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Datasheet for the decision of 10 October 2013

Case Number: T 1627/09 - 3.3.08

Application Number: 99965105.2

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Language of the proceedings: EN

Title of invention:
Desaturases and methods of using them for synthesis of polyunsaturated fatty acids

Patent Proprietor:
Washington State University Research Foundation

Opponent:
E.I. Du Pont de Nemours and Company

Headword:
Desaturases/WASHINGTON

Relevant legal provisions:
EPC Art. 83
EPC R. 76(2)(c)
RPBA Art. 13(1)

Keyword:
Admissibility of opposition - (yes)
Admissibility of Auxiliary requests 1 and 6 (yes)
Admissibility of Auxiliary Requests 2 to 5, 7 and 8 (no)
Sufficiency of disclosure of Main Request and Auxiliary Requests 1 and 6 (no)

Decisions cited:
T 0653/99, T 0426/08

Catchword:

Europäisches Patentamt
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Case Number: T 1627/09 - 3.3.08

D E C I S I O N
of Technical Board of Appeal 3.3.08
of 10 October 2013

Appellant: (Patent Proprietor)	WASHINGTON STATE UNIVERSITY RESEARCH FOUNDATION NE 1615, Eastgate Boulevard Pullman, WA 99163 (US)
Representative:	Niedermeyer, Joachim Herzog Fiesser & Partner Patentanwälte Dudenstraße 46 68167 Mannheim (DE)
Respondent: (Opponent)	E.I. DU PONT DE NEMOURS AND COMPANY 1007 Market Street Wilmington DE 19898 (US)
Representative:	Dzieglewska, Hanna Eva Dehns St Bride's House 10 Salisbury Square London EC4Y 8JD (GB)
Decision under appeal:	**Decision of the Opposition Division of the European Patent Office posted on 12 June 2009 revoking European patent No. 1147174 pursuant to Article 101(3)(b) EPC.**

Composition of the Board:

Chairman: B. Stolz
Members: T. J. H. Mennessier
D. Rogers

Summary of Facts and Submissions

I. The patent proprietor (appellant) lodged an appeal against the decision of the opposition division dated 12 June 2009, whereby European patent No. 1 147 174, which had been granted on European patent application No. 99965105.2 (PCT/US99/28655) published under the international publication No. WO 00/34439, was revoked.

II. The main and the first auxiliary request before the opposition division were held unallowable for reasons of insufficiency of disclosure (Article 83 EPC), and the second auxiliary request for reasons of non-compliance with Article 123(2) EPC.

III. The patent was opposed by one opponent (respondent) on the grounds as set forth in Article 100(a) EPC (for lack of inventive step and industrial application), and Article 100(b) EPC.

IV. The appellant filed its statement of grounds of appeal which was accompanied by three new auxiliary requests. The main request corresponded to the claims as granted.

V. The opponent (respondent) replied by filing submissions which were accompanied by a new document.

VI. The Board issued a communication pursuant to Rule 15(1) of the Rules of Procedure of the Boards of Appeal (RPBA) expressing its preliminary and non-binding views.

VII. The appellant replied to the Board's communication on 10 September 2013 by filing further submissions which were accompanied by a new document and seven auxiliary requests which replaced the previous auxiliary

requests. The first and sixth auxiliary requests corresponded to respectively the first and third auxiliary requests filed with the statement of grounds. The other auxiliary requests were new.

VIII. Oral proceedings took place in the presence of the parties as scheduled on 10 October 2013. The appellant filed an eighth auxiliary request at the oral proceedings.

IX. Claim 1 of each of the requests on file reads or is drafted as follows:

Main request (claims as granted)

"1. A purified protein having desaturase activity, and comprising an amino acid sequence selected from the group consisting of:
(a) an amino acid sequence as shown in SEQ ID NO: 4; and
(b) an amino acid sequence having at least 60% sequence identity to the sequence specified in (a)."

First auxiliary request

"1. A purified protein having delta 8 desaturase activity, and comprising an amino acid sequence selected from the group consisting of an amino acid sequence having at least 80% sequence identity to the sequence specified in SEQ ID NO: 4."

Second auxiliary request

"1. An isolated nucleic acid molecule encoding a delta 8 desaturase having at least 80% amino acid sequence identity to the sequence specified in

SEQ ID NO. 4 and comprising a section amplifiable by using primers SEQ ID NO. 5 and SEQ ID NO. 6, by

i) 5 preliminary cycles at 30 seconds at 94°C, 1-minute ramp to 37°C, 45 seconds at 37°C, 3-minute ramp to 72°C; followed by

ii) 30 cycles at 30 seconds at 94°C, 1-minute ramp to 50°C, 45 seconds at 50°C, 3-minute ramp to 72°C,

wherein in steps i) and ii), 3mM magnesium and each primer at 4 micromolecular are employed."

Third auxiliary request

"1. An isolated nucleic acid molecule encoding a delta 8 desaturase having at least 80% amino acid sequence identity to the sequence specified in SEQ ID NO. 4,

a) obtainable or obtained by a method comprising the steps of:

i) total RNA isolation from heterotrophic cultures of *Euglena gracilis*,

ii) purification of mRNAs from the isolated RNA,

iii) reverse transcription of mRNAs of step ii),

iv) amplification of a core region using the primers according to SEQ ID NO. 5 and SEQ ID NO. 6, by
- 5 preliminary cycles at 30 seconds at 94°C, 1-minute ramp to 37°C, 45 seconds at 37°C, 3-minute ramp to 72°C; followed by
- 30 cycles at 30 seconds at 94°C, 1-minute ramp to 50°C, 45 seconds at 50°C, 3-minute ramp to 72°C, wherein in both steps, 3mM magnesium and each primer at 4 micromolecular are employed and

v) isolation of amplification products having 350-750 bp length,

vi) obtaining the complete sequence using a RACE system; or

b) obtainable or obtained by a method comprising the step of amplification of a nucleic acid molecule using primers comprising 20 consecutive nucleotides of SEQ ID NO. 3; or
c) hybridizing to a probe of 600 bp of SEQ ID NO. 3 a nucleic acid molecule under the following conditions:
(i) hybridization in SSC at 65°C-75°C for 16-20 hours;
(ii) wash twice in SSC at room temperature for 5-20 minutes each; and
(iii) wash twice in SSC at 55°C-70°C for 30 minutes each."

Fourth auxiliary request

Claim 1 differs from claim 1 of the third auxiliary request only in that the term "80%"has been replaced by the term "90%" and in that the conditions of hybridization in option c) have been changed.

Fifth auxiliary request

Claim 1 differs from claim 1 of the fourth auxiliary request only in that options b) and c) have been deleted.

Sixth auxiliary request

"1. A method of identifying a nucleic acid sequence, comprising:
(a) hybridizing the nucleic acid sequence to at least 15 contiguous nucleotides of a sequence as shown in SEQ ID NO: 3; and
(b) identifying the nucleic acid sequence as one that encodes a desaturase."

Seventh auxiliary request

Claim 1 differs from claim 1 of the fifth auxiliary request only in that a disclaimer reading "wherein the nucleic acid molecule is not SEQ ID NO. 3" has been added at the end of the claim.

Eighth auxiliary request

"1. A method of identifying a nucleic acid sequence, comprising:
(a) hybridizing the nucleic acid sequence to **a sequence selected from SEQ ID NO. 5 and SEQ ID NO. 6**; and
(b) identifying the nucleic acid sequence as one that encodes a desaturase."

(emphasis by the Board to show the difference with claim 1 of the sixth auxiliary request)

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

(D1) US 7,256,033 B2 (published on 14 August 2007)

(D2) US 6,825,017 B1 (published on 30 November 2004)

(D3) Pages 7 to 9 of a patent attorney's written statement of facts dated 11 June 2003 extracted from the file wrapper at the USPTO for application 09/857,583 (PCT/US99/28655) on which patent US 6,825,017 B1 (document D2) was granted

(D4) Document showing sequence alignments prepared by the respondent and submitted together with its notice of opposition

(D16) Declaration of Quinn Zhu dated 5 March 2009

XI. The submissions made by the appellant, insofar as they are relevant to the present decision, may be summarised as follows:

Admissibility of the opposition

Document D1, filed together with the notice of opposition, did not disclose experimental data which could serve as a proof that desaturases having the amino acid sequence of SEQ ID NO:4 were inactive but rather contained an unsupported statement (see columns 41 and 42). The results of document D1 were not verifiable and not credible as such, and confirmation by document D16, submitted by the respondent later in the course of the opposition proceedings, was needed. Therefore, in the notice of opposition, the objection had not been properly substantiated and the opposition should have been deemed inadmissible.

Admissibility of document D16

Document D16 was late-filed to substantiate an objection which lacked proper substantiation in the opposition brief. It should not have been admitted into the opposition proceedings.

Admissibility of the second to eighth auxiliary requests

The sixth auxiliary request was filed with the grounds of appeal in response to the reasons of the decision under appeal.

The second to fifth and seventh auxiliary requests did not contain major amendments. The Board and the respondent had sufficient time to consider them without disruption of the proceedings. The subject of the proceedings was not changed. Neither new issues nor a shift of the subject-matter of the invention arose. The requests merely considered the reasons given in the decision under appeal, arguments newly raised by the respondent and hints provided in the Board's communication pursuant to Article 15(1) RPBA. Therefore, they could not have been filed earlier than by letter of 10 September 2013.

The eighth auxiliary request was filed in direct reply to objections by the Board against the sixth auxiliary request which had only been raised at the oral proceedings. Therefore, it should be admitted into the proceedings.

Main request (Article 83 EPC)

The evidence provided in document D1 was insufficient and the respondent had not proved but only alleged that a protein defined by SEQ ID NO:4 had no desaturase activity. The substantiation in the notice of opposition was insufficient and the teaching contained in document D16, which any way should not be admitted into the proceedings, needed to be confirmed. The respondent had therefore not discharged its burden of proof.

First auxiliary request (Article 83 EPC)

The provision of a correct sequence, either as a nucleic acid sequence or as a protein sequence, was not a *conditio sine qua non* for sufficiency of disclosure.

The delta 8-desaturase protein or a polynucleotide encoding it could also be characterised by other means than by structural features such as for instance by its biological activity and a way of obtaining it.

The pivotal question was whether the skilled person was able at the priority date to provide a biologically active delta 8-desaturase based on (i) the teachings of the disclosure contained in the patent at issue and (ii) the common technical knowledge.

There was a sufficiently clear and complete disclosure in the application as filed of the delta 8-desaturase in light of (i) the sequence information for the delta 8-desaturase, (ii) the delta 8-desaturase activity tests and (iii) the way of obtaining the delta 8-desaturase.

The skilled person would have learnt from the description that a delta 8-desaturase existed in *Euglena gracilis* and that the said organism was a suitable source for cloning it.

There were at least three different general cloning approaches disclosed in the description, namely cloning the cDNA from *Euglena gracilis* (see Example 3) , cloning via a hybridization approach and cloning via a genomic PCR approach.

Example 3 described the construction of a cDNA library in three steps. First, mRNA was reverse transcribed from *E. gracilis*. Second, targeted amplification was performed using two primers (SEQ ID NOs:5 and 6) suitable for amplifying the Hix-boxes. Third, the amplification products were sequenced to produce a

start primer for a prolongation in 5' and 3' directions. Examples 9 and 16 disclosed how to test a cloned sequence for delta 8-desaturase activity and, thereby, verify the cloned nucleic acid as encoding a delta 8-desaturase.

The application as filed disclosed conditions to reach a stringent or highly stringent hybridization of a probe having 600 nucleotides in length corresponding to any part of sequence SEQ ID NO:3 (see pages 11 and 12). Under the conditions mentioned, each fragment comprising 600 nucleotides of the nucleic acid sequence of the invention would specifically bind to the correct gene sequence. Manufacturing a genomic library and Southern hybridization with a probe of 600 nucleotides in length were routine measures.

The application as filed disclosed that 20 nucleotides of the sequence shown in SEQ ID NO:3 were sufficient to amplify the gene (see page 11, lines 19 to 26). Thus, the skilled person would also have obtained the gene with the correct sequence by genomic PCR from *Euglena gracilis* with the primers indicated in the description.

In light of the minor differences between the correct and allegedly incorrect sequence of the enzyme, the latter one could serve as a reference structure for the correct sequence since most of it was correct.

There was no need for a deposit of the plasmid pJW541 referred to in the examples. The use of that plasmid was not required to obtain the delta 8-desaturase from *Euglena gracilis*.

Sixth auxiliary request (Article 83 EPC)

The method of claim 1 was directed to a method of identifying a nucleic acid molecule. The molecule could be assayed for its desaturase activity using the tests described in the experimental part of the application as filed. The skilled person would have known that the nucleic acid sequence to be used as a probe could be selected from the specific His-box regions of SEQ ID NO:3. The experimental part of the description provided a clear and complete disclosure of the claimed method.

XII. The submissions made by the respondent, insofar as they are relevant to the present decision, may be summarised as follows:

Admissibility of the opposition

The notice of opposition contained a statement of the extent to which the patent was opposed and of the grounds on which the opposition was based, as well as an indication of the facts and evidence in support of these grounds, in particular that of insufficiency of disclosure. Therefore, the requirements of Rule 76(2)(c) EPC were met.

Admissibility of document D16

Document D16 was submitted on 20 February 2009 in response to submissions made by the appellant. It was thus responsive to issues raised and there was no reason for it not to be admitted by the opposition division into the proceedings.

Admissibility of the second to eighth auxiliary requests

The second to fifth, seventh and eighth requests were amendments to the appellant's case after it had filed its grounds of appeal. Thus, they were late-filed. The product of claim 1 of the second to fifth and seventh auxiliary requests was defined in terms of both structural features and features of a process for obtaining the product. Such a combination of features had never been discussed. It rendered the case more complex. Moreover, these requests could have been filed earlier. Therefore, they should not be admitted into the proceedings.

The sixth auxiliary request, filed with the statement of grounds of appeal, consisted of five claims corresponding to claims 14 to 18 as granted which were referred to in the arguments presented in support of the ground of insufficient disclosure in the notice of opposition. The eighth auxiliary request submitted in the course of the oral proceedings was filed as an amendment of the sixth auxiliary request. It was very late-filed.

The appellant could have filed the sixth and eighth auxiliary requests earlier in the opposition proceedings as fallback positions. Furthermore, the newly introduced feature "*a sequence selected from SEQ ID NO. 5 and SEQ ID NO. 6*" of the eighth auxiliary request was taken from the description out of its context, and gave rise to new objections under Article 123(2) EPC. Therefore, the sixth and eighth auxiliary requests should not be admitted into the proceedings.

Main request (Article 83 EPC)

Example 1 of document D1 showed that a polypeptide having the amino acid sequence of SEQ ID NO:4 of the patent at issue had no biological activity. A synthetic gene was made, designated D8S-3, which encoded a protein that was identical to the amino acid sequence of SEQ ID NO:4. It was transferred into *Yarrowia lipolytica* host cells, and feeding experiments were conducted from which it was concluded that no desaturation of EDA to DGLA by D8S-3 took place. The experimental results established lack of desaturase activity of a protein having the amino acid sequence of SEQ ID NO:4. The appellant gave no credible reason to doubt the statement that no activity had been observed. Document D16 confirmed this conclusion. Therefore, the protein defined by SEQ ID NO:4 had no desaturase activity. The plasmid pJW541 containing a cDNA encoding the protein of Example 7 of the patent at issue had not been deposited. With documents D1 and D16, the respondent had discharged its onus of proof.

First auxiliary request (Article 83 EPC)

A functional definition of an invention was only acceptable as long as the alternatives embraced by a claim were available and achieved the desired result. No specific examples of sequence variants having 80% identity with SEQ ID NO:4 were given in the patent at issue and no active sequence was disclosed. It was neither predictable from the disclosure in the patent at issue, nor on the basis of sequence identity nor on the basis of specific sequence characteristics, such as the presence of conserved His-box sequences, that the amino acid sequence of SEQ ID NO:4 could be modified, let alone which modifications should be made to restore

delta 8-desaturase activity. No evidence had been provided that proteins having a degree of 80% identity with SEQ ID NO:4 could retain said activity.

Claim 1 was nothing more than an invitation to perform a research program. This was an undue burden. It was not sufficient simply to provide a nucleic acid sequence encoding an inactive enzyme and a test for desaturase activity. Guidance as to how the sequence should be modified to restore activity was required.

Sixth auxiliary request (Article 83 EPC)

Claim 1 was directed to a method of identifying a nucleic acid sequence as one that encodes a desaturase. The method did not include any cloning step. The identification relied only on a successful hybridization of the tested sequence with a sequence of at least 15 contiguous nucleotides taken from the sequence of SEQ ID NO:3. The method did not comprise a step of determination of the desaturase activity. It was not workable for the reason that the foreseen hybridization would lack specificity in view of the poorly characterized oligonucleotide.

XIII. The appellant (patent proprietor) requested that the decision under appeal be set aside, and that the patent be maintained upon the basis of the main request, or alternatively upon the basis of any of the first to seventh auxiliary requests, all requests filed under cover of the letter dated 10 September 2013, or alternatively, upon the basis of the eighth auxiliary request filed at the oral proceedings before the Board.

XIV. The respondent (opponent) requested that the appeal be dismissed.

Reasons for the Decision

Admissibility of the opposition

1. In substance, the appellant has argued that the notice of opposition did not comply with Rule 76(2)(c) EPC (see Section X, *supra*).

2. According to Rule 76(2)(c) EPC, the notice of opposition must contain a statement of the extent to which the European patent is opposed and of the grounds on which the opposition is based, as well as an indication of the facts, evidence and arguments presented in support of these grounds, i.e. the substantiation.

3. The appellant's objection is directed to the substantiation of the ground of insufficient disclosure only, its argument being that the respondent had not provided any proof in this respect. The appellant has not objected as regards the substantiation of the other grounds for opposition (in the present case, lack of inventive step and of industrial application, pursuant to Article 100(a) EPC).

4. For the opposition to be admissible, it is sufficient that the notice of opposition fulfills the requirements of Rule 76(2)(c) EPC in respect of one of the grounds of opposition (see decision T 653/99 of 18 September 2002; point 2 of the Reasons). On this basis alone the opposition is to be considered as admissible.

5. Furthermore, the appellant has not disputed the fact that the notice of opposition contains an **indication** of the facts, evidence and arguments presented by the respondent in support of its objection of insufficient disclosure (see *inter alia* Example 1 of document D1) but argues that a proof of this assertion is lacking in the notice of opposition. In this respect, the Board notes that document D1 contains a clear statement that "*No desaturation of EDA to DGLA was observed with D8S-3*" (column 42, lines 36 to 37) but no experimental evidence. However, Rule 76(2)(c) EPC does not require that **proof** of the facts be contained in the notice of opposition. An indication thereof is necessary and sufficient (see decision T 426/08 of 1 December 2008; see point 5.1.3 of the Reasons).

6. Therefore, the requirements of Rule 76(2)(c) EPC have been met and the opposition division correctly decided that the opposition was admissible.

Admissibility of document D16

7. The appellant has argued that the opposition division erred in admitting document D16 into opposition proceedings.

8. Document D16 was enclosed with the respondent's letter submitted on 6 March 2009 in preparation for the oral proceedings before the opposition division. It was filed in direct response to the appellant's argument of 20 February 2009 that no experimental data were disclosed in columns 41 to 42 of document D1 (see point 5, *supra*).

9. If the way in which the department of first instance has exercised its discretion on a procedural matter is

challenged in an appeal, it is not the function of the Board of appeal to review all the facts and circumstances of the case as if it were in the place of the department of first instance, and to decide whether or not it would have exercised such discretion in the same way as the department of first instance. A Board of appeal should only overrule the way in which a department of first instance has exercised its discretion if the Board concludes it has done so according to the wrong principles, or without taking into account the right principles, or in an unreasonable way (cf. Case law of the Board's of Appeal, 7th edition, IV.E.3.6, page 983; and decisions cited therein).

10. The opposition division held that document D16 was submitted within the time limit set in the summons to attend oral proceedings in order to support facts and arguments already on file, and decided to admit it.

11. The Board concludes that the opposition division correctly exercised its discretionary power and sees no reason to overturn its decision.

Admissibility of the auxiliary requests

12. The admissibility of the first auxiliary request was not disputed, and the amendments in it do not raise any new issues. Therefore, the Board decides to admit it into the appeal proceedings.

13. The second to fifth and seventh auxiliary requests which were filed on 10 September 2013 are amendments to the appellant's case after it had filed its grounds of appeal. They may be admitted and considered at the Board's discretion. The discretion shall be exercised

in view of *inter alia* the complexity of the new subject-matter submitted, the current state of the proceedings and the need for procedural economy (see Article 13(1) RPBA).

14. In claim 1 of each of the second to fifth and seventh auxiliary requests, the protein or the nucleic acid sequence for which protection is sought is defined by reference to SEQ ID NO:4 and by further features relating to a process for obtaining it.

15. The appellant was perfectly aware from the onset of the opposition proceedings of the fundamental deficiencies associated with SEQ ID NO:4. As can be seen from document D3, it must even have been aware of the sequencing errors in SEQ ID NOs: 3 and 4 when the patent application was still pending.

Therefore, it could have been foreseen, when preparing for the oral proceedings before the opposition division, that a claim in which the desaturase was defined by reference to SEQ ID NO:4 only was at a high risk of being refused (see the summons of 15 December 2008). However, neither in the opposition proceedings nor with its grounds of appeal did the appellant take the opportunity to file any request in which the claimed protein or nucleotide acid would have been defined in another way, for example by reference to a process of manufacture.

16. Under these circumstances, the Board regards the filing of the second to fifth and seventh auxiliary requests one month before oral proceedings as the clearly belated introduction of fresh cases rendering the case more complex and giving rise to new objections under Articles 84 and 123 EPC.

17. Therefore, using the discretionary power conferred to it by Article 13(1) RPBA, the Board decides not to admit the second to fifth and seventh auxiliary requests into the appeal proceedings.

18. The sixth auxiliary request was filed together with the grounds of appeal. It comprises claims 1 to 5, corresponding to claims 14 to 18 as granted. The respondent has been aware of those claims from the onset of the opposition proceedings and admission of the sixth auxiliary request does not create any new issues. Therefore, the Board decides to admit the sixth auxiliary request into the appeal proceedings.

19. The eighth auxiliary request was filed in direct response to the refusal by the Board of the sixth auxiliary request (see paragraph 42 below) for reasons of insufficient disclosure. Claim 1 of the eighth auxiliary request differs from claim 1 of the sixth auxiliary request by its reference to "*a sequence selected from SEQ ID NO:5 and SEQ ID NO:6*", while the former feature "*at least 15 contiguous nucleotides of a sequence shown in SEQ ID NO:3*" was deleted.

20. The oligonucleotides of SEQ ID NO:5 and SEQ ID NO:6 are primers designed to be completely degenerate to sequences overlapping the first and the third of the His-box regions of the delta 6-desaturase of *Caenorhabditis elegans (*see bottom of page 21 of the patent application WO 00/34439). They were used **in combination** to amplify by PCR and clone a delta 8-desaturase from *E. gracilis* (see page 6, lines 29 to 30 and Example 3 on pages 21 to 22 of patent application WO 00/34439). They were however not used in any hybridization assay. Therefore, the feature

introduced in claim 1 of the eighth auxiliary request has been taken out of its context and would, if the request were admitted into the proceedings, give rise to an objection under Article 123(2) EPC. Moreover, issues under Article 123(3) EPC might arise in view of the fact that the primers defined by SEQ ID NO:5 and SEQ ID NO:6, due to their degenerate nature, encompass a sizeable number of primer sequences which are not present in SEQ ID NO:3.

21. Therefore, using the discretional power conferred to it by Article 13(1) RPBA, the Board decides not to admit the eighth auxiliary request into the appeal proceedings.

Main request (claims as granted)(Article 83 EPC)

22. Claim 1 is directed to an active desaturase comprising an amino acid sequence consisting of the sequence of SEQ ID NO:4 or having at least 60% identity thereto.

23. The pivotal question to be answered is whether such an active desaturase is actually disclosed in the patent application (WO 00/34439).

24. Example 7 in the patent application describes how the inventors have put into practice the teaching of Examples 3 and 4 to prepare a plasmid (pJW541) containing an open reading frame encoding a protein designated EFD1 (stating for *Euglena* Fatty Acid Desaturase 1). Example 8 reports that the translation of that open reading frame indicated a protein of 422 amino acids, the sequence of which is represented in Figure 3 under the designation "egd1" together with the sequences of FAT-3 and FAT-4, two desaturases of *C. elegans*. This 422 amino acid sequence is further

designated as SEQ ID NO:4 in the sequence listing. Example 9 reports that, to confirm the presumed desaturase activity of the encoded protein, the EDF1 cDNA was transferred from plasmid pJW541 to a yeast expression vector, pYES2, under the control of a galactose-inducible promoter. The resulting expression vector, pYES2-541, was introduced into *S. cerevisiae*. Yeast cultures were supplemented with various fatty acid soaps and the fatty acids of the cultures were analysed by methyl-ester derivatization and gas chromatography. The patterns of desaturation activity indicated that pYES2-541 expressed a delta 8-desaturase activity.

25. The respondent has argued that the protein defined by SEQ ID NO:4 does not exhibit any desaturase activity. In support of its argument, it has referred to documents D1 and D16.

26. Example 1 of document D1 (see columns 40 to 42) describes *inter alia* the preparation of a plasmid, pDMW261, comprising an *in vitro* synthesized codon-optimized gene for expression in *Yarrowia.* The synthetic gene sequence, designated **D8S-3**, encodes a protein having an amino acid sequence identical to the amino acid sequence of SEQ ID NO:4 of the patent application (see SEQ ID NO:7 of document D1). Following transformation of the pDMW261 construct into *Yarrowia lipolytica*, a feeding experiment using eicosadienoic acid (EDA) was conducted (see column 42, lines 1 to 12 and 32 to 35 together with column 3, line 38). The cells were collected by centrifugation, lipids were extracted, and fatty acid methyl esters were prepared by trans-esterification and subsequently analyzed. No experimental evidence is provided in this last respect. However, it is clearly stated that no desaturation of

EDA to dihomo-gamma-linoleic acids (DGLA) was observed (see column 42, lines 35 to 36 together with Table 2 on column 11). This has led the respondent to the obvious conclusion that the protein having the sequence of SEQ ID NO:4 has no desaturase activity. Detailed experimental evidence supporting this conclusion is provided by document D16 (see the Section entitled "*Co-Expression of the synthetic Delta-8 Desaturase "D8S-1" And A Synthetic Delta-9 Elongase*" on pages 3 to 4). With the submission of document D16, the respondent has discharged its burden of proof.

27. The appellant has not presented any evidence to the contrary. Its argument that the results of D1 were not verifiable and as such not credible is not tenable in view of the convincing explanations provided by document D16.

28. The Board concludes that the protein defined by SEQ ID NO:4 has no desaturase activity.

29. The appellant has argued that the information contained in the application as filed would have been sufficient to allow the skilled person to prepare a cDNA encoding the delta 8-desaturase obtainable from *Euglena gracilis*, to express it and to confirm its enzymatic activity. It referred to three cloning approaches which would have been at the disposal of the skilled person. The first approach would have used the teaching of the Examples. The second approach would have relied on hybridization assays using the general information given on pages 11 to 12 of the patent application. The third approach would have been a genomic PCR approach relying on the general information given at lines 13 to 19 on page 11.

30. Since the skilled person relying on the patent application was not informed that the protein defined by SEQ ID NO:4 was inactive, and since the patent application neither disclosed any active sequence variants having at least 60% sequence identity nor which positions of SEQ ID NO:4 had to be modified in order to obtain a functional desaturase, it had to go back to *E. gracilis* and reclone the desaturase in order to put the claimed invention into practice. Even though each of the steps necessary for recloning could be performed by a person skilled in the art, it is the combination of all the necessary steps (isolation of total mRNA, PCR amplification and selection of a group of amplification products with homology to known desaturases, completion of the 5' and 3' ends by RACE amplification, cloning and expression of the full length sequence to assess its function) which creates an undue burden on the skilled person trying to perform the invention. The same applies to the two alternative approaches mentioned by the appellant.

31. Thus, contrary to the requirements of Article 83 EPC, the skilled person would not have been in a position to perform the claimed invention readily and without undue burden across essentially the entire scope of claim 1.

32. Therefore, the main request does not meet the requirements of Article 83 EPC.

First auxiliary request (Article 83 EPC)

33. Claim 1 is directed to an active delta 8-desaturase which comprises an amino acid sequence having at least 80% identity to the sequence SEQ ID NO:4.

34. In the patent application, such proteins are referred to once (see bottom of page 13, reading "*Proteins of the invention also include proteins showing at least 60%, at least 70%,* ***at least 80%,*** *at least 90%, and at least 95%* ***similarity*** *(****to the sequence of*** *FIG. 6A or* ***FIG. 7A****) using blastp with default parameters)*" (emphasis by the Board); the sequence represented in FIG. 7A is the sequence SEQ ID NO:4).

35. Thus, the only criterion provided to the skilled person trying to identify proteins according to claim 1 is the self-contained reference to a protein of sequence SEQ ID NO:4. However, as discussed in points 26 and 27 (*supra*), the protein encoded by SEQ ID NO: 4 has no delta 8-desaturase activity. As for the main request, the skilled person trying to perform the invention according to the first auxiliary request was therefore left with the undue burden of embarking on a research program with the aim of first cloning a protein with delta 8-desaturase activity and then locating in its sequence those portions which could be altered to the extent of no more than 20% of the total sequence without prejudicing the enzymatic activity.

36. The Board reaches the conclusion that the patent fails to disclose the subject matter of claim 1 of auxiliary request 1 in a manner sufficiently clear and complete for it to be carried out by a person skilled in the art. Therefore, this request does not meet the requirements of Article 83 EPC.

Sixth auxiliary request (Article 83 EPC)

37. Claim 1 of the sixth auxiliary request is directed to a method of identifying a nucleic acid sequence as one that encodes a desaturase. It comprises two steps,

namely a step of hybridizing the sequence to at least 15 contiguous nucleotides of a sequence as shown in SEQ ID NO:3 and a step of identification as a desaturase for which no technical features have been indicated.

38. According to the claim language, as soon as hybridization occurs, a tested sequence is identified as encoding a desaturase, i.e. a protein having desaturase activity.

39. The appellant has argued that the step of identification was only a first step and that further confirmation steps as disclosed in the patent specification, i.e. cloning of a complete ORF and functional assays with the encoded protein, would be performed by the skilled person to confirm the function.

40. These confirmation steps are however not part of the claim and the question is whether the skilled person, taking into account the general knowledge and the disclosure in the patent application, was in a position to readily identify a nucleic acid sequence encoding a protein having desaturase activity by simply performing the steps of claim 1.

41. According to claim 1, the probe comprises any sequence having at least 15 contiguous nucleotides from anywhere within SEQ ID NO:3 and is not limited to e.g. sequences from the conserved His-box regions. The claim encompasses thus the use of short probes comprising sequences contributing to the inactivity of the protein encoded by SEQ ID NO:3 (see document D4 which provides a nucleotide sequence alignment of the coding region of SEQ ID NO:3 of the patent application against the

corresponding coding region of SEQ ID NO:3 as shown in patent US 6,825,017 B1 - document D2), as well as probes comprising sequences from areas with high homology to e.g. the cytochrome b5 like motif at its N-terminus (see the paragraph bridging pages 25 and 26 of the patent application). In view of the inclusion of probes detecting inactive sequences, probes with high homology to cytochrome b5, and the limited specificity of probes as short as 15 nucleic acids, the Board concludes that the skilled person was not in a position to readily, and without undue burden, perform the invention across essentially the entire scope of the claim.

42. The Board decides that the sixth auxiliary request does not meet the requirements of Article 83 EPC.

43. In the absence of any allowable request, the appeal must be dismissed.

Order

For these reasons it is decided that:

The appeal is dismissed.

The Registrar: The Chairman:

A. Wolinski B. Stolz

Decision electronically authenticated