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Datasheet for the decision of 25 February 2014

Case Number: T 0110/11 - 3.3.08

Application Number: 03005540.4

Publication Number: 1318196

IPC: C12N15/52, C12N1/21, C12N9/12, C12P13/04

Language of the proceedings: EN

Title of invention:
Amino acid producing strains belonging to the genus Escherichia and method for producing an amino acid

Patent Proprietor:
Ajinomoto Co., Inc.

Opponent:
Evonik Degussa GmbH

Headword:
Amino acid production/AJINOMOTO

Relevant legal provisions:
EPC Art. 123(2), 84, 83, 56

Keyword:
Main request - sufficiency of disclosure (no)
Auxiliary request - inventive step (no)

Decisions cited:
G 0009/91, T 0409/91, T 0932/92, T 0459/09

Catchword:

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Case Number: T 0110/11 - 3.3.08

D E C I S I O N
of Technical Board of Appeal 3.3.08
of 25 February 2014

Appellant: (Opponent)	Evonik Degussa GmbH Intellectual Property Rodenbacher Chaussee 4 63457 Hanau (DE)
Respondent: (Patent Proprietor)	Ajinomoto Co., Inc. 15-1, Kyobashi 1-chome, Chuo-ku Tokyo (JP)
Representative:	J. Tack Strehl Schübel-Hopf & Partner Maximilianstrasse 54 80538 München (DE)
Decision under appeal:	**Interlocutory decision of the Opposition Division of the European Patent Office posted on 17 November 2010 concerning maintenance of the European Patent No. 1318196 in amended form.**

Composition of the Board:

Chairman: M. Wieser
Members: B. Stolz
J. Geschwind

Summary of Facts and Submissions

I. The appeal lies against the decision of the opposition division, posted 17 November 2010, whereby European patent No. 1318196 was maintained in amended form.

II. The opposition division decided that claims 1 to 3 of the main request, filed on 27 January 2009, met the requirements of the EPC.

III. In its statement of the grounds of appeal, the opponent (appellant) raised objections under Articles 123(2), 83 and 56 EPC.

IV. The patent proprietor (respondent) filed its response to the statement of grounds of appeal.

V. The parties were summoned to oral proceedings. A communication pursuant to Article 15(1) of the Rules of Procedure of the Boards of Appeal (RPBA) annexed to the summons, informed them of the preliminary non-binding opinion of the board on some of the issues of the appeal proceedings.

VI. The appellant made further submissions in writing.

VII. Oral proceedings were held on 25 February 2014. During the oral proceedings, the respondent filed an auxiliary request comprising claims 1 and 2.

VIII. Claims 1 and 2 of the main request read:

"1. A method for producing an amino acid comprising the step of cultivating a bacterium belonging to the genus Escherichia in a culture medium which contains sucrose

as a main carbon source, and producing and accumulating the amino acid in the culture medium,

wherein the bacterium has been constructed from a sucrose non-assimilative strain belonging to the genus Escherichia, wherein the bacterium harbors sucrose non-PTS genes comprising genes coding for permease, invertase and fructokinase and has an ability to produce and accumulate the amino acid in a culture medium, and is capable of utilizing sucrose as a sole carbon source,

wherein the amino acid is selected from the group consisting of threonine and isoleucine.

2. The method according to claim 1, wherein the sucrose non-PTS genes are derived from the microorganism having the accession number VKPM B-7914."

IX. Claim 1 of auxiliary request 1 is the result of the combination of claims 1 and 2 of the main request and reads:

"1. A method for producing an amino acid comprising the step of cultivating a bacterium belonging to the genus Escherichia in a culture medium which contains sucrose as a main carbon source, and producing and accumulating the amino acid in the culture medium,

wherein the bacterium has been constructed from a sucrose non-assimilative strain belonging to the genus Escherichia, wherein the bacterium harbors sucrose non-PTS genes comprising genes coding for permease, invertase and fructokinase and has an ability to produce and accumulate the amino acid in a culture

medium, and is capable of utilizing sucrose as a sole carbon source,

wherein the amino acid is selected from the group consisting of threonine and isoleucine, and

wherein the sucrose non-PTS genes are derived from the microorganism having the accession number VKPM B-7914."

Claim 2 of the auxiliary request defines the bacterium as being Escherichia coli.

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

D2: P. J. Cowan, Thesis, University of Melbourne, 1992: Controlled production of Tryptophan by genetically manipulated strains of E. coli”

D4: Bockmann J. et al.,.Characterization of a chromosomally encoded, non-PTS metabolic pathway for sucrose utilization in Escherichia Coli EC3132, Molecular and General Genetics, Vol. 235, No. 1, 1. October 1992, pages 22-32,

D5: Debabov V.,.Construction of strains producing L-threonine, Proceedings of the IVth International Symposium on Genetics of Industrial Microorganisms, 1982, pages 254-258

D8: US 5,705,371

XI. The appellant's arguments as far as relevant for this decision can be summarized as follows:

Main request

Article 123(2) EPC

There was no basis in the application as originally filed for the features "non-PTS genes comprising genes coding for permease, invertase and fructokinase" and "has an ability to produce and accumulate the amino acid in a culture medium" of claim 1.

Article 83 EPC

Claim 1 did not comprise all essential steps of the claimed method. It lacked the step of recovering an amino acid from the culture medium.

Claim 1 referred to the use of non-PTS genes encoding a permease, invertase and fructokinase in general but the patent disclosed only the genes of the csc system from strain VKPM B-7914.

As far as genes "derived" from the microorganism with accession number VKPM B-7914 were concerned, there was no disclosure of suitable modifications of the genes. According to decisions T 923/92 (OJ EPO 1996, 564) and T 409/91 (OJ EPO 1994, 653) and contrary to the opinion of the opposition division, this objection equally concerned clarity and sufficiency of disclosure. The claim language allowed an unlimited number of modifications leaving the skilled person in the dark as to how one could establish whether or not a gene was derived from the cited strain.

Auxiliary request

Articles 83 and 84 EPC

In the absence of structural limitations, the feature "genes ... derived from" was unclear. Following decision T 459/09 of 13 December 2012, the combination of independent claim 1 with dependent claim 2 opened up the way to a complete examination of the claims, also with regard to the requirements of Article 84 EPC. Should the board disagree with this opinion, questions of law should be referred to the Enlarged Board of Appeal and the procedure should be stayed.

Upon a broad interpretation of the term "genes derived from", the subject matter of claim 1 was insufficiently disclosed across the full scope of the claim.

Article 56 EPC

Document D5, or alternatively document D8, represented the closest prior art. The technical problem underlying the claimed invention could be defined as the provision of an alternative way of producing threonine or isoleucine with a strain of E. coli growing on sucrose. Document D4 disclosed the only known non-PTS pathway for sucrose utilization in E. coli. Since this was the only alternative known, the claimed solution was obvious in view of document D5 or D8 in combination with document D4. In addition, document D2 showed that the sucrose pathway described in document D4 had been successfully used for the production of tryptophan.

XII. The arguments of the respondent as far as relevant for this decision can be summarized as follows:

Main request

Article 123(2)

Objections under Article 123(2) EPC represented a new ground of opposition and should be dismissed.

Article 83 EPC

As far as the objections concerned the use of the term "genes derived from" and the fact that claim 1 did not mention the step of collecting an amino acid, they were inadmissible objections under Article 84 EPC.

The patent put the skilled person in a position to perform the invention as claimed. Claim 1 did not only refer to the use of broadly defined genes encoding a permease, an invertase and a fructokinase but contained also the functional limitation that the resulting strain had to be capable of producing the amino acid and of growing on sucrose as the sole carbon source. The prior art described two metabolic pathways for sucrose utilization in E. coli, the known PTS system and the non-PTS system described in document D4. This and the patent specification provided sufficient guidance.

Auxiliary request

Articles 83 and 84 EPC

The number of possible modifications of the genes coding for the permease, invertase and fructokinase was limited by the functional requirements specified in claim 1. Moreover, decision T 459/09, referred to by the appellant, stood alone against a large number of

decisions by the boards of appeal reaching a different conclusion and should not be followed.

Article 56 EPC

Document D5 disclosed a successful system for the production of threonine by E. coli growing on sucrose. The technical problem consisted of providing an improved system for the production of threonine or isoleucine by E. coli growing on sucrose. Document D4 did not refer to the production of amino acids at all. Moreover, the experimental results given on pages 27 to 30 of document D4, shed doubt on the suitability of the csc system. Therefore, based on document D5 in combination with document D4, the skilled person had no reasonable expectation of success.

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

XIV. The respondent requested that the appeal be dismissed or in the alternative that the decision under appeal be set aside and the patent be maintained on the basis of the set of claims of the auxiliary request filed during the oral proceedings.

Reasons for the Decision

Main Request

Admissibility of objections under Article 100(c) EPC

1. The appellant submitted its objections under Article 100(c) EPC only with the statement setting out the grounds of appeal. They concerned features of claim 1

which were already present in claim 1 as granted. Article 100(c) EPC had however not been invoked as a ground of opposition, and during opposition proceedings the opponent had agreed that the claims of the main request were acceptable with regard to Articles 123(2) and 123(3) EPC (cf. minutes of the oral proceedings, page 1).

2. The respondent requested that objections on the ground of 100(c) EPC not be admitted.

3. As stated in decision G 9/91 (OJ EPO 1993,408), "[t]he purpose of the appeal procedure inter partes is mainly to give the losing party a possibility to challenge the decision of the Opposition Division on its merits. It is not in conformity with this purpose to consider grounds for opposition on which the decision of the Opposition Division have not been based. Furthermore, in contrast to the merely administrative character of the opposition procedure, the appeal procedure is to be considered as a judicial procedure, as explained by the Enlarged Board in its recently issued decisions in the cases G 7/91 and G 8/91 (see point 7 of the reasons). Such procedure is by its very nature less investigative than an administrative procedure. Although Article 114(1) EPC formally covers also the appeal procedure, it is therefore justified to apply this provision generally in a more restrictive manner in such procedure than in opposition procedure. In particular with regard to fresh grounds for opposition, for the above reasons the Enlarged Board considers that such grounds may in principle not be introduced at the appeal stage. However, an exception to the above principle is justified in case the patentee agrees that a fresh ground for opposition may be considered".

4. In view of the respondent's request not to admit objections on the ground of Article 100(c) EPC against the main request, this fresh ground of opposition is not considered by the board.

Article 84 EPC

5. Claim 1 of the main request results from the introduction of the features of claim 2 as granted into claim 1 as granted. The appellant did not raise any objections under the provisions of Article 84 EPC and the board has no reason to raise any on its own.

Article 83 EPC

6. The majority of sucrose positive bacteria take up and phosphorylate sucrose via a phosphoenolpyruvate (PEP) dependent phosphotransferase system, known as the *PTS* system. An alternative, PTS independent, sucrose uptake and phosphorylation system, known as the *csc* system, comprises a proton symport transport system, an invertase and a fructokinase (cf. document D4, page 22).

7. Claim 1 refers to the use of a bacterium of the genus Escherichia harboring sucrose non-PTS genes coding for a permease, an invertase and a fructokinase for the production of threonine or isoleucine in a medium containing sucrose as its main carbon source. Moreover, the strain has to be capable of growing on sucrose as the sole carbon source.

8. The patent discloses the use of the respective non-PTS genes from E.coli K 12 strain W3350csc (VKPM B-7914) which contains the "csc genes" of E. coli strain EC3132

as described in document D4 (cf. [0047] of the patent specification and Examples 3 and 5).

9. Thus, the patent provides sufficient disclosure only for methods based on the use of the csc genes from E. coli K12 strain VKPM B-7914 and genes derived therefrom.

10. The respondent submitted that the skilled person, at the date of filing was aware of two pathways for sucrose utilization in E. coli, i.e. the sucrose PTS system and the csc system disclosed in document D4. Since the functional properties resulting from the presence of non-PTS genes were readily testable, the disclosure of the patent put the skilled person in a position to readily perform the claimed invention across the full scope of the claim.

11. Claim 1 is however not limited to the use of E. coli strains comprising the csc genes. The claim language merely requires the presence of genes encoding a permease, an invertase and a fructokinase which do not encode the proteins of the PTS system and which render Escherichia capable of growing on sucrose. Also, according to [0018] of the description, "sucrose non-PTS genes are not particularly limited so long as they can function in a bacterium belonging to the genus Escherichia". Thus, the claim encompasses any solution, also not yet known solutions, which is not based on the PTS system.

12. The disclosure of one way of performing an invention is only sufficient if it allows the invention to be performed in the whole range claimed rather than only in some members of the claimed class to be obtained. Sufficiency of disclosure thus presupposes that the

skilled person is able to obtain substantially all embodiments falling within the ambit of the claims (Case law of the Boards of Appeal, 7th edition, 2013, II.C.4.4, and decisions cited therein).

13. In the present case, the claimed solution refers to the use of non-PTS genes which are merely defined in functional terms, i.e. the use of a permease, an invertase, and a fructokinase rendering the strain capable of growing on sucrose as the sole carbon source. While the skilled person is in a position to readily make use of the csc genes as disclosed by the patent, it is not in a position to readily perform the invention by using any other alternative set of genes since, as mentioned by the respondent, none is known or readily available.

14. The board therefore decides that claim 1 of the main request does not meet the requirements of Article 83 EPC.

Auxiliary request

Admissibility of the Auxiliary request

15. The respondent filed its auxiliary request during the oral proceedings, in response to the board's decision that the main request was not allowable under Article 83 EPC. Claim 1 of the auxiliary request is the result of the combination of independent claim 1 with dependent claim 2 of the main request. This amendment does not create a fresh case. Moreover, in the communication attached to the summons to oral proceedings, the board had indicated preliminary doubts that objections against the main request under Article 83 EPC were sufficiently substantiated. It was only at

the oral proceedings that the board was finally convinced by the appellant's arguments.

Under these circumstances, the board, exercising its discretion under Article 13(1) RPBA, decides to admit the auxiliary request.

Articles 123 and 84 EPC

16. The appellant raised no objections under Article 123 EPC.

17. However, an objection was raised under Article 84 EPC in view of decision T 459/09 of 13 December 2012. The appellant argued that the characterizing feature of previously dependent claim 2 ("genes ... derived from") was unclear and that an objection under Article 84 EPC was admissible because claim 1 of the auxiliary request represents an amended claim, arising from the combination of previously dependent claim 2 with independent claim 1. The appellant requested that the procedure should be stayed and questions of law should be referred to the Enlarged Board of Appeal should the board not admit this objection.

18. The respondent requested that the objection not be admitted.

19. In the light of its decision with regard to the requirements of Article 56 EPC (see points (20) to (36) below), the board refrains from giving a reasoned decision on this issue.

Article 56 EPC

20. Document D8 represents the closest prior art. It discloses a method of producing threonine by E. coli strain BKIIM B-3996. The strain was constructed by transferring into it genetic determinants allowing sucrose (saccharose) assimilation. The strain produced 85 g/l threonine when grown on sucrose at 37 degrees C for 36 hours. According to paragraph [0007] of the patent in suit the strain used in document D8 comprises the PTS genes.

21. The technical problem underlying the invention is seen in the provision of an alternative method of producing threonine or isoleucine by a strain of the genus Escherichia using sucrose as the main carbon source.

22. The respondent submitted that the technical problem should be seen in the provision of an improved method of producing these amino acids. It referred to Tables 2 and 4 of the patent according to which strains VL2055csc and 44-3-15csc yielded more threonine and isoleucine, respectively, when grown on sucrose than when grown on glucose. The figures are 27.9 g/l of threonine and 13.6 g/l of isoleucine when grown on sucrose for 72 hours at 37 degrees C.

23. Claim 1 neither refers to any particular strain of the genus Escherichia nor, apart from growth on sucrose, to any other particular features of the strain to be used. According to established case law, an unexpected or advantageous effect has to be shown by comparison with the closest prior art (Case law of the Boards of Appeal, 7th edition, 2013, I.D.10.9). In the present case, the claimed method has therefore to be compared with the method disclosed in document D8. On this

basis, the claimed method does not lead to an improvement over the prior art but constitutes an alternative method.

24. Referring to chapter I.D.9.2.2 of the Case Law of the Boards of Appeal of the EPO (7th Edition, 2013), the appellant argued that the proposed solution was in fact a combination of two independent solutions to two unrelated problems. The first relating to the provision of a microorganism belonging to the genus Escherichia capable of growing on sucrose, and the second relating to the provision of a microorganism belonging to the genus Escherichia and capable of producing threonine or isoleucine. Since the features relating to these two properties were functionally independent, inventive step had to be assessed independently for both of them.

25. This argument is flawed, because sucrose, which is taken up by the microorganism is metabolised to phosphoenol-pyruvate which is one of the major building blocks of several metabolic pathways, inter alia of the threonine and isoleucine biosynthetic pathways (cf. [0005] of the patent in suit). Thus, there is a functional link between the two properties.

26. As a solution of the problem mentioned in point 21, the patent, according to claim 1, proposes the use of a bacterium belonging to the genus Escherichia which comprises non-PTS genes derived from the microorganism having the accession number VKPM B-7914.

27. The results in Tables 2 and 4 of the patent in suit demonstrate that the method as defined in claim 1 indeed solves this technical problem.

28. It remains to be established if the claimed solution involves an inventive step.

29. The appellant argued that the proposed solution was obvious in view of documents D8 and D4.

30. Document D4, entitled "Characterization of a chromosomally encoded, non-PTS metabolic pathway for sucrose utilization in Escherichia coli EC3132", discloses the csc genes. According to [0047] of the patent in suit, the source strain of the csc genes which was used in the Examples, E. coli K12 W3350csc, contains the csc genes of E. coli EC3132. Thus, the genes derived from the microorganism VKPM B-7914 are the genes derived from E. coli strain EC3132 described in document D4.

31. The respondent argued that document D8 made no mention of alternative sucrose uptake systems and that document D4 neither contained a pointer nor any other incentive to the use of the csc system for the production of amino acids. Moreover, document D4 (Table 3, "transport activity" of strain EC3132, and pages 27 to 30) provided data that the sucrose permease encoded by cscB was not sufficiently active and therefore unsuitable for the production of amino acids. Therefore, the skilled person would not have had a reasonable expectation of successfully solving the technical problem by using the csc system.

32. The skilled person in the field of amino acid production by E. coli is aware of the general physiological properties of E. coli, one of the work horses of molecular biology. Therefore, document D4, describing the csc pathway for sucrose utilization represents general knowledge.

In the present situation, where the only known alternative sucrose uptake pathway in E.coli to the PTS-system was the csc pathway disclosed in document D4, the skilled person looking for a solution to the underlying problem could not choose from among several available alternatives. The board is therefore convinced that the skilled person not only could but indeed would have tried to use the csc system.

33. The respondent argued that the experimental data relating to the kinetic properties of the sucrose permease described in document D4 would have kept away the skilled person from trying to solve the technical problem by means of the csc pathway.

34. It is true that document D4 refers to the inefficiency of the cscB encoded sucrose permease expressed by strain EC3132 (Table 3). It is, for instance, stated that transport activity sufficient for a thorough kinetic analysis could not be obtained (page 27, right column) and that the poor expression of cscB seemed to be the result of the inefficient sucrose transport (page 28, right column). On the other hand, document D4 also discloses that strains like ECB1, a spontaneous mutant of strain EC3132, showed a low but reproducible permease activity (Table 3), and that strain HCB101/ R'Csc211, obtained after transformation with a plasmid carrying the csc genes, constitutively expressed all three enzymes of the csc system (Table 3 and page 26, right column). Finally, experiments with E. coli K12 strains carrying all csc genes except for cscB, the sucrose permease, demonstrated an essential role for this permease in sucrose metabolism (page 27, right column, 2nd paragraph). Document D4 also shows that the invertase and fructokinase activities encoded

by cscA and cscK are clearly induced by the presence of sucrose in the growth medium (cf. Table 3).

35. In view of the fact that document D4 provided unambiguous evidence for the function of the csc encoded pathway and its three enzymes, the skilled person not only would have been prompted, as set out in point 31 above, to try to solve the problem by using the csc pathway but he would even have done so with the reasonable expectation of successfully obtaining at least some sucrose utilization capacity in a threonine or isoleucine producing strain of the genus Escherichia upon transfer of the csc genes.

36. Since the subject-matter of claim 1 can be derived in an obvious way from the disclosure of document D8 upon combination with that of document D4, it does not involve an inventive step. It follows that the auxiliary request does not meet the requirements of Article 56 EPC.

Order

For these reasons it is decided that:

1. The decision under appeal is set aside.

2. The patent is revoked.

The Registrar: The Chairman:

A. Wolinski M. Wieser

Decision electronically authenticated