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**Datasheet for the decision
of 23 February 2018**

Case Number: T 1224/12 - 3.3.08

Application Number: 05010216.9

Publication Number: 1724340

IPC: C12N15/01, C12R1/01

Language of the proceedings: EN

Title of invention:

Antibiotic-sensitive lactic acid bacteria strains

Patent Proprietor:

Chr. Hansen A/S

Opponent:

DuPont Nutrition Biosciences ApS

Headword:

Tetracycline sensitive Bifidobacterium/CHR HANSEN

Relevant legal provisions:

EPC Art. 54, 56, 83, 100(a), 100(b), 114(2)
RPBA Art. 12(4)

Keyword:

Admission of new evidence into the appeal (no)
Granted claims - sufficiency of disclosure (yes)
Granted claims - novelty (yes)
Granted claims - inventive step (yes)
Appeal dismissed (yes)

Decisions cited:

T 0019/90, T 0177/01, T 0799/02, T 1277/08, T 2461/11,
T 0646/13, T 0647/15

Catchword:



Beschwerdekammern

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Case Number: T 1224/12 - 3.3.08

D E C I S I O N
of Technical Board of Appeal 3.3.08
of 23 February 2018

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Decision under appeal: **Decision of the Opposition Division of the
European Patent Office posted on 29 March 2012
rejecting the opposition filed against European
patent No. 1724340 pursuant to Article 101(2)
EPC.**

Composition of the Board:

Chairman B. Stolz
Members: P. Julià
D. Rogers

Summary of Facts and Submissions

- I. European patent No. 1 724 340 was opposed on the grounds of Articles 100(a) and (b) EPC. An opposition division of the European Patent Office decided that none of the grounds of opposition prejudiced the maintenance of the patent and, accordingly, rejected the opposition.
- II. Independent claims 1, 3 and 6 of the patent as granted read as follows:
- "1. A method of isolating a strain of *Bifidobacterium* sp. containing a mutated *tetW* on its chromosome, said mutation renders the strain sensitive to tetracyclines and said strain is isolated from a tetracycline-resistant bacterial progenitor strain wherein the antibiotic resistant phenotype is caused by the expression of *tetW* stably integrated in its chromosome, said method comprising subjecting the cells to a chemical mutagen and a physical mutagen, and wherein the method comprises the steps of:
- 1) culture the progenitor cells to obtain a culture of exponential growing cells,
 - 2) transfer an aliquot of the cells to fresh medium containing ethidium bromide (EtBr),
 - 3) transfer the culture to one or more containers to form a 0.5 - 10 mm thick layer of culture;
 - 4) subject the culture to a UV treatment,
 - 5) culture the mutated cells to obtain a culture of exponential growing cells,
 - 6) transfer an aliquot of bacteria to one or more petridishes containing a suitable agar growth medium, the aliquot of bacteria are selected to give single colonies,

7) identify those colonies that have acquired antibiotic sensitivity by replica plating to petridishes with and without tetracycline antibiotic, and

8) isolate, expand and keep those tetracycline antibiotic sensitive colonies identified as a new tetracycline antibiotic sensitive strain; and

wherein the Minimum inhibitive Concentration (MIC) of tetracycline of the progenitor strain is at least 10 microgram tetracycline/ml and the MIC of tetracycline of the antibiotic sensitive strain is 1.5 µg tetracycline/ml or less.

3. A *Bifidobacterium* strain which is sensitive to tetracyclines due to an inactivating mutation in *tetW* located on the chromosome, wherein the *tetW* gene comprises at least one sequence selected from the group of SEQ ID 3 [GGA TAC TGA ACC] and SEQ ID NO: 6 [GTG GTT TAG TCT]; and wherein the Minimum inhibitive Concentration (MIC) of tetracycline of the sensitive strain is 1.5 µg tetracycline/ml or less.

6. A *Bifidobacterium animalis* subspecies *lactis* strain, which is sensitive to tetracycline due to an inactivating mutation in *tetW* located on the chromosome, and wherein the Minimum inhibitive Concentration (MIC) of tetracycline of the sensitive strain is 1.5 µg tetracycline/ml or less."

Claims 2, 4-5 and 7 are directed to preferred embodiments of claims 1, 3 and 6, respectively. Claim 8 is directed to the use of a *Bifidobacterium* strain of any of claims 3 to 7 for the preparation of an ingestible material or a bacterial culture. Claim 9 is directed to the use of the products of claim 8.

- III. An appeal was lodged by the opponent (appellant). With the statement of grounds of appeal, the appellant filed new evidence and maintained the objections raised under Articles 100(a) and (b) EPC in opposition proceedings.
- IV. The patent proprietor (respondent) filed auxiliary requests I to VII and submitted new evidence, requesting to admit this evidence into the proceedings, if appellant's new evidence was admitted.
- V. The appellant replied to respondent's submissions and the respondent filed new submissions in reply. As an auxiliary measure, both parties requested oral proceedings.
- VI. The parties were summoned to oral proceedings. In a communication pursuant to Article 15(1) of the Rules of procedure of the Boards of Appeal (RPBA), the parties were informed of the board's provisional, non-binding opinion on substantive matters of the case. In particular, the board was not minded to admit the new evidence and was also of the provisional opinion that all claims were open to appeal on the grounds of Article 100(b) EPC and that the grounds based on Articles 100(a) and (b) EPC were not convincing. The board was thus minded to dismiss the appeal.
- VII. Both parties replied and announced their intention to attend the oral proceedings. The appellant filed submissions concerning Article 56 EPC. The respondent did not submit any substantive arguments on this issue.
- VIII. Oral proceedings took place on 23 February 2018 in the presence of both parties.

IX. The following documents are cited in this decision:

- (1) : C. Moubareck *et al.*, J. Antimicrobial Chemotherapy, January 2005, Vol. 55, pages 38 to 44;
- (2) : S. Delgado *et al.*, Current Microbiology, April 2005, Vol. 50, pages 202 to 207;
- (3) : K.P. Scott *et al.*, Antimicrobial Agents and Chemotherapy, March 2000, Vol. 44, No. 3, pages 775 to 777;
- (7) : J.S. Zhou *et al.*, Int. J. Food Microbiology, February 2005, Vol. 98, pages 211 to 217;
- (8) : K.S. Lim *et al.*, J. Dairy Science, 1993, Vol. 76, No. 8, pages 2168 to 2174;
- (11): M. Danielsen and A. Wind, Int. J. Food Microbiology, 2003, Vol. 82, pages 1 to 11;
- (19): A.K.S. Martins *et al.*, Arch. Microbiol., 2010, Vol. 192, pages 995 to 1003;
- (24): "Summary of attempts to inactivate the tet(W) gene in *Bifidobacterium animalis* subsp *lactis* BB-12", signed by P. Strøman, on 22 December 2011;
- (28): Declaration of E. Johansen (Chr. Hansen A/S) dated 22 December 2011.

X. The submissions made by the appellant, insofar as relevant to the present decision, may be summarised as follows:

Article 100(b) EPC - Article 83 EPC

Insufficiency of disclosure had been substantiated for at least one claim. Thus, all claims could validly be the subject of the opposition and appeal proceedings.

Claim 1 required the measurement of the Minimum inhibitive Concentration (MIC) of tetracycline of the progenitor and of the antibiotic sensitive strain. Nevertheless, claim 1 contained no indication of the test used for measuring this parameter. According to the patent application, tetracycline sensitivity was determined by the Etest method described in document (11). In this document, reference was made only to the manufacturer's instructions. However, instructions contemporaneous with the patent application were not available. Document (11) stated that no standards existed for testing *lactobacilli* and described specific conditions for MIC testing. A skilled person was thus confronted with the dilemma to use either the manufacturer's instructions or the conditions described in document (11), even though they were for *Lactobacilli*, not for *Bifidobacterium*. Moreover, since no test was defined in claim 1, the skilled person could never be aware of whether he/she was working within the scope of the claim. Thus, in line with the case law on insufficiency arising from an ambiguous feature, claim 1 contravened Article 83 EPC.

Document (24) showed that, for a method using a chemical (EtBr) and a physical (UV) mutagen, a screening of 1000 colonies was standard. Whilst 1000 colonies were screened for one (CHCC7158) strain used in Example 2 of the patent application, it was necessary to screen 4000 colonies for the other

(CHCC5445) strain exemplified, showing thereby that an undue amount of work was required to obtain a strain with the desired properties. Moreover, document (24) showed that the method of claim 1 was not reproducible because, after screening 1000 colonies of cells treated with EtBr/UV, none of the isolated strains had the desired properties.

Article 100(a) EPC - Article 54 EPC

The feature related to the MIC could not be taken into account for the assessment of novelty because a skilled person was unable to determine it. Therefore, any known tetracycline sensitive strains of *Bifidobacterium* sp., such as those described in documents (7) and (8), anticipated the claimed strains.

Article 100(a) EPC - Article 56 EPC

Claim 1

The closest state of the art document (1) referred to the benefits of probiotic organisms, such as *Bifidobacterium*, and the negative consequences of probiotics with resistance traits. This document described the presence of acquired resistance to tetracycline in 14% of the tested *Bifidobacterium* strains and the functional relationship between a chromosomal *tetW* gene and tetracycline resistance. In view of the importance of probiotics, there was a motivation for the skilled person to identify *Bifidobacterium* strains with probiotic properties within the large pool of tetracycline resistant strains and revert them to tetracycline sensitive strains while retaining the advantageous probiotic properties. The objective technical problem was thus the provision of a method for producing further tetracycline sensitive

strains of *Bifidobacterium* sp. The method of claim 1, a combination of chemical and physical mutagenesis, was known in the art, conventional routine practice, and obvious. The large number of *Bifidobacterium* strains with a MIC of tetracycline of 1.5 µg/ml or less shown in, *inter alia*, documents (1) and (8), proved that a reasonable expectation of success was given. Although no reversion to tetracycline sensitive strains of *Bifidobacterium* was identified in document (24), not even when using chemical and physical mutagenesis, the number of colonies screened (1000) was lower than the 4000 colonies screened for one of the strains exemplified in the patent. Sensitive strains could well have been identified by screening more colonies. No advantages were provided by the method of claim 1. It was a mere alternative to other conventional, routine methods.

Claim 3

The closest state of the art document (1) identified probiotic strains of *Bifidobacterium* with no antibiotic resistance and reported the presence of a chromosomal *tetW* gene in tetracycline resistant strains of *Bifidobacterium*. Table 2 of document (1) showed *B. longum*, *B. bifidum* and *B. pseudocatenulatum* with a MIC of tetracycline of 1.5 µg/ml or less. The objective technical problem was the provision of further strains of *Bifidobacterium* with a MIC of tetracycline of 1.5 µg/ml or less. This problem was not solved over the whole scope of claim 3. The patent showed that the opal stop codon (SEQ ID NO: 3) and the amber (SEQ ID NO: 6) mutations in the chromosomal *tetW* gene of two specific strains of *Bifidobacterium* resulted in tetracycline sensitive strains with a MIC as defined in claim 3. However, claim 3 was directed to *Bifidobacterium* in

general and was not limited to the specific strains exemplified: *B. animalis* subspecies *lactis* BB-12[®] (CHCC5445) and HN019/DR10[™] (CHCC7158). There was no evidence on file that the two specific mutations defined in claim 3 resulted in a MIC of tetracycline of 1.5 µg/ml or less in strains of *Bifidobacterium* other than those exemplified.

Document (1) referred to the benefits of probiotic organisms and the negative consequences of probiotics with resistance traits. Since these traits were present in a large (14%) proportion of tested bifidobacterial strains, a skilled person would not have discarded this large group. He/she would have been motivated to look for strains with probiotic properties within this group, checked their evolution and identified those reverting to tetracycline sensitive while retaining their advantageous properties. Tetracycline resistance was identified as arising from a chromosomal *tetW* gene which, as shown in document (3), was widespread among *Bifidobacterium*. Table 2 of document (1) showed that a large number of tetracycline sensitive strains of *Bifidobacterium* had a MIC of tetracycline of 1.5 µg/ml or less which, according to the respondent, was directly linked to - and indicated the presence of - an inactivating mutation in the chromosomal *tetW* gene. In view of this large number of strains, it was inevitable or at least highly probable that at least one of the inactivating mutations defined in claim 3, if not both, were present in some of these strains. Thus, a skilled person had a reasonable expectation of isolating a *Bifidobacterium* with the features defined in claim 3.

According to the patent, both the opal and the amber mutation resulted in tetracycline sensitive strains of *Bifidobacterium* with a MIC of tetracycline of 1.5 µg/ml

or less, but no further advantage was associated with these mutations. They represented thus an arbitrary selection from among all possible inactivating mutations in the chromosomal *tetW* gene that resulted in tetracycline sensitive strains with a low MIC of tetracycline.

The strain of *Bifidobacterium* referred to in post-published document (19) was not clearly identified, not even in association with document (28); this strain did not have any effect/advantages other than these of the known probiotic strains of *Bifidobacterium*.

Claim 6

Table 2 of the closest state of the art document (1) showed *B. bifidum* strains with a MIC of tetracycline of 1 to 64 µg/ml. Thus, at least one of these strains, if not more, had a MIC as defined in claim 6. As stated in paragraph [0038] of the patent, *Bifidobacterium* BB-12[®] was first described as a *B. bifidum* and subsequently as a *B. animalis*. The skilled person considered *B. bifidum* and *B. animalis* to be highly related and teachings/disclosures on the former to be directly applicable to the latter. The objective technical problem was the provision of tetracycline sensitive strains of *B. animalis* subspecies *lactis* with a MIC of tetracycline of 1.5 µg/ml or less.

As stated for claim 3, document (1) itself provided the skilled person with a motivation to solve this problem, made him/her aware of the widespread presence of the chromosomal *tetW* gene and of the relevance of inactivating mutations in this gene. In view of the close relationship between *B. bifidum* and *B. animalis*, the presence of *B. bifidum* with a low MIC of

tetracycline shown in Table 2 of document (1) and in Table 5 of document (8) (3 out of 7 strains of *B. bifidum* had a MIC of tetracycline of about 1.5 µg/ml), a reasonable expectation of obtaining a strain as defined in claim 6 existed.

XI. The submissions made by the respondent, insofar as relevant to the present decision, may be summarised as follows:

Article 100(b) EPC - Article 83 EPC

In the Notice of opposition only claims 3 to 6 and 9 had been attacked for insufficiency of disclosure, the remaining claims were thus not open to appeal on the grounds of Article 100(b) EPC.

For determination of the MIC, the patent application referred to the Etest method performed as described in document (11). Although the Etest method was described for *Lactobacillus* rather than *Bifidobacteria*, in absence of any indication to the contrary, it was understood that what was taught for the former applied to the latter. The Etest was known to provide reliable results for determining antibiotic susceptibility of many different species. The allegations on the Etest and the difficulties for providing manufacturer's instructions had not been substantiated as required by the case law (T 19/90, OJ EPO 1990, 476). The objection concerning the boundaries of claim 1 was an objection under Article 84 EPC, not a ground for opposition.

The experiments of document (24) were not performed in accordance with the steps/conditions defined in claim 1. As reported in this document, when these steps/conditions were used, mutants with the desired

properties were obtained. The alleged low efficiency of the method was not relevant for the issue of reproducibility. Screening 4000 colonies did not represent an undue burden as shown by document (24) reporting the screening of a higher number of colonies (4500 for UV, 7000 for heat shock at 70°C).

Article 100(a) EPC - Article 54 EPC

The feature related to the MIC had to be considered for the assessment of novelty. Neither document (7) nor document (8) described a tetracycline sensitive strain of *Bifidobacterium* with a mutated chromosomal *tetW* gene.

Article 100(a) EPC - Article 56 EPC

Claim 1

No relevance was given to a MIC of tetracycline of 1.5 µg/ml or less in the closest state of the art document (1). This document did not state that all tetracycline resistant strains of *Bifidobacterium* had a chromosomal *tetW* gene; on the contrary, it referred to other (*tetM*) genes that could also provide for this resistance. Nor did document (1) state that all tetracycline sensitive strains of *Bifidobacterium* had an inactivating mutation in a chromosomal *tetW* gene, and that all tetracycline sensitive strains of *Bifidobacterium* having such a mutation had a MIC of tetracycline of 1.5 µg/ml or less. Document (1) did not suggest a link between a mutated chromosomal *tetW* gene and the low MIC disclosed in the patent. Document (1) was concerned with the isolation of *Bifidobacterium* strains by conventional means, not by mutagenesis.

If the technical problem was the provision of further tetracycline sensitive strains of *Bifidobacterium*, a skilled person had, as a first option, the isolation of further tetracycline sensitive strains with no acquired resistance. The isolation/selection of *Bifidobacterium* strains with acquired tetracycline resistance as a starting material and the reversion of this resistance by mutagenesis was not obvious, unless with hindsight knowledge of the patent. As stated in the patent, the presence of an inactivating mutation in a chromosomal *tetW* gene of the - erroneously believed to be tetracycline sensitive - strains of *Bifidobacterium* BB-12[®] and DR10[™] was fully unexpected. It was not obvious to look for tetracycline sensitive *Bifidobacterium* strains with an inactivated chromosomal *tetW* gene, let alone to produce them by the method of claim 1. Contrary to other standard methods for which, as shown in document (24), no results were obtained, the method of claim 1 comprised specific steps/ conditions that were far from conventional and which resulted in *Bifidobacterium* strains with an inactivating mutation in the chromosomal *tetW* gene and a MIC of tetracycline of 1.5 µg/ml or less.

Claim 3

For the same reasons, the subject matter of claim 3 was inventive. There was no information on the presence or absence of a chromosomal *tetW* gene in the tetracycline sensitive strains of *Bifidobacterium* - with a MIC of tetracycline of 1.5 µg/ml or less - shown in Table 2 of the closest state of the art document (1). If the technical problem was the provision of further tetracycline sensitive strains of *Bifidobacterium*, a skilled person would have looked for further strains with no acquired tetracycline resistance. Although

documents (1) and (3) reported the presence of chromosomal *tetW* genes in tetracycline resistant strains of *Bifidobacterium*, there was no reason for a skilled person to look for strains having this gene and to expect a reversion of the associated resistance. This would have required hindsight knowledge of the patent. Likewise, the opal and amber mutations defined in claim 3 were not obvious and there was no evidence on file that they could be found in nature. Although Example 7 of the patent was of a prophetic nature, post-published document (19) in combination with document (28) showed strains of *Bifidobacterium* with the MIC of tetracycline as defined in claim 3 and with the probiotic properties predicted in Example 7. There was no evidence on file showing that the technical problem was not solved over the whole scope of claim 3.

Claim 6

The strains of *B. animalis* subspecies *lactis* shown in Table 2 of the closest state of the art document (1) had a MIC of tetracycline of 4 to 8 µg/ml, far removed from the 1.5 µg/ml or less defined in claim 6. Although *Bifidobacterium* BB-12[®] was first described as a *B. bifidum* and subsequently as *B. animalis*, these strains, *B. bifidum* and *B. animalis*, were different and not closely related. If the technical problem was the provision of tetracycline sensitive strains of *B. animalis* subspecies *lactis* with a MIC of tetracycline of 1.5 µg/ml or less, the solution proposed by claim 6 was not obvious.

There was no evidence in document (1) showing that tetracycline sensitive strains of *B. animalis* subspecies *lactis* or *B. bifidum* had an inactivating mutation in a chromosomal *tetW* gene; these strains

could be strains with no acquired antibiotic resistance. Indeed, *B. animalis* subspecies *lactis* BB-12[®] was erroneously identified as tetracycline sensitive in document (7); the presence of a chromosomal *tetW* gene in this strain was fully unexpected. The reversion of acquired resistance in tetracycline sensitive strains of *Bifidobacterium* and the presence of inactivating mutations in the chromosomal *tetW* gene, was again unexpected and not derivable from document (1) or from any other prior art without hindsight knowledge of the patent. There was no motivation in the art to look for strains of *B. animalis* subspecies *lactis* with a chromosomal *tetW* gene as starting point for solving the technical problem. Moreover, the chromosomal *tetW* gene was not the sole gene that could confer tetracycline resistance, other (*tetM*) genes could also confer this resistance. Thus, the tetracycline sensitive strains of *B. animalis* subspecies *lactis* with a MIC of tetracycline of 1.5 µg/ml or less described in the art could be strains with no acquired resistance or, in analogy to the reversion of the *tetW* gene, derived from strains in which other (*tetM*) genes had an inactivating mutation. None of these strains had a chromosomal *tetW* gene as defined in claim 6.

- XII. The appellant (opponent) requested to set aside the decision under appeal and to revoke the patent.
- XIII. The respondent (patent proprietor) requested the dismissal of the appeal.

Reasons for the Decision

Admission of new evidence into the appeal proceedings

1. In a communication pursuant to Article 15(1) RPBA, the board, with reference to the established case law on the admissibility of evidence not admitted in earlier proceedings, informed the parties that it was of the provisional opinion that the opposition division had neither applied the wrong principles nor exercised its discretion in an unreasonable way when, in the exercise of its discretion, it did not admit documents (30) to (42) into the opposition procedure. Likewise, the board, with reference to the established case law on the function of an appeal, informed the parties that neither the documents filed with the appellant's grounds of appeal nor those filed by the respondent in reply thereto (documents (43) to (52) and documents (53) to (57), respectively) could be admitted into the appeal proceedings (cf. points 6 to 12, 15, 16 and 34.5 of the board's communication).

2. At the oral proceedings before the board, no arguments were put forward by any of the parties to challenge the board's provisional opinion. Therefore, the board, exercising its discretion under Article 114(2) EPC governed by the principles laid down in Article 12(4) RPBA, decides not to admit any of documents (30) to (57) into the appeal proceedings.

Main request (claims as granted)

Article 100(b) EPC - Sufficiency of disclosure

The extent of the objection

3. In the reply to the appellant's grounds of appeal, the respondent argued that, since the objections for lack

of sufficiency raised in the "Notice of opposition" were only against granted claims 3 to 6 and 9, the remaining claims were not open to appeal on the grounds of Article 100(b) EPC.

4. In the communication pursuant to Article 15(1) RPBA, the board observed that, according to the "Minutes of the oral proceedings before the opposition division", the opposition division had considered that the sufficiency of disclosure of the subject matter of granted claim 1 was open to discussion (cf. page 2, penultimate paragraph of the Minutes), that both parties had put forward their arguments on Article 100(b) EPC as regards granted claim 1 and that the opposition division had decided thereupon (cf. points 4.2, 4.6 and 4.11 of the decision under appeal). Thus, the board was of the provisional opinion that there was no reason for not reviewing the decision under appeal in respect of claim 1 (cf. point 14 of the board's communication).
5. In its communication, the board also observed that, in the statement of grounds of appeal, the appellant had not contested the decision of the opposition division as regards sufficiency of disclosure of the subject matter of claims 3, 6 and 9 (cf. points 4.12 and 4.13 of the decision under appeal; point 24 of the board's communication).
6. At the oral proceedings before the board, the parties did not challenge the board's provisional opinion. Thus, only claim 1 is open to an objection for lack of sufficient disclosure on the grounds of Article 100(b) EPC.

Substantive reasons

7. Claim 1 defines specific ranges for the Minimum inhibitive Concentration (MIC) of tetracycline of both the tetracycline resistant progenitor strain of *Bifidobacterium* sp. ("at least 10 microgram tetracycline/ml") and the tetracycline sensitive isolated strain ("1.5 µg tetracycline/ml or less"). The definition of the MIC of tetracycline in claim 1 is thus of a comparative nature, i.e. the particular MIC test and conditions used for characterizing and selecting the tetracycline resistant progenitor strain have also to be necessarily used for the tetracycline sensitive isolated strain. Claim 1, however, does not define any particular test for measuring the MIC of tetracycline. Thus, in line with the established case law concerning technical features not present in the claims, there is no reason to limit the measure of the tetracycline MIC range given in claim 1 to a particular MIC test, such as the Etest referred to in the patent application.

8. There is evidence on file that methods for measuring the MICs of antibiotics, including disc-diffusion methods for *Bifidobacterium* sp., are well-known in the field (cf. paragraph bridging left and right-hand columns on page 39 of document (1)). Document (2) reports the use of the "E-test technique" for measuring the "real MIC" of several *bifidobacteria* and *lactobacilli* strains. It refers also to another MIC test commercially available, namely the "Sensititre Anaero3 kit" (cf. page 203, left-hand column, first full paragraph of document (2)). On page 205 of document (2), the MICs of tetracycline susceptibility of four *Bifidobacterium* sp. (*B. longum*, *B. bifidum*, *B. pseudocatenuatum* and *catenuatum*) are shown. Most

strains of these species have a MIC of tetracycline lower than 2 µg/ml and there is no indication that any technical problem or difficulty was encountered when these measurements were carried out. Documents (1) and (2) were both published - in January and April 2005, respectively - before the filing date of the patent application.

9. According to paragraphs [0023] and [0083] of the patent application, tetracycline sensitivity is determined by the "Etest" susceptibility screening method described by M. Danielsen and A. Wind (2003), which corresponds to document (11) in these proceedings. In the paragraph bridging pages 3 and 4 of document (11), it is stated that the "Etest (ABBiodysk) was used according to the manufacturer's instructions". The appellant states that it has not been able to obtain the manufacturer's instructions contemporaneous with the patent application. The appellant further argues that, since document (11) acknowledges that "no standards exist for susceptibility testing of *Lactobacilli*" and further describes specific conditions for MIC testing, the skilled person is faced with the dilemma of whether to use manufacturer's instructions or whether to carry out some modifications. The confusion, according to the appellant, is further compounded by the fact that the modification in document (11) is in relation to *Lactobacilli* and not to any *Bifidobacterium* sp. In this context, it is worth noting that document (11) also states that conditions ensuring semi-confluent growth allow optimal susceptibility testing (cf. page 4, left column, first three lines). The development of conditions for obtaining semi-confluent growth of a *Bifidobacterium* sp. strain does not, however, require an undue amount of experimentation from a skilled person.

10. In the light of these considerations and taking into account that claim 1 is not limited to any particular MIC test or to any specific conditions for measuring the MIC of tetracycline, appellant's arguments on this issue are not convincing.
11. With reference to the case law concerned with an insufficiency of disclosure arising from an ambiguous feature in a claim, the appellant argues that, in view of the ambiguity associated with measuring the MIC of tetracycline, a skilled person cannot be aware of whether he/she is working within the scope of the claims and therefore, sufficiency of disclosure is not given. The respondent considers this objection to be an objection of lack of clarity under Article 84 EPC and thus not open to appeal proceedings.
12. In point 21 of its communication pursuant to Article 15(1) RPBA, the board drew the parties' attention to point 2.3.1 of the Reasons of decision T 647/15 of 8 September 2016 wherein reference is made to a consensus or a largely predominant opinion among the Boards of Appeal that objections concerning an unclear definition of the boundaries of a claim, relate to Article 84 EPC and not to Article 83 EPC (see also decision T 646/13 of 28 July 2017, in particular point 4 of the Reasons). In the present case and in line with this jurisprudence, the board considers appellant's objection to concern Article 84 EPC and therefore, not to be open for discussion in opposition appeal proceedings.
13. In view of the experimental results described in document (24), the appellant further argues that the method of claim 1 is *prima facie* not reproducible. The

board does not however share appellant's view. None of the experiments reported in document (24) reproduce the specific steps and conditions defined in claim 1. Moreover, the alleged low yield or degree of efficiency of the method of claim 1 is not relevant for the issue of reproducibility nor a reason in itself for an objection of insufficiency. The board also considers that the numbers of colonies screened in Example 2 of the patent application (1000 and 4000) as well as the 7000 colonies screened in document (24), is not unduly high but is common and standard laboratory practice.

14. In the light of the above considerations, the board concludes that the requirements of Article 83 EPC are fulfilled.

Article 100(a) EPC (Article 54 EPC)

15. Appellant's objection of lack of novelty concerns only independent product claims 3 and 6, which share some technical features and differ in others. These claims are directed to tetracycline sensitive strains of *Bifidobacterium* with a MIC of tetracycline of 1.5 µg/ml or less and an inactivating mutation in a chromosomal *tetW* gene. Whilst claim 3 is directed to a strain of *Bifidobacterium* in general containing at least one of two specific inactivating mutations in a chromosomal *tetW* gene (opal, SEQ ID NO: 3; amber, SEQ ID NO:6), claim 6 is directed to a strain of *Bifidobacterium animalis* subspecies *lactis* containing an unspecified inactivating mutation in a chromosomal *tetW* gene.
16. Appellant's objection against claims 3 and 6 is based on the argument that the threshold MIC value cannot be reliably determined and must therefore be disregarded when examining novelty.

17. The board does not share this view (cf. points 8 to 10, *supra*) and considers that the MIC value may be relevant for the assessment of novelty.
18. There is no prior art on file disclosing the two specific inactivating mutations as defined in claim 3. Thus, appellant's objection under Article 54 EPC, based on documents (7) and (8), against claim 3 fails for this reason alone.
19. Document (7) identifies the *Bifidobacterium lactis* strains Bb12 and HN019 (DR10TM) as tetracycline sensitive (cf. Table 2 on page 214). Although document (7) refers to antimicrobial susceptibility tests (disk diffusion tests and Bauer method; cf. paragraph bridging pages 212 and 213), there is no information on the value of the MIC of tetracycline for any of these strains. Likewise, document (7) is completely silent about the presence/absence of a chromosomal *tetW* gene, let alone about a possible (inactivating) mutation in said gene. It is only in the patent that, contrary to what is stated in document (7), the Bb12 and HN019 strains are characterized as being tetracycline resistant and unexpectedly carrying a functional *tetW* gene (cf. paragraph [0014] of the patent). Although in point 27 of the communication pursuant to Article 15(1) RPBA (see also page 3, point 3.3 of the decision under appeal), the board drew the appellant's attention to this contradiction, no evidence has been put forward for questioning the assertions made in the patent or for demonstrating that the Bb12 and/or HN019 strains have the technical features defined in claim 6. In view thereof, the board considers that the claimed strain is

not anticipated by any of the strains described in document (7).

20. In Table 5 of document (8), two strains of *B. longum* are identified with a MIC of tetracycline of less than 1.5 µg/ml. In this table, there are also other strains of *Bifidobacterium* with a MIC of tetracycline of 1.56 µg/ml (3 from *B. bifidum*, 5 from *B. longum*, 1 from *B. infantis*, and 2 from "other" *Bifidobacterium* sp.), a value which falls within the range given in claim 6 when account is taken of the standard error of MIC measurements (cf. Table 5, page 2172 of document (8)). None of the strains identified in Table 5 is a *Bifidobacterium animalis* subspecies *lactis* as required by claim 6. According to document (8), a *B. animalis* strain was purchased from a commercial supplier (*B. animalis* ATCC 25527; cf. page 2169, left-hand column, second paragraph). However, there is no information on whether this strain is of the subspecies *lactis* and whether it is among the two "other" strains described in Table 5 as having a MIC of tetracycline of 1.56 µg/ml. Moreover, there is no information in document (8) about the presence of a chromosomal *tetW* gene in any of these strains. Thus, the claimed strain is not anticipated by any of the strains described in document (8).

21. Therefore, the requirements of Article 54 EPC are fulfilled.

Article 100(a) EPC (Article 56 EPC)

Claim 1

22. The closest state of the art document (1) refers to the beneficial roles of probiotic organisms and to the negative consequences of probiotics containing

resistance traits (cf. page 38). The document reports the susceptibility of eight species of *Bifidobacteria* to 30 antibiotics, including tetracycline (cf. page 39, left-hand column under the heading "Bacterial strains"). Tables 1 and 2 show the MICs of several antibiotics measured by disc-diffusion (Table 1) and agar-dilution (Table 2) (cf. page 39, left-hand column under the heading "Antimicrobial susceptibility tests"). Three strains of *B. pseudocatenulatum* (out of 11 strains), two of *B. longum* (out of 14 strains), and two of *B. bifidum* (out of 8 strains) are identified as tetracycline resistant, with MICs equal to either 16 or 64 mg/l (cf. page 42, right-hand column). Document (1) concludes that "*in the present study, only potentially acquired resistance to tetracycline ... was observed in a proportion of 14% of tested bifidobacterial strains ... We identified, for the first time, tet(W) as the gene responsible for tetracycline resistance in B. pseudocatenulatum and B. bifidum. The tet(W) gene was previously found in a human B. longum ...*" (cf. page 43, right-hand column, second full paragraph).

23. Starting from this prior art, the opposition division formulated the objective technical problem as the provision of "*a further method of isolating a tetracycline sensitive Bifidobacterium strain which does not have a (transferable) tetracycline resistance marker*" (cf. page 9, point 5.11 of the decision under appeal). The appellant formulates this problem in different terms, namely as the provision of a method for producing further tetracycline sensitive strains of *Bifidobacterium* sp. (cf. point X *supra*).

24. However, in the board's view, none of these formulations is in line with the established case law

regarding the formulation of the objective technical problem. This requires avoiding any pointer to the solution, or partially anticipating the solution, in the formulation of said problem (cf. "Case Law of the Boards of Appeal of the EPO", 8th edition 2016, I.D.4.3.1, page 176; *inter alia*, T 2461/11 of 10 March 2017, point 2.3.3 of the Reasons; T 799/02 of 27 July 2004, point 4.3 of the Reasons; T 177/01 of 11 September 2003, point 4.3 of the Reasons). The technical problem formulated by both, the opposition division and the appellant, contains pointers to the solution and results from an *ex post facto* analysis based on inappropriate hindsight knowledge of the patent.

25. Document (1) refers to human/animal habitats from which the tested bifidobacterial strains are isolated but does not provide any information on the methods used for their isolation. Document (1) describes culture conditions for bifidobacterial strains, assays for assessing antibiotic susceptibility (cf. page 39, under the headings "Media" and "Antimicrobial susceptibility tests") and the results of these assays (cf. pages 40 to 42, Tables 1 and 2), but there is no description of any isolation method, let alone a method for producing a tetracycline sensitive strain - in the interpretation given by the appellant to the term "producing", i.e. actively mutating a strain. Likewise, document (1) identifies the chromosomal *tetW* gene as responsible for tetracycline resistance (cf. page 42, left-hand column, first paragraph, and right-hand column second paragraph; page 43, right-hand column, third paragraph) but there is no reference to a possible relevance of (inactivating) mutations within antibiotic resistance genes. Reference to any of these elements in the formulation of the objective technical problem

requires, in the board's view, inappropriate hindsight knowledge of the patent.

26. In view of the actual teaching of document (1), in particular the relevance of probiotic strains free of antibiotic resistance determinants (cf. page 38 and page 43, right-hand column, fourth paragraph), the sole technical problem derivable from document (1) without using inappropriate hindsight, is the provision of further tetracycline sensitive strains of *Bifidobacterium*. However, this problem (provision of a product), which is also formulated in these terms by the respondent (cf. point XI *supra*), is far removed from - and not related to - the claimed subject-matter (a method for production).

27. In the board's view, the objective technical problem cannot be derived from document (1) identified as the closest state of the art without applying inappropriate hindsight. In view of the prior art on file, the board does not see a different outcome when starting from any other of the prior art documents on file. Moreover, according to the established case law of the Boards of Appeal, if the objective or relevant technical problem cannot be derived from any of the alleged closest prior art documents, the measures taken for its solution cannot be derivable either (cf. T 1277/08 of 17 November 2011, point 30 of the Reasons).

28. In the present case, the appellant has raised several issues which, in this context, are relevant and need to be considered by the board before taking a decision on inventive step of the method of claim 1.
 - 28.1 Based on the results described in document (24), the appellant argues that the method of claim 1 is not

reproducible, implying also that the technical problem is not actually solved by the claimed subject-matter. Appellant's argument has been dealt with by the board under Article 100(b) EPC and considered not to be relevant (cf. point 13 *supra*). In view of the results disclosed in the examples of the patent and the evidence on file, there is no reason for the board to consider that the technical problem is not solved by the claimed subject-matter.

28.2 The board is in no doubt that, as argued by the appellant, the combination of (EtBr) chemical and (UV) physical mutagenesis is a routine practice when intending to produce mutant strains with desired or improved properties; the synergistic effect of such a combination for enhancing the efficiency of mutagenesis is known in the art. In the present case, however, the decisive question is not whether such a mutagenesis could have been carried out by a skilled person but whether a skilled person would have done so in the hope of solving the underlying objective technical problem or in the expectation of some improvement or advantage (cf. "Case Law", *supra*, I.D.5, page 183). This question can only be answered in the negative because, as stated above, the relevant technical problem is not even derivable from the closest prior art document and there was no reason for a skilled person to select antibiotic resistant strains as starting material.

28.3 The appellant argues that the proportion of tested bifidobacterial strains resistant to tetracycline is so large, namely 14% according to document (1) (cf. page 43, right-hand column, third paragraph), that a skilled person would not have discarded these strains when looking for strains with probiotic properties.

28.4 The board does not agree with the appellant's view and considers that, on the contrary, this information makes the skilled person aware of the very large number of tetracycline sensitive strains (86%) available at the filing date of the patent. The information a skilled person would draw from document (1) is in line with what is provided by other prior art on file, such as document (7), in which all tested bifidobacterial strains are described as tetracycline sensitive (cf. page 214, Table 2). In view of the associated risks and the negative consequences of antibiotic resistant strains explicitly referred to in document (1) (cf. page 38, right-hand column), there was no reason for a skilled person, trying to solve the underlying technical problem, to consider tetracycline resistant strains as the starting material for obtaining a tetracycline sensitive strain of *Bifidobacterium*.

29. Therefore, claim 1 fulfils the requirements of Article 56 EPC.

Claim 3

30. The closest state of the art document (1) refers to the relevance of bifidobacterial strains having no resistance traits and further identifies tetracycline sensitive strains of *Bifidobacterium* with a MIC of tetracycline of 1.5 µg/ml or less. Starting from this prior art, the objective technical problem has been formulated as the provision of further tetracycline sensitive strains of *Bifidobacterium* with a MIC of tetracycline of 1.5 µg/ml or less.

31. As a first issue, it is questionable whether the reference to the specific value of the MIC of tetracycline in the formulation of the technical

problem is not already a pointer to the solution. Although document (1) refers to low MIC values in general as indicative of antibiotic susceptibility, there is no reason, in the board's view, to select strains of *Bifidobacterium* having a specific MIC of tetracycline of 1.5 µg/ml or less when starting from this prior art. Leaving this question aside, the appellant argues that the technical problem is not solved over the whole breadth of claim 3 because this claim is directed to any strain of *Bifidobacterium* sp. whilst, in the patent, the specific effect of a MIC of tetracycline of 1.5 µg/ml or less - achieved by the opal or the amber mutation in the chromosomal *tetW* gene - is demonstrated only for *Bifidobacterium animalis* subspecies *lactis*. In view of the role of the chromosomal *tetW* gene identified in document (1) as the gene responsible for tetracycline resistance (see also document (3) in this context) and the results disclosed in the patent, the board, in the absence of further evidence, cannot follow appellant's argumentation. There is no plausible reason and no evidence on file to support the argument that the presence of one of the two inactivating mutations in the chromosomal *tetW* gene of a tetracycline resistant strain of *Bifidobacterium*, other than the exemplified *Bifidobacterium animalis* subspecies *lactis* strain, does not result in a tetracycline sensitive strain with a MIC of tetracycline of 1.5 µg/ml or less. In the absence of any evidence, appellant's argument on inventive step must be dismissed.

32. Whilst it is clearly derivable from the studies reported in the prior art on file that there is a large number of tetracycline sensitive strains of *Bifidobacterium*, there is no evidence on file to show that the specific inactivating mutations defined in

claim 3 are actually found in nature. Indeed, there is no information at all on the actual nature of the tetracycline sensitive strains of *Bifidobacterium* described in the prior art. In particular there is no information on whether the tetracycline susceptibility arises from not having an acquired resistance gene (free of a tetracycline resistance gene), or from a reversion of the effect of the chromosomal *tetW* gene - due to the inactivating mutations defined in claim 3 or to other inactivating mutations - or, in analogy thereto, from a reversion of another (chromosomal) gene responsible for tetracycline resistance, such as the *tetM* gene cited in document (1) (cf. page 42, left-hand column, first paragraph and right-hand column, second paragraph). This is also true for the tetracycline-sensitive *Bifidobacterium* sp. strains with a MIC of tetracycline of 1.5 µg/ml or less described in document (8) (cf. page 2172, Table 5). In the absence of pertinent evidence, there is no reason to assume that reversion of a tetracycline resistance arising from a chromosomal *tetW* gene is the reason for the tetracycline sensitivity of any of the strains of *Bifidobacterium* described in the prior art, let alone that such a reversion arises from the specific inactivating mutations defined in claim 3.

33. The board considers that, in the absence of a hint or indication in the prior art, the production, isolation and/or selection of tetracycline sensitive strains of *Bifidobacterium* having one of the inactivating (opal or amber) mutations defined in claim 3 is not obvious from the available prior art unless with hindsight. In view thereof, the board does not consider it necessary to discuss the post-published document (19) and the declaration of E. Johansen (document (28)), because the contribution of the patent over the prior art is not

based on the provision of an unexpected improvement or advantage but on the provision of a non-obvious alternative group of tetracycline sensitive strains of *Bifidobacterium*.

34. Therefore, claim 3 fulfils the requirements of Article 56 EPC.

Claim 6

35. Claim 6 is directed to specific strains of *Bifidobacterium animalis* subspecies *lactis* having a specific MIC of tetracycline of 1.5 µg/ml or less, wherein the inactivating mutation in the chromosomal *tetW* gene is defined in general terms.
36. Document (8) refers to *B. animalis* but the subspecies of this strain is not characterized and the MIC of tetracycline is not given (cf. page 2169, left-hand column, second paragraph, and page 2172, Table 5). The closest state of the art, document (1), refers to a strain of *B. animalis* subspecies *lactis* with a MIC of tetracycline which is however higher than the threshold value defined in claim 6 (cf. page 41, Table 2).
37. Starting from this prior art, the objective technical problem has been formulated as the provision of a further tetracycline sensitive strain of *Bifidobacterium animalis* subspecies *lactis* having a low MIC of tetracycline, in particular 1.5 µg/ml or less. It is not disputed that the subject matter of claim 6 solves this problem.
38. As for the formulation of the technical problem in relation to claim 3 above, a first issue to consider is whether the presence of the specific value of the MIC

of tetracycline in the formulation of the technical problem is not already a pointer to the solution. However, in view of the analysis and the conclusion drawn for claim 3, the board does not consider it necessary to enter into a discussion of further details. As stated for claim 3 and for the same reasons as those given in the context of this claim (cf. point 31 *supra*), there is no reason to assume that the reversion of a tetracycline resistance (such as arising from a chromosomal *tetW* gene) of a *Bifidobacterium* strain, in particular a *B. animalis* subspecies *lactis*, is the cause of the tetracycline sensitivity of the strains of *Bifidobacterium* described in the prior art. In the board's view, the subject-matter of claim 6 is not obvious in view of the prior art on file.

39. This conclusion cannot be questioned by reference to the *B. bifidum* strains which are described in the prior art as having a MIC of tetracycline of 1.5 µg/ml or less (cf. page 41, Table 2 of document (1); and page 2172, Table 5 of document (8)). None of these strains has been characterized. In addition, there is no evidence on file demonstrating the presence of a chromosomal *tetW* gene with an inactivating mutation in any of these strains or in any other tetracycline sensitive strain of *Bifidobacterium* (*supra*). The board also does not agree with appellant's allegation that information in the art relating to the properties of *B. bifidum* applies directly to *B. animalis*. The fact that a particular strain of *Bifidobacterium* (BB-12®) was originally defined as a *B. bifidum* and only subsequently characterized as *B. animalis*, cannot be generalized to the extent that both, *B. bifidum* and *B. animalis*, are always directly interchangeable so that properties of the former must be inevitably found in the latter. The presence of tetracycline sensitive

strains of *B. bifidum* with a MIC of tetracycline of 1.5 µg/ml or less does not necessarily imply or anticipate the presence of *B. animalis* subspecies *lactis* strains with an identical MIC of tetracycline.

40. Therefore, claim 6 fulfils the requirements of Article 56 EPC.

Conclusion

41. In view of the above considerations, the board concludes that the main request fulfils the requirements of the 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