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D E C I S I O N
of 18 May 2006

Case Number: T 0268/05 - 3.3.08

Application Number: 99941978.1

Publication Number: 1100513

IPC: A61K 31/70

Language of the proceedings: EN

Title of invention:
Autoinducer synthase modulating compounds and uses therefor

Applicant:
UNIVERSITY OF IOWA RESEARCH FOUNDATION

Opponent:
-

Headword:
Autoinducer synthase/IOWA UNIVERSITY

Relevant legal provisions:
EPC Art. 54, 56, 64(2)

Keyword:
"Main request - novelty (yes)"
"Inventive step (yes)"

Decisions cited:
-

Catchword:
-

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Case Number: T 0268/05 - 3.3.08

D E C I S I O N
of the Technical Board of Appeal 3.3.08
of 18 May 2006

Appellant: UNIVERSITY OF IOWA RESEARCH FOUNDATION
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Decision under appeal: **Decision of the Examining Division of the European Patent Office posted 28 September 2004 refusing European application No. 99941978.1 pursuant to Article 97(1) EPC.**

Composition of the Board:

Chairman: L. Galligani
Members: F. Davison-Brunel
C. Rennie-Smith

Summary of Facts and Submissions

I. European patent application No. 99 941 978.1 published as International application No. WO 00/06177 with the title "Autoinducer synthase modulating compounds and uses therefor", with the earliest priority date of 31 July 1998 (US 60/094 988) was refused by the examining division for lack of novelty and inventive step. Basis for the refusal were claims 1 to 28 filed on 16 August 2004. Claims 1, 15 and 20 read as follows:

"1. A method for producing a highly soluble, highly active bacterial autoinducer synthase molecule comprising:

introducing DNA encoding said bacterial autoinducer synthase molecule into a bacterial host cell, wherein said bacterial cell and said autoinducer synthase molecule are from identical bacterial species;

growing said host cell at a temperature ranging from 20°C ± 3°C to 30 °C ± 3°C that is sufficient to overexpress said bacterial autoinducer synthase molecule in highly soluble, highly active form; and

isolating said autoinducer synthase molecule from an extract of said bacterial host cell, thereby producing the highly soluble, highly active autoinducer synthase molecule.

15. A purified bacterial autoinducer synthase molecule, which is at least about 50% pure and being biologically active.

20. The autoinducer synthase molecule according to any one of claims 15 to 19 which is RhlI."

The examining division concluded that the subject-matter of claims 15 to 18 and 20 lacked novelty over the teaching in document (9) (Section VII, infra), of active RhlI purified to at least 95% purity. As for the subject-matter of claim 1, it lacked inventive step over the combination of the teaching of document (9) which provided means of obtaining highly active isolated autoinducer synthase, with those of documents (2), (4) or (8) relating to the cloning and expression of recombinant autoinducer synthase molecules wherein the bacterial cells and the autoinducer synthase were from the same bacterial species.

II. The appellant (applicant) filed a notice of appeal against this decision, paid the appeal fee and submitted a statement of grounds of appeal together with a new main request (claims 1 to 27). The appellant also requested a refund of the appeal fee.

III. The appealed decision was not rectified by the examining division and the case was remitted to the board of appeal (Article 109(2) EPC).

IV. The board sent a communication pursuant to Article 11(1) of the Rules of Procedure of the Boards of Appeal, raising a number of objections under Articles 123(2) and 84 EPC and stating its preliminary non-binding opinion with regard to novelty, inventive step and sufficiency of disclosure.

V. On 18 April 2006, the appellant filed a further submission in answer to this communication together with a new main request and four auxiliary requests.

VI. During the oral proceedings which took place on 18 May 2006, the appellant filed a new main request comprising 26 claims and an auxiliary request. The request for reimbursement of the appeal fee was withdrawn.

Claims 1 and 21 to 26 of the **new main request** read as follows:

"1. A method for producing a soluble bacterial autoinducer synthase comprising:

introducing DNA encoding said bacterial autoinducer synthase into a bacterial host cell, wherein said bacterial cell and said autoinducer synthase are from identical bacterial species such that the autoinducer synthase is overexpressed;

growing said host cell at a temperature ranging from 20°C ± 3°C to 30°C, such that the bacterial autoinducer synthase is overexpressed in soluble form; and

isolating said autoinducer synthase from an extract of said bacterial host cell, thereby producing the soluble autoinducer synthase.

21. A purified, soluble autoinducer synthase molecule having an amino acid sequence comprising amino acids 24-73 of SEQ ID NO.1.

22. The purified, soluble autoinducer synthase molecule of claim 21 comprising amino acids 24-104 of SEQ ID NO.1.

23. A preparation comprising purified soluble Pseudomonas aeruginosa quorum sensing system autoinducer synthase molecules, wherein at least 50% of the molecules in the preparation are autoinducer synthase molecules as determined by SDS-PAGE.

24. The preparation of claim 23, wherein at least 95% of the molecules in the preparation are autoinducer synthase molecules as determined by SDS-PAGE.

25. The preparation of claim 23 or 24 wherein the preparation is free of inclusion bodies.

26. The preparation of any one of claims 23 to 25 wherein the autoinducer synthase molecule is RhlI."

Dependent claims 2 to 20 related to further features of the method of claim 1.

VII. The following documents are mentioned in this decision:

(2): Latifi, A. et al., Molecular Microbiology, Vol. 17, No. 2, pages 333 to 343, 1995;

(3): Davies, D.G. et al., Science, Vol.280, pages 295 to 298, 10 April 1998;

(4): Hwang, I. et al., Proc.Natl.Acad.Sci.USA, Vol. 91, pages 4639 to 4643, May 1994;

(8): Schaefer, A.L. et al., Proc.Natl.Acad.Sci.USA, Vol. 93, pages 9505 to 9509, September 1996;

(9): Jiang, Y. et al., Molecular Microbiology, Vol. 28, No.1, pages 193 to 203, 1998.

VIII. The appellant's arguments in writing and during oral proceedings insofar as relevant to the present decision may be summarized as follows:

Main request
Article 84 EPC

The wording of the claims had been amended in order to eliminate the expressions "highly soluble", "highly active", "biologically active", "at least about" which were regarded as having little or no meaning in the board's communication pursuant to Article 11(1) RPBA. The wording of claim 1 accurately reflected the teaching of the application concerning the temperature at which host cells had to be grown in order that the bacterial autoinducer synthase would be expressed in soluble form. The purity of the claimed preparations was defined with reference to the assay used to measure it.

The requirements of Article 84 EPC were fulfilled.

Article 54 EPC; novelty
Claims 21 and 23

Document (2) disclosed the transformation of a *Pseudomonas aeruginosa* strain by a recombinant vector carrying the RhlI gene. It also provided the sequence of the RhlI autoinducer synthase with the same amino acids in position 24 to 73 as the presently claimed RhlI enzyme (claim 21). Yet, the protein itself had never been purified. Document (2) was, thus, not relevant to the novelty of a claim directed to a purified, soluble molecule.

There were no documents on file disclosing a preparation comprising purified, soluble *Pseudomonas aeruginosa* quorum sensing system autoinducer synthases (claim 23).

For these reasons, the subject-matter of claims 21 and 23 was novel.

Article 56 EPC; inventive step

Document (8) was the closest prior art. It taught that the *Vibrio fischeri* Lux I autoinducer synthase was insoluble when expressed in recombinant form in *E.coli* and that in order to obtain it in soluble form, it was necessary to express it as a hybrid protein, eg. fused with Maltose Binding Protein (MBP). Yet, MBP interfered with the protein folding and activity of LuxI. The authors had, thus, introduced a cleavage site into the soluble fusion protein for the purpose of isolating the LuxI protein itself in soluble form. However, they were

unable to cleave MBP and LuxI despite repeated attempts.

The objective problem to be solved could be defined as finding an improved method for producing a soluble autosynthase molecule.

The provided solution went in a different direction from that used in document (8), namely it involved a change in the host cells to the same cells where the autoinducer synthase was naturally expressed, **and** a specific choice of cell growth temperatures.

Document (8) taught away from the claimed invention when teaching that to obtain overexpression of a soluble protein it was necessary to express it as a fusion protein. A skilled person working from the teaching of document (8) could have had no reasonable expectation of success in achieving overexpression of a soluble bacterial autoinducer synthase in the way taught by the applicant.

None of the prior art references, alone or in combination, taught or suggested the isolation and/or purification of the highly soluble bacterial autoinducer synthase molecules of the present invention.

Inventive step had to be acknowledged.

Article 83 EPC; sufficiency of disclosure

The application provided general instructions for producing soluble autoinducer synthase together with a detailed explanation of the procedures carried out to overexpress and purify RhlI. This teaching could equally be applied to other bacterial species and autoinducer synthase genes without undue burden. The wording of the claims reflected the actual contribution to the state of the art made by the applicant's disclosure.

IX. The appellant requested that the decision under appeal be set aside and that a patent be granted on the basis of the main or alternatively the first auxiliary request filed during the oral proceedings.

Reasons for the Decision

Main request
Articles 123(2)and 57 EPC; amendments, clarity

1. The subject-matter of claim 1 finds a basis in originally filed claims 38 and 39 together with page 7 of the application as filed, lines 16 and 17. Dependent claims 2 to 10 correspond to originally filed claims 39 (together with page 7) and 40 to 49. The subject-matter of claims 11 to 15 is found on page 3, lines 20 to 28 together with page 23, lines 7 and 8; that of claims 16 to 20 and 26 is found in Example 1 and Table 2. Claims 21 and 22 correspond to originally filed claims 52 and 53. The subject-matter of claims 23 to 25

finds a basis on page 6, lines 11 to 24 of the application as filed.

2. In the board's judgment, the claims are clearly worded and the claimed subject-matter finds support in the description.

3. The requirements of Articles 123(2) and 84 EPC are fulfilled.

Article 54 EPC; novelty
Method claims 1 to 20

4. The methods of claims 1 to 20 are characterised in particular by the two features that the soluble autoinducer synthase is produced in recombinant form in the same host cells where it was originally synthesized and within a range of temperatures below or equal to 30°C (see Section VI).

5. Document (2) (page 336, left-hand column) teaches the transformation of *Pseudomonas aeruginosa* with the *Pseudomonas aeruginosa* rhlI autoinducer synthase gene. Yet, the experiment is carried out at 37°C (experimental procedures, page 340, right-hand column).Document (4) describes the transfer in *Agrobacterium tumefaciens* of a plasmid carrying the *Agrobacterium tumefaciens* traI gene encoding a protein which is said to be related to proteins responsible for autoinducer production in other bacteria (Abstract). There is no mention of the temperature at which the recombinant host cells are grown. Nor does any of the quoted documents refer to the production of a soluble molecule.

6. None of the other documents on file disclose the transfer and recombinant expression of an autoinducer synthase gene in its original genetic background.

7. The subject-matter of claims 1 to 20 is, thus, novel.

Product claims 21 to 26

8. In document (2), Figure 5 provides the sequence of the *Pseudomonas* autoinducer synthase as deduced from the encoding DNA. On page 336, right-hand column, the transformation of *E.coli* JM107 with a plasmid, pMW407.1, carrying a *Pseudomonas* 2.1kb fragment is disclosed. Spent cell-free supernatants of the recombinant host cells are said to activate both the *lux* and *C.violaceum* bioassays. These results lead the authors to conclude that the 2.1Kb DNA fragment is capable of directing the synthesis of autoinducers, thus implying that the culture supernatant contains a *Pseudomonas* autoinducer synthase (RhlI). Yet, the presence of the enzyme in that supernatant is not directly shown, a fortiori the enzyme has not been purified. In the board's judgment, document (2) does not provide a clear and unambiguous disclosure of a purified and soluble RhlI autoinducer synthase enzyme and, consequently, is not detrimental to the novelty of the subject-matter of claims 21 to 26.

9. There are no other documents on file relating to soluble Pseudomonas autoinducer synthases per se.

10. The requirements of Article 54 EPC are fulfilled by the claims of the main request.

Article 56 EPC; inventive step
Method claims 1 to 20

11. The closest prior art is document (8) which presents a study of the autoinducer synthase LuxI produced by *Vibrio fischeri*. The aim of this study is to find out whether or not the enzyme is directly responsible for the biosynthesis of the autoinducer: N-(3-oxohexanoyl)homoserine lactone in a reaction involving S-adenosylmethionine as the substrate in the presence of 3-oxyhexanoyl coenzyme A (introduction). Plasmids carrying the luxI gene are constructed for Lux expression in *E.coli*. It is found that expression from the luxI open-reading frame leads to the production of a LuxI protein which is essentially insoluble, ie in the form of inclusion bodies. The lack of solubility is avoided by expressing LuxI in fused form - to an *E.coli* MBP (page 9506, right-hand column). MBP-LuxI shows good activity at temperatures between 20°C and 30°C (page 9507) but the fused enzyme has a low V_{max}. It is mentioned in the discussion on page 9508, left-hand column that this feature may not reflect a property of the LuxI protein per se but could be due to the addition of MBP at the N-terminus (page 9508, left-hand column). On page 9506, it is stated that: "*Although the fusion protein contains a factor X_A cleavage site, we were unable to cleave MBP and LuxI in repeated attempts with the factor X_A protease.*"

12. Starting from the closest prior art, the problem to be solved can be defined as providing a method for producing "bona fide" autoinducer synthase in soluble form.

13. The solution is a method whereby the recombinant host cell and the autoinducer synthase DNA are from identical bacterial species **and** the growth temperature of said cells is comprised within 20°C ± 3°C to 30°C.

14. In the board's judgment, it would have been obvious to the skilled person wanting to develop a method for producing a soluble form of an autoinducer synthase molecule to adapt the method disclosed in document (8) for LuxI - namely, to fit the fusion protein with another protease cleavage site. Indeed, the failure observed with factor X_A may only have been specific to that protease and, thus, did not preclude that cleavage would occur with other proteolytic enzymes. In contrast, the method of the present invention significantly departs from the above mentioned teaching. The concept of producing a fusion protein is abandoned in favour of the concept of changing the host cell.

15. There is no suggestion in the art that such a course of action might be advantageous, not even in the prior art document (9) which describes an attempt at purifying the *Pseudomonas aeruginosa* RhlI autoinducer synthase. There, the enzyme per se is expressed in *E.coli* and retrieved from the cells in insoluble form. The work is then pursued on suspensions of the enzyme which exhibit some autoinducer synthase activity (page 194, right-hand column).

16. Furthermore, the skilled person could not expect that the recombinant *Pseudomonas aeruginosa* autoinducer synthase would necessarily be soluble when expressed in *Pseudomonas aeruginosa*, even if it was naturally produced in soluble form by these bacteria, the reason

being that the two forms of the enzyme are biosynthesized in different ways. Whereas, as was known from the prior art (see patent application, page 1), the natural expression of the synthase occurs in a regulated manner, the recombinant expression from the transformed vector would not be subject to such regulation. Doubts would, thus, exist as to whether the recombinant protein expressed in higher quantities through time would remain soluble.

17. The inventive aspect of the invention resides in imparting solubility and is, thus, to be acknowledged over the scope of the claims if there is a solubility problem to be solved with all bacterial autoinducer synthases mentioned in the claims. In this context, the board remarks that *Pseudomonas* RhlI autoinducer synthase is insoluble just as LuxI exemplified in the present invention. Furthermore, it is mentioned in document (4) (page 4642, right-hand column) that autoinducer synthases belong to a family of proteins which are homologous to each other. For this reason, the board is prepared to accept that the problem to be solved exists for all specifically mentioned autoinducer synthases and, thus, concludes that the reasoning on inventive step is valid over the whole scope of the claims.

Product claims 21 to 26

18. These claims are all directed to soluble *Pseudomonas aeruginosa* quorum sensing system autoinducer synthases which are purified to various degrees.

19. The prior art document (9), which concerns the production of *Pseudomonas aeruginosa* synthase RhlI in a recombinant system, indicates that the product obtained is insoluble (see point 15, supra). The other prior art document which is concerned with an autoinducer synthase from a different source, ie document (8), indicates that soluble forms can be obtained in a recombinant DNA system but only in a fused form which cannot thereafter be cleaved to produce bona fide autoinducer synthase (see point 11 above).

20. In the board's judgment, neither of those documents taken alone nor the two combined would, if used as starting point, have led the skilled person in a straightforward manner to the soluble, purified products of claims 21 to 26. Indeed, if the skilled person had wanted to obtain the claimed products, he or she would have taken the route of isolating a fused autoinducer synthase and then retrieving the enzyme from the fusion, a course of action which would in any case have necessitated some yet to be established modifications in view of the failure to cleave reported in document (8). Whether the product would remain soluble once cleaved was also unpredictable.

21. Under these circumstances, the products of claims 21 to 26 which are conveniently obtained in unfused form by way of the method of claims 1 to 20 are considered to be non-obvious, their inventive step being also a reflection of that of the method claims (see points 11 to 17, supra).

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 grant a patent on the basis of the main request filed during the oral proceedings and a description and drawings to be adapted thereto.

The Registrar: The Chairman:

A. Wolinski L. Galligani