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D E C I S I O N
of 11 November 1999

Case Number: T 0430/96 - 3.3.4

Application Number: 85308827.6

Publication Number: 0184438

IPC: C12N 15/80

Language of the proceedings: EN

Title of invention:

Transforming aspergillus niger, and plasmids for use therein

Patentee:

Gist-Brocades N.V.

Opponent:

Röhm GmbH
Novo Nordisk A/S

Headword:

Aspergillus/GIST-BROCADES N.V.

Relevant legal provisions:

EPC Art. 54, 56

Keyword:

"Main request - novelty (no)"
"Auxiliary requests - inventive step (no)"

Decisions cited:

-

Catchword:

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Boards of Appeal

Chambres de recours

Case Number: T 0430/96 - 3.3.4

D E C I S I O N
of the Technical Board of Appeal 3.3.4
of 11 November 1999

Appellant: Novo Nordisk A/S
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Decision under appeal: Decision of the Opposition Division of the
European Patent Office posted 20 February 1996
rejecting the opposition filed against European
patent No. 0 184 438 pursuant to Article 102(2)
EPC.

Composition of the Board:

Chairman: U. M. Kinkeldey
Members: F. L. Davison-Brunel
C. Holtz

Summary of Facts and Submissions

I. The patent in suit was granted with 15 claims.

Claims 1, 13 and 14 read as follows:

"1. A process for preparing transformants of the fungal species Aspergillus niger, which comprises treating an A.niger strain lacking a selectable marker with a DNA vector containing said selectable marker and other DNA sequences, under conditions permitting at least some of the A.niger cells to take up the DNA vector."

"13. Transformants of the fungal species Aspergillus niger which contain foreign DNA conferring modified properties of expression on the Aspergillus niger, and having a rapidly selectable phenotype permitting them to be readily differentiated from the original Aspergillus niger."

"14. Transformed Aspergillus niger according to claim 13, comprising DNA foreign to wild-type Aspergillus niger."

II. Notices of opposition were filed against the patent in suit by four parties. Revocation of the patent was requested on the grounds of Article 100(a)(lack of novelty and of inventive step) and Article 100(b) EPC (lack of sufficient disclosure).

III. By a decision within the meaning of Article 106(3) EPC, the opposition division maintained the patent as granted according to Article 102(2) EPC.

IV. Opponents 2 to 4 appealed. Opponents 2 and 4 later

withdrew their oppositions.

- V. Oral proceedings were held and three auxiliary requests were introduced for consideration by the Board. In the three auxiliary requests, claim 1 remained as granted. Claim 13 of the first auxiliary request read as follows:

"13. Transformants of the fungal species Aspergillus niger which are transformed by recombinant DNA containing foreign DNA conferring modified properties of expression on the Aspergillus niger, and having a rapidly selectable phenotype permitting them to be readily differentiated from the original Aspergillus niger."

- VI. The following documents are referred to in this decision:

- (3) Ballance D. J. et al., Biochem. and Biophys. Res. Comm. Vol. 112, No. 1, pages 284 to 289, 1983,
- (25) Sen K. et al., J. Gen. Microbiol. Vol. 55, pages 195 to 200, 1969,
- (27) Boel E. et al., Abstract P26 from EMBO workshop from 17 to 19 April 1984,
- (28) Kos A. et al., Abstract P33 from EMBO workshop from 17 to 19 April 1984,
- (30) EP-A-0 191 221,

(34) Lasure L. and J. W. Bennett, Gene Manipulations in fungi, pages 531 to 534, 1985, Academic Press, Inc.,

(39) Goosen T. et al., Mol.Gen.Genet Vol. 219, pages 282 to 288, 1989.

VII. The submissions in writing and during oral proceedings by the Appellants (Opponents 3) and the parties as of right (Opponents 1, 2 and 4) can be summarized as follows:

Novelty (Article 54 EPC)

Main request: claim 13

The subject-matter of claim 13 lacked novelty over the teachings of document (25). This document described a process whereby a mutant strain of A.niger was treated with fragmented genomic DNA from wild type A.niger and transformants were, thus, obtained which were readily distinguishable from the original mutant strain in that they exhibited the wild-type phenotype. These transformants were to be considered as transformants of the fungal species A.niger within the meaning of claim 13 because the definition of foreign DNA according to the patent in suit comprised wild-type A.niger DNA.

Auxiliary request 1: claims 1 and 13

The amendment to the wording of claim 13 from "Transformants...which contain foreign DNA..." to "Transformants...which are transformed by recombinant DNA containing foreign DNA..." did not help in

establishing novelty over document (25) because the latter type of transformants could not be distinguished from the earlier type.

Document (30) (page 12) described a process for transforming A.niger with a vector comprising a replicon and a selectable marker. This process was identical to the process of claim 1. Document (30) was, thus, novelty destroying for the subject-matter of said claim. Furthermore, as the direct product of said process would necessarily be transformants with the features given in claim 13, document (30) was also novelty destroying for claim 13.

Inventive step (Article 56 EPC):

Claim 1 in all requests

The closest prior art was document (3) which described the transformation of A.nidulans with a gene from Neurospora crassa by a process, the features of which were essentially identical to those of the process of claim 1, the only difference being in the use of fungal recipient cells of a different species. The authors stated that this "experience gained in A.nidulans transformation will facilitate the extension of this technique to the industrially important Aspergillus niger." It was, thus, obvious to apply the same procedure to A.niger as had worked with A.nidulans. Furthermore, the skilled person would have had a reasonable expectation of success that such a process could be carried out seeing that document (25) taught that it had been possible to transform conidia of A.niger, ie that DNA was able to cross the cell

membrane. It was thus, all the more evident that DNA would enter protoplasts (ie cells having lost this membrane) as used in the method of document (3).

XI. The submission by the Respondents can be summarized as follows:

Novelty (Article 54 EPC)

Main request, claim 13

Claim 13 was novel over the teachings of document (25) because these teachings would have been disregarded as being wrong. Indeed, no DNA would be expected to enter conidia which had an impermeable cellular membrane. Furthermore, the frequency of transformation obtained was inordinately high as could be seen in Table 1 where as many as 162 cho⁺ transformants were obtained for a concentration of 25 µg/ml of sheared genomic DNA. The person skilled in the art would have been aware that these had to be contaminants. In this context, the declaration by Prof. Scazzocchio dated 20 December 1995 was important as he stated that the author of document (25) was unable to reproduce the transformation experiment in A.nidulans when working in his laboratory. It was also of relevance that document (25) was never referred to in the scientific literature and, finally, there was also the fact that scientists would not have gone to the trouble of preparing spheroplasts, had it been possible to transform conidia.

Auxiliary request, claims 1 and 13

It was possible to distinguish a "transformant containing foreign DNA" from a "transformant containing

a recombinant DNA containing foreign DNA" as the presence of recombinant DNA other than foreign DNA within the genome of the latter could be probed for. Thus, claim 13 was novel over the teachings of document (25) which disclosed transformants which only contained foreign DNA.

Document (30) claimed a priority date earlier than that of the patent in suit and was, thus, relevant to novelty under Article 54(3)(4) EPC. Yet, the Appellants had not provided the corresponding priority application. Thus, document (30) had to be disregarded because priority rights could not be acknowledged in the absence of any evidence as to the content of the priority application. And, besides, the skilled person reading document (30) at the priority date would not have been enabled to carry out a transformation with the only vector therein described, carrying the trpC marker, seeing that no A.niger trpC mutants were available at the time.

Inventive step

Claim 1 in all requests

The closest prior art was document (3) which dealt with the transformation of another *Aspergillus* species, A.nidulans. The fact that it was stated at the end of the document that "the experience gained in *A.nidulans* transformation would facilitate the extension of the technique to *Apergillus niger*" at the very best indicated to the skilled person a protocol for transformation. Yet, everything remained to be done, in particular:

- (a) a vector containing an appropriate marker had to be constructed,
- (b) a corresponding A.niger mutant strain had to be isolated and,
- (c) a transformation protocol specific for A.niger had to be set up.

The skilled person would have had no reasonable expectation of success with regard to any of these steps.

The patent provided a teaching which opened the door to further developments in the handling of the industrially important strain A.niger.

XII. The Appellants requested that the decision under appeal be set aside and that the European patent No. 0 184 438 be revoked.

The Respondents requested that the appeal be dismissed, alternatively, that the decision under appeal be set aside and the patent be maintained on the basis of either of auxiliary requests I, II or III as submitted in the oral proceedings.

Reasons for the Decision

1. The appeal is admissible.

Main request, claim 13

Novelty (Article 54 EPC).

2. Document (25) was argued to be novelty-destroying for the subject-matter of claim 13. It discloses the transformation of nutritionally deficient mutants of A.niger by fragments of wild-type A.niger DNA. The transformants obtained are able to grow on a medium lacking the nutrient, which the mutant A.niger host cells needed for growth. They are, thus, transformants within the meaning of claim 13 if the wild-type A.niger DNA is to be considered as a DNA foreign to the mutant A.niger host strain.

3. The description of the patent in suit does not provide any definition of the term "foreign DNA". Yet, it is possible to understand which kind of DNA is meant by reading claim 14. This claim is addressed to transformed A.niger **comprising** DNA which is foreign to wild-type A.niger. This wording necessarily implies that under the expression "foreign DNA", DNA is also comprised which is **not** foreign to wild-type A.niger ie A.niger DNA. This interpretation is confirmed by the statement on page 4, lines 16 and 17: "Whilst it is preferred...to utilize a selectable marker which is natural to wild-type A.niger...". Thus, as wild-type A.niger DNA is to be regarded as a DNA foreign to mutant A.niger strains, claim 13 covers transformants of mutant A.niger strains which contain wild type A.niger DNA. Therefore, all of the features of the claimed transformants are properties of the transformants described in document (25).

4. It was also argued that document (25) would have been

- disregarded by the skilled person who would not have believed that conidia of A.niger could be penetrated by DNA, nor that such a high frequency of transformation could be obtained.
5. The Board notices that the frequency of transformation was considered by the authors of document (25) to be low (page 195, summary) and also that they were aware of the possibility that the transformants may in fact be spontaneous revertants or contaminants. Indeed, they took great pain to show a direct link between the appearance of transformants and the addition of DNA to the conidia. For example, it was shown that the frequency of spontaneous reverse mutations was below 10^{-8} (10^5 times lower than the frequency of transformants; page 196). Controls were run to ascertain that any treatment which destroyed the DNA resulted in a decrease in the number of transformants whereas an increase in DNA concentration increased this number (pages 197 to 200).
 6. The Board would accept that these results could be challenged by a repeat of the experiments providing evidence for the Respondents' position. Yet, there are no such data on file. The declaration by Prof. Scazzocchio that no transformants could be obtained by the method of document (25) was not in relation to transforming A.nidulans but rather in relation to A.niger and Prof. Scazzocchio himself does not eliminate the hypothesis that "A.niger and A.nidulans are quite different regarding the transformation techniques".
 7. As for the facts that document (25) was never made

reference to in the scientific literature and that scientists would not go to the trouble of making spheroplasts of A.niger, had it been possible to transform conidia directly, the Board is unable to give them such a significance that document (25) cannot be taken into account in the assessment of novelty. Firstly, there might be many different reasons why a scientific report is not mentioned in the later scientific literature. Secondly, scientists may have gone to the trouble of making spheroplasts for example because they are more easily transformed (patent in suit, 300 argB⁺ transformants) than conidia (document (25), 35 arg⁺ transformants).

8. In view of this, the Board comes to the conclusion that document (25) is novelty-destroying for the subject-matter of claim 13. The main request is, thus, rejected for lack of novelty.

Auxiliary request 1

Formal requirements (Articles 84 and 123 EPC)

9. Claim 13 has been amended to a claim to "Transformants...which are transformed by recombinant DNA containing foreign DNA...". The basis for the term "recombinant DNA" is found in the patent as filed on page 9. The recombinant DNA therein described contains foreign DNA as it is made of E.coli DNA (vector part) and of A.nidulans DNA (selectable marker).
10. The amendment amounts to a restriction of the scope of the claim to a transforming DNA containing genetic information additional to the foreign DNA.

11. At the priority date, the skilled person would have had no difficulty in understanding the term "recombinant DNA" which is a commonly used term.
12. Accordingly, the requirements of Article 123(2)(3) EPC and Article 84 EPC are fulfilled.

Novelty (Article 54 EPC)

13. Document (25) does not disclose a process according to claim 1 nor does it disclose transformants according to claim 13 because the DNA which was transferred in the nutritionally deficient A.niger mutants was not recombined to any other DNA i.e. does not answer to the definition of a "DNA vector" or a "recombinant DNA" in the generally accepted meaning of these two expressions. In addition, the Board cannot accept the objection by the Appellants that the transformants according to document (25) would be undistinguishable from the claimed transformants but is rather convinced by the submission of the Respondents that at the priority date, it was already a matter of common knowledge to probe transformants for the DNA they contained. Thus, it would have been possible to distinguish between transformants containing only foreign DNA and transformants containing DNA sequences in addition to the foreign DNA. Document (25) does not affect the novelty of claim 1 nor of claim 13.
14. Document (30) was also cited as affecting the novelty of both claims 1 and 13 under Article 54(3)(4) EPC. It discloses a process for transforming ascomycetes exemplified with A.nidulans. A.niger is mentioned on page 3 and on page 12, it is stated: " The desirability

of, and the techniques involved in, this process can be best understood in the context of an **hypothetical** example. Certain industrial strains of Aspergillus niger are capable of synthesizing antibiotics by, for example, methylation of a particular organic nucleus. It may be desirable to broaden the specificity of this methylase so that additional substrates are capable of being utilized by this enzyme" (emphasis added). There are no further references made to A.niger in the document. In the Board's judgment, the expression of a desire cannot be taken as an enabling disclosure of the process of claim 1 or of the transformants of claim 13. Thus, novelty is not destroyed by document (30), quite independently from whether or not it enjoys valid priority rights.

15. The requirements of Article 54 EPC are fulfilled.

Inventive step (Article 56 EPC)

16. Document (3) discloses the successful transformation of an A.nidulans mutant requiring uridine to grow by a plasmid carrying the corresponding pyr4 gene of Neurospora crassa. Protoplasts of the A.nidulans mutant strain are put into contact with the transforming DNA, regenerated and the transformed cells are selected for their ability to grow in the absence of uridine. On page 288, it is stated: "In addition, it seems likely that experience gained in *A.nidulans* transformation will facilitate the extension of this technique to the industrially important *Aspergillus niger*". Thus, it is considered by the Board as the closest prior art document to the subject-matter of claim 1.

17. Staring from this closest prior art, the technical problem to be solved can be defined as setting up a transformation system for a different *Aspergillus* species: *Aspergillus niger*.

18. The solution provided is the process of claim 1, ie an *A.niger* strain lacking a selectable marker (*argB*⁻ in Example 1) is treated with a DNA vector containing a gene encoding said marker (*argB* gene from *A.nidulans* in Example 1) under conditions (protoplasts formation, contacting the transforming DNA to said protoplasts, regeneration) permitting some of the *A.niger* cells to take up DNA. The Board is satisfied that the claimed process solves the technical problem as Example 1 shows that transformants are, thus, recovered.

19. Since document (3) suggested that the transformation method it disclosed ought to be applied to *A.niger*, it was obvious to try this method. The question which remains to be decided is whether the skilled person might have had a reasonable expectation that it could be carried out to a successful end.

20. The Respondents have identified three steps in the method which, according to them, would have created such difficulties as to jeopardise a reasonable expectation of success:
 - the construction of the vector carrying a gene from another species, the expression of which in *A.niger* could serve as a means for selection: document (3) disclosed for example, that the *ura3* gene of yeast would not be expressed in *Neurospora crassa*.

- the isolation of the corresponding A.niger mutant host cells. Document (39) showed that trpC mutants of A.niger were difficult to isolate.

- the protocol of transformation set up for A.nidulans would not necessarily be expected to work in A.niger as both Aspergilli were taxonomically far apart, even belonging to different subdivisions of the fungi (document (39)).

21. With regard to the first of these steps, the Board notices that in the patent in suit, the isolation of the A.nidulans Arg gene is said to be achievable by known techniques (page 5, lines 14 and 15). Furthermore, although document (3) mentions the lack of expression of the yeast ura3 gene in N.crassa (page 288), it also discloses that the ura3 gene from N.crassa is expressed in yeast. Documents (27) and (28) disclose further examples of the expression of genes amongst different species: the A.niger Leu gene is expressed in E.coli and the A.niger trpC gene is expressed in A.nidulans.

22. With regard to the second of these steps, the Board again notices that in the patent in suit, the preparation of mutant A.niger strains was considered to be feasible by usual non-specific techniques (page 4, lines 10 to 13). The Respondents emphasize that according to document(39) (page 282, bottom of right-hand column) published in 1989, A.niger trpC mutants may not have been isolatable by enrichment after UV mutagenesis. But, as this was not known before the priority date, it would not have influenced the

perception the skilled person would have had of his/her chances to succeed. In addition, it is apparent from document (25) (page 195, Methods) that at the priority date, many mutants were already available in the metabolic pathways for the synthesis of such nutrients as amino-acids. Thus, there only remained to identify which genes of the pathway had been altered to obtain genetically defined mutants.

23. Taking into account these findings, the Board concludes that only routine steps known by the skilled person were required to prepare the tools for the transformation protocol for A.niger knowing that for A.nidulans described in document (3).

24. It is accepted that a favourable issue may not necessarily have been **predicted** for the performance of the transformation protocol with these tools. Yet, prediction of success is not the standard for the establishment of inventive step but rather a reasonable expectation of success. In the Board's judgment, the skilled person aware of the teaching of document (3) would have been strongly encouraged to try the transformation protocol by applying routine steps.

25. As no alterations of the protocol of document (3) were necessary to make it effective in A.niger, inventive step can also not be justified by the solving of unexpected difficulties while carrying out the invention. Accordingly, the subject-matter of claim 1 is found non-inventive.

26. These findings are based on the same approach leading to the finding of lack of inventive step in earlier

decisions by the Boards of appeal such as T 386/94 (OJ EPO 1996, 658) and T 207/94 (OJ EPO 1999, 273). In T 386/94, for example, the then competent Board decided that inventive step was lacking because carrying out the claimed invention would have been perceived as an endeavour likely to succeed and achieving it did not pose such problems as to prove that this assumption was wrong. In other appeal decisions such as T 923/92 (OJ EPO 1996, 564), or T 223/92 (dated 20 July 1993), inventive step was acknowledged because evidence existed of factual obstacles on the way to putting the invention into practice.

27. For these reasons, the Board decides to reject auxiliary request I as failing to fulfill the requirements of Article 56 EPC.

Other auxiliary requests

28. Claim 1 of auxiliary requests II and III is the same as claim 1 of auxiliary request I. Accordingly, said requests must equally be rejected for lack of inventive step.

Order

For these reasons it is decided that:

1. The decision under appeal is set aside.
2. The patent is revoked.

The Registrar:

The Chairwoman:

A. Townend

U. Kinkeldey