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Datasheet for the decision of 9 March 2010

Case Number: T 0542/08 - 3.3.08

Application Number: 95935394.7

Publication Number: 0792363

IPC: C12N 15/32

Language of the proceedings: EN

Title of invention:
Novel pesticidal proteins and strains

Patentee:
Syngenta Participations AG

Opponent:
Bayer S.A.S

Headword:
Vegetative Insecticidal Proteins/SYNGENTA

Relevant legal provisions:
EPC Art. 123(2)

Relevant legal provisions (EPC 1973):
EPC Art. 54(1)(2), 56, 83

Keyword:
"Main request - added subject-matter (no)"
"Sufficiency of disclosure (yes)"
"Novelty (yes)"
"Inventive step (yes)"

Decisions cited:
-

Catchword:
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Case Number: T 0542/08 **-** 3.3.08

D E C I S I O N
of the Technical Board of Appeal 3.3.08
of 9 March 2010

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Decision under appeal:
Decision of the Opposition Division of the European Patent Office posted 22 November 2007 revoking European patent No. 0792363 pursuant to Article 102(1) EPC 1973.

Composition of the Board:

Chairman: L. Galligani
Members: P. Julià
T. Karamanli

Summary of Facts and Submissions

I. European patent no. 0 792 363 with the title "Novel pesticidal proteins and strains", based on the published international application WO 96/10083 (referred to as "*the application as filed*" hereinafter) and having the filing date of 27 September 1995, was granted with a set of 32 claims. The patent claimed two priorities, namely from US 314594 (28 September 1994) and from US 463483 (5 June 1995).

II. An opposition was filed raising grounds under Article 100(a) to (c) EPC 1973. The opposition division considered that the main request, the first auxiliary request and the second auxiliary request then on file did not fulfil the requirements of Article 123(2) EPC 1973. The third and fourth auxiliary requests were considered to contravene Article 56 EPC 1973. Consequently, the patent was revoked (Article 102(1) EPC 1973).

III. The patentee (appellant) filed a notice of appeal with letter dated 25 January 2008. The statement setting out the grounds of appeal together with a new main request and a declaration of J.J. Estruch (D14, *infra*) were filed on 1 April 2008.

IV. On 7 August 2008, the opponent (respondent) filed observations on the appellant's grounds of appeal.

V. On 2 December 2009, the board summoned the parties to oral proceedings. A communication pursuant to Article 15(1) of the Rules of Procedure of the Boards of Appeal (RPBA), annexed to the summons, informed the

parties of the preliminary, non-binding opinion of the board on the issues of the appeal proceedings.

VI. On 9 February 2010, the appellant replied to the board's communication and filed a new main request and auxiliary requests 1 to 4. The **main request** contained 27 claims of which claims 1 and 7 read as follows:

"1. A DNA molecule encoding a vegetative insecticidal protein isolatable during the vegetative growth phase of *Bacillus spp.*, wherein said protein is isolatable from liquid culture media, and wherein said protein is encoded by a nucleotide sequence that hybridizes to a nucleotide sequence of SEQ ID NOs: 1, 3 or 4 at 65°C in a buffer comprising 7 % SDS and 0.5 M sodium phosphate."

"7. A DNA molecule according to claim 1 obtainable by a process comprising
a) obtaining a DNA molecule comprising a nucleotide sequence encoding a vegetative insecticidal protein; and
b) hybridizing said DNA molecule with an oligonucleotide probe comprising a contiguous portion of the coding sequence for the said insect-specific protein at least 10 nucleotides in length obtainable from a DNA molecule defined in SEQ ID NO: 1, SEQ ID NO: 3, or SEQ ID NO: 4; and
c) isolating said hybridized DNA."

Claims 2 to 6 were embodiments of claim 1. Claims 8 and 9 were directed to an expression cassette comprising a DNA molecule according to any one of claims 1 to 7. Claim 10 related to a vector molecule comprising an

expression vector according to claim 8. Claims 11 to 14 were directed to a host organism comprising a DNA molecule according to claims 1 to 7. Claims 15 to 21 related to a transgenic plant as well as progeny and seed thereof comprising a DNA molecule of any one of claims 1 to 7 or an expression cassette according to claim 9, stably incorporated into the plant genome. Claim 22 concerned a seed of a plant according to any one of claims 15 to 20 treated with a seed protecting coating. Claim 23 was directed to a method for isolating a DNA molecule according to claim 1, wherein said method comprised the steps of claim 7. Claims 24 and 25 related to a method of increasing insect target range. Claims 26 and 27 related, respectively, to a method of protecting plants against damage caused by an insect pest and to a method of producing a plant or plant cell.

VII. Oral proceedings took place on 9 March 2010.

VIII. The following documents are cited in this decision:

D1: WO 94/21795 (publication date: 29 September 1994);

D3: V. Sekar et al., Proc. Natl. Acad. Sci. USA, October 1987, Vol. 84, pages 7036 to 7040;

D8: T. Thanabalu et al., J. Bacteriol., May 1991, Vol. 173(9), pages 2776 to 2785;

D14: Declaration of J.J. Estruch, Ph.D., signed on 31 March 2008;

D17: M. Jucovic et al., Protein Engineering, Design & Selection, 2008, Vol. 21(10), pages 631 to 638.

IX. The appellant's arguments may be summarised as follows:

Admissibility of the main request

The main request was filed in direct reply to the board's communication and intended to overcome the objections raised therein.

Main request
Article 123(2) EPC

Claims 7 and 23 had a basis in the application as filed. Page 7 of the description referred to a DNA molecule which was defined as in the main request and which hybridized to a DNA molecule of the invention or to an oligonucleotide probe obtainable from that DNA molecule. Claim 109 of the application as filed referred to an oligonucleotide probe according to claim 107 derived from SEQ ID NO: 28, 30 or 31 which corresponded to SEQ ID NO: 1, 3 or 4 of the main request. The probe was defined in claim 107 as having a contiguous portion of the coding sequence of at least 10 nucleotides in length. Since none of the specific SEQ ID NO given in the claims had a contiguous sequence of at least 10 nucleotides common to the DNA molecule encoding the mosquitocidal toxin from *B. sphaericus* SSII-1, the absence in the main request of the limitation present in claim 107 of the application as filed was irrelevant.

Articles 84, 87 and 54(1),(2) EPC 1973

Although none of the claims of the main request was entitled to any of the claimed priorities, this finding was of no consequence on the issue of novelty because no relevant prior art was cited under Article 54(2) EPC 1973.

Article 56 EPC 1973

The closest prior art was D1 which disclosed a new class of (vegetative) insecticidal proteins (VIPs) produced during the vegetative growth phase of ***Bacillus*** which were secreted into the cultivation medium. These VIPs were different from other known insecticidal proteins from ***Bacillus***, such as the ***B. thuringiensis*** (Bt) insecticidal crystal proteins (ICPs) produced during sporulation. D1 showed these VIPs to be multimeric, i.e. a binary system comprising two proteins: a large insecticidal protein which exhibited full insecticidal activity only in the presence of a smaller auxiliary protein. Examples 1 to 12 disclosed the VIPs from the ***B. cereus*** (Bc) strain AB78: VIP1 (80 kDa) and VIP2 (35 kDa). Examples 16 to 18 described the presence of VIPs in the Bt strain AB88: a large 80 kDa and a smaller 35 kDa protein.

The patent-in-suit disclosed the cloning of a DNA sequence encoding an insecticidal protein (VIP3) from the Bt strain AB88. The VIP3 protein was different from the known ICPs and the VIP1/VIP2 system disclosed in D1 and had a different mode of action and a unique insecticidal range that made it extremely useful and valuable as shown by its commercial success.

Starting from D1, the problem to be solved was the provision of genes encoding alternative proteins conferring insecticidal activities to the supernatants of *Bacillus* strains. The solution was represented by the claimed VIP3A(a) and VIP3A(b) from the Bt strains AB88 and AB424, respectively.

Nothing in D1 suggested that the 80 kDa protein from the Bt strain AB88 had by itself insecticidal activity. Indeed, by way of analogy to the VIP1/VIP2 system of the Bc strain AB78, the insecticidal activity of the Bt strain AB88 was expected to be found only in the presence of both the 80 kDa and the 35 kDa protein. Although Examples 4 and 5 of D1 referred to VIP1 as having insecticidal activity, these examples disclosed only a preliminary characterization of the biologically active principle. However, once sufficient data were available to come to a final conclusion, VIP2 was included as a mandatory component of the VIP1/VIP2 system as shown in Example 11. Both VIP1 and VIP2 were required for insecticidal activity and their genes were closely linked as shown in SEQ ID NO: 1, where the sequence encoding VIP2 was separated from that encoding VIP1 by only four nucleotides. The presence of a minimal insecticidal activity in a plasmid having a full-length gene encoding VIP1 but without a gene encoding VIP2 (pCIB6023) was likely due to the presence of a still significant part of the latter gene. From the results shown in Table 19 and the explanations given in Example 11, it was evident that neither VIP2 nor VIP1 alone had any insecticidal activity - as also shown in the post-published evidence D17 on file. Thus, the skilled person (defined in the case law as being

cautious) would not have focused his or her interest on cloning a single gene encoding the 80 kDa protein from the Bt strain AB88 but would have rather tried the cosmid cloning strategy outlined in Examples 9 to 11 of D1 for the VIP1/VIP2 system from the *Bc* strain AB78 in order to identify a contiguous sequence in the genome of the Bt strain AB88 containing the nucleotide sequences encoding both the 80 kDa and the 35 kDa protein. Any other approach could only be based on hindsight knowledge of the patent-in-suit.

Dr Estruch in his declaration (cf. document D14) showed that the cosmid cloning strategy failed. The combination of an expectation for an inexistent contiguous sequence, a low representation of the VIP3 gene encoding the 80 kDa protein in the cosmid library and the inherent shortcomings of the probes designed on the basis of the N-terminal sequence of the 80 kDa protein disclosed in D1 (with an error at position nine), made it impossible to identify a positive clone. In particular, the failures reported in D14 were explained by the high content of A and T and the high degree of degeneracy of any oligonucleotide probe derived from the N-terminal sequence disclosed in D1, and the fact that the high degeneracy affected the middle of these oligonucleotide probes. No alternative strategies for designing probes would have been contemplated, since their outcome was unpredictable. Nor was any evidence on file showing that any of these possible alternative probes, in particular one derived from the first seven residues of the N-terminal sequence of the 80 kDa protein disclosed in D1, would have resulted in the identification of a positive clone comprising a nucleotide sequence encoding the VIP3

protein. As stated in the declaration of Dr Estruch, it was only by shear luck and after many attempts that a longer N-terminal sequence was obtained and a suitable seven amino acid sequence identified which allowed the cloning of a gene encoding the VIP3 protein.

There was no reason to put in doubt the statements made in D14. Nor had any contradicting evidence been put forward during appeal proceedings. The report in D14 was not in contradiction with the results of the patent-in-suit, which described in Example 7 the cloning of the genes encoding the VIP3 proteins from the Bt strains AB88 and AB424. Example 7 showed the use of a longer probe derived from the first 11 residues of the correct N-terminal sequence of the 80 kDa protein in an enriched *XbaI* restriction library. According to D14, the use of a restriction library and a new cloning strategy based on a single gene was developed only after analyzing the results obtained by way of a cosmid cloning library. These results were not mentioned in the patent-in-suit. However, they were essential for devising the cloning strategy described in Example 7.

As for claim 15, it had to be read with a mind willing to understand, as required by the case law. If so, it was immediately apparent that the technical effect upon which the inventive step of the patent-in-suit was based underlay the subject-matter of that claim.

Article 83 EPC 1973

The patent-in-suit disclosed the VIP3 sequences which allowed the skilled person to achieve the subject-matter of claims 7 and 23 without undue burden.

No technical difficulties were encountered to identify and select fragments from these VIP3 sequences of at least 10 nucleotides in length and fulfilling the requirements of those claims.

X. The respondent's arguments may be summarised as follows:

Admissibility of the main request

There were no objections to the admissibility of the main request.

Main request
Article 123(2) EPC

There was no support in the application as filed for claims 7 and 23 which related to a family of DNA molecules having the properties cited in claim 1 and being obtainable by using an oligonucleotide probe of at least 10 nucleotides in length obtainable from a specific DNA molecule defined in SEQ ID NO: 1, 3 or 4. No support could be derived from the generic disclosure found on page 7 of the application because there were no references therein to these specific sequences nor was a support derived from the original claims because they referred to oligonucleotide probes which were defined as being not derived from the mosquitocidal toxin of ***B. sphaericus*** SSII-1, a limitation which was not present in claims 7 or 23.

Articles 84, 87 and 54(1) EPC 1973

There were no objections under Articles 84 and 54(1) EPC 1973.

Article 56 EPC 1973

The closest prior art was D1 which disclosed the cloning of a gene encoding the insecticidal protein VIP1 from the Bc strain AB78. Example 11 mentioned the presence of an auxiliary protein VIP2, but little emphasis was placed on its alleged crucial role for the activity of the VIP1 protein. D1 also disclosed the insecticidal activity of a secreted fraction from the Bt strain AB88 against the Black Cutworm, ***Agrostis ipsilon*** (BCW). This was of utmost importance because no insecticidal proteins had been identified so far to control that insect. Thereby, the skilled person was made aware of a new class of insecticidal proteins. D1 further disclosed the N-terminal sequences of two proteins, one of 80 kDa and one of 35 kDa, present in said active secreted fraction from the Bt strain AB88. In Example 18, these two proteins were stated to be possibly responsible - singly or in combination - for the activity against the BCW. Nothing in D1 indicated that the insecticidal activity had to be necessarily associated only to the combination of both proteins. Thus, D1 clearly indicated that a single protein could be responsible for the insecticidal activity against BCW.

Starting from this prior art, the technical problem to be solved was the cloning of the gene encoding the insecticidal protein active against BCW. The claimed VIP3A(a) and VIP3A(b) genes from the Bt strains AB88 and AB424, respectively, represented the allegedly inventive solution.

In Example 11 and Table 19 of D1, the insecticidal activity of the VIP1/VIP2 system was associated with VIP1 and the role of VIP2 was defined only in a vague manner. The activity of plasmid pCIB6023 was attributed to "only VIP-1" without any reference to VIP2. Even if the presence of part of the gene encoding VIP2 was identified, its relevance on the activity of VIP1 was unknown and not disclosed in D1. This was in line with the results obtained with plasmid pCIB6203, for which a frameshift mutation in the gene encoding VIP1 resulted in a total loss of insecticidal activity. The statement in Example 11 that both VIP1 and VIP2 were required to obtain maximal activity did not necessarily imply that the genes encoding these two proteins were genetically linked. Nor was any reference in D1 to SEQ ID NO: 1 as showing that linkage. In fact, D1 was mainly concerned with VIP1 and contained only a few information (molecular weight, gene location, etc.) on the auxiliary protein VIP2 and its gene.

Thus, based on the knowledge of VIP1, which in Example 4 of D1 was also identified as the biologically active protein from the Bc strain AB78 with a molecular weight of 80 kDa, the skilled person, when faced with the above technical problem, would have obviously undertaken the cloning of the gene encoding the 80 kDa protein from the Bt strain AB88 thereby arriving at the present invention. At the priority date, technical knowledge was available for cloning that gene. In view of the unique insecticidal active of the secreted fraction from the Bt strain AB88, a motivation was also there for the skilled person to undertake it. There was a reasonable expectation of success because no technical difficulties were foreseeable based on D1 nor

obstacles would have been encountered by the skilled person when following standard cloning methods.

Although Dr Estruch in his declaration D14 reported that initial standard cloning strategies failed, the main reason given for that failure, namely the high degeneracy of the designed probe, was not correct on the facts. The information provided in D1 made it possible to design an oligonucleotide probe - based on the first seven residues of the N-terminal sequence of the 80 kDa protein (and thus, without having any error in its sequence), which had the same level of degeneracy as the probe derived from a longer N-terminal sequence referred to in D14. Indeed, a probe with the same level of degeneracy had been used in Example 6 of D1 to clone the VIP1 gene. In any case, if degeneracy was a problem for designing a suitable probe, methods were known to the skilled person for overcoming that problem, such as designing a set of degenerate probes, decreasing the number of possible codons by selecting those known to be mostly used by the concerned species from which the gene was cloned (as shown in Example 6 of D1), etc. There were thus doubts about the reliability of the statements made in the said declaration.

Alternative cloning strategies were also known to the skilled person, such as screening for positive clones using a bioassay based on the insecticidal activity of the recombinant protein as shown in prior art document D8. Example 10 of D1 indicated that the gene(s) were in a single fragment of only 6 kb which resulted in the production of insecticidal activity. Thus, D1 did not necessarily direct the skilled person to the building

of a cosmid cloning library. In view of the size of that 6 kb fragment, the use of a restriction library could also have been contemplated for cloning the gene(s) encoding the insecticidal protein(s) from the Bt strain AB88. The declaration of Dr Estruch indeed confirmed that the gene encoding the VIP3 protein was better represented in a restriction library than in a cosmid library and that no technical problems were encountered when screening that library with a probe derived from the first seven residues of the N-terminal sequence of the 80 kDa protein and/or to detect its insecticidal activity. There was also prior art on file showing the use of restriction libraries for cloning insecticidal proteins from other Bt strains (cf. ***inter alia*** D3). In fact, the skilled person, having a cautious attitude, could also have contemplated two possible cloning strategies, one for large and one for small fragments, the former using a cosmid cloning library and the latter a restriction library. Another possible approach was the purification of the 80 kDa protein from the Bt strain AB88 so as to obtain internal peptides and a longer N-terminal sequence and to derive therefrom a suitable probe. No inventive skill was required for carrying out this approach, which was also explicitly mentioned in D1 as a possible alternative. Example 7 of the patent-in-suit showed that no problems were encountered for cloning the VIP3A(a) gene using a 33 bp long oligonucleotide probe based on the first 11 residues of the N-terminal sequence of the 80 kDa protein active against ***Agrotis ipsilon***. Thus, standard cloning methods were available to the skilled person at the priority date as well as means and alternatives to overcome any possible initial failures or difficulties.

As for claim 15, there was no requirement for the transgenic plants (and parts, progeny and seed thereof) to express the DNA molecule encoding the VIP3 protein. Therefore, the claim covered embodiments that could not rely on the technical effect on which the inventive step of the patent-in-suit was based. These embodiments did not solve any technical problem and thus, they did not fulfil the requirements of Article 56 EPC 1973.

Article 83 EPC 1973

Article 100(c) EPC 1973 was an initial ground of opposition and, since claims 7 and 23 had been amended, objections against these claims under Article 83 EPC 1973 had to be admitted into the proceedings. It was stated by Dr Estruch in his declaration D14 that none of the short oligonucleotide probes used was suitable for cloning a nucleotide sequence encoding the VIP3 protein. Thus, the selection of short probes as those defined in claim 7 could not be performed without undue burden. The selection of a probe having a length of at least 10 nucleotides obtainable from SEQ ID NO: 1, 3 or 4 for isolating a DNA molecule according to claim 1 required undue burden. The more so because the experiments referred to in D14 were performed at a low hybridization temperature (32°C, low stringency), whereas claims 7 and 23 contemplated higher hybridization temperatures (65°C, high stringency, as defined in claim 1).

XI. The appellant (patentee) requested that the decision under appeal be set aside and the patent be maintained on the basis of the main request or one of the

auxiliary requests 1 to 4, all filed with letter dated 9 February 2010.

XII. The respondent (opponent) requested that the appeal be dismissed.

Reasons for the Decision

Main request
Admissibility

1. The main request was filed in reply to the board's communication pursuant to Article 15(1) RPBA within the time limit set therein for receipt of written submissions (cf. point V ***supra***). In that communication, the board drew the attention of the parties to several objections concerning ***inter alia*** Article 123(2) EPC. The main request intends to overcome them. The respondent has not argued against admitting this request (cf. point X ***supra***) nor did the board see any reason against its admission. Thus, the main request was admitted into the appeal proceedings.

Article 123(2) EPC

2. Although claims 7 and 23 were present in the main request filed with the statement of grounds of appeal, the objection against their subject-matter was raised for the first time at the oral proceedings before the board. However, Article 100(c) EPC 1973 was an initial ground of opposition and the respondent's objection arises from an amendment of the granted claims. Moreover, since the respondent's objection does not

raise any complex technical issue and the appellant has not protested against its introduction into the proceedings, the board sees no reason for not admitting it into the appeal proceedings.

3. Claim 109 of the application as filed was concerned with a DNA molecule encoding an insecticidal protein as claims 7 and 23 of the main request, said protein being obtainable by hybridisation with an oligonucleotide probe obtained from a DNA molecule comprising a nucleotide sequence defined by the same SEQ ID NOs as in claims 7 and 23 of the main request (Seq.ID No. 28, 30 and 31 referred to in the said claim 109 are the same as Seq.ID No. 1, 3 and 4, respectively). The said probe was in accordance with original claim 107, i.e. an oligonucleotide probe comprising a contiguous portion of the coding sequence for an insect-protein isolatable during the vegetative growth phase of ***Bacillus*** spp at least 10 nucleotides in length. However, as noted by the respondent, claim 107 of the application as filed required that "***said protein is not the mosquitocidal toxin from B. sphaericus SSII-1***". There is no such requirement in claims 7 or 23 of the main request. Thus, the question arises whether in the absence of this feature, claims 7 and 23 offend against Article 123(2) EPC.

4. It is noted that on page 7 of the application as filed (cf. page 7, third paragraph from the bottom), there is a generic disclosure for these DNA molecules which are characterized by hybridizing to "***a DNA molecule of the invention as defined hereinbefore***" - including thereby SEQ ID NOs: 1, 3 and 4 of claims 7 and 23 - under the hybridization conditions of claim 1 of the main request

and using an oligonucleotide probe that comprises a contiguous portion of the coding sequence for an insect-specific protein defined as above and of at least 10 nucleotides in length. Here there is no specific reference to the mosquitocidal toxin from *B. spahericus* SSII-1.

5. Moreover, the appellant's statement that none of the specific SEQ ID NOs of the main request has a contiguous sequence of at least 10 nucleotides common to the DNA molecule encoding the mosquitocidal toxin from *B. spahericus* has not been contested. Nor has any evidence been put forward against the statement of the opposition division that there is only a very low degree of sequence identity between the DNA molecule encoding the mosquitocidal toxin and the specific sequences of the main request (cf. page 8, lines 1 to 7 of the decision under appeal).

6. In the light of the above, the board considers that claims 7 and 23 comply with the requirements of Article 123(2) EPC.

Articles 84, 87 and 54(1),(2) EPC 1973

7. No objections have been raised under Article 84 EPC 1973. Nor does the board see a reason to raise any objection under this Article.

8. None of the claims is entitled to any of the two claimed priority dates (28 September 1994 and 5 June 1995). SEQ ID NO: 1 and 2 are different from those sequences disclosed in the first and second priority documents and differences are also found between SEQ ID

NO: 3 and the sequence present in the second priority document. Hence, their effective date is the filing date of the international application, namely 27 September 1995 (cf. point I *supra*).

9. Document D1, with publication date 29 September 1994, is thus prior art according to Article 54(2) EPC 1973. However, no objections have been raised under Article 54(1) EPC 1973 against the subject-matter of the main request. Nor does the board see a reason to raise any objection against novelty. The requirements of Article 54(1) EPC 1973 are thus fulfilled.

Article 56 EPC 1973

The closest prior art

10. The closest prior art is represented by document D1 which aims at overcoming the limitations of the known insecticidal proteins, such as the insecticidal crystal proteins (ICPs) from *Bacillus thuringiensis* (Bt) produced during sporulation, in particular their absence of significant effects on the genus *Diabrotica* (cf. page 1, line 21 to page 2, line 5). To this extent, D1 discloses the isolation of vegetative insecticidal proteins (called VIPs) produced during the vegetative growth phase of *Bacillus* strains, such as Bt and *Bacillus cereus* (Bc) (cf. *inter alia* page 2, lines 15 to 18 and page 13, lines 1 to 13). Reference is made to the use of the VIPs alone or in combination with auxiliary proteins, the latter being able to enhance the insecticidal activity of the former. Both insecticidal and auxiliary proteins may be components of a multimeric protein (cf. page 17, line 14 to page 18, line 18). D1 refers to nucleotide sequences

from Bacillus encoding these proteins, their optimization for expression in plants (cf. ***inter alia*** page 19, lines 4 to 25), expression cassettes, host organisms, etc.

11. Examples 1 to 4 and 7 disclose the isolation of the Bc strain AB78 (NRRL B-21058), which is active against several ***Diabrotica*** spp. in particular against Western corn rootworm (WCW) (cf. page 32, Table 14), and the purification and characterization of a 80 kDa protein (VIP1) identified as the biologically active protein. Examples 5 and 6 disclose the first 16 amino acid residues of the N-terminal sequence of VIP1 and the construction of an oligonucleotide probe derived from that sequence. Example 8 provides PCR data of the Bc strain AB78 and Example 9 discloses the cosmid cloning of total DNA from said strain and the isolation of five clones active against WCW - by screening first for that activity and further corroboration by hybridization with the VIP1 probe (cf. page 41, Table 18). A 6kb region active against WCW (pCIB6022, NRRL B-21222, SEQ ID NO: 1) is disclosed in Example 10 and the functional dissection of that VIP1 DNA region by subcloning is shown in Example 11 (cf. page 44, Table 19).

12. The isolation and characterization of the insecticidal activity from other ***Bacillus*** strains is disclosed in Examples 14 to 16 and 19. In particular, Example 16 describes the isolation and characterization of the Bt strain AB88 (NRRL B-21225) and Examples 17 and 18 report the purification and characterization of the VIPs from that strain. The activity of that strain against ***Agrotis ipsilon*** is found to be associated with a 80 kDa protein "***and or***" a 35 kDa protein "***singly or***

in combination", wherein these proteins are not related to any Bt δ-endotoxin as evidenced by the lack of sequence homology of their N-terminal sequence to those of the known toxins (cf. page 50, line 21 to page 51, line 10).

The patent-in-suit

13. The patent-in-suit provides the full disclosure of the nucleotide and amino acid sequences of the 80 kDa proteins designated as VIP3A(a) and VIP3A(b) which were respectively isolated from the Bt strains AB88 (the same strain as in D1) and AB424 (not disclosed in D1) and were defined as a new class VIP3 of insect-specific proteins with an unique spectum of insecticidal activity (cf. paragraph [0073]). The two VIP3A proteins are identical except for the following changes: Leu206Arg, Gln284Lys, Thr291Pro, Glu406Gly (VIP3A(a)/VIP3A(b)).

Analysis of inventive step

14. Starting from document D1, the underlying technical problem may be seen in the provision of genes encoding alternative active VIPs with a broad spectrum of insecticidal activity. The claimed DNA molecules encoding the VIP3A(a) and VIP3A(b) from, respectively, the Bt strains AB88 and AB424 represent a valid solution to said technical problem.

15. The board is convinced that the skilled person faced with the stated problem would have had a strong motivation to continue the work of document D1 with the aim of expanding the biological control agents, in

particular by further investigating the VIPs from the Bt strain AB88 reported therein, i.e. by trying to fully identify and characterise the genes encoding them. In said document, the VIPs from strain AB88 were disclosed together with the analogous VIP1/VIP2 system from the Bc strain AB78, and were defined as further members of the newly identified class of VIPs (an assumption later shown to be erroneous - cf. D17 - as they represented yet another class of insecticidal proteins). The motivation was further fuelled by the observation of the detected unique, advantageous activity of said VIPs against *A. ipsilon*. Since the Bt strain AB88 had been deposited and was thus available to the skilled person, it would have been obvious to start from that strain and try to clone and identify the genes encoding its insecticidal proteins. Special attention would have been put on the gene encoding the 80 kDa protein because: i) the activity of the Bt strain AB88 against *A. ipsilon* was described as being associated with a 80 kDa protein "*and or*" a 35 kDa protein "*singly or in combination*" (cf. Example 18); ii) the experimental work on the VIP1/VIP2 system in Example 4 identified VIP1 from the Bc strain AB78 (with a molecular weight in the range of 80 kDa) as the active protein; and iii) Example 11, which described plasmid pCIB6023 as containing "*only VIP-1*" and showed it in Table 19 to be active against western corn rootworm (WCRW), supported that conclusion. In the board's judgement, the cloning method of choice for pursuing the work with Bt strain AB88 would have been that described in detail in Examples 9 and 10 of the same document in relation to the analogous VIP1/VIP2 system from the Bc strain AB78, i.e. a cosmid cloning library (cf. point 11 *supra*).

16. Thus, the technical circumstances of this case were as follows: to the person skilled in the art faced with the problem of finding alternative VIPs the prior art document D1 indicated in the 80 kDa VIP from Bt strain AB88 a promising candidate; the N-terminal sequence thereof was disclosed and the experimental protocol for cloning was also available from the examples dealing with a similar VIP from Bc strain AB78. Therefore, from a purely theoretical point of view, it can be said that for the skilled person it was obvious to try to complete the work described in document D1 and that ***a priori*** he or she would not have expected any major obstacle/difficulty in carrying out the work.

17. However, the declaration of Dr Estruch on file (cf. document D14) indicates that "in real life", i.e. when trying to clone the gene by using the above standard approach, cloning was not so straightforward as one might have expected and that a different approach had to be taken by which success was finally achieved. Dr Estruch refers to the earlier attempts made by using conventional methods of cosmid library construction and screening which all resulted in dead-ends, and to a second approach in which recourse was made to the N-terminal sequence of the 80 kDa and 35 kDa proteins from the Bt strain AB88 described in Example 18 of D1 which also resulted in a failure. He then describes that attempts were made to obtain further information about larger N-terminal sequences and refers in particular to a 38 amino acid long N-terminal sequence which luckily contained a stretch of 7 amino acids that showed no degeneracy and thus permitted the synthesis of a more suitable and stable

probe which allowed the detection by Southern blot analysis of a distinctive hybridization band of 4.5 kb XbaI. The identification of that band led to the use of an AB88 DNA enriched library (using XbaI restriction enzyme) and, using the above referred stable probe, to the identification and characterisation of the gene.

18. The above statements of Dr Estruch were not essentially contradicted by the respondent which, without relying on any specific expert declaration or experimental evidence, in short submitted that:

a) Degeneracy could not have represented a problem for the skilled person because the N-terminal sequence of VIP 80 kDa disclosed in Example 18 of D1 allowed the design of suitable probes based also on the approach outlined in Example 6 of the same document as well as on general technical knowledge (use of shorter probe, codon usage of Bt, sets of degenerate probes);

b) Apart from the cosmid library approach, there were other known approaches such as screening for positive clones from the cosmid library by using a bioassay based on insecticidal activity of the recombinant protein as shown in prior art document D8 or using a restriction library as done in prior art document D3 for cloning insecticidal proteins from other Bt strains. The size of DNA fragment encoding the insecticidal protein (6kb) reported in Example 10 of D1 was compatible with the use of such alternative approaches so that the skilled person was not compelled to use the cosmid library approach only as described in D1. This was further demonstrated by the fact that Example 7 of

the patent-in-suit which indeed relied on the restriction library approach (cf. point X *supra*).

19. For the reasons given in points 20 to 32 hereinafter, the board does not agree with the respondent's view.

As regards the issue of the probe design

20. Example 6 of D1 exemplifies the synthesis of a probe for the gene encoding VIP1 from the Bc strain AB78 based on the N-terminal sequence disclosed in Example 5. The region encoding amino acids 3 to 9 from the first 16 residues of that N-terminal sequence was selected for its low degeneracy and used to synthesize the probe based on the codon usage of a Bt δ-endotoxin gene (cf. page 36, Example 6). Without departing from these teachings of D1, an oligonucleotide probe could also be synthesized based on the disclosed first 14 residues of the N-terminal sequence of the 80 kDa protein from the Bt strain AB88 (cf. page 51, Example 18). In line with these teachings, a region of low degeneracy would be selected and, as rightly identified by the respondent (cf. point X *supra*), found to be present in the first seven residues of that N-terminal sequence. However, only ***a posteriori*** and with the knowledge of the patent-in-suit, it is now known that these first seven residues are correct and that they do not comprise the erroneously identified amino acid proline (instead of serine) at the position nine of the N-terminal sequence shown in Example 18 of D1.

21. From the appellant's submissions (cf. point IX *supra*), the board understands that such an oligonucleotide probe was also contemplated in the assays reported by

Dr Estruch in his declaration. In particular, his references to "***designed and used degenerated oligos derived from these N-terminal sequences***" (i.e. from the 80 kDa and 35 kDa proteins from the Bt strain AB88) and to "***using oligos whose sequence was directly derived from the N-terminal sequence that had been analyzed up to that moment***" (which were identical to those shown in D1) as well as to the assays "***modifying the hybridization conditions***" and "***using the above oligos as primers in a PCR reaction to potentially amplify any sequence related to the protein or proteins of interest***" (cf. paragraphs bridging pages 2 and 3 in D14), indicate that all efforts were undertaken to succeed when screening the cosmid cloning library with oligonucleotide probes but to no avail.

22. As likely causes for the negative results, the declaration of Dr Estruch refers to ***inter alia*** the presence of high amounts of A or T in the synthesized oligonucleotide probes (more than 80% of the sequence, known to have a low annealing stability) and to their high degeneracy (cf. page 3, third full paragraph). According to the declaration, it was only after achieving a larger (38 residues) N-terminal sequence with an advantageous stretch of amino acids that a more stable probe with lower degeneracy could be obtained (cf. paragraphs bridging pages 3 and 4). Nevertheless, the respondent argues that the amount of A or T and the level of degeneracy of a probe derived from the seven first residues of the N-terminal sequence of the 80 kDa protein disclosed in D1 were in all similar to those of the probe referred to in the declaration and, therefore, if no problems were found with this latter probe, none

should be encountered with the latter probe (cf. point X *supra*).

23. Whereas the level of degeneracy of a probe might be of importance, its specificity will also depend on the quality of the sample used, i.e. on the number of target sequences (positive clones) and of non-target sequences which nevertheless still hybridize to the probe (false positive clones) present in that sample. Even though the specificity of a probe might be enhanced by increasing its quality (lower degeneracy and therefore, lower false positive clones), for a probe with a nucleotide sequence closely related to other non-target sequences which are broadly distributed in a cosmid library, the specificity will remain low or not significant at all. The screening of a cosmid library with that probe will result in the identification of a high number of false positive clones. This could explain the different results obtained when using the probe referred to by Dr Estruch D14 and that suggested by the respondent. Indeed, Dr Estruch refers to the identification of a positive clone with probes derived from the N-terminal sequence of the 80 kDa protein disclosed in D1 which, after isolation and characterization, turned out to be a PhoB gene (an alkaline phosphate regulator) completely different from an insecticidal protein (cf. page 3, second paragraph). In fact, this is also a reason not to rely on a single screening method but to use at least two different methods, such as the hybridization with probes and, when possible, a bioassay for activity. The chances to identify real positive clones and to discard the false ones at an early stage of the cloning

method are increased, avoiding thereby unnecessary time-consuming and costly expensive work.

24. Although, as stated by the respondent (cf. point X *supra*), several approaches were known to the skilled person at the filing date of the patent-in-suit for optimizing a probe based on the N-terminal sequence of the 80 kDa protein disclosed in D1, there is no evidence on file showing positive results with any of them for screening a cosmid cloning library wherein, according to the appellant, the gene encoding the 80 kDa protein from the Bt strain AB88 was under-represented. Nor were, according to Dr Estruch, those positive results obtained when "*using conventional methods of cosmid library construction and screening*" which might include some of these alternative standard approaches. As a matter of fact, in "real life" as stated by Dr Estruch in his outline of the history of the cloning, it was only after achieving a larger (38 residues) N-terminal sequence of the 80 kDa protein from the Bt strain AB88 and the use of a restriction cloning library that allowed cloning of the desired gene.

As regards an alternative method for screening the cosmid cloning library: bioassay for insecticidal activity

25. The use of (insecticidal) bioassays for screening cosmid cloning libraries and identifying toxic clones was a well-known method in the art as shown by D8, in which cosmid clones (20 to 40 kb) and restriction subclones (4.5 to 9.0 kb) were both assayed for insecticidal toxicity for isolating the gene encoding the 100 kDa mosquitocidal toxin from *B. sphaericus*

SSII-1 (cf. page 2776, right-hand column, last paragraph to page 2777, left-hand column, second full paragraph). The use of a VIP1/VIP2 insecticidal assay for screening the cosmid library and the (subcloning) restriction fragments from the Bc strain AB78 is also described in D1 (cf. Examples 9 and 10, respectively). Whereas in the cosmid library, hybridization with an oligonucleotide probe is carried out to corroborate the positive clones (cf. page 40, last paragraph), for sub-cloning, the activity is only tested after hybridization to a PCR probe (cf. page 42, lines 7 to 10). A similar approach was described in D3 for screening a restriction library and isolating the gene encoding a Bt ICP (cf. page 7036, right-hand column, last paragraph to page 7037, left-hand column, first full paragraph).

26. Although there is no reference in the declaration of Dr Estruch to any bioassay when describing the screening of the cosmid cloning library, the board understands from the appellant's submissions (supported by the reference to the use of "***conventional methods of cosmid library construction and screening***" on page 2 of the declaration) that such a bioassay (would have) resulted in the detection of no positive clones, the reason given being the low representation of the gene encoding the 80 kDa protein in the cosmid cloning library from the Bt strain AB88 (cf. point IX ***supra***). The failure to detect insecticidal activity would have been associated to the absence of a cosmid fragment large enough to comprise the genes encoding both the 80 kDa and the 35 kDa proteins, which - in analogy to the VIP1/VIP2 system - were (erroneously) expected to be the insecticidal and the auxiliary proteins from the Bt

strain AB88 (*supra*). The wrong explanation for that failure and the erroneous expectations would have discouraged the skilled person from using a restriction cloning library with even shorter restriction fragments. Only with the hindsight knowledge of the patent-in-suit would it have been foreseeable that the insecticidal activity was associated, only and exclusively, with the 80 kDa protein, that protein from the Bt strain AB88 being completely different from the VIP1 of the Bc strain AB78 disclosed in D1.

As regards the restriction library approach

27. In the board's judgement, the skilled person would not have immediately chosen a restriction library to isolate the gene encoding that 80 kDa protein from the Bt strain AB88.

28. First, there was no evidence that in the absence of an auxiliary protein (which in all probability would have been identified with the 35 kDa protein mentioned in Example 18 of D1), the level of insecticidal activity of the 80 kDa protein alone would have been sufficient to screen the cloning library as done for the VIP1/VIP2 system in Example 9. Indeed, D1 refers explicitly to insecticidal proteins for which no activity is found in the absence of an auxiliary protein (cf. page 18, lines 9 to 12) and the fact is, as shown by post-published D17, that this is what would actually have been found for VIP1 if the skilled person had decided to further analyze the activity of that protein, such as, for instance, in order to quantify the results shown in Table 19 of D1.

29. Second, the selection of a restriction cloning library based solely on the results shown in Example 10 (a 6 kb *Cla* I fragment encoding the whole VIP1/VIP2 system) merely disregards that these results were obtained only after the isolation of appropriate cosmid clones (cf. Table 18 of D1) and that there is no reason to assume the presence of similar clones in the Bt strain AB88. The less so because there is no explicit statement in D1 acknowledging the presence - let alone the relevance - of a genetic link between the genes encoding VIP1 and VIP2. Nor can the use of a restriction library be based on prior art concerned with other known (sporulation) insecticidal proteins, such as the Bt ICP described in D3, since they are clearly not related to - and have different modes of action than - those of the VIP1/VIP2 system disclosed for the first time in D1.

As regards Example 7 of the patent-in-suit

30. Example 7 discloses a restriction cloning library obtained by digestion of total DNA isolate AB88 with the restriction enzyme *Xba*I (cf. paragraph [0202] of the patent-in-suit), wherein this enzyme is directly derived from the experiments referred to in D14 (cf. page 4, second paragraph). The oligonucleotide probe used in Example 7 is longer (33 bases long, based on the first 11 N-terminal amino acids) than that suggested by the respondent (21 bases long, based on the first 7 N-terminal residues) and, the screening of the restriction cloning library, which according to D14 "*significantly enriched the presence of the potential gene of interest*" (cf. page 4, sixth and seventh paragraph of D14), cannot be compared with the screening of a cosmid cloning library in which that

gene is not enriched but under-represented. This is corroborated in Example 7 by the successful performance of insect bioassays in the restriction library showing again the advantageous use of two screening methods (cf. paragraph [0202] of the patent).

Conclusions on the obviousness of the cloning exercise

31. For the above reasons, the board concludes that the skilled person would not have departed from the teachings of D1 and that, in the absence of any indication to the contrary, a cosmid cloning library would be selected for cloning a gene encoding the insecticidal protein from the Bt strain AB88 (cf. point 15 *supra*). However, Dr Estruch in his declaration states that technical difficulties were encountered when using such a standard cosmid cloning library approach, the skilled person being led only to dead-ends when screening for positive recombinant clones and that success was achieved by a change of strategy (cf. point 17 *supra*).

32. The board considers that the alternative approaches, which according to the respondent would have been adopted in a straightforward manner by the skilled person, depart from the more immediate information and from the teachings directly derivable from the closest prior art D1 and that they could have been contemplated only if failures would have been encountered when applying this immediate information and teachings. Yet, these failures are to be interpreted in the context of that closest prior art, namely the finding of a new class of vegetative insecticidal proteins (VIPs) with the VIP1/VIP2 system from the Bc strain AB78, and,

obviously, without the knowledge of the patent-in-suit, namely that the VIP3 is a further new class of vegetative insecticidal proteins entirely different from the known ICPs and the VIPs disclosed for the first time in D1. It is in that context that the interpretation of the encountered failures (absence of insecticidal activity in a cosmid cloning library and no hybridization with the probe suggested by the respondent) and the possible use of other alternative approaches are considered by the board not to be straightforward for the skilled person.

The subject-matter of claim 15

33. The subject-matter of claim 15 is directed to a transgenic plant (as well as parts, progeny and seeds thereof) comprising a DNA molecule encoding VIP3 or an expression cassette comprising such a DNA molecule stably incorporated into the plant genome (cf. point VI ***supra***). There can be no doubt that the purpose of an expression cassette is the expression of the encoded VIP3 protein as it is also clearly derivable from the description of the patent-in-suit (cf. ***inter alia*** paragraphs [0093], [0095]). Although not stated explicitly, it is also implicitly derivable from the description when taken as a whole that the ultimate purpose of the DNA molecule in the claimed transgenic plant is the expression of the encoded VIP3 protein. The skilled person is aware that the expression of the encoded VIP3 protein depends on the expression system used, i.e. the transcriptional and translational regulatory sequences. The use of different promoters (inducible, constitutive, temporally regulated or developmentally regulated, tissue-preferred or

tissue-specific promoters) might direct or restrict the expression of the encoded VIP3 protein to a selected plant tissue, development stage, etc. (cf. *inter alia* paragraph [0098] of the patent-in-suit). The targeted introduction of the DNA molecule into a plant and the use of endogenous plant expression systems might also be contemplated by the skilled person (cf. paragraph [0094] of the patent-in-suit). For some of those expression systems, the encoded VIP3 protein will not necessarily be always expressed but only under certain conditions. It is thus the presence of the DNA molecule encoding the VIP3 protein or of an expression cassette comprising such a DNA molecule stably incorporated into the plant genome that is considered to be essential and necessary to provide the technical effect of the patent-in-suit. Therefore, the respondent's argument on the subject-matter of claim 15 (cf. point X *supra*) cannot be followed by the board.

Conclusion on inventive step

34. From all the above, it is concluded that the main request fulfils the requirements of Article 56 EPC 1973.

Article 83 EPC 1973

35. Although the objection against claims 7 and 23 under Article 83 EPC 1973 was raised at a late stage of the appeal proceedings, Article 100(b) EPC 1973 was an original ground of opposition (cf. point II *supra*) and the objection arises directly from the amendments introduced into those claims, namely the requirement of the oligonucleotide probe to be of at least 10

nucleotides in length (cf. point VI *supra*). Therefore, the objection is admitted into the appeal proceedings.

36. For carrying out the subject-matter of claims 7 and 23 in the sense of Article 83 EPC 1973, a skilled person has the complete knowledge of the disclosure of the patent-in-suit (DNA molecules defined in SEQ ID NO: 1, 3 and 4 encoding VIP3 proteins) and of all the prior art concerned with (crystal) insecticidal proteins, including the mosquitocidal toxin from *B. spahericus* SSII-1 and the vegetative insecticidal proteins (VIP1 and VIP2) of D3 and D1, respectively. The difficulties described in D14 and referred to by the respondent arose originally from the selection of an appropriate cloning library and from the methods used to screen that library. These difficulties were mainly originated from a wrong assumption, namely that the insecticidal proteins produced during the vegetative growth phase of *Bacillus* strains had to be similar to the VIP1/VIP2 system disclosed in D1. That assumption is clearly dispelled by the patent-in-suit in which the disclosed VIP3 is shown to be different from that VIP1/VIP2 system. In view of that prior art and the disclosure of the patent-in-suit, the board is convinced that the skilled person would have no problem in carrying out the invention as claimed, including claims 7 and 23.

37. No other objections have been raised under Article 83 EPC 1973 nor does the board see any reason to raise any objection under this Article. The requirements of Article 83 EPC 1973 are thus fulfilled.

Order

For these reasons it is decided that:

1. The decision under appeal is set aside.

2. The case is remitted to the first instance with the order to maintain the patent with the following claims and a description to be adapted: claims No. 1 to 27 of the main request filed on 9 February 2010.

The Registrar: The Chairman:

A. Wolinski L. Galligani