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Datasheet for the decision of 3 June 2014

Case Number: T 0655/11 - 3.3.08

Application Number: 01109779.7

Publication Number: 1149911

IPC: C12N15/52, C12N1/21, C12N9/12, C12P13/08

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:
Sucrose PTS Genes/AJINOMOTO

Relevant legal provisions:
EPC Art. 83, 56

Keyword:
Main request - requirements of the EPC met (yes)

Decisions cited:
G 0009/91

Catchword:

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Case Number: T 0655/11 - 3.3.08

D E C I S I O N
of Technical Board of Appeal 3.3.08
of 3 June 2014

Appellant: (Opponent)	Evonik Degussa GmbH Intellectual Property Rodenbacher Chaussee 4 63457 Hanau (DE)
Representative:	Ioannidis, Johannes Evonik Industries AG DG-IPM-PAT Postcode 84/339 Rodenbacher Chaussee 4 63457 Hanau (DE)
Respondent: (Patent Proprietor)	Ajinomoto Co., Inc. 15-1 Kyobashi 1-chome, Chuo-ku Tokyo 104-0031 (JP)
Representative:	Fischer, Heinrich Strehl Schübel-Hopf & Partner Maximilianstrasse 54 80538 München (DE)
Decision under appeal:	**Decision of the Opposition Division of the European Patent Office posted on 15 December 2010 rejecting the opposition filed against European patent No. 1149911 pursuant to Article 101(2) EPC.**

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 15 December 2010, whereby the opposition filed against European patent number 1 149 911 was rejected.

The patent was granted with 2 claims and opposed on the grounds of Article 100(a) EPC in conjunction with Article 56 EPC, and Articles 100(b) and (c) EPC. The opposition division decided that the objection under Article 100(c) EPC was not sufficiently substantiated, and that the claims as granted met the requirements of Articles 83 and 56 EPC.

II. With the statement setting out the grounds of appeal, the appellant (opponent) filed a new document, D15, an excerpt from a textbook.

III. The patent proprietor (respondent) responded to the grounds of appeal.

IV. The appellant was 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 it of the preliminary non-binding opinion of the board on some of the issues of the appeal proceedings.

V. In response to the board's communication, the appellant made further submissions.

VI. Claims 1 as 2 as granted read as follows:

"1. A method for producing lysine comprising the step of cultivating a bacterium belonging to the genus

Escherichia in a culture medium which contains sucrose as a main carbon source, wherein the bacterium has been constructed from a sucrose non-assimilative strain belonging to the genus Escherichia, the bacterium harboring sucrose PTS genes from Escherichia coli VKPM B-7915 and having an ability to produce and accumulate lysine in a culture medium which contains sucrose as a sole carbon source.

2. The method according to claim 1, wherein the bacterium belonging to the genus Escherichia is Escherichia coli."

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

D1: US 5,175,107

D4: Mitteilung an das Deutsche Patentamt vom 6. Mai 1997 während eines Einspruchverfahrens gegen DE3891417

D7: US 4,806,480

D8: Doroshenko et al. (1988) Molec. Bio., 22, 645-658 (in English 506-517)

D11: Tsunekawa H et al.: ..Acquisition of a sucrose utilization system in Escherichia-coli K-12 derivatives and its applications to industry" Applied and Environmental Microbiology, vol. 58, no. 6, 1992, pages 2081-2088

D13: US 4,346,170

VIII. The arguments of the opponent, as far as relevant for this decision, can be summarised as follows:

Article 100(c) EPC

The combination of features contained in claims 1 and 2 was not directly and unambiguously disclosed in the application as filed. In view of the discretion given to the opposition division by virtue of Article 114(1) EPC, and in view of the clear non-compliance with the requirements of Article 123(2) EPC, the opposition division could and should have examined the claims ex officio.

Articles 83 and 84 EPC

According to established case law, the clarity of the wording of a claim could affect the assessment of sufficiency of disclosure. Therefore, the decision under appeal should have addressed a number of objections arising from the use of the ambiguous terms "saccharose non-assimilative strain", "the bacterium harbouring sucrose PTS genes", and "medium which contains sucrose as a main carbon source". Furthermore, the claimed method lacked the essential feature of collecting the amino acid from the culture medium.

Article 56 EPC

Starting from document D13 as closest prior art, the technical problem was seen in the provision of an improved method of producing lysine using a medium comprising carbohydrates. The solution to this problem comprised the use of strains harbouring sucrose PTS genes from E. coli VKPM B-7915 and the growth of these strains on sucrose as a main carbon source. In fact,

the patent addressed two independent technical problems, (i) the use of a cheaper carbon source, and (ii) an improved lysine production. The solution to the first problem was not inventive in view of document D8, referring to the use of strains comprising the sucrose PTS genes for the production of biologically active substances. As a consequence of the introduction of the sucrose PTS genes, the yield of lysine increased. The solution to the second problem was the necessary consequence of the use of the PTS genes, hence a bonus effect.

The patent disclosed only a single example using a medium with a very low percentage of non-sucrose carbon source. The claim language allowed, however, for a vast range of sucrose concentrations, the only requirement being that sucrose represented the main carbon source. There was no evidence on file that the claimed increase in lysine production could be obtained throughout the entire scope of the claim, i.e. throughout the entire range of sucrose concentrations.

The claimed solution was also not inventive in view of document D13 in combination with document D1. Document D1 disclosed the use of the sucrose PTS genes to improve threonine production. The PTS genes of document D1 were the same as those of the opposed patent. Since the biosynthesis of lysine and threonine was based on the same precursor molecule, oxalo acetate, and since document D1 disclosed the production of increased amounts of threonine, the same could reasonably be expected to happen upon the transformation of lysine producing strains with sucrose PTS genes. The increase of about 50% in lysine production shown in Table 5 of the opposed patent could be expected on the basis of theoretical considerations. It was general knowledge

that less PEP (phospho-enol-pyruvate) was consumed for the import of a sucrose molecule than for the import of two glucose molecules, resulting in a 1.5 fold increase in the amount of PEP available as a source for the biosynthesis of carbon backbones upon growth on sucrose.

Respondent's argument that the strains of document D13 did not represent sucrose non-assimilitative strains according to claim 1, and that document D13 could therefore not represent the closest prior art, was for the first time presented on the day of the oral proceedings before the board. Should the board give any weight to this argument, the appellant should have the right to develop a new line of arguments on the basis of document D14 as the closest prior art.

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

Article 100(c) EPC

No substantiation of this ground of opposition was provided in the written opposition procedure and no objection was raised in the oral proceedings. The minutes of the oral proceedings before the opposition division showed that the appellant had abandoned this ground of opposition. Appellant's arguments in this respect represented therefore a new ground of opposition which should not be admitted.

Articles 83 and 84 EPC

Appellant's objections were in fact objections under Article 84 EPC and should be disregarded solely for this reason. The term "sucrose non-assimilitative

strain" meant that such strains could not utilize sucrose at all. Regarding the question which sucrose PTS genes were necessary for transforming a non-assimilitative strain into a sucrose assimilating strain, the claim comprised a functional limitation which specified that the strain had to be able to grow on sucrose as the sole carbon source. In addition. strain VKPM B-7915, the source of the sucrose PTS genes, had been deposited. The skilled person knew what carbon sources other than sucrose could be used to cultivate bacteria in a medium.

As far as the objections related to Article 83 EPC, there was no evidence on file that the skilled person could not rework the claimed invention.

Article 56 EPC

The important aspect of the invention as demonstrated by Table 5 was the 50% increase in yield calculated as g lysine produced/g sugar consumed and not just any kind of increase of the lysine concentration in the culture medium. Based on the molecular mass, 50 g of sucrose comprised only 5-10% more carbon atoms than 50 g of glucose. Thus, the skilled person would at best have expected an increase in yield of 5 to 10%.

Starting from document D13, the technical problem could be defined as the provision of a cheaper method for the production of lysine with an increased yield. Document D13 referred to alternative carbon sources but the E. coli strains described in document D13 were derived from E. coli K12 and comprised alternative mechanisms for sucrose uptake in the form of weakly permeable non-sucrose PTS uptake systems. Therefore, document D13 did not disclose the use of sucrose non-assimilating E.

coli strains for the production of lysine. The weak permeability of E. coli K12 strains was evident from table 3 of document D11. Document D1 did not disclose an increase in the yield of threonine upon introduction of the PTS genes. As shown in document D4 (page, 6, comparative experiments) the yield of threonine produced by strain 472T23, disclosed in column 2 of document D1, increased by about 10% when grown on sucrose instead of glucose. Therefore, the claimed subject matter was not obvious in view of document D13 in combination with document D1. Furthermore, based on document D11, which related to the production of tryptophane and did not show any improvement at all upon the introduction of the PTS genes, the skilled person would not have had any expectation of success. Finally, the skilled person trying to solve the technical problem had no motivation or incentive to use strain VKPM B-7915 as the source of the PTS genes.

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

XI. The respondent requested that the appeal be dismissed.

Reasons for the Decision

Admissibility of objections under Article 100(c) EPC

1. With its statement of grounds of appeal, the appellant submitted objections on the ground of Article 100(c) EPC.

2. The notice of opposition mentioned Article 100(c) EPC as a ground of opposition but did not contain any arguments to substantiate it. The appellant (opponent) raised an objection at the oral proceedings before the

opposition division but did not substantiate it and finally abandoned it (cf. minutes of the oral proceedings before the opposition division, page 2).

3. As stated in point 18 of decision G 9/91 of the EBA (OJ 1993, p. 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 above and the respondent's request that Article 100(c) EPC not be admitted as a ground of opposition, the board decides that objections under this Article are not admitted.

Article 83 EPC

5. Appellant's first objection relates to the use of the terms "sucrose non assimilitative strain belonging to the genus Escherichia" and "ability to produce and accumulate lysine". It submitted that the patent in suit does not sufficiently disclose which strains can be used as a starting material and how much lysine these strains produce.

6. As demonstrated by the patent and the cited prior art, assays for assessing sucrose assimilation were state of the art.

The patent itself describes the determination of a sucrose assimilating (suc+) phenotype in paragraph [0036] by the assessment of growth on M9 agar plates containing 0.2% sucrose as the sole carbon source.

Document D7 (column 4, lines 35 to 50) describes the screening for a suc+ phenotype using minimal agar plates comprising 0.1% of sucrose and an indicator molecule.

Document D8 (page 6, 2nd paragraph, and section "Nutrient media, Concentrations of Antibiotics") describes the identification of clones with a Sac+ (suc +) phenotype on M9 agar plates comprising 0.1-0.2% sucrose.

Document D11 (page 2082, left column, last paragraph) describes the identification of Scr+ (suc+) transformants on McKonkey sucrose agar plates.

Thus, the person of skill knew how to assess whether a strain of Escherichia is sucrose assimilative or not.

7. As far as the objection concerned the absence of an indication of the amount of lysine produced by a strain of the genus Escherichia, the board cannot find any reason why the absence of such an indication would prevent the skilled person from readily assessing lysine accumulation in a culture medium.

8. The second objection concerned the feature "the bacterium harboring sucrose PTS genes from Escherichia coli VKPM B-7915". According to the appellant, this wording leaves it open which of the several PTS genes present in Escherichia coli VKPM B-7915 have to be transferred to a lysine producing strain.

9. It is true that the claim does not specify whether all sucrose PTS genes of strain VKPM B-7915 have to be transferred or not. However, the functional feature that the resulting bacterium has to be able to produce and accumulate lysine with sucrose as a sole carbon source provides sufficient guidance to create and isolate sucrose assimilating strains because this property can be tested readily (cf. point 6 above).

10. The third objection related to the fact that the claim does not specify which additional carbon sources may be present in the medium. This is however not a convincing argument why the skilled person would not be in a position to readily perform the claimed invention. There is no evidence on file that the presence of any other carbon source would prevent the skilled man from readily carrying out the method of claim 1.

11. The last objection was that the claim, by omission of the feature "recovering the lysine from the culture medium", did not specify all steps necessary for the

production of lysine. The board notes that the claim specifies a method for producing lysine **comprising** the step of cultivating bacteria with certain properties. The use of the term "comprising" signals that the claimed method is not limited to the steps specified in the claim but may include further steps. The skilled person is perfectly aware that lysine has to be recovered in a method of producing lysine.

12. In view of the above, appellant's objections under Article 83 EPC are without merit.

Article 56 EPC

13. Document D13 represents the closest state of the art. It discloses the production of increased amounts of lysine by strains of E. coli with reduced resistance to high concentrations of lysine in the culture medium. According to the table in column 5, an optimised strain grown on glucose produced 0.28 g lysine per liter of culture medium.

14. The appellant submitted that a first technical problem underlying the present invention should be seen in the provision of a cheaper carbon source for the production of lysine by E. coli and an independent second problem in the provision of a method of increasing the yield of lysine produced by E. coli.

15. The board does not agree with the splitting of the problem to be solved into two independent and unconnected problems, because the difference between the claimed method and the method of the prior art lies in a single feature, i.e. the use of an E. coli strain capable of growing on sucrose as the carbon source. This situation is different from a situation where an

invention is characterized by a combination of new features and where the relevant question could be whether individual elements (or features) defined as solutions to partial problems and their combination were known or obvious from the prior art (cf Case law of the Boards of Appeal, 7th edition, 2013, I.D. 9.2, "Combination invention"). Since the two effects are the consequence of the same technical modification, there cannot be two independent technical problems.

16. Accordingly, the board, in agreement with the respondent, sees the technical problem underlying the present invention in the provision of a cheaper method of producing lysine by E. coli with an increased yield.

17. As a solution to this problem the patent proposes the method of claim 1, comprising the use of a bacterium of the genus Escherichia transformed with the sucrose PTS genes from strain VKPM B-7915.

18. According to Table 5 of Example 6 of the opposed patent, the yield of lysine produced by a strain of E. coli increased from 5.4% when grown on glucose to 8.4% when the same strain was transformed with sucrose PTS genes and grown on sucrose. This corresponds to an increase of about 55%.

19. The appellant argued that the evidence in Example 6 was not sufficient to demonstrate improved lysine production because the strains were grown on sucrose as the sole carbon source, whereas the claim required cultivation in a medium containing sucrose as the main carbon source.

20. According to Example 6, 0.3 ml of a culture of strain VL613 grown in a nutrient broth were inoculated into 3

ml of fermentation medium comprising sucrose as the sole carbon source. The fermentation medium of Example 6 thus comprised multiple carbon sources due to the carry-over of the nutrient broth which by definition comprises multiple carbon sources.

21. The appellant also submitted that the term "harboring sucrose PTS genes" required merely the presence of at least two of the genes from strain VKMP B-7915. There was however no evidence, that each of the possible combinations of at least two genes led to an improved yield.

22. The strains used in the claimed method are not only characterized by the presence of genes from strain VKPM B-7915 but also by the functional property of having the ability to produce and accumulate lysine when grown in a culture medium containing sucrose as a sole carbon source. As stated in point 9 (above), this functional limitation provides guidance in the creation of suitable strains, and excludes strains merely containing two arbitrarily selected genes which do not provide the property of growing on sucrose as the sole carbon source. Moreover, the appellant has only argued that the claimed effect could not be achieved across the entire scope of the claimed method but not presented any evidence in this respect.

23. The board is therefore satisfied that the technical problem is indeed solved.

24. It remains to be established whether the claimed solution involves an inventive step.

25. The respondent submitted that the claimed invention could not be obvious when starting from document D13

because the claimed method required that the strain be constructed from a sucrose non-assimilitative strain, i.e. from a strain unable to take up any sucrose at all. The E. coli strains disclosed in document D13 were however derived from E. coli K12 strains which were capable of taking up some sucrose via alternative non-sucrose PTS transporters. This could be seen in Table 3 of document D11 and in column 1, lines 18 to 20 of document D7.

26. This was altogether a new argument which was for the first time presented by the respondent at the oral proceedings before the board of appeal.

For the reasons given below, the board disagrees with it and it is of no further relevance for the board's assessment of inventive step.

27. The skilled person does not interpret the term "sucrose non-assimilitative" strain as excluding the presence of any residual sucrose uptake and metabolism. Document D11 refers for instance to strain JC1557 as non-sucrose fermenting, i.e. non-assimilitative, (cf. p. 2085, last sentence), despite the fact that it shows some residual sucrase activity and sucrose uptake (cf. Table 2, and page 2086, left column: "As expected, E. coli JC1557 showed no significant sucrase activity, implying that 0.2 micromol/20 min/mg of dry cell with the cells cultured in both glucose and sucrose might be regarded as a control.", and "Shown are specific uptake rate data for each strain with respect to sucrose and glucose after the rates for JC1557 (non-sucrose fermenting strain) were subtracted ..."). Thus, the term "sucrose non-assimilitative" excludes only the presence of significant capacities for sucrose assimilation.

Moreover, while strain SGIII1032 of document D11 showed some residual assimilitative capacity for sucrose, there is no evidence on file that this is a property shared by the strains of document D13. Document D13 merely discloses that the strains were derived from E. coli K12 (column 4, line 26) and, in the general description (column 3, line 12), refers to the use of alternative carbon sources such as sucrose. It does however not disclose significant sucrose assimilation by the strains used in the examples.

28. The board therefore concludes that the E. coli strains disclosed in document D13 are sucrose non-assimilitative strains within the meaning of claim 1.

29. The respondent also submitted that the claimed solution was inventive because none of the prior art referred to the use of the genes of strain VKPM B-7915.

30. According to [0037] and [0067] of the opposed patent, the genes used for the transduction of strain VL612 of Example 6 were the genes comprised in transposon Tn2555. This transposon was known in the art (document D8) and comprises the known sucrose PTS genes ([0032, line 9]). The fact that strain VKPM B-7915 was used as the donor of the well known sucrose PTS genes does not contribute to inventive step because the use of this strain does not result in any properties going beyond what the skilled person would expect on the basis of document D8.

31. The appellant submitted that the claimed solution was obvious in view of document D13 in combination with document D7 or D8.

32. Document D7 discloses a plasmid conferring sucrose fermenting capacity upon transformation into E. coli K12 cells. The plasmid could additionally be used to carry industrially profitable genetic information (cf. abstract). When grown on sucrose as the sole carbon source, only cells comprising the plasmid would be able to grow.

Document D8 discloses an analysis of transposon Tn2555 carrying sucrose PTS genes (sac genes). The document concludes with the statement: "Thus, in the creation of strains that produce biologically active substances on the basis of laboratory strains of E. coli (that do not grow on sucrose), it is advisable to introduce sac genes into the chromosome of these strains."

While these two documents contain general statements about the utility of the sucrose PTS genes for the production of biologically active substances, they contain no information or hint pointing to the effect of their introduction upon yields of any biologically active substances. Thus, while the skilled person, in retrospect, could have followed these suggestions, he would have had no reasonable expectation of successfully solving the underlying technical problem.

33. The appellant further submitted that the invention was obvious in view of document D13 in combination with document D1 which related to the production of threonine by E. coli and disclosed the use of sucrose PTS genes. According to [0007] and [0032] of the opposed patent the PTS genes used in document D1 were almost identical to those used in the claimed invention. The data of document D1 showed that strain VKPM B-3996 produced about 85 g/l of threonine compared to 30 g/l produced by sucrose non-assimilating strain

M1 which was used as the starting material. Thus, according to the appellant, a significant increase in yield was to be expected upon application of the teaching of document D1 to the method disclosed in document D13.

34. The board is not convinced by this argument.

35. Document D1 discloses the construction of a strain for the production of threonine comprising (a) the introduction of genes conferring the capacity to assimilate sucrose (column 1, lines 14 to 20) into strain M1, (b) the selection of mutants resistant to threonine in the culture medium (column 1, lines 25-30), resulting in strain 472T23, (c) the inactivation of a gene encoding a threonine dehydrogenase (column 1, lines 32-38), and (d) the introduction of 3 genes coding for enzymes involved in threonine biosynthesis. Only the strain resulting from these multiple modifications produced 85 g/l of threonine in the culture medium. A meaningful and significant calculation of the improvement of the yield resulting from the introduction of the sucrose PTS genes alone is therefore not possible from the disclosure in document D1.

Data in document D4 (a submission made by the proprietor of patent document D1 in different proceedings) support this conclusion. Comparative experiments (page 6) show that strain 472T23, which differs from original strain M-1 only by the modifications of steps (a) and (b), yielded 40.5% of threonine when grown on sucrose, as opposed to 36.3% when grown on glucose. This brings the gain in yield into the realm of only 10% but it is not possible to attribute it to the introduction of the PTS genes alone

(step (a)) because the strain was also selected for increased resistance to threonine (step (b)).

36. Thus, document D1, although disclosing the introduction of sucrose PTS genes into E. coli strains producing threonine, did not provide a basis upon which the skilled person would have had a reasonable expectation to solve the underlying technical problem when starting from document D13.

37. Finally, the appellant argued that an increase in yield of about 50% could be expected on the basis of theoretical considerations. As generally known and stated in [0006] of the opposed patent, the amount of PEP available as a source of carbon backbone increased by 1.5 fold when strains were grown on sucrose instead of glucose. This corresponded well with the increase of about 50% described in Example 6.

38. This argument is based on the assumption that the additional 50% of PEP obtained as a result of the growth on sucrose is funnelled into lysine biosynthesis. PEP is one of the major building blocks in several biosynthetic pathways and plays a central role in glycolysis based energy production. There is however no evidence that or why significant amounts of the extra PEP resulting from the import of sucrose would be made available to the biosynthesis of lysine.

39. Thus, while the skilled person could have tried to switch to using sucrose as a cheap carbon source for the production of lysine, it had no reasonable expectation of increasing the yield.

40. The claimed subject matter is therefore inventive.

Order

For these reasons it is decided that:

1. The appeal is dismissed.

The Registrar: The Chairman:

A. Wolinski M. Wieser

Decision electronically authenticated