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Datasheet for the decision of 13 August 2024

Case Number: T 1134/22 - 3.3.08

Application Number: 12199502.1

Publication Number: 2749644

IPC: C12N9/88, C12P7/24, C12N15/52

Language of the proceedings: EN

Title of invention:
Recombinant host cell for biosynthetic production of vanillin

Patent Proprietor:
SPECIALTY OPERATIONS FRANCE

Opponents:
Ennolys
Lesaffre International

Headword:
Vanillin production

Relevant legal provisions:
EPC Art. 56, 83, 123(2)
RPBA 2020 Art. 12(2), 12(6)

Keyword:
Inventive step - non-obvious alternative
Sufficiency of disclosure - (yes)
Amendments - extension beyond the content of the application as filed (no)
Late-filed evidence - error in use of discretion at first instance (no)

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Case Number: T 1134/22 - 3.3.08

D E C I S I O N
of Technical Board of Appeal 3.3.08
of 13 August 2024

Appellant: (Opponent 2)	Lesaffre International 137 rue Gabriel Péri 59700 Marcq-en-Baroeul (FR)
Representative:	Bandpay & Greuter 11 rue Christophe Colomb 75008 Paris (FR)
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Party as of right: (Opponent 1)	Ennolys Zone Artisanale FR-40140 SOUSTONS (FR)
Representative:	Bandpay & Greuter 11 rue Christophe Colomb 75008 Paris (FR)
Decision under appeal:	**Interlocutory decision of the Opposition Division of the European Patent Office posted on 9 March 2022 concerning maintenance of the European Patent No. 2749644 in amended form**

Composition of the Board:

Chair	T. Sommerfeld
Members:	A. Schmitt
	D. Rogers

Summary of Facts and Submissions

I. The appeal lodged by opponent 2 (appellant) lies from the opposition division's interlocutory decision according to which European patent No. 2 749 644 (the patent) in the version of auxiliary request 1, and the invention to which it relates, meet the requirements of the EPC. The patent proprietor is the respondent, opponent 1 is a party as of right in the appeal proceedings.

II. The patent entitled "*Recombinant host cell for biosynthetic production of vanillin*" was granted on European patent application No. 12 199 502.1, published as EP 2 749 644 A1 (the application).

III. The opposition proceedings were based on the grounds for opposition in Article 100(a) EPC, in relation to inventive step (Article 56 EPC), and those in Article 100(b) and (c) EPC.

IV. In reply to the appeal, the respondent submitted sets of claims of a main request and auxiliary requests 1 to 10. The set of claims of the main request consists of 14 claims and is identical to the set of claims of auxiliary request 1 underlying the opposition division's decision.

Claim 1 of the main request is the only independent claim and reads as follows:

"1. A cell comprising heterologous polynucleotides encoding a multienzyme complex involved in the metabolic pathway of phenylpropanoids and biosynthesis of a vanilloid or a hydroxybenzaldehyde precursor

thereof, which multienzyme complex comprises enzymes for the biosynthesis of coumaric acid and a crotonase; characterized in that the multienzyme complex comprises a crotonase, a CoA ligase, a 3-monooxygenase and a methyltransferase;
wherein the crotonase is suitable for performing a chain reduction reaction on feruloylCoA or coumaroylCoA; wherein the 3-monooxygenase is a phenol hydroxylase (PheA) and a flavin reductase (FLARED), or a hydroxybenzoic acid hydroxylase (HBH); and wherein the methyltransferase is a 3-O-methyltransferase."

V. The board summoned the parties to oral proceedings in accordance with their requests and, in a communication pursuant to Article 15(1) RPBA, expressed its preliminary opinion that the appeal would likely be dismissed.

VI. On 24 May 2024, the appellant and the party as of right informed the board that they would not attend the scheduled oral proceedings. The appellant requested a decision based on their written observations presented in the statement of grounds of appeal and the submission dated 9 June 2023.

VII. The board then cancelled the oral proceedings and informed the parties that the proceedings would be continued in writing.

VIII. Documents referred to in this decision:

D12 R. B. Nair et al., 2002, Plant Physiology 130, 210-20

D43 M. Berner et al., 2006, Journal of Bacteriology 188(7), 2666-73

D45 U. Kirchner et al., 2003, Journal of Biological Chemistry 278(48), 47545-53

D48 R.B. Hamed et al., 2008, Cell. Mol. Life Sci. 65, 2507-27

D56 Extracts of pages from the International Genetically Engineered Machine (iGEM) Foundation, including "Edinburgh/Yoghurt/Design" retrieved from http://2007.igem.org/wiki/index.php/Edinburgh/Yoghurt/Design, 28-34

D62 Phenol hydroxylase, GenBank AAC38324.1

D63 Phenol hydroxylase component 2, GenBank AAQ04677.1

D64 F.M. Duffner and R. Müller, 1998, FEMS Microbiology Letters 161, 37-45

IX. The arguments of the parties relevant to the board's decision are set out in the reasons for the decision.

X. The parties' requests relevant for the decision are as follows:

The appellant requests that the decision under appeal be set aside and that the patent be revoked and that documents D62 to D64 be considered in the appeal proceedings.

The respondent requests that the appeal be dismissed, i.e. that the patent be maintained on the basis of the claims of auxiliary request 1 submitted in the opposition proceedings and re-submitted as main request with the reply to the appeal, and that documents D62 to D64 not be considered in the appeal proceedings.

Reasons for the Decision

Admittance and consideration of documents (Article 12 RPBA)
Documents D62 to D64

1. Documents D62 to 64 had been filed by the appellant on the penultimate working day prior to the oral proceedings before the opposition division and were thus filed late in opposition. Their admittance was hence at the discretion of the opposition division, which decided not to admit them into the opposition proceedings.

2. Pursuant to Article 12(6) RPBA, the board must not admit, *inter alia*, evidence which was not admitted in the proceedings leading to the decision under appeal, unless the decision not to admit them suffered from an error in the use of discretion or unless the circumstances of the appeal case justify their admittance.

3. The board finds that the opposition division's discretionary decision not to admit documents D62 to D64 for having been filed very late, for not adding any new information to the case and for not being a (timely) response to the new auxiliary requests submitted two months prior to the oral proceedings under Rule 116 EPC, represents a correct exercise of its discretion.

4. Claim 1 of each of the new auxiliary requests filed under Rule 116 EPC was amended, *inter alia*, by further defining the enzyme 3-monooxigenase as being a phenol hydroxylase (PheA) and a flavin reductase (FLARED), or a hydroxybenzoic acid hydroxylase (HBH). These 3-monooxigenases were recited in dependent claim 5 of

the patent as granted and are the only 3-monooxigenases exemplified in the patent. The appellant could therefore have been expected to submit any evidence relating to these preferred enzymes earlier in the opposition proceedings.

5. On appeal, the appellant asserted that documents D62 to D64 reflected common general knowledge and complemented the teaching in document D43. They demonstrated that the two-component enzyme PheA/FLARED was known to the skilled person. As the GenBank accession numbers of documents D62 and D63 were also cited in Table 7 of the patent, documents D62 and D63 were already known to the respondent.

6. However, the respondent neither contested that the two-component enzyme PheA/FLARED was known in the prior art nor that the GenBank accession number recited in the first entry of Table 1 of D43 referred to PheA. In this respect, document D62 to D64 do not contain any additional information. Moreover, each of these documents identifies the two-component enzyme PheA/FLARED as a phenol hydroxylase and/or catechol 2,3-dioxygenase, but not that coumaric acid is a substrate for this enzyme. Hence, also in this respect, these documents do not add any new information to the case.

7. The board therefore neither sees any persuasive reasons to overrule the opposition division's discretionary decision on this issue nor any particular circumstances of the appeal case that would justify admittance of documents D62 to D64 in appeal and thus decides not to consider these documents in the appeal proceedings.

Main request
Amendments (Article 123(2) EPC) - claim 1

8. The claim concerns a cell comprising heterologous polynucleotides encoding a multienzyme complex that comprises a crotonase, a CoA ligase, a 3-monooxygenase and a methyltransferase, wherein the crotonase is suitable for performing a chain reduction reaction on feruloylCoA or coumaroylCoA, the 3-monooxygenase is a PheA and a FLARED, or a HBH and the methyltransferase is a 3-O-methyltransferase (see section IV. for a full wording of the claim).

9. The opposition division was right in that the claim has a basis in claims 1, 4 and paragraph [0083] of the application (point 25 of the decision under appeal).

10. Claims 1 and 4 of the application read as follows:

"1. A cell comprising heterologous polynucleotides encoding a multienzyme complex involved in the metabolic pathway of phenylpropanoids and biosynthesis of a vanilloid or a hydroxybenzaldehyde precursor thereof, which multienzyme complex comprises enzymes for the biosynthesis of coumaric acid and a crotonase."

"4. Cell according to claim 1 to 3, wherein the multienzyme complex comprises
a) a crotonase, preferably enoyl-CoA hydratase (ECH);
b) a CoA ligase, preferably 4-coumarate-CoA ligase (4CL);
c) a 3-monooxygenase, preferably phenolhydroxylase (PheA) and flavinreductase (FLARED), or hydroxybenzoic acid hydroxylase (HBH); and/or
d) a methyltransferase, preferably an O-methyltransferase, preferably a 3-O-methyltransferase

or a 4-O-methyltransferase, preferably caffeic acid O-methyltransferase (COMT)."

11. Thus, claim 4 of the application explicitly discloses a combination of all the four enzyme classes recited in the claim (a crotonase, a CoA ligase, a 3-monooxygenase "and" a methyltransferase). The different enzyme combinations encompassed by the "or" conjunction also present in this claim are irrelevant for the explicit disclosure of a combination of all four enzymes. Contrary to the appellant's assertion, the combination of the four enzymes is hence not the result of multiple selections from a list of enzyme combinations disclosed in claim 4 of the application.

12. As claim 4 of the application is dependent on *inter alia* claim 1 of the application, the disclosure in other claims of the application, including claim 3, is also irrelevant for the teaching in claim 4 that the multienzyme complex comprises a combination of the four enzyme classes recited in the claim.

13. The board is not persuaded either by the appellant's assertion that the selection of the specific enzymes for each of the four enzyme classes that are recited in the claim (a crotonase that is suitable for performing a chain reduction reaction on feruloylCoA or coumaroylCoA, a 3-monooxygenase that is PheA and FLARED, or HBH, a methyltransferase that is a 3-O-methyltransferase) was an intermediate generalisation that resulted in a new teaching not disclosed in the application.

14. In fact, with the exception of the methyltransferase, for which claim 4 of the application recites four options, including three independent alternatives (a O-

methyl-transferase, a 3-O-methyltransferase, a 4-O-methyl-transferase and caffeic acid O-methyltransferase), none of the other enzyme definitions is selected from a list of independent alternatives of considerable length.

15. The CoA ligase is defined in claim 1 as in claim 4 of the application, with the exception that the optional feature defining the preferred enzyme subclass has been deleted. For the 3-monooxygenase, claim 4 of the application recites only two preferred options, which are both included in claim 1, so no selection from a list was necessary for the definition of this enzyme, either.

16. Compared to the disclosure in claim 4 of the application, the crotonase is additionally functionally defined as being suitable for performing a chain reduction reaction on feruloylCoA or coumaroylCoA. This functional definition has a basis in paragraph [0083] of the application, which discloses that "[e]*nzymes in the crotonase superfamily include those catalytically performing a chain reduction reaction on feruloylCoA or coumaroylCoA, e.g. enoyl-CoA hydratase (ECH, crotonase; EC 4.2.1.17), which catalyses the hydratation of 2-trans-enoyl-CoA into 3-hydroxyacyl-CoA*".

17. This sentence hence describes a single functional definition of the crotonase and a single exemplary enzyme class (ECH) that has this enzymatic activity. ECH is also the only preferred enzyme class disclosed in claim 4 of the application. The inclusion of the functional definition of the crotonase from paragraph [0083] into the claim is therefore neither the result of a selection from a list of independent alternatives nor results, in combination with the other

enzyme definitions in the claim, in an unallowable intermediate generalisation.

18. The requirements of Article 123(2) EPC are met.

Sufficiency of disclosure (Article 83 EPC)

19. The appellant's assertion that the terms "crotonase" and "CoA ligase" used in the claim covered a multitude of enzymes which were not suitable for the required enzymatic reactions and that the skilled person therefore was not able to reproduce the claimed invention across the entire scope encompassed by the claim without undue burden, is not persuasive.

20. It is true that the term "crotonase" encompasses a superfamily of different enzymes catalysing diverse enzymatic reactions (see e.g. Table 1 and Figures 1 and 2 of D48 and paragraph [0089] of the patent). However, the crotonase is further defined in the claim as being suitable for performing a chain reduction reaction on feruloylCoA or coumaroylCoA (see point 16. above) and hence does not encompass any enzyme which does not have this functionality. In view of this functional restriction present in the claim, the broader and diverse definitions of the term "crotonase" in document D48 and paragraph [0089] of the patent are irrelevant.

21. In addition, the application discloses a crotonase that possesses this functionality (ECH; see e.g. paragraph [0083] and claim 4 of the application). In view of this example, there can be no doubt that the skilled person can produce a cell comprising a heterologous polynucleotide encoding a crotonase as defined in the claim that is suitable for the required

enzymatic reaction without undue burden. The appellant has not submitted any persuasive evidence or arguments that the provision of any other crotonase having this functionality would involve undue burden.

22. The same is true for the "CoA ligase" recited in the claim. The application defines the enzymatic reaction which the CoA ligase should catalyse (the CoA esterification of coumaric acid or ferulic acid; see paragraph [0088] of the application) and provides a suitable example (4-coumarate-CoA ligase; see e.g. paragraph [0088] and claim 4 of the application). Hence, the skilled person can produce a cell comprising a heterologous polynucleotide encoding a suitable CoA ligase without undue burden.

23. The requirements of Article 83 EPC are met.

Inventive step (Article 56 EPC) - claim 1

24. Document D56 was seen as the most suitable starting point for the assessment of inventive step by the opposition division and the parties. Document D56 presents the creation of a bacterial cell comprising the five heterologous polynucleotides *sam8*, *sam5*, *COMT*, *fcs* and *ech* for the production of vanilla flavour (chapter "Vanilla Flavour Production" on pages 30 to 32 of D56).

25. The claimed cell differs from this bacterial cell in the heterologous 3-monooxygenase gene(s) inserted into the cell. While in document D56 it is the 4-coumarate 3-hydroxylase gene *sam5* (Figure 3 and second paragraph on page 31 of D56), in the claim it is the genes for PheA and FLARED, or a gene encoding HBH.

26. It is undisputed that in the absence of any comparative data, the monooxygenase gene(s) recited in the claim are alternatives to the monooxygenase gene disclosed in document D56. The objective technical problem may therefore be formulated as the provision of an alternative cell comprising heterologous polynucleotides involved in the biosynthesis of a vanilloid.

27. In a first line of argument, the appellant disputed that a cell that comprised the heterologous polynucleotides encoding PheA and FLARED was suitable for the production of a vanilloid since this had not been demonstrated in Example 2 of patent. In the absence of this technical effect associated with the claimed cell, the selection of the genes for PheA and FLARED was an arbitrary choice.

28. This line of argument is not persuasive however. Two different enzymatic pathways for the synthesis of vanilloids were investigated in the patent (see Figures 1 and 8 and Examples 1 and 2 of the patent). Example 1 of the patent is concerned with the first pathway and demonstrates that yeast cells comprising, *inter alia*, heterologous polynucleotides encoding a multienzyme complex comprising the crotonase ECH, the CoA ligase 4CL, the 3-monooxygenase HBH and the 3-O-methyltransferase COMT produced the vanilloid vanillic acid (paragraphs [0208] to [0209] and Figure 5 of the patent). This was not disputed.

29. Example 2 of the patent is concerned with the second pathway. It describes the enzymes required for this pathway and their assembly in yeast host cells (paragraphs [0215] and [0216] and Figure 8 of the patent). It moreover demonstrates that the two-

component enzyme PheA/FLARED can indeed use coumaric acid as substrate and convert it to caffeic acid when expressed in a yeast cell (paragraph [0217] of the patent).

30. In view of this teaching and the fact that most other enzymes are common to both pathways (Figures 1 and 8 and paragraph [0215) of the patent), the skilled person had no reasons to doubt that a cell comprising the heterologous polynucleotides necessary for implementing the second pathway in a cell, as proposed in Example 2 of the patent, was suitable for the production of a vanilloid, as demonstrated in Example 1 for a cell comprising the (largely identical) heterologous polynucleotides necessary for implementing the first pathway.

31. For the alternative 3-monooxigenase HBH recited in the claim, no objections were presented by the appellant either in opposition or in appeal. As mentioned above, (see point 28.), the patent demonstrates in Example 1 that yeast cells comprising heterologous polynucleotides encoding a multienzyme complex comprising, *inter alia*, the enzymes ECH, 4CL, COMT and HBH produced vanillic acid. In the absence of any arguments or evidence to the contrary, this alternative of the cell as defined in the claim was not obvious to the skilled person.

32. The assessment of inventive step of the claimed cell therefore hinges on the question whether the second alternative expressed in the claim was obvious to the skilled person, i.e. whether the skilled person would have replaced the enzyme Sam5 with the two-component enzyme PheA/FLARED.

33. Sam5 is a 4-coumarate 3-hydroxylase that catalyses the conversion of p-coumaric acid to caffeic acid in the vanillin biosynthesis pathway depicted in Figure 3 of document D56 (see also the section entitled "*sam5*" on page 31 of D56 and Table 1 of D43). Since the conversion of coumaric acid to caffeic acid is a necessary step in this pathway (see D56, *supra*, and Figure 8 of the patent), the skilled person would have replaced Sam5 with another hydroxylase only if they could have reasonably expected that this other hydroxylase was able to convert coumaric acid to caffeic acid.

34. The board is not persuaded that this was the case for the two-component enzyme PheA/FLARED. None of the documents cited by the appellant in support of their arguments discloses or suggests that the two-component enzyme PheA/FLARED has this enzymatic activity (see also point 6. above). Indeed, document D45 describes the identification of PheA as part of a two-component phenol hydroxylase that can convert phenol to catechol (abstract, last paragraph of the right-hand column on page 47545, Figure 1 of D45), but does not identify coumaric acid as a substrate of the two-component enzyme PheA/FLARED.

35. Document D43 identifies the enzymes involved in caffeic acid biosynthesis in an actinomycete, including Sam5, and proposes that Sam5 is a 4-coumarate 3-hydroxylase (see the first entry in Table 1 on page 2669 of D43). Since document D43 also identifies PheA as the protein to which Sam5 has the closest amino acid sequence identity and similarity (*supra*; see also patent, Table 7 on page 26), the appellant asserted that document D43 inferred that PheA had the same enzymatic activity as that proposed for Sam5.

36. However, the sequence identity and similarity of the two proteins disclosed in Table 1 of document D43 are merely 62% and 76%, respectively. This degree of sequence identity and similarity is not sufficiently high for reasonably expecting the same enzymatic activity for both proteins. This view is supported by the teaching in document D12 which discloses that in a particular group of monooxigenases (P450), even nearly identical enzymes do not necessarily have the same substrate specificity (first full sentence of the left-hand column on page 215 of D12).

37. In conclusion, it was neither known to the skilled person nor could have been reasonably expected that the two-component enzyme PheA/FLARED converted coumaric acid to caffeic acid. The skilled person thus did not have any incentive to select this two-component enzyme as alternative 3-monooxigenase in the cell of document D56.

38. The claimed subject-matter involves an inventive step (Article 56 EPC).

Order

For these reasons it is decided that:

The appeal is dismissed.

The Registrar: The Chair:

L. Malécot-Grob T. Sommerfeld

Decision electronically authenticated