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D E C I S I O N
of 17 March 2005

Case Number: T 0090/03 - 3.3.8

Application Number: 96202943.5

Publication Number: 0779037

IPC: A23K 1/165

Language of the proceedings: EN

Title of invention:
Cloning and expression of microbial phytase

Applicant:
BASF AKTIENGESELLSCHAFT

Opponent:
-

Headword:
Phytase/BASF

Relevant legal provisions:
EPC Art. 76(1), 123(2), 54

Keyword:
"Subject-matter extending beyond the content of earlier application (no)"
"Added subject-matter (no)"
"Novelty (yes)"

Decisions cited:
T 0767/95, T 0990/96, T 0797/02

Catchword:
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Case Number: T 0090/03 - 3.3.8

D E C I S I O N
of the Technical Board of Appeal 3.3.8
of 17 March 2005

Appellant: BASF AKTIENGESELLSCHAFT
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Decision under appeal: **Decision of the Examining Division of the European Patent Office posted 26 August 2002 refusing European application No. 96202943.5 pursuant to Article 97(1) EPC.**

Composition of the Board:

Chairman: F. L. Davison-Brunel
Members: P. Julia
S. C. Perryman

Summary of Facts and Submissions

I. The applicant (appellant) lodged an appeal against the decision of the examining division dated 26 August 2002 whereby the European patent application No. 96 202 943.5 (published as EP-A-0 779 037) with the title "Cloning and expression of microbial phytase" was refused pursuant to Article 97(1) EPC on grounds of lack of novelty (Article 54 EPC). The application was a divisional application of the earlier application No. 90 202 565.9 (published as EP-A-0 420 358) in accordance with Article 76 EPC.

II. Claim 1 of the divisional application as filed read as follows:

"1. *A composition comprising a fungal phytase which catalyses the liberation of at least one inorganic phosphate from a myoinositol phosphate, and wherein the phytase is encoded by a DNA sequence that hybridizes under conditions of low stringency (6 x SSC; 50°C overnight) with a probe comprising nucleotide positions 1 - 818 of Figure 8, and characterized in that the composition is substantially free of an Aspergillus acid phosphatase with an apparent molecular weight on SDS-PAGE of 100 kDa and with at its N-terminus the amino acid sequence: Val Val Asp Glu Arg Phe Pro Tyr Thr Gly.*"

III. The decision under appeal was based on claims 1 to 4 filed on 24 May 2002, wherein claim 1 read as follows:

"1. *An Aspergillus ficuum phytase exhibiting the following characteristics:*

(a) it provides a single band at 85 kDa on SDS-PAGE;
(b) it has a molecular weight after deglycosylation in the range of about 48-56.5 kDa;
(c) it has a specific activity of about 100 U/mg protein, wherein a unit (U) is defined as that amount of enzyme which liberates inorganic phosphorus from 1.5 mM sodium phytate at the rate of 1 µmol/min at 37°C and at pH 5.50."

Claim 2 was directed to a composition comprising an *Aspergillus ficuum* phytase which exhibited the characteristics (a) and (b) of claim 1 and wherein the composition had the specific activity defined in part (c) of claim 1. Claims 3 and 4 were further embodiments of claims 1 and 2 defining a (first and second) pH optimum of the phytase.

IV. The appellant filed an appeal and submitted a statement of grounds of appeal, wherein claims 1 to 4 before the examining division were maintained.

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

VI. Oral proceedings took place on 17 March 2005. At the beginning of these proceedings, the appellant filed a new request which differed from the previous request by deletion of claim 2 (cf. point III *supra*).

VII. The documents cited in the present decision are the following:

D1: A.H.J. Ullah, Prep. Biochem., 1988, Vol. 18(4), pages 459 to 471;

D5: A.H.J. Ullah and D.M. Gibson, Prep. Biochem., 1987, Vol. 17(1), pages 63 to 91;

D6: W.D. MacRae et al., Gene, 1988, Vol. 71, pages 339 to 348.

VIII. The appellant's arguments in writing and during oral proceedings may be summarised as follows:

Article 54 EPC (Novelty)

Document D1 disclosed the preliminary structure, substrate selectivity and kinetic characterization of the *Aspergillus ficuum* phytase. Reference was made on page 460 to document D5 which described the purification of the enzyme, allegedly to homogeneity. However, as was evident from the latter document, the "pure" phytase migrated on SDS-PAGE as a broad diffused band from 85 kDa to 100 kDa. Similarly, the native phytase was shown as a broad band above the 97.4 kDa marker in a Western blot (immunoblotting) assay (Figure 7, lanes 3 and 4).

Thus, document D5 did not identify the 85 kDa band as a phytase. To the contrary, the occurrence of a band which reached up to 100 kDa could be explained by the presence of contaminants in the phytase preparation such as an acid phosphatase as disclosed in document D6. The presence of a protein contaminant had been confirmed by the fact that peptide IV identified in

document D1 as being part of the phytase had a sequence which corresponded to a peptide sequence of the acid phosphatase disclosed in document D6. This contaminant was also clearly identified in the present application (partial sequence E in Figures 1B and 1C). The authors of document D5 had simply failed to recognize the presence of a contaminant in the (homogeneously) purified phytase.

As regards the specific activity of the phytase, Table 1 of the application provided a comparison between the phytase disclosed in the application (100 U/mg protein) and the one obtained in document D5 (50 U/mg protein). The result clearly demonstrated that both enzymes were of different purity grades.

Thus, the allegedly pure form of phytase disclosed in document D5 and/or D1 did not anticipate the subject-matter of claim 1, since it did not provide a single band at 85 kDa on SDS-PAGE and its specific activity was not of about 100 U/mg protein.

The reference to decision T 990/96 (OJ EPO 1998, 489) could not support the opinion of the examining division, since this decision only dealt with organic compounds of low molecular weight and not with bio-molecules of high molecular weight, such as the phytase. For the organic compounds conventional purification methods (distillation, (re)crystallisation, chromatography, etc.) were, as a rule, within the common general knowledge of the skilled person. Conversely, it was known that every protein required a specific purification protocol in order to be successfully separated from contaminants and to be obtained with the

desired degree of purity. These specific protocols could not simply be equated to conventional methods within the common general knowledge of the skilled person. In fact, document D5 referred to further purification of the "pure and homogeneous" phytase by an additional chromatography step. However, no significant increase (only 10%) in the specific activity of the enzyme was achieved. Thus, the rather conventional attempt of the experts in the field of phytase purification failed and led them to conclude that their phytase preparation was pure and that no further purification was possible.

In fact, the factual situation in the present case was different from that underlying decision T 990/96 (*supra*). The present case constituted such an exceptional situation as argued in T 990/96 to deserve recognition of novelty because all prior art attempts to achieve a particular degree of purity by conventional purification process had failed, i.e. the prior art did not make this compound available to the public in the desired degree of purity. In the present case, the prior art taught that the phytase could not be further purified. Therefore, for this reason alone, the claimed "further purified" phytase was novel. Contrary to the decision of the examining division, it was of no importance whether the phytase - with a higher specific activity - provided a new technical effect (use) when compared with the phytase disclosed in the prior art.

IX. The appellant requested that the decision under appeal be set aside and that the case be remitted to the first instance for further examination on the basis of

claims 1 to 3 submitted at oral proceedings on 17 March 2005.

Reasons for the Decision

Articles 76(1) and 123(2) EPC; claims 1 to 3

1. The application in suit is a divisional application of the parental application published under No EP-A-0 420 358. Claim 1 of the divisional application as filed is directed to **compositions** comprising a fungal phytase defined by the following criteria: "*catalyses the liberation of at least one inorganic phosphate from a myoinositol phosphate, and wherein the phytase is encoded by a DNA sequence that hybridizes under conditions of low stringency (6 x SSC; 50°C overnight) with a probe comprising nucleotide positions 1 - 818 of Figure 8, and characterized in that the composition is substantially free of an Aspergillus acid phosphatase with an apparent molecular weight on SDS-PAGE of 100 kDa and with at its N-terminus the amino acid sequence: Val Val Asp Glu Arg Phe Pro Tyr Thr Gly*" (cf. section II, *supra*).

2. In accordance with the case law (eg. T 797/02 of 23 September 2004), the invention or group of inventions defined in the **claims** of a divisional application determines the **content** of the divisional application per se. (Note: in that earlier case, what was at stake was the content of a parental application compared to that of a grandparental application, but the findings are directly applicable to a divisional versus a parental application). Thus, the content of

the divisional application which is to be taken into account for the purpose of assessing whether the requirements of Article 123(2) EPC are fulfilled by present claim 1 (cf. section III, *supra*) is that which relates to phytase compositions.

3. On page 3, lines 20 to 22, of the originally filed divisional application (as published), a phytase is disclosed with a molecular weight of 85 kDa and an apparent molecular weight of the deglycosylated form in the range of 48 to 56 kDa. In Table I, this phytase is described as having a specific activity of 100 U/mg of protein. Most importantly, it is this phytase which is used as a starting material for obtaining the phytase composition as claimed in claim 1 of the divisional application as filed.

4. Accordingly, although claim 1 of the divisional application as filed does not define the phytase comprised in the claimed composition by the same parameters as present claim 1, given that the relevant parts of the description (those relating to making the composition of claim 1 as filed) leave no doubt that the phytase as now claimed is an enzyme comprised within the composition, the board accepts that there is a basis in said parts of the description for a phytase as now claimed.

5. The pH optima of the enzyme of claim 1 which are the subject-matter of present dependent claims 2 and 3 are equally found in Table I of the divisional application as filed. The same reasoning as just developed for the subject-matter of claim 1 applies to claims 2 and 3.

6. Thus, the requirements of Article 123(2) EPC are fulfilled.

7. The description of the parental application is identical to the description of the divisional application. It, thus, discloses a phytase with the properties given in present claims 1 to 3 (cf. points 3 to 5, *supra*). The requirements of Article 76(1) EPC are fulfilled.

Article 54 EPC; novelty

8. Document D5 discloses the purification and characterization of a phytase from *Aspergillus ficuum* NRRL 3135. Four purification steps are referred to in this document, namely a culture filtrate centrifugation, a cationic chromatography followed by an anionic chromatography and, as a last step, a chromatofocusing (Figures 1A to 1C). The purified enzyme, when analyzed by SDS-PAGE, shows "*two broad diffused bands at approximately 85-KDa and 100-KDa (Figure 2A)*", a protein banding pattern typical of glycoproteins (cf. page 75, lines 2 to 7). Document D5 refers to this phytase as present in two different forms, both of them positively stained for carbohydrate, "*although the larger form (approximate MW 100-KDa) was stained more heavily than the smaller form (MW 85-KDa)*" (cf. page 81, first full paragraph). Deglycosylation by Endo H digestion results in "*only one broad band migrating at approximately MW 76-KDa ... only slightly smaller in molecular size than the smaller form of undigested enzyme (MW 85-KDa)*" (cf. page 81, last full paragraph). These large and small forms of phytase are identified in a Western blot (immunoblotting) too (cf. page 82).

The purified phytase has a specific activity of 2094 nKat/mg, which corresponds to 50 U/mg in the enzymatic units of the present application. An additional chromatography step results in an increase of the specific activity of only 10% (cf. page 74, last paragraph).

9. The disclosure in document D1 does not add anything to that in document D5 insofar as purification is concerned, simply making reference to the method described in said document (cf. page 460, last full paragraph). It teaches a slightly lower molecular weight for the deglycosylated phytase form (61.7 kDa; page 463, Table II), confirms the specific activity disclosed in document D1 (Table IV: 2100 nKat/mg of protein corresponding to 50 U/mg of protein), shows kinetic studies of the enzyme under various conditions and provides the primary amino acid sequence of four peptides which are said to have been obtained by reacting CNBr with the phytase preparation.

10. It follows from the foregoing that none of the three features which characterize the claimed enzyme (MW: 85 kDa, MW of the deglycosylated form: 48 - 56.5 kDa; specific activity: 100 U/mg of protein) are disclosed for the phytase preparation of the prior art. This preparation is clearly different if only because of lower degree of purity (as evidenced by a lower specific activity).

11. The decision under appeal refers to decision T 990/96 (OJ EPO 1998, 489) according to which a change in purity does not render novel a further purified product. The invention then under review was concerned with the

purification of low molecular weight organic compounds (erythro acid and related erythro compounds), which are prepared by **chemical reactions**. It is stated in T 990/96 that in preparative organic chemistry, "*conventional methods for the purification of* ***low molecular organic reaction products*** *such as recrystallisation, distillation, chromatography, etc., which normally can be successfully applied in purification steps, are within the common general knowledge of those skilled in the art*" and therefore, "*it follows that, in general, a document disclosing a* ***low molecular chemical compound*** *and its manufacture makes available this compound to the public in the sense of Article 54 EPC in all grades of purity as desired by a person skilled in the art*" (cf. point 7 of the Reasons; emphasis added by the board).

12. In contrast, the present application relates to the purification of a **high molecular weight** enzyme obtained from a crude native **biological material**. Although methods for protein purification are known to the skilled person, they are far from standardized, in the sense that a protocol adapted to the specific properties of the protein has to be established for every protein. In fact, evidence to support this point may be found in document D5 itself which shows that an additional (conventional) purification step, namely a cationic chromatography, only provides a slight increase in the degree of purity and specific activity of the purified enzyme (cf. page 74, last paragraph). Decision T 990/96 is, thus, of no relevance to this case.

13. The appropriate jurisprudence is represented by decision T 767/95 of 5 September 2000 concerning the purification of interleukin-1β (IL-1β), a high molecular weight protein (17.5 kDa). In that decision a purified homogeneous preparation of IL-1β was found to be novel over a semi-purified mixture of proteins containing IL-1β. A relevant consideration in that case was the provision of IL-1β in a degree of purity that allowed the determination of its (partial) amino acid sequence, whereas "*no analysis of the amino acid sequence of IL-1 that would provide definitive proof of the homogeneity of IL-1 preparations*" was found in the prior art (cf. point 6 of the Reasons).

14. In the present case, the skilled person is aware that the phytase preparation of document D5 is only purified to "*virtual homegeneity*" or "*near homogeneity*" (cf. pages 65 and 84, first full paragraph and last line, respectively). In Figure 1C, showing the elution profile of the last purification step, the phytase protein peak elutes as a distinct shoulder of two earlier protein peaks. The maximum of the phytase protein peak is also slightly shifted with respect to the maximum of phytase activity, a clear indication of the presence of possible contaminants (cf. page 73). Reference is also made to a phytase preparation of an even higher degree of purity after an additional purification step (cf. page 74, last paragraph). Thus, there is a very strong suspicion that the phytase is not the only protein in the phytase preparation.

15. The phytase preparation of the prior art could only be considered as being the same as the phytase of claim 1 if its sequence was the same. Yet, it was not sequenced

per se. What was done instead was to sequence isolated peptides originating from the CNBr cleavage of the "phytase preparation". Of course, one cannot be sure that each and every one of them belongs to the phytase protein and not to some protein contaminant present in the preparation. In fact, the application shows that two of the internal peptides disclosed in document D1, namely the peptides III and IV (i.e. the ones of a worse quality judging from their short length and the presence of gaps in their sequence), are not derived from phytase but from other possible contaminants. In particular, peptide IV is found in the amino acid sequence of an acid phosphatase from *A. niger* of document D6 (cf. residues 23 to 32 in Figure 5, page 343). Thus the determination of the peptide sequences in fact proves that the phytase preparation of the prior art is not in such a state of purity as to make it possible to sequence. Otherwise stated, the present situation is not different from that encountered in decision T 767/95 (*supra*) and, as in this earlier case, the board concludes that the mixture of proteins comprising phytase disclosed in the prior art does not destroy novelty.

16. The requirements of Article 54 EPC are fulfilled.

Article 56 EPC; inventive step

17. Having reached a conclusion of lack of novelty, the examining division did not assess inventive step. In order to give the party an opportunity to have this issue considered by two instances, the board decides to use its power under Article 111(1) EPC to remit the case to the first instance for further prosecution.

Order

For these reasons it is decided that:

1. The decision under appeal is set aside.

2. The case is remitted to the first instance for further examination on the basis of claims 1 to 3 submitted at oral proceedings on 17 March 2005.

The Registrar: The Chairman:

A. Wolinski F. Davison-Brunel