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Title of invention:
Microorganisms and methods for producing substituted phenols

Patent Proprietor:
Symrise AG

Opponent:
Ennolys

Headword:
Vanillin-producing microorganism/SYMRISE

Relevant legal provisions:
EPC Art. 56, 83

Keyword:
Sufficiency of disclosure - auxiliary request 1 (no) - undue burden (yes) - auxiliary request 2 (yes) - undue burden (no)
Inventive step - reasonable expectation of success (no)

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Case Number: T 2251/22 - 3.3.08

D E C I S I O N
of Technical Board of Appeal 3.3.08
of 12 March 2025

Appellant: (Opponent)	Ennolys Zone Artisanale FR-40140 SOUSTONS (FR)
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Decision under appeal:	**Interlocutory decision of the Opposition Division of the European Patent Office posted on 28 July 2022 concerning maintenance of the European Patent No. 2721148 in amended form**

Composition of the Board:

Chairwoman	T. Sommerfeld
Members:	A. Schmitt
	L. Bühler

Summary of Facts and Submissions

I. The appeal lodged by the opponent (appellant) is against the opposition division's interlocutory decision that European patent No. 2 721 148 (the patent), as amended in the version of auxiliary request 1, and the invention to which it relates meet the requirements of the EPC.

II. The opposition proceedings were based on the grounds for opposition under Article 100(a) EPC in relation to novelty (Article 54 EPC) and inventive step (Article 56 EPC) and those under Article 100(b) and (c) EPC.

III. In the statement of grounds of appeal, the appellant submitted arguments to the effect that the invention as defined in the claims of auxiliary request 1 considered allowable by the opposition division was not sufficiently disclosed (Article 83 EPC) and that the claimed subject-matter did not involve an inventive step (Article 56 EPC).

IV. With its reply to the appeal, the patent proprietor (respondent) re-filed auxiliary request 1 considered by the opposition division, re-filed auxiliary requests 2 to 10 as filed before the opposition division, and filed new auxiliary requests 11 to 13. A corrected set of claims for auxiliary request 12 was filed with a subsequent submission.

V. The board summoned the parties to oral proceedings in accordance with their requests and issued a communication under Article 15(1) RPBA setting out its preliminary opinion.

VI. During the oral proceedings, the respondent renumbered auxiliary request 6 as auxiliary request 2.

Claim 1 of auxiliary request 1 reads as follows:

"1. Method for producing vanillin, comprising the step of adding an educt selected from eugenol, coniferyl alcohol, coniferyl aldehyde and ferulic acid to a microorganism of genus Amycolatopsis, wherein the microorganism does not comprise a gene coding for a vanillin dehydrogenase."

Claim 1 of auxiliary request 2 reads as follows:

"1. Method for producing vanillin, comprising the step of adding an educt selected from eugenol, coniferyl alcohol, coniferyl aldehyde and ferulic acid to a microorganism of Amycolatopsis sp. ATCC 39116, wherein the microorganism does not comprise a gene coding for a vanillin dehydrogenase."

VII. The following documents are referred to in this decision:

D1 Muheim, A. et Lerch, K., Appl Microbiol Biotechnol 51, 1999, 456-61

D3 US 2011/0065156 A1

D5 Plaggenborg, R. et al., Appl Microbiol Biotechnol 72, 2006, 745-55

D7 Overhage, J. et al., Appl Microbiol Biotechnol 52, 1999, 820-28

D9 WO 00/26355 A2

D10 Overhage, J. et al., J Biotechnol 125, 2006, 369-76

D13 Priefert, H. et al., Appl Microbiol Biotechnol 56, 2001, 296-314

D14 Brown, M. E. et al., J. Am. Chem. Soc. 133, 2011, 18006-9

D15 EP 0 761 817 B1

D16 EP 0 885 968 B1

D21 Achterholt, S. et al., Appl Microbiol Biotechnol 54, 1999, 799-807

D23 Achterholt, S., 2001, Dissertation "Untersuchungen zur Produktion von Vanillin durch Amycolatopsis sp. HR167: Ferulasaurekatabolismus und Etablierung eines Transformationssystems"

D24 Yoon, S.-H. et al., Biotechnol. Bioprocess Eng. 10, 2005, 378-84

D53 Labuda, I., "Biotechnology of Vanillin: Vanillin from Microbial Sources", in Handbook of Vanilla Science and Technology. John Wiley & Sons, Ltd., 2010, 301-331

D54 Xu, P. et al., Trends in Biotechnology 25, 2007, 571-76

VIII. The appellant's arguments, insofar as relevant to the decision, are summarised as follows.

Auxiliary request 1

Sufficiency of disclosure (Article 83 EPC)

It was not possible to provide, without undue burden, a microorganism of genus *Amycolatopsis* that did "*not comprise a gene coding for a vanillin dehydrogenase*". This expression meant that the microorganism must not comprise any gene coding for any enzyme that was able to catalyse the conversion of vanillin to vanillic acid under suitable conditions. Suppressing the gene coding

for vanillin dehydrogenase (*vdh gene*) was not sufficient as microorganisms comprised other enzymes able to catalyse this reaction.

The patent contained data for a single bacterial strain with unusual and uncommon vanillin production properties (second paragraph of right-hand column on page 458 and second, third and last paragraphs of right-hand column on page 460 of D1). These properties could not be generalised to each of the more than 70 other different species of genus *Amycolatopsis*, as evident from e.g. document D23 (Tables 3.1 and 3.2), and from the fact that vanillin was toxic to microorganisms. It was therefore unlikely that the claimed method could be carried out with each of the 70 species of genus *Amycolatopsis*.

Neither the patent nor the skilled person's common general knowledge enabled the skilled person to identify, without undue burden, those microorganisms of genus *Amycolatopsis* with which the claimed method could be carried out. No evidence to the contrary was provided by the respondent. The denominations ATCC 39116, *Streptomyces setonii (S. setonii)*, HR167, DSM9991 and DSM9992 were different names for the same or phylogenetically extremely closely related bacterial strains (e.g. D1, D3, D13, D14, D16, D23 and D24).

Auxiliary request 2

Sufficiency of disclosure (Article 83 EPC)

Amycolatopsis strain ATCC 39116 did not contain any of the genes coding for the enzymes necessary to process eugenol, coniferyl alcohol and/or coniferyl aldehyde. It was hence necessary to express exogenous genes

encoding these enzymes, a fact that required the implementation of a research programme and constituted an undue burden in view of the large number of possible gene combinations taught in the patent and the fact that the prior art disclosed a single recombinant ATCC 39116 strain (D10).

Inventive step (Article Article 56 EPC) - claim 1

Any of documents D1, D21 and D54 was a suitable starting point for the assessment of inventive step as they all described methods for producing vanillin using *Amycolatopsis* strain ATCC 39116 or the identical or at least phylogenetically extremely closely related strain HR167. The claimed method differed from those taught in these documents in that this strain did not comprise a gene coding for a vanillin dehydrogenase.

As strain ATCC 39116 was not able to produce vanillin from the educts eugenol, coniferyl alcohol and coniferyl aldehyde, inactivating the *vdh* gene could not, *a priori*, improve vanillin production from these educts. Hence, no technical effect was associated with this difference, and the claimed method was merely an alternative.

Even if an increased production of vanillin and decreased production of vanillic acid were accepted as technical effects of the difference, and the objective technical problem was the provision of an improved method for producing vanillin, the solution proposed in the claim was obvious.

The skilled person would have considered that a vanillin dehydrogenase must also be responsible for the degradation of vanillin to vanillic acid in strain

ATCC 39116, as it contained the same CoA-dependent non-beta-oxidative metabolic pathway for producing vanillin from ferulic acid as other microorganisms, and vanillin was degraded to vanillic acid by vanillin dehydrogenase in this pathway (for example Figure 4 of D1; Figure 1 of D21; paragraph that bridges pages 379 and 380 and Figure 1 of D24; section 19.2.1.1 on page 304 of D53). The "*special metabolic flow*" in this strain (first full paragraph of right-hand column on page 460 of D1) was due to a different regulation mechanism but not a different pathway.

The oxidation of vanillin to vanillic acid was a known problem in the microbial production of vanillin, and inactivation of vanillin dehydrogenase reduced this reaction (middle paragraph in left-hand column page 460 of D1; paragraph bridging left- and right-hand columns on page 753 of D5; second sentence in left-hand column on page 821 of D7; first to third paragraph on page 3 of D9; last sentence of right-hand column on page 799 of D21; left-hand column on page 380 of D24; paragraph that bridges pages 574 to 575 of D54).

The skilled person was hence motivated to inactivate vanillin dehydrogenase in strain ATCC 39116 to avoid vanillin degradation and could reasonably expect to thereby increase vanillin production. At very least, it was a "try and see" situation. The toxicity of vanillin was not a concern in this highly tolerant strain.

Document D5 taught the skilled person how to inactivate a gene coding for a vanillin dehydrogenase in *Actinomycetales* (strains *Rhodococcus* I24 and PD630) without prior identification of the gene in the strain's genome or prior knowledge of the gene's sequence (Figure 1; page 749). The skilled person would

have applied this technique to the strain ATCC 39116 without any inventive effort by using degenerated primers based on two vanillin dehydrogenase consensus sequences identified in document D5 that were indeed also present in the vanillin dehydrogenase amino acid sequence of ATCC 39116 identified in the patent.

The claimed method hence lacked an inventive step in view of the disclosure in any of documents D1, D21, and D54 combined with that in document D5.

IX. The respondent's arguments, insofar as relevant to the decision, are summarised as follows.

Auxiliary request 1

Sufficiency of disclosure (Article 83 EPC)

Since the term "vanillin dehydrogenase" was understood by the skilled person as referring to a specific enzyme class having the EC number 1.2.1.67, the appellant's claim interpretation that any enzyme having an activity that could possibly convert vanillin to vanillic acid fell under this term was incorrect.

The objection that not all *Amycolatopsis* strains were able to produce vanillin was not supported by any evidence and not credible in light of the teaching in the prior art that different microorganisms of the genus *Amycolatopsis* were able to produce vanillin (*S. setonii* or ATCC 39116 (e.g. D1, D3, D16); HR167 (e.g. D21, D23); DSM 9991 and DSM 9992 (e.g. D15)). The patent demonstrated, for an exemplary strain, that this was the case, and the appellant did not provide any proof to the contrary. The claimed method could be carried out using the teaching of the patent.

This objection was in any case irrelevant to the question of sufficiency of disclosure since only methods for producing vanillin fell under the ambit of the claim. Non-working embodiments were excluded, and the skilled person would exclude any other unreasonable embodiments. This objection concerned the scope of protection and the clarity of the claim, but not the question of whether or not the skilled person could carry out the claimed method.

Auxiliary request 2

Sufficiency of disclosure (Article 83 EPC)

Paragraphs [0022] and [0035] of the patent taught which exogenous genes encoding which enzymes from which microorganisms would allow strain ATCC 39116 to catalyse the conversion of eugenol to coniferyl alcohol, coniferyl alcohol to coniferyl aldehyde, and coniferyl aldehyde to ferulic acid. Based on this teaching in the patent and on common general knowledge, the skilled person could produce vanillin from each of the educts recited in the claim using strain ATCC 39116 without undue burden. Document D10 supported this view.

Inventive step (Article Article 56 EPC) - claim 1

The claimed method differed from those taught in documents D1, D21 and D54 at least in that the microorganism of the genus *Amycolatopsis* did not contain a gene coding for a vanillin dehydrogenase.

The technical effect of this difference was improved production of vanillin and reduced production of vanillic acid, as evident from Example 6 of the patent.

Since the production of vanillin was a technical feature of the claimed method, the objection that strain ATCC 39116 could not produce vanillin from the educts eugenol, coniferyl alcohol and coniferyl aldehyde was irrelevant to the assessment of inventive step. The objective technical problem was the provision of an improved method of producing vanillin and reducing vanillic acid production.

The skilled person had no motivation to inactivate the *vdh* gene in strain ATCC 39116 in the expectation of increased vanillin production, as it was neither known nor could be assumed that this strain possessed a *vdh* gene at all.

Even if the skilled person assumed that microorganisms of genus *Pseudomonas*, *Rhodococcus* and *Amycolatopsis* had the same CoA-dependent, non-beta-oxidative pathway for converting ferulic acid into vanillin, this did not mean that the same catabolic pathway was used for further metabolising vanillin.

Document D21, which described the cloning of two genes involved in the degradation of ferulic acid to vanillin in *Amycolatopsis sp.* strain HR167, speculated in Figure 1 that vanillin might be degraded to vanillic acid in a pathway similar to that described for, *inter alia*, *Pseudomonas sp.* strain HR199, but did not propose that this degradation occurred via vanillin dehydrogenase, which was not mentioned in document D21 in the context of strain HR167.

In fact, document D5 published five years later by the same group demonstrated that, unlike *Pseudomonas sp.* and *Rhodococcus sp.*, the genomic structure of the genes

involved in ferulic acid catabolism in *Amycolatopsis sp.* HR167 did not comprise a *vdh* gene (Figure 3). Findings concerning vanillin degradation by a vanillin dehydrogenase in these former species could hence not be transferred to *Amycolatopsis sp.*.

The fact that strain ATCC 39116 was able to produce vanillic acid was not evidence for an active vanillin dehydrogenase in this strain since vanillin degradation could be due to other enzymes.

The documents cited by the appellant that discussed reducing or inactivating vanillin dehydrogenase activity (D1, D5, D7, D9, D21, D24, D54) did not provide any incentive to inactivate this gene in strain ATCC 39116 as the recited references in these documents concerned other vanillin-producing strains. Although D1 and D21 described inactivation of the *vdh* gene in other microorganisms, they did not suggest this measure for the strains *S. setonii* and HR167. In fact, D1 suggested other measures for improving vanillin production in *S. setonii* (first full and last paragraph of right-hand column on page 460).

The skilled person hence could neither have reasonably expected that strain ATCC 39116 possessed a gene for a vanillin dehydrogenase at all nor that inactivation of this enzyme would necessarily have increased vanillin production in this particular strain, which had a vanillin biosynthesis pathway different from other microorganisms and already accumulated a large quantity of vanillin. In view of this and the fact that vanillin was toxic, a further increase in vanillin production in this strain was surprising.

X. The parties' requests of relevance to the decision were as follows.

The appellant requested that the decision under appeal be set aside and the patent be revoked.

The respondent requested that the appeal be dismissed with the consequence that the patent be maintained in amended form based on the set of claims of auxiliary request 1 or, in the alternative, that the patent be maintained in amended form on the basis of the set of claims of auxiliary request 2.

Reasons for the Decision

Auxiliary request 1

Sufficiency of disclosure (Article 83 EPC)

1. Claim 1 of auxiliary request 1 concerns a method for producing vanillin using a microorganism of genus *Amycolatopsis* that "*does not comprise a gene coding for a vanillin dehydrogenase*" (see section VI. for the full wording of the claim).

2. The term "vanillin dehydrogenase" is associated with the Enzyme Commission number (EC number) 1.2.1.67. and identifies the enzyme class that catalyses the chemical reaction identified by this EC number. It does not, therefore, include any other enzyme that is or may be, under suitable conditions, also able to catalyse the conversion of vanillin to vanillic acid. In view of this, the board does not agree with the appellant's claim interpretation that a microorganism that "*does not comprise a gene coding for a vanillin*

dehydrogenase" means that the microorganism must not comprise *any* gene coding for *any* enzyme that is able to catalyse the conversion of vanillin to vanillic acid.

3. In consequence, the appellant's first line of argument under Article 83 EPC, that it was impossible to reproduce a bacterial strain that did not comprise *any* gene coding for *any* enzyme that was able to catalyse the conversion of vanillin to vanillic acid under suitable conditions, is without merit.

4. In a second line of argument under Article 83 EPC, the appellant asserted that the invention could not be reproduced across its claimed scope without undue burden. This line of argument is persuasive.

5. *Amycolatopsis* is a genus of bacteria within the order of *Actinomycetales* that consists of more than 70 different species. The production of vanillin from ferulic acid is, however, not a property common to many microorganisms, including *Amycolatopsis* strains. This is not surprising since, as undisputed, vanillin is known to be toxic to most microorganisms (e.g. paragraph that bridges pages 456 and 457 of D1; paragraph [0005] of D16).

6. In line with this fact, document D23 teaches that different *Amycolatopsis* strains do not have the same capability to grow on different carbon sources and to produce the same products, i.e. not all of them are able to metabolise ferulic acid and/or vanillin, or to produce vanillin (Tables 3.1 and 3.2 of D23). This fact is also supported by document D1 which discloses that, when investigating the ferulic acid degradation pattern of over 120 *Actinomycetes* strains, only four strains showed weak formation of vanillic acid, and only for

Streptomyces setonii (*S. setonii*) was the formation of vanillin detected at all (second paragraph of right-hand column on page 458 of D1).

7. This strain - *S. setonii* - possesses an unusual and uncommon vanillin tolerance and ability to accumulate vanillin, and not vanillic acid, that the authors of D1 found "*surprising*" (second paragraph of right-hand column on page 458), and to be evidence of a "*special metabolic flow*" (second paragraph of right-hand column on page 460) and a "*unique metabolism*" in this strain (last paragraph of right-hand column on page 460).

8. The *S. setonii* strain ATCC 39116 was also the single *Amycolatopsis* strain that was analysed in the patent. In view of this, it is unlikely that many strains other than *S. setonii* within the more than 70 known *Amycolatopsis* species could produce vanillin from ferulic acid or the other educts recited in the claim, nor did the skilled person know with which *Amycolatopsis* strain, other than *S. setonii*, the claimed method could be carried out.

9. The respondent pointed out that several different microorganisms of the genus *Amycolatopsis* were known in the prior art as vanillin producers. However, each of these allegedly different strains - ATCC 39116, HR167, DSM9991, DSM9992 - are merely different denominations for the same or for phylogenetically closely related strains, as evident from, e.g. D13 (last full paragraph of left-hand column on page 309), D14 (abstract); D15 (paragraph [0012]); D16 (paragraph [0016]); D23 (Table 3.1) and D24 (first full paragraph on left-hand column on page 379). This argument is therefore not persuasive.

10. In order to carry out the claimed method, the skilled person must, however, be able to identify and select those of the 70 different *Amycolatopsis* strains that are able to produce vanillin according to the claimed method. Contrary to the respondent's assertion, the patent, which teaches a single strain - ATCC 39116 - suitable for carrying out the claimed method, does not disclose any criteria based on which the skilled person could assess and identify which other *Amycolatopsis* strains were suitable for use in the claimed method. The identification and selection of suitable *Amycolatopsis* strains hence requires extensive research based on trial and error, without any expectation of success, a fact that amounts to undue burden.

11. The board cannot agree with the respondent's assertion that this objection related to the scope and clarity of the claim and could not be raised under Article 83 EPC, as only methods with which vanillin could be produced fell under the ambit of the claim, but not any non-working embodiments. Functional features restrict the ambit of a claim, but do not result in the claimed invention being - automatically - sufficiently disclosed by excluding non-working embodiments. As explained above, the skilled person must be able to identify, without undue burden, those microorganisms with which the claimed method can be carried out. If this is not the case, the skilled person cannot carry out the claimed method without undue burden and the requirements of Article 83 EPC are not met.

12. In the case at hand, as assessed in point 10. above, the skilled person neither knows from the teaching in the patent nor from common general knowledge with which *Amycolatopsis* strain, other than ATCC 39116, the claimed method could be carried out, and is not

provided in the patent with any criteria as to how further suitable *Amycolatopsis* strains could be identified other than by testing each strain separately. This requires an undue research effort.

13. The invention as defined in claim 1 of auxiliary request 1 is not sufficiently disclosed in the application, contrary to the requirements of Article 83 EPC.

Auxiliary request 2

Sufficiency of disclosure (Article 83 EPC)

14. Claim 1 of auxiliary request 2 differs from claim 1 of auxiliary request 1 in that the microorganism of the genus *Amycolatopsis* is *Amycolatopsis* sp. ATCC 39116. This is the bacterial strain for which the patent describes the cloning and analysis of a vanillin dehydrogenase (*vdh*) gene, the generation of a *vdh* deletion mutant strain, and the production of vanillin from the educt ferulic acid (Examples 1 to 6 on pages 9 to 14).

15. In addition to ferulic acid, the claim recites eugenol, coniferyl aldehyde and coniferyl alcohol as educts in the production of vanillin. Possibly, strain ATCC 39116 is not able to metabolise these educts without the expression of exogenous genes encoding suitable enzymes.

16. However, paragraphs [0022] to [0025] and [0035] of the patent disclose which exogenous genes coding for which enzymes could be expressed in this microorganism in order to enable it to produce vanillin from each of these educts. Moreover, document D10 discloses that the

expression of the gene for the vanillyl alcohol oxidase gene from *Penicillium simplicissimum* in the closely related vanillin-tolerant strain Amycolatopsis HR167 allowed this strain to metabolise eugenol to, *inter alia*, coniferyl alcohol, coniferyl aldehyde and ferulic acid (e.g. abstract of D10) and therefore supports the feasibility of this approach.

17. In view of this, the board finds the teaching in paragraphs [0022] to [0025] and [0035] of the patent sufficient to allow the skilled person to provide recombinant ATCC 39116 strains expressing the exogenous genes necessary for the strain to produce vanillin from each of the educts recited in the claim without undue burden. The requirements of Article 83 EPC are met.

Inventive step (Article 56 EPC) - claim 1

Closest prior art

18. Documents D1, D21 and D54 were proposed as starting points for the assessment of inventive step.

Document D1 discloses a method of producing vanillin from ferulic acid using *S. setonii*, which is identified in document D1 by ATCC number "391161" (second full paragraph of left-hand column of page 457 of D1). Since the ATCC number "391161" does not exist and each commercially available *S. setonii* strain mentioned in the prior art is consistently referenced to as "ATCC 39116" (e.g. paragraph [0012] of D3; second full paragraph of right-hand column on page 309 of D13; abstract of D14; paragraph [0016] of D16), the *Amycolatopsis* sp. strains used in D1 and the patent are identical.

Document D21 discloses a method of producing vanillin from ferulic acid using *Amycolatopsis* sp. strain HR167, i.e. a strain that is phylogenetically closely related, if not identical, to strain ATCC 39116 (see point 9. above).

Document D54 is a review article which teaches, *inter alia*, the production of vanillin from eugenol and isoeugenol by different microorganisms, including *Amycolatopsis* sp. HR167, i.e. the same strain that is used in document D21 (Table 2 on page 574 of D54).

19. Neither the appellant nor the respondent identified any difference between the claimed method and the methods disclosed in any of documents D1, D21 and D54 other than that the microorganism of the genus *Amycolatopsis* does not comprise a gene coding for a vanillin dehydrogenase.

Technical effect and objective technical problem

20. The patent discloses the cloning and characterisation of a gene and corresponding gene product for vanillin dehydrogenase of *Amycolatopsis* strain ATCC 39116 (Examples 1 to 4 of the patent) and demonstrates that deletion of this gene in this strain leads to increased vanillin and decreased vanillic acid production from the educt ferulic acid compared to the wild-type strain (Examples 5 and 6; Figures 7 and 8). Based on these data, the technical effects associated with the difference are increased vanillin production and decreased vanillic acid production by strain ATCC 39116.

21. In a first line of argument, the appellant asserted that this technical effect could not be accepted for

the production of vanillin from educts other than ferulic acid as strain ATCC 39116 could not produce vanillin from any of these educts. However, this objection, which was also raised under Article 83 EPC, was found to be without merit as the skilled person was able to provide ATCC 39116 strains that could produce vanillin from each of the educts recited in the claim based on the teaching in the patent (see points 15. to 17. above). The technical effects of increased vanillin production and decreased vanillic acid production demonstrated in the patent can therefore be acknowledged.

22. The objective technical problem is hence formulated as proposed by the respondent as the provision of an improved method for producing vanillin with an increased yield in vanillin and decreased yield in vanillic acid.

Obviousness

23. The appellant asserted that the skilled person was motivated to inactivate the *vdh* gene in strain ATCC 39116 to solve the problem posed because they knew that this strain must have a *vdh* gene, and because inactivation of the v*dh* gene in other microorganisms had the recited technical effects. Inactivation of the *vdh* gene in strain ATCC 39116, although this gene had not yet been cloned, could be achieved by the method taught in document D5.

24. This line of argument is however not persuasive. As undisputed, the *vdh* gene had not been cloned in ATCC 39116 or other related *Amycolatopsis* species such as HR167. It is true that the CoA-dependent non-beta-oxidative metabolic pathway for the cleavage of ferulic

acid to, *inter alia*, vanillin and vanillic acid has also been proposed for strains ATCC 39116 (Figure 4 of D1) and HR167 (e.g. Figure 1 of D21). In fact, document D21 discloses the cloning of the genes *ech*, encoding enoyl-CoA hydratase/aldolase, and *fcs*, encoding feruloyl-CoA synthetase, which in the CoA-dependent non-beta-oxidative metabolic pathway are responsible for converting ferulic acid to vanillin (Figure 1 of D21; see also D53, section 19.2.1.1 on page 304 and Figure 19.2 on page 305).

25. It is also true that vanillin may further be degraded to vanillic acid, that this reaction may be catalysed by the *vdh* gene product, and that inactivation of the *vdh* gene has improved vanillin production in other microorganisms (e.g. middle paragraph in left-hand column on page 460 of D1; paragraph that bridges the left-and right-hand columns on page 753 of D5; second sentence in left-hand column on page 821 of D7; page 3 of D9; last paragraph of right-hand column on page 799 of D21; paragraph that bridges pages 379 and 380 and Figure 1 of D24; paragraph that bridges pages 574 and 575 of D54).

26. However, none of the prior art documents explicitly teaches that a *vdh* gene exists in *Amycolatopsis* strains ATCC 39116 and HR167. On the contrary, Figure 1 of document D21 discloses the enzymes responsible for the conversion of ferulic acid to vanillin (*ech* and *fcs*; see point 24. above), but conspicuously lacks any proposal for the enzyme responsible for the further degradation of vanillin to vanillic acid, despite the fact that document D21 mentions inactivation of the *vdh* gene in another microorganism (*Pseudomonas sp.* strain HR199; see last sentence of right-hand column on page 799 of D21).

27. Figure 1 of document D1 discloses the metabolites detected in strain ATCC 39116, including vanillic acid, but does not propose any enzymes responsible for converting the respective metabolites into each other. The fact that vanillic acid is produced by this strain is not sufficient, either, to conclude that the strain must comprise a *vdh* gene, as vanillic acid could also be produced by other aldehyde dehydrogenases (e.g. last sentences of the paragraph that bridges the left- and right-hand columns on page 753 of D5).

28. Likewise, review article D54, which was also proposed as a starting point for the assessment of inventive step, touches on vanillin production by, *inter alia*, *Amycolatopsis sp*. HR167 (last full paragraph on right-hand column of page 573; Table 2) and, in the paragraph that bridges pages 574 and 575, mentions that one possible solution for preventing oxidation of vanillin to vanillic acid could be inactivation of relevant enzymes "*such as vanillin dehydrogenase*". However, the latter remark is made in the context of different bacterial species and hence is neither evidence that a *vdh* gene is present in strain HR167 nor constitutes a proposal for attempting a similar approach in this strain.

29. In addition, there is evidence on file that the (activity of the) genes and gene products involved in ferulic acid catabolism in *Amycolatopsis* strains *S. setonii* and HR167 differ from those in other microorganisms, for which the CoA-dependent non-beta-oxidative metabolic pathway has been investigated and a *vdh* gene was cloned.

30. Firstly, as discussed above in the context of sufficiency of disclosure (see point 7.), *S. setonii* has a special metabolic flow that allows a surprising accumulation of vanillin during the degradation of ferulic acid, a fact that document D1 characterises as a "*unique metabolism*" (last paragraph of right-hand column on page 460 of D1). This is contrasted in D1 with the second vanillin-producing microorganism discussed in this document, *Pseudomonas putida*, which rapidly degrades vanillin to vanillic acid due to a relatively high vanillin dehydrogenase activity (first and second paragraphs of right-hand column on page 458 of D1). Although D1 discusses the inactivation of the *vdh* gene in this latter strain, it does not propose this measure for improving vanillin production by *S. setonii*, but, on the contrary, explicitly proposes other measures (last paragraph of right-hand column on page 460).

31. Secondly, document D5, which compared the organisation of structural genes involved in ferulic acid catabolism in different bacteria, including *Pseudomonas putida*, *Pseudomonas* HR199 and *Amycolatopsis* sp. HR167, demonstrates that unlike a *Pseudomonas putida* strain and *Pseudomonas* sp. HR199, for which the effects of deletion of the *vdh* gene on vanillin production had been assessed (e.g. last sentence of right-hand column on page 799 of D21), HR167 lacks a *vdh* gene between the *ech* and *fcs* genes (figure 3 of D5). In line with this teaching, document D21 disclosed cloning only of the genes *ech* and *fcs* responsible for producing vanillin from ferulic acid, and not of any of the genes involved in the further degradation of vanillin in the CoA-dependent non-beta-oxidative metabolic pathway.

32. Hence, contrary to the appellant's view, there was no clear evidence that strain ATCC 39116 contained a *vdh* gene, let alone a *vdh* gene similar to other microorganisms for which this gene had been cloned. In view of these uncertainties, the skilled person could not have reasonably expected that inactivation of this hitherto unknown gene would improve vanillin production in strain ATCC 39116. The skilled person was not in a so-called "try and see" situation, either. A "try and see" situation could only arise if an approach was suggested in the prior art and if there were no particular technical difficulties in implementing this approach (Case Law of the Boards of Appeal of the European Patent Office, 10th edition 2022, I.D.7.2. and the decisions cited there). However, as outlined above, none of the cited documents suggested this approach for *Amycolatopsis* strain ATCC 39116.

33. In view of these facts, the claimed method was not obvious to the skilled person from the teaching in any of documents D1, D21 and D54 combined with that of document D5. The claimed method hence involves an inventive step according to Article 56 EPC.

Order

For these reasons it is decided that:

1. The decision under appeal is set aside.

2. The case is remitted to the opposition division with the order to maintain the patent in amended form according to claims 1 to 9 of auxiliary request 2, filed as auxiliary request 6 with the reply to the appeal, and the description and drawings possibly to be adapted thereto.

The Registrar: The Chairwoman:

C. Rodríguez Rodríguez T. Sommerfeld

Decision electronically authenticated