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Datasheet for the decision of 1 December 2020

Case Number: T 2277/16 - 3.3.08

Application Number: 04703147.1

Publication Number: 1587909

IPC: C12N1/00, C12N1/04

Language of the proceedings: EN

Title of invention:
Storage stable frozen lactic acid bacteria culture

Patent Proprietor:
Chr. Hansen A/S

Opponents:
DuPont Nutrition BioSciences ApS
DSM IP Assets B.V.
CSK food enrichment B.V.

Headword:
Stable frozen lactic acid bacteria/CHR. HANSEN

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

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It can be changed at any time and without notic

Keyword:
Main request - Novelty (no)
Auxiliary request 3 - Added subject-matter (no), Inventive step (no)
Auxiliary request 7 - Inventive step (no)

Decisions cited:
T 0688/14

Catchword:

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Case Number: T 2277/16 - 3.3.08

D E C I S I O N
of Technical Board of Appeal 3.3.08
of 1 December 2020

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Decision under appeal: **Decision of the Opposition Division of the European Patent Office posted on 4 August 2016 revoking European patent No. 1587909 pursuant to Article 101(3)(b) EPC.**

Composition of the Board:

Chairman P. Julià
Members: M. Montrone
D. Rogers

Summary of Facts and Submissions

I. The appeal lies against the decision of an opposition division to revoke the European patent No. 1 587 909. The patent was filed under the PCT and published as international patent application WO 2004/065584 (hereinafter the "patent application").

II. The opposition division held *inter alia* that the main request and auxiliary requests 1 and 2 lacked novelty, while auxiliary requests 3 to 9 comprised added subject-matter, and auxiliary request 10 lacked an inventive step. Auxiliary request 11 was not admitted into the proceedings.

III. With its statement of grounds of appeal, the patent proprietor (hereinafter "appellant") filed new evidence and auxiliary requests 2A, 3A, 9A, 10 and 11. The main request and auxiliary requests 1 to 11 corresponded to the respective claims dealt with in the decision under appeal. Auxiliary requests 2A, 3A and 9A were new to the proceedings.

IV. Opponents 01 to 03 withdrew their oppositions, and hence, ceased to be parties to the present proceedings.

V. In a communication pursuant to Article 15(1) RPBA, the appellant was informed of the board's provisional, non-binding opinion.

VI. Oral proceedings before the board were held on 1 December 2020 by video conference as requested by the appellant. During the oral proceedings, the appellant withdrew auxiliary requests 1, 2, 2A, 3A, 4 to 6, 8, 9,

9A, 10 and 11. Thus only the main request and auxiliary requests 3 and 7 remained in the proceedings.

VII. Claims 1 and 6 of the main request read:

"1. A frozen lactic acid bacteria (LAB) culture that comprises LAB that are able to utilize sucrose and is a mixed mesophilic culture consisting of mesophilic bacteria having optimum growth temperatures at about 30°C, has a weight of at least 50g frozen material and a content of viable bacteria of at least 10^9 colony forming units (CFU) per g frozen material and characterized in that the frozen culture comprises from 0.5% to 80% of a cryoprotective agent measured as w/w of the frozen material".

"6. A method for making a frozen lactic acid bacteria (LAB) culture that comprises LAB that are able to utilize sucrose, has a weight of at least 50g frozen material and a content of viable bacteria of at least 10^9 colony forming units (CFU) per g frozen material comprising following steps:

(i) adding a cryoprotective agent to viable bacteria to get at least 50g of material with a content of viable bacteria of at least 10^9 colony forming units (CFU) per g material and comprising the cryoprotective agent in an amount from 0.5% to 80% measured as w/w of the material,
(ii) freezing the material to get frozen material, and
(iii) packing the frozen material in a suitable way".

VIII. Claims 1 and 2 of auxiliary request 3 read:

"1. A frozen lactic acid bacteria (LAB) culture that comprises LAB that are able to utilize sucrose and is a

mixed mesophilic culture consisting of mesophilic bacteria having optimum growth temperatures at about 30°C, has a weight of at least 50g frozen material and a content of viable bacteria of at least 10^9 colony forming units (CFU) per g frozen material and characterized in that the frozen culture comprises from 0.5% to 80% of a cryoprotective agent measured as w/w of the frozen material,
wherein the culture is a *LD-culture* that comprises *Lactococcus lactis* subsp. *lactis, Lactococcus lactis* subsp. *cremoris, Lactococcus lactis* subsp. *lactis* biovar. *diacetylactis* and *Leuconostoc mesenteroides* subsp. *cremoris*, and
wherein the cryoprotective agent is sucrose or a mixture of sucrose and maltodextrine".

"2. A method for making a frozen lactic acid bacteria (LAB) culture that comprises LAB that are able to utilize sucrose, has a weight of at least 50g frozen material and a content of viable bacteria of at least 10^9 colony forming units (CFU) per g frozen material and wherein the culture is a *LD-culture* that comprises *Lactococcus lactis* subsp. *lactis, Lactococcus lactis* subsp. *cremoris, Lactococcus lactis* subsp. *lactis* biovar. *diacetylactis* and *Leuconostoc mesenteroides* subsp. *cremoris* comprising following steps:

(i) adding a cryoprotective agent to viable bacteria to get at least 50g of material with a content of viable bacteria of at least 10^9 colony forming units (CFU) per g material and comprising the cryoprotective agent in an amount from 0.5% to 80% measured as w/w of the material, wherein the cryoprotective agent is sucrose or a mixture of sucrose and maltodextrine,
(ii) freezing the material to get frozen material, and
(iii) packing the frozen material in a suitable way".

IX. Claim 1 of auxiliary request 7 reads:

"1. A method for making a frozen lactic acid bacteria (LAB) culture that comprises LAB that are able to utilize sucrose, has a weight of at least 50g frozen material and a content of viable bacteria of at least 10^9 colony forming units (CFU) per g frozen material and wherein the culture is a *LD-culture* that comprises *Lactococcus lactis* subsp. *lactis*, *Lactococcus lactis* subsp. *cremoris*, *Lactococcus lactis* subsp. *lactis* biovar. *diacetylactis* and *Leuconostoc mesenteroides* subsp. *cremoris* comprising following steps:

(i) adding a cryoprotective agent to viable bacteria to get at least 50g of material with a content of viable bacteria of at least 10^9 colony forming units (CFU) per g material and comprising the cryoprotective agent in an amount from 0.5% to 80% measured as w/w of the material, wherein the cryoprotective agent is sucrose or a mixture of sucrose and maltodextrine,
(ii) freezing the material in liquid nitrogen to get frozen material, and
(iii) packing the frozen material in a suitable way".

X. The following documents are referred to in this decision:

D3: US 4,262,023 (publication date: 14 April 1981);

D5: Cárcoba R. and Rodríguez A., European Food Research Technology, 2000, Vol. 211, 433-437;

D40: Milliere J.B. *et al.*, Journal of Applied Bacteriology, 1989, Vol. 67, 529-542;

D41: Bridge P.D. and Sneath P.H., Journal of General Microbiology, 1983, Vol. 129, 565-597;

D42: API 50 CHL Medium kit instructions, 1998;

D50: Experimental report of Mr Klassen, dated 9 May, 2016;

D66: Bergey's Manual of Systematic Bacteriology, 2009, Vol. 3, Vos P.D. *et al.* [Eds.], 2nd Edition, NY, Springer, 626 and 631;

D67: Advances in the Microbiology and Biochemistry of Cheese and fermented Milk, 1984, Davies F.L. and Law B.A. [Eds.], London and NY, Elsevier Applied Science Publishers, 52-55.

XI. The appellant's submissions, insofar as relevant to the present decision, may be summarised as follows:

Main request

Novelty - claim 1

According to the case law of the Boards of Appeal, in order to establish that the disclosure in a prior art document inherently discloses the claimed subject-matter, the document has to provide a clear, unambiguous and enabling disclosure that inevitably leads to the claimed properties or features (see Case Law of the Boards of Appeal of the EPO, 9th edition 2019, hereinafter "Case Law", I.C.4.4, 119).

The disclosure of document D3 did not fulfil this criterion and thus, the frozen lactic acid bacteria

(LAB) culture of claim 1 was novel. Example 4 of document D3 disclosed neither a LAB that was able to utilise sucrose, nor a concentration of viable LAB of at least 10^9 CFU/g of frozen material.

The blend of LAB strains mentioned in Example 4 of document D3 referred to the ATCC 19254 strain of "*Leuconostoc cremoris*" (*Lm. cremoris*) (currently named *Leuconostoc mesenteroides* subsp*. cremoris*) as an alternative only, and document D3 failed to indicate whether this strain used sucrose or not. The secondary evidence reported in documents D40, D41 and D50 disclosed an inconsistent metabolic activity of the ATCC 19254 strains analysed, namely on their ability to grow on lactose and sucrose. This indicated that different ATCC 19254 strains existed. Consequently, the identity between the strain disclosed in document D3 with those in documents D40, D41 and D50 was unclear, and hence its ability to use sucrose. The more so since evidence was on file showing that either 25% of the *Lm. cremoris* strains were able to use sucrose only (see document D42, Table), or none of them (see documents D66, Table 122, document D67, Table 5 on page 54). Since the available evidence was contradictory, it was not possible to arrive with certainty at any conclusion regarding the inherent sucrose utilisation ability of the ATCC 19254 strain cited in document D3.

Furthermore, document D3 in the passage starting in column 4, line 47 described in the present tense the method of making frozen LAB cultures, which meant it was prophetic. This method consisted of three scaling-up fermentations, wherein the culture volume (140 l) of the second fermentation - including the LAB contained therein - was diluted by a factor of 50 in the last

fermentation (7000 l) to obtain the viable LAB to be frozen. While the LAB concentration at the end of the second cultivation was indicated to be "*2-3x10^{9} cfu.ml^{-1}*", document D3 was silent on the LAB concentration at the end of the third fermentation. Since the LABs were diluted by a factor of 50 at the beginning of the third fermentation, it was merely speculative that their concentration at the time of harvesting was equal to that observed at the end of the second fermentation ("*2-3x10^{9} cfu.ml^{-1}*"). Accordingly, the legal standard of the Boards of Appeal, namely a disclosure with certainty up to the hilt, was not met for an implicit disclosure of a concentration in document D3 that fell within the scope of claim 1.

Moreover, a concentration falling within the scope of claim 1 had to be obtained with each of the different LAB disclosed in Example 4 of document D3. These strains were produced separately, i.e. not in a mixture, and mixed in a composition only after their freezing in form of single pellets. Since the fermentation conditions reported in columns 4 and 5 of document D3 did not relate to a specific LAB, but to LAB in general, the final concentration of each single strain obtained was a matter of speculation. This added further uncertainty to the disclosure of document D3.

Auxiliary request 3

Added subject-matter - claim 2

The method of claim 2 had *inter alia* a basis in claim 8 as originally filed in combination with the disclosure starting on page 8, line 10 to page 9, line 3, in particular on page 8, lines 24 and 25, and on page 9, lines 1 to 3 of the patent application.

Inventive step - claim 1

Document D3 represented the closest prior art. The frozen LAB culture of claim 1 differed from the culture disclosed in document D3 in that it required (i) the presence of at least one strain that was able to utilise sucrose, (ii) a minimum concentration of viable bacteria of at least 10^9 CFU/g of frozen material, and (iii) the use of either sucrose or a mixture of sucrose and maltodextrin as cryoprotectants. These distinguishing features allowed the provision of an improved frozen LAB culture. However, since there were no data on file comparing the cryoprotective effect of sucrose or a mixture of sucrose and maltodextrin as referred to in claim 1 with that of lactose mentioned in document D3, a potential improvement of the frozen LAB culture based on this difference alone could not be substantiated in the form required by the case law. Therefore, the frozen LAB culture of claim 1 was a non-obvious alternative to the frozen LAB culture disclosed in document D3.

Document D3 disclosed a prophetic example of producing frozen LAB cultures only. Starting therefrom, the skilled person had no reason or motivation to combine this teaching with that of document D5. Document D5 related to the stabilisation of a single LAB strain only, i.e. not of a mixture of several LAB species, and this strain (*Lactococcus lactis* ssp. *lactis* CECT 5180) was different from those used in document D3. Moreover, document D5 reported that the cryoprotectants tested had different effects on protecting the viability of the frozen LAB strain, including negative ones. In other words, the specific effects of cryoprotectants on a single LAB strain could not be extrapolated to the

mixed frozen LAB culture of claim 1. Thus, the skilled person would have combined the teaching of document D3 with that of document D5 only with the hindsight knowledge of the claimed method. And, even if the skilled person would have combined the teachings of these documents, (s)he would have had no reasonable expectation of success in arriving at the subject-matter of claim 1.

Auxiliary request 7

Inventive step - claim 1

In addition to the differences (i) to (iii) mentioned in the context of auxiliary request 3 above, the method of claim 1 further differed from the method disclosed in the closest prior art document D3 by using liquid nitrogen for freezing the LAB culture. Document D3 disclosed in column 5, line 47 that a "*nitrogen vapour tunnel freezer*" was used for this purpose, but it was silent on using liquid nitrogen. The Examples in the patent in suit showed that the frozen LAB culture obtained by the claimed method had a good viability. However, in line with the case law, in the absence of comparative data, a particular effect (improved viability) could not be ascribed to this additional distinguishing feature. Therefore, the objective technical problem was the provision of an alternative method for making a frozen LAB culture to that disclosed in document D3. Since none of the other prior art documents cited under inventive step pointed to the use of liquid nitrogen for freezing, the claimed method was a non-obvious alternative to the method disclosed in document D3.

XII. The appellant requested that the decision under appeal be set aside and that the patent be maintained upon the basis of the main request, or alternatively upon the basis of either auxiliary request 3 or 7.

Reasons for the Decision

Main request

Claim construction - claim 1

1. Claim 1 is directed to a frozen lactic acid bacteria (LAB) culture which is characterised by the following features:

(i) "*comprises LAB that are able to utilize sucrose*";
(ii) is a mixture of mesophilic LAB, i.e. it contains at least two LAB, having an optimum growth temperature at about 30°C;
(iii) has a weight of at least 50g of frozen material;
(iv) has a content of viable bacteria of at least 10^9 colony forming units (CFU) per gram of frozen material; and
(v) comprises from 0.5% to 80% of a cryoprotective agent measured as w/w of the frozen material.

2. The term "*mesophilic*" in claim 1 means that the LAB are characterised by an optimum growth temperature of about 30°C. Due to the use of the comprising language, claim 1 encompasses a frozen LAB culture that contains a mixture of mesophilic lactic acid bacteria, wherein not all strains are necessarily able to use sucrose as a growth substrate. In other words, all LAB strains in the frozen mixture have to be mesophilic, and at least one of them must have the ability to use sucrose. Moreover, the culture may contain further components.

This construction of the claim has not been contested in the appeal proceedings.

Novelty - claim 1

3. The appellant contested that Example 4 of document D3 disclosed a mixture of LAB species containing at least one LAB that was able to use sucrose, and a frozen LAB culture with a content of viable bacteria of at least 10^9 CFU/g frozen material.

4. Document D3 discloses frozen concentrated cheese starter compositions and a method for their production (see column 2, lines 43 to 60; column 4, line 45 to column 6, line 16). Example 4 of this document discloses a blend for the manufacture of Gouda and Edam cheese types containing "*Streptococcus lactis*" ("*ML8*" strain), "*Streptococcus cremoris*" ("*AM1*" strain) and "*Leuconostoc cremoris*" ("*LnC*" strain). These LAB have been renamed since to *Lactococcus lactis* ssp. *lactis* (*L. lactis*), *Lactococcus lactis* ssp. *cremoris* (*L. cremoris*), and *Leuconostoc mesenteroides* subsp. *cremoris* (*Lm. cremoris*), respectively. If a buttery flavour is desired, a "*Streptococcus diacetilactis*" strain is added to the blend (see column 4, lines 19 to 35), a strain renamed to *Lactococcus lactis* subsp. *lactis* biovar. *diacetylactis* (*L. diacetylactis*).

4.1 Example 4 of document D3 further mentions that the "*ATCC 19254*" strain of *Lm. cremoris* can be used as an alternative to the LnC strain (see footnote of the Table in column 4, line 29). The document is however silent on a potential ability of the ATCC 19254 strain to use sucrose.

4.2 It is uncontested that Example 4 of document D3 neither explicitly discloses a LAB strain that is able to use sucrose as a substrate, nor that the blend of LAB strains has a concentration of viable bacteria of at least 10^9 CFU/g frozen material. This concentration is also not explicitly disclosed in column 4, line 45 to column 6, line 16 of document D3 which describes a method for producing the frozen LAB culture.

5. According to the established case law, a prior art document anticipates the novelty of claimed subject-matter if the latter is directly and unambiguously derivable from that document, including any features implicit to a person skilled in the art. A disclosure can only be considered "implicit" if it is immediately apparent to the skilled person that nothing other than the alleged implicit feature forms part of the subject-matter disclosed (see Case Law, I.C.4.3, 116).

6. Thus, the first issue to be assessed is whether document D3 implicitly discloses a LAB strain that can utilise sucrose. An implicit property of a disclosed product, here the specific *Lm. cremoris* strain ATCC 19254, can be established by evidence in other documents.

7. Document D40 mentions that a bacterium designated as "*DSM 20346, type strain coded as NCDO 543*" is "*able to weakly ferment saccharose*" (see page 539, right-hand column, third paragraph; saccharose is the synonym of sucrose, comment added by the board). Table 1 on page 533 of this document further indicates that, for the serial No. "*55*", the *Lm. cremoris* strain with the DSM number *"20346*" is identical to the strain designated as ATCC 19254 or as NCDO 543.

8. This finding is corroborated by document D41, which discloses in Table 1 on page 570 that the *Lm. cremoris* strain "*PB164*" is the strain designated as ATCC 19254 or as NCDO 543. This strain belongs to a group of *Leuconostoc* bacteria designated as "*Phenon 19*" of which 100% of the members can utilise sucrose (see Table 1 on page 570, first paragraph, Table 3 on page 582 of document D41). Document D50 likewise reports that the ATCC 19254 strain uses sucrose (see page 5, point 3.2).

9. Whilst the appellant did not contest that documents D40, D41 and D50 disclose a *Lm. cremoris* strain designated as ATCC 19254 which uses sucrose, it contested that the ATCC 19254 strain disclosed in all of these documents and in document D3 is the same (identical) strain. An indication for this was the reported failure of the ATCC 19254 strain in document D40 to metabolise lactose, whilst documents D41 and D50 reported that this strain grows on lactose. In other words, the reported metabolic property of the "same" strain on using lactose in these three documents is inconsistent, thereby raising doubts that the disclosed strain is the identical strain. Since a direct and unambiguous disclosure required certainty, and the identity of the ATCC 19254 strain in document D3 was unclear, the appellant argued that this document did not disclose a strain with the required property. The more so, since evidence was on file showing that either only 25% of the *Lm. cremoris* strains are able to use sucrose (see document D42, Table), or none of them (see document D66, Table 122, document D67, Table 5 on page 54).

9.1 The board is not convinced by this argument. Claim 1 only requires that at least one LAB in the culture can use "*sucrose*", there is no requirement for the presence

of a strain that is able to use lactose. Documents D40, D41 and D50 consistently demonstrate that a LAB strain designated as ATCC 19254, i.e. the strain disclosed in Example 4 of document D3, is able to use sucrose. Since all strains with this ATCC number are reported to metabolise sucrose, i.e. the property required by claim 1, the question is irrelevant whether the ATCC 19254 strain is a single *Lm. cremoris* strain or a mixture of several sub-strains with different properties as regards their ability to use lactose. In other words, the identity of the strain disclosed in documents D3, D40, D41 and D50 is irrelevant, since documents D40, D41 and D50 demonstrate that **all** ATCC 19254 strains have the metabolic property defined in claim 1. Likewise, the fact that documents D42, D66 and D67 state that most of the *Lm. cremoris* strains are unable to grow on sucrose is irrelevant, because none of these documents reports on the particular properties of the *Lm. cremoris* ATCC 19254 strain.

9.2 In this context, the appellant referred to the legal standard established by the Boards of Appeal for assessing novelty, and submitted that the assessment cannot rely on probability or possibility, since certainty was required, i.e. a fact has to be proven with certainty or, as sometimes expressed, "up to the hilt". The board agrees with the appellant that, for the purpose of assessing novelty, a stricter standard than the so-called "balance of probabilities" has to be applied. However, the standard to be applied is not absolute certainty, or "beyond any (possible) doubt", but "beyond any reasonable doubt" (see Case Law, I.C. 3.5.2, 110). Based on the evidence on file (see above), the board is convinced beyond any reasonable doubt that the *Lm. cremoris* ATCC 19254 strain cited in document D3 is able to utilise sucrose.

10. The second issue to be assessed is whether document D3 implicitly discloses a frozen LAB culture with a content of viable cells of at least 10^9 CFU/g frozen material.

11. The appellant submitted that the description of the process for the production of a frozen LAB culture in column 4, line 45 to column 6, line 16 of document D3 is prophetic, since it is written in the present tense. Thus, document D3 provided no evidence that, by carrying out said process, a frozen LAB culture with the concentration of claim 1 was obtained. Furthermore, claim 1 of document D3 required that each lactic acid bacterium was separately cultivated and frozen before being mixed in a starter composition. However, the fermentation conditions disclosed in column 4, line 45 to column 6, line 16 of document D3 were described in general terms only, i.e. not specific for the different LAB species disclosed in Example 4. Thus, it was doubtful whether the cell concentrations disclosed in these passages could be obtained for each of the individual lactic acid bacterium cited in Example 4.

11.1 The board is not convinced by these arguments. Firstly, the use of the present tense in the passage of document D3 indicated above provides no indication whether or not the process is prophetic. In the board's view, it suffices that the conditions disclosed in document D3 for cultivating LAB are the ones known in the art and commonly used by the skilled person for growing LAB strains.

11.2 Secondly, "*Streptococcus cremoris* or *Streptococcus lactis*", i.e. two of the strains referred to and used in the blend of Example 4, are mentioned in column 4,

lines 66 to 67 of document D3, as examples of bacteria "*suitable*" for the process. Instructions for cultivating LAB other than the ones disclosed in the passage starting in column 4, line 45 are not mentioned in document D3. The LAB strains in Example 4 all belong to a so-called "LD-culture". These strains are commonly cultivated under the same conditions, since their growth requirements are the same (see e.g. patent in suit, paragraph [0072]). Thus, the skilled person would have taken the instructions and conditions disclosed in column 4 of document D3 at face value for cultivating all the LAB strains referred to in Example 4.

12. In a further line of argument, the appellant submitted that the up-scaling from the second fermentation to the third fermentation results in a dilution of the "inoculum" or starter LAB concentration by a factor of 50. In these circumstances, the concentration of "*2-3x10^{9} cfu.ml^{-1}*" (see column 5, line 8) would be below the required concentration of at least 10^{9} CFU/g in claim 1. Moreover, there was no guarantee that the concentration of cells at the end of the third fermentation would be near or equal to the concentration used at the start of the fermentation.

12.1 As set out above, there is no evidence on file, nor any indication available to the board, showing that the fermentation conditions described in column 4, line 45 *et seq.* in document D3 are not suitable for cultivating the individual LAB strains cited in Example 4. In the absence of any doubts, the board is convinced that each of these strains reaches the concentrations disclosed in document D3 after the first and second fermentation ("*5 to 10x10^{8}*" CFU/ml, i.e. 5 x 10^{8} to 1 x 10^{9} CFU/ml, see column 5, line 4; and "*2-3x10^{9} cfu.ml^{-1}*", see column 5, line 8).

12.2 In the third fermentation, the LAB are fermented in "*the pasteurised medium*" for another "*6 to 10 hours*" by "*avoiding onset of culture senescence*". In other words, the bacteria are grown to their maximum density before reaching the plateau/stationary phase. In the board's view, the indication of the term "*the pasteurised medium*" in line 9 of column 5, refers back to the skim milk-based medium indicated in column 4, lines 47 to 65, which mentions the pasteurisation of the medium. Therefore, the first and the third fermentation are carried out in a reconstituted skim milk-based medium, i.e. under identical conditions (see column 4, lines 48 and 49; and column 5, line 1). In view thereof, the board has no doubts that the disclosure in column 5, lines 4 to 27 necessarily implies that, at the end of the third fermentation, each LAB strain reaches at least the same cell concentration as that disclosed for the first fermentation, i.e. 5×10^8 CFU/ml to 1×10^9 CFU/ml (see above). It is irrelevant that, due to the dilution of the inoculum, the cell concentration is lower at the start of the fermentation, because the cell number necessarily increases again during the fermentation until the end of the exponential growth phase.

12.3 Document D3 further discloses that, at the end of the third fermentation, the cells are harvested and concentrated in a ratio of "*40:1 to 60:1*" (see column 5, lines 28 to 36), i.e. by a factor of 40 to 60. If the minimum cell concentration indicated above (5×10^8 CFU/ml) is multiplied by the factor of at least 40, these passages in document D3 necessarily disclose that the viable cell concentration is at least 2×10^{10} CFU/ml. This concentration is comparable to that of the LD-cultures described in the patent in suit

(see, e.g. paragraph [0057]), and falls within the scope of claim 1. Thus, the board is convinced, beyond any reasonable doubts, that document D3 implicitly discloses a frozen LAB culture with a content of viable cells of at least 10^9 CFU/g frozen material.

13. Since document D3 discloses all the features of claim 1, the main request lacks novelty (Article 54 EPC).

Auxiliary request 3

14. Claims 1 and 2 of auxiliary request 3 differ from the respective claims 1 and 6 of the main request in that the features "*wherein the culture is a LD-culture that comprises Lactococcus lactis* subsp*. lactis, Lactococcus lactis* subsp*. cremoris, Lactococcus lactis* subsp*. lactis* biovar*. diacetylactis* and *Leuconostoc mesenteroides* subsp*. cremoris*", and "*wherein the cryoprotective agent is sucrose or a mixture of sucrose and maltodextrine*" have been added.

Added subject-matter - claim 2

15. The opposition division held in the decision under appeal that claim 2 of auxiliary request 3 comprised added subject-matter. The opposition division was of the view that the term "LD" in a LD-culture was derived from the presence in the culture of "***L****euconostoc and L.* ***d****iacetylactis (=Lactococcus lactis* subsp*. lactis* biovar*. diacetylactis), but that other bacteria may be present*". Therefore, the absence of the limitation "*mixed mesophilic culture*" in claim 2 was considered to allow the presence of non-mesophilic bacteria in the culture too. Since no basis was found in the patent application for a claim to a method of freezing a LD-

culture which was not a mixed mesophilic culture, claim 2 contravened Article 123(2) EPC (see page 15, second paragraph of the decision under appeal).

16. As a basis for claim 2, the appellant referred to, *inter alia*, claim 8 as originally filed in combination with the disclosure on page 9, lines 1 to 3.

17. Claim 8 as originally filed reads:

"*8. A method for making a frozen lactic acid bacteria (LAB) culture that comprises LAB that are that are (sic) able to utilize sucrose, has a weight of at least 50 g frozen material and a content of viable bacteria of at least 10^9 colony forming units (CFU) per g frozen material comprising following steps:*

(iv) adding a cryoprotective agent to viable bacteria to get at least 50 g of material with a content of viable bacteria of at least 10^9 colony forming units (CFU) per g material and comprising the cryoprotective agent in an amount from 0.5% to 80% measured as w/w of the material,
(v) freezing the material to get frozen material, and
(vi) packing the frozen material in a suitable way."

18. Claim 8 as originally filed neither defines the culture as "*mixed*", nor as "*mesophilic*". The LAB culture prepared by the method of claim 8 solely requires the presence of LAB that are able to utilise sucrose. The presence of further LAB is not excluded from such a culture due to the use of the comprising language, i.e the culture may include non-mesophilic bacteria.

19. Page 8, line 24 of the patent application mentions that a L-culture and a LD-culture are examples of mesophilic cultures, and page 9, lines 1 to 3 further states:

"*A LD-culture comprises Lactococcus lactis* subsp. *lactis, Lactococcus lactis* subsp. *cremoris, Lactococcus lactis* subsp. *lactis* biovar. *diacetylactis and Leuconostoc mesenteroides* subsp. *cremoris*."

20. This passage on page 9, lines 1 to 3 of the patent application is identical to the feature introduced into claim 2 of auxiliary request 3. It discloses a LD-culture that is solely characterised by the presence of at least the four cited LAB strains. If, due to the comprising language and in the absence of any limitation, the LD-culture of claim 2 is considered to comprise further LAB strains, including LAB strains that are not mesophilic, the same interpretation must be applied to the disclosure on page 9, lines 1 to 3 of the patent application. Therefore, the disclosure on page 9, lines 1 to 3 and claim 8 of the patent application provide a basis for the subject-matter of claim 2 of auxiliary request 3.

21. Consequently, the board cannot agree with the opposition division's finding that the patent application provides no basis for the method of claim 2. Auxiliary request 3 complies with Article 123(2) EPC.

Inventive step - claim 1

Closest prior art and objective technical problem

22. It is uncontested that document D3 represents the closest prior art for the subject-matter of claim 1.

23. The appellant submitted that the claimed frozen LAB culture differed from that disclosed in the closest prior art document D3 in the following features:
(i) at least one LAB was able to use sucrose,
(ii) in a minimum concentration of viable bacteria of 10^9 CFU/g frozen material, and
(iii) in the use of sucrose or a mixture of sucrose and maltodextrin as cryoprotectants.

24. However, as set out above under novelty, document D3 discloses features (i) and (ii) of the claimed frozen LAB culture. Accordingly, the sole distinguishing feature is the type of cryoprotective agent used, namely sucrose or a mixture of sucrose and maltodextrin instead of lactose, i.e. feature (iii) above (see column 5, lines 40 to 42 in conjunction with claim 1 of document D3).

25. It is uncontested that, due to the absence of any comparative data (see Case Law, I.D.10.9, 271), no advantageous technical effects can be ascribed to this difference.

26. Thus, the board shares the opposition division's view in the decision under appeal that, starting from the closest prior art, the objective technical problem is the provision of a frozen LAB culture with an alternative cryoprotective agent.

27. The claimed frozen LAB culture with sucrose or a mixture of sucrose and maltodextrin as cryoprotective agents solves this problem as shown by the data disclosed in Example 1 in combination with Figures 1 to 4 of the patent.

Obviousness

28. It remains to be assessed whether or not the skilled person, starting from the frozen LAB culture disclosed in the closest prior art document D3 and faced with the technical problem identified above, would have arrived at the claimed frozen LAB culture in an obvious manner.

29. The teaching of document D3 as regards the use of cryoprotectants is exemplified by using lactose and glycerophosphate, alone or in combination (see column 5, lines 40 to 42 in conjunction with claim 1). Document D3 does not hint at other specific cryoprotectants, in particular not sucrose, and thus, the claimed frozen LAB culture cannot be considered obvious based on the teaching of document D3 alone. Nevertheless, claim 1 of this document refers to a "*multiple-strain cheese starter bacteria composition*" containing "*at least one cryoprotective agent*", i.e. at such agents in general. Although the case law defines the skilled person as having a conservative attitude (see Case Law, I.D.8.1.3, 205), it is established that furthering the existing state of the art belongs to the normal tasks of the skilled person, and that the use of known alternatives does not go beyond what may be normally expected from an average skilled person (see Case Law, I.D.9.6, 247, and I.D.9.11, 254, and e.g., T 688/14 of 24 July 2019, point 25.1 of the Reasons).

30. Therefore, starting from document D3, the skilled person was motivated to turn her/his attention to other documents of the prior art disclosing alternative cryoprotectants suitable for stabilising frozen LAB cultures.

31. Document D5, for example, assesses the influence of various cryoprotectants on the viability of a frozen *L. lactis* ssp. *lactis* strain (see abstract), i.e. one of the four LAB species referred to in claim 1. Sucrose and lactose as cryoprotectants are *inter alia* mentioned (see page 434, left-hand column, fifth paragraph).

31.1 Document D5 reports that "[O]*n the whole, our results indicate than the lower the temperature of freezing (-80°C), the higher the survival rate and acidifying activity in milk obtained, whatever the storage time. In addition, whatever the freezing temperature, sugars (lactose, sucrose and trehalose), glutamic acid and gelatin provided the best results (67-74% survival at -80°C and 65-73% at —20°C for 120 days of frozen storage). Surprisingly, meat extract that stood out as one of the most advisable cryoprotectants for freezing cells at —80°C (74% survival for 120 days), was very harmful for freezing at —20°C (with the longest storage time (120 days) as an exception)*" (see page 434, right-hand column, fifth paragraph, emphasis added).

31.2 Thus, document D5 recommends the use of, *inter alia*, sucrose or lactose as cryoprotectants to maintain the viability of a frozen strain of *L. lactis* ssp. *lactis*.

32. The appellant submitted that document D5 taught the use of, *inter alia*, sucrose for a single, specific LAB strain only. Furthermore, it was evident from the reported failure of meat extract to protect the frozen LAB strain at a temperature of -20°C (see above) that cryoprotectants acted differently on different bacterial species, i.e. their protective effect could not be extrapolated to the mixed LAB culture referred to in claim 1. Accordingly, the teaching of documents D3 and D5 would have been combined with hindsight of

the claimed culture only. And, even if they were combined, the skilled person had no expectation of success in arriving at the claimed frozen LAB culture.

32.1 The board is not convinced by these arguments. Firstly, the teaching of document D5, as regards the use of sugars, in particular, sucrose for cryoprotection, is not limited to *L. lactis* ssp. *lactis* only. This is derivable from the disclosure in document D5 that the cryoprotectants used in the study, including sucrose, have been selected because they were "*previously described as cryoprotectants in LAB*", i.e. in lactic acid bacteria in general (see page 434, left-hand column 1, fifth paragraph; emphasis added). Furthermore, the document states that "*... in accordance with results previously cited in the literature [16, 17], both the good cell viability and acidifying activity observed when freeze-dried and stored frozen cultures were supplemented with sugars is worth noting*". Moreover, trehalose and sucrose are selected because their role "*in protecting cell membranes from fusion and breakage [21], and stabilising proteins from denaturation [22] during freeze and freeze-drying, has already been studied*" (see page 436, left-hand column, first full paragraph).

32.2 Secondly, as shown above, sucrose is mentioned in document D5 - together with lactose (the cryoprotective agent exemplified in document D3) and trehalose - as one of three cryoprotective sugars that "*provided the best results*". Thus, no inventive skill would have been required from a skilled person to select sucrose as an alternative cryoprotectant to lactose in a frozen LAB culture. In the board's view, this conclusion is not altered by the reported failure of meat extract at

certain storage temperatures (see above), since this cryoprotective agent is of a nature completely different from that of the lactose used in document D3. Moreover, document D3 neither refers to meat extract nor provides hints for its use.

32.3 In conclusion, the board is convinced that the teaching of document D5 is not limited to the cryopreservation of a single, specific LAB, but can be extrapolated to other LAB cultures, including mixed ones. Accordingly, the combination of the teachings of documents D3 with D5 concerning the use of sucrose as an alternative for lactose neither requires hindsight knowledge of the claimed frozen LAB culture, nor are technical problems described in document D5 indicating that sucrose is not a suitable cryoprotectant for frozen LAB cultures, rather on the contrary. The skilled person has therefore a clear expectation of success in using sucrose as an alternative to lactose.

33. Consequently, the skilled person would have combined the teachings of documents D3 and D5, and would have arrived at the claimed frozen LAB culture in an obvious manner. Auxiliary request 3 lacks an inventive step (Article 56 EPC).

Auxiliary request 7

34. The method for making a frozen LAB culture of claim 1 in auxiliary request 7 differs from the corresponding method of claim 2 in auxiliary request 3, in that the feature "*in liquid nitrogen*" has been added in step (ii) of claim 1 to further specify the way of freezing the LAB culture.

Article 56 EPC - claim 1

Closest prior art and objective technical problem

35. Document D3 remains the closest prior art for the method of claim 1, since it discloses a method for making a frozen LAB culture (see above, hereinafter "closest prior art method").

36. The method of claim 1 differs from the closest prior art method in the following two respects: (i) the use of sucrose or a mixture of sucrose and maltodextrin instead of lactose as a cryoprotectant (see above), and (ii) the use of liquid nitrogen instead of "*nitrogen vapour*" for freezing the LAB culture (see column 5, line 47 of document D3).

37. No comparative data is available demonstrating an advantageous or surprising technical effect for these two distinguishing features.

38. Therefore, starting from the closest prior art, the objective technical problem to be solved is the provision of an alternative method for the preparation of a frozen LAB culture.

39. The claimed method for making a frozen LAB culture solves this problem as shown by the data disclosed in the patent.

Obviousness

40. The question to be assessed is thus whether or not the skilled person, starting from the closest prior art method and faced with the technical problem defined

above, would have arrived at the claimed method in an obvious manner.

41. As set out above, the use of known alternatives, like the use of sucrose instead of lactose as a cryoprotectant, does not go beyond what may be normally expected from an average skilled person. Likewise, the use of liquid nitrogen to quickly freeze bacteria for storage purposes belongs, since many decades, to the common general knowledge of the skilled person. This use is considered to be so fundamentally established in the art that there is no need for providing documentary proof (see Case Law, I.C.2.8.5, 79). Consequently, also the use of liquid nitrogen instead of the nitrogen vapour disclosed in document D3 is a known alternative for freezing LAB strains.

42. Therefore, the claimed method is considered obvious for the skilled person using common general knowledge and when combining the teachings of documents D3 and D5. Thus, auxiliary request 7 lacks an inventive step (Article 56 EPC).

Order

For these reasons it is decided that:

The appeal is dismissed.

The Registrar: The Chairman:

L. Malécot-Grob P. Julià

Decision electronically authenticated