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Datasheet for the decision of 21 February 2019

Case Number: T 0067/14 - 3.3.08

Application Number: 06700045.5

Publication Number: 1841855

IPC: C12N1/38, C12N1/20, A23C19/032

Language of the proceedings: EN

Title of invention:
USE OF COMPOUNDS INVOLVED IN BIOSYNTHESIS OF NUCLEIC ACIDS TO INCREASE YIELD OF BACTERIAL CULTURES

Patent Proprietor:
Chr. Hansen A/S

Opponent:
DuPont Nutrition Biosciences ApS

Headword:
Increasing yield of bacterial culture / CHR. HANSEN

Relevant legal provisions:
EPC Art. 54, 56, 100(a), 100(b), 100(c), 114(2)
EPC R. 116
RPBA Art. 12(4)

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

Decisions cited:
T 1002/92, T 1119/05

Catchword:

Europäisches Patentamt
European Patent Office
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Beschwerdekammern

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Case Number: T 0067/14 - 3.3.08

D E C I S I O N
of Technical Board of Appeal 3.3.08
of 21 February 2019

Appellant: (Opponent)	DuPont Nutrition Biosciences ApS Langebrogade 1 P.O. Box 17 1001 Copenhagen K (DK)
Representative:	McConchie, Connor D Young & Co LLP Briton House Briton Street Southampton SO14 3EB (GB)
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Representative:	von Menges, Albrecht Uexküll & Stolberg Partnerschaft von Patent- und Rechtsanwälten mbB Beselerstraße 4 22607 Hamburg (DE)
Decision under appeal:	**Decision of the Opposition Division of the European Patent Office posted on 6 November 2013 rejecting the opposition filed against European patent No. 1841855 pursuant to Article 101(2) EPC.**

Composition of the Board:

Chairman	B. Stolz
Members:	D. Pilat
	D. Rogers

Summary of Facts and Submissions

I. The opponent (appellant) lodged an appeal against the decision of an opposition division, dated 6 November 2013, rejecting its opposition against European patent No. 1 841 855 based on the grounds of Article 100(a) in conjunction with Articles 54 and 56 EPC, and Articles 100(b) and (c) EPC. Together with its statement of grounds of appeal, the appellant filed new evidence (documents D12 to D17 and D24 to D26).

II. The patent proprietor (respondent) replied to the statement of grounds of appeal and filed a set of auxiliary requests 1 to 9.

III. The appellant commented on the respondent's reply and on the new auxiliary requests in a written submission dated 19 February 2016.

IV. The parties were summoned to oral proceedings. In a communication pursuant to Article 15(1) RPBA, the parties were informed of the board's provisional, non-binding opinion on some of the legal and substantive matters of the case.

V. The appellant informed the board that it would not attend oral proceedings.The respondent replied to the board's communication.

VI. The Board informed the parties that the oral proceedings scheduled for 7 December 2018 were cancelled.

VII. Independent claim 1 as granted reads as follows:

"1. A method for obtaining increased yields of a lactic acid bacteria culture fermented under aeration and high Optical Density-conditions, said method comprising the steps of

i) culturing a lactic acid bacteria in a culture medium and at conditions that allows the fermentation to proceed beyond an Optical Density measured at 600 nm (OD_{600}) of 10, wherein said culture medium comprises at least one yield-enhancing agent selected from the group consisting of a purine base, a pyrimidine base, a nucleoside, a nucleotide at a concentration that ensures that the culture medium comprises at least 1 µM of said at least one yield-enhancing agent at the termination of the fermentation, wherein the OD_{600} is at least 10 at the termination of the fermentation; and

ii) harvesting said lactic acid bacteria to obtain the lactic acid bacteria culture,

wherein the yield-enhancing agent results in an increased yield of harvested lactic acid bacteria as compared to culturing the microorganism at identical conditions and in an similar medium which comprise less than 1 µM of each yield-enhancing agent at end of the fermentation, and
wherein said culture medium initially comprises at least 1 mM of said at least one yield-enhancing agent, and
wherein the fermentation of the microbial culture was performed under aeration and in a nutrient medium, in which at least one porphyrin compound is present or is added."

Dependent claims 2 to 12 define specific embodiments of the method of claim 1.

VIII. The following documents are cited in this decision:

D1: WO 00/05342 A1, published on 3 February 2000;

D2: WO 01/52668 A2, published on 26 July 2001;

D5: Smith et al., J. Dairy Res. 1975, Feb:42(1) pages 123-138;

D6: Potvin et al., J. of Microbiological Methods vol 29 (1997), pages 153-160;

D7: Kilstrup et al., FEMS Microbiology Review vol. 29 (2005), pages 555-590;

D8: Koburger et al., J. Bacteriol. vol. 85 (1963), pages 1051-1055;

D12: Matlock et al., Technical Note: 52236, Thermo Scientific 2011;

D13: Sigma-Aldrich product catalog, excerpt inosine or IMP, submitted by the opponent with letter dated 12 July 2013;

D14a: European Association for Specialty Yeast products (EURASYP), Yeast extract, not dated;

D14b: European Association for Specialty Yeast products (EURASYP), Yeast extract with natural nucleotides not dated;

D15: Angel Yeast Co. Ltd., Extract for peptide

17 April 2012;

D16: WO 03/063613 A1 published on 7 August 2003;

D17: BioSpringer, Product information Springer 2000 Bakers yeast extract 23 September 2004;

D24: Expert declaration and experimental evidence repeating Example 1 of EP 1 841 855 and measuring OD according to common parameters, submitted by the opponent with letter dated 14 March 2014.

IX. The submissions made by the appellant as far as relevant to this decision were essentially as follows:

Admission of documents D12 to D17 filed with opponent's submission dated 12 July 2013.

The date for making final submissions according to Rule 116 EPC, as indicated in the summons to attend oral proceedings in opposition, was 17 July 2012, while documents D12 to D17 were submitted on 12 July 2012. Therefore the decision of the opposition division to refuse their admission under Rule 116 EPC was incorrect and constituted a procedural violation. An opportunity to discuss admission of documents D12 to D17 was only granted by the opposition division after opponent had protested. Opponent's right to be heard was therefore only formally observed. This constituted another procedural violation.

The Opposition division erred in its assessment of the prima facie relevance of documents D12 to D17. They all prejudiced the maintenance of the patent for the following reasons:

Document D12 was highly relevant with regard to Article 83 EPC and the measurement of OD_{600}.

Document D13 provided the molecular weight of inosine and IMP.

Documents D14 to D17 were filed to support the lack of novelty argument based on document D2. They demonstrated common general knowledge that had been challenged by the patent proprietor.

Furthermore, the decision under appeal gave no reason why these documents were no more relevant than the documents already on file.

Admission of new documents D24 to D26 filed with appellant's submission dated 14 March 2014.

Document D24 was filed in reaction to the decision taken by the opposition division that the claimed invention was sufficiently disclosed. Document D24 showed that the selection of particular conditions for measuring the optical density at OD_{600} of fermented lactic acid bacteria according to example 1 resulted in variable results.

Document D30 was filed in response to respondent's document D28.

Article 100(c) EPC

The patent application did not directly and unambiguously disclose a method according to claim 1 "wherein the OD_{600} is at least 10 at the termination of the fermentation" (last feature of claim 1 i)). There

was a distinction between the termination (or end) of a fermentation and the termination (or end) of a fermentation process. The termination of the fermentation was the point at which culturing or propagation was terminated, whereas, by contrast, a fermentation process included the actual culturing step and possible additional steps, such as cell concentration steps. The application as filed described that the OD_{600} was measured at the end of the fermentation process, yet the granted claim required it to be measured at the end of the fermentation. Likewise, the paragraph on page 2, lines 7 to 11, of the patent application did not refer to the fermentation as such but to a fermentation process. Dependent claim 25 of the patent application was also of no help, as it was ambiguous about the point in time when a certain OD had to be reached. Finally, claim 1 of the application as filed merely stated that culture conditions had to be such that they allowed the fermentation to proceed beyond an OD_{600} of 10, but did not say anything about the actual optical densities that had to be obtained.

Therefore, claim 1 contained subject matter not directly and unambiguously derivable from the application as filed, thereby offending against the provisions of Articles 100(c) EPC.

Article 100(b) EPC - Insufficiency of disclosure

The claimed subject matter was insufficiently disclosed, because measurement of OD_{600} inherently depended on many factors, such as the make/type of the spectrophotometer and the medium used for diluting samples before measurement. None of these factors were however described in the patent. Furthermore, Example 1

of the patent lacked technical information to such an extent that it could not be readily reproduced. Indeed, a skilled person reproducing example 1 of the patent and measuring an optical density of the culture medium at the end of the fermentation of below 10 would have been unable to determine the reason(s) for this failure. Parameters affecting the optical density measurement of a sample were held to stem from the fermentation method and/or the method for measuring the sample. Thus the skilled person would, when measuring an OD_{600} below 10 at the end of the fermentation, have had to embark on a research project to determine which appropriate parameters had to be applied to reproduce the invention. Another insufficiency arose from the fact that many lactic acid bacteria were only able to respire when menaquinone was specifically added. Hence, at the priority date, the skilled person would not have understood why essentially no growth would have been observed, when, for example, Lactobacillus brevis was cultured. The claimed invention was therefore shown to be insufficiently disclosed over the whole range claimed.

Articles 100(a) and 54 EPC - Novelty

Experiment 1 of document D2 disclosed a method with all the features of claim 1. The culture medium used in "fermentation D" comprised a porphyrin compound (10 mg/l haemin) (cf. point 1.2 medium composition of Experiment 1) and had to comprise yeast extract, since, according to the respondent's own words, example 1 of the patent compared the fermentation process of document D2 with the fermentation process claimed.

Paragraph [0052] of the patent mentioned that a fermentation medium could be formulated by using

components which were particularly rich with respect to yield enhancing agents. One such component could be provided by yeast extract, in particularly so-called "enriched" or "fortified" yeast extract preparations, which were particularly rich in purines and/or pyrimidines. Since it was acknowledged that "yeast peptone" of high quality was obtained through enrichment of yeast protein from yeast cells, yeast peptone had to be seen to refer to an enriched yeast extract, which was estimated to contain 5'-nucleotides at a concentration of about 5% w/w. Finally, since yeast peptone occurred at 15 g/L in the fermentation medium of D2, said medium had to contain at the start of the fermentation a yield enhancing compound concentration above the one required in claim 1.

Articles 100(a) and 56 EPC - Inventive step

Document D1 represented the closest prior art. It disclosed the fermentation of lactic acid bacteria under aeration and in a nutrient medium comprising at least one porphyrin compound. Document D1 was specifically related to yields. Alternatively, document D2 could be considered as closest prior art.

The sole difference between claim 1 of the patent and D1 was the presence of at least 1 mM at the start of the fermentation of a particular yield enhancing agent. Claim 1 required also the optical density measured at OD_{600} to be at least 10 at the “termination of the fermentation” and that at least one yield enhancing agent concentration is at least 1 µM at the “termination of the fermentation”.

Because of the unreliable determination of the optical density of the cultured medium, this feature was not

able to impart any technical character to the subject matter of claim 1.

No data had been found in the patent of any improvement resulting from the presence of a "yield enhancing agent" such as inosine in a fermentation medium. Since Example 1 of the patent did not provide information on the amount of the yield enhancing agent at the end of the fermentation, there was no evidence that the technical effect was achieved across the entire scope of the claim. Therefore, based on decision T 939/92, the problem had to be reformulated less ambitiously as the provision of an alternative method of culturing lactic acid bacteria under aeration conditions.

Regardless of whether the objective technical problem to be solved was to provide an improved or an alternative method for culturing lactic acid bacteria, the solution of incorporating nucleotides at a certain concentration was obvious in the light of the technical teaching of documents D5 to D8. The patent did not provide any evidence that the amount of yield enhancing agent required in claim 1 at the end of fermentation led to a particular technical effect.

It was furthermore well known that purine and pyrimidine bases in yeast extracts stimulated cell growth (see documents D5 and D6). Likewise, document D7 (page 557, LHC 3rd full paragraph) and document D8 (page 1054, LHC, lines 5-11) reported that at least purines could be added to growth media of lactic acid bacteria to enhance their growth and nucleosides to stimulate growth of Streptococcus lactis (later renamed as Lactococcus lactis) respectively.
It was therefore obvious for the skilled person to add the above mentioned growth enhancing components to

obtain an alternative or improved method of culturing lactic acid bacteria under aeration conditions.

X. The submissions made by the respondent as far as relevant to this decision were essentially as follows:

Admission of documents D12 to D17 filed with opponent's submission dated 12 July 2013 and of new documents D24 to D26 filed with appellant's submission dated 14 March 2014.

Documents D12 to D17, D24 to D26 and D30 were filed late and without excuse for their late filing. No reason was provided as to why these documents were prima facie relevant for the case under appeal. Document D12 appeared to have been printed in 2011. However, its publication date was unclear. Documents D14a, D14b, D15 and D17 did not provide a publication date at all. Documents D14, D16 and D17 related to yeast extracts and were prima facie filed to support the novelty objection based on document D2 using yeast peptone containing media. Document D30 reported that different lactic acid bacterial cells in the human intestine competed for binding to human Caco-2 cells under conditions found in the intestine. The cells tended to form chains and aggregates under these conditions. This tendency was not shown to occur under different conditions such as in D28.

Article 100(c) EPC

In the context of the present application, fermentation was always a process of propagating or cultivating a microbial cell under aerobic or anaerobic conditions. This was stated in the first full paragraph on page 6 of the patent application. Thus, there was no

difference between a fermentation and a fermentation process. The patent application as originally filed already included the feature wherein the culture of the lactic acid bacteria should be carried out under conditions that allowed "the fermentation to proceed beyond an optical density measured at 600 nm (OD_{600}) of 10" in claim 1. Thus, the insertion of this feature did not contravene Article 100(c) EPC.

Article 100(b) EPC - Insufficiency of disclosure

The patent provided a detailed disclosure of the claimed invention which included a number of examples to illustrate different aspects of the invention. Example 1 showed that the addition of a yield enhancing agent provided a significant increase in terms of biomass, as determined by OD_{600} and as determined by measurement of kilograms of F-DVS per 100 liter medium providing an acidification activity in the range of 4.8 to 5.1 according to the Pearce test (Example 1, page 12 of the present patent). Example 2 explained the procedure used to determine the activity of the lactic acid bacteria. Example 3 provided a negative control showing that the yield enhancing effect was only obtained under aerobic conditions. Example 4 and Figure 1 confirmed this conclusion. Example 5 illustrated the depletion of nucleo compounds (purine and pyrimidine sources) in different fermentation processes (see also Figures 2 and 3). Example 6 showed that proteomics can be additionally used to analyze the effect of the depletion of nucleo compounds in fermentation processes.

Optical density, OD_{600}, was commonly used to determine cell density of microbial cultures, such as lactic acid bacterial cultures. It represented common general

knowledge. The yields in example 1 of the patent were not only indicated in terms of OD_{600} but also in kg F-DVS (Table 2 of the patent). The yield in terms of kg F-DVS provided a basis to obtain reliable and comparable OD_{600} values.

Articles 100(a) and 54 EPC – Novelty

Document D2 disclosed that lactic acid bacteria comprising an increased amount of a porphyrin compound could be used as starter cultures (D2, page 5, line 3 onwards). In the examples of D2 Lactococcus lactis subspecies lactis was fermented in a complex fermentation medium described in some detail (on page 18). The fermentation medium D comprised yeast peptone at a concentration of 15 g/L (D2, page 18, line 16) and haemin as a porphyrin compound (see Table 3 of D2). Yeast extract was chemically different from yeast peptone. Document D2 did not disclose the concentration of one of the yield-enhancing agents of the present patent in the medium, neither at the initiation nor at the termination of the fermentation. Nor did it disclose the use of a yeast extract in fermentation medium D, let alone in combination with a yield enhancing compound at a concentration of 1 mM at the start of the fermentation. As a consequence, the subject matter of claim 1 was novel.

Articles 100(a) and 56 EPC – Inventive step

Documents D1 or D2 could be considered to represent the closest prior art as they both related to the use of a porphyrin compound in aerobic fermentation processes of lactic acid bacteria cultures. The objective technical problem in view of this prior art resided in providing an improved method for culturing lactic acid bacteria .

The improvement was an increase in biomass as determined by a higher optical density at OD_{600}.

The problem was solved by the method of claim 1, as the patent showed in the examples that the addition of a high concentration of a yield enhancing agent led to a significant increase of optical density, when the fermentation was carried out under aeration.

Neither document D1 nor D2 contained any indication that a further improvement could be achieved.

Documents D5, D6, D7 or D8 would not have been combined with the teaching of Dl or D2, as they all taught that nucleotides precursor compounds acted as yield enhancing agents in a medium where these compounds were missing. However, Dl used a complete medium, that already included nucleotide precursor compounds in a concentration that was sufficient for anaerobic fermentation. Thus, since it was unknown in the prior art that a depletion of the nucleo compounds, during lactic acid bacteria fermentation under aeration in a medium comprising at least one porphyrin compound, led to a reduced yield of lactic acid bacteria, the skilled person faced with the technical problem identified above would not have arrived at the method of claim 1 in an obvious way.

XI. The appellant requested that the decision under appeal be set aside, the patent be revoked and documents D12 to D17, D24 to D26 and D30 be admitted into the proceedings.

XII. The respondent requested that the appeal be dismissed, documents D12 to D17, D24 to D26, and D30 not be admitted, and in case the Board of Appeal admitted any

of these documents into the proceedings, that documents D18 to D23 and D27 to D29 be admitted too.

Reasons for the Decision

Admission of documents D12 to D17 filed with opponent's submission dated 12 July 2013.

1. At the oral proceedings, the opposition division decided not to admit documents D12 to D17 into the proceedings (cf. pages 1 and 2 of the minutes of the oral proceedings, and point 2 of the reasons for the decision under appeal).

2. The appellant submits that this decision should be reversed, first on the ground that it was wrongly based on Rule 116 EPC ("Preparation of oral proceedings"), and second that the opposition division was wrong to decide that the documents were not prima facie relevant.

3. The view that Rule 116 EPC cannot provide a legal basis for the non-admission of evidence submitted before a date set according to that Rule is correct.

   However, although the opposition division mentions Rule 116 EPC in the header of point 2 of its Reasons for the decision, the reasons given in point 2.3. of its decision, justifying the exclusion of documents D12 to D17, explicitly refer to their late filing with regard to the nine month period for filing the notice of opposition and to their relevance to the case.

4. In opposition proceedings, facts and evidence should normally be filed with the notice of opposition (cf. Rule 76(2)(c) EPC). While the filing of facts and

evidence by parties to opposition and opposition appeal proceedings is not precluded at any stage of such proceedings, the admissibility of facts and evidence filed at a late stage in such proceedings is always a matter of discretion for the EPO (Article 114(2) EPC) (cf. point 4(a) of the Reasons for the decision G 4/95, OJ EPO 7/1996). Late-filed facts, evidence and related arguments which go beyond the "indication of the facts, evidence and arguments" presented in the notice of opposition are only exceptionally admitted into the proceedings if, prima facie, there are clear reasons to suspect that such late-filed material would prejudice the maintenance of the European patent in suit (cf. point 3.3 of the Reasons for the decision T 1002/92, OJ 1995, 665, point 3).

5. According to the principles laid down in the above mentioned decisions, documents D12 to D17 were late filed in the opposition proceedings. Their admission was therefore at the discretion of the opposition division (Article 114(2) EPC). As reported on pages 1 and 2 of the minutes of the oral proceedings, both parties had the opportunity to present their arguments on the admission of the documents, before the opposition division's deliberation and before the opposition division decided not to admit the documents. In view of the fact that the opposition division heard the parties on the admission of documents D12 to D17 and took into consideration their prima facie relevance before deciding on their admission, the board concludes that the opposition division did not commit a procedural violation but took its decision on the basis of the right principles and in a reasonable way, and that this exercise of discretion should therefore not be overturned (cf. e.g. point 3.2 of decision T 1119/05 of 8 January 2008).

6. In view of the above, the board sees no reason to re-assess the admissibility of documents D12 to D17, re-submitted with the notice of appeal, into the appeal proceedings. Exercising its discretion under Article 114(2) EPC in conjunction with Article 12(4) RPBA, the board decides not to admit them.

Admission of documents D24 to D26 and D30 filed with appellant's submissions and D18 to D23, and D27 to D29 filed by the respondent

7. Appellant stated that document D24 was submitted in response to the reasons given in the decision under appeal regarding sufficiency of disclosure of the claimed invention.

8. In its communication in preparation for the oral proceedings, the board observed that document D24, an expert declaration filed with appellant's statement of grounds of appeal, describing the reproduction of Example 1 of the patent, was prima facie unsuitable to support appellant's objection under Article 100(b) EPC, because it did not describe the fermentation in sufficient detail. In particular, based on the data presented in document D24, it was prima facie impossible to conclude that the OD_{600} measurements were taken at the end of the fermentation and that a skilled person carrying out example 1 was incapable, under any circumstances, to achieve an OD_{600} of at least 10.

9. Despite the board explicitly mentioning this issue in its communication according to Article 15(1) RPBA, no further arguments were provided by the appellant. Thus, the board has no reason to deviate from its provisional

assessment of the relevance of document D24 and decides not to admit it.

10. No reason was provided by the appellant as to why documents D25 and D26 could not have been presented in opposition proceedings, neither with the statement of grounds of appeal nor in reply to the board's communication explicitly mentioning this issue. They are thus not admitted into the proceedings.

11. Since none of documents D12 to D17 nor any of documents D24 to D26 are admitted into the proceedings, respondent's conditional request for the admission of documents D18 to D23 and D27 to D29 needs no further consideration.

Main Request

Article 100(c) EPC - Added-matter

12. The issue to be examined is whether the patent application, either explicitly or implicitly, directly and unambiguously, discloses a method for obtaining increased yields of lactic acid bacteria according to claim 1 as granted, comprising culturing such bacteria in a culture medium where "the OD_{600} is at least 10 at the termination of the fermentation".

13. The appellant argued that the patent application described the measurement of OD_{600} at the end of the fermentation process while claim 1 required it to be measured at the end of fermentation. These two terms referred to different points in time.

14. The preamble of claim 1 of the patent application as originally filed (and of granted claim 1) reads:

- "A method for obtaining increased yields of a lactic acid bacteria culture fermented under aeration and high Optical Density conditions".

15. In the decision under appeal, the opposition division concluded that the paragraph on page 2, lines 7 to 11, relating to "high Optical-Density-conditions" clearly and unambiguously referred to the fermentation as such without any additional downstream processing steps.

The cited paragraph reads:

- "The optical density of liquid medium at 600 nm (OD_{600}) is an accurate means of evaluating the density of bacterial cells in a sample of culture. By the term a "high Optical Density-conditions" is referred to fermentations which is characterized by that the concentration of the propagated cells sufficiently high to result in an OD_{600}, which is 10, or more at the end of the fermentation process."

16. The board considers the expression "end of the fermentation process" at the end of this paragraph to refer to the end of the cultivation or propagation (process) of the cells. The board arrives at this conclusion from the fact that the second sentence explicitly refers to fermentations as such, i.e. propagations of cells in culture, which are characterized by the fact that the concentration of the propagated cells is sufficiently high to result in an OD_{600} which is 10 or more at the end of the fermentation or, in synonymous words, at the end of the fermentation process. The meaning of the term "fermentation" as used in the application is provided on page 6, lines 11-12,

of the application as filed and refers explicitly to a process of propagating or cultivating the cells, which concurs with the board's interpretation.

17. If the appellant's interpretation were correct, there would be no need to characterize fermentation as being under high Optical density-conditions, as in the first part of the sentence, since bacterial cultures, irrespective of whether they are fermented under high Optical Density-conditions or not, can be concentrated to an OD_{600} of 10 or more by harvesting the cells. This sentence therefore directly and unambiguously indicates to the skilled reader that the method of claim 1 of the patent application ("for obtaining increased yields of a lactic acid bacteria culture fermented under aerobic and high Optical Density-conditions") is a method characterized by an OD_{600} of 10 or more (in other words at least 10) at the end of the fermentation.

18. The board's interpretation is further supported by claim 25 of the patent application which indicates that the optical density of the culture medium reached an "OD_{600} = 10 to OD_{600} =200". Read in conjunction with, for instance claim 1 of the patent application, it directly and unambiguously discloses a method for obtaining increased yields of lactic acid bacteria comprising culturing the bacteria to an optical density between 10 and 200 at the end of fermentation. Even if claim 25 in combination with claim 1 does not provide a basis for an OD_{600} of "at least 10", it confirms that the OD is measured at the termination of the fermentation.

18.1 The main request does not contravene Article 100(c) EPC.

Article 100(b) EPC - Sufficiency of disclosure

19. The appellant submitted that the claimed subject matter was insufficiently disclosed, first because measurement of OD_{600} inherently depended on many factors such as the make/type of the spectrophotometer and the medium used for diluting samples before measurement. None of these factors was however described in the patent. Second, Example 1 of the patent lacked technical information to such an extent that it could not be readily reproduced. Third, many lactic acid bacteria were only able to respire when menaquinone was specifically added. At the priority date, the skilled person would not have understood why, when using *Lactobacillus brevis*, essentially no growth was observed.

20. The third argument, that menaquinone is required for aerobic growth of certain lactic acid bacteria, is a new argument based on new facts (document D26) which was not presented in the opposition proceedings. The board is not aware of any reasons that may have prevented the appellant from presenting this document in opposition proceedings. In view of the fact that the purpose of appeal proceedings is primarily to review a decision under appeal and not to advance new facts and arguments which could have been presented in the first instance proceedings, the board does not admit the third objection based on a document not admitted into appeal proceedings at this stage of the proceedings (Article 114(2) EPC in conjunction with Article 12(4) RPBA).

21. As to the first objection, it is undisputed that the measurement of OD_{600} belonged to the general knowledge of a skilled microbiologist (cf. for instance document

D4, paragraph [0106]). Since the measurement is based on the scattering/the dispersion of light, it is also undisputed that the raw data obtained when measuring OD_{600} depend on the optical arrangement/set-up and thus the brand/make of the measuring instrument and the medium used for diluting samples before measuring.

22. Appellant's first objection concerns therefore the (in)sufficient definition of how the Optical Density should be measured. The proper definition of the claimed subject matter or a step of the claimed method is however an issue under Article 84 EPC which is not a ground for opposition.

23. The second objection concerns an alleged lack of technical information in the patent. Appellant submitted document D24 to support its argument that under no circumstances a skilled person could achieve an OD_{600} of at least 10. As set out in point 8 (above), said document was however not admitted because it was not only late filed but also lacked prima facie relevance in view of the lack of sufficient experimental details.

24. According to established case law of the Boards of Appeal an objection based on lack of sufficient disclosure presupposes that there are serious doubts, substantiated by verifiable facts.

25. No such verifiable facts leading to serious doubts are identifiable by the board in the present case. Therefore, appellant's objections under Article 100(b) EPC fail to convince the board.

Articles 100(a) and 54 EPC - Novelty

26. The appellant submitted that document D2 deprived the claimed invention of novelty for the reason that a method as defined in claim 1 was disclosed in Experiment 1 describing "fermentation D". Based on the composition of yeast extract and a number of assumptions concerning the composition of yeast peptone described as one of the components of the fermentation medium, the appellant estimated the initial IMP concentration in the culture medium to lie well above 1 mM.

26.1 A key issue is to determine whether the medium composition used in fermentation D of document D2 includes implicitly at least 1 mM of at least one yield-enhancing agent selected from the group consisting of a purine base, a pyrimidine base, a nucleoside, and a nucleotide at a concentration that ensures that the culture medium comprises at least 1 µM of said at least one yield-enhancing agent at the termination of the fermentation.

27. The medium described in point 1.2 of document D2 mentions "yeast peptone" (page 18, line 15)), but does not mention "yeast extract" upon which the appellant based its arguments.

28. Yeast peptone is obtained by proteolysis of an enriched proteinaceous fraction, whereas a yeast extract is obtained by proteolysis/autolysis of whole cells (cf. document D6, paragraph bridging pages 153/154). Thus, the chemical composition of a yeast peptone differs significantly from the composition of a yeast extract. Given the difference between the chemical composition

of a yeast peptone and a yeast extract, an "enriched" or "fortified" yeast extract preparation cannot be equated with a yeast peptone. It follows that appellant's arguments based on its assumption that the chemical composition of yeast peptone is identical to the composition of fortified yeast extract fail to convince the board.

29. Thus, document D2 does not disclose a culture medium comprising at least one yield-enhancing agent at a concentration of at least 1 mM. Therefore, the main request fulfills the requirements of Article 54 EPC.

Articles 100(a) and 56 EPC - Inventive step

30. Both documents, D1 and D2, have been cited as closest prior art documents. Both documents describe methods of cultivating lactic acid bacteria under aerobic conditions in the presence of a porphyrin compound.

31. Document D1 describes that the addition of a porphyrin compound increases the yield of lactic acid bacteria (Table 1, page 3, lines 9-11, page 11, lines 1-2, page 13, lines 18-21). After 24 hours of growth with shaking but without control of pH (page 9, lines 6-11), an OD_{600} of about 6 is reported (Table I).

Document D2 relates to culturally modified lactic acid bacteria having an increased content of a porphyrin compound that is useful to reduce the oxygen content in a food or feed product. It describes that when lactic acid bacteria are grown under aerobic conditions in the presence of a porphyrin compound, they are capable of maintaining their increased oxygen reducing activity when inoculated into milk or other media. Fermentations of lactic acid bacteria were carried out using

different media (Table 3, e.g. fermentations C and D). Cultures were allowed to acidify to pH 6.2 (page 19, line 12) and continued until no further base consumption was observed (page 19, lines 19 and 20). An OD_{600} of about 13 was measured for fermentations C and D, wherein said OD_{600} was slightly higher in the presence of a porphyrin compound (Table 3).

32. The method of present claim 1 aims at increasing the yield of lactic acid bacteria grown under aerobic conditions. Since document D1 describes an increased yield upon fermentation of the cells under aerobic conditions and in the presence of hemin (a porphyrin compound), whereas document D2 is silent about it (although such an effect may be derivable from Table 3), the board considers document D1 to represent the closest state of the art.

33. Starting from document D1 as closest prior art, the technical problem underlying the claimed invention is defined as the provision of a method for further increasing the yield of lactic acid bacteria fermented under aeration.

34. As a solution to this problem, the patent proposes the method of claim 1.

35. Example 1 of the patent provides a direct comparison of the medium of D1 and the medium of the present invention. The medium of D1 is a basic culture medium designated BD-5-ex3* (see page 12 Table 1 of the present patent) supplemented with additional compounds. Paragraph [0105] of the present patent (above Table 1) confirms that all types of cultures were carried out in a nutrient medium comprising at least one porphyrin compound. Table 2 shows further that a dramatic

increase of lactic acid bacteria yields is achieved by the addition of yield-enhancing agents at an initial concentration of at least 1 mM, i.e.0.2% w/v IMP and 0.2% w/v inosine ("Super EMIL") to said medium, rendering it significantly more productive in terms of OD_{600} (or kg of F-DVS) per 100 L of medium, from 45 (8.33 kg F-DVS) ("New EMIL") to 76 (16.67 kg F-DVS) ("Super EMIL") than the method of D1 measured.

36. The appellant submitted that the patent did not provide evidence that the concentration of the yield enhancing agents at the end of fermentation was indeed above 1 µM. There is however also no evidence that the concentration at the end of the fermentation described in Example 1 is below said concentration. What is however clear from Example 1 is that the claimed method, irrespective of the final concentration of the yield enhancing agents, provides an increased yield compared to the methods described in document D1.

37. The board is therefore satisfied that the technical problem is indeed solved by the method of claim 1.

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

39. Document D1 is silent about any further improvements. Thus, a skilled person faced with the above mentioned technical problem would not have arrived at the claimed solution in an obvious way based on document D1 alone.

40. Document D5 relates to methods of culturing Streptococcus lactis in a medium comprising yeast extract. In one experiment the growth stimulating activity of nucleotide mixtures on Streptococcus lactis was assayed under anaerobic conditions (D5 page 132,

figure 7 and first three paragraphs), but when added to culture media comprising yeast extract, the nucleotides did not stimulate bacterial growth any further (D5, page 132, first full paragraph).

Document D6 evaluated an automated turbidimetric method as a screening tool to determine the growth stimulating effect of different lots of yeast extracts on cultures of lactic acid bacteria (page 154, last full paragraph). It was concluded that the method is indeed suitable. There is however no mention of any other growth stimulating agents.

Document D7 was published after the first two priority dates but before the filing date of the patent in suit. In appeal proceedings, none of the parties addressed the issue whether the claimed subject matter enjoys priority rights from the first two priority applications. Instead, both parties discussed the technical contents of document D7. In view of the final conclusion in respect of inventive step (infra), the board sees no need to give further consideration to this issue. Document D7 reviews the nucleotide metabolism and its control in lactic acid bacteria. In the introductory section it is stated that many lactobacilli are auxotrophic for purines and pyrimidines and that lactic acid bacteria were apparently stimulated by the addition of purines to the growth medium (see D7, page 557 col.1).

Document D8 relates to growth-stimulatory properties of pancreas extract to accelerate acid production by Streptococcus lactis growing in milk. The stimulatory factors in the pancreas extract were isolated, and identified as nucleic acid derivatives.

41. None of the documents D5 to D8 pointed to or suggested in any other way that the yield of lactic acid bacteria cultured under aerobic conditions in a fermentation medium containing porphyrin as a yield enhancing compound could be further improved by supplementing the medium so that at least 1 mM of at least one yield-enhancing agent selected from the group consisting of a purine base, a pyrimidine base, a nucleoside, a nucleotide is initially present in said medium, a concentration which is above the concentration of such compounds in culture media comprising yeast extracts at the start of the fermentation.

42. Thus, unless with hindsight, the skilled person faced with the technical problem identified above, had no motivation to combine the teaching of document D1 with the teaching of any one of documents D5 to D8.

43. In the alternative, if as proposed by the parties, document D2 is considered to represent the closest prior art, the board arrives at the same conclusion.

44. Document D2 relates to culturally modified lactic acid bacteria having an increased content of a porphyrin compound, that is useful to reduce the oxygen content in a food or feed product. There is no mention of growth stimulation and nothing suggesting that the addition of porphyrin to lactic acid bacteria fermented aerobically leads to the depletion of nucleotides to the extent of becoming growth-limiting. The skilled person starting from document D2 as closest prior art had therefore no motivation to turn to any of documents D5 to D8.

45. In view of the above, the board concludes that the subject-matter of independent claim 1 and dependent

claims 2-12 of the main request meets the requirements of Article 56 EPC.

Order

For these reasons it is decided that:

The appeal is dismissed.

The Registrar: The Chairman:

L. Malécot-Grob B. Stolz

Decision electronically authenticated