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Datasheet for the decision of 20 November 2008

Case Number: T 0390/07 - 3.3.08

Application Number: 97108764.8

Publication Number: 0811682

IPC: C12N 9/06

Language of the proceedings: EN

Title of invention:
Method of producing L-lysine

Patentee:
Ajinomoto Co., Inc.

Opponent:
BASF Aktiengesellschaft

Headword:
Lysine production/AJINOMOTO

Relevant legal provisions:
EPC Art. 123(3), 83, 84, 56
RPBA Art. 13

Relevant legal provisions (EPC 1973):
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Keyword:
"Admissibility of the main request (no)"
"Admissibility of experimental evidence submitted by a third party (no)"
"Admissibility of a new document filed by a third party (no)"
"Auxiliary request - extension of protection (no), clarity (yes), inventive step (yes), sufficiency of disclosure (yes)"

Decisions cited:
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Headnote:

1. A claim request which was replaced by another request in proceedings before the opposition division because it was manifest that it would fail thereby avoiding a formal decision, and which was filed on appeal, was considered inadmissible (cf. points 1 to 3 of the Reasons).

2. A third party within the meaning of Article 115 EPC is not a party to any proceedings and has no more than an opportunity to "present observations", the admissibility of which is entirely a matter for the board (cf. points 4 to 7 of the Reasons).

Case Number: T 0390/07 - 3.3.08

D E C I S I O N
of the Technical Board of Appeal 3.3.08
of 20 November 2008

Appellant I: (Patent Proprietor)	Ajinomoto co., Inc. No. 15-1, Kyobashi 1-chome Chuo-ku Tokyo 104 (JP)
Representative:	Polz, Leo Hoffmann Eitle Postfach 81 04 20 D-81904 München (DE)
Appellant II: (Opponent)	BASF Aktiengesellschaft -Patentabteilung - C6- Carl-Bosch-Strasse 38 D-67056 Ludwigshafen (DE)
Representative:	Bühler, Dirk Maiwald Patentanwalts GmbH Elisenhof Eliesenstraße 3 D-80335 München (DE)
Decision under appeal:	**Interlocutory decision of the Opposition Division of the European Patent Office posted 11 December 2006 concerning maintenance of European patent No. 0811682 in amended form.**

Composition of the Board:

Chairman: L. Galligani
Members: P. Julià
C. Rennie-Smith

Summary of Facts and Submissions

I. European patent no. 0 811 682 was opposed on the grounds of Articles 100(a),(b),(c) EPC. The opposition division considered the main request to contravene Article 56 EPC and maintained the patent on the basis of a first auxiliary request filed on 22 August 2006 at oral proceedings before the opposition division, wherein claim 1 read as follows:

"1. A coryneform bacterium which has a DNA sequence coding for an aspartokinase which is desensitized in feedback inhibition by L-Lysine and L-Threonine and in which the intracellular enzymatic activities of dihydrodipicolinate reductase, dihydrodipicolinate synthase, diaminopimelate decarboxylase and diaminopimelate dehydrogenase are raised by increasing a copy number of the DNA sequences coding for said enzymes."

II. Notices of appeal were filed by both parties and, with letters dated 20 and 23 April 2007, the patentee (appellant I) and the opponent (appellant II) submitted, respectively, the statements setting out their grounds of appeal. Appellant I filed thereby a **new main request**.

III. Both parties replied to the other's grounds of appeal, on 19 and 21 September 2007 respectively.

IV. Observations of a third party under Article 115 EPC 1973 were filed on 16 June 2007 and on 28 February 2008. In the latter submissions, reference was made to a document relevant under Article 54(3),(4) EPC 1973.

V. With the summons to oral proceedings, the board sent a communication dated 7 August 2008 pursuant to Article 15(1) of the Rules of Procedure of the Boards of Appeal (RPBA), indicating its preliminary, non-binding opinion to the parties.

VI. In a letter dated 19 September 2008, the third party filed further observations and experimental data under Article 115 EPC 1973. In a letter dated 8 October 2008, appellant I requested the board to exercise its discretion to reject this experimental evidence or, in the alternative, to postpone oral proceedings. In a communication dated 20 October 2008, the parties were informed that oral proceedings were not postponed and that the experimental evidence of the third party was considered to be inadmissible.

VII. In letters dated 20 and 17 October 2008, appellants I and II filed, respectively, their reply to the board's communications. Appellant I filed five documents and a collated list of the documents on file. Appellant II filed a declaration of Prof. C. Wittmann dated 12 September 2008.

VIII. Oral proceedings took place on 20 November 2008.

IX. Claim 1 of the **main request** read as follows:

"1. A coryneform bacterium which has a DNA sequence coding for an aspartokinase which is desensitized in feedback inhibition by L-Lysine and L-Threonine and in which the intracellular enzymatic activities of dihydrodipicolinate reductase, dihydrodipicolinate synthase, diaminopimelate decarboxylase and

diaminopimelate dehydrogenase are raised by increasing a copy number of the DNA sequences coding for said enzymes, or using a strong promoter, or combination thereof."

Claims 2 to 6 were directed to embodiments of claim 1 defining the DNA sequences coding for the different enzymes. Claim 7 was directed to a method for producing lysine using the coryneform bacterium of claims 1 to 6.

X. The main request at issue filed with the statement of grounds of appeal (cf. point II *supra*) is identical to the so-called "unamended first auxiliary request" which - as reported in the "Minutes of the oral proceedings before the opposition division" - was replaced by the "amended first auxiliary request" on the basis of which the opposition division maintained the patent (cf. point I *supra*), the "unamended" request being considered unallowable under Article 83 EPC.

XI. The following documents are cited in this decision:

D4: J. Cremer et al., Appl. Environ. Microbiol., 1991, Vol. 57(6), pages 1746 to 1752;

D5: JP 07-75579 (publication date: 20 March 1995);

D6: JP 05-284970 (publication date: 2 November 1993);

D19: US 4 861 722 (publication date: 29 August 1989);

D39: Experimental data from BASF dated 19 April 2007;

D47: E. Menkel et al., Appl. Environ. Microbiol., 1989, Vol. 55(3), pages 684 to 688.

XII. The appellant I's arguments may be summarized as follows:

Procedural issues
Admissibility of the main request

It was clear from the minutes of the oral proceedings before the opposition division that the introduction of an amendment to the first auxiliary request was not a deliberate action made in order to avoid a decision on the unamended first auxiliary request, which had been extensively discussed (ca. 5 hours), in particular in respect of the feature later deleted in the amended first auxiliary request ("*a strong promoter, or combinations thereof*"). Appellant I did not withdraw this request nor waive its right to a decision thereon but, in view of the very long proceedings and for procedural economy, agreed to an amended form of the request in response to the opinion expressed by the opposition division on the matter (see the minutes of the oral proceedings, page 25), namely that it was considered not to fulfil the requirements of Article 83 EPC. Although there was no formal decision on the request, it was clear what the decision would have been if written down.

New requests could be introduced on appeal and there was no regulation in the EPC which restricted the appeal only to the previous auxiliary requests. As a result of the extensive discussion in the opposition proceedings, the new main request did not come as a

surprise, as confirmed by the lack of any objection from the opponent until the board raised this issue. The new main request was further included in the original grounds of appeal and thus at the first possible opportunity in the appeal proceedings and not at an advanced stage of the proceedings.

Admissibility of the experimental evidence filed by the third party in a letter dated 19 September 2008

The experimental evidence data was late-filed and the third party did not give any reason for the late filing. The evidence related to technical data obtained from microbial experiments which could not be accomplished within a short period of time. Neither the board nor the parties were informed by the third party that further experiments were being conducted. The submissions were filed one week after the date when the document was allegedly prepared and even then the annexes were not enclosed. They were filed at the EPO only four days later but not provided directly to the patentee. In view of the complicated technical nature of these experiments and the fact that it was not sufficient to look at the results but their quality had to be assessed (how they were obtained and performed), a *prima facie* evaluation of the evidence (cumbersome genetic manipulations on microorganisms, cultivation experiments) was not possible. Costs incurred by dealing with late-filed evidence could not be awarded against a third party as might happen in the case of a party to the proceedings. In essence, the third party's behaviour amounted to an abuse of procedure. The board's communication of 20 October 2008 (cf. point VI *supra*) was to be regarded as final.

Admissibility of document EP 0 756 007 filed by the third party under Article 54(3),(4) EPC 1973

Document EP 0 756 007 was *prima facie* irrelevant, since it did not disclose the claimed subject-matter. Its priority document disclosed only a lysC* gene encoding an aspartokinase resistant to feedback inhibition (AK*) but it did not disclose any other gene involved in the biosynthesis of lysine.

Auxiliary request (as maintained by the opposition division)
Article 123(3) EPC

Claim 4 as granted was directed to a coryneform bacterium in which the activity of diaminopicolinate decarboxylase (DCC) and diaminopicolinate dehydrogenase (DDH) were raised by increasing a copy number of the DNA sequences coding for these enzymes (lysA and ddh), using a strong promoter, or a combination thereof. There was no limitation to the starting coryneform bacterium in regard to any other enzyme of the lysine biosynthetic pathway, namely aspartokinase (AK encoded by lysC), dihydrodipicolinate synthetase (DDPS encoded by dapA) and dihydrodipicolinate reductase (DDPR encoded by dapB). Granted claim 4 embraced many possible starting coryneform bacteria, including coryneform bacteria enhanced in lysine production by genetic engineering. The claimed subject-matter required an increase in all enzymatic activities and, therefore, was more limited than claim 4 as granted.

Article 84 EPC

The patent in suit referred to an improved growth and rate of lysine production for a coryneform bacterium over-expressing the lysA and dhh genes when compared to coryneform bacteria in which these genes were enhanced singly. The rate of lysine production could be further improved (although the improvement was not quantified) by enhancing these two genes in a coryneform bacterium which was already enhanced in lysine production by genetic engineering. Table 1 showed these effects when comparing strain AJ11082/pDL (over-expressing lysA and ddh) with strains AJ11082/pLYSAB and AJ11082/pPK4D (singly over-expressing lysA or ddh, respectively), and when comparing strain AJ11082/pCABDL (over-expressing lysA and ddh with lysC*, dapA and dapB) with strain AJ11082/pCAB (over-expressing only lysC*, dapA and dapB). Even though it was not the right comparison, these effects were also shown for strain AJ11082/pCABDL when compared to strain AJ11082/pCABL (over-expressing all genes except ddh). In this case the effects were small but nevertheless of industrial relevance. Thus, the claimed subject-matter was technically supported by the patent.

Article 56 EPC

Document D4 showed strain 52-5/pJC50 (chromosomal lysC* and plasmid lysC* and dapA) to produce the highest amount of lysine as compared to other strains, such as strains 52-5/pJC24 and 52-5/pJC33 (both with a chromosomal lysC* and with plasmids dapA and lysC*, respectively). Although no disadvantageous effects were observed in seven recombinant strains harbouring a

plasmid containing each a single gene of the lysine biosynthesis pathway, there was no information addressing the introduction of a plurality of genes and their effect on growth. This was only addressed in the patent in suit which showed that growth was maintained when all genes involved in lysine biosynthesis were introduced into a parental strain. The claimed coryneform bacteria differed from strain 52-5/pJC50 of document D4 by introduction of the genes encoding the other enzymes of the lysine biosynthetic pathway (dapB, lysA and ddh). Strain AJ11082/pCABDL in Table 1 of the patent in suit exemplified this subject-matter.

Starting from this closest prior art, the technical problem to be solved was the provision of an improved method of lysine production. The patent in suit provided this improved method with regard both to the rate of lysine production and to the overall amount of lysine produced (faster rate and higher yield). Strain AJ11082/pCABDL in Table 1 showed this effect, which was demonstrated only when the correct prior art was chosen, namely strain 52-5/pJC50, the best strain available in the art. This strain 52-5/pJC50 was characterized by the same features as those characterizing strain AJ11082/pCRCAB of the patent in suit, namely over-expression of lysC* and dapA. In line with the case law, strains 52-5/pJC50 and AJ11082/pCRCAB were the closest possible structural approximations required for assessing the contribution of the patent over the art and for showing that the effect disclosed in the patent had its origin only in its distinguishing features, i.e. increased enzymatic activity of DDPR (dapB), DDH (ddh), and DDC (lysA) in a coryneform bacteria having an increased AK* (chromosomal and

plasmid lysC*) and DDPS (dapA). Table 1 showed that, when compared to strain AJ11082/pCRCAB, strain AJ11082/pCABDL had a faster rate (26.5 g/l vs. 19.7 g/L at 40 hours) and an overall higher amount of lysine production (47.0 g/l vs. 36.5 g/l at 72 hours).

The experimental report of BASF (cf. document D39), which did not disclose any of the experimental conditions used, did not provide an appropriate starting strain for comparison, since it disclosed only the strain (lysC*, Psod dapA) similar to strain 52-5/pJC24 of document D4 (chromosomal lysC* and plasmid dapA) but not to strain 52-5/pJC50 (chromosomal lysC* and plasmid lysC* and dapA). The strong Psod promoter was used to enhance dapA and dapB, and there were no strains in which all genes were enhanced by increasing only their copy number. Document D39 was not appropriate to demonstrate whether or not the claimed subject-matter provided an effect over the strains disclosed in the art. Moreover, this document did not show increased enzymatic activities as required by the claimed subject-matter. The duplication of a gene or the introduction of a strong promoter did not always result in increased enzymatic activity. In spite of these deficiencies, document D39 showed that, when compared to strain (lysC*, Psod dapA), the four strains over-expressing all the genes cited in the claimed subject-matter had an increased rate of lysine production at 32 hours and, except for one of these four strains, at 40 hours as well. Two of these four strains also produced higher amounts of lysine when compared to strain (lysC*, Psod dapA). Notwithstanding faulty experimental design (use of strong promoter Psod) and lack of basic controls (no demonstration of

increased enzymatic activity), document D39 confirmed the effect disclosed in the patent in suit.

There were no pointers in the art nor in document D4 suggesting that a combined increase in the enzymatic activities of DDPR, DDH and DDC with those of DDPS and AK^* could result in enhanced lysine production, let alone in a faster production rate. Indeed, document D4 was silent on the rate of lysine production and, since it explicitly referred to dapB, ddh and lysA as having no relevance for increasing lysine production, it taught away from the claimed subject-matter. Nor could any encouragement be seen in any of documents D5, D6 or D19, since none of them referred to a transformation of a coryneform bacteria with a plurality of genes involved in the lysine biosynthetic pathway. Neither document D5 nor document D6 reported production of lysine, they measured only enzymatic activities of DDC (lysA) and DDH (dhh), respectively. However, Table 2 of document D4 showed that increased enzymatic activity did not always result in increased lysine production. The biosynthetic pathway of lysine was not like a simple system of sluices (as in appellant II's analogy) since it branched at several points, where the components were used for purposes other than lysine production (homoserine production, cell wall synthesis). There was no indication in the art suggesting that more lysine could be produced by increasing the activity of all the enzymes involved in this pathway. In fact, document D4 explicitly stated that the relevant enzymes were only AK (lysC) and DDPS (dapA).

Article 83 EPC

There was no limitation in the claimed subject-matter to any particular method for increasing the copy number of the DNA sequences nor, if the method was chromosomal integration, on the location of their incorporation into the genome of the coryneform bacteria. Chromosomal integration using transposons (DNA transposition), homologous recombination and conjugation were known and routinely used by the skilled person. Means and methods were also available in the art for overcoming feedback inhibition of enzymes isolated from other bacteria and known to have this type of inhibition, such as the DDPS (dapA) and DDC (lysA) of *E. coli*.

XIII. The appellant II's arguments may be summarized as follows:

Procedural issues
Admissibility of the main request

The admissibility of new requests on appeal was in the board's discretion. This request was withdrawn in the first instance proceedings, either for reasons of procedural economy as appellant I argued or because it did not want a decision. In fact there was no procedural economy because it was clear what the decision on that request would have been. While appellant I argued that this request was filed at the outset of the appeal proceedings, in the opposition it was only filed four weeks before the oral proceedings, too late for the opponent to adapt evidence already prepared to deal with other requests. Appellant I sought a tactical advantage. While appellant II did not

previously object to this request in the appeal proceedings, it was entitled to do so even at the last minute.

Admissibility of the experimental evidence filed by the third party in a letter dated 19 September 2008

This evidence was to be admitted. It was *prima facie* relevant in that, while it did not open up a new line of argument, it supported appellant II's pre-existing argument and its own experimental data and related to matters raised by the board in its communication of 7 August 2008. It was not filed late and could not have surprised appellant I, being within the deadline set by the board for receipt of further submissions. It was very detailed and understandable. The opponent (appellant II) had already filed experimental evidence and the patentee (appellant I) had chosen not to file any such evidence in reply. There was too much laborious work for just appellant II to do, so the third party took the burden for it. The board's communication of 20 October 2008 was not to be treated as final since appellant II was to be allowed to make its submissions on this issue before the admissibility of the third party's evidence was decided.

Admissibility of document EP 0 756 007 filed by the third party under Article 54(3),(4) EPC 1973

No submissions were made on the admissibility of this document.

Auxiliary request (as maintained by the opposition division)
Articles 123(2),(3) and 54 EPC

No submissions were made under any of these articles.

Article 84 EPC

According to the patent in suit, the growth of coryneform bacteria and the rate of lysine production were improved by concomitant over-expression of lysA and ddh. The claimed subject-matter was a selection of coryneform bacteria in which the other genes involved in lysine biosynthesis (lysC*, dapA and dapB) were also over-expressed. However, Table 1 of the patent showed that concomitant over-expression of lysA and ddh did not provide the claimed effects. Strains AJ11082/pCABL (all genes over-expressed except ddh) and AJ11082/pCABDL (all genes over-expressed including ddh) produced similar amounts of lysine, the latter strain being the sole example of the claimed subject-matter. Since this example did not show the claimed effect, there was no technical support in the contested patent for the claimed subject-matter.

Article 56 EPC

Document D4 studied the regulation of lysine biosynthesis in *C. glutamicum* by over-expressing the individual genes involved in this biosynthetic pathway (lysC*, dapA, dapB, ddh, lysA) in strains transformed with appropriate plasmids (pJC33, pJC24, pJC25, pJC40, pCT4-1). The highest amounts of lysine were produced by strain 52-5/pJC50 with a chromosomal lysC* and plasmid

lysC* and dapA. Starting from this closest prior art, the technical problem to be solved was the provision of an improved method of lysine production in coryneform bacteria. The claimed subject-matter did not provide a solution to this problem over the whole scope of the claim.

There was no example in the patent showing that lysine production (rate or yield) was improved in a coryneform bacterium over-expressing all genes (dapA, dapB, ddh and lysA) except lysC*, although such a bacterium was an embodiment of the claimed subject-matter (minimal embodiment). Improvement (if any) was only demonstrated when over-expressing all five genes, including lysC*. The exemplified strain AJ11082/pCABDL in Table 1 of the patent in suit had chromosomal and plasmid lysC*, i.e. over-expressed lysC*. Both documents D4 (52-5/pJC50) and D39 (2xlysC*) showed that the best results were always obtained with lysC* (AK*) over-expression. According to the case law, when the claimed effect was shown only in some of the claimed compounds but not in substantially all of them, the invention as broadly defined in the claims was not a solution to the technical problem.

Document D39 disclosed *C. glutamicum* strain 2xlysC* (two chromosomal lysC*), comparable to strain 52-5/pJC33 of document D4 (chromosomal and plasmid lysC*), and four *C. glutamicum* strains in which all the genes involved in lysine biosynthesis (lysC*, dapA, dapB, lysA and ddh) were over-expressed, comparable thus to strain AJ11082/pCABDL of Table 1 in the patent in suit. Strain 2xlysC* produced higher amounts of lysine than, and had a similar production rate to,

these four strains. Indeed, two of these four strains produced less lysine than strain lysC*, Psod dapA (chromosomal lysC* and dapA over-expression) which was comparable to strain 52-5/pJC24 of document D4 (chromosomal lysC* and plasmid dapA). Since strains 52-5/pJC33 and 52-5/pJC24 produced less lysine than strain 52-5/pJC50, it was reasonable to expect that none of these four strains produced better results than strain 52-5/pJC50. Strains lysC*, 2xdapB and lysC*, Psod dapB produced similar results, showing thereby that strong promoters (Psod dapB) and increased gene copy number (2xdapB) could be similarly used for over-expression. This was also in line with the teachings of the patent in suit. Thus, although the four strains over-expressing all the genes involved in lysine biosynthesis always had a strong promoter for over-expressing at least one of these genes, they were nevertheless comparable to the claimed coryneform bacteria which required to increase the copy number of all these genes. The claimed subject-matter embraced coryneform bacteria that did not provide any improvement over the bacteria disclosed in document D4. In fact, they were even worse than those of document D4.

Moreover, in line with document D4, which stated that the growth of strains was not affected by the introduction of plasmids with the individual genes involved in lysine biosynthesis, no disadvantageous effects (bacterial growth, lysine production) were shown in document D39 when these individual genes were over-expressed in a lysC* strain. These results were contrary to those reported in Table 1 of the patent in suit, where the introduction of single genes always resulted in lower lysine production at 40 hours when

compared to the non-transformed strain AJ11082. The quality of the experimental data of document D39 was never disputed and the experimental conditions used therein were identical to those disclosed in great detail for related experimental evidence submitted during the opposition proceedings. Contrary to the strain AJ11082 used in the patent in suit, which was not available to repeat the experiments presented there, the strain ATCC 13032 used in the experimental report D39 was available and used in document D4 as well and no objections had been raised against the repeatability of those experiments.

Document D39 demonstrated thus that the results shown in Table 1 of the patent in suit were strain specific and could not be extrapolated to other coryneform bacteria. Since the patent did not disclose any criteria allowing the skilled person to select other coryneform bacteria for which the same results as those of the specific strain disclosed in the patent could be expected, the contribution of the patent did not justify the claimed scope of protection. Moreover, there was no example in the patent showing the introduction of DNA sequences into the chromosome of a coryneform bacterium, all examples were performed using plasmids. Similarly, there were no measurements of enzymatic activities, Table 1 reporting only the amounts of lysine produced.

There was no disincentive in the art to consider the over-expression of additional genes aside from the concomitant over-expression of lysC* and dapA. Document D4 explicitly taught that the over-expression of dapB, lysA and ddh had no negative impact on bacterial growth.

There was evidence on file, such as documents D5 (lysA, DDC), D6 (ddh, DDH) and in particular D19 (lysA/DDC in a lysC*/AK* strain), confirming that the skilled person considered the concomitant over-expression of dapA, dapB, lysA and ddh in strains over-expressing lysC* as an obvious alternative to the strains disclosed in document D4. To use a simple analogy, the lysine biosynthetic pathway could be equated to a sequence of sluices regulating the flow of water through them. Once the first key sluice (AK*) had been opened, other sluices downstream of that key regulation point (the other enzymes involved in lysine production) had to be opened in order to accommodate the increased volume of moving water.

Article 83 EPC

The patent in suit was exemplified by a single specific coryneform bacterium over-expressing all genes lysC*, dapA, dapB, ddh and lysA. However, document D39 showed that the teachings of the patent did not always result in increased production of lysine in all coryneform bacteria. Therefore, it was not credible that the same effect could be obtained as a matter of routine and without undue burden with other coryneform bacteria. The less so for chromosomal integration for which no examples, indications or guidance, were provided in the patent, which referred in general terms to transposon technology. There were no indications in the patent to lead the skilled person to achieve the desired results (chromosomal integration and increased production of lysine) without undue burden. Moreover, there was no demonstration showing that the effects claimed in the patent could be achieved without lysC* over-expression.

XIV. The third party's observations under Article 115 EPC may be summarized as follows:

Auxiliary request (as maintained by the opposition division)
Article 54(3),(4) EPC 1973

Document EP 0 756 007 concerned the improvement of lysine production by over-expression of genes involved in the lysine biosynthetic pathway and suggested to over-express lysC, dapA, dapB, lysA and dhh, in particular using lysC*. Although it was exemplified only by a plasmid containing lysC*, dapA, dapB and lysA, this example showed the feasibility of the suggestion made for improving lysine production.

Article 56 EPC

Document D4 disclosed that over-expression of each of the genes (lysC, dapA, dapB, lysA, ddh) involved in the biosynthesis of lysine did not impair the growth of transformed coryneform bacteria. The claimed subject-matter required the over-expression of all these genes in a transformed coryneform bacterium. Since there was no demonstration that this subject-matter resulted in increased lysine production, the technical problem to be solved was the provision of an alternative method of lysine production in coryneform bacteria. Table 1 of the patent in suit showed that, as regards lysine production and bacterial growth at 72 hours of culture, the results of strain AJ11082/pCABDL (exemplifying the claimed subject-matter) were worse than those of strain AJ11082/pCABL (all

genes over-expressed except dhh). Therefore, the claimed subject-matter did not solve the problem.

Document D4 disclosed that the transformation of the *C. glutamicum* 52-5 strain (chromosomal lysC*) by plasmid pJC50 with lysC* and dapA resulted in strain 52-5/pJC50 with the highest increased lysine production. In the light thereof, it was obvious for the skilled person to expect an increased lysine production by further over-expressing all the other genes involved in the lysine biosynthetic pathway, the more so since document D4 stated that the over-expression of each of these genes did not impair the growth of the resulting transformed strains. The relevance of all these genes (lysC, dapA, dapB, lysA and ddh) in the production of lysine was known to the skilled person. The combined over-expression of all these genes did not amount to an inventive contribution, it was an obvious alternative for producing lysine easily derivable from the prior art. No prejudice whatsoever existed in the prior art against such alternative.

Article 83 EPC

The disclosure of a single desensibilized aspartokinase (AK*) did not allow the skilled person to obtain other AK* without undue burden. The selection of coryneform bacteria with an AK* by mutagenesis was a random method with no guarantee of success and required an unreasonable amount of work and time. Since the claims were not limited to the exemplified AK*, their scope was not justified. These other AK* mutants represented by themselves new inventions. Similarly, in the selection of other (dapA, lysA, dhh, etc.) genes, the

source of their origin had to be taken into account. There was evidence on file showing that the dapA and lysA genes from *E. coli* were inhibited or regulated by lysine. The production and selection of other mutants resistant to this inhibition was also in itself inventive. Moreover, the prior art described the contribution of the dhh gene to the lysine production as being NH^{4+} dependent. There was, however, no indication of this in the patent. On the contrary, it even mentioned the possible use of soybean hydrolysate as a culture source of nitrogen for replacing NH^{4+}. Thus, in order to achieve the claimed subject-matter and the effect disclosed in the patent, selections (culture conditions, source of genes, etc.) had to be made for which, however, no guidance was found in the patent to assist the skilled person to make them.

XV. The appellant I (patentee) requested that the decision under appeal be set aside and the patent be maintained on the basis of the main request filed on 20 April 2007 with the statement of grounds of appeal or that the appeal by the opponent (appellant II) be dismissed.

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

Reasons for the Decision

Procedural issues
Admissibility of Appellant I's main request

1. The submissions of both appellant I, that new requests can be filed on appeal, and of appellant II, that the

admissibility of such requests is a matter for the board to decide in its discretion, are entirely correct. However, the request at issue here is not a new request but one which was before the opposition division and on which no decision was made because it was withdrawn. The board accepts appellant I's submission that it was only withdrawn because the opposition division expressed the opinion that, as long as claim 1 referred to a strong promoter, that claim would not be allowable under Article 83 EPC. It thus also follows that, as appellant I further submits, it was clear what the opposition division's decision on this request would have been if it had not been withdrawn - it would have been refused. That is not of course to say that the decision was predictable other than in general terms: all that one can confidently say is that the request would have been refused because the reference to strong promoters led to an insufficiency of disclosure under Article 83 EPC, but one cannot say exactly what the detailed reasons would have been. While the request may not have been withdrawn with the intention of avoiding a decision thereon, that is the necessary and inevitable result of the withdrawal of a request as appellant I must have known at the time. The appellant I's submission that the request was withdrawn to save time necessarily means to save the time it would have taken to obtain a formal refusal in the oral proceedings which would in turn have required the opposition division to provide written reasons for refusing the request. Once admitted, requests are either refused or withdrawn and, respectively, the subject of a decision or not. There is no possible "hybrid" or "intermediate" result according to the

circumstances of the case or the intention of the party withdrawing its request.

2. The purpose of an appeal is to review what has been decided at first instance (and, by necessary corollary, not to review what has not been decided; see, in the present context of a withdrawn request, T 528/93 of 23 October 1996, point 1 of the Reasons). The only basis on which a request withdrawn at first instance may be admitted on appeal is that, as in the case of any other request filed on appeal, it may overcome the reasons for the decision actually given on another request. It must often be unlikely that a request, which was withdrawn at first instance because it became obvious it would fail, will succeed in overcoming the reasons for dismissal of a less obviously objectionable request but it is always open to an appellant to seek to persuade a board that it may. However, in its statement of grounds of appeal appellant I did not make a case why its previously withdrawn request (now renamed "main request") should overcome the reasons for the decision under appeal but instead made a case why the decision to refuse that request - a decision which had not been taken - should be set aside. It appears that to this end the grounds of appeal were extremely carefully drafted, so carefully in fact that on a first perusal the board did not notice this and it only emerged on a subsequent reading when the significance of the reference (on page 5 of the grounds of appeal) to page 25 of the minutes of the opposition division oral proceedings as "page 25 of the interlocutory decision" emerged. That is the only written statement of the opposition division's opinion on the request in question and is **not** a decision or part of a decision

but, of course, without a decision there can be no appeal and thus the grounds of appeal had to refer to that as the decision. It would appear that appellant II also did not notice this since it made no objection until after the board had addressed the issue in its communication of 7 August 2008.

3. In those circumstances it could be said that appellant I's appeal was inadmissible and, indeed, if the mis-reference mentioned above had been noticed earlier by the board or objected to earlier by appellant II, the admissibility of appellant I's appeal could have been dealt with as a preliminary issue. However, since appellant I had no other requests (apart from dismissal of the appeal of appellant II), it makes no difference to find that the main request itself is inadmissible. The board further notes that its decision in this respect conforms with similar earlier decisions (see T 848/00 of 13 November 2002, point 2 of the Reasons; T 506/91 of 3 April 1992, point 2 of the Reasons; T 613/97 of 26 May 1998, points 1 and 2 of the Reasons; T 54/00 of 19 December 2000, point 4 of the Reasons; T 434/00 of 29 June 2001, points 2 and 3 of the Reasons).

Admissibility of the experimental evidence filed by the third party in a letter dated 19 September 2008

4. The parties, and appellant II in particular, appear to have misunderstood the procedural position with regard to evidence filed by third parties. A third party is, despite the use of the word "party", not a party to the proceedings and has no more than an opportunity to "present observations" (Article 115 EPC). It is clear

from Article 115 EPC that, since a third party cannot be a party to any proceedings, it cannot be a party to appeal proceedings (see also Article 107 EPC). While it is well-established by case-law that third party observations can be considered, both at first instance and on appeal, there is no obligation on the board beyond such consideration and no right of a third party to be heard on the admissibility of its observations and of any evidence in support of observations. While, of course, the actual parties to proceedings have the right to be heard in relation to such observations if they might (in whole or in part) form the basis of a decision, that right arises quite independently under Article 113(1) EPC. Thus, the admissibility of third party observations is entirely a matter for the board. It may of course consider submissions from the parties to the proceedings (as it did at the oral proceedings in the present case) but that is a courtesy extended by the board and not a right of the parties. If a party to the proceedings wishes to make submissions on the admissibility of third party observations, it can only ensure this by adopting those observations (and accompanying evidence) as its own.

5. In the present case appellant II, the party to the proceedings arguing for admissibility of the third party's experimental evidence, did exactly the opposite. While claiming to be entitled to argue for the admissibility of that evidence, it made it abundantly clear that it was the third party's own evidence: it had been prepared by the third party because it was "laborious" and "too much" for the appellant to do itself; it "supported" the appellant's case. All this

shows that it was not the appellant's own evidence and not part of its own case.

6. Even if the third party's experimental evidence had been the evidence of appellant II itself, the board would not have admitted it into the proceedings. The evidence was not, as appellant II submitted, filed in time. The third party's letter of 19 September was received by fax just before the deadline in the board's communication of 7 August 2008 for submissions in response to the communication but the annexes containing the actual evidence were only sent by post and received after that deadline. Even assuming all the evidence had been filed by that deadline and was a true response to the communication, it would still fall to be decided whether it would be admissible in view of Article 13 RPBA and Article 13(3) RPBA in particular and the nature and volume of the evidence alone would make it inadmissible. It would be simply unfair to appellant I to admit new evidence when there would have been insufficient time or opportunity for it to consider the new evidence and if necessary prepare its own evidence or other submissions in reply. Appellant II submitted the evidence did deal with matters raised in the board's communication which, if correct, means the third party had two months from mid-August until mid-October 2008 to conduct its experiments and prepare its evidence. It would be manifestly unfair to allow appellant I only one month to reply. Adjournment of the oral proceedings, even if that were to be allowed in view of Article 13(3) RPBA, would not be an alternative because, as appellant I rightly observed, the third party could not be ordered to pay appellant I's costs of the adjournment (again, the position would have

become fairer if appellant II had adopted the third party's evidence as its own).

7. The only correct course was to refuse to admit the third party's experimental evidence since to do otherwise would accord a third party more favourable treatment than would be given to an actual party seeking to introduce such evidence at such a stage of the proceedings.

Admissibility of document EP 0 756 007 filed by the third party under Article 54(3),(4) EPC 1973

8. Document EP 0 756 007 was not cited in the opposition proceedings and it has been filed in the appeal proceedings as part of the third party observations after the appellants had filed their statements of grounds of appeal. This document discloses a method of amplifying genes in coryneform bacteria, in particular genes participating in the biosynthesis of amino acids, such as lysine. Reference is made to genes encoding the enzymes AK, DDPS, DDPR, DDH and DDC (genes lysC, dapA, dapB, ddh and lysA, respectively) (cf. page 4, lines 28 to 39). The examples show the use of an AK desensitized in feedback inhibition by lysine and threonine (AK*, encoded by lysC*) (cf. *inter alia* page 7, lines 52 to 54 and page 14, Example 4) and the construction of plasmid pCABL containing the lysC*, dapA, dapB, and lysA genes. The introduction of this plasmid into the wild-type strain *Brevibacterium lactofermentum* AJ12036 (cf. page 19, lines 13 to 40) results in transformant AJ12036/pCABL, which produces high amounts of L-lysine (cf. page 20, Table 5). However, there is no example of a coryneform bacteria transformed with a plasmid

containing all the enzymes involved in the biosynthesis of lysine.

9. Document EP 0 756 007 is not entitled to the claimed priority. The reference found in this document to genes encoding all the enzymes involved in the biosynthesis of lysine does not have any counterpart in the priority document. Moreover, the priority document discloses only the construction of a transposon containing a tetracycline resistance gene and a lysC* gene. There is, however, no example of a plasmid containing the genes involved in lysine biosynthesis nor of plasmid pCABL (AJ12036/pCABL) disclosed in document EP 0 756 007.

10. In view of these deficiencies, document EP 0 756 007 is considered not to be relevant and therefore it is not admitted into the appeal proceedings (Article 13(1) RPBA).

Auxiliary request (as maintained by the opposition division, cf. points I and X *supra*)

Article 123(3) EPC

11. Granted claim 4 is directed to a coryneform bacterium in which the enzymatic activities of DDC and DDH are raised. As starting strains, the patent in suit contemplates the use of wild-type coryneform bacteria as well as artificial mutant strains and coryneform bacteria enhanced in lysine productivity by genetic engineering, including mutant strains with an AK* (cf. paragraphs [0034] to [0038]). The activity of the other enzymes involved in the biosynthesis of lysine is thus completely open and undefined. The introduction of the

other enzymes involved in the biosynthesis of lysine in the claimed subject-matter represents a limitation of the subject-matter of granted claim 4. The board is satisfied that the requirements of Article 123(3) EPC are met.

Article 84 EPC

12. Table 1 of the patent discloses a coryneform bacterium that contains all the features that characterize the claimed subject-matter, i.e. strain AJ11082/pCABDL with a raised copy number of all genes involved in the biosynthesis of lysine. The construction of a plasmid containing all these genes, the transformation of a (parent) coryneform bacterium with this plasmid and the production of lysine are exemplified in the patent. There is no reference to any functional requirement or technical effect in the claimed coryneform bacterium (claims 1 to 6) or in the method for producing lysine (claim 7). Nor is any comparison required in the claims or directly implied by any characterizing feature. Under these circumstances, the board considers that the assessment of the technical effect achieved by the claimed subject-matter and the possible advantages associated therewith has to be performed under Articles 56 and 83 EPC. The requirements of Article 84 EPC are considered to be fulfilled.

Articles 123(2) and 54 EPC

13. There is no substantiation on file for any objection under these Articles. Nor does the board see any reason to raise any objection under these articles of its own motion.

Article 56 EPC

The closest prior art and the technical differences in comparison with the claimed subject-matter

14. Document D4, identified as the closest prior art, discloses the effect of over-expressing individually each of the six genes involved in the biosynthesis of lysine, including a feedback-resistant aspartate kinase (AK* encoded by lysC*), in *C. glutamicum* (cf. page 1749, Table 2). This enzyme (AK) is identified as the sole enzyme in the biosynthetic pathway to lysine that is inhibited by lysine and threonine, the activities of the other five enzymes are neither repressed nor inhibited by any amino acid (cf. page 1746, left-hand column, last sentence). Over-expression of lysC* (by transformation with plasmid pJC33 containing a lysC* gene; cf. page 1747, Table 1) results in high production of lysine when using the wild-type strain ATCC 13032 (38 mM) or strain 52-5 (48 mM), a mutated strain with a chromosomal lysC* gene (cf. page 1749, Table 2). Strain 52-5 produces high amounts of lysine by itself and without any transformation (40 mM). Over-expression of the dapA gene (by transformation with plasmid pJC24 containing a dapA gene; cf. page 1747, Table 1) also increases the production of lysine both in the wild-type strain ATCC 13032 (11 mM) and in the mutated strain 52-5 (48 mM) (cf. page 1749, Table 2). The individual over-expression of the genes encoding each of the other enzymes involved in lysine biosynthesis does not result in lysine production when using the wild-type strain ATCC 13032 (0 mM) nor does it increase lysine production in strain 52-5 (about 40 mM) (cf. page 1749, Table 2). Document D4 states

that the growth of the seven recombinant strains - each strain containing a plasmid encoding one of the enzymes involved in the biosynthetic pathway to lysine - is not affected by the presence of the plasmid (cf. page 1749, right-hand column, last sentence).

15. A cumulative increase in the overall amount of lysine is obtained when the genes encoding the relevant enzymes (AK* and DDPS) are over-expressed in the wild-type strain ATCC 13032 (45 mM) and in strain 52-5 (68 mM) (by transformation with plasmid pJC50 containing the lysC* and dapA genes) (cf. page 1750, left-hand column, last full paragraph and Table 3). In fact, strain 52-5/pJC50, containing the lysC* gene both in its chromosome and in plasmid pJC50, is identified as the strain producing the highest amount of lysine.

16. The claimed subject-matter relates to a coryneform bacterium wherein the intracellular activities of the enzymes DDPR, DDPS, DDC and DDH are raised by increasing the copy number of the DNA sequences coding for these enzymes (dapB, dapA, lysA and ddh genes, respectively) and, wherein the coryneform bacteria must also have a DNA sequence (lysC*) coding for an AK* (cf. point I *supra*). Although the claimed subject-matter only requires the presence of an AK* (and over-expression of the other four enzymes of the biosynthetic pathway to lysine), the over-expression of the lysC* gene is not excluded.

17. The claimed subject-matter is exemplified in the patent in suit by *B. lactofermentum* strain AJ11082 transformed with plasmid pCABDL containing the lysC*, dapA, dapB, lysA and ddh genes (AJ11082/pCABDL) (cf. page 15,

Table 1). The parental strain AJ11082 is described as an artificial mutant resistant to the L-Lysine analogue S-(2-aminoethyl)-L-cysteine (AEC) and therefore, containing a chromosomal lysC* gene (cf. page 5, paragraph [0036]). Strain AJ11082/pCABDL, with a lysC* gene in its chromosome and in plasmid pCABDL, over-expresses the lysC* gene and differs from strain 52-5/pJC50 of document D4 solely by the additional over-expression of the dapB, lysA and ddh genes.

The technical problem to be solved and the solution proposed by the patent in suit

18. Starting from the closest prior art document D4, the technical problem to be solved is seen in the provision of an improved method for producing lysine, both in the rate (speed) and in the yield (amount) of lysine production. The solution proposed for this problem is a coryneform bacterium having the features of claim 1 (cf. point 16 *supra*).

19. Table 1 of the patent shows that, when compared to the parental strain AJ11082, strain AJ11082/pCABDL, which exemplifies the claimed subject-matter, results in an improved rate and yield of lysine production (26.5 g/L vs. 22.0 g/l and 47.0 g/l vs. 29.8 g/l of lysine at 40 and 72 hours of culture, respectively). Similar results are obtained when comparing strain AJ11082/pCABDL with strain AJ11082/pCRCAB (19.7 g/l and 36.5 g/l of lysine at 40 and 72 hours of culture), a strain with a plasmid having the lysC* and dapA genes and thus similar to strain *C. glutamicum* 52-5/pJC50, which produced the highest amount of lysine in the closest prior art document D4 (cf. point 15 *supra*).

20. Thus, Table 1 of the patent in suit demonstrates that the claimed subject-matter results in an improved rate and yield of lysine production over the parental non-transformed strain and over a strain over-expressing the genes which, according to the closest prior art document D4, result in the highest production of lysine. No other comparisons are required for demonstrating that the technical problem is solved.

Is the technical problem solved over the whole scope of the claim?

21. Appellant II has argued that the claimed subject-matter does not provide a solution over the whole scope of the claims. Whereas a first line of argumentation is based on the so-called "minimal embodiment", a second line is based on the experimental results shown in the BASF report D39 (cf. point XIII *supra*).

22. As stated in point 16 above, although the claimed subject-matter requires an over-expression of the four enzymes involved in the four last steps of the biosynthetic pathway to lysine, it does not require - even though not excluded - an over-expression of AK*. There is no evidence on file showing that the effects obtained with strain AJ11082/pCABDL cannot also be obtained with the parental strain AJ11082 (with chromosomal lysC* gene) when over-expressing the dapA, dapB, lysA and ddh genes but not the lysC* gene, i.e. the "minimal embodiment".

23. Document D4 shows that a strain over-expressing the lysC* gene (52-5/pJC33, with chromosomal and plasmid

lysC*) produces more lysine than a strain expressing only the lysC* gene (13032/pJC33, with only plasmid lysC*) (48 mM vs. 38 mM) (cf. page 1749, Table 1). Similarly, a strain over-expressing the lysC* and dapA genes (52-5/pJC50) produces higher amounts of lysine than a strain over-expressing only the dapA gene (13032/pJC50) (68 mM vs. 45 mM) (cf. page 1750, Table 3). However, there is no evidence on file showing that the introduction and over-expression of the dapB, dhh and lysA genes in this latter strain (13032/pJC50) does not result in an improved lysine production when compared to strain 52-5/pJC50, as indicated in the patent in suit - even though not exemplified therein. In the absence of such evidence, it cannot be convincingly concluded that the "minimal embodiment" does not solve the technical problem.

24. The appellant II has nevertheless argued that, since the preferred embodiments of the contested patent as exemplified in document D39 do not solve the technical problem, there are even less reasons to expect the "minimal embodiment" to solve it (cf. point XIII *supra*). Document D39 discloses several strains derived from the strain *C. glutamicum* ATCC 13032 used in document D4. The introduction of a mutation (T311I) in the chromosomal lysC gene of this strain results in a desensitized aspartokinase and in a low lysine producer strain (8.74 g/l, 11.03 g/l and 16.29 g/l of lysine at 32, 40 and 72 hours, respectively), with a genetic background similar to that of the high lysine producer *B. lactofermentum* AJ11082 used in Table 1 of the patent. Document D39 discloses further strains in which the copy number of the DNA sequences of several genes encoding the enzymes of the biosynthetic pathway to

lysine has been increased (lysC*, 2xargSlysA; lysC*, 2xddh; lysC*, 2xdapB; 2xlysC*) or in which the endogenous promoter of these genes has been replaced by the strong promoter Psod (lysC*, Psod dapB; lysC* Psod dapA) or combinations thereof (2xargSlysA, 2xddh, Psod dapB, Psod dapA, Psod lysC*; 2xargSlysA, 2xddh, Psod dapB, Psod dapA, 2xlysC*; 2xargSlysA, 2xddh, 2xdapB, Psod dapA, Psod lysC*; 2xargSlysA, 2xddh, 2xdapB, Psod dapA, 2xlysC*). The board notes, however, several deficiencies in this experimental evidence.

25. First of all, while the description of the patent in suit contemplates raising the activity of the intracellular enzymatic activities either by increasing the copy number of the DNA sequences coding for the corresponding enzymes, or using strong promoters, or a combination thereof, the claims now under consideration do not specifically require the use of strong promoters but only an increase in the copy number of the DNA sequences (cf. point I *supra*). None of the experiments in document D39 was carried out by increasing only the copy number of the DNA sequences coding for all those enzymes. There is always a replacement of an endogenous promoter by a strong promoter in at least one of these DNA sequences, namely the Psod dapA (cf. point 24 *supra*).

26. While for the dapB gene, the results of both an increase in the DNA copy number (lysC*, 2xdapB) and the use of a strong promoter (lysC*, Psod dapB) are reported, there is no such comparison for the dapA gene in document D39. According to document D4, it is indeed an increase in the number of copies of the dapA gene (when the number of copies of the lysC* gene is also

increased) which results in strain 52-50/pJC50 producing the highest amount of lysine (cf. page 1750, Table 3). Document D39 does not describe a strain with an increased number of copies of only the lysC* and dapA genes. The copy number of the dapA gene was not increased in any strain, but rather its endogenous promoter was replaced by the strong promoter Psod. Although similar results are reported for the dapB gene (and the lysC* gene) when using a strong promoter or increased copy numbers, there is no direct evidence that the same holds true for the dapA gene.

27. In any case, the BASF report D39 does not include any detailed information on the products and conditions used to achieve the strains and the results shown in the Tables disclosed therein. During the oral proceedings before the board, appellant II referred to technical information related to other experimental evidence filed either with the grounds of opposition or later during the opposition proceedings for completing the original experimental evidence and only as a reply to the patentee's observations, none of which disclosed the specific examples of document D39. None of this information is found in document D39 nor in the statement setting out the grounds of appeal of appellant II, which refer only in general terms to the submissions made by appellant II during the opposition proceedings. In the board's view, if evidence against the presence of inventive step is to be based on experimental data, such data should then be available in a form which is in itself complete and which provides sufficient detail for a critical scrutiny.

28. In view of the above considerations, the experimental evidence on file is found not to be conclusive enough to decide that the effect disclosed in the patent, in particular the results shown in Table 1, cannot be generalised to other coryneform bacteria. The claimed subject-matter is thus considered to solve the technical problem over the whole scope of the claim.

Obviousness of the claimed subject-matter

29. Appellant II has argued that, once the key modification has been carried out (introduction of an AK*), it would be obvious to maintain the increased metabolic flux through the branch point of the lysine biosynthetic pathway by concomitantly expressing all other genes of this pathway located downstream of the key lysC* gene. In this context, appellant II has made an analogy of the lysine biosynthetic pathway to a sequence of inter-connecting sluices regulating the flow of water (cf. point XIII *supra*). The board cannot follow this argumentation.

30. In line with appellant II's simplified picture, the flow of water might be controlled both by key sluices (enzymes with feedback inhibition) as well as by the size or volume of the sluices (amount of enzyme or degree of enzyme saturation by the substrate, i.e. incoming flow of water). However, once the key sluices have been opened, the size of the other sluices is only important if they are not big enough to contain the incoming flow of water. If the size is enough for the incoming flow, there is no reason to further increase their size, the less so since such a change usually implies additional costs than those absolutely

necessary (manufacture, maintenance). Both types of regulation of a biosynthetic pathway were known to the skilled person, particularly in connection with the lysine biosynthetic pathway (cf. page 688, left-hand column, first paragraph in document D47 to which some of the authors of document D4 contributed).

31. In fact, both types of regulation are addressed in document D4, which identifies the AK as the sole enzyme controlled by feedback inhibition in the lysine biosynthetic pathway (key sluice) (cf. page 1746, left-hand column, last paragraph) and the contribution of the other enzymes in the wild-type strain 13032 (AK with feedback inhibition, key sluice closed), and in the mutant strain 52-5 (feedback-resistant AK*, key sluice opened) (cf. page 1749, Table 2). Document D4 identifies DDPS as involved in the flow control of the wild-type strain 13032 and states that "*since the enzyme is not regulated, the amount itself is of relevance, and not the catalytic state, as with the kinase*" and "*the other reactions investigated by our genetic approach are apparently not involved in flow control of the wild-type to meet its demand for protein synthesis*" (cf. page 1751, left-hand column). Similar results are reported for the "*flow control*" in strain 52-5, namely "*the chromosomally encoded kinase activity still limits the total flow to external lysine*" and the synthase is also limiting "*which could mean that the enzyme is still not saturated*" and suggesting that this enzyme is of "*secondary importance for flow control in the simple producer 52-5*" (cf. page 1751, paragraph bridging right and left-hand column). Both relevant enzymes are concomitantly over-expressed and the resulting strain 52-5/pJC50 is identified as the strain

producing the highest amount of lysine (cf. page 1750, Table 3). Notwithstanding the general knowledge of the mechanisms of biosynthetic pathway regulation, there is no suggestion in document D4 to over-express the other enzymes of the lysine biosynthetic pathway, which are explicitly identified as having no effect on lysine secretion. Nor can a motivation be directly derived from documents D5, D6 or D19 cited by appellant II.

32. Documents D5 and D6 disclose, respectively, the cloning and sequences of the lysA and dhh genes from *B. flavum* MJ-233. The introduction of plasmids containing these genes results in strains *B. flavum* MJ233-lysA and *B. flavum* MJ233-dapY, which have, respectively, more DDC and DDH enzymatic activity than the parental strain MJ-233. Based on these results both documents suggest the use of these plasmids for increasing the efficiency of coryneform bacteria producing lysine (cf. paragraphs [0038] and [0041] in document D5 and paragraphs [0075], [0081] and [0082] in document D6). However, neither of these documents measures the actual amount of lysine produced by any of the disclosed strains. This is only done in document D19, which discloses the cloning and sequencing of the lysA gene from *B. lactofermentum* ATCC 13869. The introduction of a plasmid containing the lysA gene into the lysine producing strains *B. lactofermentum* AJ12019 and *C. glutamicum* ATCC 13287 results in a slight increase of lysine production. A greater increase in lysine production is reported when the plasmid is introduced into strain *B. lactofermentum* AJ 3789 with a lysC* gene (AK*) (cf. columns 9 and 10, Tables 2 and 3). There is, however, no suggestion in any of these documents to over-express these enzymes

concomitantly with any of the other enzymes involved in the biosynthetic pathway to lysine.

33. As a matter of fact, the results disclosed in these documents are comparable to those reported in Table 2 of document D4. The introduction of each of the genes encoding the enzymes involved in the lysine biosynthetic pathway results in increased intracellular activity of all the corresponding enzymes in the wild-type strain 13032 and in the mutated strain 52-5. However, the actual production of lysine in both strains is increased only when the lysC gene or the dapA gene are introduced. There are no significant changes when the lysA gene or the ddh gene are introduced into any of these strains. And accordingly, there is no suggestion to over-express these other enzymes of the lysine biosynthetic pathway.

34. Hence, none of these documents cited by appellant II add nothing of significance to the disclosure of the closest prior art document D4. In view of this, it is concluded that the requirements of Article 56 EPC are fulfilled.

Article 83 EPC

35. Appellant II has argued that the effects disclosed in the contested patent are strain specific and that the results disclosed in Table 1 of the patent cannot be repeated in other coryneform bacteria without undue burden (cf. point XIII *supra*). This objection is based on the experimental evidence of document D39, which has been analyzed in detail in points 24 to 27 *supra*. In line with the results of this analysis, the evidence of

document D39 is considered not to be conclusive enough to decide in appellant II's favour.

36. The claimed subject-matter requires raising the intracellular activities of DDPR, DDPS, DDC and DDH (when compared to those of the wild-type strains) by increasing the copy number of the DNA sequences coding for these enzymes. Although the contested patent only reports the amount of lysine produced and does not measure any enzymatic activity, these activities are directly associated with the amount of lysine produced. Table 2 of document D4 shows that the increase in the copy numbers of these DNA sequences always results in increased activity of the corresponding encoded enzymes, even though the increase does not always result in (increased) lysine production (cf. page 1749, Table 2). Document D4 discloses growth conditions and methods for measuring these enzymatic activities (cf. page 1747, left-hand column, last paragraph to page 1748, left-hand column, last paragraph but one). It also lies within the normal technical abilities of the skilled person to achieve the optimal conditions for bacterial growth and for measuring these enzymatic activities, thereby avoiding possible disadvantageous or detrimental effects known from the prior art (such as an absence of NH^{4+}).

37. The increase in copy number of the DNA sequences required by the claimed subject-matter might be achieved both by introducing DNA vectors/plasmids or by chromosomal DNA integration. Although there are no examples of chromosome DNA integration in the contested patent, the description explicitly refers to prior art related to transposons derived from coryneform bacteria

(cf. paragraph [0056]) and there is also evidence on file showing that methods and means to achieve chromosomal DNA integration in coryneform bacteria were available to the skilled person. Moreover, DNA sequences encoding the enzymes referred to in the claims were also known to the skilled person (cf. *inter alia* documents D4 to D6 and D19); the patent discloses the specific DNA sequences of the lysC, dapA, dapB, ddh and lysA of the *B. lactofermentum* ATCC 13869 (cf. Sequence Listing, paragraph [0117]). The prior art on file also refers to well-known methods for preparing altered feedback resistant aspartate kinase (cf. *inter alia* page 1746, left-hand column, first full paragraph in document D4 and references cited therein).

38. Thus, the requirements of Article 83 EPC are considered to be fulfilled.

Order

For these reasons it is decided that:

The appeals of both appellants are dismissed.

The Registrar: The Chairman:

A. Wolinski L. Galligani