Final Review report for the active substance *Bacillus amyloliquefaciens* strain FZB24

Finalised in the Standing Committee on the Food Chain and Animal Health at its meeting on 23 March 2017 in view of the approval of *Bacillus amyloliquefaciens* strain FZB24 as low risk active substance, in accordance with Regulation (EC) No 1107/2009\(^2\)

1. **Procedure followed for the evaluation process**

This review report has been established as a result of the evaluation of the new active substance *Bacillus amyloliquefaciens* strain FZB24, made in the context of the work provided for in Articles 7 to 13 of Regulation (EC) No 1107/2009 concerning the placing of plant protection products on the market and repealing Council Directives 79/117/EEC\(^3\) and 91/414/EEC\(^4\), with a view to the possible approval of this substance for the use in plant protection products.

In accordance with the provisions of Article 7 of that Regulation, the authorities of France received on 19 June 2013 an application from Novozymes Biologicals France (since November 2015: Novozymes France), hereafter referred to as the applicant, for the approval of the active substance *Bacillus amyloliquefaciens* strain FZB24 for use in plant protection products. The French authorities indicated to the Commission on 4 September 2013 the results of their examination of the completeness of the dossier satisfying the requirements of Article 8, according to the provisions of Article 9 of the Regulation. Subsequently, and in accordance with the requirements of Article 9(3), a dossier on *Bacillus amyloliquefaciens* strain FZB24 was distributed to the Member States, the European Food Safety Authority (EFSA) and the Commission.

Thereupon, France as rapporteur Member State started the detailed examination of the dossier provided by the applicant. According to the provisions of Article 11, the rapporteur Member State shall prepare and submit it to the Commission and EFSA within twelve months a report (the draft assessment report), assessing whether the active substance can be expected to meet the criteria provided for in Article 4 of the Regulation.

France submitted that draft assessment report to the Commission and EFSA on 13 April 2015.

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\(^1\) Does not necessarily represent the views of the Commission services.


\(^3\) OJ L 33, 8.2.1979, p. 36.

On 20 May 2015, EFSA circulated the draft assessment report to Member States and the applicant and, in addition, organised a public consultation on it, in line with the provisions of Article 12(1) of the Regulation.

EFSA organised a consultation to review the draft assessment report and the comments received thereon (peer review) in accordance with the provisions of Article 12(2) and 12(3). In this framework, EFSA decided to request additional information from the applicant.

According to the provisions of Article 12(2) of the Regulation, EFSA sent to the Commission its conclusion on the risk assessment [Conclusion on the peer review of the pesticide risk assessment of the active substance *Bacillus amyloliquefaciens* strain FZB24 (approved: 29 April 2016)](https://doi.org/10.2903/j.efsa.2016.4494). This conclusion refers to background document A (draft assessment report) and background document B (EFSA peer review report).

According to the provisions of Article 13 of the Regulation, the Commission produced a draft review report and a draft Regulation on *Bacillus amyloliquefaciens* strain FZB24. The Commission referred the draft review report to the applicant for commenting on 29 September 2016 and on 6 October 2016 to the Standing Committee on Plants, Animals, Food and Feed, for final examination. The draft review report was finalised in the meeting of the Standing Committee on 23 March 2017.

The present review report contains the conclusions of the final examination by the Standing Committee. Given the importance of the conclusion of EFSA, and background documents A and B, these documents are also considered to be part of this review report.

2. **Purposes of this review report**

This review report, including the background documents and appendices hereto, has been developed and finalised in support of [Commission Implementing Regulation (EU) 2017/806](https://eur-lex.europa.eu/legal-content/EN/TXT/?uri=uriserv:OJ.L.2017.121.01.0031.01.ENG) concerning the approval of *Bacillus amyloliquefaciens* strain FZB24 as active substance under Regulation (EC) No 1107/2009, and to assist the Member States in decisions on individual plant protection products containing *Bacillus amyloliquefaciens* strain FZB24 they have to take in accordance with the provisions of that Regulation, and in particular the provisions of Article 29(1) of Regulation (EC) No 1107/2009 and the uniform principles laid down in Regulation (EU) No 546/2011.

This review report provides also for the evaluation required under part II, Section A.2(b) of the above mentioned uniform principles, as well as under several specific sections of chapter B of these principles. In these sections it is provided that Member States, in evaluating applications and granting authorisations, shall take into account the information concerning the requirements of Regulation (EU) No 544/2011, submitted for the purpose of approval of the active substances, as well as the result of the evaluation of those data.

This review report will be made available to the public by the Commission.

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The information in this review report is, at least partly, based on information which is confidential and/or protected under the provisions of Regulation (EC) No 1107/2009. It is therefore recommended that this review report would not be accepted to support any registration outside the context of that Regulation, e.g. in third countries, for which the applicant has not demonstrated to have regulatory access to the information on which this review report is based.


The overall conclusion from the evaluation is that it may be expected that plant protection products containing *Bacillus amyloliquefaciens* strain FZB24 will fulfil the safety requirements laid down in Article 4(1) – (3) of Regulation (EC) No 1107/2009 and the criteria for low risk substances provided in Article 22 of this Regulation. This conclusion is however subject to compliance with the particular requirements in sections 4, 5, 6 and 7 of this report, as well as to the implementation of the provisions of Article 29(1) of Regulation (EC) No 1107/2009 and the uniform principles laid down in Regulation (EU) No 546/2011, for each *Bacillus amyloliquefaciens* strain FZB24 containing plant protection product for which Member States will grant or review the authorisation.

Furthermore, these conclusions were reached within the framework of the uses which were proposed and supported by the data submitter and mentioned in the list of uses supported by available data (attached as Appendix II to this review report).

Extension of the use pattern beyond those described above will require an evaluation at Member State level in order to establish whether the proposed extensions of use can satisfy the requirements of Article 29(1) of Regulation (EC) No 1107/2009 and of the uniform principles laid down in Regulation (EU) No 546/2011. This might in particular be the case for the risk to consumers from metabolites produced after application of the active substance, which is considered low for the uses supported by available data, but might be different for uses where there is a higher metabolite secretion.

Based on the lack of significant toxicity, infectivity or pathogenicity in the available toxicological studies, the setting of health-based reference values for the microorganism *Bacillus amyloliquefaciens* strain FZB24 is not needed. *Bacillus amyloliquefaciens* is recommended for the Qualified Presumption of Safety list (2013) if it is qualified for the absence of toxigenic activity, and if the strain does not harbour any acquired antimicrobial resistance genes to clinically relevant antibiotics. In a test for antimicrobial resistance, *Bacillus amyloliquefaciens* FZB24 was susceptible to all tested antibiotics; additionally, the review established that no significant level of toxins is expected in the end-use product.

With particular regard to residues, the microorganism itself is not a mammalian pathogen and therefore a quantitative risk assessment is not necessary. For the uses supported by data currently no harmful effects on human or animal health from the residues are expected, taking into account the route and rate of application and the weight of evidence that *Bacillus amyloliquefaciens* strain FZB24 does not produce relevant metabolites of significant toxicity or at levels leading to exposure levels higher than negligible.

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On the same basis, the review has identified several acceptable exposure scenarios for operators, workers and bystanders, which require however to be confirmed for each plant protection product in accordance with the relevant sections of the above mentioned uniform principles, taking into account the need for adequate personal protective equipment.

The review has also concluded that under the proposed and supported conditions of use there are no unacceptable effects on the environment, as provided for in Article 4(3)(e) of Regulation (EC) No 1107/2009, provided that certain conditions are taken into account as detailed in section 6 of this report.

The following points were considered as open by EFSA (2016) for *Bacillus amyloliquefaciens* strain FZB24, however their concern is considered low or negligible for the following reasons:

1) **Pending on further investigations of toxins/secondary metabolites produced after application, the risk assessment for the re-entry workers and consumers cannot be concluded.**

*Bacillus amyloliquefaciens* strain FZB24 is not known to produce any kind of toxins of concern to human health. *Bacillus amyloliquefaciens* strain FZB24 is known to produce secondary metabolites involved in the control of fungal pathogens such as cyclic lipopeptides (CLPs) of the iturins, fengycin and surfactin families, however, it is not reported to produce the well-known mammalian toxins. The analysis of its genome sequence revealed no sequences with significant identity to known virulence factors or genes encoding known toxins produced by pathogenic species like *Bacillus anthracis* and *Bacillus cereus*. Furthermore, it does not include any plasmids that could include genes involved in toxin production. The EFSA Conclusion mentioned a case of food poisoning caused by the toxic peptide amylosin which can be produced by *Bacillus amyloliquefaciens*, however, the potential of *Bacillus amyloliquefaciens* strain FZB24 to produce amylosin has not been resolved.

The levels of the metabolites iturins, fengycins and surfactins cumulatively found in the technical and in the end-use product Taegro were very low.

Levels of *Bacillus amyloliquefaciens* strain FZB24 are not expected to increase on crops and in soil following applications according to the intended uses. Additionally, as the strain is widely distributed in the environment and is wild type, the expression of metabolites under the proposed conditions is very unlikely to differ from the naturally occurring expression. Therefore, the exposure to *Bacillus amyloliquefaciens strain* FZB24 and metabolites due to use in plant protection products are expected to be within the same magnitude as background exposure.

Furthermore, given the QPS (Qualified Presumption of Safety) status of *Bacillus amyloliquefaciens*, the experience of safe production and application of *Bacillus amyloliquefaciens*–based plant protection products and that the strain is present naturally in the environment, the overall concern with regard to human health is low.

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2) The information available was insufficient to demonstrate that Bacillus amyloliquefaciens strain FZB24 would respect the uniform principles criterion of not being expected to persist in soil in concentrations considerably higher than the natural background levels, taking into account repeated applications over the years and should this not be the case, satisfy the uniform principles associated unless clause, in the context of soil organisms.

*Bacillus amyloliquefaciens* is a naturally occurring, ubiquitous soil organism and occurs without geographical restriction. *Bacillus amyloliquefaciens* is unlikely to multiply when applied on aerial parts of plants. Following introduction into soil, *Bacillus amyloliquefaciens* spores may persist for a certain period of time and multiply given the sufficient availability of nutrients. However, local effects resulting from the use of products containing *Bacillus amyloliquefaciens* FZB24 are likely to be transient as populations of *Bacillus* spp. are subject to competition and antagonism in the soil. Therefore, the risk for soil microorganisms and soil function is considered negligible.

3) Satisfactory information to demonstrate that, under the conditions of use, any secondary metabolites/toxins produced by *Bacillus amyloliquefaciens* strain FZB24 will not occur in the environmental compartments in concentrations considerably higher than under natural conditions was missing. Consequently, further data on the persistence, transformation and mobility of these compounds may be needed in order to assess the potential level of environmental exposure including the exposure of groundwater and the effects to non-target organisms.

*Bacillus amyloliquefaciens* spores may persist for a prolonged period of time in soil, however, in the form which is not metabolically active. In the availability of nutrients spores are expected to germinate and multiply, however, the potential increase in levels of *Bacillus amyloliquefaciens* strain FZB24 following applications according to the intended uses is likely to be transient due to competition and antagonism in the soil.

*Bacillus amyloliquefaciens* strain FZB24 can be mobile in soil predominantly through passive transport of spores, however, spores reaching groundwater environments do not germinate and grow due to insufficient nutrient availability. Dilution as a result of continuous water flux and predation by groundwater (micro-) organisms will cause a continuous decline of the spore populations. Thus, groundwater accumulation is unlikely.

Given that *Bacillus amyloliquefaciens* is commonly found in soils, including agricultural settings, metabolites which are involved in the mode of action and might be produced transiently at the treatment site are not considered to pose any risk to non-target organisms. Additionally, the expression of metabolites under the proposed conditions is very unlikely to differ from the naturally occurring expression.

Overall, due to the absence of toxicity and the low exposure, the risk for non-target organisms and groundwater due to a possible production of metabolites is considered low.

4. **Identity and Physical/chemical properties**

The main identity of *Bacillus amyloliquefaciens* strain FZB24 is given in Appendix I.
Bacillus amyloliquefaciens strain FZB24 is a bacterium deposited at the culture collection of the ‘Deutsche Sammlung von Mikroorganism’ (DSM), Germany, under the accession number 10271 and also at the Agricultural Research Service Culture Collection (NRRL), USA, under the accession number B-50304.

The minimum content of *Bacillus amyloliquefaciens* strain FZB24 in the microbial pest control agent (MPCA) is $2 \times 10^{14}$ CFU/kg (775 g/kg).

It has been established that for the active substance notified by the applicant Novozymes France none of the manufacturing impurities considered are, on the basis of information currently available, of toxicological or environmental concern. Member States should pay particular attention to ensuring that a full consideration is given to the specification of technical material used in plant protection products, including a full characterisation and quantification of impurities and secondary metabolites.

Strict maintenance of environmental conditions and quality control analysis during the manufacturing process shall be assured by the producer, in order to ensure the fulfilment of the limits on microbiological contamination as referred to in the Working Document SANCO/12116/2012.

5. **Endpoints and related information**

In order to facilitate Member States, in granting or reviewing authorisations, to apply adequately the provisions of Article 29(1) of Regulation (EC) No 1107/2009 and the uniform principles laid down in Regulation (EU) No 546/2011, the most important endpoints were identified during the re-evaluation process. These endpoints are listed in the conclusion of EFSA.

6. **Particular conditions to be taken into account on short term basis by Member States in relation to the granting of authorisations of plant protection products containing *Bacillus amyloliquefaciens* strain FZB24.**

On the basis of the proposed and supported uses (as listed in Appendix II), the following issue has been identified as requiring particular and short term attention from all Member States, in the framework of any authorisations to be granted, varied or withdrawn, as appropriate.

The protection of operators and workers, taking into account that microorganisms are considered as potential sensitizers, ensuring that adequate personal protective equipment is included as a condition of use.

As mentioned in Section 4 of this report, strict maintenance of environmental conditions and quality control analysis during the manufacturing process shall be assured by the producer, in order to ensure the fulfilment of the limits on microbiological contamination as referred to in the Working Document SANCO/12116/2012.

Member States should pay particular attention to ensuring that a full consideration is given to the specification of technical material used in plant protection products, including a full characterisation and quantification of impurities and secondary metabolites.
Conditions of use shall include risk mitigation measures where appropriate.

7. **List of studies to be generated**

No further studies were identified which were at this stage considered necessary in relation to the approval of *Bacillus amyloliquefaciens* strain FZB24 under the current approval conditions.

Some endpoints however may require the generation or submission of additional studies to be submitted to the Member States in order to ensure authorisations for use under certain conditions. A complete list of studies to be generated, still ongoing or available but not peer reviewed can be found in the relevant part of the EFSA Conclusion (pages 11-12).

8. **Information on studies with claimed data protection**

For information of any interested parties, the rapporteur Member State will keep available a document which gives information about the studies for which the main data submitter has claimed data protection and which during the re-evaluation process were considered as essential with a view to approval under Regulation (EC) No 1107/2009. This information is only given to facilitate the operation of the provisions of Article 62 of Regulation (EC) No 1107/2009 in the Member States. It is based on the best information available but it does not prejudice any rights or obligations of Member States or operators with regard to its uses in the implementation of the provisions of Article 62 of Regulation (EC) No 1107/2009 and neither does it commit the Commission.

9. **Updating of this review report**

The information in this report may require to be updated from time to time in order to take account of technical and scientific developments as well as of the results of the examination of any information referred to the Commission in the framework of Articles 13, 21, 38, 44, 56 of Regulation (EC) No 1107/2009. Any such adaptation will be finalised in the Standing Committee on Plants, Animals, Food and Feed, in connection with any amendment of the approval conditions for *Bacillus amyloliquefaciens* strain FZB24.
**APPENDIX I**

**Main identity**

**BACILLUS AMYLOLIQUEFACIENS STRAIN FZB24**

<table>
<thead>
<tr>
<th>Name of the organism:</th>
<th><em>Bacillus amyloliquefaciens</em> strain FZB24</th>
</tr>
</thead>
<tbody>
<tr>
<td>Taxonomy:</td>
<td>Species: <em>Bacillus amyloliquefaciens</em>; Strain: FZB24; Genus: <em>Bacillus</em>; Family: <em>Bacillaceae</em>; Division: Bacteria</td>
</tr>
<tr>
<td>Species, subspecies, strain:</td>
<td><em>Bacillus amyloliquefaciens</em> strain FZB24</td>
</tr>
<tr>
<td>Identification / detection:</td>
<td>The identification is based on the genome sequencing methods (SNP analysis, ion torrent sequencing). Four different SNPs between <em>B. amyloliquefaciens</em> FZB24 and <em>B. amyloliquefaciens</em> FZB42 were demonstrated which can distinguish <em>B. amyloliquefaciens</em> FZB24 from <em>B. amyloliquefaciens</em> FZB42. Furthermore, it was demonstrated that genes ssrSA, yocJ, and yocK are present in <em>B. subtilis</em> 168 and in <em>B. amyloliquefaciens</em> FZB24 and not in <em>B. amyloliquefaciens</em> FZB42 and can be used to differentiate <em>B. amyloliquefaciens</em> FZB24 from <em>B. amyloliquefaciens</em> FZB42.</td>
</tr>
<tr>
<td>Culture collection:</td>
<td>German Collection &quot;Deutsche Sammlung von Mikroorganism&quot; (DSM) accession number 10271 Agricultural research Service (ARS) Culture and Patent Culture Collections NRRL accession number B-50304</td>
</tr>
<tr>
<td>Minimum concentration of the MPCA (microbial pest control agent) used for manufacturing of the formulated product (wettable powder (WP)):</td>
<td>$2 \times 10^{14}$ CFU/kg (775 g/kg).</td>
</tr>
<tr>
<td>Identity and content of relevant impurities, additives, contaminating organisms in the technical grade of MPCA:</td>
<td>No relevant impurities.</td>
</tr>
<tr>
<td>Is the MPCA genetically modified; if so provide type of modification</td>
<td>No</td>
</tr>
</tbody>
</table>
## APPENDIX II

List of uses supported by available data

**BACILLUS AMYLOLIQUEFACIENS STRAIN FZB24**

<table>
<thead>
<tr>
<th>Crop and/or situation</th>
<th>Membe r state or Country</th>
<th>Product name</th>
<th>F G or I</th>
<th>Pest or group of pests controlled</th>
<th>Formulation</th>
<th>Application</th>
<th>Application rate per treatment</th>
<th>PHI (days)</th>
<th>Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Cucurbits</strong></td>
<td>EU-member states</td>
<td>TAEGRO</td>
<td>G</td>
<td>Pseudoperonospora sp. (downy mildew)</td>
<td>WP 13% = 1x10^{13} CFU/kg MCPP</td>
<td>foliar spray all growth stages crop season</td>
<td>3 12</td>
<td>0.0185-0.206 1.85x10^{11}-2.06 x10^{12} 180-1000 L 0.185-0.370 1.85x10^{12}-3.7 x10^{12}</td>
<td>4 hours</td>
</tr>
<tr>
<td><strong>Potato</strong></td>
<td>EU-member states</td>
<td>TAEGRO</td>
<td>F</td>
<td>Phytophthora infestans (late blight)</td>
<td>WP 13%= 1x10^{13} CFU/kg MCPP</td>
<td>foliar spray all growth stages crop season</td>
<td>3 10</td>
<td>0.0185-0.206 1.85x10^{11}-2.06 x10^{12} 180-1000 L 0.185-0.370 1.85x10^{12}-3.7 x10^{12}</td>
<td>4 hours</td>
</tr>
<tr>
<td><strong>Grapevine</strong></td>
<td>EU-member states</td>
<td>TAEGRO</td>
<td>F</td>
<td>Botrytis cinerea (grey mould)</td>
<td>WP 13% = 1x10^{13} CFU/kg MCPP</td>
<td>foliar spray all growth stages crop season</td>
<td>3 10</td>
<td>0.0185-0.206 1.85x10^{11}-2.06 x10^{12} 180-1000 L 0.185-0.370 1.85x10^{12}-3.7 x10^{12}</td>
<td>4 hours</td>
</tr>
</tbody>
</table>

**Remarks:**

(a) For crops, the EU and Codex classifications (both) should be used; where relevant, the use situation should be described (e.g. fumigation of a structure)
(b) Outdoor or field use (F), glasshouse application (G) or indoor application (I)
(c) e.g. biting and sucking insects, soil born insects, foliar fungi, weeds
(d) e.g. wettable powder (WP), emulsifiable concentrate (EC), granule (GR)
(e) GCPF Codes - GIFAP Technical Monograph No 2, 1989
(f) All abbreviations used must be explained
(g) Method, e.g. high volume spraying, low volume spraying, spreading, dusting, drench
(h) Kind, e.g. overall, broadcast, aerial spraying, row, individual plant, between the plants - type of equipment used must be indicated
(i) g/kg or g/L
(j) Growth stage at last treatment (BBCH Monograph, Growth Stages of Plants, 1997, Blackwell, ISBN 3-8263-3152-4), including where relevant, information on season at time of application
(k) The minimum and maximum number of application possible under practical conditions of use must be provided
(l) PHI - minimum pre-harvest interval
(m) Remarks may include: Extent of use/economic importance/restrictions