over a semi-purified protein mixture which contains the pure protein (cf. *inter alia* T 767/95 of 5 September 2000, point 6 of the Reasons and T 90/03 of 17 March 2005, points 12 to 15 of the Reasons).

8. In the present case, there is no doubt that document D1 discloses only a semi-purified PVA preparation. Evidence is also on file showing the presence of contaminant proteins in this PVA preparation. Based thereon it has been further argued that the PVA preparation disclosed in document D1 may only be of about 50% purity. This has not been disputed by the examining division which has not raised any objection against the novelty of the claimed subject-matter. Nor does the board, in the light of the documents on file, see any reason to raise such an objection.

9. Thus, the requirements of Article 54 EPC are fulfilled.

Article 56 EPC

10. In assessing inventive step the Boards of Appeal apply the "problem-solution" approach, which essentially involves identifying the closest prior art, determining in the light thereof the technical problem which the claimed invention addresses and successfully solves, and examining whether or not the claimed solution to this problem is obvious to the skilled person (cf. "Case Law", *supra*, I.D.2., 101). In those cases where the suggested approach or solution would have been obvious for the skilled person to try, then it still has to be assessed whether there was a reasonable expectation of success and whether this expectation was put in jeopardy by real difficulties, i.e. based upon technical facts (cf. "Case Law", *supra*, I.D.6.2, 117 and *inter alia* T 207/94, OJ EPO 1999, 273).

11. Document D1 has been identified as the closest prior art. This document discloses the extraction and partial purification of soluble PVA from *Fusarium oxysporum* by some centrifugation, precipitation and dialysis steps (cf. page 153, Table 1). Two PVA samples taken at different stages of the purification process are subjected to size exclusion chromatography for determination of the apparent molecular weight and quaternary structure (cf. page 154, fourth paragraph). Document D1 always refers to the PVA preparation as being only partially purified and the purification process is also acknowledged to be "*developed around processes that could be scaled-up for large scale enzyme isolation*" (cf. page 154, third paragraph).

12. Starting from this closest prior art, the problem to be solved may be defined as the provision of large amounts of isolated PVA. The solution provided in the application is the production of recombinant PVA and the provision of the products required therefor, i.e. isolated PVA polypeptide and nucleic acid molecules encoding the PVA, expression vectors, etc.

13. At the priority date, it was a matter of common general knowledge - as shown by *inter alia* the handbook cited in the application itself (J. Sambrook et al., 1988, *supra*) - that the best route to produce a protein in large quantities was the recombinant route. Thus, the approach followed in the application, i.e. the isolation of homogeneous PVA and cloning of the corresponding PVA gene, was obvious to try. The question remains whether it would be achievable with a reasonable expectation of success and whether the skilled person would have been confronted with real technical difficulties when following this approach (cf. point 10 *supra*).

14. In fact, appellant's argument in favour of inventive step is based on the presence of these (alleged) difficulties, which according to appellant's submissions may be summarized as i) difficulties in the selection of a purification strategy - a specific combination of chromatography steps - for obtaining homogeneous PVA free of (micro)heterogeneous contaminant proteins, i.e. sufficiently pure for direct sequencing, ii) difficulties in sequencing pure PVA (presence of N-terminus heterogeneity and of a blocked N-terminus, presence of amino acids difficult to analyse or of modified amino acids, as for example,

glycosylated amino acids) and iii) problems in cloning the PVA gene - difficulties in obtaining a full-length DNA clone encoding the pre-PVA gene and in the determination of the sequence encoding the mature PVA, in the selection of expression vectors and hosts for producing enzymatically active PVA, etc. (cf. point XXII *supra*).

15. In response to these arguments, the examining division referred to the common general knowledge of the skilled person and to the techniques and methods that were routine and well-known in the art (cf. point XXI *supra*). However, no evidence was provided by the examining division for substantiating this allegation. There is no document on file showing whether the specific combination of chromatographies referred to by the appellant had been used in the purification of other fungal proteins or, alternatively, whether similar and/or comparable combinations were at the disposal of the skilled person and known, or at least expected, to provide the same results, i.e. a homogeneously pure protein from a fungal strain. Nor is any information on file as regards the alleged problems (let alone possible solutions) on the purification and sequencing of fungal proteins having glycosylated isoforms (glycoforms) and/or signal sequences that might contribute to the (micro)heterogeneity of the obtained preparations. More strikingly, none of the documents cited by the examining division relates to the cloning of a fungal gene, not to mention a gene from *Fusarium*. None of these documents thus reflects the common general knowledge of the skilled person in the field of recombinant production of fungal proteins (gene expression systems, vectors and hosts, transformation

systems, etc.) at the priority date of the present application, namely 23 December 1994.

16. Nevertheless, it is well established by the jurisprudence of the Boards of Appeal that substantiation of an allegation that something is common general knowledge is required when this is challenged by a party (cf. "Case Law", *supra*, I.D.5.3, 114). This is the situation arising in the present case as rightly noted by the appellant in its letter of 2 May 2003 in preparation of the oral proceedings before the examining division. In this letter, after summarizing the "*numerous well documented difficulties in the art*", the appellant refers to the absence of any written evidence in support of the allegations of the examining division (cf. point IX *supra*). The reasoning of the examining division cannot be accepted by the board in the absence of any appropriate substantiation or evidential support for the challenged common general knowledge.

17. For the sake of completeness, the board also observes that no such evidence of what was common general knowledge can be derived from any of the documents cited in the European Search Report (ESR). Those documents are mainly concerned with the production of recombinant PVA derived from bacterial sources, such as *Bacillus sphaericus*, *Alcaligenes faecalis* and *Escherichia coli*. Although two documents are referred to in the ESR as defining the general state of the art or the technical background of the invention (category of the documents "A"), they are completely inappropriate for supporting the arguments of the examining division based on common general knowledge

(cf. point 15 *supra*). Nor is any such evidence provided by the application itself, which refers to a handbook for cloning techniques in general and to particular scientific publications concerned with transformation systems and selectable markers for very specific fungi (cf. page 10, lines 1 to 5 and page 9, lines 34 and 37 of the application as published, respectively).

18. Accordingly, in view of the number and nature of the assumptions made by the examining division, the board sees itself unable to uphold the decision of the examining division.

19. Under these circumstances, the board accepts the appellant's arguments on inventive step. Thus, the main request is considered to fulfil the requirements of Article 56 EPC.

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 claims 1 to 30 filed on 2 March 2006 and a description to be adapted thereto.

The Registrar: The Chairman:

A. Wolinski L. Galligani