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D E C I S I O N
of 12 December 1994

Case Number: T 0782/91 - 3.3.2

Application Number: 82112072.2

Publication Number: 0088166

IPC: C12N 15/00

Language of the proceedings: EN

Title of invention:
Method for expressing a gene

Patentee:
KYOWA HAKKO KOGYO CO., LTD.

Opponent:
(01) Degussa AG, Frankfurt - Zweigniederlassung Wolfgang - Zentrale Abteilung Patente
(02) Kernforschungsanlage Jülich GmbH RPA-PT

Headword:
Expressing a gene/KYOWA

Relevant legal provisions:
EPC Art. 54, 56

Keyword:
Main request: "Novelty (no) - prior art disclosure enabling - onus of proof not discharged"
Auxiliary request: "Novelty (yes) - Inventive step (yes)"

Decisions cited:
T 0455/91, T 0158/91

Catchword:
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Beschwerdekammern | Boards of Appeal | Chambres de recours

Case Number: T 0782/91 - 3.3.2

D E C I S I O N
of the Technical Board of Appeal 3.3.2
of 12 December 1994

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Representative: -

Decision under appeal: **Decision of the Opposition Division of the European Patent Office dated 20 August 1991 rejecting the opposition filed against European patent No. 0 088 166 pursuant to Article 102(2) EPC.**

Composition of the Board:

Chairman: A. J. Nuss
Members: L. Galligani
S. C. Perryman

Summary of Facts and Submissions

I. European patent application No. 82 112 072.2 was granted as European patent No. 0 088 166 on 22 March 1989 with nine claims.

Claim 1 read as follows:

" A process for producing a metabolic product which comprises transforming a host microorganism belonging to the genus *Corynebacterium* or *Brevibacterium* with a recombinant DNA wherein a DNA fragment containing at least one gene responsible for the biosynthesis of the metabolic product and obtained from a microorganism of *Escherichia coli* or *Bacillus subtilis* is inserted into a vector DNA, culturing the transformant in a nutrient medium, accumulating the metabolite resulting from the gene in the culture medium and thereafter recovering the metabolite therefrom."

Dependent Claims 2 to 5 concerned the specific embodiments of L-threonine and L-glutamic acid production.

Independent Claim 6 read as follows:

" A process for producing L-lysine which comprises culturing in a medium a microorganism obtained by transforming a host microorganism belonging to the genus *Corynebacterium* or *Brevibacterium* with a recombinant DNA wherein a DNA fragment conferring the resistance to a lysine analogue, S-(2-aminoethyl)-cysteine, and lysine producing ability is inserted into a vector DNA, accumulating L-lysine in the culture medium and recovering L-lysine therefrom."

Claim 7 concerned a specific embodiment of the process according to Claim 6, namely a process wherein the recombinant DNA is plasmid pAec5 contained in Corynebacterium glutamicum K17 ATCC 39032.

Claims 8 to 9 concerned a process for producing L-tryptophan.

II. Notice of opposition against the European patent was filed by two Opponents who requested the revocation of the patent on the grounds of Article 100(a) EPC, in particular lack of novelty and inventive step. During the opposition proceedings the parties relied on a number of documents, in particular on the following:

1) EP-A-0 082 485, cited under Article 54(3) and (4) EPC;
2) FR-A-2 482 622.

III. With decision dated 20 August 1991, the Opposition Division rejected the oppositions pursuant to Article 102(2) EPC and, thus, maintained the patent on the basis of the claims as granted. The main reasons given for the decision were as follows:

a) Document (1), did not affect the novelty of any of the Claims 1 to 5 under Article 54(3) and (4) EPC because it disclosed neither a method which resulted in the "...accumulation of the metabolite resulting from the gene in the culture medium..." nor the production of L-glutamic acid.

b) Document (2) did not affect the novelty of the subject-matter of Claims 6 and 7 because its disclosure was unclear in respect of the cloned DNA fragment conferring S-(2-aminoethyl)-cysteine resistance (AEC).

c) None of the cited documents showed or suggested that nucleotide sequences isolated from Escherichia coli could have been used to transform Corynebacterium or Brevibacterium in order to produce increased amounts of metabolites of interest. For these reasons, the subject-matter of Claim 1 involved an inventive step.

IV. The Appellant (Opponent I) lodged an appeal against this decision.

V. In response thereto, the Respondent (Patentee) filed the report of an experiment in which an attempt to reproduce the results reported in document (2) had failed. Based on this, the Respondent argued that document (2) could not be used as an anticipation of the subject-matter of Claim 6.

VI. The Appellant objected that in the quoted experimental report the AEC resistance/sensitivity had not been tested under the proper conditions.

VII. In a communication dated 15 October 1993, the Board made some preliminary remarks on novelty and drew the parties' attention to Example 2 in document (2) which seemed to be prejudicial to the patentability of the subject-matter of granted Claims 6 and 7.

VIII. With the letter dated 14 December 1993, the Appellant stated its agreement with the evaluation of the facts made by the Board.

By letter dated 26 April 1994, the Respondent submitted a report of a further experiment in which an attempt to reproduce the results given in Example 2 of document (2) had failed.

IX. In a further communication, the Board expressed the provisional opinion that, in view of the submissions by the Respondent, the Appellant would have to demonstrate the relevance of document (2) for the novelty issue.

X. Oral proceedings took place on 12 December 1994.

During oral proceedings the Respondent submitted an auxiliary request which differed from the main request by incorporating into granted Claim 6 the features of dependent Claim 7 and by renumbering the following claims. Minor obvious clerical errors were also corrected.

XI. The Appellant argued essentially as follows:

a) document (1), which like the patent-in-suit dealt with the problem of the production of useful substances in Corynebacterium or Brevibacterium (see page 4, lines 13 to 25 and page 6, lines 13 to 19), disclosed transformants of Corynebacterium or Brevibacterium that expressed genes derived from Escherichia coli and other microorganisms. One of the exemplified plasmids of document (1), namely pEthr1 (see Example 4), was also identically used in the patent-in-suit (see Example 2). The step of

accumulating and recovering the useful substance were necessarily implied in document (1) in view of its aim. Thus, the said document took away the novelty of Claims 1 to 5 pursuant to Article 54(3) EPC.

b) document (2) disclosed L-lysine producing microorganisms belonging to the genus Corynebacterium or Brevibacterium which incorporated a DNA fragment that controlled resistance to AEC and production of L-lysine. The process for obtaining such microorganisms was described, the exemplified microorganisms were made generally available by way of deposition and their yields in L-lysine were reported (see Tables I and II). It was not credible that the applicants of document (2) would have deposited microorganisms not having, at least to some extent, the claimed features. The inability by the Respondent to reproduce the results of document (2) in respect of the deposited microorganisms was partly to be explained by the fact that the Respondent did not use the same experimental conditions. For example, since the host microorganism B.lactofermentum No. 28 was methionine-dependent, it should not have been tested on an agar medium which contains no methionine. As regards the evaluation of the AEC resistance, it was observed that poor growth (+/-) could not be equated to no growth, but was rather indicative of some resistance. Since the Respondent had not shown that the process described in document (2) was not workable, the subject-matter of Claim 6, which related to an identical process, lacked novelty.

XII. The Respondent's arguments were essentially as follows:

a) the main purpose of document (1) was to facilitate recombinant DNA technology in the Corynebacteria and Brevibacteria. To this end, foreign genes of microorganisms such as Escherichia coli were inserted into a plasmid autonomously replicable in Corynebacterium and Brevibacterium cells in order to create selective markers and new cleavage sites for restriction endonucleases in the said plasmid (see passage bridging pages 4 and 5). None of the plasmids disclosed in document (1) was used for the production of a metabolic product that accumulated in the culture medium and was recovered therefrom. Thus, document (1) did not anticipate any of the claims of the present patent.

b) As shown by the tests performed by the Respondent, the microorganisms resulting from the processes described in document (2) did not have the stated features. Consequently, the disclosure of document (2) was not enabling and could not be used as an anticipation of the subject-matter of Claim 6.

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

The Respondent requested as main request that the appeal be dismissed, and as auxiliary request that the decision under appeal be set aside and that the patent be maintained on the basis of the claims of the auxiliary request submitted during oral proceedings.

Reasons for the Decision

1. The appeal is admissible.

2. *The main request*

2.1 Novelty vis-à-vis document (1) [Article 54(3) and (4) EPC]

Document (1) constitutes prior art under the provisions of Article 54(3) and (4) EPC.

This document is indeed concerned with the expression in Gram positive microorganisms of the genus Corynebacterium or Brevibacterium of genes derived from other microorganisms, such as E.coli, in order to improve the production of useful substances in the said Gram positive microorganisms (see page 6, lines 13 to 25). On page 6, lines 12 to 23 it is stated inter alia that "preferably the genes...responsible for the biosynthesis of cell components such as amino acids, nucleic acids and vitamins...derived from...bacteria belonging to the genera Escherichia,...and Bacillus... are employed".

The examples of document (1) relate to the preparation of a series of autonomously replicable vectors which contain a gene (or genes) encoding a selection marker (antibiotic resistance, complementation of amino acid requirement) to be used for the transformation of Corynebacterium or Brevibacterium host cells. None of the examples describe the accumulation of any particular metabolic product in the culture medium of transformant cells and its recovery therefrom.

As remarked by the Appellant, document (1) describes inter alia in Example 4 the preparation of plasmid pEthr1, which contains the threonine operon, and its use in the transformation of Corynebacterium glutamicum LA-201. The same plasmid is used in the patent-in-suit (see Example 2) for the preparation of L-threonine in Corynebacterium glutamicum LA-106 (see Table 2), i.e. as an embodiment of the invention related to Claims 1 to 3. However, the process according to Claims 1 to 3 of the patent-in-suit contains the additional steps of accumulating L-threonine in the culture medium and recovering it therefrom. Such steps are neither described nor implied in document (1) which is limited to the testing of the complementation of the homoserine requirement.

The Board noted also that Example 5 in the patent-in-suit was taken identically from document (1) (see Example 3 on pages 23 to 24). The said example, which relates to the expression of the kanamycin-resistance gene from pUB110 isolated from B.subtilis in C.glutamicum LA-103, does neither describe nor imply the accumulation and recovery of any particular product from the culture medium. Thus, it must be concluded that the said example does not concern an embodiment of the invention as claimed in Claim 1, but merely the preparation of a vector containing a selection marker. This was also confirmed by the Respondent during oral proceedings before the Board.

The novelty of a multistep process can only be considered to be anticipated by a prior art document if the whole of the process, i.e. the entire sequence of all its steps, is disclosed therein, either directly or by way of implication. Such is not the case here. Thus, the Board concludes that

document (1) does not destroy the novelty of the process according to Claims 1 to 5 under Article 54(3) and (4) EPC.

2.2 Novelty vis-à-vis document (2) [Article 54(1)(2) EPC]

2.2.1 Document (2) discloses (see page 1) a method for producing L-lysine by fermentation which is characterized by

a) the cultivation of a Brevibacterium or a Corynebacterium in which a plasmid containing a DNA fragment that controls the resistance to AEC and the production of L-lysine is inserted and

b) the recovery of the accumulated L-lysine from the culture medium (see Claim 1).

Document (2) gives on page 2 a list of deposited microorganisms from which suitable DNA fragments can be obtained, and describes on pages 2 to 4 methods suitable for each required step. On pages 4 to 8 there follow two examples giving a detailed protocol, one directed to Corynebacterium, the other to Brevibacterium. The said examples describe the preparation of transformants starting from known deposited microorganisms. The resulting transformants, which were selected from a medium containing AEC (see, for example, page 6, lines 12 to 21), are shown to accumulate in the medium more L-lysine than the respective parent strains (see Tables I and II). Such transformants were also made available in the form of deposited strains [namely Corynebacterium glutamicum AJ 11575 (FERM-P 5501, NRRL B-12418) and Brevibacterium lactofermentum AJ 11590 (FERM-P 5518, NRRL B-12421)].

2.2.2 The Respondent maintained that the teaching of document (2) was not enabling on the basis of experimental reports which showed that:

(i) strain NRRL B-12418 (final product of Example 1) did not carry a plasmid;

(ii) strain No. 97, an intermediate strain in Example 1, was an L-lysine requiring strain;

(iii) strains NRRL-B-12418 (final product of Example 1) and No. 97/pClysA (obtained from No. 97 by introducing the diaminopimelate decarboxylase gene) were AEC sensitive;

(iv) strain NRRL-B-12418 (final product of Example 1) was a low lysine producer (10 mg/dl);

(v) strain NRRL-B-12421 (final product of Example 2) was AEC sensitive.

In the Respondent's submissions, the above data demonstrated that strains resulting from the processes disclosed in the examples of document (2) did not possess the stated features, i.e. resistance to AEC and ability to produce high amounts of L-lysine. The Respondent concluded that, since the examples of document (2) were not workable, the said document could not be used as an anticipation of the subject-matter of Claim 6.

On the other hand, the Appellant maintained that the tests were not performed under the same experimental conditions as given in document (2) and, thus, they could not be used to contradict the disclosure of the document in question.

2.2.3 The Board observes that the experimental reports submitted by the Respondent are essentially limited to the testing of the final products of the Examples 1 and 2 of document (2).

The question whether the reported experiments were carried out under the proper conditions need not to be decided because even if correct, it does not follow that document (2) is not an enabling disclosure.

As suggested by the Respondent (see letter dated 20 October 1992, page 5), a possible explanation for the quoted experiments is that the harboured plasmids which had conferred the features of "L-lysine production" and "AEC resistance" to the transformed strains of Examples 1 and 2 were lost during the long period that the deposited microorganisms had been stored. However, the disclosure of document (2) is not limited to the deposited products of Examples 1 and 2, but enables the skilled person to repeat the method for himself. Prima facie it appears to the Board that only routine experimentation and trials would be involved and could be repeated by a skilled person without undue burden, the desired results being directly verifiable (AEC resistance, L-lysine production). The Respondent did not put forward any evidence to contradict this.

To accept the Respondent's argument that document (2) is not enabling as far as the general method is concerned would be to apply a different standard to the disclosure of this piece of prior art than to the patent-in-suit which would be contrary to the established case law (see in this respect, for example, T 158/91 of 30 July 1991, not published in the OJ EPO, in particular point 2.2 of the Reasons).

It would, therefore, be untenable to maintain that virtually the same amount of detail as to a method as given in a patent, is inadequate in the prior art. In the present case, the Respondent was unable to point to any critical information given in the patent-in-suit which was not already given in document (2). An expert witness on behalf of the Respondent did indeed give some technical details of difficulties which had to be avoided if the method of document (2) was not to fail. But these were not referred to in the patent-in-suit, so the Board can only infer that overcoming them would be routine for the person skilled in the art. If it was critical to have a deposit of the desired microorganism, then to be consistent the patent-in-suit should also be confined to claims to deposited microorganisms. When a process claim is put forward which is not limited to the use of a particular deposited strain, in the absence of strong evidence to the contrary, it is to be accepted that it is within the skill of the person in the art to carry out the process even without access to that particular microorganism. It would be untenable to say that prior art should be disregarded, because occasionally some particular microorganism did not give the expected result.

For these reasons, in the Board's judgement, document (2) contains an enabling disclosure and can be opposed to the patent-in-suit under the provisions of Article 54(1) and (2) EPC. Since a process comprising all the steps of that of Claim 6 is disclosed in document (2), the said claim lacks novelty. Consequently, the main request, of which the said claim is part, is not allowable under Article 54(1) and (2) EPC.

3. *The auxiliary request*

3.1 Formal allowability of the amendments [Article 123(2)(3) EPC]

The auxiliary request differs from the main request (claims as granted) merely in that Claim 6 incorporates the features of dependent Claim 7, the following claims being correspondingly renumbered. As these amendments result neither in an extension of the protection conferred nor in subject-matter extending beyond the contents of the original application (see Example 1 therein), there are no objections in respect of the formal admissibility under Article 123 (2) and (3) EPC of this request. This was not contested by the Appellant.

3.2 Novelty (Article 54 EPC)

The novelty of Claim 6 of this request was no longer contested by the Appellant nor does the Board see any objection thereto because a L-lysine production process wherein the recombinant DNA is plasmid pAec5 contained in Corynebacterium glutamicum K17 ATCC 39032 is not disclosed in document (2) or in any other available prior art document.

3.3 Inventive step (Article 56 EPC)

3.3.1 The closest prior art

Document (2) (see point 2.2 supra) represents the closest prior art also for Claims 1 to 5 as well as for Claim 6 of this request.

3.3.2 The technical problem

In the light of document (2), the technical problem underlying the present application can be seen in the provision of an alternative method for the production of a desired metabolite, in particular - in the case of L-lysine - an improved method for its production.

3.3.3 The solution(s)

As a solution to the stated technical problem, Claim 1 (and dependent Claims 2 to 5) provide(s) a process based on the expression in Corynebacterium or Brevibacterium cells of a DNA fragment containing at least one gene responsible for the biosynthesis of the metabolic product, said gene being derived from other microorganisms, such as Escherichia coli, and being inserted in a vector DNA.

Claim 6 provides, on the other hand, an L-lysine production process wherein use is made of the strain Corynebacterium glutamicum K17 ATCC 39032 which contains the specific plasmid pAec5.

The working examples show that the claimed processes provide indeed a solution to the underlying technical problem since they allow accumulation and recovery of good amounts of the desired metabolite, in particular L-lysine, L-threonine and L-glutamic acid. As regards L-lysine, Table 1 of the patent-in-suit reports a remarkably high yield (7.2 mg/ml) in connection with process according to Claim 6. Thus, the Board is satisfied that the said problem has been solved.

3.3.4 Assessment of inventive step

3.3.4.1 As regards Claim 1, the Appellant maintained at oral proceedings that it lacked an inventive step because the solution based on foreign gene expression was more complex and no better than the solution offered by document (2) which was based on a simpler self cloning system.

As for Claim 6, the Appellant acknowledged that the solution proposed therein was not obvious having regard to document (2), especially in consideration of the high L-lysine yields reported in Table 1 of the present patent specification.

3.3.4.2 For the actual assessment of inventive step of the subject-matter of Claim 1, the relevant question to be asked is whether the skilled person, faced with the problem of finding an alternative process for the production of a desired metabolite, starting from the teaching of document (2), would have arrived at a process such as that claimed.

The Board observes that at the relevant priority date the application of recombinant DNA technology to Corynebacteria or Brevibacteria was essentially limited to the transfer of DNA fragments within the same genus (self cloning systems) in order to improve amino acid production [see, for example, document (2)]. In spite of the fact that recombinant DNA technology was established for Escherichia coli, in the Board's view, none of the available prior art documents would have given to the skilled person any hints or suggestions leading to the introduction of one or more genes derived from Escherichia coli or Bacillus subtilis into a microorganism belonging to the genus Corynebacterium or Brevibacterium. The use of non-self cloning systems in the latter microorganisms was a yet unexplored area into which

an average skilled person would not have entered in order to find an alternative process for the production of a metabolite because it would have involved too many uncertainties and incalculable risks (see in this respect decision T 455/91 of 20 June 1994, to be published in the OJ EPO, in particular point 5.1.3.3), and thus doing so could not be regarded as obvious.

The argument put forward by the Appellant at oral proceedings that the proposed solution based on foreign gene expression in Corynebacterium or Brevibacterium was more complex than the solution offered by the prior art document (2), far from supporting the obviousness of the claimed solution, confirms the Board's view that the cautious attitude of the skilled person (cf. T 455/91 supra, loc.cit.) would not have induced him or her to attempt any non-self cloning in Brevibacteria or Corynebacteria.

For these reasons, the Board considers that the subject-matter of Claim 1 as well as that of dependent Claims 2 to 5 involves an inventive step.

3.3.4.3 As regards Claim 6, the relevant question to be asked is whether the skilled person, faced with the problem of finding an improved process for the production of L-lysine, starting from the teaching of document (2), would have arrived at a process involving the use of plasmid pAec5 as contained in Corynebacterium glutamicum K17 ATCC 39032.

In the Board's view, within the normal design procedures, the skilled person would have readily sought to improve the protocols disclosed in document (2) in order to render them more reliable or to increase the yield of L-lysine. To this end, the

said skilled person would, for example, have attempted the construction of alternative recombinant vectors. However, neither document (2) nor any other document suggested to the skilled person the construction of the specific vector pAec5 and, consequently, of the strain Corynebacterium glutamicum K17 ATCC 39032. Nor would the skilled person have foreseen that this would have resulted in a process for producing L-lysine with the remarkable yield reported in Table I of the patent-in-suit. In fact, the use of the specific vector pAec5 as contained in Corynebacterium glutamicum K17 ATCC 39032 results in the accumulation and recovery of L-lysine amounts (7.2 mg/ml of culture medium; see Table 1) which are by far superior to those reported in document (2) (cf. 2.35 mg/ml and 1.89 mg/ml, respectively, in Tables I and II). This is to be regarded as a truly unexpected effect, which constitutes a decisive indication in favour of inventive step. This is acknowledged also by the Appellant.

For these reasons, the Board considers that the subject-matter of Claim 6 involves an inventive step.

3.4 The patentability of the subject-matter of Claims 7 to 8 (corresponding to the granted Claims 8 to 9) was not challenged at any stage by the Appellant.

3.5 In view of the above conclusions, the auxiliary request is allowable.

Order

For these reasons it is decided that:

1. The decision under appeal is set aside.

2. The case is remitted to the first instance with the order to maintain the patent on the basis of the claims of the auxiliary request submitted during the oral proceedings, and a description to be adapted.

The Registrar: The Chairman:

E. Görgmaier A. Nuss