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**Datasheet for the decision
of 4 May 2011**

Case Number: T 2224/08 - 3.3.08

Application Number: 02008316.8

Publication Number: 1254957

IPC: C12N 15/31

Language of the proceedings: EN

Title of invention:

Method for producing an L-amino-acid substance by fermentation

Patentee:

Ajinomoto Co., Inc.

Opponent:

Evonik Degussa GmbH

Headword:

Producing L-amino acids with MalK mutants/AJINOMOTO

Relevant legal provisions:

EPC Art. 83, 54, 56

Relevant legal provisions (EPC 1973):

-

Keyword:

"Main and sole request - sufficiency of disclosure - (yes)"

"Novelty and inventive step - (yes)"

Decisions cited:

T 0032/85, T 0010/86, T 0019/90, T 0923/92, T 0644/97,

T 0608/07

Catchword:

-



Case Number: T 2224/08 - 3.3.08

D E C I S I O N
of the Technical Board of Appeal 3.3.08
of 4 May 2011

Appellant:
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Decision under appeal: Interlocutory decision of the Opposition
Division of the European Patent Office posted
26 August 2008 concerning maintenance of
European patent No. 1254957 in amended form.

Composition of the Board:

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

Summary of Facts and Submissions

- I. The opponent (appellant) filed an appeal against the interlocutory decision of the opposition division dated 26 August 2008 whereby the European patent no. 1 254 957 was maintained in amended form (Article 101(3)(a) EPC)
- II. The opposition division, finding that the main request before it, claims 1 to 7 as granted, did not meet the requirements of Article 54 EPC, decided that auxiliary request 1, claims 1 to 6 filed on 27 May 2008, met all requirements of the EPC.

Claim 1 of auxiliary request 1 read as follows:

"A method for producing an L-amino acid utilizing a microorganism and comprising culturing the microorganism in a medium to produce and accumulate the L-amino acid in the medium and collecting the L-amino acid from the culture, wherein the microorganism is a mutant or recombinant strain of a microorganism in which maltose assimilation is controlled by an interaction between IIA^{Glc} protein of glucose PTS and Malk protein, and the interaction between IIA^{Glc} protein and Malk protein of the mutant or recombinant strain is reduced or eliminated, and the strain can take up glucose and maltose, and the medium contains glucose and oligosaccharide as carbon sources."

Dependent claims 2 to 6 referred to preferred embodiments of the method according to claim 1.

- III. With its grounds of appeal, submitted with letter dated 16 December 2008, the appellant introduced two new prior art documents.
- IV. With letter dated 13 May 2009, the patentee (respondent) submitted its response to the grounds of appeal.
- V. The board expressed its preliminary opinion in a communication pursuant to Article 15(1) of the Rules of Procedure of the Boards of Appeal (RPBA).
- VI. Oral proceedings were held on 4 May 2011. At the beginning of the oral proceedings, the appellant withdrew the two prior art documents introduced with its grounds of appeal.
- VII. The following documents are referred to in this decision:
- (D1) J. Bacteriol., vol.169, no.8, 1987,
pages 3539 to 3545;
- (D2) Journal of Biological Chemistry, vol.265,
no.34, 1990, pages 21005 to 21010;
- (D3) Research in Microbiology, vol.143, 1992,
pages 251 to 261;
- (D9) J. Bacteriol., vol.173, no.7, 1991,
pages 2180 to 2186.
- VIII. The appellant's arguments, insofar as they are relevant to the present decision, may be summarized as follows:

Article 83 EPC

Lack of clarity could lead to objections under Article 83 EPC. The patent provided no instructions how to assess the reduction or elimination of the interaction between IIA^{Glc} protein and MalK protein. In the absence of clear definitions in the claims, the term "reduced interaction" was open to interpretation. Therefore it was necessary to consult the description for more specific instructions. From paragraph [0020] of the patent specification, the skilled reader could take that the interaction was affected by mutations. The effect of mutations had however only been shown for one specific bacterial strain.

In order to find further suitable strains the patent specification suggested to measure growth in a medium containing glucose and maltose and to use strains showing diauxie. However, in the absence of clear instructions under what conditions to measure diauxie, this was an unreliable means. Identifying suitable mutants thus constituted an undue burden.

Article 56 EPC

Document (D2) represented the closest prior art. It referred to an interaction between IIA^{Glc} and MalK and disclosed inducer exclusion-resistant mutants of E.coli. The problem to be solved was the provision of a method for producing L-amino acids with microorganisms having improved assimilation of maltose. In the alternative, the problem to be solved could be defined as finding a new use for the microorganisms of document (D2).

The use of the strains disclosed in document (D2) for the production of L-amino acids represented an arbitrary selection from different known uses of *E. coli* and did not therefore involve an inventive step.

Moreover, the patent provided only one example solving the underlying technical problem. Accordingly, the technical problem was not plausibly solved across the entire breadth of the claims.

- IX. Respondent's arguments, insofar as they are relevant to the present decision, can be summarised as follows:

Article 83 EPC

Appellant's objection that the claimed subject-matter was insufficiently disclosed was not based on verifiable facts but was merely speculative.

Measuring diauxie provided a simple means for assessing the interaction between IIA^{Glc} and MalK in strains differing only in this property. Appellant's statement that the link between the claimed procedure and diauxie was shown for one strain only confirmed that diauxie could be used as a suitable parameter. The skilled person was aware of other generally known methods, such as gel filtration or spectroscopic methods, to assess reduced or eliminated interactions.

Article 56 EPC

The closest prior art should relate to the same technical area, in this case the production of amino acids. None of the documents cited in appellant's

grounds of appeal related thereto. The objective technical problem was the production of L-amino acids using cheaper carbon sources such as e.g. maltose. As shown by the examples, this problem was solved. Examples 1 to 3 showed faster uptake of maltose providing the advantage of reaching higher cell densities after 15 to 18 hours of growth. The prior art documents on file did not suggest any relationship between the production of L-amino acids and the reduction or elimination of the interaction between IIA^{Glc} and MalK.

- X. The appellant requested the decision under appeal to be set aside and the patent to be revoked.
- XI. The respondent requested the appeal to be dismissed.

Reasons for the Decision

Article 54 EPC - Novelty

- 1. The appellant raised in writing objections under Article 54 EPC which were entirely based on the two prior art documents which were introduced with its grounds of appeal. These two documents were withdrawn at the onset of the oral proceedings (see section VI above). As no further novelty objections were raised, the board sees no reason to comment on this issue.

Article 83 EPC - Sufficiency of disclosure

- 2. Example 4 of the patent discloses an evaluation of the productivity of L-amino acids by malK mutant strains.

Table 1 shows L-amino acid production by several E. coli strains derived from W3110(tyrA)malk comprising a mutated malK gene with an L327Q (cf. example 3) substitution. Figure 4 shows monophasic growth of a strain with this mutation in the presence of maltose and glucose, demonstrating reduced inducer exclusion as a result of the reduced or eliminated interaction between IIA^{Glc} and MalK protein. The table also shows the uptake of glucose and maltose in those mutants.

3. Thus, Example 4 discloses a method having all technically characterizing features of the claimed invention. Moreover, the patent discloses further examples of malK mutants and of IIA^{Glc} mutants which per se were known from documents (D2) and (D3), respectively, to show reduced or eliminated inducer exclusion. These mutants were introduced into E. coli strains which appear to be suitable to be used in the claimed method.

4. The appellant did not contest that the patent disclosed in the examples one way of producing amino acids by a microorganism having the features required by the claims. However, it argued that the patent disclosed a specific example with one particular strain of E. coli only. This did not put the skilled person in a position to carry out the claimed invention readily and without undue burden across the entire breadth of the claims, as set out e.g. in decision T 923/92 (OJ EPO 1996, 564), because the functional feature, requiring that "the interaction between IIA^{Glc} protein and MalK protein of the mutant or recombinant strain is reduced or eliminated", was insufficiently defined and could not be easily tested in other microorganisms.

The Board does not agree. Firstly, isolating mutants in which either the IIA^{Glc} or the MalK gene is mutated lies well within the capabilities of a person skilled in the art. Document (D9) describes methods of inducing mutations in the malK gene by standard treatment with UV light or hydroxylamine (page 2181, right column, first paragraph). Document (D3) discloses mutagenesis of the crr gene, encoding the IIA^{Glc} protein, by hydroxylamine (page 254, "Results", first paragraph). Methods of inducing further mutations more specifically in the MalK protein of E. coli are disclosed in document (D2) (page 21006, "Experimental Procedures", first paragraph).

Secondly, reduced or eliminated interaction between IIA^{Glc} and MalK can be readily assessed and is not limited to measuring diauxie. Documents (D2), (D3) and (D9) disclose methods for identifying mutants with reduced or eliminated interaction between IIA^{Glc} and MalK using selective growth media. In document (D9) strains comprising the mutant proteins were tested for their ability to metabolize maltose in the presence of the non-metabolizable glucose analogue alpha-methyl glucoside (page 2181, right column, first paragraph). Document (D3) discloses a screening procedure for identifying mutants with reduced or eliminated inducer exclusion (page 253, left column, first paragraph). Document (D2) discloses a further screening procedure to identify mutations in MalK affecting the interaction between IIA^{Glc} and MalK (page 21006, "Experimental Procedures", first paragraph).

There is no evidence on file, and the board sees no reasons, why these procedures would not work with any of the alternative strains listed in paragraphs [0016] and [0017] of the patent. The standard procedures used for mutagenesis in combination with the screening procedures known in the art put the skilled person in a position to isolate and test readily and without undue burden strains in which the interaction between IIA^{Glc} and MalK is reduced or eliminated.

5. The patent shows not only how to produce mutants in the IIA^{Glc} protein and the MalK protein which lead to reduced or eliminated interaction between the two proteins, but also how the absence of biphasic growth (diauxie) in a medium comprising glucose and maltose can be used to identify suitable mutants.

The appellant submitted that the patent did not provide sufficient technical information about the growth assays to reliably test for the absence of diauxie. This made it difficult, if not impossible, to establish whether the interaction between the two proteins was reduced or not because the loss of diauxie might depend on the conditions used.

Measuring diauxie requires growth of the microorganisms in a medium comprising glucose and maltose in concentrations suitable for showing a biphasic growth behaviour. The board has no doubts that the skilled person can readily establish such conditions for any given microorganism in which maltose assimilation is controlled by the interaction between IIA^{Glc} protein and MalK protein. Once the conditions are established, mutant IIA^{Glc} or MalK proteins can readily be tested.

Regarding the objection that the absence of diauxie might not prove a reliable means for identifying suitable strains in all cases, the board notes that the claims require a microorganism with reduced or eliminated interaction between IIA^{Glc} protein and MalK protein. Documents (D2) (page 21006, left hand, section "Bacterial Strains and Growth Conditions"), (D3) (page 253, left hand, first paragraph), and (D9) (page 2181, right hand, lines 6 to 13) disclose screening assays to test this interaction based on selective media. Thus, even if assessing the loss of diauxie proved in individual cases difficult, the person of skill had alternative means at its disposition to readily identify suitable mutants.

6. The appellant also argued that document (D9) disclosed that three functionally distinct classes of MalK mutants could be generated. Only one thereof showed loss of inducer exclusion. Therefore it constituted an undue burden to identify those mutants with altered the interaction between IIA^{Glc} and MalK protein and which were therefore suitable for the method according to claim 1.

In the board's view, this conclusion is not warranted. Document (D9) describes three functionally distinct classes of molecules, of which only class III mutants are insensitive to inducer exclusion. Only this third class shows the required reduction or elimination of the interaction between the IIA^{Glc} and MalK proteins. These mutants were selected for their ability to grow on maltose in the presence of the non-metabolizable glucose analogue alpha-methylglucoside (page 2181,

right column, first paragraph). The person of skill, looking for mutants with reduced or eliminated interaction between the IIA^{G1c} and the MalK proteins would undoubtedly choose members of this class III, and would directly arrive at mutants with the desired properties.

7. Regarding the standards set for sufficiency of disclosure, the appellant referred to decision T 10/86 of 1 September 1988. However, this decision relates to a situation where the only means disclosed for solving the technical problem was considered unsuitable, i.e. non-working, and alternative means were not available to the skilled man on the basis of his general knowledge. Contrary to this, the patent under appeal discloses a working embodiment in example 4, it lists several alternative embodiments in examples 1 to 3, and it suggests a number of strains suitable for the claimed use (paragraphs [0016] and [0017]).

The appellant also referred to decision T 32/85 of 5 June 1986 relating to a case where the skilled person, trying to achieve a functionally defined result, had to establish numerous parameters by trial and error. This was considered an undue burden. In the present case, there is only one parameter which in the board's view can be readily assessed by several methods (see point 5 above).

Finally, the appellant also referred to decision T 608/07 of 27 April 2009 in which a limiting functional term was found to be unclear to such an extent that the skilled person did not know when he was working within the area of the claim. This was the

consequence of multiple definitions of the limiting term leading to contradictory results depending on the definition used. In the present case, the board cannot recognize any contradictory definitions that could render ambiguous whether the interaction between IIA^{Glc} and MalK is reduced or eliminated.

The board concludes that the case law of the Boards of Appeal, relied on by the appellant to substantiate its arguments does not apply to the present case.

8. According to established case law of the Boards of Appeal the objection based on lack of sufficient disclosure presupposes that there are serious doubts, substantiated by verifiable facts. The mere fact that a claim is broad is not in itself a ground for considering the patent as not complying with the requirement of sufficient disclosure under Article 83 EPC (cf. decision T 19/90 OJ EPO 1990, 476, point 3.3 of the reasons).

No such verifiable facts leading to serious doubts are identified by the Board in the present case. Therefore it decides that the requirements of Article 83 EPC are met.

Article 56 EPC

9. Claim 1 refers to a method for producing an L-amino acid utilizing a microorganism (see section II above).
10. In accordance with the problem and solution approach and the relevant case law developed by the Boards of Appeal, the closest prior art which provides the best

starting point for assessing inventive step should be prior art conceived for the same purpose or aiming at the same objective as the claimed invention (cf. Case Law of the boards of Appeal of the European Patent Office, 6th edition 2010, Chapter I.D.3.1).

None of the prior art documents (D1) to (D10) relates to the production of amino acids. Document (D2), which the appellant considered to represent the closest prior art, describes the regulation of the maltose uptake system in *E. coli*. It discloses an analysis of MalK mutants insensitive to inducer exclusion through their interaction with IIA^{Glc}. Document (D2), as well as documents (D1) and (D3) to (D10), does not however mention the production of L-amino acids.

On the other hand, the production of L-amino acids by microorganisms and especially by *E. coli*, is well known in the art, as e.g. demonstrated by the patent documents listed in paragraph [0017] of the patent in suit.

The board considers the disclosure in the documents cited in paragraph [0017] of the present patent, which obviously belongs to the general knowledge of a person skilled in the art of microbial L-amino acid production, to be more relevant and thus closer related to the claimed subject-matter than the disclosure in document (D2) or in any of the other prior art documents on file.

11. In the present case it is therefore appropriate to apply considerations developed by the boards of appeal for cases in which none of the cited prior art

documents relates to the same purpose as the claimed invention. In such cases, it is extremely important not to formulate the problem in terms containing pointers to the solution (cf. decision T 644/97 of 22 April 1999; point 2.6.1 of the reasons of decision).

Bearing this in mind, the board defines the technical problem underlying the present invention as the provision of a method for the production of L-amino acids by using microorganisms having an improved ability to assimilate oligosaccharides.

12. Example 4 of the patent shows a method of producing L-amino acids. Table 1 shows uptake of glucose and maltose by *E. coli* strains derived from strain W3110(*tyrA*) comprising a mutation in the MalK protein and growing in a medium comprising glucose and maltose. Example 3, in particular Figure 4, shows a loss of biphasic growth (diauxie) in strains carrying this mutation. In the absence of any genetic differences other than the mutation in MalK, this loss of diauxie is the result of a reduced or eliminated interaction between the IIAGlc protein and the MalK protein. Thus, example 4 of the patent provides solutions with all the features of claim 1.

13. With reference to decisions T 1329/04 of 28 June 2005, T 893/02 of 26 Mai 2004, and T 210/02 of 1 October 2004, the appellant considered it implausible that the teaching of the patent indeed solved the problem over the whole area claimed.

The circumstances of the present case differ from those cases underlying the cited decisions. The patent in

suit explicitly discloses various embodiments of the invention which actually are solutions of the underlying problem. Contrary to this, the decisions cited by the appellant refer to cases wherein the claimed technical effect was not proven but was merely based on assumptions.

Theoretically, appellant's argument might prevail in a case where claim 1 referred to the use of strains merely characterised by their ability to take up glucose and maltose without any further specification of the strain. However, this is not the case, the strains used in the method according to claim 1 are defined as, and thus limited to, strains "in which maltose assimilation is controlled by an interaction between IIA^{Glc} protein of glucose PTS and MalK protein, and the interaction between IIA^{Glc} protein and MalK protein is reduced or eliminated". This functional feature excludes all strains wherein maltose assimilation in the presence of glucose might be governed by alternative mechanisms.

The present situation is therefore not comparable with that of the cases underlying the above cited decisions, which are not therefore relevant for the present case.

Accordingly, the board is convinced that the technical problem underlying the patent (see point 11 above) is solved over the entire scope of the claims.

14. It remains to be examined if the claimed solution to this problem involves an inventive step as required by Article 56 EPC.

The skilled person starting from the disclosure in the documents cited in paragraph [0017] of the patent in suit and trying to solve the problem was in a position where he/she could have chosen between various approaches which possibly could have led to a satisfying result. However, in order to arrive at the conclusion that the approach finally chosen, which resulted in the method according to claim 1, was arrived at in an obvious way, the board has to be sure that the skilled person had a reasonable expectation of success to consider that the interaction between IIA^{Glc} and MalK protein would improve the ability of a microorganism to assimilate oligosaccharides in a method for producing an L-amino acid.

The cited prior art documents (D2), (D3) and (D9) all are concerned with the interaction between IIA^{Glc} protein and MalK protein and how this affects inducer exclusion. Although these documents disclose mutants with properties which would make them useful for the claimed method, they do not make any mention of a method for producing L-amino acids.

The appellant argued that the use of the mutated microorganisms of document (D2) for the production of L-amino acids represented one possible use of such microorganisms which was well known to a person skilled in the art. Therefore, the claimed method represented an arbitrary selection from a well known number of possible applications of known microorganisms and did not involve an inventive step.

The board observes that the prior art documents on file do not contain any hint that could be seen as a

motivation to combine the teaching of any of documents (D2), (D3) or (D9) with the skilled person's general knowledge about the production of L-amino acids by microorganisms as described in the prior art documents cited in [0017] and to use the microorganisms disclosed therein for the claimed method.

Appellant's conclusion is therefore considered to be based on hindsight.

15. Thus, the board decides that the subject matter of claims 1 to 6 involves an inventive step within the meaning of Article 56 EPC.

Order

For these reasons it is decided that:

The appeal is dismissed.

The Registrar:

The Chairman:

A. Wolinski

M. Wieser