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D E C I S I O N
of 18 January 2006

Case Number: T 0717/03 - 3.3.09

Application Number: 98940861.2

Publication Number: 1003379

IPC: A23B 7/10

Language of the proceedings: EN

Title of invention:
Novel phytase

Applicant:
DIVERSA CORPORATION

Opponent:
-

Headword:
-

Relevant legal provisions:
EPC Art. 54, 56

Keyword:
"Novelty (yes - after amendment)"
"Inventive step (yes, new experimental results)"

Decisions cited:
-

Catchword:
-

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Case Number: T 0717/03 - 3.3.09

D E C I S I O N
of the Technical Board of Appeal 3.3.09
of 18 January 2006

Appellant: DIVERSA CORPORATION
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Decision under appeal: **Decision of the Examining Division of the European Patent Office posted 17 February 2003 refusing European application No. 98940861.2 pursuant to Article 97(1) EPC.**

Composition of the Board:

Chairman: P. Kitzmantel
Members: J. Jardón Alvarez
M.-B. Tardo-Dino

Summary of Facts and Submissions

I. This appeal lies from the decision of the Examining Division dated 17 February 2003, refusing European patent application 98 940 861.2, published as WO-99/08539 on 25 February 1999.

II. The decision under appeal was based on Claims 1 to 18 filed on 30 April 2002. Claims 1 and 18 read as follows:

"1. Substantially pure phytase having an amino acid sequence consisting of SEQ ID NO:2.

18. An enzyme selected from the group consisting of:

a) an enzyme comprising an amino acid sequence which is identical to the amino acid sequence set forth in SEQ ID NO:2; and

b) an enzyme which comprises at least 30 consecutive amino acid residues homologous with an enzyme of a); wherein the enzyme is a phytase."

III. According to the decision of the Examining Division, the claimed subject-matter was not novel and did not involve an inventive step, in the light of the disclosures of:

D1: R. Greiner et al., Archives of Biochemistry and Biophysics, vol. 303, No. 1, 15 May 1993, pages 107 to 113;

D4: J. Dassa et al., Journal of Bacteriology, Sept. 1990, pages 5497 to 5500; and

D7: S. Golovan et al., Can. J. Microbiol. 46: 2000, 59 to 71.

In particular, the Examining Division held that the subject-matter of Claim 18b) lacked novelty in view of D4, which disclosed the nucleotide sequence of the E. coli gene appA, which encoded a periplasmic acid phosphatase. Having in mind that the phytase claimed in the application and said phosphatase have more than 99% amino acid sequence identity, they must have the same biological activity and consequently D4 was novelty destroying for this subject-matter. The Examining Division pointed out that it was not relevant that the phytase activity was not explicitly disclosed in D4, since it was an inherent property of the enzyme. The Examining Division also noted that post-published document D7 confirmed the phytase activity of the phosphatase of D4.

Concerning inventive step, the Examining Division held that starting from D1 as the closest prior art the problem to be solved by the application was the provision of a polynucleotide sequence and of the encoded polypeptide sequence from E. coli, this polypeptide having phytase activity.

In its opinion the skilled person confronted with this problem would be prompted, in view of the teaching of D1 relating to the purification and characterization of two phytases for E. coli, to make use of the sequence data available from D4 in order to identify closely related sequences. In the absence of any advantageous and/or surprising properties as compared to the

phytases known e.g. from D1, an inventive step could not be recognized for the claimed subject-matter.

IV. The Notice of Appeal was filed on 22 April 2003 and the appeal fee was paid on the same day. The statement setting out the Grounds of Appeal was filed on 17 June 2003.

V. In the communication of 17 October 2005 pursuant to Article 110(2) EPC the Board informed the Appellant that the subject-matter of Claim 18b) was considered to lack novelty having regard to the disclosure of D4. The Board also indicated that the subject-matter of the other claims involved an inventive step.

VI. In reply thereto, on 30 November 2005, the Appellant submitted three sets of claims as bases for a new main and two auxiliary requests, all amended in a manner to deal with the aforementioned lack of novelty objection.

By letter dated 15 December 2005 the Appellant withdrew these main and first auxiliary requests and maintained the second auxiliary request as the only request.

The 17 claims of this request read as follows:

"1. Substantially pure phytase having an amino acid sequence consisting of SEQ ID NO:2.

2. An isolated polynucleotide sequence encoding a phytase of claim 1.

3. An isolated polynucleotide selected from the group consisting of:

a) SEQ ID NO:l;

b) SEQ ID NO:1, wherein T can also be U.

4. The polynucleotide of claim 2, wherein the polynucleotide is isolated from a prokaryote.

5. An expression vector including the polynucleotide of claim 2.

6. The vector of claim 5, wherein the vector is a plasmid.

7. The vector of claim 5, wherein the vector is a virus-derived.

8. A host cell transformed with the vector of claim 5.

9. The host cell of claim 8, wherein the cell is prokaryotic.

10. Antibodies that bind to the polypeptide of claim 1.

11. The antibodies of claim 10, wherein the antibodies are polyclonal.

12. The antibodies of claim 10, wherein the antibodies are monoclonal.

13. A method for producing an enzyme comprising growing a host cell of claim 8 under conditions which allow the expression of the nucleic acid and isolating the enzyme encoded by the nucleic acid.

14. A method for degrading phytate comprising contacting phytate with an amount of the enzyme of claim 1 required to degrade at least 50% of the phytate as compared to phytate not contacted with the enzyme.

15. A method for hydrolyzing the phospho-mono-ester bond in phytate comprising contacting the phytate with an amount of the enzyme of claim 1 required to hydrolyze at least 50% of the phospho-mono-ester bonds as compared to phytate not contacted with the enzyme.

16. An animal feed composition comprising a phytase having an amino acid sequence as set forth in SEQ ID NO:2.

17. The antibody of claim 10, wherein the antibody is detectably labeled."

VII. The arguments put forward by the Appellant in its written submissions can be summarized as follows:

D4 did not describe the phytase activity of the phosphatase therein disclosed. Such phytase activity was only disclosed 10 years later, when the authors of D7 analyzed the catalytic properties of the acid phosphatase from E. coli. Moreover the phytase of the present invention and the enzyme disclosed in D4 differed from each other since they were derived from different E. coli strains.

Concerning inventive step the Appellant submitted that the claimed phytase showed improved properties vis-à-vis the combined teaching of D1 and D4. The Appellant filed summary charts of predicted protease cleavage

sites showing that the claimed phytase has additional chymotrypsin, pepsin, thermolysin and trypsin protease cleavage sites as compared to the phytase derived from E. coli K12. In the Appellant's view, these additional protease cleavage sites provide biological, commercial and environmental advantages over the phytase of the prior art.

VIII. Since the Board intended to allow the new main request, it was no longer necessary to schedule an oral procedure.

IX. The Appellant requested that the decision of the Examining Division be set aside and that a patent be granted on the basis of Claims 1 to 17 filed as second auxiliary request with letter of 30 November 2005 and on the basis of the description pages 1, 2, 4, 6, 7, 10 to 14, 17 to 26, 28 to 31 and 34 as originally filed; and pages 3, 5, 8, 9, 15, 16, 27, 32 and 33 as filed with letter of 26 October 2001 and drawings 1/3, 2/3 as originally filed and 3/3 as filed with letter of 26 October 2001. Alternatively, it requested to remit the application to the Examining Division for further prosecution.

Reasons for the Decision

1. The appeal is admissible.

2. *Amendments (Article 123(2) EPC)*

2.1 Claims 1 to 13, 16 and 17 correspond to originally filed Claims 1 to 13, 17 (in combination with 16) and

28 respectively. Claims 14 and 15 correspond to Claims 14 and 15 as originally filed wherein the "effective amount of enzyme" has been defined in accordance with page 31, lines 13 to 16 and 20 to 22 of the description.

2.2 Therefore, the subject-matter of the claims meets the requirements of Article 123(2) EPC.

3. *Novelty (Article 54 EPC)*

3.1 The Examining Division rejected the application because of lack of novelty of the subject-matter of the then pending Claim 18b), relating to "an enzyme comprising at least 30 consecutive amino acid residues homologous of an enzyme comprising an amino acid sequence which is identical to the amino acid sequence set forth in SEQ ID NO:2". This subject-matter is no longer comprised by the present claims.

3.2 The Examining Division did not raise any novelty objections against the remaining claims, which are the basis for the present set of claims. The Board agrees with this finding of the Examining Division because none of the cited documents discloses a phytase having an amino acid sequence consisting of SEQ ID NO:2 or a polynucleotide sequence (SEQ ID NO:1) as now claimed.

3.3 The subject-matter of the claims is thus novel (Article 54 EPC).

4. *Inventive step (Article 56 EPC)*

4.1 Closest prior art

4.1.1 The patent in suit concerns a recombinant phytase isolated from *Escherichia coli* B. Phytases are found naturally in plants and microorganisms, particularly fungi. They are capable of catalyzing hydrolysis of *myo*-inositol hexaphosphate to D-myo-inositol 1,2,4,5,6-pentaphosphate and orthophosphate. As acknowledged in the description of the present application (see pages 1 to 3) phytases are used as feed supplements to enhance plant phosphorus utilization.

4.1.2 The Board agrees with the finding in the appealed decision that document D1 represents the closest prior art. It describes the purification and characterization of two phytases, called P1 and P2, from *Escherichia coli* (see abstract and pages 112 to 113 under Discussion). The phytase P2 corresponds very closely to the enzyme described in D4 as a "pH 2,5 acid phosphatase" (see D1, page 112, left column, second paragraph; see also D4 Abstract). The complete nucleotide sequence of this phosphatase is given in D4 (see Figure 2).

4.2 Problem and solution

4.2.1 The phytase according to Claim 1 of the present application differs from said known phytase in the amino acid sequence. Although the claimed amino acid sequence presents a high degree of homology with the amino acid sequence of D4, they differ at positions 298 and 299, in that the claimed sequence has the residues

methionine and alanine while the sequence in D4 has lysine and threonine. Moreover the phytase of the present application is derived from *Escherichia coli* B while the enzyme disclosed in D4 is derived from *Escherichia coli* strain K12, as can be seen from post-published document D7 (see page 60, right column, penultimate paragraph).

4.2.2 During the appeal proceedings the Appellant submitted summary charts comparing the predicted protease cleavage sites of both protein sequences in order to demonstrate the advantageous properties of the claimed phytase. Thus, the claimed phytase has additional chymotrypsin, pepsin, thermolysin and trypsin protease cleavage sites and the presence of these additional cleavage sites results in less release of undigested phytase.

4.2.3 The technical problem underlying the present application can thus be seen as the provision of a novel phytase to be used as animal feed, which phytase increases the availability of phosphorous while at the same time resulting in less undigested phytase being released to the ecosystem.

4.2.4 The Examining Division did not have the benefit of the further evidence submitted by the Appellant during the appeal proceedings (see above 4.2.2.) and defined the technical problem to be solved merely as the provision of a polynucleotide sequence and the encoded polypeptide sequence from E. coli, this polypeptide having phytase activity. This definition of the problem is no longer valid having regard to the new evidence on file.

4.2.5 The problem underlying the present application is credibly solved by the recombinant phytase according to Claim 1. The claimed phytase can be used as a supplement in animal feed to catalyze the conversion of phytate to inositol and inorganic phosphate, significantly reducing the need for inorganic phosphorous supplementation in monogastric animal feed. The presence of additional protease cleavage sites, when compared with the known phytase according to D4/D1, which may be broken down further in gastric juices (e.g. by pepsin) avoids the potential spread of recombinant genetic material to the ecosystem.

4.3 Inventive step

4.3.1 There is no hint to these advantageous properties of the claimed phytase in the available prior art. On the contrary, having regard to the high degree of amino acid sequence homology between both phytases, the skilled person would have expected similar properties.

4.3.2 For these reasons the subject-matter of Claim 1, involves an inventive step (Article 56 EPC).

4.3.3 The subject-matter of the remaining claims, which also include the use of the phytase of Claim 1, involves an inventive step for the same reasons.

5. For the reasons given above, the present claims can form the basis for grant. However, it remains necessary to adapt the description to the claims and to acknowledge the closest prior art document. In the circumstances, it appears expedient that this should be

done before the Examining Division in accordance with the alternative request of the Appellant.

Order

For these reasons it is decided that:

1. The decision under appeal is set aside.

2. The case is remitted to the Examining Division with the order to grant the patent on the basis of the claims filed 30 November 2005, after appropriate amendments to the description.

The Registrar: The Chairman:

G. Röhn P. Kitzmantel