BESCHWERDEKAMMERN DES EUROPÄISCHEN PATENTAMTS | BOARDS OF APPEAL OF THE EUROPEAN PATENT OFFICE | CHAMBRES DE RECOURS DE L'OFFICE EUROPEEN DES BREVETS

Internal distribution code:
(A) [ ] Publication in OJ
(B) [ ] To Chairmen and Members
(C) [X] To Chairmen
(D) [ ] No distribution

D E C I S I O N
of 2 October 2003

Case Number: T 0036/00 - 3.3.8

Application Number: 89908939.5

Publication Number: 0429490

IPC: C12N 1/15

Language of the proceedings: EN

Title of invention:
Aspartic proteinase deficient filamentous fungi

Patentee:
GENENCOR INTERNATIONAL, INC.

Opponents:
(01) Unilever N.V.
(02) NOVOZYMES A/S
(03) Primalco Ltd.

Headword:
Proteinase deficient fungi/GENENCOR

Relevant legal provisions:
EPC Art. 54, 56, 83, 123(3)

Keyword:
"Main request - broadening of claims (no)"
"Novelty and inventive step (yes)"
"Sufficiency of disclosure (yes)"

Decisions cited:
T 0019/90

Catchword:
-

Case Number: T 0036/00 - 3.3.8

D E C I S I O N
of the Technical Board of Appeal 3.3.8
of 2 October 2003

Appellant I: (Opponent 02)	NOVOZYMES A/S Krogshoejvej 36 DK-2880 Bagsvaerd (DK)
Representative:	Bassett, Richard Eric Potter Clarkson Parkview House 58 The Ropewalk Nottingham NG1 5DD (GB)
Appellant II: (Opponent 03)	Primalco Ltd. Valta-akseli SF-05200 Rajamäki (FI)
Representative:	Jaenicken, Hans-Rainer, Dr VOSSIUS & PARTNER Postfach 86 07 67 D-81634 München (DE)
Respondent: (Proprietor of the patent)	GENENCOR INTERNATIONAL, INC. 4 Cambridge Place 1870 South Winton Road Rochester, New York 14618 (US)
Representative:	Armitage, Ian Michael MEWBURN ELLIS York House 23 Kingsway London WC2B 6HP (GB)

Other Party: (Opponent 01)
Unilever N.V.
Weena 455
NL-3013 AL Rotterdam (NL)

Respondent:
van der Toorren, Johannes, Drs
UNILEVER N.V.
Patent Division
Postbus 137
NL-3130 AC Vlaardingen (NL)

Decision under appeal: **Decision of the Opposition Division of the European Patent Office posted 29 October 1999 rejecting the opposition filed against European patent No. 0429490 pursuant to Article 102(2) EPC.**

Composition of the Board:

Chairman: L. Galligani
Members: P. Julia
S. C. Perryman

Summary of Facts and Submissions

I. European patent No. 0 429 490 with the title "Aspartic proteinase deficient filamentous fungi" was granted with 36 claims based on the International application No. PCT/US89/02891 published as WO 90/00192.

II. Three notices of opposition were filed requesting the revocation of the patent under Article 100(a) EPC (lack of novelty and inventive step) and Article 100(b) EPC (insufficiency of disclosure). The opposition division rejected the opposition and maintained the patent as granted.

III. Independent claims 1, 29, 30 and 32 as granted read:

"1. A mutant filamentous fungus suitable for the production of heterologous polypeptides therefrom wherein a gene for aspartic proteinase in the fungus is non-revertibly inactivated or eliminated so that the mutant thereby produced is incapable of excreting enzymatically active aspartic proteinase."

"29. A method of producing a heterologous polypeptide in a filamentous fungus which comprises culturing a filamentous fungus, which is capable of expressing the heterologous polypeptide and which contains a non-revertible site-selected deletion of amino acids that results in the filamentous fungus being incapable of excreting enzymatically active aspartic proteinase, until an amount of the heterologous polypeptide has accumulated in the culture broth, and then recovering the polypeptide."

"30. A heterologous polypeptide preparation comprising a culture broth made according to claim 29."

"32. A method of making cheese comprising:

a) Selecting an active chymosin preparation obtained from a host filamentous fungus expressing the DNA for chymosin and incapable of excreting active aspartic proteinase;

b) Adding the chymosin preparation to milk;

c) Allowing the milk to coagulate to produce curd and whey; and

d) Converting the curd of step c) into cheese."

Claims 2 to 21 were dependent on claim 1 and defined specific embodiments thereof (non-revertible deletion of the gene for aspartic proteinase, fungus species, aspartic proteinase, heterologous polypeptide, etc…). Independent claim 22 concerned a filamentous fungus culture which in its mutated form was free of any gene capable of expressing enzymatically active aspartic proteinase. Claims 23 to 28 were dependent thereon and defined specific embodiments thereof (fungus species, heterologous polypeptide, etc…). Claim 31 defined the heterologous polypeptide of the preparation of claim 30 as being chymosin. Claim 33 was dependent on claim 32 and defined the chymosin preparation. Independent claim 34 was directed to a gene replacement vector, whereas claims 35 to 36 were dependent thereon and further characterized the gene replacement vector.

IV. The appellants I (opponent 02) and II (opponent 03) filed an appeal against the decision of the opposition division. The respondent (patentee) filed observations in reply to the statements of grounds of appeal. The

appellant II submitted further comments as a reply to respondent's observations.

V. The board sent a communication pursuant to Article 11(2) of the Rules of procedure of the Boards of Appeal indicating its preliminary opinion.

VI. The appellants I and II as well as the respondent filed observations relating to the board's communication. A main request was also filed by the respondent. Opponent 01 (party as of right under Article 107 EPC) informed the board that it would not attend the oral proceedings.

VII. Oral proceedings took place on 2 October 2003. During the oral proceedings the respondent filed a new main request.

VIII. The main request before the board of appeal comprised 29 claims. Claims 1 to 21 were as the corresponding granted claims, whereas claims 22 to 24 and claims 27 to 29 corresponded respectively to granted claims 29 to 31 and claims 34 to 36. Claims 25 and 26 of the main request corresponded to the method of making cheese of granted claim 32, wherein step (a) had been amended as follows:

"a) Producing an active chymosin preparation by expression from a host filamentous fungus expressing the DNA for chymosin and incapable of excreting active aspartic proteinase;" (claim 25),

"a) Selecting an active chymosin preparation obtained from a host filamentous fungus expressing the DNA for

chymosin and incapable of excreting active aspartic proteinase, wherein the active chymosin preparation is the culture broth used for the filamentous fungus expressing the polypeptide;" (claim 26)

IX. The following documents are referred to in the present decision:

(D17) EP-A-0 206 783;

(D20) S. Hayashida and P.Q. Flor, Agric. Biol. Chem., 1981, Vol. 45, No. 12, pages 2675 to 2681;

(D26) WO-A-86/01825;

(D27) A. Upshall, BioTechniques, 1986, Vol. 4, No. 2, pages 158 to 166;

(D28) V.I. Ostoslavskaya et al., Soviet J. Bioorg. Chem., 1986, Vol. 12, No. 8, pages 548 to 563;

(D34) S. Murao and K. Oda, in "Aspartic Proteinases and their Inhibitors", 1985, Walter de Gruyter & Co., Berlin, pages 379 to 399;

(D40) J.L. Smith and R.Y. Yada, Can. Inst. Food Sci. Technol. J., 1991, Vol. 24, pages 48 to 56;

(D42) I.E. Mattern et al., Mol. Gen. Genet., 1992, Vol. 234, pages 332 to 336;

(D44) V. Barkholt, Eur. J. Biochem., 1987, Vol. 167, pages 327 to 338;

(D47) K. Sakka et al., J. Ferment. Technol., 1985, Vol. 63, No. 5, pages 479 to 483;

(D53) W-J. Chang et al., J. Biochem., 1976, Vol. 80, pages 975 to 981.

X. The appellants' arguments in writing and during oral proceedings, insofar as they are relevant to the present decision, may be summarized as follows:

Appellant I (Opponent 02)

Articles 123(2),(3) and 84 EPC. Rule 57a EPC

No comments were made to the amendments introduced into the subject-matter of claims 25 and 26.

Sufficiency of disclosure

In order to achieve a non-revertible elimination of a gene for aspartic proteinase, the opposed patent disclosed the deletion of a specific aspartic proteinase gene. However, the claimed mutants were not solely limited to deletion mutants. Claim 1 embraced mutants obtained by other methods and having mutations other than in the aspartic proteinase gene itself, such as in a regulatory gene of the aspartic proteinase. This subject-matter was not disclosed in the patent. Moreover, the claimed mutants were required to be incapable of excreting any aspartic proteinase and not only the exemplified aspergillopepsin A. However, no teaching was given for the deletion of more than one aspartic proteinase gene and suitable means (sequence

information) for deleting a second aspartic proteinase gene were not disclosed.

Article 54 EPC

Claim 1 did not define the conditions under which the claimed fungi were incapable of excreting active aspartic proteinase. Document D20 showed a protease-less Aspergillus mutant (HF-15) without extracellular proteinase activity at 24 and 120 hrs of culture (Figure 5). Document D42 (post-published expert evidence), using the method of document D20 and obtaining similar mutants, showed that the aspartic proteinase from Aspergillus (aspergillopepsin A) was responsible for 80 to 85% of the total extracellular protease activity. Since document D20 showed that mutant HF-15 had a 84% decrease of acid protease activity, under certain conditions even 93%, this mutant had to be deficient in aspergillopepsin A. Moreover, since the frequency of spontaneous reversion was as low as 10^{-7}, mutant HF-15 had a non-revertibly inactivated gene for aspartic proteinase and it was incapable of excreting enzymatically active aspartic proteinase as required by the claims. The fact that it could be mutated in a (regulatory) gene different from the aspartic proteinase gene was irrelevant. The claimed mutants were suitable for the production of heterologous polypeptides and document D20 showed mutant HF-15 to produce greater amounts of a desired polypeptide (glucoamylase). There was no difference between the claimed mutant filamentous fungi and the mutant HF-15 of document D20. The unclear term "non-revertible site-selected deletion" could not distinguish the mutant used in the method of claim 22

from mutant HF-15. Claim 23 defined a heterologous polypeptide preparation as a product-by-process. The presence of a culture broth could not differentiate this preparation from polypeptides produced by culturing filamentous fungi under conditions wherein the extracellular proteinases were repressed (document D27).

Article 56 EPC

The closest prior art was document D27, which recognized the protease problem and proposed an empirical, initial solution, namely the use of agents known to repress protease production at the genetic level. However, this solution had many technical problems (cf Declaration of K. Hansen, appellant's I letter of 1 September 2003) and thus, the skilled person was prompted to improve it. It was obvious to improve on a temporary gene inactivation by making a more permanent one, ie to permanently inactivate the protease gene by a deletion. In view of the fact that proteases were unselective in their action and not essential for the viability of the fungi and, knowing that the deletion of such a gene had already been used for improving the production of heterologous polypeptides in different species (Bacillus, document D26 and Saccharomyces, document D17), the skilled person had a reasonable expectation of success. This expectation was supported by document D20, which showed that a protease-less Aspergillus was viable. It was known that Aspergillus produced almost solely an acid protease as a component of its extracellular proteolytic enzyme system (document D47) and, since the only known amino acid sequence of this proteolytic

system was the aspartic proteinase (document D28), it was a matter of routine work to delete the corresponding gene so as to obtain an Aspergillus mutant as the claimed one. The deletion of this specific aspartic proteinase was an obvious selection. Moreover, claim 1 referred to a general aspartic proteinase and did not exclude the deletion of other (aspartic, acid, alkaline, neutral) proteinases.

Appellant II (Opponent 03)

Articles 123(2),(3) and 84 EPC. Rule 57a EPC

The amendments introduced in granted claim 32 extended the scope of protection. Claims 25 and 26 were not allowable under Article 123(3) EPC.

Sufficiency of disclosure

The claims were not sufficiently disclosed in so far as they extended beyond the specific example disclosed in the patent. There was no information as regards the sequences of genes encoding aspartic proteinases from filamentous fungi other than Aspergillus and there was no evidence that these other filamentous fungi with a deletion in those genes were viable and capable of producing heterologous polypeptides. In view of the fact that filamentous fungi could contain more than one aspartic proteinase (document D40), it was not possible to know which one had to be inactivated for obtaining enhanced amounts of heterologous polypeptides. The patent was not enabling for filamentous fungi having more than one aspartic proteinase gene. For these cases, it did not teach how to achieve a mutant

completely incapable of excreting enzymatically active aspartic proteinase by a non-revertible inactivation of only one aspartic proteinase gene as required by claim 1. The characterization of other aspartic proteinase genes could only be done with undue experimentation.

Article 54 EPC

Document D27 mentioned the production of chymosin using filamentous fungi as hosts and referred to conditions for repressing the production of proteases so as to obtain improved yields. In line with claim 22, a culture broth (without fungi cells) was different from a filamentous fungi culture (with fungi cells). The presence of an undefined culture broth (without fungi cells) in claims 23 and 24 did not differentiate the polypeptide preparations of these claims from a polypeptide preparation obtained by the method indicated in document D27.

Article 56 EPC

Starting from document D27, the closest prior art, the technical problem was to provide a filamentous fungus having reduced levels of extracellular proteases. Document D27 prompted the skilled person to come up with a final, permanent solution. This solution had already been anticipated by document D26 disclosing the deletion of a protease gene in Bacillus. It was known that the main secreted protease in Aspergillus was an acid protease (document D47) and that the inactivation of extracellular proteases in Aspergillus, particularly acid proteases, resulted in viable mutants with a

decreased overall proteolysis and increased production of desired polypeptides (document D20). Acid proteases were equated to aspartic proteinases in the prior art (document D34) and the amino acid sequence of the aspartic proteinase from Aspergillus was also known (document D28), actually being the only (acid) protease sequence available to the skilled person. Thus, there was a motivation to generate an Aspergillus mutant deficient in this aspartic proteinase by applying known gene replacement techniques rather than generating a strain with an altered (unknown) regulatory mechanism as regards protease gene expression, with mutations in all protease genes (unknown sequences, random mutagenesis associated with deleterious mutations, etc…) or expensive, unspecific repression of protease activity by supplementing the culture medium with protease inhibitors (document D27). In the light of the prior art showing the viability of organisms with protease gene deletions (documents D17 and D26) or with reduced level of acid (aspartic) proteases (document D20), as well as the enhanced production of desired (heterologous) polypeptides by those organisms (documents D20 and D26), there was a reasonable expectation of success.

XI. Respondent's arguments in writing and during oral proceedings, insofar as they are relevant to the present decision, may be summarized as follows:

Articles 123(2),(3) and 84 EPC. Rule 57a EPC.

The amendments were introduced to overcome a ground of opposition and they only made the scope of protection narrower than the one of the granted claims.

Article 83 EPC

The patent showed that the inactivation of an aspartic proteinase left a viable filamentous fungus suitable for the expression of heterologous polypeptides. This teaching could be applied to other fungi and it was not rendered insufficient by having to clone other aspartic proteinase genes. The deletion of the specific aspartic proteinase gene shown in the patent was only an example for the non-revertible inactivation of this gene. Other methods could be easily envisaged for achieving the same effect even if not exemplified. Possible theoretical methods could not be of relevance for assessing whether the patent was enabling or not. There was no prior art showing the presence of more than one aspartic proteinase in filamentous fungi.

Article 54 EPC

Document D20 disclosed a mutant with low protease activity but not a protease-less mutant. The acid protease activity was not completely inactivated but it was dependent on the culture conditions used. The mutation in mutant HF-15 was probably at the level of gene regulation rather than a non-revertible inactivation or gene deletion. The acid protease activity of this mutant was not equated to aspartic proteinase activity and there was no certainty that the inactivated acid protease was an aspartic proteinase. Document D20 disclosed the production of an endogenous polypeptide and, in view of the method used for producing mutant HF-15 (random mutagenesis) and the absence of a genetic characterization, there was no

certainty whether this mutant was suitable for the production of heterologous polypeptides. Cultured (fungi) cells were present in the culture broth and thus, the presence of the mutant fungi of claim 22 in the culture broth of claims 23 and 24 differentiated the claimed heterologous polypeptide preparations from the ones of document D27.

Article 56 EPC

The closest prior art, document D27, identified the protease problem and referred to copious amounts and many types of proteases in filamentous fungi. Their wide variety, different modes of action and cellular location suggested an essential function for fungi. Document D27 indicated a satisfactory solution (use of agents repressing the production of proteases) and other solutions were also available to the skilled person (pH medium, removal products, etc…). However, there was no suggestion of deleting a protease gene let alone an aspartic proteinase gene. There was a substantial difference between the shotgun approach of document D27 and the targeted approach of the patent. Document D20 disclosed a shotgun approach (random mutagenesis) to obtain mutant HF-15 with a protease activity partially inactivated. The inactivation was probably the result of an altered regulation of protease genes together with other mutations that allowed the mutant to survive. The document disclosed the activities of three classes of proteases (neutral, alkaline and acid) under particular culture conditions and it did not allow to draw any conclusions on their relative importance. Document D47 identified an acid proteinase as a major component of its proteolytic

system but it also showed an enhanced production of serine proteinase. There was no motivation to use the amino acid sequence of the Aspergillus aspartic proteinase (document D28) in the manner of the patent. No prior art had shown that aspartic proteinase was not essential for filamentous fungus. Document D26 disclosed the deletion of a different protease (subtilisin) from a different organism (Bacillus) and the viability of the resulting mutant was said to be uncertain. Document D17 referred to a non-filamentous fungus with impared proteolytic function but neither the strain nor the secreted protease were characterized. Thus, in the light of this prior art, there was no reasonable expectation of success.

XII. The appellants (opponents 02 and 03) requested that the decision under appeal be set aside and that the European patent No. 0 429 490 be revoked.

XIII. The respondent (patentee) requested that the decision under appeal be set aside and that the patent be maintained on the basis of the main request filed at the oral proceedings on 2 October 2003.

Reasons for the Decision

Articles 123(2),(3) and 84 EPC. Rule 57a EPC

1. Claim 25 requires as a first step in a method of making cheese the actual **production** of an active chymosin preparation by expression from a host filamentous fungus which is incapable of excreting active aspartic proteinase, whereas granted claim 32 only required the

selection of an active chymosin preparation obtained by said method. Thus, the scope of claim 25 is narrower than the one of granted claim 32. Claim 26 represents a combination of independent claim 32 with dependent claim 33 both as granted. No extension of protection can be seen in this combination. Thus, the requirements of Article 123(3) EPC are fulfilled.

2. The amendments have been introduced in order to overcome a ground of opposition as required by Rule 57a EPC. No objections have been raised under Articles 84 and 123(2) EPC and the board sees none.

Article 83 EPC

3. Claim 1 requires the non-revertible inactivation or elimination of a gene for aspartic proteinase so that the mutant filamentous fungus produced is incapable of excreting enzymatically active proteinase. Aspartic proteinases are defined in the patent as exhibiting proteolytic activity at low pH, containing at least two aspartic residues in the active site (cf *inter alia* page 4, lines 34 to 39 and page 5, line 5) and being inhibited by pepstatin (cf page 14, lines 54 to 56). A residual proteolytic activity is found in transformants)AP3 and)AP4 which have a deleted aspartic proteinase gene. This residual activity is, however, pepstatin-insensitive and it is associated to "*one or more secreted proteinases other than the deleted aspergillopepsin*", presumably to "*a pepstatin-insensitive aspartyl proteinase*" similar to that described in document D34 (cf page 15, lines 1 to 8). Document D34 defines aspartic proteinases by the properties cited in the patent and pepstatin-

insensitive acid proteinases are identified as a new subclass different from aspartic proteinases (cf page 379, second full paragraph and page 380, first full paragraph). Similarly, document D53, referring to type B (aspergillopepsin A) and type A (aspergillopepsin B) proteases from Aspergillus, states that "*the nature of the active site of the Type A enzyme is rather different from that of the type B enzyme and hence the Type A enzyme belongs to a different class of acid proteases from the type B enzyme*" (cf page 975, three lines from the bottom). Thus, the residual proteolytic activity of transformants)AP3 and)AP4 cannot be associated to an aspartic proteinase and these transformants are incapable of excreting enzymatically active aspartic proteinase as required by claim 1.

4. Moreover, on the evidence on file, the presence of a single aspartic proteinase gene in Aspergillus can be generalised to other if not all filamentous fungi. Two post-published documents have been cited as expert evidence for demonstrating that this is not the case, particularly for Mucor and Rhizopus, which are referred to in claims 8 to 12: document D40 discloses two different pepstatin-sensitive aspartyl proteinases from Mucor miehei and document M. Ward and K.H. Kodama (in "Structure and Function of the Aspartic Proteinases", Ed. B.M. Dunn, Plenum Press, N.Y., 1991, pages 149 to 160) refers to two separate genes encoding secreted aspartic proteinases in Rhizopus niveus (cf Table 1 and page 151, lines 5 to 6). However, document D40 states that the presence of two aspartyl proteinases had not been previously reported and concludes that several reasons could explain their presence, particularly the

use of a commercial preparation instead of a pure culture of fungus (cf page 55, left-hand column). The board also notes that the reference in Ward and Kodama (cf *supra*) to the aspartic proteinase genes is found in the context of isozymes and splicing (intron) variants of secreted aspartic proteinases. The publication cited therein (Horiuchi et al, 1990) has not been filed in the appeal proceedings and thus, it is not possible to assess its relevance with certainty.

Thus, the two documents in question cannot alter the conclusion that at the time of the invention the view in the art was that a single aspartic proteinase was present in filamentous fungi. Consequently, the board has to conclude that the teaching of the patent, namely the non-revertible inactivation of a (single) aspartic proteinase gene as exemplified in Aspergillus, would enable the skilled person to obtain generally a mutant filamentous fungus incapable of excreting enzymatically active aspartic proteinase.

5. Claim 1 is not limited to mutants obtained by the exemplified non-revertible deletion of the gene for aspartic proteinase (claim 2) but it covers mutants obtained by other methods which result in a non-revertible inactivation of this gene. Once the viability of the mutants deficient in aspartic proteinase as well as their advantageous properties for the production of heterologous polypeptides are known, nothing prevents a skilled person from using other methods available in the prior art for achieving the same result, such as gene disruption by insertion of one or more foreign DNA fragments into the aspartic proteinase gene, removal of promoter regions, etc…

Similarly, nothing prevents a skilled person from using the aspartic proteinase genes of other filamentous fungi in the same manner as in the opposed patent. As shown in the patent (cf page 3, lines 10 to 31), these genes are already known, such as in Endothia parasitica (cf document D44), or else they can readily be determined by known methods. The generalisation of the exemplified deletion of the aspartic proteinase gene in Aspergillus to a more general non-revertible inactivation of this gene in filamentous fungi does not represent an unjustified extension to subject-matter not enabled by the patent.

6. Reference has been made to other methods that could theoretically achieve the same result as the opposed patent and for which, however, there is no sufficient teaching in the patent itself nor any guidance in the prior art, such as the non-revertible inactivation of a regulatory gene of the aspartic proteinase. However, no claim, or part of a claim is specifically directed to such subject matter. The complaint is merely that the wording of the broadest claims would *inter alia* cover this possibility, though it is not discussed in the patent or in the prior art, nor even in post-published documents. In theory such an approach might work, though there is no evidence that such a putative regulatory region exists, or that the approach would be feasible in practice. If the subject matter of a claim can be made to work in numerous ways in the manner described, which the board accepts for the present claims, under the case law of the Boards of Appeal Article 83 EPC has not been interpreted as requiring the claim to be limited to exclude certain only hypothetically conceivable other embodiments which

might also fall under the claims. It would be different if there were some verifiable facts that raised serious doubts on the enabling character of the patent (cf T 19/90, OJ EPO 1990, 476).

7. Thus, the board considers that the requirements of Article 83 EPC are met.

Article 54 EPC

8. Document D20 refers to the degradation of endogenous glucoamylase by the presence of proteases in the filamentous fungus Aspergillus. The document discloses mutant HF-15 which is produced by random mutagenesis and selected for significantly low protease activity and high amount of glucoamylase. Three different protease activities (acid, neutral and alkaline) are identified and their presence is shown to be dependent on the conditions of culture. In particular, mutant HF-15 shows a reduction in protease activity of 93%, 84% and 50% in solid wheat bran culture, shaking medium B and wheat bran culture, respectively. On submerged culture in synthetic liquid medium B the protease activity of the mutant decreases by 84% and neither neutral nor alkaline proteases are observed (cf page 2679, left-hand column and Figure 5). Under the same conditions the parent strain presents all three activities, wherein the acid protease activity is the most important one (cf Figure 5).

9. There is no reference in document D20 to aspartic proteinase nor to its specific contribution to the disclosed acid protease activity in terms of pepstatin-sensitivity (aspartic proteinase) (cf item 3 *supra*). It

cannot be directly deduced from this document that the gene for the aspartic proteinase is non-revertibly inactivated in mutant HF-15 and that the residual acid protease activity present under all conditions is solely due to an acid protease other than an aspartic proteinase. Even if assuming that the major protease and, in particular the major acid protease of Aspergillus, is an aspartic protease, so that the total (acid) protease activity referred to in document D20 could be equated to aspartic protease (cf page 2677, right-hand column, last sentence of the second full paragraph), mutant HF-15 is not incapable of excreting enzymatically active aspartic proteinase as it shows a residual protease activity as high as 50% on submerged culture in a wheat bran medium (cf page 2679, left-hand column). Thus, the inactivation of mutant HF-15 is revertible. The board cannot follow appellants' interpretation that, due to the absence of culture conditions in claim 1, this claim embraces mutants incapable of excreting enzymatically active aspartic proteinase only under certain conditions but capable of excreting the proteinase under other conditions. This interpretation goes against the normal understanding of a non-revertible inactivation and there is nothing in the patent that could lead to believe that this unusual interpretation is the one actually intended.

10. Mutant HF-15 differs from the parent strain not only by having a low proteinase activity but it also fails to produce mannosidase and glucosaminidase (cf Figure 6) and by differences in conidia formation that are suggested to be associated with high levels of amyloglucosidase and low levels of transglucosidase (cf page 2677, right-hand column, last full paragraph). In

the absence of a complete characterization of the mutations present in the genome of mutant HF-15, there is no reliable basis for assuming that the aspartic proteinase gene itself, its regulatory region or else an (unknown) regulatory gene was (non-revertibly) eliminated. Moreover, there is no reference in document D20 to heterologous polypeptides and, in the light of the above mentioned differences between mutant HF-15 and the parental Aspergillus strain as well as the failure to fully characterize the mutations of HF-15, it is not possible to ascertain whether this mutant is suitable for producing heterologous polypeptides, as it might well have other mutations that prevent this production, such as an impaired DNA transformation, impaired integration, stability or expression of heterologous DNA, etc… .

11. In the absence of a genetic characterization of mutant HF-15, there is no evidence that it contains a "non-revertible site-seleted deletion of amino acids" (or the corresponding gene coding thereof) as required for the mutants used in the method of claim 22.

12. Whereas "broth" alone is understood as a medium or a culture medium, ie the liquid prepared and used for culturing cells, "culture broth" comprises the cultured cells, ie the cells in a sample of broth used as culture medium. Claim 22 refers to the accumulation of heterologous polypeptide in the "culture broth", ie in the culture medium in presence of the cultured mutant fungi. There is no reference to a centrifugation, filtration, etc… in order to separate the cultured fungi. The presence of mutant filamentous fungi in the culture broth of the heterologous polypeptide

preparations of claim 23 differentiates these preparations from other known preparations and in particular from the ones cited in document D27 (growth of filamentous fungi under culture conditions repressing the production of extracellular proteases).

13. Thus, the board considers that the claimed subject-matter fulfils the requirements of Article 54 EPC.

Article 56 EPC

14. Document D27, the closest prior art, identifies "*the protease problem*" for the expression of heterologous polypeptides using filamentous fungi as recombinant hosts. This document discloses an "*empirical, initial solution*", namely "*to ensure that the culture medium contains sufficient quantities of the agents known to repress protease production*". It is also stated that "*copious amounts of these protein degrading enzymes are produced, especially under derepressing conditions*" with reference to literature documents reporting the repression of Aspergillus extracellular proteases by ammonium as well as the effect of sulfur on the formation and synthesis of these proteases (cf page 164, left-hand column).

15. Starting from this closest prior art, the objective technical problem underlying the opposed patent may be defined as the provision of an alternative solution to this "protease problem". In the light of the example disclosed in the patent, the board is convinced that the non-revertible inactivation or elimination of the gene for aspartic proteinase provides a solution to this problem.

16. Document D27, a review on filamentous fungi in biotechnology, refers to recombinant DNA techniques as providing "*a logical extension of the array of methods used for improving the productivity of fungal strains*" and states that "*the classic methods of mutation followed by selection and/or screening, although empirical in scientific approach, have proven immensely successful*" (cf page 159, left-hand column, last paragraph). It is further said that "*recombinant DNA methodology allows manipulation at the single gene level, thereby avoiding ... to expose a balanced genome to heavy dose of mutagen ...*(so as to have)... *precisely defined changes*" (cf page 159, middle column, full paragraph) and that "*gene disruption, gene replacement and cotransformation have been developed*" (cf page 159, right-hand column, last paragraph). Nevertheless, there is no suggestion to apply these techniques to the protease problem nor an indication of the possible advantages or drawbacks of using such an approach. Thus, the question arises whether the skilled person would have derived the suggestion to use these techniques from any other prior art document.

17. As said above (cf point 14 *supra*), document D27 refers to "*copious amounts of these degrading enzymes, especially under derepressing conditions" and that in spite of "much literature on the presence of proteases and some on their activity ... they are genetically undefined*" citing literature documents concerned with neutral proteinases from Aspergillus (cf page 164, left-hand column, second full paragragh). The presence of large amounts of proteases as well as their dependency on the culture conditions was well-known,

however, neither their genetic organization nor the relative contribution of each protease to the total protease activity, let alone the variability of these relative contributions on the conditions of culture, were known. Document D47, concerned with the isolation of a serine proteinase, refers to Aspergillus niger as producing "*almost solely an acid proteinase as a component of its extracellular proteolytic enzyme system*" (cf page 479, left-hand column, first paragraph). However, there is no disclosure of the culture conditions used (ammonium salts, sulfur) nor an identification of the acid proteinase in terms of pepstatin-sensitivity. Similarly, document D20 shows the presence of an important acid proteinase activity in both parent and mutant HF-15 strains. However, significant variability of acid, neutral and alkaline protease activities on culture conditions is also shown and the acid protease activity is not characterized (cf point 9 *supra*).

18. In the absence of a clear identification of a specific protease as the main component of the proteolytic system of filamentous fungi, there could be no clear motivation for the skilled to use the gene disruption or replacement techniques mentioned in document D27. It has been argued that document D28, by making available the amino acid sequence of the aspartic proteinase of Aspergillus awamori, would have prompted the skilled person to use those techniques. However, this document is completely silent on the importance of this enzyme in the proteolytic system of Aspergillus and thus, it cannot provide the missing incentive for the skilled person. The board considers that the critical question is whether a targeted approach would be obvious to the

skilled person and not whether possible technical means to achieve this approach were obvious. It is only if the first question can be answered positively, which it is not in the present case, that it becomes necessary to assess the second one. But even if, for the sake of argumentation, the board follows appellants' approach and considers that it is obvious to use the information of document D28, the board concludes that, in view of the prior art, there is no reasonable expectation of success.

19. Neither document D26 nor document D17 provide such expectation.

Document D17 refers to strains of non-filamentous fungus (Saccharomyces cerevisiae) with impaired proteolytic function (cf abstract, page 10, lines 26 to 28). Strain SB7-5D bears a mutation which renders the cells deficient in a secreted protease (cf page 59, lines 34 to 36). However, neither the secreted protease nor the specific culture conditions or the method for obtaining this mutant strain are disclosed therein.

Document D26 concerns a different organism (Bacilli) which produces large quantities of extracellular proteases, wherein the most abundant are a neutral metalloproteinase and an alkaline serine protease (subtilisin), the latter being the one inactivated by gene deletion for obtaining a strain with reduced protease levels. Thus, neither the protease system nor the most abundant proteases are similar to the ones found in Aspergillus. Moreover, document D26 states that "*since the contribution of the enzyme subtilisin to the viability of Bacilli was uncertain, it was an*

unpredictable finding that the genetic alteration which produced the Bacilli strains of the invention was not lethal to the organism" (cf page 8, lines 4 to 8).

In fact, there is no prior art on file that would have allowed the person skilled in the art to expect filamentous fungi with a non-revertible inactivation of aspartic proteinase to be viable. There is only evidence that these fungi are viable in presence of a revertible elimination of (acid) proteases, such as by general repression (cf document D27) or by random mutation with other possible compensatory mutations (cf document D20).

20. The importance and specific contribution of the aspartic proteinase to the extracellular proteolytic activity of Aspergillus (80 to 85% of total protease activity under the culture conditions used in document D42, post-published expert evidence) as well as the viability of an aspartic proteinase-deleted Aspergillus was only shown by providing a mutant having the characteristics of the claimed subject-matter (cf document D42). In the absence of this information, it is the board's opinion that the general solution suggested in the closest prior art (cf point 14 *supra*) or a similar general (shotgun) approach (cf document D20), even if associated with possible shortcomings (cf declaration of K. Hansen, appellant's I letter of 1 September 2003), would have been considered satisfactory by the person skilled in the art. Therefore, the targeted solution proposed by the claims was **not obvious**.

21. Thus, the claimed subject-matter fulfils the requirements of Article 56 EPC.

Order

For these reasons it is decided that:

1. The decision under appeal is set aside.

2. The matter is remitted to the first instance with the order to maintain the patent on the basis of the claims filed at the oral proceedings on 2 October 2003 and the description and Figures as granted.

The Registrar: The Chairman:

A. Wolinski L. Galligani