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Datasheet for the decision of 8 November 2021

Case Number: T 1023/18 - 3.3.08

Application Number: 11809152.9

Publication Number: 2597152

IPC: C12N1/21, C12N15/63, C12N15/76, C12R1/465, C12P19/62

Language of the proceedings: EN

Title of invention:
GENETICALLY ENGINEERED STRAIN WSJ-IA FOR PRODUCING ISOVALERYL SPIRAMYCIN I.

Applicant:
Shen Yang Fuyang Medicine Technology Co., Ltd

Headword:
Isovaleryl spiramycin I producing strain/SHEN YANG FUYANG MEDICINE TECHNOLOGY

Relevant legal provisions:
EPC Art. 56

Keyword:
Main Request - requirements of the EPC met (yes)

Decisions cited:

Catchword:

Europäisches Patentamt
European Patent Office
Office européen des brevets

Beschwerdekammern

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Case Number: T 1023/18 - 3.3.08

D E C I S I O N
of Technical Board of Appeal 3.3.08
of 8 November 2021

Appellant: (Applicant)	Shen Yang Fuyang Medicine Technology Co., Ltd No. 18-12, Yaoyang Road Shenbei New Area Shen Yang City, 110000 Liaoning Province (CN)
Representative:	Exner, Torsten Michalski Hüttermann & Partner Patentanwälte mbB Speditionstraße 21 40221 Düsseldorf (DE)
Decision under appeal:	**Decision of the Examining Division of the European Patent Office posted on 30 November 2017 refusing European patent application No. 11809152.9 pursuant to Article 97(2) EPC.**

Composition of the Board:

Chairman	B. Stolz
Members:	D. Pilat
	A. Bacchin

Summary of Facts and Submissions

I. European patent application No. 11 809 152.9 with the title "Genetically engineered strain WSJ-IA for producing isovaleryl spiramycin I" was published under the PCT as International patent application WO 2012/009963 and in accordance with Article 153(4) EPC as EP 2 597 152 A1 (hereinafter "the patent application"). The examining division found that the main request before it did not fulfil the requirements of Article 56 EPC and, accordingly, refused the application.

II. The examining division, in its decision, considered document D1 and alternatively document D2 to represent the closest prior art with regard to claims 1 and 2 of the main request.

III. The applicant (appellant) lodged an appeal, filed new documentary evidence, maintained its main request filed with a letter dated 2 October 2014 and filed new auxiliary requests I and II.

IV. It requested that, in case a favourable decision on neither the Main Request nor the first or second Auxiliary Request can be given in the course of written proceedings, oral proceedings pursuant to Rule 116 EPC be held.

V. Claims 1 and 2 of the main request read as follows:

"1. A genetically engineered Streptomyces strain for producing isovaleryl spiramycin I, designated as WSJ-IA, and deposited under the accession number CGMCC 3942.

2. A method for producing isovaleryl spiramycin I, the method comprising recovering the isovaleryl spiramycin I from a fermentation broth produced by culturing the genetically engineered Streptomyces strain according to claim 1 in a culture medium."

VI. The following documents are cited in this decision:

D1: Chunyan Ma *et al*. "Construction of 4"-isovalerylspiramycin-I-producing strain by in-frame partial deletion of 3-O-acyltransferase gene in Streptomyces spiramyceticus WSJ-1, the bitespiramycin producer." Current microbiology, vol. 62 (1), pages 16-20, 19 May 2010;

D2: JP H06 121677 A (MERCIAN CORP) 6 May 1994 (1994-05-06);

D3: EP 0345546 Bl (published on 13 December 1989).

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

Main request
Article 56 EPC

It was contested that document D2 was an alternative closest prior art with regard to the claimed subject-matter and that the claimed subject matter lacked an inventive step vis-a-vis a combination of documents D1 and D2.

Document D2's purpose was to efficiently manifest the activity of an enzyme acylating the 4"-position of a macrolide antibiotic. A microorganism was transduced

with a DNA fragment containing the *acyB2* gene obtained from a bacterium belonging to the genus *Streptomyces* to amplify the enzyme gene capable of acylating the 4"-position of macrolide antibiotics (tylosin, spiramycin, angolamycin or deltamycin) (see abstract and paragraph [0005]). Examples 1 to 5 of document D2 disclosed the construction of vectors which were then introduced into *Streptomyces lividans*, known to lack on its own the ability of acylating the 4"-position of tylosin (see document D3, paragraph spanning pages 11 and 12). Example 6 measured the expression of *acyBl* mRNA and the conversion of leucomycin U to its leucomycin U-4"-O-isovalerylated form (leucomycin A3). The leucomycin U conversion rates in % in recombinant *S. lividans* was shown on the right side of Figure 8. First, unlike spiramycin, leucomycin U carried no sugar moiety at position 14 of the lactone ring. Second, any deletion within the *acyB2* gene strongly reduced the conversion activity, while any deletion within the *acyBl* gene abolished the conversion activity completely. Finally, Example 7 described the generation of *Streptomyces thermotolerans acyB2* and *acyB2*$^{-}$ mutants, and its transformation with pMAB-B2. In contrast to *S. lividans*, *S. thermotolerans* was capable of acylating the 4"-position of tylosin. Mutants 2257 and 2398 of *S. thermotolerans* achieved a low acylation of leucomycin U, while mutants transformed with a plasmid pMAB-B2 comprising a complete *acyB2* gene restored the acylation of leucomycin U to values between 0.3- and 2.5-fold of what was achieved by the *S. thermotolerans* parent strain.

Thus, document D2 explored the role of each of the heterologous acyBl and acyB2 on the acylation of the 4" position of leucomycin U in *S. lividans* comprising a plasmid encoding them.

Document D1 disclosed a modified *Streptomyces* strain (WSJ-2) comprising a 4"-isovaleryltransferase gene (ist) integrated in its chromosome (see WSJ-1 strain) and in which the 3-O-acyltransferase gene was inactivated by deletion so that the 3-O-position of the lactone ring of spiramycin could no longer be acylated.

VIII. The appellant requested that the decision under appeal be set aside and that a patent be granted on the basis of the main request or auxiliary requests I or II.

Reasons for the Decision

Admission of the main request

1. The main request is identical to the main request underlying the decision under appeal. Thus, since the aim of appeal proceedings is to review the decision under appeal in a judicial manner (Article 12(2) RPBA 2020), the main request forms part of the appeal proceedings.

Article 56 EPC

2. In the course of the examination procedure only an objection under Article 56 EPC was maintained by the examining division. The board sees no reason to raise other objections of its own motion.

3. In the decision under appeal, the examining division held that, in view of the teaching of documents D1 and D2, the claimed subject-matter did not involve an inventive step within the meaning of Article 56 EPC (see section 15 of the decision).

4. The examining division in the decision under appeal considered that document D1 or alternatively document D2 represented the closest prior art. Document D1 addressed the problem of producing isovaleryl spiramycin I (see item 15.2), while document D2 addressed the problem of increasing the yield in 4"-acylated macrolide antibiotic production (see item 15.7.1).

5. The appellant agreed that document D1 represented the closest state of the art, but disputed that document D2 did.

6. The subject-matter of claim 1 is a genetically engineered *Streptomyces spiramyceticus* strain deposited under the accession number CGMCC 3942 capable of producing isovaleryl spiramycin I at high yield and having a high productivity (see patent application paragraph [0008]).

7. Document D1 discloses a recombinant strain of *Streptomyces spiramyceticus* F21 harboring a 4"-O-acyltransferase gene (*ist*) - synonymous of *acyB1* - from *Streptomyces mycarofaciens* 1748, which produces bitespiramycin consisting of 4"-isovalerylspiramycin I, II, and III as its major components (BT, Fig. 1) (strain WSJ-1). The complexity of the BT produced by WSJ-1 was reduced in that the gene (*sspA*) encoding the 3-O-acyltransferase, responsible for the acylation of spiramycin I to spiramycin II and III, was inactivated by in-frame partial deletion which resulted in a genetically engineered *Streptomyces spiramyceticus* WSJ-2 strain producing 4"-isovalerylspiramycin-I only (see Title, abstract). The *Streptomyces spiramyceticus* WSJ-2 strain is a 4"-isovalerylspiramycin-I-producing strain (see abstract; Fig.1 : R_1=H and

R_2=$COCH_2CH(CH_3)_2$; Fig.3 WSJ-2 and Fig. 5b). The fermentation titer of WSJ-2 was ca. 15% that of WSJ-1. A similar drastic decrease of spiramycin productivity was also observed in *Streptomyces spiramyceticus* F21, a spiramycin producer strain, in which the whole *sspA* gene was deleted. The exact reason for this was not clear, but it implied that *Streptomyces spiramyceticus* F21 was a hardly genetically manageable strain (see page 19, col.2 last paragraph).

8. Document D2 explores the role of heterologous acyBl and acyB2 on the acylation at position 4"- of leucomycin U in *Streptomyces lividans* comprising a plasmid encoding each or both of them. It discloses also *acyB2*$^-$ *Streptomyces thermotolerans* mutant strains transformed with a functional *acyB2* encoding gene thereby producing again 4"-isovaleryl leucomycin U. Although the production of leucomycin U can be considered representative of other macrolide antibiotics including tylosin, spiramycin (see paragraph 4), this is not explicitly stated anywhere.

9. It is established case law that for assessing inventive step the closest prior art is normally a prior art document disclosing subject-matter conceived for the same purpose or aiming at the same objective as the claimed invention and having the most relevant technical features in common (see Case Law of the Boards of Appeal of the European Patent Office, 9th Edition, July 2019, Chapter I.D.3, 178).

9.1 Thus, document D2 explores the role of *acyB1* and *acyB2* genes in acylating the 4"-position on leucomycin U, irrespective of whether the C_3-OH macrolide core is acylated or not, whereas document D1 describes a genetically engineered *Streptomyces spiramyceticus*

WSJ-2 strain producing only isovaleryl spiramycin-I. Since the objective of the application is to obtain bacterial strains yielding high concentrations of isovaleryl spiramycin-I, document D1 must be regarded as the closest state of the art (see paragraph [0008] of the patent application).

9.2 The *Streptomyces* strain of claim 1 as deposited under the accession number CGMCC 3942 differs from the strain WSJ-2 of document D1 in that it comprises an *ist* gene and the regulatory *acyB2 gene* derived from *Streptomyces thermotolarences* CGMCC4.1501 in its bacterial chromosome. The WSJ-IA strain is obtained by transformation of the WSJ-2 strain with an integrative vector incorporating the dual *ist-acyB2* gene fragment amplified by PCR, using appropriate primers and total DNA of *Streptomyces thermotolarences* CGMCC4.1501. As stated above, the WSJ-2 strain is not conducive to large-scale production.

9.3 The technical effect of this difference is that the WSJ-IA strain produces a higher fermentation titer of spiramycin, namely by a factor of 4.14, and a higher percentage of isovaleryl spiramycin I, namely by a factor of 1.7 (i.e. wherein the 3-O-position of the macrolide lactone ring is not acylated) compared to the spiramycin produced by WSJ-2 (see patent application [0040] and Table 5).

9.4 The technical problem to be solved starting from the closest prior art can thus be defined as to provide a *S. spiramyceticus* strain having an improved productivity of isovaleryl spiramycin I (see patent application [0008]).

9.5 As a solution to this problem, the patent proposes the *Streptomyces* strain deposited under the accession number CGMCC 3942 (see patent application example 4 and Table 5).

9.6 The board is satisfied that the technical problem is solved by the deposited strain (see Table 5 of the patent application).

9.7 For the determination of the obviousness or non-obviousness of the subject-matter of claim 1, it must be decided whether or not the skilled person would have combined the teachings of documents D1 and D2 to arrive at the claimed subject-matter when attempting to solve the underlying technical problem.

9.7.1 Starting from the content of document D1, the skilled person would have derived that the genetically engineered *Streptomyces spiramyceticus* strain WSJ-1, having integrated the 4"-isovaleryltransferase gene (*ist*) into the chromosome, is capable of producing isovaleryl spiramycin I, II and III. The isovaleryl spiramycin I level produced by strain WSJ-2, comprising an integrated *ist* gene and an in-frame deleted *sspA* gene, is however ca. 15% that of the isovaleryl spiramycin level produced by WSJ-1 (see Fig.5A and 5B). A similar result was obtained when the whole *sspA* gene was deleted in the parent *Streptomyces spiramyceticus* strain F21 of WSJ-1.

9.8 Faced with the technical problem cited above, the skilled person, based on the teaching of document D1, would be motivated to investigate how to overcome or reverse the low productivity caused by the integration of the 3-O-acyltransferase into the *Streptomyces spiramyceticus* WSJ-1 strain.

9.8.1 However, document D1 is silent about how to overcome the drastic decrease in spiramycin productivity observed in strains WSJ-2 (*acyB1/ist*$^{+}$; *sspA*$^{-}$) and F21 (*sspA*$^{-}$). Thus, a skilled person faced with the above technical problem would not have arrived at the claimed solution in an obvious way based on document D1 alone.

9.9 Document D2 describes that the co-expression of the transformed *acyB2* and *acyB1/ist* genes in *Streptomyces lividans* TK24 (see paragraph [0022]) results in higher expression of *acyB1/ist,* which acylates the 4"-position of a macrolide antibiotic (tylosin, spiramycin, angoramycin, deltamycin, etc.), than when said cell is transformed with an *acyB1/ist* gene alone (see paragraph [0030]; Fig.8 and 10). The expression of the 4"-position acylating enzyme in a host transformed with the *acyB1/ist* gene alone was weak and insufficient for the industrial production of 4"-O-isovalerylated macrolides (see paragraph [0004]). The expression of the *acyB1/ist* gene in a host transformed in addition with a DNA fragment encoding the acyB2 regulator generated a 4"-position acylated product with high efficiency (see paragraphs [0003], [0005], [0006]). The DNA fragment shown in Fig. 1 contains the *acyB2* gene derived from *Streptomyces thermotolerans* strain ATCC 11416. "This gene is useful for industrially producing the 4"-position acylated tylosin using the 4"-position acyl-transferase gene and macrolide antibiotics of Streptomyces"(see paragraph [0007]). The *acyBl* gene expression level and the leucomycin U conversion level were assayed (see example 6). *Streptomyces lividans* TK 24 mutant strains, having lost their acyB2 activity, because of the deletion of most of the *acyB2* gene or because of a frame shift in the coding region of *acyB2,* show a decreased expression of

acyB1 and a lower level of conversion of leucomycin U compared to the *Streptomyces lividans* TK 24 strain transformed with a plasmid pMAB8 comprising *Streptomyces thermotolerans acyB1* and *acyB2* genes (see Figures 8 and 10). The conversion level of leucomycin U of *Streptomyces thermotolerant* acyB2$^-$ mutant strains was low but could be restored when they were transformed with pMAB-B2 expressing a functional acyB2 gene.

9.10 In point 15.7.1 of the decision under appeal, the examining division considered that document D2 describes that the co-expression of heterologous *acyB1* and *acyB2* genes in *Streptomyces lividans* TK 24 strain results in higher expression of acyB1 and in an increased conversion activity of leucomycin U and of its yield compared to strains transformed with only a heterologous *acyB1* gene (see paragraph [0030]; Fig.8 and 10). Thus, document D2 proposed said co-expression to lead to an increased yield in 4"-acylated macrolide antibiotics.

9.11 The board considers that the increased expression of *acyB1* mRNA and the overall increased conversion of leucomycin U macrolide antibiotic in document D2, regardless of its form, i.e. acylated or non-acylated at position 3-OH of the macrolide core, could have motivated the skilled person to use the non-spiramycin-producing *Streptomyces lividans* TK 24 mutant strain to increase the overall yield of 4"-acylated spiramycins, regardless of the isovaleryted forms, but it could not have derived from document D2 that the reduced yield observed in spiramycin-producing Streptomyces spiramyceticus parent and daughter strains F21 and WSJ-2, both with an inactivated *sspA* gene encoding the 3-O-acyltransferase, could be overcome and increased by

the mere co-expression of a heterologous *acyB1/ist* and *acyB2* gene.

9.11.1 For example, Figure 8 of document D2 shows that the leucomycin U conversion level in non-spiramycin-producing *Streptomyces lividans* TK 24 strains transformed with a plasmid encoding a heterologous *acyB1/ist* gene was lower than when it was transformed with a plasmid co-expressing *acyB1* and *acyB2*.

9.11.2 From Table 1 of document D2 the skilled person derives that the conversion level from leucomycin U to its 4"-isovalerylated form(s) (leucomycin A3) in *Streptomyces thermotolerans* is 38 % while in a *acyB2* mutant strains the conversion was less than 1 %. Transformation of these *acyB2*$^-$ mutants with a vector encoding *acyB2* restores the conversion level to some degree. The results in Table 1 vary however widely (13% conversion rate and 95% conversion rate, respectively).

The board is therefore of the opinion that the skilled person, in view of the data presented in document D2, would not have expected the co-expression of acyB1 and acyB2 in *Streptomyces spiramyceticus* F21 and WSJ-2 to successfully solve the technical problem underlying the claimed invention.

9.11.3 In point 15.7.2 of the decision under appeal, the examining division argued that the skilled person would have considered applying the teaching of document D2 because the HPLC profile in Figure 5a and b of document D1 showed a similar isovaleryl spiramycin I peak for strains WSJ-1 and WSJ-2. This suggested that the negative effect of the inactivation of *sspA* would not jeopardize the beneficial effect described in document

D2 (i.e. increased yield upon co-expression of acyB1 and acyB2).

9.11.4 There may by many reasons why the yield of isovaleryl spiramycin I produced by *Streptomyces spiramyceticus* F21 and WSJ-2 mutant strains after inactivation of the *sspA* gene - encoding a 3-O-acyltransferase - is low. The low yield may or may not be related to a low level of 4"-isovaleryltransferase acyB1/ist.

9.11.5 Even if document D2 used heterologous *acyB1-acyB2* genes in antibiotic-producing strains and obtained an increased overall yield of acylated and non-acylated macrolide antibiotics, no conclusion can be drawn with regard to the yield of other macrolide antibiotics produced in different *Streptomyces* strains incapable of producing acylated macrolide antibiotics to high yield because their *sspA* gene was inactivated.

9.12 In point 15.8 of the decision under appeal, the examining division addressed appellant's argument that the skilled person had no reasonable expectation of success when applying the teaching of document D2, transformation of S. lividans with heterologous genes from S. thermotolerans, to S. spiramyceticus due to the different genetic background. The examining division was not convinced by this argument "since D2 and the present application both rely on the linked acyB1-acyB2 genes from *S. thermotolerans* (see e.g. last paragraph on page 5 of the present application; and paragraph 7 in D2), and the description in D2 of increased yield in a *S. lividans* strain can thus be considered to provide a reasonable expectation of also obtaining an increasing yield in yet another strain of *Streptomyces* than *S. thermotolerans* or *S. lividans*, while the

selection of an *S. spiramyceticus* strain originates from closest prior art D1".

9.13 The board is not convinced by this argument. It appears that an argument based on the fact that both strains, *S. lividans* and *S. spiramyceticus*, were successfully transformed with the same heterologous element to produce a high yield of 4"-acylated macrolide antibiotics, particularly the isovaleryl spiramycin I, relies on experimental results described in the patent application, and thus can only be based on hindsight.

9.14 Thus, the skilled person starting with the teaching of document D1, faced with the above technical problem would not have arrived at the claimed solution in an obvious way and with a reasonable expectation of success based on document D1 in combination with document D2.

10. Also if, for the sake of the argument, the skilled person would have started from document D2 as the closest prior art, the claimed invention is not obvious.

10.1 Document D2 addresses the problem of increasing the yield of 4" acylated leucomycin U or of macrolide antibiotic in general. The subject-matter of claim 1 is a Streptomyces spiramyceticus strain producing isovaleryl spiramycin I with good yield. The technical effect resulting from this difference is the production of a different and particular form of a macrolide antibiotic with good yield and with improved purity. Starting from document D2, the technical problem may thus be defined as the production of an alternative particular form of a macrolide antibiotic with good yield and improved purity. The board finds no reason

why the skilled person would be motivated to combine the teaching of documents D2 and D1 to solve the technical problem identified above with a reasonable expectation of success. After all, isovaleryl spiramycin I is just one of many possible alternatives. Moreover, the board is of the opinion that a skilled person would not turn its attention to a *Streptomyces* strain known to produce the alternative antibiotic in insufficient amounts. The skilled person could have tried but would not have had a reasonable expectation of success when combining the teaching of documents D2 and D1 as they relate to different *Streptomyces* strains producing different macrolide antibiotics, modified differently, i.e. unacylated 3-OH form I, by prima facie independent steps in the biosynthesis of the antibiotic.

10.2 Hence, the board concludes that the product of claim 1 and also the method of claim 2 using the product of claim 1 meet the requirements of Article 56 EPC.

Order

For these reasons it is decided that:

1. The decision under appeal is set aside.

2. The case is remitted to the examining division with the order to grant a patent on the basis of claims 1 and 2 of the main request and a description to be adapted.

The Registrar: The Chairman:

L. Malécot-Grob B. Stolz

Decision electronically authenticated