Peer review of the pesticide risk assessment of the active substance *Bacillus amyloliquefaciens* strain QST 713 (formerly *Bacillus subtilis* strain QST 713)

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Abstract

The conclusions of the EFSA following the peer review of the initial risk assessments carried out by the competent authorities of the rapporteur Member State, Germany, and co-rapporteur Member State, Denmark, for the pesticide active substance *Bacillus amyloliquefaciens* strain QST 713, formerly *Bacillus subtilis* strain QST 713, are reported. The context of the peer review was that required by Commission Implementing Regulation (EU) No 844/2012, as amended by Commission Implementing Regulation (EU) No 2018/1659. The conclusions were reached on the basis of the evaluation of the representative uses of *Bacillus amyloliquefaciens* strain QST 713 as a fungicide on strawberry (field and greenhouse uses) and grapes (field use). The reliable end points, appropriate for use in regulatory risk assessment, are presented. Missing information identified as being required by the regulatory framework is listed. Concerns are identified.

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Summary

Commission Implementing Regulation (EU) No 844/2012, as amended by Commission Implementing Regulation (EU) No 2018/1659, lays down the procedure for the renewal of the approval of active substances submitted under Article 14 of Regulation (EC) No 1107/2009. The list of those substances is established in Commission Implementing Regulation (EU) No 686/2012. Bacillus amyloliquefaciens strain QST 713 is one of the active substances listed in Regulation (EU) No 686/2012.

In accordance with Article 1 of Regulation (EU) No 844/2012, the rapporteur Member State (RMS), Germany, and co-rapporteur Member State (co-RMS), Denmark, received an application from Bayer CropScience AG for the renewal of approval of the active substance Bacillus amyloliquefaciens strain QST 713.

An initial evaluation of the dossier on Bacillus amyloliquefaciens strain QST 713 was provided by the RMS in the renewal assessment report (RAR), and subsequently, a peer review of the pesticide risk assessment on the RMS evaluation was conducted by EFSA in accordance with Article 13 of Commission Implementing Regulation (EU) No 844/2012, as amended by Commission Implementing Regulation (EU) No 2018/1659. The following conclusions are derived.

The uses of Bacillus amyloliquefaciens strain QST 713 according to the representative uses as a fungicide on field and protected strawberries and on field grapes, as proposed at EU level, result in a sufficient fungicidal efficacy against Botrytis cinerea.

In the area of mammalian toxicology, considering that the non-dietary exposure to secondary metabolites (present in the product and secreted by Bacillus amyloliquefaciens strain QST 713 after application) has not been determined, and that their potential toxicity has not been fully characterised, the risk assessment for operators, workers, bystanders and residents cannot be concluded.

Bacillus amyloliquefaciens strain QST 713 may produce a range of metabolites; however, information is not available as to whether these are produced on plants under good agricultural practice (GAP) directed use and/or on the quantity of the metabolites on edible commodities at harvest. Therefore, a consumer risk assessment cannot be finalised.

Bacillus amyloliquefaciens strain QST 713 is not proposed to be included into Annex IV of Regulation (EC) No 396/2005.

In the fate section, the production of relevant toxins/secondary metabolites known to be of concern for the environment cannot be excluded. Therefore, the risk assessment cannot be finalised for the environment including the assessment of potential groundwater exposure.

In the area of ecotoxicology, low risk was concluded for birds, wild mammals, aquatic organisms (excluding plants) and non-target arthropods. Low risk to bees could not be concluded for all the representative uses leading to a critical area of concern (it was not confirmed if the use in protected structures is limited to permanent greenhouses, for which no exposure to bees is expected with the exception of bees introduced as part of Integrated Pest Management (IPM), and low risk can be concluded. Thus, in that case the identified concern for bees would be lifted). No suitable data were available for earthworms and soil microorganisms, and thus, the assessment could not be finalised.
# Peer review of the pesticide risk assessment of the active substance *Bacillus amyloliquefaciens* strain QST 713

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Background

Commission Implementing Regulation (EU) No 844/2012\(^1\), as amended by Commission Implementing Regulation (EU) No 2018/1659\(^2\) (hereinafter referred to as ‘the Regulation’), lays down the provisions for the procedure of the renewal of the approval of active substances, submitted under Article 14 of Regulation (EC) No 1107/2009\(^3\). This regulates for the European Food Safety Authority (EFSA) the procedure for organising the consultation of Member States, the applicant(s) and the public on the initial evaluation provided by the rapporteur Member State (RMS) and/or co-rapporteur Member State (co-RMS) in the renewal assessment report (RAR), and the organisation of an expert consultation where appropriate.

In accordance with Article 13 of the Regulation, unless formally informed by the European Commission that a conclusion is not necessary, EFSA is required to adopt a conclusion on whether the active substance can be expected to meet the approval criteria provided for in Article 4 of Regulation (EC) No 1107/2009 within 5 months from the end of the period provided for the submission of written comments, subject to an extension of an additional 3 months where additional information is required to be submitted by the applicant(s) in accordance with Article 13(3).

In accordance with Article 1 of the Regulation, the RMS, Germany, and co-RMS, Denmark, received an application from Bayer CropScience AG for the renewal of approval of the active substance *Bacillus amyloliquefaciens* strain QST 713, formerly *Bacillus subtilis* QST 713. Complying with Article 8 of the Regulation, the RMS checked the completeness of the dossier and informed the applicant, the co-RMS (Denmark), the European Commission and EFSA about the admissibility.

The RMS provided its initial evaluation of the dossier on *Bacillus amyloliquefaciens* strain QST 713 in the RAR, which was received by EFSA on 4 June 2018. In accordance with Article 12 of the Regulation, EFSA distributed the RAR to the Member States and the applicant, Bayer CropScience AG, for consultation and comments on 5 September 2018. EFSA also provided comments. In addition, EFSA conducted a public consultation on the RAR. EFSA collated and forwarded all comments received to the European Commission on 5 November 2018. At the same time, the collated comments were forwarded to the RMS for compilation and evaluation in the format of a reporting table. The applicant was invited to respond to the comments in column 3 of the reporting table. The comments and the applicant’s response were evaluated by the RMS in column 3.

The need for expert consultation and the necessity for additional information to be submitted by the applicant in accordance with Article 13(3) of the Regulation were considered in a telephone conference between EFSA and the RMS on 8 April 2019. On the basis of the comments received, the applicant’s response to the comments and the RMS’s evaluation thereof, it was concluded that additional information should be requested from the applicant and that EFSA should conduct an expert consultation in the area of ecotoxicology.

The outcome of the telephone conference, together with EFSA’s further consideration of the comments, is reflected in the conclusions set out in column 4 of the reporting table. All points that were identified as unresolved at the end of the comment evaluation phase and which required further consideration, including those issues to be considered in an expert consultation, were compiled by EFSA in the format of an evaluation table.

The conclusions arising from the consideration by EFSA, and as appropriate by the RMS, of the points identified in the evaluation table, together with the outcome of the expert consultation and the written consultation on the assessment of additional information, where these took place, were reported in the final column of the evaluation table.

A final consultation on the conclusions arising from the peer review of the risk assessment took place with Member States via a written procedure in September–November 2020.

This conclusion report summarises the outcome of the peer review of the risk assessment of the active substance and the representative formulation, evaluated on the basis of the representative uses

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1. Commission Implementing Regulation (EU) No 844/2012 of 18 September 2012 setting out the provisions necessary for the implementation of the renewal procedure for active substances, as provided for in Regulation (EC) No 1107/2009 of the European Parliament and of the Council concerning the placing of plant protection products on the market. OJ L 252, 19.9.2012, p. 26–32.

2. Commission Implementing Regulation (EU) No 2018/1659 of 7 November 2018 amending Implementing Regulation (EU) No 844/2012 in view of the scientific criteria for the determination of endocrine-disrupting properties introduced by Regulation (EU) 2018/605.

3. Regulation (EC) No 1107/2009 of 21 October 2009 of the European Parliament and of the Council concerning the placing of plant protection products on the market and repealing Council Directives 79/117/EEC and 91/414/EEC. OJ L 309, 24.11.2009, p. 1–50.
of *Bacillus amyloliquefaciens* strain QST 713 as a fungicide on strawberry (field and greenhouse uses) and grapes (field use), as proposed by the applicant. In accordance with Article 12(2) of Regulation (EC) No 1107/2009, risk mitigation options identified in the RAR and considered during the peer review are presented in the conclusion. A list of the relevant end points for the active substance and the formulation is provided in Appendix A.

In addition, a key supporting document to this conclusion is the peer review report (EFSA, 2020), which is a compilation of the documentation developed to evaluate and address all issues raised in the peer review, from the initial commenting phase to the conclusion. The peer review report comprises the following documents, in which all views expressed during the course of the peer review, including minority views, where applicable, can be found:

- the comments received on the RAR;
- the reporting table (8 April 2019);
- the evaluation table (30 November 2020);
- the report(s) of the scientific consultation with Member State experts (where relevant);
- the comments received on the assessment of the additional information (where relevant);
- the comments received on the draft EFSA conclusion.

Given the importance of the RAR, including its revisions (Germany, 2020), and the peer review report, both documents are considered as background documents to this conclusion and thus are made publicly available.

It is recommended that this conclusion report and its background documents would not be accepted to support any registration outside the EU for which the applicant has not demonstrated that it has regulatory access to the information on which this conclusion report is based.

**The identity of the microorganism and the properties of the formulated product**

*Bacillus amyloliquefaciens* strain QST 713 (formerly *Bacillus subtilis* strain QST 713) is a bacterium deposited at the Agriculture Research Culture Collection (Northern Regional Research Laboratory; NRRL), at the Microbial Properties Research Unit, National Centre for Agricultural Utilization Research, Agricultural Research Service, U.S. Department of Agriculture Peoria, Illinois, USA, under the deposit number NRRL B-21661. The strain *Bacillus amyloliquefaciens* QST 713 is a naturally occurring, indigenous wild-type bacterium, initially isolated from the soil in a peach tree orchard in Fresno County, California (USA) in 1995.

Due to availability of new data, the taxonomy of the active substance was changed from the time of submission. It was proposed that the *Bacillus amyloliquefaciens* clade should be considered as a taxonomic unit above species level, designated as ‘operational group *Bacillus amyloliquefaciens*’, consisting of the soil-borne *Bacillus amyloliquefaciens*, and plant associated *Bacillus siamensis* and *Bacillus velezensis*, whose members are closely related.

The representative formulated product for the evaluation was ‘Serenade ASO’, a suspension concentrate (SC) containing 967 g/kg (minimum content $1.0 \times 10^{12}$ CFU/kg, maximum content: $3.0 \times 10^{13}$ CFU/kg) *Bacillus amyloliquefaciens* strain QST 713.

The representative uses evaluated comprise applications by spraying on field and protected strawberries and on grapes (field applications), as a fungicide against *Botrytis cinerea*. Full details of the GAPs can be found in the list of end points in Appendix A. The applicant did not explicitly clarify which protected cropping systems were included in the intended use on protected strawberries.

Data were submitted to conclude that the uses of *Bacillus amyloliquefaciens* strain QST 713 according to the representative uses proposed at EU level result in a sufficient fungicidal efficacy against grey mould, following the guidance document SANCO/2012/11251-rev. 4 (European Commission, 2014).

**Conclusions of the evaluation**

1. **Identity of the microorganism/biological properties/physical and technical properties and methods of analysis**

The following guidance documents were followed in the production of this conclusion: SANCO/3030/99-rev. 4 (European Commission, 2000), SANCO/12116/2012-rev. 0 (European Commission, 2012) and EFSA FEEDAP Panel, 2018.
**Bacillus amyloliquefaciens** strain QST 713 was previously designated as *Bacillus subtilis* QST 713 (European Commission, 2006). The taxonomy of the *Bacillus subtilis* group is dynamically changing due to availability of new data (mostly due to fast sequencing tools).

Due to newer information on genetic data, *Bacillus subtilis* strain QST 713 was reclassified as *Bacillus amyloliquefaciens* strain QST 713 and subsequently as *Bacillus amyloliquefaciens* ssp. *plantarum* strain QST 713. Based on more recent phylogenetic analysis from additional literature identified, the RMS considered necessary a further reclassification as *Bacillus velezensis* strain QST 713. *Bacillus velezensis* and *Bacillus amyloliquefaciens* ssp. *plantarum* were considered synonymous taxonomic designations.

If a consensus on the ongoing debate on the taxonomy of the strain to be used in the conclusion cannot be reached to refer to strain QST 713 as *Bacillus velezensis*, then the RMS proposed *Bacillus amyloliquefaciens* subsp. *plantarum* strain QST 713 to be used. It was decided to follow the same approach used with the previously published conclusion of a *Bacillus amyloliquefaciens* strain, maintaining the name provided by the applicant in the dossier and used in the RAR. As a consequence, the originally proposed name of the active substance is used in the conclusion.

The technical grade microbial pest control agent (MPCA) is only a hypothetical stage in the continuous production process of the end-use product (MPCP). As a consequence, the specification is given only for the formulated product ‘Serenade ASO’ of minimum content of $1.0 \times 10^{12}$ CFU/kg (max. content: $3.0 \times 10^{13}$ CFU/kg).

*Bacillus amyloliquefaciens* strain QST 713 can be identified by its biochemical and morphological characteristics and by molecular biological methods. The complete genome sequence of *Bacillus amyloliquefaciens* strain QST 713 has been deposited at National Center for Biotechnology Information (NCBI) under the GenBank accession number CP025079.

The analysis of contaminating microorganisms in commercially produced batches complies with the requirements of SANCO/12116/2012 rev.0 (European Commission, 2012).

*Bacillus amyloliquefaciens* strain QST 713 has the genetic capacity to produce the cyclic lipopeptides: iturin A, bacillomycin, fengycin and surfactin; the polyketides: macrolactin, bacillaene and diffidicin; the iron siderophore bacillibactin; the antimicrobial dipeptide bacilysin, the antibiotics ericin A and ericin S, the volatile compound 2,3-butanediol; and the plant growth hormone indole-3-acetic acid. The secondary metabolites detected in Serenade ASO were iturin A, fengycin A, fengycin B, surfactin, bacillaene, diffidicin, ericin A and ericin S. Even though indicated by genome analysis, bacilysin and 2,3-butanediol were not produced by strain QST 713. Bacillomycin and its variants and indole-3-acetic acid have not been investigated by chemical analysis. The strain does not have the genetic capacity to produce the following secondary metabolites: amylosin, subtilisin, plantazolicin, mersacidin, other bacteriocins, halobacillin, methyl-halobacillin and mixirin.

Production of iturins, fengycins, surfactin, bacillaene, diffidicin and ericins may constitute part of the mode of action of *Bacillus amyloliquefaciens* strain QST 713.

The growth temperature range of *Bacillus amyloliquefaciens* strain QST 713 is between 15°C and 55°C. It is able to grow at a pH in the range of 5.0–8.0 and it is sensitive to ultra-violet light. *Bacillus amyloliquefaciens* strain QST 713 was shown to be sensitive to all relevant antibiotics as provided in the EFSA FEEDAP Panel (2018) guidance document (chloramphenicol, tetracycline, streptomycin, clindamycin, erythromycin, kanamycin, gentamicin and vancomycin), except bacitracin. This resistance apparently seems not plasmid encoded and is therefore unlikely to be transferred to other microorganisms.

The supported shelf-life of the product is 2 years at 20°C in the original packaging (high-density polyethylene; HDPE). Acceptable methods are available for the determination of the microorganism in the technical material and for the determination of the content of contaminating microorganisms. Appropriate analytical methods were available to identify secondary metabolites present in the formulated product.

Residue definitions were not applicable for *Bacillus amyloliquefaciens* strain QST 713; therefore, post-registration monitoring methods are not needed.

2. **Mammalian toxicity**

**General data**

From the literature review including species closely related to *Bacillus amyloliquefaciens*, the species *Bacillus subtilis* has been isolated on several occasions from human local infections (species not always clearly identified) or in immunodepressed patients, or in food poisoning cases (with large numbers of
spores in contaminated food). *Bacillus subtilis* is also broadly used in industrial setting (e.g. enzyme or vaccine production).

No indications of any toxicological or allergenic effects to the workers involved in the research laboratory or production plant for *Bacillus amyloliquefaciens* strain QST 713 since 1995 have been observed.

*Bacillus amyloliquefaciens* is recommended for the Qualified Presumption of Safety list (EFSA BIOHAZ Panel, 2020) 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. Based on the available data, it cannot be concluded that these qualifications are met (see also Sections 1 and 3).

**Toxicity/Infectivity/Pathogenicity studies**

As the available methods for testing dermal sensitisation are not suitable for testing microorganisms and there are no validated test methods for sensitisation by inhalation, *Bacillus amyloliquefaciens* strain QST 713 may have the potential to provoke sensitising reactions.

In acute toxicity, pathogenicity and infectivity studies with rats, *Bacillus amyloliquefaciens* strain QST 713 did not induce any clinical signs or mortality after oral, intratracheal or intravenous administration. After intratracheal and intravenous administration, an extended clearance time was observed with no evidence of pathogenicity, further spore germination or vegetative growth. A slight irritating potential for the skin and the eyes was observed in studies with rabbits.

After repeated inhalation exposure in rats with the product Serenade ASO no clinical signs but minor organ weight changes were observed, with a complete clearance from all organs during the recovery period (8-week after final exposure).

**Secondary metabolites/toxins**

*Bacillus amyloliquefaciens* strain QST 713 has the genetic potential to produce different secondary metabolites, and some of them are present in the manufactured product (see Section 1). No information has been given on which metabolites will be formed after application.

It is noted that iturins and surfactins are strong surfactants showing membrane damaging properties (lytic activity) *in vitro*. Toxicity studies with surfactin C, produced by other strains of *Bacillus amyloliquefaciens* or *Bacillus subtilis*, were found in the literature. In a rat 28-day oral study with surfactin C (produced by *Bacillus subtilis*), the no observed adverse effect level (NOAEL) was 500 mg/kg body weight (bw) per day based on decreased body weight and liver toxicity. Similarly, surfactin C showed no genotoxic potential *in vitro* in the bacterial reverse mutation and chromosome aberration assays, or *in vivo* in the bone marrow micronucleus test (considered supplementary due to lack of evidence of bone marrow exposure). In a developmental study in mice, surfactin C did not show maternal toxicity or teratogenicity potential.

Pending on further investigations of toxins/secondary metabolites produced after application (data gap), further assessment of their toxicological profile could be triggered.

**Reference values and exposure**

Based on the lack of significant toxicity, infectivity and pathogenicity in the available studies with the microorganism and considering the ubiquitous presence of *Bacillus amyloliquefaciens* strain QST 713 in the environment, the setting of health-based reference values is considered not necessary. In the absence of a quantitative risk assessment, the use of personal protective equipment (PPE) for operators might be considered to reduce the exposure.

Considering that the non-dietary exposure to secondary metabolites (present in the product and/or produced after application) has not been determined, and that their toxicity profile has not been fully characterised, the risk assessment for operators, workers, bystanders and residents cannot be concluded (issue not finalised). One of the Member States (MSs) disagreed and was of the opinion that adequate information was provided to conclude that there is not foreseeable risk for operators, workers, bystanders and residents.

### 3. Residues

The representative uses are foliar applications by spraying up to a BBCH of 89 for the last treatment which represents the growth stage of ripe fruits at harvest on strawberries (maximally six applications with $2.52 \times 10^{14}$ CFU/ha) outdoor, or on strawberries ($6 \times 3.15 \times 10^{14}$ CFU/ha) in a
greenhouse or on grapes (maximally nine applications with $2.52 \times 10^{14}$ CFU/ha) outdoor. A preharvest interval (PHI) is not reported for any of the representative uses (see GAP table in Appendix A).

For *Bacillus amyloliquefaciens* strain QST 713, studies on grapes and pepper leaves were provided to demonstrate that viable counts were declining following application and did not persist or multiply. On grapes, it was demonstrated that following field application (up to $1.44 \times 10^{14}$ CFU/ha), viable spore counts were declining (initially around $9 \times 10^8$ CFU/g grape berries to $7 \times 10^5$ CFU/g) within 28 days. In an indoor study on pepper leaves, following foliar spray, it was demonstrated that viable counts declined within 21 days to around 1% of the measured viable counts after treatment (around $3.8 \times 10^5$ CFU/g; AS formulation), however, for a wettable powder (WP) formulation viable counts did not decline below levels of $10^5$ CFU/g within this time (concentration after treatment: $4.7 \times 10^5$ CFU/g).

Since information on viable counts on strawberries and their residue behaviour was not provided, it is recommended to provide this information and to establish the number of viable spores at the time of harvest for information and characterisation of the treated plant produce intended for human consumption.

With regard to non-viable residues, *Bacillus amyloliquefaciens* strain QST 713 has a known potential to form several secondary metabolites (see Section 1). However, information on the formation of these metabolites and on their expected quantities on strawberries and grapes under GAP directed conditions of use and particularly at harvest is not provided. Furthermore, the toxicological profiles of these metabolites are not conclusively elucidated (see Section 2).

The production of iturin was investigated both under greenhouse and field conditions on wheat ($3 \times 10^9$ and $2.3 \times 10^8$ CFU/ear). According to the RMS, the relevance of this study is difficult to decide on because of the use of a different *Bacillus* strain of the species *Bacillus amyloliquefaciens* and the fact that no reliable relation to GAP rates under consideration can be made. Nevertheless, iturin concentrations were highest directly after application (10 mg/100 ears) and were shown to decline by a factor of 5 (to 2 mg/100 ears) within 3 days.

However, qualitative and quantitative information on iturin and other relevant metabolites potentially formed by *Bacillus amyloliquefaciens* strain QST 713 (see Sections 1 and 2) under GAP directed conditions for the representative uses on strawberries and grapes is not available and considered necessary (data gap for non-viable residues).

Since according to the representative uses on strawberries and grapes the last treatment can be performed on ripe fruits (BBCH of 89), consumer exposure to metabolites potentially formed by *Bacillus amyloliquefaciens* strain QST 713 cannot be excluded.

Considering the uncertainties related to the occurrence of non-viable residues (metabolites) of *Bacillus amyloliquefaciens* strain QST 713 and particularly regarding their amounts (quantitative information) on edible commodities at harvest, the consumer risk assessment cannot be finalised.

*Bacillus amyloliquefaciens* strain QST 713 is therefore not proposed to be included into Annex IV of Regulation (EC) No 396/2005.

It is to be noted that one of the MSs is of the opinion that to list the risk assessment for consumers for this microorganism as an issue that could not be finalised based on available information is not scientifically justified. No toxic/pathogenic effects were observed in studies with the technical grade active ingredient (i.e. the microbial active substance as manufactured). No toxic effects were found in the literature for the metabolites known to be produced. Therefore, toxic effects due to exposure to any metabolites present in the product upon application are therefore not a foreseeable risk. Furthermore, population numbers upon application were shown to decrease; a massive *in situ* production of metabolites upon application which would be necessary to result in consumer products is also not a foreseeable risk. Therefore, it is the opinion of the MS that based on a weight-of-evidence approach using available information, no further assessment of consumers’ exposure is needed, and the assessment is therefore finalised.

The MS expressed the view that although it is (scientifically) not possible to show that no other metabolites can be produced under any environmental conditions, and thus, a data gap for this issue is by default identified, adequate information was provided to perform a risk assessment.

4. Environmental fate and behaviour

Satisfactory information has been provided in relation to potential interference of *Bacillus amyloliquefaciens* strain QST 713 with the analytical systems for the control of the quality of drinking
water provided for in Directive 98/83/EC⁴ (see specific Annex VI decision-making criteria in Part II Commission Regulation (EU) No 546/2011⁵). The provided information support that these methods utilise chromogenic agents to which *Bacillus amyloliquefaciens* strain QST 713 does not give a response. Therefore, it was considered unlikely that *Bacillus amyloliquefaciens* strain QST 713 would interfere with the methodologies used for such determinations.

*Bacillus amyloliquefaciens* strain QST 713 is a 'wild type' and there are no marker genes in the strain which would permit analysis of a frequency of genetic exchange. As the genetic diversity and drift in the wild-type population have not been ascertained, it would not be possible to distinguish any genetic drift from that in the wild population based on the information provided. Though it is acknowledged that the possibility and effects of transfer of genetic material are not different for *Bacillus amyloliquefaciens* strain QST 713 than for other naturally occurring *Bacillus amyloliquefaciens* strains, transfer of genetic material by *Bacillus amyloliquefaciens* strain QST 713 after application is possible and could not be excluded based on the information in the dossier. The transfer of antibiotic resistance genes is considered unlikely (see Section 1).

### 4.1. Fate and behaviour in the environment of the microorganism

Information was derived from published literature on different strains of *Bacillus amyloliquefaciens* in relation to its **persistence and multiplication in soil**. A peer-reviewed published paper was available with *Bacillus amyloliquefaciens* strain QST 713 where colony-forming units declined in seedlings in soil after 28 days. The information on other strains of *Bacillus amyloliquefaciens* and on *Bacillus amyloliquefaciens* strain QST 713 supports that the strain will likely decline in soil after application. The information was considered sufficient to conclude on the likely competitiveness, persistence and multiplication of *Bacillus amyloliquefaciens* strain QST 713 in soil. Introduced endospores can survive in soil for extended periods. PEC soil for field use in grapes has been calculated (see Appendix A).

With respect to the **persistence and multiplication in surface water**, a published paper was available providing information on the persistence of *Bacillus velezensis* in surface water. There were no specific studies available for *Bacillus amyloliquefaciens* strain QST 713. Consequently, EFSA concluded that the information is insufficient to address the uniform principles criterion of the strain not being expected to persist and multiply in surface water in concentrations considerably higher than the natural background levels, taking into account repeated applications over the years. The information on the presence of *Bacillus velezensis* in natural surface water was considered insufficient to demonstrate that *Bacillus amyloliquefaciens* strain QST 713 is likely to decline in surface water. This conclusion identifies a data gap. PEC surface water for field use in grapes (that covers the uses on strawberries in the field, in walk-in tunnels and other non-permanent protected structures) and use on strawberries in permanent greenhouse have been calculated (see Appendix A).

The literature search according to the EFSA guidance (EFSA, 2011) on *Bacillus amyloliquefaciens* provided limited information on occurrence and behaviour in **air**. Multiplication and survival of *Bacillus amyloliquefaciens* strain QST 713 in air are not expected due to environmental conditions e.g. UV-radiation.

Regarding **mobility** in general, vertical distribution of the microbial organism through soil is unlikely to happen based on the available information.

### 4.2. Fate and behaviour in the environment of any relevant metabolite formed by the microorganism under relevant environmental conditions

According to scientific papers from the literature search, the species *Bacillus amyloliquefaciens* is able to produce secondary metabolites. Several secondary metabolites have been confirmed as being present in the product (see Sections 1 and 2). *Bacillus amyloliquefaciens* strain QST 713 has the genes for the production of additional secondary metabolites, but these were not identified in production material analysis (see Sections 1 and 2).

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⁴ Council Directive 98/83/EC of 3 November 1998 on the quality of water intended for human consumption. OJ L 330, 5.12.1998, p. 32–54.
⁵ Commission Regulation (EU) 546/2011 of 10 June 2011 implementing Regulation (EC) No 1107/2009 of the European Parliament and of the Council as regards uniform principles for evaluation and authorisation of plant protection products. OJ L 155, 11.6.2011, p. 127–175.
It is not known to what extent *Bacillus amyloliquefaciens* strain QST 713 will produce any metabolites following its application once the spores reach the soil, should they grow. Adequate information to address the potential concentrations of secondary metabolites/toxins to be produced by *Bacillus amyloliquefaciens* strain QST 713 in all environmental compartments was not available. These exposure assessments for the secondary metabolites/toxins measured as present in the product were also not available. Therefore, a data gap was identified. Consequently, it is not clear if such metabolites might fulfill the criteria according to Part B section 7 (iv) of Commission Regulation (EU) 283/2013 namely:

- the relevant metabolite is stable outside the microorganism;
- a toxic effect of the relevant metabolite is independent of the presence of the microorganism;
- the relevant metabolite is expected to occur in the environment in concentrations considerably higher than under natural conditions.

Therefore, data on the potential for *Bacillus amyloliquefaciens* strain QST 713 to produce metabolites in relation to these criteria are necessary to assess if the further data requirements and the corresponding risk assessment according to Commission Regulation (EU) No 283/2013, part A, section 7 (standard data requirements and assessment mandatory for chemical plant protections active substances) are triggered. Consequently, this resulted in a data gap leading to an assessment not finalised.

5. Ecotoxicology

No information was available on the potential effects of secondary metabolites on non-target organisms (issue not finalised).

A toxicity study of suitable length with birds and the active substance was available. No signs of toxicity, infectivity and pathogenicity were observed. Consequently, low risk to birds was concluded for all the relevant routes of exposure and representative uses.

Toxicity studies were available on mammals both with the active substance and the representative formulation. Based on those data, no signs of toxicity, infectivity and pathogenicity were observed, and low risk was concluded for mammals for all the relevant routes of exposure and representative uses.

The potential for toxicity, infectivity and pathogenicity was investigated in fish and aquatic invertebrates. The available studies were discussed at the Pesticide Peer Review Meeting Teleconference 25 (March 2020). Based on those studies, no signs of infectivity and pathogenicity were observed in both fish and aquatic invertebrates. However, in both studies, a high mortality was observed. Since similar effects were observed both in the attenuated control (microbe-free or non-viable microbe comprising material from the culture system used for propagation) and in the broth concentrate (treatment concentrations), the experts excluded a treatment-related effect of the active substance. The observed mortality could be attributed to several causes like cloudiness of the medium, change in environmental conditions or exposure to secondary metabolites which could not be excluded based on the lack of analytical verifications in the available studies. When considering a risk assessment based on the lowest available endpoint based on mortality, low risk was concluded. A toxicity study was available on algae. The study was not completely valid. However, by taking into consideration the lack of effects in the study and available literature, low risk to algae was also concluded. No studies were available on plants (data gap).

Several studies, including laboratory and field studies, were available on bees and were all discussed at the Pesticide Peer Review Meeting Teleconference 25 (March 2020). The laboratory studies with honeybees did not show any toxic effect. However, those were too short to conclude on infectivity and pathogenicity. Two field studies were available: one feeding study for assessing larval development and a field study on alfalfa where the substance was applied by helicopter. Based on the feeding study, high risk to honeybee larvae could not be excluded. The field study did not show notable effects on colony size or foragers mortality. However, exposure of bees could not be fully confirmed based on the available information, and thus, low risk could not be concluded. A laboratory study with bumblebees was also available. Although the study showed several deficiencies, the experts

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6 Commission Regulation (EU) 283/2013 of 1 March 2013 setting out the data requirements for active substances in accordance with Regulation (EC) No 1107/2009 of the European Parliament and of the Council concerning the placing of plant protection products on the market. OJ L 93, 3.4.2013, p. 1–84.

7 See expert consultation 1 of the Report of Pesticide Peer Review Meeting Teleconference 25.

8 See expert consultation 2 of the Report of Pesticide Peer Review Meeting Teleconference 25.
agreed that in lack of any other information and based on the available evidence, low risk to bumblebees cannot be concluded. Overall, high risk to honeybees could not be excluded for all the representative uses (strawberry and grapes) leading to a critical area of concern. In the case of permanent greenhouses, no exposure to bees is expected with the exception of bees introduced as part of Integrated Pest Management (IPM), and low risk can be concluded. Thus, in that case the identified concern for bees would be lifted. An assessment with inclusion of mitigation measures was not available. However, mitigation measures may be further considered at MS level.

Several studies with different species of non-target arthropods other than bees were available. No sign of infectivity and pathogenicity were reported. Furthermore, based on the toxicity endpoint, low risk was concluded for all the representative uses.

No suitable studies were available on earthworms and soil microorganisms (data gap) and the risk assessment could not be finalised. If the use is limited to permanent greenhouses, low risk to earthworms and soil microorganisms can be concluded based on the lack of exposure. The identified issue not finalised would not apply in that case.
6. Overview of the risk assessment of compounds listed in residue definitions triggering assessment of effects data for the environmental compartments (Tables 1–4)

Table 1: Soil

| Compound (name and/or code)                      | Persistence                                                                 | Ecotoxicology   |
|-------------------------------------------------|------------------------------------------------------------------------------|-----------------|
| Bacillus amyloliquefaciens strain QST 713       | Though endospores may remain viable in soil for extended periods, decline in colony-forming units was demonstrated for the strain | Data gap        |
| Toxins/secondary metabolites such as iturins, fengycins, surfactins, bacilysin, bacillaene, diffidicin, ericins, macrolactin and bacillibactin | Data gap pending on their identification and quantification                   | Data gap        |

Table 2: Groundwater

| Compound (name and/or code)                      | Mobility in soil | > 0.1 μg/L at 1 m depth for the representative uses(a) | Pesticidal activity | Toxicological relevance |
|-------------------------------------------------|------------------|-------------------------------------------------------|---------------------|------------------------|
| Toxins/secondary metabolites such as iturins, fengycins, surfactins, bacilysin, bacillaene, diffidicin, ericins, macrolactin and bacillibactin | Open, possible data gap pending on their identification and quantification | Open               | Open                  |

(a): FOCUS scenarios or relevant lysimeter.

Table 3: Surface water and sediment

| Compound (name and/or code)                      | Ecotoxicology   |
|-------------------------------------------------|-----------------|
| Bacillus amyloliquefaciens strain QST 713       | Low risk        |
| Toxins/secondary metabolites such as iturins, fengycins, surfactins, bacilysin, bacillaene, diffidicin, ericins, macrolactin and bacillibactin | Data gap        |

Table 4: Air

| Compound (name and/or code)                      | Toxicology                                                                 |
|-------------------------------------------------|----------------------------------------------------------------------------|
| Bacillus amyloliquefaciens strain QST 713       | No mortality, no toxic effects and no pathogenicity were observed in rats following intratracheal instillation of Bacillus amyloliquefaciens strain QST 713 at $5 \times 10^8$ cfu/kg bw |
| Toxins/secondary metabolites such as iturins, fengycins, surfactins, bacillaene, diffidicin and ericins | No data on toxicity by inhalation                                           |
7. Data gaps

This is a list of data gaps identified during the peer review process, including those areas in which a study may have been made available during the peer review process but not considered for procedural reasons (without prejudice to the provisions of Article 56 of Regulation (EC) No 1107/2009 concerning information on potentially harmful effects).

- Pending on further investigations of the production of toxins/secondary metabolites after application, and related exposure of humans (operators, workers, bystanders/residents and consumers), non-target organisms and groundwater; further toxicological assessment of these toxins/secondary metabolites might be triggered to conclude on the risk assessment (relevant for all representative uses; see Sections 2, 3, 4 and 5).
- Quantitative information on non-viable residues under GAP-directed conditions in particular at harvest (relevant for all representative uses, see residue Section 3).
- Adequate information to address the uniform principles criterion of the strain not being expected to persist and multiply in surface water in concentrations considerably higher than the natural background levels, provided that repeated applications over the years were not available (relevant for all representative uses evaluated; see Section 4).
- Additional information on the effects on aquatic plants should be submitted (relevant for all the representative uses evaluated; see Section 5).
- Additional information on the toxicity, infectiveness and pathogenicity to earthworms should be submitted (relevant for all the representative uses evaluated; see Section 5).
- Additional information on the impact on soil microorganisms should be submitted (relevant for all the representative uses evaluated; see Section 5).

8. Particular conditions proposed to be taken into account to manage the risk(s) identified

In the absence of a quantitative risk assessment, the use of personal protective equipment (PPE) for operators might be considered during mixing/loading and application to reduce the exposure (see Section 2).

9. Concerns

9.1. Issues that could not be finalised

An issue is listed as ‘could not be finalised’ if there is not enough information available to perform an assessment, even at the lowest tier level, for the representative uses in line with the uniform principles in accordance with Article 29(6) of Regulation (EC) No 1107/2009 and as set out in Commission Regulation (EU) No 546/2011\(^9\) and if the issue is of such importance that it could, when finalised, become a concern (which would also be listed as a critical area of concern if it is of relevance to all representative uses).

An issue is also listed as ‘could not be finalised’ if the available information is considered insufficient to conclude on whether the active substance can be expected to meet the approval criteria provided for in Article 4 of Regulation (EC) No 1107/2009.

1) The production of relevant toxins/secondary metabolites known to be of concern for humans and the environment cannot be excluded. Therefore, the risk assessment cannot be finalised for operators, workers, bystanders, residents, consumers and the environment including the assessment of potential groundwater exposure (see Sections 2, 3, 4 and 5).

2) The risk assessment could not be finalised for earthworms and soil microorganisms for all the representative uses (see Section 5).\(^{10}\)

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\(^9\) Commission Regulation (EU) No 546/2011 of 10 June 2011 implementing Regulation (EC) No 1107/2009 of the European Parliament and of the Council as regards uniform principles for evaluation and authorisation of plant protection products. OJ L 155, 11.6.2011, p. 127–175.

\(^{10}\) If the use is limited to permanent greenhouses, low risk to earthworms and soil microorganisms can be concluded based on the lack of exposure. The identified issue not finalised would not apply in that case.
9.2. **Critical areas of concern**

An issue is listed as a critical area of concern if there is enough information available to perform an assessment for the representative uses in line with the uniform principles in accordance with Article 29(6) of Regulation (EC) No 1107/2009 and as set out in Commission Regulation (EU) No 546/2011, and if this assessment does not permit the conclusion that, for at least one of the representative uses, it may be expected that a plant protection product containing the active substance will not have any harmful effect on human or animal health or on groundwater, or any unacceptable influence on the environment.

An issue is also listed as a critical area of concern if the assessment at a higher tier level could not be finalised due to lack of information, and if the assessment performed at the lower tier level does not permit the conclusion that, for at least one of the representative uses, it may be expected that a plant protection product containing the active substance will not have any harmful effect on human or animal health or on groundwater, or any unacceptable influence on the environment.

An issue is also listed as a critical area of concern if, in the light of current scientific and technical knowledge using guidance documents available at the time of application, the active substance is not expected to meet the approval criteria provided for in Article 4 of Regulation (EC) No 1107/2009.

3) High risk to bees (honeybees and bumblebees) could not be excluded (see Section 5).\(^{11}\)

9.3. **Overview of the concerns identified for each representative use considered**

(If a particular condition proposed to be taken into account to manage an identified risk, as listed in Section 8, has been evaluated as being effective, then 'risk identified' is not indicated in Table 5).

| Representative use                                      | Grapes | Strawberry field | Strawberry greenhouse |
|--------------------------------------------------------|--------|------------------|-----------------------|
| **Operator risk**                                      | Risk identified |                  |                       |
|                                                        | Assessment not finalised | X1 | X1 | X1 |
| **Worker risk**                                        | Risk identified |                  |                       |
|                                                        | Assessment not finalised | X1 | X1 | X1 |
| **Resident/bystander risk**                            | Risk identified |                  |                       |
|                                                        | Assessment not finalised | X1 | X1 | X1 |
| **Consumer risk**                                      | Risk identified |                  |                       |
|                                                        | Assessment not finalised | X1 | X1 | X1 |
| **Risk to wild non-target terrestrial vertebrates**    | Risk identified |                  |                       |
|                                                        | Assessment not finalised |             |                       |
| **Risk to wild non-target terrestrial organisms other than vertebrates** | Risk identified |                  |                       |
|                                                        | Assessment not finalised | X2 | X2 | X2(c) |
| **Risk to aquatic organisms**                          | Risk identified |                  |                       |
|                                                        | Assessment not finalised |              |                       |
| **Groundwater exposure to active substance**           | Legal parametric value breached |                  |                       |
|                                                        | Assessment not finalised |              |                       |
| **Groundwater exposure to metabolites**                | Legal parametric value breached(a) |                  |                       |
|                                                        | Parametric value of 10 μg/L(b) breached |                  |                       |
|                                                        | Assessment not finalised | X1 | X1 | X1 |

\(^{11}\) It was not confirmed if the use is limited to permanent greenhouses. In the case of permanent greenhouses, no exposure to bees is expected with the exception of bees introduced as part of Integrated Pest Management (IPM), and low risk can be concluded. Thus, in that case the identified concern for bees would be lifted.
Columns are grey if no safe use can be identified. The superscript numbers relate to the numbered points indicated in Sections 9.1 and 9.2. Where there is no superscript number, see Sections 2 to 6 for further information.

(a): When the consideration for classification made in the context of this evaluation under Regulation (EC) No 1107/2009 is confirmed under Regulation (EC) No 1272/2008 of the European Parliament and of the Council of 16 December 2008.

(b): Value for non-relevant metabolites prescribed in SANCO/221/2000-rev. 10 final, European Commission, 2003.

(c): In the case of permanent greenhouses, no exposure to bees is expected with the exception of bees introduced as part of integrated pest management (IPM), and low risk can be concluded. Thus, in that case the identified concern for bees would be lifted.

(d): If the use is limited to permanent greenhouses, low risk to earthworms and soil microorganisms can be concluded based on the lack of exposure. The identified issue not finalised would not apply in that case.

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Abbreviations

\(1/n\) slope of Freundlich isotherm

\(\varepsilon\) decadic molar extinction coefficient

ADE actual dermal exposure

AF assessment factor

AV avoidance factor

bw body weight

CAS Chemical Abstracts Service

CFU colony-forming units

CHO Chinese hamster ovary cells

CI confidence interval

CL confidence limits

DAR draft assessment report

DAT days after treatment

www.efsa.europa.eu/efsajournal 16 EFSA Journal 2021;19(1):6381
Peer review of the pesticide risk assessment of the active substance *Bacillus amyloliquefaciens* strain QST 713
Appendix A – List of end points for the active substance and the representative formulation

Appendix A can be found in the online version of this output ('Supporting information' section): https://doi.org/10.2903/j.efsa.2021.6381
### Appendix B – Used compound codes

| Code/trivial name(a) | Chemical name/SMILES notation(b) | Structural formula(c) |
|---------------------|--------------------------------|-----------------------|
| iturin A            | 3-[(3R,6S,13S,16R,19R,22S,27aS)-3,13,19-tris(2-amino-2-oxoethyl)-6-(hydroxymethyl)-16-[(4-hydroxyphenyl)methyl]-9-(9-methyldecyl)-1,4,7,11,14,17,20,23-octaoxohexacosahydro-1H-pyrrolo[2,1-][1,4,7,10,13,16,19,22]octaazacyclopentacosin-22-yl]propanamide | ![Structural formula of iturin A](image1) |
| surfactin C         | 3-[(3S,6R,9S,12S,15R,18S,21S)-9-(carboxymethyl)-3,6,15,18-tetraisobutyryl-12-isopropyl-25-(10-methylnonyl)-2,5,8,11,14,17,20,23-octaoxo-1-oxa-4,7,10,13,16,19,22-heptaaazacyclopentacosan-21-yl]propanoic acid | ![Structural formula of surfactin C](image2) |
| fengycin            | 3-[(3S,6R,13S,16S,22R,27aR)-3,6,13,19-tetrakis(2-amino-2-oxoethyl)-16-[(4-hydroxyphenyl)methyl]-9-(9-methylundecyl)-1,4,7,11,14,17,20,23-octaoxohexacosahydro-1H-pyrrolo[2,1-][1,4,7,10,13,16,19,22]octaazacyclopentacosin-22-yl]propanamide | ![Structural formula of fengycin](image3) |
| difficidin          | (4E,6E,12E,14E,16E)-7,19-dimethyl-20-methylene-2-[(3E)-3-methylhexa-3,5-dien-1-yl]-22-oxo-1-oxacyclodocos-4,6,12,14,16-pentaen-8-yl dihydrogen phosphate | ![Structural formula of difficidin](image4) |
| Code/trivial name<sup>a</sup> | Chemical name/SMILES notation<sup>b</sup> | Structural formula<sup>c</sup> |
|-------------------------------|---------------------------------------------|---------------------------------|
| **macrolactin**               | (3Z,5E,8R,9E,11Z,14S,16S,17E,19E,24R)-8,14,16-trihydroxy-24-methyloxacyclotetracosa-3,5,9,11,17,19-hexaen-2-one | ![Macrolactin structural formula](image1) |
|                              | O=C1C–CC–CC[C@H](O)C–CC–CC[C@H](O)C[C@H](O)C–CC–CCCCC[C@@H](C)O1 | |
|                              | XXDIJWSZFWZBRM-QCEWEWFLSA-N | |
| **bacillibactin**             | N-[2-((2R,3S,6R,10R,11S)-7,11-bis[2-(2,3-dihydroxybenzamido)acetamido]-2,6,10-trimethyl-4,8,12-trioxo-1,5,9-trioxacyclododecan-3-yl)amino)-2-oxoethyl]-2,3-dihydroxybenzamide | ![Bacillibactin structural formula](image2) |
|                              | Oc1cccc(c1O)C(=O)NCC(=O)N[C@@H]1C(=O)O[C@H](C)[C@@H](NC(=O)N[C@H](C)N)C(=O)2cccc(O)C2O[C@H](C)[C@@H](NC(=O)N[C@H](C)N)C2cccc(O)C2O[C@H](C)[C@@H]1C | |
|                              | RCQTVFEB/LUNTGM-GPTSMOPA-N | |
| **bacilysin**                 | L-alanyl-3-[(1R,2S,6R)-5-oxo-7-oxabicyclo[4.1.0]heptan-2-yl]-L-alanine | ![Bacilysin structural formula](image3) |
|                              | O=C1CC[C@@H](C[C@H](NC(=O)[C@H](C)N)(=O)O)[C@H]2O[C@H]12 | |
|                              | XFOUAXMJRHNTOP-PFQXTLEHSA-N | |

<sup>a</sup>: The metabolite name in bold is the name used in the conclusion.

<sup>b</sup>: ACD/Name 2019.1.1 ACD/Labs 2019 Release (File version N05E41, Build 110555, 18 July 2019).

<sup>c</sup>: ACD/ChemSketch 2019.1.1 ACD/Labs 2019 Release (File version C05H41, Build 110712, 24 July 2019).