BESCHWERDEKAMMERN DES EUROPÄISCHEN PATENTAMTS | BOARDS OF APPEAL OF THE EUROPEAN PATENT OFFICE | CHAMBRES DE RECOURS DE L'OFFICE EUROPEEN DES BREVETS

Internal distribution code:
(A) [ ] Publication in OJ
(B) [ ] To Chairmen and Members
(C) [X] To Chairmen

D E C I S I O N
of 7 May 1998

Case Number: T 0901/94 - 3.3.4

Application Number: 85103560.0

Publication Number: 0156355

IPC: C12P 21/02

Language of the proceedings: EN

Title of invention:
Method of improving the yield of heterologous protein produced by cultivating recombinant bacteria

Patentee:
Cetus Oncology Corporation

Opponent:
Celltech Limited

Headword:
Protein yield/CETUS ONCOLOGY CORP

Relevant legal provisions:
EPC Art. 123(2), (3), 84, 54, 56

Keyword:
"Novelty - yes"
"Inventive step - yes"

Decisions cited:
T 0433/86, T 0171/87, T 0004/80

Catchword:
-

Europäisches Patentamt | European Patent Office | Office européen des brevets

Beschwerdekammern | Boards of Appeal | Chambres de recours

Case Number: T 0901/94 - 3.3.4

D E C I S I O N
of the Technical Board of Appeal 3.3.4
of 7 May 1998

Appellant: (Opponent)
Celltech Limited
216-222 Bath Road
Slough
Berkshire SL1 4EN (GB)

Representative:
Mercer, Christopher Paul
Carpmaels & Ransford
43, Bloomsbury Square
London WC1A 2RA (GB)

Respondent: (Proprietor of the patent)
Cetus Oncology Corporation
1400 Fifty-Third Street
Emeryville
California 94608 (US)

Representative:
VOSSIUS & PARTNER
Postfach 86 07 67
81634 München (DE)

Decision under appeal: **Interlocutory decision of the Opposition Division of the European Patent Office posted 19 September 1994 concerning maintenance of European patent No. 0 156 355 in amended form.**

Composition of the Board:

Chairman: U. M. Kinkeldey
Members: F. L. Davison-Brunel
W. Moser

Summary of Facts and Submissions

I. European patent No. 0 156 355 with the title "Method of improving the yield of heterologous protein produced by cultivating recombinant bacteria" was granted with 11 claims based on European patent application No. 85 103 560.0.

Claim 1 as originally filed read:

"A method of improving the yield of heterologous protein produced by cultivating recombinant bacteria in a liquid medium characterized by supplementing the medium with an effective amount of a water soluble alkanol of 1 to 4 carbon atoms and/or an effective amount of a mixture of amino acids that supports bacterial growth during the terminal portion of the cultivation."

Claim 1 as granted differed from claim 1 as originally filed in that the expression "during the terminal portion of the cultivation" was inserted between "...characterized by supplementing the medium" and "with an effective amount..."

Dependent claims 2 to 11 as originally filed and as granted related to further embodiments of the method of claim 1.

II. A notice of opposition was filed. Revocation of the patent was requested on the grounds of Article 100(a) to (c) EPC.

III. The following documents were cited inter alia:

(1): EP-B-0 036 776 published on 30 September 1981,

(3): Japanese Patent Kookai (A) 55-124495 published on 25 September 1980,

(3T): Certified translation of document (3),

(4): Brunschede H. et al., J.Bact., vol. 129, pages 1020 to 1033, 1977.

IV. By a decision within the meaning of Article 106(3) EPC, the Opposition Division maintained the patent in amended form according to Article 102(3) EPC on the basis of the fourth auxiliary request submitted at oral proceedings.

The Opposition Division decided that claims which related to the addition to the growth medium of an effective amount of amino-acids in the absence of alkanol, lacked either novelty (main, first and second auxiliary requests) over documents (1) or (3T) or inventive step over document (3T) (third auxiliary request).

The claims of the fourth auxiliary request which related to the addition of an effective amount of a water soluble alkanol of 1 to 4 carbon atoms optionally in co-cultivation with an effective amount of a mixture of amino acids fulfilled the requirements for patentability.

V. The Appellant (Patentee) lodged an appeal against the decision of the Opposition Division, paid the appropriate fee and filed a statement of the grounds of appeal.

VI. The Respondent (Opponent) answered the Appellant's submissions.

VII. A communication was sent by the Board according to Article 11(2) of the Rules of Procedure of the Boards of Appeal setting out the Board's provisional non-binding opinion.

VIII. The Appellant sent a further submission.

IX. During oral proceedings, the Appellant filed a new request ("main request") as the sole request to be considered.

Claim 1 read:

"A method of improving the yield of heterologous protein produced by cultivating recombinant bacteria in a liquid medium characterized by supplementing the medium during the terminal portion of the cultivation with

(a) an effective amount of a water soluble alkanol of 1 to 4 carbon atoms;

(b) an effective amount of a water soluble alkanol of 1 to 4 carbon atoms and an effective amount of a mixture of amino-acids; or

(c) an effective amount of from 0.5 to 5.0% w/v of a mixture of amino acids added to the nutrient medium, wherein said terminal portion is not the stationary phase;

that supports bacterial growth."

Dependent claims 2 to 11 remained as granted but for the correction of obvious typing mistakes in claims 6, 9, 10 and 11.

X. The submissions in writing and during oral proceedings by the Appellant can be summarized as follows:

- Support could be found for part (c) of claim 1 on page 5, lines 17 to 20 of the patent specification as filed, where it was stated that "the amount of amino acid mixture added to the nutrient medium will usually be in the range of about 0.5% to 5% (w/v)...". This sentence would be understood by the skilled person as meaning that it was the final concentration of amino acids in the nutrient medium which was in the range of about 0.5% to 5% (w/v), rather than the initial concentration of the amino acid mixture to be added. This was evident in the light of example 3 which disclosed using a 20% stock solution of amino-acids.

  The disclaimer was allowable to distinguish the claimed subject-matter from the teachings of document (3T) because document (3T) incidentally disclosed the use of amino acids in place of leucine in a process the purpose of which was clearly different from that of the present invention. The wording of the disclaimer was based on the sentence on page 3 of document (3T) " ...adding the above mentioned amino acid required for propagation to the culture medium after the passage of at least one hour after the propagation of the above mentioned organism has essentially ceased and continuing the culturing again". The skilled person would have no doubt that the time when propagation of the micro-organism has ceased was the stationary phase.

- Document (1) did not destroy novelty as it disclosed adding a very low quantity of casamino acids mixture to the growth medium of the micro-organisms which produced the heterologous polypeptide (page 14: 40 µg/ml i.e. 0.004% (w/v), final concentration).

  Care had been taken that the teachings of document (3T) were not comprised within claim 1 by means of the disclaimer.

- Both documents (1) and (3T) could be considered to be closest prior art. Document (1) related to the use of the trp promoter for the expression of heterologous proteins and document (3T) described a method for protein production involving the amplification of the DNA vector carrying the gene to be expressed. However, as they explored the usefulness of quite different methods for protein production, their teachings could not be combined.

  The technical problem could be defined as producing an heterologous protein with an improved yield. The proposed solution (claim 1) solved this problem as could be seen in Table 1 of the patent in suit which showed an increased amount of interleukine being produced by the claimed process.

  The combination of document (1) or document (3T) with document (4) which disclosed that more protein was made when further nutrient was added during bacterial growth did not suggest a process leading to more protein being made in a higher concentration. The claimed process also had the definite advantage over classic processes that the growth medium never needed to be replaced with fresh medium. The claimed process was inventive.

XI. The Respondent's submissions were essentially as follows:

- The wording of claim 1(c) found no support in the patent specification as filed as the amino acid concentration mentioned on page 5, lines 17 to 20 of this specification was clearly the concentration of the mixture of amino acids to be added to the growth medium, and not the final concentration of amino acids in said growth medium.

  The use of the disclaimer was not justified as there was enough information in the patent specification to draft a claim without it, and disclaimers should only be used when no easier way existed to distinguish the claimed subject-matter from the prior art. Furthermore, the disclaimer was unclear as the skilled person would not know what was meant by the term "stationary phase" which was not mentioned in document (3T). Besides, the passage contained in document (3T) cited by the Appellant did not relate to the addition of a mixture of amino acids but rather of leucine.

- In document (1), the value of 0.004% (w/v) for the final amino acid concentration of the growth medium of the micro-organisms producing the heterologous protein failed to be plausible. The skilled person would understand without hesitation that a typing mistake had occurred. Document (1) was novelty destroying.

- With regard to inventive step, it was important that the process steps described in document (1) were those adopted by the Appellant in Example 1 of the patent in suit to demonstrate the feasibility of the claimed process. Document (3T)

provided the further information that supplementing the bacterial growth medium with leucine and casamino acids after bacterial growth had ended resulted in an improvment in the yield of protein produced. By combining the teachings of these two documents, one necessarily arrived at the claimed invention except for the additional feature in claim 1 that the addition of the mixture of amino acids should be done in the terminal portion of the cultivation but before stationary phase. Yet, the Appellant had not demonstrated that any advantages were linked to this additional feature.

Finally, it was evident from first principle that the production of proteins required amino-acids and that, therefore, amino acids would advantageously be added to the growth medium of micro-organisms used for the production of proteins whether they be heterologous or not. Support therefor, if needed, could be found in document (4) which disclosed that there was a general increase in DNA, RNA and protein syntheses when bacteria were transferred from a minimal medium to a medium enriched in casamino acids. The claimed process was obvious.

XII. The Appellant requested that the decision under appeal be set aside and that the European patent No. 0 156 355 be maintained on the basis of the following documents:

(a) Claims 1 to 11 and description, pages 1 to 9 submitted during oral proceedings,

(b) Drawings, Figures 1 and 2, as granted.

The Respondent requested that the appeal be dismissed.

Reasons for the Decision

1. The appeal is admissible.

Article 123(2)(3) EPC

2. The concentration range now claimed in claim 1 is disclosed in the patent application as filed on page 5, lines 17 to 19. Introducing it into the claim amounts to a limitation of the scope of claim 1 as granted in which the concentration range is not specified.

3. The subject-matter of the disclaimer "wherein said terminal portion is not the stationery phase" in claim 1 finds no support in the patent application as filed. However, in accordance with the case law of the Boards of Appeal, disclaimers may be applied when there is an overlap between the prior art and the claimed subject-matter **even in the absence of support for the excluded matter in the original documents**, if the subject-matter remaining in the claim cannot technically be defined directly more clearly and concisely (T 4/80 OJ EPO 1982,149, T 433/86 of 11 December 1987). This is the case here. Furthermore, the present disclaimer necessarily brings about a limitation of the scope of the claim.

4. In view of these findings, the Board considers that the requirements of Article 123(2)(3)EPC are fulfilled.

Article 84 EPC

5. The Respondent argued that the wording "an effective amount from 0.5% to 5% (w/v) of a mixture of amino-acids added to the nutrient medium" in claim 1 was unclear with regard to this range of concentration being that of the stock solution of amino acids to be

added to the nutrient medium or the final range of possible concentrations of amino acids in the nutrient medium. The Board would agree that this wording may be somewhat confusing if taken in isolation. However, Example 3 shows that the concentration of the stock solution of amino acids used to supplement the nutrient medium is 20% (page 7). Thus, if the claim is read in the context of the patent specification, it becomes clear that the claimed concentration range is the concentration range in the nutrient medium.

6. Document (3T) discloses a process for protein production by leucine requiring bacteria whereby said bacteria are grown under sub-optimal conditions (in the presence of limiting amounts of leucine) until growth ceases. Then, leucine is added to the growth medium in normal amounts so that protein synthesis starts again. In one of the examples provided, casamino acids are added together with leucine. In the Board's finding (see points 19 and 20 below), this process is conceptually different from the process of claim 1. Thus, the disclaimer serves the purpose of excluding a way of carrying out the invention which incidentally happens to fall within the claim. According to the established case law of the Boards of Appeal (see e.g. decision T 0171/87 (OJ EPO 1989, 441)), such a disclaimer is allowable.

7. Furthermore, the Board finds that the expression "stationary phase" although not mentioned in document (3T) would be understood without difficulty by the skilled person as it is the usual term to define the time when a culture has ceased to grow i.e. a culture within the meaning of document (3T) (page 3).

8. The disclaimer is of technical nature, clear and concise. It is thus allowable.

9. The requirements of Article 84 EPC are fulfilled.

Article 54 EPC

10. Document (1) was cited by the Respondent in the context of assessing novelty. It discloses the production of a heterologous protein by recombinant bacteria. The final concentration of casamino acids in the bacterial growth medium is 40 µg/ml i.e. 0.004% w/v. This concentration is outside of the claimed range. The Respondent argued that a typographical error must be the reason for this concentration being so low. This allegation is not supported by any evidence and, thus, cannot be considered by the Board. Accordingly, document (1) cannot be novelty destroying.

11. By introducing the disclaimer into claim 1, the Appellant has ensured that any incidental disclosure in the state of the art represented by document (3T) is excluded from the claim.

12. No other documents on file disclose subject-matter detrimental to novelty.

13. The requirements of Article 54 EPC are fulfilled.

Article 56 EPC

14. Document (3T) discloses a method for increasing the yield of a protein made by recombinant cells when the gene encoding said protein is carried by a plasmid. In a first step, the copy number of the plasmid is amplified. This is achieved by culturing the cells in a medium containing a sub-optimal concentration of an amino acid which the bacterial cells are not able to synthesize until this amino acid has been fully consumed. The deprived state is maintained for at least one hour during which the increase in plasmid copy

number takes place (see page 5, second paragraph). Once the plasmid is amplified, leucine alone or yeast extract and leucine are added to the culture medium, which allows protein synthesis to take place. When the experiments are carried out with a plasmid carrying the β-lactamase gene, the following results are obtained:

- 24.1 units of β-lactamase are produced by control bacteria which have been grown in the presence of a normal amount of leucine (50 µg/ml)(Example 2).

- 56.5 units of β-lactamase are produced by cells which are initially grown in the presence of 10 µg/ml of leucine and transferred after nine hours to a medium comprising 50 µg/ml of leucine (Example 2).

- 80 units of β-lactamase are produced by cells which are initially grown in the presence of 10 µg/ml of leucine and transferred after nine hours to a medium comprising 40 µg/ml of leucine and a ten fold dilution of a 10% solution of yeast extract (i.e 10mg/ml final concentration), which contains all essential amino acids (example 3).

The yield of the protein product is thus improved. The improvement is somewhat greater following the addition of yeast extract and leucine than following the addition of leucine alone.

15. Starting from this prior art, the objective technical problem to be solved can be defined as the provision of an alternative process for improving the yield of a foreign protein being made by recombinant bacteria.

16. The solution provided is to grow the protein producing bacterial cells under normal conditions whereby protein synthesis and cell multiplication occur unimpeded, and to add casamino acids during the terminal portion of the cultivation before the stationary phase.

17. By reason of the results presented in Table 1 of the patent in suit, the Board is satisfied that the claimed process solves the above stated problem.

18. The question to be answered is thus whether the knowledge of document (3T) renders the features of the process of claim 1 obvious.

19. The Board observes that document (3T) does not specify whether the increase in improvement observed in the presence of yeast extract (10 mg/ml) and leucine (40 µg/ml) compared with leucine alone (50 µg/ml) is due to the presence of all essential amino acids in the yeast extract (i.e. to the addition of a mixture of amino acids to the medium) or is due to the addition of leucine as one of the constituents of the yeast extract. The observed increase in improvement could only be attributed to the yeast extract "as a whole" if it had been shown, on the one hand, that the addition of yeast extract to a final concentration of 10 mg/ml did not amount to the addition of leucine to a final concentration superior to 50 µg/ml and that an increase in the leucine concentration above 50 µg/ml did not result in an increase in the protein yield, on the other.

20. Furthermore, the metabolic states of the bacteria at the onset of protein production seem to be quite different in both processes. In the process of document (3T), they have been left in a state of starvation which as stated by the authors may result in death if maintained for too long (page 5). In the claimed

process, all biosynthetic pathways must still be active as the stationary phase has not yet been reached. It is, thus, not obvious that the results obtained with the earlier process as disclosed in document (3T) are necessarily indicative of what would happen in the latter.

21. For these reasons, the Board concludes that the teachings of document (3T) are too remote from the claimed process to be detrimental to inventive step.

22. Document (1) is concerned with producing protein by a process and is based on the known phenomenon of derepression. The synthesis of the protein is repressed until the bacteria are grown to the required optical density and then allowed to proceed by changing the growth conditions. In view of the fact that this process is not a process for improving a protein yield and that the phenomenon of derepression is not relevant to the process of document (3T), the Board does not see any reason why the teachings of both documents should be combined nor how such a combination, if made, would render the claimed process obvious.

23. Document (4) is a mathematical study of the relative rates of synthesis of DNA, RNA and protein in non-transformed bacteria as a function of the growth conditions. It is stated on page 1020 (end of the introduction) that the translation efficiency and RNA synthesis require several hours to reach their definitive values after a shift in growth conditions comprising a change in the energy source and the addition of amino acids. Thus, this document is concerned with basic microbial cell physiology. It does neither relate to recombinant bacteria nor to improving

protein yield. Accordingly, it provides no hint that amino-acids should be added during the terminal portion of the cultivation in order to achieve an increase in protein production.

24. The Board does not see that the skilled person would ever think of combining this teaching with that of document (3) in order to arrive at the claimed process.

25. Inventive step is acknowledged.

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 with claims 1 to 11 and description, pages 1 to 9, submitted during oral proceedings, and drawings, Figures 1 and 2, as granted.

The Registrar: The Chairwoman

A. Townend U. Kinkeldey