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**Datasheet for the decision
of 10 September 2009**

Case Number: T 1941/07 - 3.3.08

Application Number: 92902669.8

Publication Number: 0562003

IPC: C12N 15/56

Language of the proceedings: EN

Title of invention:

Improved saccharification of cellulose by cloning and amplification of the beta-glucosidase gene of *Trichoderma reesei*

Patentee:

GENENCOR INTERNATIONAL, INC.

Opponent:

NOVOZYMES A/S

Headword:

beta-glucosidase/GENENCOR

Relevant legal provisions:

EPC Art. 87, 54, 56

Keyword:

"Main request - inventive step - no"
"Auxiliary request 1 - priority - yes"
"Auxiliary request 1 - novelty - yes"
"Auxiliary request 1 - inventive step - yes"

Decisions cited:

-

Catchword:

-



Case Number: T 1941/07 - 3.3.08

DECISION
of the Technical Board of Appeal 3.3.08
of 10 September 2009

Appellant I: GENENCOR INTERNATIONAL, INC.
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Decision under appeal: Interlocutory decision of the Opposition
Division of the European Patent Office posted
8 October 2007 concerning maintenance of the
European Patent No. 0562003 in amended form.

Composition of the Board:

Chairman: L. Galligani
Members: F. Davison-Brunel
C. Heath

Summary of Facts and Submissions

- I. European patent No. 0 562 003 with the title "Improved saccharification of cellulose by cloning and amplification of the beta-glucosidase gene of *Trichoderma reesei*" was granted with 25 claims, based on European patent application No. 92 902669.8.

Granted claim 1 read as follows:

"1. A process for modifying the expression of extracellular β -glucosidase in a filamentous fungus comprising transforming said fungus with an expression vector containing a fungal DNA sequence which:

(a) is capable of enhancing the expression of extracellular β -glucosidase through the presence of at least one additional copy of a fungal β -glucosidase gene; or,

(b) encodes an altered extracellular β -glucosidase i.e. an enzyme having an amino acid sequence which has been altered with respect to that encoded by the bgl1 gene derived from *Trichoderma reesei* by manipulating said bgl1 DNA sequence.

Claims 2 to 11 related to further features of the process of claim 1. Claim 12 was directed to a fungal cellulase composition and claims 13 to 16 and 18 related to various methods comprising the use of a recombinant fungal cellulase composition. Claim 17 was directed to a detergent composition comprising the recombinant fungal composition. Claims 19 and 20 respectively related to transformants produced by the process of claim 7 and to a method for using them.

Claim 21 was directed to further features of the methods of claims 18 and 20. Claim 22 related to a nucleotide sequence of a bgl1 gene for use as a probe and claims 23 to 25 related to methods of using such a probe.

- II. An opposition was filed under Article 100(a) to (c) EPC. The opposition division maintained the patent on the basis of the third auxiliary request then on file, the main request being rejected under Article 123(2) EPC and the first and second auxiliary requests under Article 56 EPC.
- III. Appellant I (patentee) and appellant II (opponent) filed notices of appeal and submitted statements of grounds of appeal. Appellant I's statement of grounds was accompanied by three auxiliary requests, the main claim request being the set of granted claims.
- IV. Each appellant replied to the other's statement of grounds of appeal. Appellant I's reply was accompanied by, in particular, two further auxiliary requests (AR4 and AR5).
- V. In a further submission, appellant II provided new arguments as regards priority entitlement.
- VI. The board sent a communication pursuant to Article 15(1) of the Rules of Procedure of the Boards of Appeal, indicating its preliminary, non-binding opinion.
- VII. Both parties commented on the situation created by the filing of the new arguments relating to priority.

- VIII. The board sent a communication to inform the parties of its intention of dealing with the priority issues.
- IX. Both parties filed further submissions in advance of oral proceedings. Appellant I's submissions on 10 August 2009 were accompanied by, in particular, a main request and four auxiliary requests, in replacement of all the requests on file.
- X. During oral proceedings which took place on 10 September 2009, appellant I withdrew auxiliary requests 1 to 3. Auxiliary request 4 was re-numbered auxiliary request 1.

Claim 1 of the main request read as follows:

1. A process for modifying the expression of extracellular β -glucosidase in a filamentous fungus comprising transforming said fungus with an expression vector containing a fungal DNA sequence which:

(a) is capable of enhancing the expression of extracellular β -glucosidase through the presence of at least one additional copy of a fungal **extracellular β -glucosidase gene wherein the filamentous fungus is Trichoderma reesei, or said fungal extracellular β -glucosidase gene is a bg11 gene derived from Trichoderma reesei; or,**

(b) encodes an altered extracellular β -glucosidase i.e. an enzyme having an amino acid sequence which has been altered with respect to that encoded by the bg11 gene derived from Trichoderma reesei by manipulating said bg11 DNA sequence." (emphasis added by the board)

Claim 1 of auxiliary request 1 read as follows:

"1. A process for modifying the expression of extracellular β -glucosidase in a filamentous fungus comprising transforming said fungus with an expression vector containing a fungal DNA sequence which:

(a) is capable of enhancing the expression of extracellular β -glucosidase through the presence of at least one additional copy of a fungal **extracellular β -glucosidase gene wherein said extracellular β -glucosidase gene is a bg11 gene derived from Trichoderma reesei**; or,

(b) encodes an altered extracellular β -glucosidase i.e. an enzyme having an amino acid sequence which has been altered with respect to that encoded by the bg11 gene derived from Trichoderma reesei by manipulating said bg11 DNA sequence." (emphasis added by the board)

In this request, claims 2 to 24 correspond to granted claims 2 to 4, 6 to 25.

XI. The documents relevant to the present decision are the following:

- (3) : Penttilä, M.E. et al., Mol.Gen.Genet., Vol. 194, pages 494 to 499, 1984;
- (8) : Chirico, W. J. and Brown, Jr., R.D., Eur.J. Biochem., Vol. 165, pages 333 to 341, 1987;
- (15) : Barnett, C.C. et al., Bio/Technology, Vol. 9, pages 562 to 567, June 1991;

- (16) : Kubicek, C.P., Journal of General Microbiology, Vol. 133, pages 1481 to 1487, 1987;
- (17) : Brown, Jr., R.D. and Gritzali, M., from Genetic Control of Environmental Pollutants, Edited by Gilbert S. Omenn and Alexander Hollaender, Plenum Publishing Corporation, pages 239 to 265, 1984;
- (18) : Sternberg, D. and Mandels, G.R., J. Bacteriol., Vol. 144, No. 3, pages 1197 to 1199, Dec. 1980;
- (22) : Sternberg, D., Appl. Envir. Microbiol., Vol. 31, No. 5, pages 648 to 654, May 1976;
- (25) : Waksman, G., Curr. Genet., Vol. 15, pages 295 to 297, 1989;
- (26) : Moranelli, F. et al., Biochem Intern., Vol. 12, No. 6, pages 905 to 912, June 1986;
- (27) : Morrison, J. et al., Biochim. Biophys. Acta, Vol. 1049, pages 27 to 32, 1990;
- (29) : Durand, H. et al., Biochemistry and Genetics of Cellulose Degradation, Academic Press Ltd., pages 135 to 151, 1988;
- (31) : Szczodrak, J., Biotechnology and Bioengineering, Vol. 33, pages 1112 to 1116, 1989;

- (32) : Penttilä, M.E. et al., Gene, Vol. 61,
pages 155 to 164, 1987;
- (33) : EP-A 0 244 234, A2 version published on
04 November 1987;
- (49) : Sambrook. J et al., Molecular Cloning;
A Laboratory Manual; second Edition, Cold
Spring Harbor Laboratory Press,
pages 14.7 to 14.8, 1989;
- (50): Declaration of Christopher C. Barnett dated
7 August 2009, filed on 10 August 2009.

XII. Appellant I's submissions in writing and during oral proceedings insofar as relevant to the present decision may be summarized as follows:

Main request and auxiliary request 1; claim 1

Articles 87 and 54 EPC; priority, novelty

The sequence of the Trichoderma reesei (T.reesei) bg11 gene shown in Figure 1 of the patent in suit was the same as that shown in Figure 1 of the priority document US 625 140. Admittedly, the copy of Figure 1 of the priority document presently on file was only readable with difficulty. Yet, at oral proceedings, appellant I was able to provide a copy of the figure on file at the US patent office in connection with the priority application. This figure clearly showed the bg11 sequence as being the same as that in Figure 1 of the patent in suit. Accordingly, the T.reesei bg11 gene

enjoyed priority rights from the filing date of the priority document US 625 140. Document (15) published in the priority interval was not prior art and could not be taken into consideration when assessing novelty or inventive step. There were no documents of the cited prior art disclosing the now claimed subject-matter. Novelty could be acknowledged.

Article 56 EPC; inventive step

*- Cloning of a β -glucosidase gene in *T.reesei**

Document (29) was concerned with "Classical and molecular genetics applied to *Trichoderma reesei* for the selection of improved cellulolytic industrial strains". It disclosed T.reesei as a good cellulase producer, nonetheless having the disadvantage that it only synthesized β -glucosidase in limiting quantity, which prevented an efficient conversion of cellulose into glucose in industrial processes. It also described the known technique of increasing β -glucosidase production by isolating T.reesei mutant strains overproducing the enzyme. On page 137, it was remarked that considering the performances achieved by some of the mutants, what further improvements could be expected by, in particular, molecular genetics was a matter of conjecture. Increasing the amount of β -glucosidase produced through increasing the number of copies of the β -glucosidase gene was also qualified as "a challenge". Furthermore, a failure in cloning and expressing the Saccharomyces cerevisiae (S.cerevisiae) invertase gene in T.reesei was reported. Thus, even if molecular genetics was mentioned in document (29) as a possible approach to an increase in the T.reesei cellulolytic abilities, the document hardly provided the incentive to choose this approach.

Nonetheless, if one was to take document (29) as the closest prior art, the problem to be solved could be defined as making available an enhanced cellulose degradation system.

The claimed invention solved this problem by providing T.reesei expressing at least one additional copy of a β -glucosidase gene.

At the priority date, the skilled person desirous to solve the above mentioned problem would have been faced with different options. Thus, the isolation of further better hyperproducing mutants could have been attempted (document (31), page 1112); alternatively, enzymes other than β -glucosidase could be used to produce glucose (document (22), abstract, last sentence). This situation was not that commonly qualified of "a one-way street". In fact, the combination of using T.reesei as a host for recombinant fungal β -glucosidase genes would have been quite unexpected. Some β -glucosidase genes from different origins had been isolated but none of them had been re-transferred to a filamentous fungus (documents (3), (25) and (27)). As for the available vector systems described in documents (32) or (33), the promoters which they carried were not suited for the expression of a β -glucosidase gene, some of them (amG, gpd) being too weak; another, the T.reesei cbh1 promoter, already belonging to the cellulolytic pathway.

In addition, what was known of the regulatory mechanisms affecting the cellulolytic pathway at the priority date would have deterred the skilled person

from increasing the amount of β -glucosidase produced by T.reesei. Sophorose was thought to induce cellulase synthesis while glucose repressed it (documents (16) or (17)) and β -glucosidase had been disclosed as cleaving sophorose (document (18)). Accordingly, if the β -glucosidase level was increased, it would have been expected that the sophorose level would decrease and the glucose level would increase, leading to an enhanced repression of the cellulolytic process overall. With this expectation the skilled person would have certainly refrained from cloning an extra copy of a β -glucosidase gene in T.reesei for the purpose of increasing cellulose degradation.

In summary, the skilled person may have embarked on the cloning of a fungal β -glucosidase gene in T.reesei for the purpose of solving the above mentioned problem but not with a reasonable expectation of success. It was only with the benefit of hindsight that the reverse conclusion could be reached. Inventive step had to be acknowledged.

- Cloning of the T.reesei bgl1 gene

Document (8) could be considered as the closest prior art as it taught how to obtain the T.reesei Bgl1 protein in purified form. Starting from document (8), the problem to be solved could be defined as providing the T.reesei bgl1 gene. There was no less than six documents on file which related to the cloning of genes encoding enzymes involved in the cellulolytic pathway (eg. documents (25), (26), (27)). All these documents described strategies which were different from that which led the inventors to the successful cloning of the T.reesei bgl1 gene. It could not be said that the

earlier strategies rendered obvious this last cloning since they had not been shown to work with said gene. In the same manner, the general outline of the cloning method used in the patent in suit may have been a matter of common general knowledge but a number of features had been included in the method which made it unique and, therefore, inventive. The inventors had used specific sets of oligonucleotides as primers. They had monitored the level of mRNA in the T.reesei culture by following the amount of carbohydrase produced, which would not have been considered useful as the regulatory pathways for the synthesis of carbohydrase and β -glucosidase were thought to be different (document (16)). Most importantly, they had grown T.reesei in a medium containing sophorose as a means of increasing the level of β -glucosidase mRNA available to start the cloning. This step had never been taken before even in document (8) which described the purification of the Bgl1 protein - which process would have benefited from a large amount of the protein ie. mRNA being synthesized. In fact, the art taught that the inducer of β -glucosidase synthesis was methyl- β glucoside and not sophorose (document (18)).

For these reasons, the cloning of the T.reesei bgl1 gene required inventive step. The claimed subject-matter which involved this gene fulfilled the requirements of Article 56 EPC.

XIII. Appellant II's submissions in writing and during oral proceedings insofar as relevant to the present decision may be summarized as follows:

Main request and auxiliary request 1; claim 1

Articles 87 and 54 EPC; priority, novelty

The T.reesei bgl1 gene sequence shown in Figure 1 of the patent in suit differed by two amino acids from that claimed in the priority document US 625140. Furthermore, as shown in a copy of the priority document certified by the USPTO, Figure 1 of this document - which also provided the bgl1 sequence and was argued by appellant I to contain these two amino acids - was hardly readable. Therefore, the bgl1 gene did not enjoy priority. The consequence thereof was that document (15) published in the priority interval was prior art. As it disclosed the same bgl1 sequence as that in Figure 1 of the patent in suit, it was detrimental to the novelty of claim 1 and of any other claims referring to the T.reesei bgl1 gene.

Article 56 EPC; inventive step

- Cloning of a β -glucosidase gene in T.reesei

The closest prior art was document (29) which related to "Classical and molecular genetics applied to *Trichoderma reesei* for the selection of improved cellulolytic industrial strains". It mentioned that T.reesei was the most efficient and best characterized producer of cellulases and that β -glucosidase was the rate limiting activity in the cellulolytic complex. It taught that improvements had been carried out in the art to alleviate the problem, namely the addition of β -glucosidase to the cellulase composition obtained from T.reesei or the isolation of T.reesei mutants which overproduced the enzyme. On pages 137, 142 and 148, a further solution was envisaged which was to construct a

strain in which β -glucosidase expression would be increased as the result of the presence in T.reesei of an extra β -glucosidase cloned gene.

The problem to be solved could be defined as putting the suggestion of document (29) into practice. This required an expression system for the β -glucosidase gene as well as such a gene.

Documents (32) and (33) described expression systems for T.reesei. In the first document, the expression plasmid comprised the amdS or argB gene of Aspergillus nidulans (A.nidulans) as the selective marker and the A.nidulans gpd promoter for the expression of the gene of interest. In the second document, the selective marker was the amdS gene and chymosin was expressed under the control of the Aspergillus niger (A.niger) amG or T.reesei cbh1 promoter. Thus, vector systems for cloning and expression in T.reesei were known in the art.

At the priority date, bgl1 genes had already been isolated from various fungal sources (document (3), from A.niger; document (25), from Sclerotinia sclerotorium (S.sclerotorium); document (27), from Talaromyces emersonii (T.emersonii)).

Taking into account the knowledge in the prior art, the cloning of a β -glucosidase gene in T.reesei could be achieved in an obvious manner.

- Cloning of the T.reesei bgl1 gene

The cloning of the T.reesei bgl1 gene remained to be achieved. Yet, the cloning method used in the patent in suit was that described in document (49), a textbook

disclosing the common general knowledge relative to cloning at the priority date. The purification of the Bgl1 protein which was necessary to design the relevant probes for cDNA cloning had already been described in document (8).

Appellant I argued that the use of sophorose to induce the expression of the T.reesei bgl1 gene in order to increase the number of β -glucosidase mRNA molecules which served as templates for synthesizing β -glucosidase cDNA was to be regarded as a key feature of the cloning process, which imparted inventive step. Yet, sophorose induction was an obvious step to take. Documents (8) and (17) both described sophorose as a β -glucosidase inducer. As for document (18), it taught that sophorose had a repressive effect on β -glucosidase induction. Yet, this was within a very specific experimental setting where another β -glucosidase inducer was used simultaneously. It did not provide a reliable teaching as to the effect of sophorose per se.

Furthermore, there were other ways in which the T.reesei bgl1 gene could be cloned. For example, it could be retrieved from T.reesei by using the DNA from other β -glucosidase genes as a probe. Indeed, document (26) taught the sequence of the yeast Schizophyllum commune Bgl1 protein and its homology to the β -glucosidase of other fungi. One would expect in a similar manner that T.reesei β -glucosidase would have homology to other β -glucosidases. This implied an homology at the DNA level which would allow the design of heterospecific probes for the cloning of any one β -glucosidase gene including that from T.reesei.

Thus, all the tools and techniques were available to transfer an extra copy of a β -glucosidase gene in T.reesei including a copy of the T.reesei bgl1 gene itself.

There was no doubt that the skilled person would have had a reasonable expectation of success when carrying out the experiment. Adding gene copies was a well tried method to increase protein production. There was nothing surprising in using T.reesei as a host. The controversy about induction by sophorose was essentially hypothetical since the teaching of document (18) would have been regarded as superseded by that of the later published document (17).

In conclusion, the cloning of a β -glucosidase gene in T.reesei was obvious from the combination of the teachings of document (29) with those of documents (32) or (33) and of documents (3), (25) or (27). The cloning of the T.reesei bgl1 gene per se was obvious starting from document (29) combined with the skilled person's general knowledge (document (49)) or from the teachings in document (26) which enabled the design of suitable DNA probes for cloning.

Neither the main request nor auxiliary request 1 fulfilled the requirements of Article 56 EPC.

XIV. Appellant I (patentee) requested that the decision under appeal be set aside and that the patent be maintained on the basis of the main request filed on 10 August 2009 or auxiliary request 1 filed as auxiliary request 4 on 10 August 2009.

Appellant II (opponent) requested that the decision under appeal is set aside and the patent be revoked.

Reasons for the decision

Main request

Articles 123(2) and 84 EPC; formal requirements

1. Claim 1 of the main request differs from granted claim 1 in that the β -glucosidase is qualified as being extracellular, the filamentous fungus is identified as T.reesei or the β -glucosidase gene is identified as a bg11 gene derived from Trichoderma reesei. The same amendments have been carried out in claims 2, 3, 5 and 12 as necessary. Claim 22 is now directed to "a nucleotide sequence of a bg11 gene which entire sequence or a portion is labelled for use as a probe" wherein the bg11 sequence has the nucleotide sequence of Figure 1 (rather than "is or is not labelled" for use as a probe in granted claim 22). Appellant II did not raise any formal objections against the amended claims. The board is of the opinion that they fulfil the requirements of Articles 123(2) and 84 EPC.

Articles 87 and 54 EPC; priority, novelty

2. The priority document US application No. 625 140 provides two different sequences for the T.reesei bg11 gene in claim 40 and in Figure 1, respectively. The sequence in claim 40 differs by two amino acids from that given in Figure 1 of the patent in suit. Figure 1 of the priority document on file is not readable. At oral proceedings, appellant I produced a copy of Figure 1 of the priority document (extracted from application US 462 090, a continuation of the priority application US 625 140) which unambiguously shows that the problem had been at the level of photocopying and

that the T.reesei bgl1 sequence in Figure 1 of the priority document is the same as the sequence in Figure 1 of the patent in suit, this being in both cases indicated as the sequence of the entire T.reesei bgl1 gene. It is, thus, concluded that priority rights may be acknowledged to the T.reesei bgl1 gene.

3. Novelty had been challenged on the basis of the disclosure of the bgl1 sequence in document (15) published in the priority interval. Since the claimed subject-matter enjoys priority, this document may not be taken into account. None of the cited prior art discloses the claimed subject-matter. Novelty is acknowledged.

Article 56 EPC; inventive step

4. Claim 1(a) comprises two separate embodiments, the inventive step of which must be assessed independently. The first embodiment reads:

"A process for modifying the expression of extracellular β -glucosidase in a filamentous fungus comprising transforming said fungus with an expression vector containing a fungal DNA sequence which:

(a) is capable of enhancing the expression of extracellular β -glucosidase through the presence of at least one additional copy of a fungal extracellular β -glucosidase gene **wherein the filamentous fungus is Trichoderma reesei**."

5. The closest prior art is document (29) concerned with "Classical and molecular genetics applied to *Trichoderma reesei* for the selection of improved

cellulolytic industrial strains". It teaches on pages 135 and 137 that:

"The fungus *Trichoderma reesei* is generally considered as the most efficient and the best characterized producer of cellulases... It is of general acceptance that β -glucosidase, the enzyme hydrolysing cellobiose and short oligocellodextrins is a rate limiting activity in *T.reesei* cellulolytic complex."

It also discloses that genetic improvement programs had been undertaken which had led to the isolation of high yielding T.reesei cellulolytic strains in the form of β -glucosidase mutant hyperproducers. The suitability of increasing the number of β -glucosidase gene copies as an alternative means for β -glucosidase overproduction is clearly envisaged on page 137:

"The recently appeared molecular genetic methodologies offer new possibilities such as introducing a novel function encoded by a foreign gene, amplifying a gene ...",

and, at the end of the article:

"A classical program of strain improvement by mutagenesis ... has resulted in the isolation of mutant strains with a two-fold increase of the β -glucosidase activity at the shake flask level. This result is a challenge for molecular biologists to construct a strain with a still better β -glucosidase activity without altering the cellulase productivities as a consequence of an amplified or a better expressed β -glucosidase cloned gene."

Nonetheless, the following question is raised on page 137:

"Considering the performances reached by some of the mutants already isolated, what further improvements could be expected by mutation and molecular genetics ?

Furthermore, a failed attempt at cloning and expressing in T.reesei the S.cerevisiae invertase gene is described (pages 134 and 135).

6. Starting from document (29), the problem to be solved can be defined as increasing the productivity of the T.reesei cellulolytic complex.
7. The solution provided is to modify the expression of β -glucosidase by expressing in the fungus at least one further copy of a β -glucosidase gene.
8. In the board's judgment, the teachings in document (29) provide a clear incentive for the skilled person to try and implement this solution. More specifically, the board does not see the question raised by the authors (see point 5 supra) as intended to throw doubts on the suitability of cloning in T.reesei an extra copy of a β -glucosidase gene in order to increase its cellulolytic capacities. It rather reflects a scientific curiosity as to how far "the system could be pushed". The same opinion is held in relation to the term "challenge" used in the conclusion part of the article in relation to cloning. As regards the failed attempt at expressing the S.cerevisiae invertase gene in T.reesei, the reasons therefor are identified in document (29) itself as being the specific experimental

conditions used (degrading enzymes, heterologous promoter). Thus, it would not have had any bearing on the skilled person's assessment of the potential relevance of cloning a β -glucosidase gene in T.reesei to enhance its cellulolytic capacities.

9. The question which must be answered is whether or not the cloning and expression of a β -glucosidase gene in T.reesei was per se an endeavour which required inventive step. In order to achieve such a goal, it is, of course, necessary to have a DNA sequence encoding the protein and a vector DNA capable of carrying it into T.reesei and allowing its expression from a regulatory sequence recognized as such by the fungus.
10. At the priority date, three DNAs encoding extracellular β -glucosidases had already been cloned from A. niger (document (3)), S. sclerotiorum (document (25)) and T. emersonii (document (27)). Nothing particular is disclosed in these documents which could have raised doubt as to the feasibility of re-isolating the genes. While it is true that none of the documents suggests to transfer the genes to T.reesei, it would be wrong to take this absence of suggestion as implicit evidence that difficulties would have been expected when doing so. It is most probably simply due to the fact that the authors of these documents had scientific interests other than T.reesei.
11. Documents (32) and (33) disclose transformation systems for T.reesei. Each of these systems is composed of a vector capable of integration into the T.reesei chromosomal DNA comprising a selection marker (A.nidulans amdS or argB) and a promoter downstream of

which the gene of interest should be cloned, namely the A.nidulans gpd promoter (document (32); expression of β -galactosidase), the A.niger amg promoter or the T.reesei cbh1 promoter (document (33); expression of chymosin). After transformation of T.reesei, expression of the cloned genes was observed in every case although to various extents, the T.reesei cbh1 promoter being the most efficient.

It is true that in these documents a β -glucosidase gene was not chosen as the gene to be expressed. Yet, as already just above remarked, the fact that one route has not been chosen does not necessarily imply that it was regarded as fraught with difficulties.

12. Thus, cloning and expression systems for T.reesei were known at the priority date. Appellant I argued that the skilled person would have been reluctant to use the efficient T.reesei cbh1 promoter to express an extra copy of a β -glucosidase gene in T.reesei because in vivo this promoter is that of a cellobiohydrolase gene which is itself part of the cellulolytic pathway. The board is not convinced by this argument in the absence of any technical evidence as to why this specific choice of promoter would have a negative incidence on overall gene expression.

13. Along the same lines, appellant I argued that the skilled person would have had no reasonable expectation of success of obtaining a T.reesei strain with enhanced cellulolytic activity when expressing an extra copy of a β -glucosidase gene in T.reesei because the regulation mechanisms governing the cellulolytic pathway were very poorly understood at the time. Nonetheless, appellant I considered that the information provided in documents

(16) to (18) would be regarded as suggesting that an increased amount of β -glucosidase - as obtained by molecular engineering - would necessarily lead to a switch-off of the cellulolytic pathway, that is to the opposite result to the one wished for.

14. The board is not convinced that the prior art including documents (16) to (18) may be interpreted in a straightforward and simple manner. Document (8) (page 333) teaches that sophorose induces the full complement of cellulolytic enzymes including β -glucosidases. Document (16), page 1481 refers to sophorose as inducing cellulases - yet apparently not including β -glucosidase - and as being formed in vivo by constitutively expressed β -glucosidase. It also teaches that β -glucosidase biosynthesis is not under the same control as cellulases (page 1486). Document (17) mentions that β -glucosidase is induced by sophorose (page 249). Document (18) teaches that sophorose can completely block the induction of β -glucosidase under very specific circumstances (page 1197). The skilled person would not have derived from such scant and somewhat confused state of the art any expectations, whether positive or negative, as to what might be the result of increasing β -glucosidase amounts in T.reesei - by expressing an extra copy of the β -glucosidase gene.
15. A contrario, the skilled person knew from document (29) that T.reesei mutants overexpressing β -glucosidase existed which produced better yields of glucose from cellulose. Thus, prima facie, and in the absence of any evidence otherwise, it would have been reasonable to expect that enhanced amounts of β -glucosidase achieved through genetic engineering may have the same effect.

16. In conclusion, the board regards document (29) as providing the incentive for improving the cellulolytic properties of T.reesei by adding and expressing at least an extra copy of the β -glucosidase gene in the fungus. The combination of the teachings of document (29) with those of document (32) or (33) (expression vectors) and of any of documents (3), (25) or (27) (fungal extracellular β -glucosidase genes) renders obvious the subject-matter of claim 1.
17. The main request is rejected for failing to fulfil the requirement of Article 56 EPC.

First auxiliary request

18. Claim 1 of this request is limited to the expression in any fungus of the T.reesei bgl1 gene (claim 1(a)) or of a derivative thereof obtained by manipulating said bgl1 DNA sequence (claim 1(b)). The conclusion reached in points 1 to 3, supra that claim 1 of the main request fulfils the requirements of Articles 123(2), 84, 87 and 54 EPC equally applies here, for the same reasons. The issue which remains to be decided is that of inventive step.
19. The closest prior art is document (29) which, as already mentioned above (point 5, supra), discloses the enzyme β -glucosidase as being a rate limiting enzyme in the T.reesei cellulolytic pathway. Document (29) suggests that this pathway may be rendered more efficient if the expression of β -glucosidase is enhanced and one possible way to do this is identified

as isolating a β -glucosidase gene, then cloning and expressing it in the cellulolytic fungus (page 148).

20. Starting from the closest prior art, the problem to be solved can be defined as putting the suggestion of document (29) into practice.
21. The solution provided is the isolation of the T.reesei bgl1 gene and its transfer and expression into a cellulolytic fungus.
22. The question to be answered is whether or not retrieving the T.reesei bgl1 gene from T.reesei involves inventive step.
23. The isolation of the bgl1 gene was achieved as follows. Total T.reesei mRNA was purified on an oligodT column and transcribed into cDNA with reverse transcriptase and DNA polymerase. Amplification of the cDNA fragments encoding a portion of the bgl1 gene was done using the polymerase chain reaction, the necessary oligonucleotide primers being designed on the basis of a selected N-terminal amino acid sequence and an internal sequence of the Bgl1 protein. A 700bp cDNA fragment was, thus, obtained which was used as a probe to isolate a 6kb genomic DNA fragment comprising the entire bgl1 coding sequence along with sequences necessary for gene transcription and translation (patent in suit, sections [0042] to [0066]).
24. Inasmuch, the cloning technique follows the common general knowledge at the priority date as described in document (49). However, it is not limited to the above general outline. The culture taken as a source of total

RNA is specifically induced for cellulase production by sophorose and care is taken that the RNA is only extracted after the induction of the cellulase pathway has taken place (confirmed by the presence of carbohydrolase 2 mRNA). Appellant I contends that this way of proceedings is one of the reasons why the cloning was successful. In this respect, document (50) is cited. This is a declaration from the scientist who undertook this cloning. It discloses that earlier attempts at cloning the gene involving the standard technique of probing a T.reesei genomic DNA library with pools of degenerate oligonucleotides designed on the basis of the amino acid sequence of a portion of the bgl1 gene had failed.

25. Here, a number of observations must be made:

- As already mentioned point 10 supra, three fungal β -glucosidase genes had already been cloned at the priority date. In documents (3) and (25), it is disclosed that the β -glucosidase genes from A.niger and S.sclerotium were obtained by cloning genomic DNA in respectively, S.cerevisiae or E.coli, the clones containing the β -glucosidase genes being screened by their ability to produce β -glucosidase. In document (27), the same approach is used except for the fact that it is a cDNA library which is made from T.emersonii. The positive recombinant clones are also screened for having β -glucosidase activity. This is akin to the technique said to have failed for the cloning of the bgl1 gene in document (50), except for the fact that in the latter case the DNA was tested for its homology to bgl1 DNA rather than for its capacity to express a functional β -glucosidase gene.

Accordingly, this prior art does not deprive the cloning of the bgl1 gene of inventive step.

- There would have been no reason for the skilled person to initiate the cloning of the β -glucosidase gene by growing the starting T.reesei strain in the presence of sophorose. As already mentioned in point 14, supra, the regulatory mechanisms affecting the biosynthesis of the various enzymes involved in the degradation of cellulose to glucose were not well understood at the priority date. Even if sophorose was then considered as a cellulase inducer, its status in the regulation of β -glucosidase was not clear.

- Finally, it was argued that the cloning of the bgl1 gene could have been achieved in a much simpler way by taking advantage of the fact that β -glucosidase proteins from different fungi were known to have homology (document (26)) and, therefore, it was to be expected that the encoding DNA for anyone of them - including T.reesei β -glucosidase could be cloned by hybridisation with the DNA encoding another one as a probe. In this respect, the board notices that while Figure 4 of document (26) shows homology between β -glucosidases of C.pelliculosa and S.commune, it fails to provide information on T.reesei β -glucosidase. In any case there is no technical evidence on file that the cloning of the bgl1 gene could indeed be achieved in this way.

26. For these reasons, the board concludes that the cloning of the T.reesei bgl1 gene is inventive and, therefore, that the claimed subject-matter which involves said gene (claim 1(a)) or a derivative obtained by

manipulating its sequence (claim 1(b)) fulfils the requirements of Article 56 EPC.

27. The decision of the opposition division contained a section on sufficiency of disclosure which did not concern the T.reesei β -glucosidase gene per se. During appeal proceedings, the sufficiency of disclosure as regards this specific gene (derivative thereof) was not challenged. The board also considers that the claimed subject-matter can be reproduced on the basis of the teachings of the patent in suit comprising, in particular, the sequence of the bgl1 gene.

Order:

For these reasons, it is decided that:

1. The decision under appeal is set aside.
2. The case is remitted back to the first instance with the order to maintain the patent based on auxiliary request 1 and a description and drawings to be adapted thereto.

The Registrar

The Chairman

A. Wolinski

L. Galligani