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D E C I S I O N
of 16 February 2006

Case Number: T 0275/04 - 3.3.08

Application Number: 89202106.4

Publication Number: 0357127

IPC: C12N 15/80

Language of the proceedings: EN

Title of invention:
Gene replacement as a tool for the construction of aspergillus strains

Patentee:
DSM IP Assets B.V.

Opponent:
AB Enzymes GmbH

Headword:
Aspergillus/DSM

Relevant legal provisions:
EPC Art. 83, 54, 56

Keyword:
"Sufficiency of disclosure - yes"
"Novelty - yes"
"Inventive step - yes"

Decisions cited:
T 0019/90, T 0793/93, T 0511/92, T 0464/94

Catchword:
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Beschwerdekammern | Boards of Appeal | Chambres de recours

Case Number: T 0275/04 - 3.3.08

D E C I S I O N
of the Technical Board of Appeal 3.3.08
of 16 February 2006

Appellant: (Opponent)
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Decision under appeal: **Decision of the Opposition Division of the European Patent Office posted 15 January 2004 rejecting the opposition filed against European patent No. 0357127 pursuant to Article 102(2) EPC.**

Composition of the Board:

Chairman: L. Galligani
Members: F. Davison-Brunel
M. B. Günzel

Summary of Facts and Submissions

I. European patent No. 0 357 127 with the title "Gene replacement as a tool for the construction of aspergillus strains" was granted with 15 claims for all designated Contracting States, based on European patent application No. 89 202 106.4.

Granted claim 1 read as follows:

"1. A transformed filamentous fungus host comprising an expression cassette originating by *in vitro* recombination and comprising a transcriptional initiation regulatory region, an open reading frame encoding a signal sequence for secretion in frame with a structural gene of interest and a transcriptional termination regulatory region,

the transformed filamentous fungus host being characterized in that the expression cassette is integrated in a chromosome of the filamentous fungus host at a predetermined target locus comprising a gene whose expression product is secreted to a concentration of at least about 0.1 g/l,

the gene of the target locus being further characterized in that it has been inactivated."

Dependent claims 2 to 8 related to further features of the transformed filamentous fungus of claim 1. Independent claim 9 related to a DNA construct comprising an heterologous gene expression and secretion cassette for insertion at a predetermined locus in the fungal chromosome. Dependent claims 10 to

14 related to further features of the DNA construct of claim 9. Claim 15 related to a method for producing a protein of interest comprising growing in a nutrient medium a transformed filamentous fungus host as defined in any one of claims 1 to 8.

II. An opposition was filed under Article 100(a) and (b) EPC for lack of novelty, lack of inventive step and lack of sufficient disclosure. It was rejected by the opposition division and the patent was maintained as granted.

III. The appellant (opponent) filed a notice of appeal, paid the appeal fee and submitted a statement of grounds of appeal.

IV. The respondent (patentee) submitted arguments in answer to the grounds of appeal.

V. The appellant answered to the respondent's submissions.

VI. The board sent a communication pursuant to Article 11(1) of the Rules of Procedure of the Boards of Appeal indicating its preliminary, non-binding opinion.

VII. The appellant and the respondent sent further submissions in answer to this communication. The respondent's submissions were accompanied by a main request and four auxiliary requests.

VIII. At oral proceedings, the respondent withdrew the main request. Patentability was thus assessed on the basis of the first auxiliary request (having then become the

new main request) which was identical to the claims as granted.

IX. The following documents are mentioned in the present decision:

(1) : EP-A- 0 244 234;

(2) : EP-A- 0 249 350;

(3) : Rambosek, J. and Leach, J., CRC Critical reviews in Biotechnology, Vol.6, No.4, pages 357 to 393, 1987;

X. The appellant's arguments in writing and at oral proceedings which are relevant to the present decision may be summarized as follows:

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

The object of the claimed invention was to facilitate the purification of a desired protein from a fermentation broth. There were doubts that this could be achieved as Table 1 of the patent in suit showed that strains transformed according to the invention only had a slightly lower secretion level in terms of total protein concentration than an untransformed strain. This result could not be taken as a proof that purification had been facilitated. As for Table 2, it showed that proteases produced by the fungal host would degrade the heterologous protein. As the claim covered all possible fungi, it could not be excluded that a particular fungus would produce so much proteases that

the foreign protein would, in fact, never be obtained in any sizeable quantity.

Article 54 EPC; novelty of claim 1

Document (1) disclosed a generic construct for achieving a high level of expression and secretion of a desired protein in filamentous fungi. The transformation of a fungal strain with such a construct led to its random integration in the fungal DNA. Thus, integration into the locus of a gene encoding a highly expressed and secreted protein was not excluded.

In accordance with the case law (T 793/93 of 27 September 1995), availability in the sense of Article 54 may be established if the inevitable outcome of what was literally or explicitly disclosed fell within the ambit of the claim. Since a transformed filamentous fungus host such as claimed in claim 1 was the inevitable outcome of what was literally disclosed in document (1), this document destroyed the novelty of said claim.

Alternatively, consideration should be given to the fact that document (1) also disclosed the targeted integration of an expression cassette to a predetermined locus on the fungal chromosome (Example 6). Admittedly, this targeting could not have led to the production of a foreign protein. Yet, nothing at all would prevent the skilled person to work according to the full teaching of document (1) comprising the expression of heterologous genes, targeted integration and, in claim 25, the teaching that the protein could be produced from a fungal host

strain which was deficient in a gene encoding an undesired product. In this manner, he/she would necessarily obtain a transformed fungal strain such as now claimed. Thus, claim 1 lacked novelty.

Article 56 EPC; inventive step of claim 1

The closest prior art was document (2) which was concerned with developing filamentous fungal expression systems. On page 2 of this document, an expression cassette was described which contained the same elements as the expression cassette in claim 1. On page 4, it was mentioned that the cassette could be joined to other sequences, in particular to a DNA sequence homologous with a DNA sequence in the host cell. This disclosure unambiguously pointed to a cassette which would integrate at a specific location in the chromosome of the filamentous fungus.

The objective problem to be solved was to develop an efficient production and secretion system in filamentous fungi (patent in suit, page 2, line 30, page 3, lines 48 to 50).

Document (3) was a review on recombinant DNA and filamentous fungi. When discussing improvement of industrial production strains, it disclosed on page 359 that: *"For example, it may be possible to use fungi for the production of heterologous proteins by redirecting the enzyme production and secretion capacity of these microorganisms."* This disclosure combined with that on page 4 of document (2) made it obvious to solve the above mentioned problem as was now claimed, namely by isolating a transformed filamentous fungal host strain

wherein a gene encoding a highly expressed protein was replaced with the gene encoding the desired protein. Alternatively, the teachings of document (3), which could be taken into account, were the disclosure just mentioned together with the information on page 370 that gene replacement could be achieved by homologous recombination. The combination of this overall teaching with that in document (2) also led to a conclusion of lack of inventive step.

Defining the problem as being the development of an alternative expression system did not change the findings of obviousness. Indeed at the priority date, there already existed very good expression systems and the one provided in the patent was not in any way advantageous compared to these earlier ones.

XI. The respondent's arguments in writing and at oral proceedings which are relevant to the present decision may be summarized as follows:

Article 83 EPC; sufficiency of disclosure in relation to the subject-matter of claim 1.

It had never been disputed that the claimed transformed fungal strain could be isolated without undue burden. In fact, the appellant's objections amounted to raising doubts that the exemplified strain and also those comprised within the scope of the claim would enable an efficient production of heterologous proteins. Even if these objections were taken into account within the framework of assessing sufficiency of disclosure in relation to obtaining the transformed fungal host, there remained that doubts were not the appropriate

standard for assessment of sufficiency of disclosure. What was needed was evidence substantiated by verifiable facts. For this reason, the appellant had failed to discharge their burden of proof that the requirement of Article 83 EPC was not complied with.

Article 54 EPC; novelty of claim 1

The appellant's first argument, namely that it could not be excluded that some of the transformed fungal hosts disclosed in document (1) would in fact carry the cassette at a locus comprising a gene encoding a highly secreted protein, did not fulfil the correct standard of proof to be achieved for the purpose of destroying novelty. While decision T 793/93 (supra) stated that availability in the sense of Article 54 may be established if the inevitable outcome of what was literally or explicitly disclosed fell within the ambit of the claim, it also established that inevitability precluded the existence of a credible or valid alternative outcome or choice, in other words was tantamount to 100% probability. Here, it was simply not credible that the multiple integration events of the heterologous gene in the fungal chromosome which occurred at random would always necessarily comprise an integration event in a locus comprising a gene encoding a highly secreted protein.

As for the second argument ie. that by working according to the full teaching of document (1), one would obtain a transformed fungal strain such as claimed, it was purely speculative and speculations were not sufficient to reach a conclusion of lack of novelty.

Article 56 EPC, inventive step of claim 1

Document (2) disclosed heterologous protein expression and secretion in transformed fungal hosts by integrating the expression cassette carrying the heterologous gene in multiple copies and at random in the fungal chromosome.

The problem to be solved could be defined as being the development of an alternative expression system.

The solution provided in claim 1 was a transformed fungal host wherein the expression cassette comprising the foreign gene was targeted to a locus on the fungal chromosome comprising a gene encoding a highly secreted protein, which protein was no more produced as a consequence.

There may have been a pointer in document (2), page 4, lines 3 and 4 to the possibility of targeted rather than random integration to produce a transformed fungal host. Yet, at no point did document (2) suggest that the insertion of the heterologous gene should lead to the replacement of a gene encoding a highly secreted protein. Document (3), page 370 taught gene replacement for the purpose of studying gene expression. On page 359, the very general statement was also made that it could be possible to use fungi for the production of heterologous proteins by redirecting the enzyme production and secretion capacity of the microorganisms. The combination of these two isolated pieces of information to arrive at the conclusion that document (3) suggested the replacement of a gene

encoding a secreted protein by the gene encoding the protein of interest as a mean to achieve heterologous protein production, could only be done with hindsight knowledge of the invention. This was all the more true of the combination of the suggestion in document (2) with the teachings on pages 359 and 370 of document (3).

For these reasons, the appellant had failed to demonstrate that isolating a transformed fungal strain such as claimed was obvious.

XII. The appellant requested that the decision under appeal be set aside and the patent be revoked.

As main request, the respondent requested that the appeal be dismissed. As auxiliary requests 1, 2 and 3, the respondent requested that the decision under appeal be set aside and the patent be maintained on the basis of one of the second, third or fourth auxiliary requests filed with the letter dated 16 January 2006.

Reasons for the decision

Main request; granted claims
Sufficiency of disclosure in relation to the subject-matter of claim 1

1. Claim 1 is directed to a product: a transformed filamentous fungus host. Example 1 shows how to construct by in vitro recombination a cassette to be inserted in said host comprising the heterologous gene flanked by the relevant regulatory sequences. Example 2

describes the transformation of a fungus (*Aspergillus niger)* by a vector carrying this cassette while Example 3 provides evidence of its integration at the intended locus on the chromosome together with evidence of the elimination of the gene encoding the highly secreted protein (glucoamylase) initially present at this locus. In Example 4, the secretion of the heterologous protein (bovine chymosin) is demonstrated. There is thus no doubt that the description provides the information necessary to carry out the invention with at least one fungus. In fact, it was not challenged that at the priority date, the cassette could be constructed nor that fungi in general could be transformed nor that targeted integration of the heterologous gene occurs by homologous recombination. The board, thus, concludes that the requirements of Article 83 EPC are fulfilled.

2. Arguments as to lack of sufficient disclosure were directed to the fact that purifying the heterologous protein from the growth medium of the claimed transformed fungus would not be easier than purifying it from the growth medium of a fungus which still produced the highly secreted protein. Doubts were also expressed that sizable amounts of the heterologous protein could be recovered in the growth medium of all fungi comprised within the scope of the claim.

3. These arguments relate to a "virtual" claim to the use of the transformed fungus host rather than to its isolation. And, beside, they do not fulfil the standard which would make them relevant to the assessment of sufficiency of disclosure. In accordance with the case law (T 19/90, OJ EPO 1990, 476, point 3.3 of the

decision), *"the mere fact that a claim is broad is not in itself a ground for considering the application as not complying with the requirement for sufficiency of disclosure under Article 83 EPC. Only if there are serious doubts, substantiated by verifiable facts, may an application be objected to for lack of sufficient disclosure."* Here, the appellant failed to substantiate the doubts which they expressed by any verifiable facts.

Article 54 EPC; novelty of claim 1

4. Document (1) is a patent document which was argued to be detrimental to the novelty of the subject-matter of claim 1. It discloses a transformed filamentous fungus which expresses and secretes a heterologous protein in the culture medium (page 5 and Example 10). The method used to obtain the transformants involves **random integration** in the fungal chromosome of **multiple copies** of the cassette carrying an heterologous **functional** gene (Example 3).

5. A second teaching is contained in document (1): that of a transformed filamentous fungal host wherein a wild-type gene has been mutated by insertion at its locus of a defective allele (page 8 and Example 6). The method used to obtain this mutant involves the **targeted integration** in the fungal chromosome of **one copy** of the **defective** allele, by homologous recombination between the wild-type gene and what is left of it in the defective allele.

6. Document (1), however, does not disclose a transformed fungal host for expressing and secreting a heterologous protein wherein the heterologous gene is inserted at a

specific locus in the chromosome by targeted integration, let alone that the specific locus should be that of a gene encoding a highly secreted protein.

7. It was pointed out by the appellant that the inevitable outcome of random and multiple integrations of the expression cassette in the fungal chromosome as described in document (1) (point 4, supra) was that some transformants would have inserted the heterologous gene at the locus of a gene encoding a highly secreted protein ie. the teachings of document (1) inevitably led to a transformed fungal strain falling within the scope of claim 1. In its view, this fact seen in the light of the following sentence in the first paragraph, point 2.1 of decision T 793/93 (supra) dealing with "General legal observations" on the issue of novelty:

"In the case where a prior art document fails explicitly to disclose something falling within a claim, availability in the sense of Article 54 may still be established if the inevitable outcome of what is literally or explicitly disclosed falls within the ambit of that claim."

led to a conclusion of lack of novelty.

8. The board is definitely not convinced by this argument which seems to be founded on an incomplete reading of point 2.1 of said decision. Indeed, in the third paragraph, the term inevitable is defined:

" ... the term "inevitable" means unavoidable, sure to happen, something that is bound to occur or appear, so true to nature as to preclude alternatives or solutions

(see Concise Oxford Dictionary). It is therefore self-evident that inevitability precludes the existence of a credible or valid alternative outcome or choice: in other words, it is tantamount to 100% probability."

9. It is clearly not the case that the **random** integration of the expression cassette into the fungal chromosome, even if comprising multiple events, will **always** lead to transformants which will **all** have the cassette inserted at the locus of a gene encoding a highly secreted protein, and this comes from the very notion of randomisation which in this context means "anywhere in the chromosome". Otherwise stated, some transformants will have integrated the cassette somewhere else than at the above mentioned locus. Thus, the method of document (1) will produce alternative transformants to those falling within the scope of claim 1 and obtaining a transformed fungus host as now claimed is not the inevitable outcome - as understood in the case law - of carrying said method.

10. It was also argued that by working in accordance with the full teaching of document (1) ie. by combining the disclosures respectively relating to heterologous protein expression (point 4, supra) and to mutagenesis by targeted integration (point 5, supra) and possibly also with the subject-matter of claim 25 of this document which relates to a method of heterologous gene expression in a transformed strain deficient in any gene encoding an undesired product, the skilled person would necessarily isolate a transformed fungus strain such as now claimed in claim 1.

11. This line of arguments cannot be followed as it fails to comply with the principles well-established in the case law that for an invention to lack novelty, its subject-matter must be clearly and directly derivable from the prior art (eg. T 511/92 of 27 Mai 1993) and that speculations as to what may be done on the basis of the teaching of a prior art document do not fulfil the standard of proof to be applied when assessing novelty (eg. T 464/94 of 21 Mai 1997). The above mentioned combination is nowhere described in document (1) nor is it suggested, and the assertion that this combination would necessarily lead to a transformed strain such as claimed is fully hypothetical and speculative.

12. For these reasons, novelty is acknowledged.

Article 56 EPC; inventive step of claim 1

13. Documents (1) and (2) were indifferently cited as closest prior art. Both are concerned with developing filamentous fungi expression systems. Their disclosures are not different in any manner susceptible to bring a different outcome to the assessment of inventive step. In the following, document (2) is used as closest prior art. Document (1), however, will be given brief consideration at the end of the section.

14. Document (2) describes a transformed filamentous fungus host for the expression of a desired protein. The gene encoding said protein is contained in an expression cassette carrying all of the elements necessary for expression and secretion: a transcriptional initiation regulatory region, a signal sequence for secretion and

a transcriptional termination regulatory region (page 2, lines 41 to 48). After transformation of the fungus host with a vector containing the cassette, random integration of the cassette occurs at multiple locations in the fungal chromosome (page 3, lines 4 to 6). Expression and secretion of the desired protein ensue (page 4, lines 25 to 51). On page 4, lines 1 to 4, it is mentioned that:

" The expression construct including the gene may be used by itself for transformation, particularly where integration is desired, or may be joined to other DNA sequences for a variety of purposes. One DNA sequence with which it may be joined is a DNA sequence homologous with a DNA sequence of the host cell."

15. Starting from the closest prior art, the problem to be solved may be defined as being the provision of an alternative filamentous fungus expression system.

16. The solution provided is a transformed fungus host in which the expression cassette is inserted in the fungal chromosome by targeted integration (ie. homologous recombination) to the locus of a gene encoding a highly secreted protein. As this latter gene is replaced by the heterologous gene, the highly secreted protein is no more expressed.

17. The information given on page 4 of document (2) (point 14, supra) was argued to provide the skilled person with a clear hint that targeted rather than random integration may be used for the purpose of obtaining a fungus for the production of heterologous proteins. Taking as an assumption that this is indeed

the case - it was freely admitted by the respondent that at the priority date, targeted and random integrations were both well-tried techniques for introducing foreign DNA into a fungal chromosome -, there remains to be assessed whether or not the technical characteristic of the transformed fungus that it expresses the desired protein instead of expressing the endogenous highly secreted protein is obvious, ie whether or not it was obvious to target the desired gene in such a way as to inactivate said endogenous gene.

18. Document (3), a thirty-six pages review on "Recombinant DNA in filamentous fungi: progress and prospects", was cited in this respect. In the introductory part, passage bridging pages 357 and 358, the objective of the review is defined as providing a synthetic view of the molecular biology of filamentous fungi. In the context of introducing sections on prospects for industrial production strains, it is mentioned on page 359 that:

"Recombinant DNA methods offer a powerful set of additional techniques for strain improvement for industrial filamentous fungi.... For example, it may be possible to use fungi for the production of heterologous proteins by redirecting the enzyme production and secretion capacity of these microorganisms."

19. This passage taken together with the suggestion in document (2), page 4 (point 14, supra) was argued to destroy inventive step. In order to reach this conclusion, the expression "redirecting the secretory

capacity" must necessarily be interpreted as meaning "inactivating a gene encoding a highly secreted protein by the very same mechanism which leads to the integration of the heterologous gene in the fungal chromosome". In the board's judgement, this interpretation undoubtedly requires hindsight knowledge of the present invention. Indeed, redirecting the secretory capacity is a very vague concept. It may have any meaning, for example, expressing the heterologous protein preceded by a signal sequence for secretion from any position in the fungal chromosome will readily redirect the secretory capacity of the fungus since this pathway will then have to secrete this protein as well as all other proteins normally secreted by the organism.

20. A further attempt at challenging inventive step was made on the basis of a combination between the suggestion in document (2), page 4 (point 14, supra), the above mentioned information on page 359 of document (3) (point 18, supra) and section D of document (3), page 370 onwards. In this section, carrying out gene replacement is acknowledged as a powerful technique for studying gene expression because genes that have been altered in vitro can be reintroduced to their normal location and the effect of the specific mutation can be assessed in situ. At oral proceedings, it was, thus, argued that the combination of the two passages of document (3) made it obvious to carry out gene replacement at a secretory locus and that, in turn, the combination of this combination with the above mentioned suggestion in document (2) rendered obvious the transformed fungal host of claim 1.

21. The board is not convinced by this somewhat sophisticated argument if only for the reason that document (3) provides absolutely no incentive to combine the very general statement on page 359 with the information in section D, let alone for the purpose of heterologous gene expression. In fact, document (3) has a specific section on heterologous gene expression (section IV B, page 384 onwards) which mentions that the heterologous gene may be transformed into the fungal host cell by an autonomously replicating plasmid or by multiple insertions in the chromosome, but fails to refer to targeted gene replacement. This last option is also not suggested in section V dealing with future prospects.

22. For these reasons, the board concludes that the combination of the teachings of documents (2) and (3) is not detrimental to inventive step.

23. The teachings of document (1) relative to a transformed fungal host for the expression of a desired heterologous protein correspond to those of document (2) (point 14, supra). As already discussed in points 10 and 11, there is no pointer in document (1) to using targeted rather than random integration of the heterologous gene in the fungal chromosome since targeted integration is mentioned in a totally different context. In this situation, the same reasoning which led to a finding of inventive step over the combined teachings of documents (2) and (3), applies all the more so to the combination of the teachings of documents (1) and (3).

24. The appellant also argued that the problem to be solved was to provide an efficient system for secretion rather than an alternative one and that inventive step could not be acknowledged because this problem had not been solved by the claimed transformed fungus host. In this respect, the board remarks that the problem to be solved is to be defined starting from the closest prior art. Thus, if the closest prior art may be interpreted as providing a suggestion that expression systems other than the ones it offers may be devised (point 17, supra) and if the alternative solution proposed by the invention is not obvious, then it is irrelevant whether the invention provides a more efficient system. Thus, the formulation of the problem as suggested by the appellant is not justified, which renders irrelevant any argument presented in relation to it.

25. The requirements for patentability are fulfilled.

Order

For these reasons, it is decided that:

The appeal is dismissed.

The Registrar The Chairman

A. Wolinski L. Galligani