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Datasheet for the decision of 2 December 2020

Case Number: T 0713/14 - 3.3.08

Application Number: 04734158.1

Publication Number: 1692271

IPC: C12N1/21, C12P7/46, C12N15/74, C12R1/00

Language of the proceedings: EN

Title of invention:
NOVEL RUMEN BACTERIA VARIANTS AND PROCESS FOR PREPARING SUCCINIC ACID EMPLOYING THE SAME

Patent Proprietor:
Korea Advanced Institute of Science and Technology

Opponent:
BASF SE

Headword:
SUCCINIC ACID PRODUCING RUMEN BACTERIA MUTANTS/KOREA ADVANCED INSTITUTE OF SCIENCE AND TECHNOLOGY

Relevant legal provisions:
EPC Art. 84, 56, 113(1)
EPC R. 80, 111(2)
RPBA Art. 13
RPBA 2020 Art. 13(2)

Keyword:
Main request - clarity (no)
Auxiliary request I - admitted - clarity (no)
Auxiliary request II - admitted - inventive step (yes)
Substantial procedural violation - appealed decision sufficiently reasoned (no)
Reimbursement of the appeal fee (yes)

Decisions cited:
T 1340/10

Catchword:

Europäisches Patentamt
European Patent Office
Office européen des brevets

Beschwerdekammern

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Case Number: T 0713/14 - 3.3.08

D E C I S I O N
of Technical Board of Appeal 3.3.08
of 2 December 2020

Appellant: (Opponent)	BASF SE 67056 Ludwigshafen (DE)
Representative:	Neuhierl, Bernhard Altmann Stößel Dick Patentanwälte PartG mbB Dudenstrasse 46 68167 Mannheim (DE)
Respondent: (Patent Proprietor)	Korea Advanced Institute of Science and Technology 373-1, Guseong-dong, Yuseong-gu 305-701 Daejeon (KR)
Representative:	Hiebl, Inge Elisabeth Kraus & Weisert Patentanwälte PartGmbB Thomas-Wimmer-Ring 15 80539 München (DE)
Decision under appeal:	**Interlocutory decision of the Opposition Division of the European Patent Office posted on 29 January 2014 concerning maintenance of the European Patent No. 1692271 in amended form.**

Composition of the Board:

Chairman	B. Stolz
Members:	D. Pilat
	R. Winkelhofer

Summary of Facts and Submissions

I. European patent N° 1 692 271 is based on European patent application N° 04734158.1 and was opposed on the grounds of Articles 100 (a), (b) and (c) EPC. An opposition division decided that the main request before it (patent as granted) extended beyond the content of the application as filed whilst auxiliary request 1 contravened Article 83 EPC. It maintained the patent on the basis of an auxiliary request 2.

II. The opponent (appellant) lodged an appeal against the decision of the opposition division.

III. The patent proprietor (respondent) replied to appellant's statement of grounds of appeal and replied to the board's communication. They submitted new auxiliary requests I to III.

IV. The parties were summoned to oral proceedings, and in a communication of 27 March 2020, pursuant to Article 17(1) RPBA 2020, they were informed of the board's provisional opinion on some of the legal and substantive matters of the case.

V. Independent claims 1 to 3 of the main request read as follows:

"1. A rumen bacterial mutant in which a lactate dehydrogenase-encoding gene(*ldhA*) and a pyruvate formate-lyase-encoding gene (*pfl*) have been disrupted, and has the property of producing succinic acid at high concentration while producing little organic acids in anaerobic conditions, wherein the rumen bacteria are

selected from the group consisting of genus *Mannheimia*, genus *Actinobacillus* and genus *Anaerobiospirillum*.

2. A rumen bacterial mutant in which a lactate dehydrogenase-encoding gene(*ldhA*), a pyruvate formate-lyase-encoding gene (*pfl*), a phosphotransacetylase encoding gene (*pta*) and a acetate kinase-encoding gene (*ackA*) have been disrupted, and has the property of producing succinic acid at high concentration while producing little organic acids in anaerobic conditions, wherein the rumen bacteria are selected from the group consisting of genus *Mannheimia*, genus *Actinobacillus* and genus *Anaerobiospirillum*.

3. A rumen bacterial mutant in which a lactate dehydrogenase-encoding gene(*ldhA*) a pyruvate formate-lyase-encoding gene (*pfl*) and a phosphoenolpyruvate carboxylase-encoding gene (*ppc*) have been disrupted, and has the property of producing succinic acid at high concentration while producing little organic acids in anaerobic conditions, wherein the rumen bacteria are selected from the group consisting of genus *Mannheimia*, genus *Actinobacillus* and genus *Anaerobiospirillum*."

Independent claims 10 to 12 and 23 relate to a method for producing the rumen bacterial mutant defined in claims 1 to 3 and to a method for producing succinic acid by culturing any one of the bacterial mutant of claims 1 to 3. Dependent claims 4 to 9, 13 to 22, 24 and 25 define specific embodiments of the rumen bacterial mutant of claims 1 to 3, of the method of producing said rumen bacterial mutant and of the method of producing succinic acid using the rumen bacterial mutant of claims 1 to 3.

VI. The following documents are cited in this decision:

D3 R. Chatterjee *et al.*, "Mutation of the ptsG gene results in increased production of succinate in fermentation of glucose by Escherichia coli" Appl. Environ. Microbiol. 2001; 67(1), p.148-154;

D5 J. G. Zeikus *et al.*, "Biotechnology of succinic acid production and markets for derived industrial products. Appl. Microbiol. Biotechnol. (1999); 51, p.545-552;

D10 WO 97/16528 (publication date 9 May 1997);

D11 G.N. Vemuri *et al.*, "Effects of growth mode and pyruvate carboxylase on succinic acid production by metabolically engineered strains of Escherichia coli" Appl. Environ. Microbiol. 2002 Apr; 68(4), p.1715-27;

D12 P.C. Lee *et al.*, "Isolation and characterization of a new succinic acid-producing bacterium, Mannheimia succiniciproducens MBEL55E, from bovine rumen" Appl. Microbiol. Biotechnol. (2002); 58, p.663-668.

VII. The substantive submissions made by the appellant, insofar as relevant to the present decision, may be summarized as follows:

Main request
Article 84 EPC

The appellant maintained that the feature "producing succinic acid at high concentration while producing little organic acids", introduced during oral proceedings before the opposition division into independent claims 1 to 3, contravened Article 84 EPC.

The patent failed to define any minimal or maximal concentrations of succinic acid and organic acids to be considered as "high" and "little", respectively. The skilled person was for this reason unable to determine whether a rumen bacterium fell within the scope of claim 1 or not. These relative terms, unlike comparative terms expressed as "higher than" or "less than", could not only mean "that one should compare the amounts of the different products with a situation where, everything else being equal, the disruptions to the two genes have not been performed" as other interpretations were possible. The patent remained silent as to which strain(s) had to be used to determine what was a high concentration of succinic acid and what was little organic acids. At least two different strains, e.g. the cells disclosed in document D3 or the rumen bacterial cells in which the *pfl* and *ldh* genes had not been disrupted, could be used for determining the meaning of the relative terms "little" and "high concentration". Claim 1 lacked clarity in the sense of Article 84 EPC. The conclusion applied mutatis mutandis to independent claims 2, 3, 10 to 12 comprising the same unclear terms.

Auxiliary requests I and II
Admission/consideration of auxiliary requests I and II

The clarity objection against the relative terms "high" and "little", were discussed during oral proceedings in opposition proceedings where they had been first introduced into the claims. The clarity objection was raised again in appellant's statement of grounds of appeal (see section 2.1, page 3 of the grounds). The respondent chose not to file any request addressing this objection until after the summons to attend oral

proceedings and the board's preliminary communication was issued.

Pursuant to Article 13(2) RPBA 2020, amendments such as new requests were not to be taken into account after a summons was issued, unless there were exceptional circumstances.

There was no exceptional situation sufficient to admit and justify late-filed requests, when a board's provisional opinion deviated from the opposition division's opinion laid down in the decision under appeal. Besides, the new auxiliary requests were not prima facie allowable as they failed to solve all the clarity issues and raised further issues not discussed yet in the proceedings. Thus, the auxiliary requests I to III should not be admitted.

Rule 80 EPC

Since the amendments introduced into claims 1 to 3 and 10 to 12 of auxiliary requests I and II only addressed an issue of clarity, which was not a ground for opposition under Article 100 EPC, they contravened Rule 80 EPC.

Auxiliary request II
Inventive step

Documents D12 and D11 represented alternative closest prior art documents.

Document Dl2 described Mannheimia sp. 55E-cells and their use for producing succinate. Mannheimia sp. 55E-cells produce formate and lactate as side-products when using glucose as the carbon source (see D12 Fig. 2A-C

on page 666 and Table 2 on page 667). The metabolic pathways responsible for the formation of succinate on the one side and formate and lactate on the other side were also disclosed. The "pyruvate kinase converts PEP to pyruvate, which is subsequently converted to several end products including acetic, lactic, and formic acids", clearly indicating that Mannheimia sp. 55E-cells expressed an enzyme that converted pyruvate to lactate (i. e. a lactate dehydrogenase) and an enzyme that converted pyruvate to formate, i.e. a pyruvate formate lyase (see bottom of the left column on page 667 of D12). Mannheimia succiniciproducens MBEL55E and E. coli were reported to share some sequence similarities with regard to their 16S ribosomal RNA genes (see Table 1), which was held to extend to its metabolic pathways. Under anaerobic conditions, PEP was channeled preferably into the reactions leading to succinate generation when CO_2 was available during fermentation, while it was preferably metabolized via pyruvate, leading to production of lactic acid and acetic acid when CO_2 was missing (for illustration see document D5, Fig. 2, left branch and right branch). In other words, document D12 taught that the distribution of PEP into different pathways could be influenced by re-adjusting availability of the respective substrates.

The difference between the subject matter of claim 1 and document Dl2 was that the Mannheimia sp. 55E-cells disclosed in document D12 had no disrupted lactate dehydrogenase (*ldh*) and pyruvate formate lyase (*pfl*).

The effect of these gene deletions resulted in an increased succinic acid production while producing little organic acids in anaerobic conditions.

Starting from document D12, the problem to be solved might be seen as the provision of an improved rumen bacterium for producing succinic acid.

As a solution to this problem, the patent application proposed the rumen bacterial mutant of claims 1 to 3.

Document D11 described a mixed acid fermentation of E. coli under anaerobic conditions with glucose as a carbon source, which was degraded via the glycolytic pathway to PEP, itself metabolized essentially to formate, lactate, ethanol, and acetate, while succinate was a minor product (p. 1715, right col. 1, full paragraph). It explicitly taught that "[d]iversion of carbon to succinate alone is not sufficient to prevent the accumulation of other undesired products." (p. 1715, right column, 2nd full paragraph) and that the "mutations in lactate- and formate-forming steps can further improve succinate production" (p. 1715, right column, 2nd full paragraph). Thus, it taught to (i) reduce the amount of by-products and (ii) improve succinate production in a bacterium by inactivating the products of the *ldh* and *pfl* genes.

As neither the bacterial growth nor the fermentation on glucose was a feature of the claims, the decreased bacterial growth observed for E. coli lacking *ldh* and *pfl* genes (e.g. strain NZN111) under anaerobic conditions on glucose was of no relevance. The dual fermentation process and the fermentation on other carbon and energy source were anyway possible. The skilled person would have immediately understood that the disruption of the *ldh* and *pfl* genes in E. coli blocked all the reactions allowing the disposal of redox equivalents (cf. Fig. 2, right arm of D5). The C_4 succinate pathway, hardly used in E. coli, could

however not make up for this loss (cf. Fig. 2, left arm of D5). Document D11 established that *ldh* and *pfl* disruptions in E. coli was however not a problem when an alternative way of disposing reducing equivalents was available. The E. coli *ldh pfl* mutant NZN111, when provided with an enzyme allowing increased flux of carbon into the C_4 (succinate) branch using recombinant (pyc, pyruvate carboxylase), grew well (consuming about half the glucose available), had a high yield of succinate (4 g/L), and produced only small amounts of other organic acids (see Figure 2 (open symbols) and Table 2, lines 2 and 3).

Faced with the technical problem identified above, the skilled person would have been motivated to apply and transfer the modification introduced into E. coli by analogy to at least bacteria whose C_4 metabolic pathways were known to be active, like rumen bacteria. None of the unpredictabilities could deter the skilled person from applying the teaching to the rumen bacteria, nor did it involve any particular technical difficulties.

The technical effect underlying the present invention consisting of an increased succinic acid while producing little organic acids, was moreover not achieved over the whole scope of claim 1. The prior art taught and confirmed that succinate production by rumen bacteria as claimed was only achievable under anaerobic conditions and under a CO_2 atmosphere, while under an N_2 atmosphere other products were obtained (see document D5. p. 549. 1st paragraph and Fig. 2 "high CO_2" and a "low CO_2" branch; document D12, Fig. 2B and 2C).

Document D3 related to recombinant E. coli cells and to their use for the fermentative production of succinic acid (see the title in D3). An E. coli *pfl ldhA*-double mutant strain (i.e. NZNlll see document D11) comprising a further deletion of the *ptsG*-gene was disclosed and named AFP111. Although anaerobic plate tests revealed that glucose failed to support the NZN111 and AFP111 fermentative growth, other tested sugars such as mannose, lactose, fructose and trehalose allowed it.

The skilled person starting from document D12 and faced with the objective problem identified above, would have been motivated to delete *pfl* and *ldh* genes in the rumen bacteria producing succinic acid, when being cultured for example in mannitol and fructose as carbon sources, to produce at least less organic acids such as lactic acid and formic acid. Document D3 established that a deletion of both genes decreased the formation of lactic acid and formic acid, without negatively affecting the anaerobic growth of the cells, at least in the presence of mannose and fructose as carbon sources.

The skilled person could have also at least adopted a "try and see" attitude depriving the subject-matter of claim 1 of an inventive step, when neither the implementation nor the testing of an approach suggested by the prior art involved any particular technical difficulties.

VIII. The substantive submissions made by the respondent, insofar as relevant to the present decision, may be summarized as follows:

Main request
Article 84 EPC

The concentrations of succinate and other organic acids had to be compared with the concentrations of the same product generated by a parent strain cultured under the same conditions (see patent application pages 18 to 21).

Prior art strains producing succinic acid at industrially useful levels were unstable or not reported yet. Thus, Mannheimia succiniciproducens 55E was used to develop mutant strains to overcome this shortcoming (see patent paragraphs [0002] and [0003]).

As the subject-matter of claim 1 related to a rumen bacterial mutant, the skilled person would have turned to page 21 of the patent application, which established that the Mannheimia sp LPK7 mutant strain, when cultured for producing succinic acid under anaerobic conditions saturated with CO_2, led to a great increase in the yield of succinic acid and improved the ratio of succinic acid:acetic acid by 9.8 in comparison to its parent strain Mannheimia succiniciproducens 55E. Both the LPK4 and LPK7 mutant strains were capable of producing high concentrations of succinic acid and generated a most favourable ratio of succinic acid:acetic acid compared to the parent Mannheimia strains 55E (see patent [0065]; "succinate" and "S/A" in Table 1).

The skilled person was most notably aware of the fundamental metabolic differences between E. coli strains and rumen bacterial strains (see patent [0005], e.g. *ptsG* gene). Documents D11 and D5 confirmed this view. Document D5 described that the physiology of succinate-producing bacteria varied significantly. For example, *A. succiniciproducens* and *A. succinogenes* used

exclusively the phosphoenolpyruvate (PEP) carboxykinase pathway, whereas E. coli utilizes multiple pathways to form succinic acid (see p.547, col.2, last paragraph, Fig.2; p.548, col.1-2 bridging paragraph). Document D11 described that only a few other bacteria than *Anaerobiospirillum succiniproducens* were studied for succinate production, such as *Actinobacillus* sp strain 130Z and *Enterococcus* sp strain RKY1 (see p.1715, 2nd paragraph), while wild type stains of *Escherichia coli* produced succinate only as a minor fermentation product under anaerobic conditions. *E. coli* NZN111 and AFP111 strains required genetic modifications, such as the overexpression of a *pyk* encoding gene and a dual phase fermentation, to achieve a succinate yield comparable to for example bacterial strain *Anaerobiospirillum succiniproducens* (see p.1716, col.1, lines 8-12; p. 1720, "discussion" section, first paragraph). The pathways used in E. coli to generate high yields of succinate were however ambiguous (see p.1716, col.1, lines 21-23).

Thus, in the light of the patent's teaching, the sole meaningful interpretation for the term "little organic acids" in claim 1 was that the concentrations of all organic acids for each strain, except succinic acid, were added up to establish whether an amount of organic acids was little or not. There was no disclosure in the patent for combining arbitrarily some selected organic acid concentrations from Table 1 but leaving out others.

Likewise, the skilled person faced with the relative terms "high concentration" of succinic acid while producing "little organic acids" could only have interpreted them as relative terms where any increase or decrease observed for the genetically modified

bacterial strains had to be compared to the corresponding concentrations obtained in the parent strain when cultured under the same experimental conditions.

Also, in the light of pages 18 to 21 of the patent application, the concentration of succinate and other organic acids had to be compared with the concentrations of the parent strain cultured under the same conditions, the physiological characteristics of which may have a certain degree of variation and may not be fixed in exact terms. This was the only meaningful interpretation and was in line with the decision under appeal.
A skilled person was therefore capable of determining whether a specific rumen bacterial mutant fell within the scope of claim 1 or not.

Admission/consideration of auxiliary requests I and II

Auxiliary request I was identical to the main request except that claims 1 to 3 and 10 to 12 were amended to rumen bacteria selected from the group consisting of the genus Mannheimia.
Auxiliary request II was identical to the main request except that claims 1 to 3 were amended to include the technical features of granted dependent claims 5, 7, and 9.

Auxiliary requests I and II were filed in direct response to the board's preliminary negative opinion on clarity with regard to the main request, which was based on a completely different interpretation of the two relative terms used in the claims of auxiliary request II which had been maintained with the decision

under appeal. A change of interpretation of the claim language was not to be expected.

It was immediately evident that the amendments to the claim requests were occasioned by the negative opinion of the board's communication concerning this aspect.

Auxiliary request I

Amended claims 1 to 3 and 10 to 12 according to auxiliary request I differed from the corresponding claims of the main request in that the genera of rumen bacteria were limited to the genus Mannheimia.

The arguments supporting the clarity of the terms producing succinic acid at "high" concentration and producing "little" organic acids submitted for the main request were maintained. Examples 7 and 8 described Mannheimia bacterial strain mutants and unambiguously taught that the relative terms "little" and "high" ought to be related to the production of the corresponding acids by the parent strain, i.e. Mannheimia succiniciproducens 55E. Hence, the requirements of Article 84 EPC were met.

Auxiliary request II
Inventive step

Document D12 represented the closest prior art, since it related to the same technical field as the present invention, namely the production of succinic acid by the rumen bacterium Mannheimia sp. 55E. Mannheimia sp. 55E cells produced formate and lactate as side products when using glucose as the carbon source (see figures 2A-C, page 666, table 2 on page 667) and the document disclosed which metabolic pathways were responsible for

the formation of succinate on the one side and formate and lactate on the other side.

The difference between the claimed rumen bacterial mutants of claim 1 and the Mannheimia sp. 55E cells disclosed in document D12 was that they had no disrupted lactate dehydrogenase (*ldh*) and pyruvate formate-lyase (*pfl*) genes.

The technical effect resulting therefrom was an increased production of succinic acid while the production of other organic acids was reduced.

Hence, the objective technical problem to be solved by the present invention starting from document D12 could be seen as the provision of an improved rumen bacterium for the production of succinic acid at high yield.

The solution thereto was the Mannheimia succiniciproducens 55E strain in which *ldhA* and a *pfl* genes were disrupted (see claims 1 to 3; patent section [0008]).

When trying to find a solution for the above identified problem, the person skilled in the art had no incentive to introduce the claimed mutations into rumen bacteria, as no prior art suggested corresponding gene modifications in rumen bacteria.
The skilled person needed for this reason to take into account solutions disclosed in a neighboring field.

Document D12 disclosed many bacteria, more related to Mannheimia strain 55E than E. coli when considering their 16S RNA gene sequence similarities (see Table 1). Document D12 provided therefore no incentive to turn especially to document D11. No incentive was derivable

from document D5 to turn to document D11 either, first because there were major physiological differences between succinic acid producing bacteria that display very high succinate yields during fermentation of glucose in complex media (see p.547, col.2, last paragraph) and second because a high variability of succinate yields depending on the experimental conditions was observed (see document D11, with and without IPTG induction; AFP111 vs AFP111Δppc in Tables 2 and 4). As E. coli strain NZN111 had a low succinic acid yield in Table 1 compared to its parent or its mutated daughter strain, document D10 confirmed this view.

Document D11 disclosed that the deletion in E. coli of both the *ldh* gene and the *pfl* gene in combination with an overexpression of the *pyc* gene led to an increased succinate production. There was, however, no incentive or motivation to apply the teaching of document D11 to the teaching of D12 with a reasonable expectation of success of arriving at the subject-matter of the present invention, as there were metabolic differences between the pathways used by E. coli and rumen bacteria to generate high yields of succinate (see p.1716, col. 1, lines 21-23), as illustrated by the cells' marginal anaerobic growth on glucose and the increase of succinate assigned to the overexpression of the pyruvate carboxylase when comparing NZN111 and NZN111/ pyc strains in Figures 1 and 2 (see p.1715, col.1, lines 24-26; Figures 1 and 2 (pTrc99A-*pyc* gene), Table 2). Thus, the skilled person would not have derived from this teaching that the disruption of *ldh* and *pfl* genes increased the production of pyruvate but decreased the production of other organic acids in E. coli as well as in rumen bacteria known to grow anaerobically.

Even if, in the alternative, document D11 represented the closest prior art, it differed from the subject-matter of the present invention in that the microorganism used for producing succinic acid were E. coli cells but not the rumen bacteria of claims 1 to 3.

The technical problem was the provision of different bacterial cells capable of producing increased amounts of succinic acid while producing reduced amounts of other organic acids.

Document D3 stated that under anaerobic conditions Escherichia coli fermented glucose to a mixture of products consisting primarily of acetate, formate, and ethanol, as well as smaller amounts of lactate and succinate, while two bacteria, Anaerobiospirillum succiniciproducens and Actinobacillus succinogenes, producing succinate naturally, were developed for commercial production of succinic acid (see p.148, col. 1, lines 1-5 and 18-22). It disclosed further that the increased production of succinic acid in E. coli was linked to a mutation of the *ptsG* gene whose effect could be extended to strains having disrupted *ldh* and *pfl* genes as well (see Table 3).
There was no hint or evidence in the prior art providing a motivation to transfer the results obtained with E. coli to rumen bacteria, let alone the specific claimed mutational pattern, with a reasonable expectation of success. The skilled person could have tried to solve the technical problem in many ways, for example by modifying the culture or fermentation conditions applied on the E. coli or Mannheimia sp. host cells to increase succinate yield production or by improving the succinate production by mutating E. coli strains already known to produce succinate at high

yield. Thus, the claimed rumen bacterial mutants involved an inventive step.

IX. The appellant requests that the decision under appeal be set aside and the patent be revoked. They requested further that the auxiliary requests I to III not be admitted into the proceedings and the appeal fee be reimbursed.

X. The respondent requests that the appeal be dismissed, and alternatively to maintain a patent based on any of the auxiliary requests I to III.

Reasons for the Decision

Main request (auxiliary request 2 as maintained by the opposition division; claims 1-25)

Clarity (Article 84 EPC)

1. The appellant argued that the feature "producing succinic acid at high concentration while producing little organic acids", introduced into independent claims 1 to 3 and 10 to 12 during oral proceedings before the opposition division, lacked clarity and contravened Article 84 EPC.

2. The respondent contended that the controversial and relative terms "high" and "little" were ambiguous. Since the patent as a whole described a selective and advantageous production of fermentation products using mutants of rumen bacteria, a comparison of the concentrations of the fermentation product obtained by the mutants of claims 1 to 3 with the concentrations of the fermentation product obtained by other prior art strains was arbitrary and unjustified. It deviated

significantly from the technically sound interpretation adhered to in the decision under appeal according to which "... one should compare the amounts of the different products with a situation where, everything else being equal, the disruptions to the two genes have not been performed". The upper limit of "little" and the lower limit of "high" concentrations was sufficiently clear, in the light of the patent's teaching, to allow the skilled person to clearly establish whether a mutant of Mannheimia, Actinobacillus or Anaerobiospirillum fell under the scope of claims 1 to 3 and 10 to 12 or not.

3. The subject-matter of claims 1 to 3 relates to rumen bacterial mutants in which a lactate dehydrogenase and a pyruvate formate lyase encoding gene is disrupted and the rumen bacteria are selected from the group consisting of genus Mannheimia, genus Actinobacillus and genus Anaerobiospirillum. In addition, the claimed strains are defined by the property of producing succinic acid at high concentration while producing little organic acids in anaerobic conditions. In view of the wide variety of genetic backgrounds of the three genera mentioned, these relative terms further define and limit the exact extent of the protection afforded by the claims.

3.1 The terms "high concentration" of succinic acid and "little organic acids" in claim 1 are relative terms, the meaning of which primarily depends on reference concentrations which are however not mentioned in claim 1. Since these terms are neither clearly defined in the claim nor do they have a well-recognised meaning in the art, the skilled person trying to interpret and identify their meaning will turn to the description.

3.2 There is no evidence supporting the respondent's view that the interpretation of a characterizing relative feature defining the subject-matter of claim 1 has to be derived from the background art mentioned in the patent which mentions that anaerobic microorganism strains producing succinic acid at industrially useful yields had not yet been reported, except for A. succiniciproducens.

3.3 Nor is there any reason why this definition should be limited by considering only references to the rumen bacterial strains recited in this section leaving out other bacterial strains because they produce succinic acid using different major metabolic pathways or include recombinant genetic modifications.

3.4 The background art provides no evidence that a "high" succinic acid concentration as mentioned in claim 1 necessarily means a concentration of succinic acid useful at an industrial level or specifically the concentration of succinic acid produced by A. succiniciproducens when fermenting glucose in the presence of excessive CO_2. Thus, the skilled person cannot establish what actually falls under the definition of the relative terms used in claim 1. In particular, it is not possible to unambiguously establish what concentrations of succinic acid and organic acids are high and little enough, respectively, to fall under the terms of the claims.

3.5 The board does also not concur with the respondent's view that the meaning of the relative terms "high" succinic acid and "little" organic acids concentrations should be established by comparison with the corresponding concentrations of organic acids produced by Mannheimia succiniciproducens 55E. After all, the

claims are not limited to mutants of said strain but refer to bacterial mutants of the genera Mannheimia, Actinobacillus and Anaerobiospirillum.

The skilled person reading the patent may understand that the mutant strains *Mannheimia* sp. LPK4 and LPK7, under anaerobic conditions, produce succinic acid at high concentrations (see Table 1). Although the specific succinic acid concentration values for mutants LPK4 and LPK7 may serve as an indication of what may be understood by a "high" succinic acid concentration, the skilled reader is left in the dark when it comes to establishing the meaning of "little organic acids". The two strains, described in Example 8 of the patent, produce different concentrations of organic acids.

3.6 In this context, it is highlighted that the term "little organic acids" used in claim 1, in its plural form, encompasses any combination of at least two organic acids including obviously all organic acids. There is no compelling reason, derivable from claim 1 or from the patent's description, to limit the meaning of this term only to the sum of all organic acid concentrations.

3.7 Since many different but equally valid interpretations can be attributed to the relative terms used to define the subject-matter of claim 1, the skilled person is not in a position to determine clearly what falls under the definition of "high" succinic acid concentration and "little" organic acids and thus to unambiguously establish whether a mutant rumen bacterial strain falls under the scope of protection or not.

3.8 To conclude, the definition of the subject matter of claims 1 to 3 and 10 to 12 is ambiguous. The main

request does not meet the requirements of Article 84 EPC.

Admission of new auxiliary requests I and II (Article 13(2) RPBA 2020)

4. Since the summons to the oral proceedings were notified on 22/23 January 2020, Article 13 RPBA 2020 is to be applied for questions regarding any amendment to a party's appeal case in response to the summons (Article 24(1) RPBA 2020).

4.1 The respondent filed auxiliary requests I and II only thereafter, and in response to the board's provisional opinion, pursuant to Article 17 RPBA 2020, on clarity with regard to the main request.

4.2 Auxiliary requests I and II represent an amendment to the respondent's case under Article 13(2) RPBA 2020 according to which any amendment to a party's appeal case made after notification of a summons to oral proceedings shall, in principle, not be taken into account unless there are exceptional circumstances, which have been justified with cogent reasons by the party concerned.

4.3 Auxiliary requests I and II are identical to the main request, except that claims 1 to 3 and 10 to 12 of auxiliary requests I were limited to rumen bacteria selected from the genus Mannheimia, whereas claims 1 to 3 of auxiliary request II were limited to the embodiments of granted dependent claims 5, 7, and 9.

4.4 Auxiliary requests I and II constitute an attempt to overcome an objection under Article 84 EPC raised for the first time during oral proceedings before the

opposition division, which were then, though, found not convincing and clarity was acknowledged. The board's provisional opinion deviated from these findings of the decision under appeal, in seeing a lack of clarity.

4.5 At the time of filing the appeal, the applicable Rules of Procedure of the Boards of Appeal were those of 2007. Article 13(3) RPBA 2007 stipulated that amendments made after oral proceedings had been arranged should not be admitted if they raised issues which the Board or the other party or parties could not reasonably be expected to deal with without adjournment of the oral proceedings (see Article 13(3) RPBA 2007).

Against this background, up until 1 January 2020, the date of entry into force of its revised version (RPBA 2020) there was no specific reason for the respondent to amend the main request upfront, on the basis of (clarity) objections held to be unjustified by the first instance.

4.6 The respondent must have been aware of the advent of the new rules (RPBA 2020), for some time ahead of 1 January 2020, which should have generally prompted them to review their patent portfolio with a view to the possible filing of submissions and claim requests in pending cases, and before a board's communication to come.

However, it may overstretch a party's obligations of due diligence to pre-empt possible communications in all pending cases, and to have possible submissions and claim requests ready on or after 1 January 2020. This is particularly true for cases like the present which have been pending for long before the entry into force of the new rules.

4.7 Moreover, in the case at hand, the board's communication was issued on 27 March 2020, thus at the outset of the Coronavirus (Covid-19) pandemic in Europe, with all the known disruptive consequences for parties' workflow and the communication with their representatives.

4.8 When exercising discretion in the framework of Article 13 RPBA 2020, inter alia, the complexity of the new subject-matter, the current state of the proceedings and the need for procedural economy have to be taken into account (cf. Article 13(1) RPBA 2020).

The deletions of alternatives from independent claims and the restriction of the subject-matter to embodiments of dependent claims - as done here - limits the case to embodiments that were explicitly present in the claims as granted, and thus do not introduce any new issues.

Since the appellant had ample opportunity to object to all embodiments claimed and therefore also to the subject matter of the amended claims, orally and in writing, there are also no issues of fair trial that would preclude the admission of the respondent's requests.

Consequently, in the present circumstances, taking the entire background of the case into consideration, there are exceptional circumstances pursuant to Art 13(2) RPBA 2020 which justify the admission of auxiliary requests I and II.

Rule 80 EPC

5. According to the appellant, the amendments introduced into claims 1 to 3 of auxiliary requests I and II only addressed an issue of clarity which did not represent a ground of opposition under Article 100 EPC. They were thus not occasioned by a ground of opposition and contravened Rule 80 EPC.

5.1 Auxiliary requests I and II were filed in direct response to the board's communication under Article 17(1) RPBA 2020. The amendments introduced into the claims were occasioned by clarity objections raised against the main request comprising amendments proposed to overcome an objection of added-matter under Article 100 (c)EPC.

In fact, these amendments, which had been proposed to overcome an objection of added-matter, lacked clarity. It follows that any attempt to clarify an amendment introduced after grant, aiming at overcoming an objection of added-matter, intends foremost to overcome this ground of opposition in a clear manner. Thus, the proposed amendments are occasioned by a ground of opposition (Rule 80 EPC).

Auxiliary request I

6. Amended claims 1 to 3 and 10 to 12 differ from the corresponding claims of the main request in that the genera of rumen bacteria are limited to the genus of Mannheimia.

7. The respondent submitted that the restriction of the claims to a rumen bacterial mutant selected from the genus Mannheimia limited the meaning of "high" succinic acid and "little" organic acids production to the corresponding acid concentrations obtained by the

parent strain used in the examples, i.e. the strain Mannheimia succiniciproducens 55E.

8. For the reasons outlined in items 3.0 to 3.8 above, the claims of auxiliary request I, directed to a rumen bacterial mutant selected from the genus of Mannheimia, also lack clarity.

8.1 In the light thereof, the requirements of Article 84 EPC are not met.

Auxiliary request II

9. Amended claims 1 to 3 differ from the corresponding claims of the main request in that they are restricted to more specifically defined mutants of the genus Mannheimia sp., set out in the examples of the description, i.e. Mannheimia sp. LPK, Mannheimia sp. LPK7 and Mannheimia sp. LPK4.

Since the amended claims 1 to 3 incorporate the wording of granted dependent claims 5, 7 and 9 respectively, these amendments are not open to an objection under Article 84 EPC (see decision G 3/14, OJ 2015, A102, catchword).

Interpretation of claims 1 to 3

10. The restriction of the subject-matter of claims 1 to 3 to rumen bacterial mutants defined as Mannheimia sp. LPK, LPK7 and LPK4 according to Figures 3, 8 and 9, imposes that the subject-matter must be obtainable by the homologous recombination processes described in Figures 3, 8 and 9 respectively, taking into account the figure legends.

10.1 Figure 3 describes the disruption of *ldhA* and *pfl* genes in Mannheimia succiniciproducens 55E, using two gene targeting vectors each undergoing a double crossover with its target gene. The resulting strain is designated LPK. Figure 8 describes the disruption of *pta-ackA* genes in Mannheimia succiniciproducens LPK, using one gene targeting vector undergoing a double crossover with its target genes. Figure 9 describes the disruption of *ppc* genes in Mannheimia succiniciproducens LPK, as explicitly specified in the Figure and its legend, using one gene targeting vector undergoing a double crossover with its target gene.

The strain of origin of Figure 3 is Mannheimia succiniciproducens 55E, known in the art as mentioned in paragraph [0003] of the patent. This strain is modified according to the process described in Figure 3. Likewise, the strain of origin of Figures 8 and 9 can only be the mutated Mannheimia strain obtainable by the process described in Figure 3 further modified by the processes described in Figures 8 and 9.

Article 84 EPC, Rule 80 EPC

11. The appellant repeated the arguments brought forward against the main request with regard to clarity and with respect to Rule 80 EPC. It was furthermore ambiguous whether the definitions of the strains according to claims 1 to 3 were open-ended or not, i.e. whether they encompassed mutant strains with further genetic modifications.

11.1 The wording "wherein the bacterial mutant strain is Mannheimia sp. LPK according to Figure 3" clearly limits the genetic make-up of the claimed strain to what is described in Figure 3. The claim does not

encompass strains obtainable by further genetic modifications. The same applies to claims 2 and 3. The subject-matter of the claims 1 to 3 are thus the Mannheimia sp. 55E mutants designated LPK, LPK7 and LPK4 obtainable by the steps of Figures 3, 8 and 9.

11.2 The board agrees with the respondent that the property of producing succinic acid at high concentration while producing little organic acids in anaerobic conditions, as recited in claims 1 to 3, is the inevitable consequence of the genetic modifications of Mannheimia succiniciproducens 55E according to Figures 3, 8 and 9. Thus, this feature merely refers to an inherent property of the claimed strains.

11.3 Hence the subject-matter of claims 1 to 3 of auxiliary request II is clearly defined in the sense of Article 84 EPC.

Article 56 EPC

12. It is common ground that document D12 represents the closest prior art for the subject-matter of claim 1.

It discloses the isolation and characterization of Mannheimia succiniciproducens sp. MBEL55E-cells and their use for the production of succinate. Mannheimia succiniciproducens is shown to produce formate and lactate as side-products when using glucose as the carbon source (see page 666, Figure 2A-C and page 667, Table 2). In Mannheimia succiniciproducens MBEL55E-cells the metabolic pathways responsible for the formation under anaerobic conditions of succinate on the one side and formate and lactate on the other side demonstrate that these cells include an enzyme capable of converting pyruvate to lactate and an enzyme capable

of converting pyruvate to formate. It further discloses that "... PEP carboxylation catalyzed by PEP carboxykinase is a key reaction for succinic acid production [...] and the availability of CO_2 controls the partition of PEP to various metabolites such as succinic, lactic and acetic acids." (see p.667 col.2 lines 3-7). This indicates that when CO_2 is available PEP is channeled into the reactions leading to succinate, while in the absence of CO_2 PEP is preferably metabolized via pyruvate, leading to the production of lactic acid and acetic acid (see document D5 Fig. 2, left and right branches respectively).

13. The difference between the subject matter of claim 1 and document Dl2 is that the Mannheimia sp. 55E-cells disclosed in document D12 do not have disrupted lactate dehydrogenase (*ldh*) and pyruvate formate lyase (*pfl*).

The effect of these deletions is that they increase succinic acid production while producing little organic acids in anaerobic conditions.

14. Starting from document D12, the problem to be solved may be seen as the provision of an improved rumen bacterium for producing succinic acid.

As solutions to this problem, the patent proposes rumen bacterial mutants as defined in claims 1 to 3.

15. It has not been disputed and examples 4 and 8 demonstrate that the claimed strains solve the underlying technical problem.

16. According to the appellant, faced with the technical problem identified above, the skilled person could have turned to document D11 and have been motivated to apply

and transfer the modifications introduced into E. coli to bacteria with an active C_4 metabolic pathway, like rumen bacteria, as neither a particular growth rate under anaerobic conditions, the use of a particular carbon source nor the need of a CO_2 saturation to achieve a high succinate concentration were mentioned in the claims. The skilled person was accordingly not deterred from applying the teaching of document D11 to the rumen bacteria described in document D12 as there were no particular technical difficulties to be expected. Documents D3 and D11 confirmed this view. Document D3 disclosed that when E. coli had both *ldh* and *pfl* genes disrupted, it produced less of the other organic acids, such as lactic and formic acids, whereas document D11 mentioned that E. coli mutated in lactate- and formate-forming steps could further improve succinate production.

17. The appellant has argued that a high concentration of succinic acid can only be obtained when the cells are grown anaerobically under high concentrations of CO_2 but not when grown under N_2. From this it concluded that the claimed effect could not be achieved over the whole breadth of the claim.

The board finds it difficult to follow this line of argument.

The subject matter of claim 1 is a fully genetically defined rumen bacterial mutant which, as shown by the patent, has the claimed property when grown anaerobically under high concentrations of CO_2. It is thus an intrinsic property of the claimed subject matter. The fact that the same mutant does not show this property when grown under unsuitable or unconducing anaerobic conditions does not change this

fact. The claimed mutant is a solution to the underlying technical problem.

The board does not share appellant's view that the same product, having a required property when assessed under certain conditions but not under others is only a partial solution to an underlying technical problem. It rather seems that the appellant considers the definition of anaerobic conditions in claim 1 unclear. This however is an objection under Article 84 EPC and not a ground of opposition.

18. A skilled person, starting from document D12, could but would not have arrived at the Mannheimia mutants according to the claims.

Indeed, no hint and clear incentive is identifiable and derivable from documents D12, D5 or D10 to combine the teaching of document D12 with document D11.

First, document D12 (Table 1) discloses many bacteria more closely related to Mannheimia succiniproducens 55E than E. coli which could be used for solving the technical problem identified above (see Table 1). Second, E. coli strain NZN111 shows a low succinic acid yield when compared to its parent or its mutated daughter strain (see document D10, Table 1) and there is a high variability of succinate yields depending on the experimental conditions used (see document D11, Tables 2 and 4, AFP111 vs AFP111Δppc with and without IPTG induction). Third, major physiological differences exist between succinic acid producing strains that display very high succinate yields during fermentation of glucose in complex media (see document D5, p.547, col.2, last paragraph).

Thus, appellant's argument that the skilled person was motivated to combine the teaching of documents D12 and D11 is unconvincing, as it leaves out all the other options how a high succinate yield could be achieved starting from the closest prior art and disregards the concomitant need to reduce the concentration of the other organic acids. There is no suggestion neither in document D12 nor D11 which provides the skilled person with a clear pointer that the modifications according to Figure 3 or according to Figures 8 and 9, solve the technical problem identified above with a reasonable expectation of success.

Even if the skilled person knew that the Mannheimia strain disclosed in document D12 had metabolic pathways for producing succinate, lactate and formate from glucose similar to those in E. coli, it usually adopts a conservative attitude.

19. That is, if not all the relevant mutated genes of E. coli exist and can be mutated in Mannheimia, the skilled person would have questioned whether the strong similarity of the metabolic pathways between the E. coli and Mannheimia was actually correct and whether the conclusions on the formation of succinate on the one side and side-products such as lactate and formate on the other side attributed to the gene disruptions were transferable.

20. Based on the teaching of document D11, the skilled person would not only have disrupted the *ldh* and *pfl* genes but also introduced a *pyc* gene encoding pyruvate carboxylase in the Mannheimia strain to achieve an increased succinate production, as demonstrated in Figures 2 and 3 for strain NZN111/pTrc99A-pyc when compared to strain NZN111 of Figure 1. Thus, the

skilled person would have transferred the entire set of genetic modifications carried out in one of the best succinic acid producing E. coli mutants to Mannheimia and thereby would not have arrived at any of the solutions of claim 1 to 3.

21. When starting with document D11 as alternative closest prior art, the difference between the subject matter of claim 1 and document D11 is that the microorganisms used for producing succinic acid are strains of Mannheimia succiniproducens in which a lactate dehydrogenase-encoding gene(*ldhA*) and a pyruvate formate-lyase-encoding gene (*pfl*) have been disrupted.

22. Starting from document D11, the objective problem to be solved may be seen in the provision of a different bacterial cell producing increased amounts of succinic acid and reduced amounts of other organic acids.

23. Starting from the content of document D11, the skilled person trying to solve the problem defined above, had to assess whether it was justified to extrapolate strong similarities between Mannheima sp. and E. coli in the metabolic pathways for the formation of succinate on the one side and side-products such as lactate and formate on the other side.

Since there is no clear hint or indication in document D11 wether or not such a correspondence existed, the skilled person, being at the limit of being creative, would have followed the teaching of document D11 and kept the genetic modifications in Mannheimia to strictly what had been described. The skilled person would have selected the highest succinic acid producing modified E. coli strain, e.g. (compare the succinate and pyruvate concentrations for NZN111 vs NZN111/

pTrc99A-pyc or AFP111 in Figures 1 to 3), for solving the technical problem of providing an alternative bacterial host having an improved succinate production and would have transferred all its genetic modifications into Mannheimia.

24. Finally, the appellant's argument that the skilled person would have at least adopted a "try and see" attitude in the light of the available prior art, is not convincing. The prior art provides no teaching which clearly envisages the specific Mannheimia sp mutants of claims 1 to 3, necessitating only the mere application of known routine tests to get the desired effects and to arrive at the claimed solution. Thus, in the present circumstances, the skilled person is and was never in a "try and see" situation.

25. Thus, even when starting from the alternative closest prior art document D11, the combination of document D11 with document D12 would not have led the skilled person to the solution of claim 1, let alone claims 2 and 3 in an obvious manner. Consequently, the claimed subject-matter involves an inventive step.

Reimbursement of the appeal fee

26. The appellant further submitted that the decision under appeal had ignored their main line of argument under Article 56 EPC that a skilled person, faced with the technical problem posed, based on documents D12 and D10 or D11, would have deleted both, the *ldh* and the *pfl* genes, and overexpressed the *pyc* gene or selected a strain with a spontaneous modification which resulted in the inactivation of the *ptsG* gene. This amounted to a substantial procedural violation justifying the

reimbursement of the appeal fee.

27. The minutes of the oral proceedings before the opposition division confirmed that the appellant had argued that these modifications were obvious and led to an increased production of succinic acid in said host.

The decision under appeal does not give any reasons as to why this important argument failed to convince the opposition division.

28. According to Rule 111(2) EPC, appealable decisions have to be reasoned. This follows from the general procedural principle that a decision may only be taken to the disadvantage of parties if it is sufficiently reasoned, to enable them to assess whether a substantive examination of their (core) arguments has taken place and whether the decision appears justified, or on which grounds an appeal could be based. Only the sufficient reasoning of a decision at first instance also enables the Boards of Appeal to carry out their proper review.

For this purpose, a reasoning does not have to deal with all the arguments of the parties in detail, but it must at least address the decisive points in dispute, so that the reasons for the decision do not have to be reconstructed or even speculated upon. It must deal with the relevant facts, evidence and arguments, and contain the logical chain that led to the formation of the final judgement (see Case Law of the Boards of Appeal[9] III.K.3.4 with further references).

The defective reasoning of a decision, in principle, amounts to a substantial procedural violation which is to be taken up ex officio, and which has to lead to the

reimbursement of the appeal fee if the appeal is held allowable (Rule 103(1) EPC; see Case Law of the Boards of Appeal[9], V.A.7.7.2, V.A.9.5.9, with further references; e.g. T 1340/10).

Such reimbursement has to take place regardless of the appeal being fully successful, or only in part (Case Law of the Boards of Appeal[9], V.A.9.4, J 18/84, T 704/96, T 129/01, T 604/01, T 363/08, J 1/13, T 443/12, T 2160/12).

29. In the present case, such a procedural violation has occurred. As no reasons in the decision under appeal addressed the appellant's arguments outlined above, they never learnt why said (important) arguments were not considered convincing by the opposition division, rendering them actually unable to understand whether their line of arguments could deprive the patent of an inventive step or not.

Hence, the appellant could but file an appeal to find out whether and why this line of arguments submitted before the opposition division was not considered convincing.

30. Against this background, and in view of the appeal turning out partly successful, the appeal fee is to be reimbursed (see again, for many, T 1340/10, reasons 1.).

Order

For these reasons it is decided that:

1. The decision under appeal is set aside.

2. The case is remitted to the opposition division with the order to maintain the patent on the basis of claims 1 to 8 of auxiliary request 2 as filed with submission of 29 October 2020, and a description to be adapted.

3. The appeal fee is reimbursed.

The Registrar: The Chairman:

L. Malécot-Grob B. Stolz

Decision electronically authenticated