Datasheet for the decision of 11 May 2016

Case Number: T 0715/11 - 3.3.08
Application Number: 98924059.3
Publication Number: 0985043
IPC: C12N15/63
Language of the proceedings: EN

Title of invention:
LACTIC ACID BACTERIAL STARTER CULTURES AND COMPOSITIONS THEREOF

Patent Proprietor:
Chr. Hansen A/S

Opponent:
DuPont Nutrition Biosciences ApS

Headword:
Lactic acid bacterial helper organisms/CHR. HANSEN

Relevant legal provisions:
EPC Art. 54(2), 54(3), 56, 83, 84, 123(2)
EPC R. 139
Keyword:
Correction of typographical error (yes)
Added matter (no)
Clarity (yes)
Sufficiency of disclosure (yes)
Novelty (yes)
Inventive step (yes)

Decisions cited:
T 0727/95

Catchword:
Case Number: T 0715/11 – 3.3.08

DECISION of Technical Board of Appeal 3.3.08 of 11 May 2016

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Decision under appeal: Interlocutory decision of the Opposition

Composition of the Board:
Chairman M. Wieser
Members: M. R. Vega Laso
O. Loizou
Summary of Facts and Submissions

I. European patent No. 985 043 with the title "Lactic acid bacterial starter cultures and compositions thereof" was granted on European application No. 98924059.3 filed under the Patent Cooperation Treaty and published as WO 98/54337 (in the following "the application as filed"). Claims 1 to 36 of the patent as granted were directed to a method of enhancing the growth and/or controlling the metabolic activity of a lactic acid bacterial strain (claims 1 to 18), a starter culture composition (claims 19 to 29) and a lactic acid bacterium (claims 30 to 36).

II. The patent was opposed on the grounds for opposition under Article 100(a) in conjunction with Articles 54 and 56, 100(b) and 100(c) EPC.

III. In an interlocutory decision under Article 101(3)(a) and 106(2) EPC posted on 11 February 2011, an opposition division of the European Patent Office found that amended claims 1 to 30 and the adapted description according to the main request filed during the oral proceedings, and the invention to which they related, met the requirements of the EPC.

IV. Claims 1, 2, 6, 17, 26 and 30 of the main request read as follows:

"1. A method of enhancing the growth rate and/or controlling the metabolic activity of a lactic acid bacterial strain, comprising cultivating the strain in association with a lactic acid bacterial helper organism, wherein said lactic acid bacterial helper organism is defective in its pyruvate metabolism."
2. A method according to claim 1 wherein the metabolic activity of the lactic acid bacterial strain that leads to an increased production of acids is enhanced.

6. A method according to claim 1 wherein the amount of oxygen present in the medium, wherein the lactic add [sic] bacterial strain and the helper organism are cultivated, is reduced by at least 1% per hour.

17. A starter culture composition comprising a lactic acid bacterium and a lactic acid bacterial helper organism, wherein said lactic acid bacterial helper organism is defective in its pyruvate metabolism and is capable of enhancing the growth rate of the lactic acid bacterial strain.

26. A lactic acid bacterium that is defective in at least one enzyme involved in the pyruvate metabolism and in which a gene coding for an enzyme that is capable of catalysing the reduction of $O_2$ to $H_2O$ or $H_2O_2$, and regenerating NAD$^+$ is over-expressed.

30. A bacterium according to claim 29 wherein the DNA fragment is selected from the group consisting of the sequence shown in SEQ ID NO:1 and a variant or derivative hereof which is at least 50% identical with said sequence, and has the same function."

Dependent claims 2 to 5 and 7 to 16 were directed to particular variants of the method of claim 1. Dependent claims 18 to 25 and 27 to 29 were directed to different embodiments of, respectively, the starter culture composition of claim 17 and the lactic acid bacterium of claim 26.
V. The opponent (appellant) filed an appeal against the decision and submitted a statement setting out the grounds of appeal including additional documentary evidence.

VI. By letter dated 27 October 2011, the patent proprietor (respondent) replied to the statement of grounds of appeal, and submitted eight sets of claims as main request and auxiliary requests I to VII, as well as additional evidence. The claims of the main request are identical to those of the main request underlying the decision under appeal, except that in claim 6 "add" has been replaced by "acid".

VII. Both the appellant and the respondent requested oral proceedings as a subsidiary request.

VIII. The board summoned the parties to oral proceedings. In a communication under Article 15(1) of the Rules of Procedure of the Boards of Appeal (RPBA) attached to the summons, the board observed that the admission of new requests or evidence into the proceedings would have to be discussed at the oral proceedings. The board also expressed a provisional opinion on some substantive issues to be discussed at the oral proceedings with respect to the main request, in particular issues relating to Articles 123(2), 84, 83, 54 and 56 EPC.

IX. The respondent replied to the board's communication and submitted an additional set of claims as new auxiliary request I. The appellant did not make any further substantive submissions.

X. Oral proceedings were held on 11 May 2016.
XI. The following documents are referred to in the present decision:


(15): M.S. Reddy et al., December 1972, Applied Microbiology, Vol. 24, No. 6, pages 953 to 957;


XII. The appellant's submissions on the issues relevant to this decision were essentially as follows:

*Article 123(2) EPC - claims 2, 17 to 25 and 30*

The opposition division had erred in finding that there was a basis for present claim 2 in page 3, lines 10 to 12 and claim 2 of the application as filed. The passage on page 3 made no reference to increased acid
production, and claim 2 of the application as filed did not indicate that the enhancement of metabolic activity of the lactic acid bacterial strain was responsible for the increase in acid production.

Claim 25 of the application as filed, which referred to "enhancing the growth rate and/or metabolic activity", did not provide a specific and unambiguous basis for the subject-matter of claim 17 and dependent claims 18 to 25. Enhancing the growth rate represented a selection from the three possibilities disclosed in claim 25.

There was no basis in the application as filed for a lactic acid bacterium over-expressing an enzyme having the sequence of SEQ ID NO:1, as specified in claim 30. Nor was there a basis for an enzyme with a sequence which was a variant or derivative of SEQ ID NO:1 having at least 50% identity with said sequence and the same function. In the absence of a specific teaching in the application as filed as to what activity was to be expected from a sequence having only 50% identity to SEQ ID NO:1, a person skilled in the art would in no way interpret the variants or derivatives mentioned in the second and third paragraphs of page 14 as having the same (i.e. identical) function as the enzyme encoded by SEQ ID NO:1.

Article 84 EPC - claim 17

The wording "and is capable of enhancing the growth rate of the lactic acid bacterial strain" in claim 17 lacked clarity and was not supported by the description. There was no way of knowing from the examples whether the decrease in pH recorded in the
culture was due to enhanced growth of the bacteria or increased metabolic activity in individual cells.

Article 83 EPC

In the patent in suit, the helper organism was defined broadly and encompassed a vast number of organisms. In view of the wording "in general" in paragraph [0023], it was clear that not all "helper organisms" had to be derived from lactic acid bacteria. Furthermore, it was not plausible that both an organism associated with decreased production of a metabolite of pyruvate and an organism associated with increased production thereof could be used in the present invention, as suggested by paragraph [0014] of the patent. Consequently, there was an undue burden on the skilled person to determine which helper organisms could be used for carrying out the invention.

The specification only exemplified two specific strains, DN223 and DN224, which were mutant strains of Lactococcus lactis subspecies lactis, but did not teach the skilled person how to obtain other helper organisms which were "defective in pyruvate metabolism", in particular as regards the specific sites that were to be subjected to site-directed mutagenesis. Moreover, the screening methods used to identify DN223 and DN224 were specific for Pfl or Ldh defective mutants and could not be used to identify other bacteria defective in the pyruvate metabolism. As stated in decision T 727/95 of 21 May 1999, relying on chance for reproducibility amounted to an undue burden in the absence of evidence that such chance events occurred and could be identified frequently enough to guarantee success.
There was no disclosure in the specification demonstrating that either the growth rate or the metabolic activity of a lactic acid bacterial strain could be enhanced by adding a helper organism defective in the pyruvate metabolism. There was also no exemplification of increased or decreased production of any metabolite. There was an undue burden on the skilled person to determine which metabolites, if any, could be increased or decreased using a particular helper strain.

The patent did not teach how to obtain a helper organism defective in its pyruvate metabolism which over-expressed a gene coding for an enzyme that was capable of catalysing the reduction of O₂ to H₂O or H₂O₂ and regenerating NAD⁺, let alone how to screen for such a helper organism. The sole gene exemplified in the patent was the NADH:H₂O oxidase encoded by the nox gene.

The specification only exemplified the cultivation of specific strains in milk. There was no disclosure as to how to obtain helper strains which could be cultured in a medium other than milk.

*Article 54(3) EPC - document (4)*

The subject-matter of claims 1 to 30 lacked novelty in view of document (4). Document (4) described the bacterial strains DN223 and DN224, which were lactic acid bacterial organisms defective in their pyruvate metabolism, and a method comprising cultivating a mixture of these two strains (see page 19, lines 7 and 8). It described also starter cultures comprising these strains (page 8, lines 9 to 11) and methods of producing a food product by adding to the food product...
starting materials a culture of DN223 and DN224 (see page 8, lines 1 to 4). The feature "capable of enhancing the growth rate and/or controlling the metabolic activity of the lactic acid bacterial strain" was a functional feature that was inherent when DN223 was added to another lactic acid bacterial strain.

Article 56 EPC

Document (4) as the closest state of the art – claims 14 to 16, 22 to 24 and 26 to 30

The subject-matter of claims 14 to 16, 22 to 24 and 26 to 30 lacked inventive step. These claims did not enjoy the claimed priority; consequently, document (4) constituted prior art under Article 54(2) EPC. The difference between the method described in document (4) and that of claim 14 was that a gene coding for an enzyme that was capable of catalysing the reduction of O2 to H2O or H2O2 and regenerating NADH+ was over-expressed in the helper organism. The problem to be solved was to arrive at a helper cell which had improved growth/survival. The solution proposed in claim 14 was suggested in document (4). It was well known in the art that reducing the oxygen content of the culture medium (such as milk) would result in more rapid growth of the lactic acid bacterium. Thus, the skilled person would be motivated to obtain strains which reduced the amount of oxygen in the medium. It was apparent from the passage on page 13, lines 10 to 16 of this document that the Pfl- Ldh- (such as DN223) was unable to grow anaerobically due to an inability to regenerate NADH+ from NADH. Hence, it was obvious for a person skilled in the art to over-express a gene coding for an NADH oxidase that regenerated NADH+
in order to allow the bacteria to grow in anaerobic conditions.

*Documents (6) and (2) as the closest state of the art - claims 1 to 30*

Claims 1 to 30 lacked inventive step in view of document (6) and/or (2) with or without common general knowledge. Starting from these documents, the problem to be solved was to enhance the metabolic activity of a lactic acid bacterial strain so as to produce, for example, diacetyl - a flavour compound having a buttery taste. Since an enhanced growth rate or control of metabolic activity would inherently occur when a lactic acid bacterial strain was cultured in the presence of a lactic acid bacterial helper organism defective in its pyruvate metabolism, the decisive question was whether a person skilled in the art would have combined a lactic acid bacterium with a helper organism which was defective in its pyruvate metabolism.

At the priority date, it was routine in the art to combine different bacterial strains to make starter cultures. Helper organisms for cultivation with starter cultures were well known in the art. For instance, the mutant strains of *Lactococcus lactis* described in document (6) were defective in the pyruvate metabolism and capable of increasing the production of diacetyl, i.e. of controlling the metabolic activity of starter culture lactic acid bacteria. Moreover, document (6) suggested that increased levels of NADH oxidase would increase the levels of the aromatic compound diacetyl (see page 2641, first paragraph). The skilled person seeking to increase the buttery flavour of a dairy product would have readily considered supplementing the normal starter culture with a helper strain which
produced high levels of the butter flavour diacetyl - such as the strains described in documents (6) and (2).

XIII. The respondent's submissions, insofar as they are relevant to the present decision, may be summarised as follows:

Article 123(2) EPC - claims 2, 17 to 25 and 30

Claim 2 had a basis in the application as filed. It was immediately apparent from the passages on page 9, lines 11 to 14, and page 4, lines 20 to 23 that the enhancement of the metabolic activity of the lactic acid bacterial strain was responsible for the increased acid production. The passage on page 3, lines 10 to 12 likewise supported claim 2.

Claim 17 was based on claim 25 of the application as filed. The selection of an embodiment from a list of three embodiments originally disclosed in claim 25 did not add subject-matter.

Claim 30 had a basis in the passage on page 12, lines 2 to 6 of the application as filed. It was clear from claim 40 of the application as filed that SEQ ID NO:1 was part of the invention. Further support was provided by the passage from page 10, line 24 to page 14, line 21.

Article 84 EPC

It was clear from Figures 7 to 9 of the patent that the acidification enhancement occurred in parallel to the growth enhancement. Thus, Article 84 EPC was not contravened.
Article 83 EPC

There could be no doubt that strains DN223 and DN224 were sufficiently disclosed in the patent. The patent also contained a very detailed description as to how lactic acid bacteria like DN223 and DN224, i.e. strains which were defective in their pyruvate metabolism, could be obtained. Even if the methods described in the patent for obtaining such mutants relied on random mutagenesis, the identification of mutants did not require an undue amount of experimentation. Moreover, since the gene sequences of the enzymes of the pyruvate metabolism were known in the art, site-directed mutagenesis, a method well known in the art, could be used for obtaining the mutants.

The feature "controlling the metabolic activity" in claim 1 could not be construed as requiring that the production of every metabolite was increased or decreased.

Enzymes capable of catalysing the reduction of O₂ to H₂O or H₂O₂ and regenerating NAD⁺ other than that encoded by the nox gene were disclosed in the passage on page 10, lines 10 to 18 which referred to document (5).

Although milk might be the most typical growth medium in the dairy industry, various other suitable media for growing lactic acid bacteria starter cultures had been known long before the effective date of the patent.
Article 54 EPC

Document (4) did not disclose a method comprising the cultivation of a lactic acid bacterial strain in association with a lactic acid bacterial helper organism defective in its pyruvate metabolism. Even assuming that document (4) described the co-cultivation of two helper strains, no functional relationship between the two strains - as required by the present invention - was apparent. Thus, the feature "capable of enhancing the growth rate of the lactic bacterial strain" was not disclosed in document (4). Nor was the over-expression of a gene capable of catalysing the reduction of O₂ to H₂O or H₂O₂ and regenerating NAD⁺ in the helper organism. Hence, the claimed subject-matter was novel over document (4).

Article 56 EPC

Document (4) as the closest state of the art - claims 14 to 16, 22 to 24 and 26 to 30

Document (4) did not teach or suggest a mixture of two bacteria, let alone a mixture of a lactic acid bacterial strain and a lactic acid bacterial helper organism. The objective technical problem to be solved starting from this document could be formulated as providing a generally applicable biological method for enhancing the growth and metabolic activity of a given lactic acid bacterial strain. The solution proposed in claims 14 to 16, 22 to 24 and 26 to 30 was not obvious to a person skilled in the art.
Documents (6) and (2) as the closest state of the art – claims 1 to 30

A mixture of two bacteria was neither described nor suggested in document (6). This document taught the use of a single strain with a mutated aldB gene for the production of diacetyl. The objective technical problem to be solved was the same as that starting from document (4). The solution suggested in document (6) was completely different from that proposed in the patent. The same applied to document (2), in which aldB<sup>−</sup> *L. lactis* subsp. *lactis* biovar. *diacetylactis* strains were shown to produce a large amount of alpha-acetolactate. Thus, in view of these documents, whether taken alone or in combination, the solution proposed in the present claims could not be considered to be obvious to a person skilled in the art.

XIV. The appellant (opponent) requested that the decision under appeal be set aside and that the patent be revoked.

XV. The respondent (patent proprietor) requested that the decision under appeal be set aside and that the patent be maintained in amended form on the basis of the claims of the main request filed on 27 October 2011.

**Reasons for the Decision**

*Rule 139 EPC – claim 6*

1. Claim 6 according to the present main request is identical to the corresponding claim of both the patent as granted and the main request underlying the decision under appeal, except that the wording "the lactic add
bacterial strain" has been corrected to read "the lactic acid bacterial strain" (emphasis added by the board). It is immediately apparent to a person skilled in the art, in the context of claim 6 as granted, that the word "add" does not fit and must, therefore, be a typographical error. Since claim 6 is dependent on claim 1, in which "a lactic acid bacterial strain" is specified (see section IV above), it is also immediately evident that the word "acid" offered instead in present claim 6 is what was actually intended.

2. For these reasons and in the absence of objections by the appellant, the board decides to allow the offered correction and admits into the proceedings the amended main request submitted together with the reply to the statement of grounds of appeal.

Article 123(2) EPC - claims 2, 17 to 25 and 30

3. Except for amended claim 6 (see point 1 above), the set of claims according to the present main request is identical to the claims of the main request underlying the decision under appeal.

4. In the decision under appeal, the opposition division found that the objections under Article 123(2) EPC raised by the opponent against claims 2, 17 to 25 and 30 were not justified (see section 9 of the decision). This finding is, in the board's view, correct.

5. A basis for present claim 2 (see point IV above) is found in claim 2 of the application as filed, read in the light of the passage on page 4, lines 20 to 24 of the description. Claim 2 of the application as filed is directed to a method of enhancing the growth rate and/
or controlling the metabolic activity of a lactic acid bacterial strain, comprising the step of cultivating the strain in association with a lactic acid bacterial helper organism defective in its pyruvate metabolism, which results in enhanced the acid production by the lactic acid bacterial strain. On page 4, lines 20 to 24 of the application as filed, the expression "controlling the metabolic activity" is defined as referring to "... the increased or decreased production of any metabolite produced by the starter culture, including the production of acids,...". From this passage, a person skilled in the art can directly and unambiguously derive a link between the metabolic activity of the lactic acid bacterial strain and the increased production of acids, as specified in present claim 2.

6. Like the opposition division in the decision under appeal, the board regards claim 25 of the application as filed as a basis for the subject-matter of present claim 17 (see point IV above). Claim 25 of the application as filed is directed to a starter culture composition comprising a helper organism capable of enhancing the growth rate of a lactic acid bacterium also included in the composition and, alternatively or additionally, capable of controlling the metabolic activity of the lactic acid bacterium ("... being capable of enhancing the growth rate and/or controlling the metabolic activity of the lactic acid bacterium", emphasis added by the board). Undisputedly, a person skilled in the art reading claim 25 can derive from its wording three distinct embodiments of the starter culture composition. However, contrary to the appellant's view, the board holds that singling out one of these embodiments, in particular a starter culture composition comprising a helper organism capable of
enhancing the growth rate of the lactic acid bacterial strain as claimed in present claim 17, does not present the skilled person with any information which is not directly and unambiguously derivable from claim 25 of the application as filed. Thus, the amendments in claim 17 do not contravene Article 123(2) EPC.

7. A lactic acid bacterium as claimed in present claim 30 (see point IV above) is directly and unambiguously derivable from the application as filed, inter alia from the disclosure on page 14, lines 12 to 30 taken together with the sequences according to SEQ ID NO:1 and 2. The passage on page 14, lines 12 to 18 reads:

"In accordance with the invention there is also provided a lactic acid bacterium that is defective in at least one enzyme involved in the pyruvate metabolism as it is described above and in which a gene capable of regenerating NAD⁺ is overexpressed, including a gene coding for an enzyme catalysing the reduction of O₂ to H₂O or H₂O₂ such as an NADH:H₂O oxidase including the enzyme having the sequence SEQ ID NO:2."

From the sequence listing in the application it is immediately apparent that the amino acid sequence SEQ ID NO:2 is encoded by the nucleotide sequence in SEQ ID NO:1.

8. As regards variants or derivatives of SEQ ID NO:1, it is stated on page 14, lines 19 to 25 of the application as filed that:

"... the invention also provides an isolated DNA fragment derived from a lactic acid bacterium comprising a gene coding for a polypeptide having NADH:H₂O oxidase activity such as a DNA fragment which
is selected from the group consisting of the sequence shown in SEQ NO ID:1 and a variant or derivative hereof which is at least 50% e.g. at least 60% including at least 70% identical with said sequence ..."

9. In the board's view, the appellant's objection that the feature "... and has the same function" extends the subject-matter of present claim 30 beyond the content of the application as filed is not justified. There is no evidence whatsoever on file of any common general knowledge which a person skilled in the art at the relevant date might have had in mind while reading the application, and which would have made him/her doubt that variants or derivatives that were only 50% identical to SEQ ID NO:1 could still code for a polypeptide having NADH:H₂O oxidase activity. In the absence of such evidence, the appellant's argument as to what the skilled person would derive from the passage on page 14, lines 19 to 25 quoted above with respect to variants of derivatives fails to convince the board.

10. Summarising the above, none of the objections raised by the appellant under Article 123(2) EPC is considered to be justified.

Article 84 EPC

11. The appellant contested the opposition division's adverse findings concerning the objection to claim 17 under Article 84 EPC (see last paragraph in section 10 of the decision under appeal).

12. Article 84 EPC requires the claims to be clear and concise and to be supported by the description. In the board's view, in particular as regards the feature
"... and is capable of enhancing the growth rate of the lactic acid bacterial strain", claim 17 is clear within the meaning of Article 84 EPC. Whether or not a helper organism as specified in claim 17 enhances the growth rate of a lactic acid bacterial strain in a starter culture composition comprising both can be easily determined using methods that are well known in the art.

13. The experiments in the examples of the patent show that co-cultivation of a lactic acid bacterial strain with a lactic acid bacterial helper organism defective in its pyruvate metabolism results in a pH decrease due to increased acid production by the lactic acid bacterial strain (see Figures 4, 5, 6A, 7A and 8A). Since lactic acid bacteria normally produce lactic acid in proportion to the cell mass (see document (24), page 204, line 2), it can be inferred from the increased acid production that the growth rate of the lactic acid bacterium is enhanced. Thus, contrary to the appellant's argument, claim 17 is in fact supported by the examples of the patent.

14. For these reasons, the board holds that claim 17 fulfils the requirements of Article 84 EPC.

Article 83 EPC

15. In the decision under appeal, the opposition division found that the claimed invention was disclosed in the patent application in a manner sufficiently clear and complete for it to be carried out by a person skilled in the art (see last paragraph in point 11 of the decision).
16. In its statement of grounds of appeal, the appellant raised an objection to claims 1 to 9, 12 to 19 and 22 to 30 based on the allegedly extremely broad definition of the helper organism in the first sentence of paragraph [0023] of the patent (see point XII above).

17. The appellant's interpretation of the wording "In general, ..." in paragraph [0023] as meaning that the helper organism does not necessarily need to be derived from a lactic acid bacterium is not shared by the board. Nor can the board accept the appellant's argument that the technical information provided in the patent does not enable the skilled person to determine which helper organisms could be used to carry out the invention.

18. The meaning given in the patent to the feature "lactic acid bacterial" characterising the helper organism is explained in paragraph [0023], and specific examples of suitable helper organisms are given in paragraphs [0024] to [0026]. It is stated in paragraph [0023] that, in general, the lactic acid bacterial helper organism is a derivative of a lactic acid bacterium, derivatives including spontaneous mutants and mutants obtained by genetic modification in vitro of a lactic acid bacterium. Paragraphs [0024] to [0026] disclose lactic acid bacteria (e.g. Lactococcus lactis) from which a helper organism can be derived. In particular, DN223 and DN224 strains derived from Lactococcus lactis subspecies lactis are mentioned as suitable helper organisms for use in the invention.

19. The appellant raised a plausibility issue concerning the definition of a lactic acid bacterial helper organism defective in its pyruvate metabolism as an organism which, compared to the wild-type, has an
increased or decreased production of one or more metabolites derived from pyruvate (see paragraph [0014] of the patent). In view of the fact that, in lactic acid bacteria, pyruvate acts as substrate in various metabolic pathways which are regulated differently by various environmental conditions (e.g. carbon source limitation or aeration; see document (8)), it is, in the board's view, not implausible that a lactic acid bacterial organism useful as a helper organism may be associated with increased production of a particular metabolite in one pathway, and another helper organism with decreased production of a different metabolite in a different pathway.

20. The appellant's objection of lack of disclosure in the application as to how to obtain a helper organism defective in its pyruvate metabolism, other than the DN223 and DN224 strains described in the examples, is not justified. Reference Example 2 illustrates how to obtain mutants of two different strains of Lactobacillus lactis which are defective in the Pfl gene encoding the enzyme pyruvate formate lyase, and in Example 3 double mutants defective in both the Pfl gene and the Ldh gene coding for lactate dehydrogenase are obtained, these two enzymes being involved in the pyruvate metabolism. It is not apparent to the board why a person skilled in the art following the technical instructions given in these examples could not obtain further mutants defective in the pyruvate metabolism, and the appellant has not provided any arguments or evidence to this effect. While the methods for obtaining mutants defective in their pyruvate metabolism disclosed in the application as filed use random mutagenesis, there is no evidence on file showing that a person skilled in the art could not
identify and select the desired mutants without an undue burden of experimentation.

21. It should be noted that, at the relevant date, further genes coding for enzymes involved in the pyruvate metabolism had been described, and their nucleotide sequences were available from public databases (see e.g. documents (6) and (11)). As disclosed in the passage on page 8, lines 13 to 23 of the application as filed (paragraph [0024] of the patent), a person skilled in the art could modify these genes to construct the desired derivatives of a lactic acid bacterium, applying techniques known in the art at the relevant date, including mutation and DNA recombination techniques.

22. Moreover, the fact that the application as filed does not exemplify a lactic acid bacterial helper organism defective in the pyruvate metabolism which over-expresses a gene for an enzyme capable of catalysing the reduction of O₂ and regenerating NAD⁺ does not mean that a person skilled in the art would not be able to obtain such a helper organism relying on the information provided in the application and methods well known in the art at the relevant date. The nox gene is mentioned in the application as a gene for a NADH oxidase capable of catalysing the reduction of O₂ and regenerating NAD⁺. It has not been disputed that the sequence of the nox gene was available at the relevant date. There is also no evidence on file showing that finding other suitable genes coding for further NADH oxidases, as mentioned in the passage on page 10, lines 10 to 18 of the application as filed, would have involved an undue burden of experimentation or a need for inventive skills.
23. Contrary to the appellant's view, the examples of the application as filed (and of the patent as granted) show that cultivation of a lactic acid bacterial strain in association with a helper strain defective in its pyruvate metabolism, in particular strains DN223 and DN224, results in an enhancement of the acidification rate, i.e. an increased production of acids, presumably lactic acid, which is a metabolite derived from pyruvate. As stated in point 12 above, it can be inferred from the increase in acid production that the growth rate of the lactic acid bacterium is enhanced, as lactic acid bacteria produce lactic acid in proportion to the cell mass (see document (24), page 204, line 2).

24. Lastly, there is no evidence on file showing that the starter culture composition of claims 17 to 25 cannot be used to inoculate any culture media other than milk.

25. For these reasons, the appellant's objection under Article 83 EPC must fail.

Article 87 EPC - priority

26. The respondent accepts that the priority of the earlier DK and US applications filed on 30 May 1997 cannot be validly claimed for the subject-matter of claims 14 to 16, 22 to 24 and 26 to 30 (see respondent's reply to the statement of grounds of appeal, pages 15 and 16). Thus, for assessing whether or not the subject-matter of these claims is novel and involves an inventive step the relevant date is the filing date, i.e. 25 May 1998.

27. Thus, the content of document (4), an international application filed on 20 August 1997 claiming the priority of an earlier US application filed on
22 August 1996, forms part of the state of the art to be considered for the purposes of assessing novelty for claims 1 and 17 (Article 54(3) EPC) and novelty and inventive step for claims 14 to 16, 22 to 24 and 26 to 30 (Article 54(2) EPC).

*Article 54(2) and (3) EPC - claims 1, 17 and 26*

28. The appellant based its objection of lack of novelty on document (4). This document relates to methods for obtaining mutants or variants of lactic acid bacteria which, when they are used in the manufacture of fermented food products, produce increased amounts of desirable metabolites or reduced amounts of less desirable metabolites (see page 1, lines 3 to 9). Document (4) describes, in a first aspect, a method of isolating a pyruvate formate lyase (Pfl) defective lactic acid bacterium (see passage from page 6, line 3 to page 7, line 3) and, in a further aspect, also a method of isolating a Pfl and lactate dehydrogenase (Ldh) defective lactic acid bacterium. Lactose dehydrogenase (Ldh) and pyruvate formate lyase (Pfl) are two enzymes involved in the pyruvate metabolism of lactic acid bacteria.

29. In the examples, several Ldh or Ldh and Pfl defective mutants of *Lactobacillus lactis* obtained by these methods, *inter alia* the DN223 (Ldh" Pfl") and DN224 (Ldh") mutants used in the examples of the patent in suit, are tested - **individually** - for growth (measured as OD₆₀₀ and/or pH of the medium) and production of various metabolites of the pyruvate metabolism (e.g. acetaldehyde, diacetyl, lactic acid or ethanol), under aerobic and anaerobic conditions (see e.g. Examples 3.2 and 4.3).
30. The appellant referred to the following passages of document (4) in support of its objection of lack of novelty:

"There is also provided a lactic acid bacterial starter culture composition comprising any of the above mentioned lactic acid bacteria." (page 8, lines 9 to 11).

"It will also be understood that the presently provided strains will be highly useful as production strains in the manufacturing of lactic acid bacterial metabolite compounds including the above aroma compounds. Accordingly, the invention encompasses in a still further aspect a method of producing a lactic acid bacterial metabolite. Such a method comprises cultivating one or more of the lactic acid bacteria as disclosed herein in a suitable medium under industrially feasible conditions where the metabolite is produced, and isolating, if required, the metabolite from the culture" (page 19, lines 1 to 10; emphasis added by the board).

31. In the board's view, neither passage anticipates the subject-matter of the claims on file. A person skilled in the art cannot derive, directly and unambiguously, from the first passage quoted above a starter culture composition which includes not only any of the Ldh⁻ or Ldh⁻ Pfl⁻ mutants described in document (4), in particular DN223 or DN224, but also - as required in claims 1 and 17 - a lactic acid bacterial strain. The second passage on which the appellant relied relates to the use of one or more of the Ldh⁻ or Ldh⁻ Pfl⁻ mutants described in document (4) as a production strain, rather than as a helper strain as specified in the
present claims. Moreover, it is more than doubtful whether - as the appellant suggested - a skilled person can derive directly and unambiguously from this passage a starter culture composition comprising, specifically, the DN223 and DN224 mutants. But even if the board were to acknowledge that this specific combination of mutants could be derived from the passage in question, there is no evidence, either in the same document or in any other document on file, showing that DN223 is capable of enhancing the growth rate of DN224, or vice versa. Nor is there any evidence on file that either mutant is capable of controlling the metabolic activity of the other mutant. For these reasons, the board holds that the subject-matter of claims 1 and 17 must be regarded as novel over document (4).

32. As regards claim 26, the appellant cited Table 3.1 on page 28 of document (4) in support of its argument that, since the specific activity of the NADH oxidase - an enzyme capable of regenerating NAD⁺ - is higher in the DN223 mutant than in the parent strain CHCC373, the gene encoding this enzyme must be over-expressed in the mutant. This is, however, not necessarily the case. In Lactobacillus lactis, pyruvate metabolism is a metabolic network in which control of the pyruvate distribution within various pathways is subject to co-ordinated regulation by both gene expression mechanisms and allosteric modulation of enzyme activity (see document (8), last sentence of the abstract on page 157). Moreover, a modified level of lactose dehydrogenase (Ldh) and pyruvate formate lyase (Pfl) gene expression, as observed in the DN223 mutant, may have an effect on the regulation of oxygen metabolism and NAD⁺ regeneration, in which different NADH oxidising enzymes, inter alia the NADH oxidase, are involved (see document (8), chapter headed "Effect of
aeration" starting on page 165). Thus, contrary to the appellant's view, it cannot be ascertained from the data in Table 3.1. of document (8) that a gene coding for an enzyme that is capable of catalysing the reduction of O₂ to H₂O or H₂O₂, and of regenerating NAD⁺, is over-expressed in the DN223 mutant.

33. In view of the above, the appellant's objection of lack of novelty based on document (4) must fail.

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Document (4) as the closest state of the art – claims 14 to 16, 22 to 24 and 26 to 30

34. Claims 14 and 22, which depend directly and/or indirectly of claims 1 and 17, respectively, as well as independent claim 26 (see point IV above) specify that the helper organism over-expresses a gene coding for an enzyme that is capable of catalysing the reduction of O₂ to H₂O or H₂O₂, and of regenerating NAD⁺. It is important to note that the subject-matter of claims 14 and 22 is further characterised by the features specified in the independent claims on which they depend.

35. The general content of document (4), which the appellant regards as the closest state of the art, has been outlined in points 28 and 29 above.

36. As stated above in the framework of assessing novelty, a person skilled in the art could not derive from document (4) a method in which a lactic acid bacterial strain is cultivated in association with a lactic acid bacterial helper organism defective in the pyruvate metabolism for the purpose of enhancing the growth rate
and/or controlling the metabolic activity of the lactic acid bacterial strain. Nor could the skilled person derive from document (4) a composition comprising a lactic acid bacterium strain and a lactic acid helper organism capable of enhancing the growth rate of the lactic acid bacterial strain. Document (4) describes the use of a mutant defective in the pyruvate metabolism as a production strain rather than as a helper strain.

37. Thus, starting from document (4), the problem to be solved can be formulated as the provision of an alternative use of the lactic acid bacterial mutants described in this document. The board is convinced that this problem has been solved by the method and compositions proposed in the present claims.

38. The board is unable to find in document (4) any hint that would motivate the skilled person to use mutants in the pyruvate metabolism as helper strains to enhance the growth rate and/or control the metabolic activity of a lactic acid bacterial strain. There is also no suggestion of a further modification of the mutants in order to over-express a gene coding for an enzyme capable of catalysing the reduction of O₂ to H₂O or H₂O₂, and of regenerating NAD⁺.

39. The passage on page 13, lines 10 to 22 of document (4), to which the appellant referred in support of its objection, explains the strategy followed by the author for isolating and selecting a mutant of a lactic acid bacterium which is not only Pfl defective but also Ldh defective. This passage reads:

"Accordingly, it was hypothesized that a double mutant having the Pfl⁻ Ldh⁻ phenotype would be unable to grow under anaerobic conditions, i.e. such a strain would
have the additional phenotype Ang\textsuperscript{−} (inability to grow anaerobically). This hypothesis was based on the assumption that such a double mutant would be unable to regenerate NAD\textsuperscript{+} from NADH under anaerobic conditions, since Pfl would be blocked by a mutation (whereas under aerobic conditions, NADH can be converted to NAD\textsuperscript{+} by NADH oxidase), PDC would be blocked due to inhibition by NADH and Ldh would be blocked by mutation. It was thus contemplated that a Pfl\textsuperscript{−} Ldh\textsuperscript{−} double mutant could grow under aerobic conditions but not under anaerobic conditions."

40. In the board's view, without knowledge of the present invention a person skilled in the art cannot derive from this passage that the over-expression of a gene coding for an enzyme capable of catalysing the reduction of O\textsubscript{2} to H\textsubscript{2}O or H\textsubscript{2}O\textsubscript{2} and of regenerating NAD\textsuperscript{+} would be in some way advantageous for the use of a mutant defective in the pyruvate metabolism as helper organism. Only with hindsight can such a measure be considered obvious.

41. Since the further claims objected to by the appellant are dependent on claims 14, 22 or 26, the same applies, mutatis mutandis, to those claims.

42. Hence, the board concludes that the objection of lack of inventive step based on document (4) is not justified.

Documents (6) and (2) as the closest state of the art - claims 1 to 30

43. Document (6) describes the genetic manipulation of the pathway for diacetyl metabolism in Lactococcus lactis, in particular the generation of mutants defective in
the gene encoding α-acetolactate decarboxylase (aldB),
an enzyme involved in the pyruvate conversion to
acetoin and 2,3-butanediol. Over-expression of genes
encoding an α-acetolactate synthase in the defective
strains lead to the production of higher levels of
α-acetolactate, acetoin and diacetyl. Document (2) too
reports a higher yield of diacetyl produced by mutants
defective in the α-acetolactate decarboxylase.

44. Starting from these documents, the problem to be solved
is similar to that starting from document (4), i.e. the
provision of an alternative use of the mutants
described in the prior art.

45. The board holds that the use of such a mutant as helper
organism in association with a lactic acid bacterial
strain to enhance the growth rate and/or control the
metabolic activity of the lactic acid bacterial strain
was not obvious to a person skilled in the art. It is
ture that, as documents (15) and (16) show, the use of
mixed starter cultures was common general knowledge at
the priority date. Thus, a person skilled in the art
could, in principle, have combined a mutant described
in document (6) or (2) with a lactic acid bacterial
strain. But without knowledge of the technical effect
underlying the present invention, i.e. that in a mixed
culture of a mutant defective in the pyruvate
metabolism and a lactic acid bacterial strain the
growth rate of the latter is enhanced and/or its
metabolic activity controlled, he/she would have no
motivation to do so.

46. It should also be stressed that the appellant's choice
of documents relating to mutants defective in the
pyruvate metabolism as the closest state of the art has
been done with hindsight, because it introduces
elements of the solution provided by the claimed invention. Already for this reason, the appellant's lines of argument in support of its objection of lack of inventive step are biased.

47. Summarising the above, the board is not persuaded that, in view of the documents brought forward by the appellant, the claimed subject-matter lacks inventive step.

Conclusion

48. The appellant's request that the patent be revoked fails.
Order

For these reasons it is decided that:

1. The decision under appeal is set aside.

2. The case is remitted to the department of first instance with the order to maintain the patent in amended form on the basis of claims 1 to 30 of the main request filed on 27 October 2011.

The Registrar: The Chairman:

A. Wolinski M. Wieser

Decision electronically authenticated