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STATEMENT

Update of the list of qualified presumption of safety (QPS) recommended microbiological agents intentionally added to food or feed as notified to EFSA 21: Suitability of taxonomic units notified to EFSA until September 2024

EFSA Panel on Biological Hazards (BIOHAZ) | Ana Allende | Avelino Alvarez-Ordóñez | Valeria Bortolaia | Sara Bover-Cid | Alessandra DeCesare | Wietske Dohmen | Laurent Guillier | Liesbeth Jacxsens | Maarten Nauta | Lapo Mughini-Gras | Jakob Ottoson | Luisa Peixe | Fernando Perez-Rodriguez | Panagiotis Skandamis | Elisabetta Suffredini | Pier Sandro Cocconcelli | Pablo Salvador FernándezEscámez | Miguel Prieto Maradona | Amparo Querol | Lolke Sijtsma | Juan Evaristo Suarez | Ingvar Sundh | Angela Botteon | Barizzone Fulvio | Sandra Correia | Lieve Herman

Correspondence: biohaz@efsa.europa.eu

The declarations of interest of all scientific experts active in EFSA's work are available at https://open.efsa.europa.eu/experts

Abstract

The qualified presumption of safety (QPS) process was developed to provide a safety assessment approach for microorganisms intended for use in food or feed chains. In the period covered by this Statement, no new information was found that would change the status of previously recommended QPS taxonomic units (TUs). The TUs in the QPS list were updated based on a verification, against their respective authoritative databases, of the correctness of the names and completeness of synonyms. Of 54 microorganisms notified to EFSA between April and September 2024 (33 as feed additives, 17 as food enzymes or additives, 4 as novel foods), 50 were not evaluated because: 12 were filamentous fungi, 1 was *Enterococcus faecium* and 8 were *Escherichia coli* (all excluded from the QPS evaluation), and 29 were TUs that already have a QPS status. One notification (*Ensifer adhaerens*) was already evaluated in a previous Panel Statement. Another notification (*Enterococcus lactis*) was already evaluated in the previous 3-year QPS cycle and was reassessed within this document. Two TUs were notified for the first time and were assessed for a possible QPS status: *Serratia plymuthica* and *Lacticaseibacillus huelsenbergensis*. *Bacillus thuringiensis* and *Bacillus nakamurai* have been assessed for a possible QPS status in response to internal requests. The following was concluded on the five assessed TUs. *L. huelsenbergensis* can be granted the QPS status based on its close relatedness to several other QPS *Lacticaseibacillus* species. *E. lactis* is not recommended for the QPS status due to insufficient information on safety. *S. plymuthica* and *B. thuringiensis* are not recommended for the QPS status due to safety concerns. *B. nakamurai* cannot be recommended for the QPS list due to a lack of body of knowledge for its use in the food and feed chain.

KEYWORDS

Bacillus nakamurai, *Bacillus thuringiensis*, *Enterococcus lactis*, *Lacticaseibacillus huelsenbergensis*, QPS, *Serratia plymuthica*

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SUMMARY

The European Food Safety Authority (EFSA) asked the Scientific Panel on Biological Hazards (BIOHAZ) to deliver a Scientific Opinion on the maintenance of the qualified presumption of safety (QPS) list. The QPS list contains microorganisms, intentionally added to food and feed, which have received QPS status. The request included three specific tasks as mentioned in the Terms of Reference (ToR).

The QPS process was developed to provide a harmonised safety assessment approach to support EFSA Scientific Panels and Units. This process assesses the taxonomic identity, body of relevant knowledge and safety of microorganisms. Safety concerns identified for a taxonomic unit (TU) are, where possible, confirmed at strain or product level, reflected as 'qualifications' that should be assessed at the strain level by EFSA's Scientific Panels. A generic qualification for all QPS bacterial TUs applies in relation to the absence of acquired genes conferring resistance to clinically relevant antimicrobials (EFSA, 2008).

The list of microorganisms is maintained and re-evaluated approximately every 6 months in a Biohazard Panel Statement. The Panel Statement also includes the evaluation of newly notified microorganisms to EFSA in the context of technical dossiers for safety assessment, within the previous 6-month period.

The first ToR requires ongoing updates of the list of microorganisms notified to EFSA, in the context of a technical dossier for safety assessment. The list 'Microbiological agents as notified to EFSA' (https://doi.org/10.5281/zenodo.3607183) was updated with the notifications received between April and September 2024 (inclusive). Within this period, 54 notifications were received by EFSA, of which 33 were proposed for use in feed, 17 as food enzymes, food additives and flavourings and 4 as novel foods. The new notifications received within that period are included in the current Statement (see Appendix G).

The second ToR concerns the revision of the TUs previously recommended for the QPS list and their qualifications. A new procedure has been established to ensure that the TUs are kept up to date in relation to recent taxonomical insights. The QPS TUs of bacteria, yeast, algae, protists and viruses are being verified every 6 months against their respective authoritative databases to ensure the accuracy for each Panel Statement.

For the revision of the QPS list, articles published from January to June 2024 were assessed. The articles were retrieved and assessed through an extensive literature search (ELS) protocol available in Appendix B (see https://doi.org/10.5281/zenodo.3607188) and the search strategies in Appendix C (see https://doi.org/10.5281/zenodo.3607192). The ELS launched for this Panel statement included the updated names/synonyms reported in the previous QPS Panel statement (EFSA BIOHAZ Panel, 2024b) as keywords. No new information was found that would affect the QPS status or the qualifications for the TUs on the QPS list.

The third ToR requires a (re)assessment of new TUs notified to EFSA, for their suitability for inclusion in the updated QPS list at the Knowledge Junction in Zenodo (https://doi.org/10.5281/zenodo.1146566, Appendix F- the link opens at the latest update of the QPS list, and also includes the links to the versions associated to each Panel Statement).

In the current period, 54 notifications were received, 50 of which were not evaluated for the following reasons: 21 notifications were related to microorganisms that are excluded from QPS evaluation (12 were notifications of filamentous fungi, 1 of *Enterococcus faecium* and 8 of *Escherichia coli*), and 29 were related to TUs that already have QPS status and did not require further evaluation. Two of the other four notifications were already evaluated for possible QPS status in previous Panel Statements: *Ensifer adhaerens* (EFSA BIOHAZ Panel, 2024b) which will not be assessed again now and *Enterococcus lactis* (EFSA BIOHAZ Panel, 2022) to be reassessed within this document as the previous assessment was done within the previous 3-years QPS cycle. The other two TUs were notified for the first time and therefore were assessed for a possible QPS status in this Panel Statement: *Serratia plymuthica* and *L. huelsenbergensis*. *Bacillus thuringiensis* has been reassessed for a possible QPS status in response to an internal request. *Bacillus nakamurai* has also been included in response to another internal ad-hoc request.

The following conclusions were drawn:

- *Bacillus thuringiensis* is not recommended for the QPS status due to safety concerns.
- *Enterococcus lactis* is not recommended for the QPS status due to insufficient information on safety.
- *Bacillus nakamurai* cannot be recommended for the QPS list due to a lack of body of knowledge for its use in the food and feed chain.
- *Lacticaseibacillus huelsenbergensis* can be granted the QPS status based on its close relatedness to several other QPS *Lacticaseibacillus* species.
- *Serratia plymuthica* is not recommended for the QPS status due to safety concerns.

1 | INTRODUCTION

The qualified presumption of safety (QPS) approach was developed by the EFSA Scientific Committee to provide a generic concept for risk assessment within the European Food Safety Authority (EFSA) for microorganisms intentionally introduced into the food and feed chains, in support of the respective Scientific Panels and Units in the context of market authorisations for their use in food and feed and the requirement for a safety assessment by EFSA (EFSA, 2007; Herman et al., 2019). The list, first established in 2007, has been continuously revised and updated. A Panel Statement is published approximately every 6 months. These Panel Statements include the results of the assessment of relevant new scientific articles related to the taxonomic units (TUs) with QPS status. They also contain the assessment of newly submitted TUs to the EFSA Units on Feed and Contaminants (FEEDCO), Food Ingredients and Packaging (FIP), Nutrition and Food Innovation (NIF), and Pesticides Peer Review (PREV). After 3 years, a QPS opinion is published summarising the results of the Panel Statements published in that period.

1.1 | Background and Terms of Reference as provided by the requestor

A wide variety of microorganisms are intentionally added at different stages to the food and feed chains. In the context of applications for market authorisation, EFSA is requested to assess the safety of microorganisms when used either directly or as sources of food and feed additives, food enzymes and plant protection products.

EFSA's work on QPS activities began in 2004, when the Scientific Committee issued a Scientific opinion in continuation of the 2003 working document '*On a generic approach to the safety assessment of microorganisms used in feed/food and feed/food production*' prepared by a working group consisting of members of the former Scientific Committee on Animal Nutrition, the Scientific Committee on Food and the Scientific Committee on Plants of the European Commission.[1] The document, made available for public consultation, proposed the introduction of the concept of Qualified Presumption of Safety (QPS), to be applied to selected groups of microorganisms. Microorganisms not considered suitable for QPS status would remain subject to a full safety assessment. EFSA management asked its Scientific Committee to consider whether the QPS approach could be applied to the safety assessment of microorganisms across the various EFSA Scientific Panels. In doing so, the Committee was required to take into account the response of stakeholders to the QPS approach. In its 2005 Opinion (EFSA, 2005), the Scientific Committee concluded that the QPS approach could provide a generic assessment system that could be applied to all requests received by EFSA for the safety assessments of microorganisms deliberately introduced into the food and feed chains. Its introduction was intended to improve transparency and ensure consistency in the approach used across the EFSA Panels. Applications involving a TU belonging to a species that falls within a QPS group do not require a full safety assessment.

Several TUs (usually species for bacteria and yeasts; families for viruses) have been included in the QPS list, either following notifications to EFSA, or proposals made initially by stakeholders during a public consultation in 2005, even if they were not yet notified to EFSA (EFSA, 2005). The EFSA Scientific Committee reviewed the range and numbers of microorganisms likely to be the subject of an EFSA Opinion and, in 2007, published a list of microorganisms recommended for the QPS list.

In their 2007 Opinion (EFSA, 2007), the Scientific Committee recommended that the QPS approach should provide a generic concept to prioritise and to harmonise safety risk assessment of microorganisms intentionally introduced into the food and feed chains, in support of the respective Scientific Panels and EFSA Units in the frame of the market authorisations for their use in the food and feed chains. The same Committee recognised that there would have to be continuing provision for reviewing and modifying the QPS list and, in line with this recommendation, the EFSA Panel on Biological Hazards (BIOHAZ) took the prime responsibility for this and started reviewing annually the existing QPS list. In 2008, the first annual QPS update was published (EFSA, 2008).

In 2014, the BIOHAZ Panel, in consultation with the Scientific Committee, decided to change the revision procedure; the overall assessment of the TUs previously recommended for the QPS list (EFSA BIOHAZ Panel, 2013) was no longer carried out annually but over a 3-year period. From 2017, the search and revision of the possible safety concerns linked to those TUs began instead to be carried out every 6 months through extensive literature searches (ELS). The update of the 2013 QPS list (EFSA BIOHAZ Panel, 2013) was done in 2016 (EFSA BIOHAZ Panel, 2017). From 2016 on, the QPS list (https://doi.org/10.5281/zenodo.1146566) and the list of notifications to EFSA (https://doi.org/10.5281/zenodo.3607183) are constantly updated, independent of the QPS Opinion, and are available at the Knowledge Junction in Zenodo. The most recent QPS Opinion (EFSA BIOHAZ Panel, 2023) summarises the main results of the 3-year ELS on the QPS TUs, together with an update of the process for granting QPS status. In the meantime, every 6 months a Panel Statement, compiling the assessments for a QPS status of the microorganisms notified to EFSA requested by the Feed and Contaminants (FEEDCO) Unit, the Food Ingredients and Packaging (FIP) Unit, the Nutrition and Food Innovation (NIF) Unit, the Pesticides Peer Review (PREV) Unit,[2] as well as the summary of each 6-month ELS exercise, has been produced and published. Each QPS Panel Statement contains the evaluations of the new notifications for microorganisms submitted for possible QPS status. It also contains the result of a standardised ELS performed every 6 months regarding possible new safety concerns related to the TUs already included in

[1] https://ec.europa.eu/food/sites/food/files/safety/docs/sci-com_scf_out178_en.pdf.

[2] Units as in December 2022.

the QPS list. The data identified are used to inform decisions on whether any TU may or may not remain on the QPS list, and whether any qualifications need to be revised.

Establishing a QPS status is based on 4 pillars: [1] the taxonomic unit (TU) for which QPS is sought ('*taxonomic identification*'); [2] whether sufficient relevant information is available about the proposed TU to conclude on human/animal exposure via food/feed ('*body of knowledge*'); [3] whether the TU proposed contains known '*safety concerns*' and, finally, [4] the intended end use ('*intended use*'). If a hazard related to a TU is identified, which can be tested at the strain or product level, a 'qualification' to exclude that hazard may be established and added. The subject of these qualifications for the microbial strain under investigation is evaluated by the EFSA Unit to which the application dossier has been allocated. Absence of acquired genes coding for resistance to antimicrobials relevant for humans and animals is a generic qualification for all bacterial TUs; the absence of antimycotic resistance should be proven if the pertinent yeasts are to be used as viable organisms in the food and/or feed chains. The qualification 'for production purpose only' implies the absence of viable cells of the production organism in the final product and can also be applied to food and feed products based on microbial biomass (EFSA BIOHAZ Panel, 2020a).

Because the QPS evaluation is, after its initial creation, only triggered through an application dossier notified to EFSA, the QPS list is not exhaustive.

In summary, the QPS evaluation provides a safety assessment approach for use within EFSA that covers safety concerns for humans, production animals and the environment. In the QPS concept, a safety assessment of a defined TU is performed independently of the legal framework under which the application is made in the course of an authorisation process. Although general human safety is part of the evaluation, specific issues relating to type and level of exposure of users handling the product (e.g. dermal contact, inhalation, ingestion) are not addressed. In the case of Genetically Modified Microorganisms (GMMs) for which the species of the recipient strain qualifies for the QPS status, and for which the genetic modification does not give rise to safety concerns, the QPS approach can be extended to genetically modified production strains (EFSA BIOHAZ Panel, 2018). The assessment of potential allergenic microbial residual components is beyond the QPS remit; however, it is reported if science-based evidence is available for a microbial species. These aspects are separately assessed, where applicable, by the EFSA Panel responsible for assessing the application.

The lowest TU for which the QPS status is granted is the species level for bacteria, yeasts and protists/algae, and family for viruses.

Filamentous fungi, bacteriophages, *Streptomycetes*, Oomycetes, *Enterococcus faecium, Escherichia coli, Clostridium butyricum* (EFSA BIOHAZ Panel, 2020a, 2020b), *Klebsiella pneumoniae* (EFSA BIOHAZ Panel, 2024a), *Actinomadura roseirufa* and *Burkholderia stagnalis* (within the previous Panel Statement (EFSA BIOHAZ Panel, 2024b)) are excluded from the QPS assessments based on an ambiguous taxonomic position or the possession of potentially harmful traits by some strains of the TU and therefore, require a specific assessment for each strain for which an application is made.

The **Terms of Reference** are as follows:

ToR 1: Keep updated the list of microorganisms being notified in the context of a technical dossier to EFSA Units such as Feed and Contaminants (FEEDCO), Pesticides Peer Review (PREV), Food Ingredients and Packaging (FIP) and Nutrition and Food Innovation (NIF),[3] for intentional use directly or as sources of food and feed additives, food enzymes and plant protection products (PPPs), as novel foods and Genetically Modified Microorganisms (GMM) for safety assessment.

ToR 2: Review taxonomic units previously recommended for the QPS list and their qualifications when new information has become available. The latter is based on an update of the ELS aiming to verify whether any new safety concern has arisen that could require the removal of a taxonomic unit from the list, and to verify if the qualifications still effectively exclude safety concerns.

ToR 3: (Re) assess the suitability of new taxonomic units notified to EFSA for their inclusion in the QPS list. These microorganisms are notified to EFSA in the context of technical dossiers for safety assessment and trigger a QPS assessment.[4]

2 | DATA AND METHODOLOGIES

2.1 | Data

In reply to ToR 3, (re)assessment of the suitability of TUs notified within the period covered by this Statement (between April and September 2024 (inclusive)) was carried out. The literature review considered the information on taxonomy, the body of knowledge, the potential safety concerns related to human and animal health and to the environment (EFSA BIOHAZ Panel, 2023) for each TU. The environmental risk assessment of a TU used in PPPs, following the legal requirements, is not included in the QPS assessment but is carried out by the Pesticide Peer Review (PPR) Unit, based on the risk assessment in the application.

[3]Units as in December 2022.

[4]Previous text 'These microorganisms are notified to EFSA and requested by the Feed Unit, the FIP Unit, the Nutrition Unit or by the Pesticides Unit'.

Relevant databases, such as PubMed, Web of Science, CAB Abstracts or Food Science Technology Abstracts (FSTA) and Scopus, were searched, based on the judgement of the experts. When needed, an ELS-based approach is applied to ensure the completeness of the information retrieved from the literature in terms of body of knowledge and possible safety concerns. The ELS follows the same methodology as used for monitoring new safety concerns related to species with QPS status but also included information on the body of knowledge. More details on the search strategy, search keys and approach for each of the assessments are described in Appendix A. Only the literature that is considered, based on expert judgement, to be relevant for the QPS assessment is reflected in the Statement.

Only valid TUs covered by the relevant international committees on the nomenclature for microorganisms are considered for the QPS assessment (EFSA BIOHAZ Panel, 2023). In order to validate this statement, it was decided to revise in a systematic way the TUs names and synonyms included in the current QPS list. The TUs of bacteria, yeasts, algae, protists and viruses present in the QPS list were checked against their respective authoritative databases to verify the correctness of the names and completeness of synonyms. The results of this exercise can be found in Section 3.4.

2.1.1 | Reassessment of a possible QPS status of *Bacillus thuringiensis* at species level

An ELS was launched to screen for possible safety concerns linked to *B. thuringiensis*. The search terms used were the ones used for the *Bacillus* species with a QPS status and some extra key-terms linked to the common end use as a microbial plant protection product. References published from January 2015 until July 2024, were searched in order to include all relevant literature since the previous BIOHAZ Panel Opinion on 'Risks for public health related to the presence of *Bacillus cereus* and other *Bacillus* spp. including *Bacillus thuringiensis* in foodstuffs' (EFSA BIOHAZ Panel, 2016). The search strings can be found in Appendix A.

A total of 5867 hits were identified and from these, 64 references were selected based on the information provided in the abstract for the article evaluation phase. From these 64 references, 24 were considered relevant for further assessment (Appendix E) according to the QPS pillars (i) taxonomic identity, (ii) *body of knowledge* (ecological aspects such as natural presence in agricultural soils, use as microbial plant protection product, attachment to edible plant components, prevalence and concentrations in the food and feed chain, environmental distribution, etc.) and (iii) safety concerns (case reports of human disease/ foodborne outbreaks, particularly foodborne infections or intoxications, presence of virulence factors in the genome sequence of the strains, in vitro and in vivo safety tests). The other 40 references were excluded because they were not relevant for the QPS assessment and/or the species identification method was not conclusive.

Besides the ELS, additional papers retrieved based on experts' knowledge were included in the assessment because of their relevance to the topic; some of these papers were published before 2015. These papers were included to interpret the data in a broader context than covered by the ELS search items.

2.2 | Methodologies

2.2.1 | Evaluation of a QPS recommendation for taxonomic units notified to EFSA

In response to ToR 1, the EFSA Units were asked to update the list of microorganisms being notified to EFSA. A total of 54 notifications were received between April and September 2024 (inclusive), of which 33 were for evaluation for use in feed, 17 for use as food enzymes, food additives and flavourings, 4 as infant formula/nutrition/novel foods and none as plant protection products (Table 1).

In response to ToR 3, 50 notifications were excluded from QPS evaluation for the following reasons: 21 notifications were related to microorganisms that are generally excluded from QPS evaluation (12 were notifications of filamentous fungi, 1 of *Enterococcus faecium* and 8 of *Escherichia coli*) and 29 were related to TUs that already had QPS status and did not require further evaluation in this mandate. Two of the other four notifications were already evaluated for a possible QPS status in previous Panel Statements: *Ensifer adhaerens* (EFSA BIOHAZ Panel, 2024a, 2024b) which will not be assessed again now and *Enterococcus lactis* (EFSA BIOHAZ Panel, 2022) to be assessed within this document as the previous assessment was done within the previous 3 years QPS cycle. The other 2 TUs were notified for the first time and therefore will be assessed for a possible QPS status in this Panel Statement: *Serratia plymuthica* and *Lacticaseibacillus huelsenbergensis*.

These 50 notifications do not include two TUs which were assessed in response to internal ad-hoc requests: *Bacillus thuringiensis* and *Bacillus nakamurai*.

TABLE 1 Notifications received by EFSA, per risk assessment area and by microbiological group, from April to September 2024.

Risk assessment area	Not evaluated in this statement		Evaluated in this statement[b]	Total
Microbiological group	**Already QPS**	**Excluded in QPS[a]**		
Feed additives	**21**	**9**	**3**	**33**
Bacteria	19	4	3	26
Filamentous fungi		5		5

TABLE 1 (Continued)

Risk assessment area	Not evaluated in this statement		Evaluated in this statement[b]	
Microbiological group	**Already QPS**	**Excluded in QPS[a]**		**Total**
Yeasts	2			2
Novel foods	**1**	**2**	**1**	**4**
Bacteria		2	1	3
Filamentous fungi				0
Protists/Algae				0
Yeasts	1			1
Plant protection products	**0**	**0**	**0**	**0**
Food enzymes, food additives and flavourings	**7**	**10**	**0**	**17**
Bacteria	6	3		9
Filamentous fungi		7		7
Yeasts	1			1
Genetically modified organism	**0**	**0**	**0**	**0**
Bacteria				0
Total	**29**	**21**	**4**	**54**

Abbreviation: QPS, qualified presumption of safety.

[a]The number includes 12 notifications of filamentous fungi, 1 of *Enterococcus faecium* (bacterium) and 8 of *Escherichia coli* (bacterium), all excluded from QPS evaluation.

[b]Four notifications corresponding to four TU, *Ensifer adhaerens* (not assessed within in this Panel statement), *Enterococcus lactis*, *Serratia plymuthica* and *Lacticaseibacillus huelsenbergensis*.

2.2.2 | Monitoring of new safety concerns related to species with QPS status

In reply to ToR 2, concerning the revision of the TUs previously recommended for the QPS list and their qualifications, an extensive literature search (ELS) was conducted as described in Appendix B – ELS protocol, see https://doi.org/10.5281/zenodo.3607188, and in Appendix C Search strategies – see https://doi.org/10.5281/zenodo.3607192, respectively. The ELS launched for this Panel statement included the updated names/synonyms reported in the previous QPS Panel statement (EFSA BIOHAZ Panel, 2024a, 2024b) as keywords.

The aim of the ELS was to identify any publicly available scientific studies reporting on safety concerns for humans, production animals, the environment, AMR or genotoxicity caused by QPS organisms since the previous QPS review (i.e. scientific articles published from January to June 2024) that would require a change in the QPS status of the TU.

The ELS was done in DistillerSR starting with a screening based on the title and the abstract followed by evaluation of the full texts of the selected abstracts.

The Title and Abstract screening step in this process was supported by a machine-assisted tool (DAISY) in DistillerSR. Details of the process followed can be found in the previous QPS Panel Statement (EFSA BIOHAZ Panel, 2024b).

The Title and Abstract screening step was performed in parallel by one Expert and the same classifier used for the QPS batch of references processed in the previous Panel Statement (EFSA BIOHAZ Panel, 2024b). An assessment of the performances of the classifier on the above-mentioned batch of references was performed. The specificity of the classifier was close to 0.99 while, when considering the results of the process up to the Article Evaluation step, the sensitivity was close to 0.86. Against this background, the classifier was considered useful when put in production as one of the reviewers at Title and Abstract screening.

To allow the potential expansion of the training set for the DistillerSR Classifier and hence continuously improve the performance of the algorithm in subsequent QPS batches, conflicts between the Experts and the classifier were solved. In case of conflicts where the answer of the classifier had to be changed (after consultation with the Expert concerned), the reply was changed manually by the EFSA Scientific Officer in charge of the assessment who had administration rights on the DistillerSR project.

For case reports of human infections or intoxications, important additional information includes whether any negative impacts are confined to people with conditions that leave the person susceptible to opportunistic infections, for example immunosuppression, and whether transmission occurred through ingestion of food, intake of probiotics or other routes (e.g. medical devices), when described. Studies indicating the presence of virulence factors (e.g. toxins and enzymes that may contribute to the pathogenicity of the microorganism) in the TU are also reported as relevant when identifying potential safety concerns.

Several of the QPS-TUs are sporadically reported as causing infections in individuals with recognised predisposing conditions for the acquisition of opportunistic infections, e.g. cardiovascular conditions associated with endocarditis, people in the lower or upper age spectrum, or with other conditions which can lead to impairment of the immune system, such as patients subjected to transplants, undergoing cancer therapy, suffering from physical trauma or tissue damage, or HIV patients. Moreover, gastrointestinal tract-related conditions with, for example, mucosal impairment and/or proton pump

inhibitors can also be predisposing factors for infection. Previous use of the microorganisms being assessed as food supplements/probiotics for humans was reported in many of these cases. The QPS assessment takes into consideration these reports, extracting relevant information whenever justified.

After removal of duplicates, 9429 records were submitted to the title and abstract screening step, which led to the exclusion of 9348 of these. The remaining 81 records were found eligible for article evaluation step (full text) and 48 were considered to report a potential safety concern and were further analysed.

The flow of records from their identification by the different search strategies (as reported in Appendix C) to their consideration as potentially relevant scientific articles for QPS is shown in Table 2.

TABLE 2 Flow of records by search strategy step.

Species	Title/abstract screening step	Article evaluation step (screening for potential relevance)	Article evaluation step (identification of potential safety concerns)
	Number of articles retrieved		
Bacteria (total)	**4893**	**19**	**14**
Bacillus spp.	1172	7	5
Bifidobacterium spp.	575	0	0
Carnobacterium divergens	7	0	0
Corynebacterium glutamicum	135	1	1
Gram negatives[a]	994[b]	2	1
Lactobacilli	1122	3	3
Lactococcus lactis	174	2	1
Leuconostoc spp.	152	3	2
Microbacterium imperiale	1	0	0
Oenococcus oeni	23	0	0
Pasteuria nishizawae	1	0	0
Clostridium tyrobutyricum	33	0	0
Pediococcus spp.	295	1	1
Propionibacterium spp.	40	0	0
Streptococcus thermophilus	169	0	0
Viruses (total)	**271**	**0**	**0**
Alphaflexiviridae/Potyviridae	140	0	0
Baculoviridae	131	0	0
Yeasts	**3640**	**62**	**34**
Protists	**20**	**0**	**0**
Algae	**605**	**0**	**0**
Total	**9429**	**81**	**48**
Excluded	9348	33	

[a]*Gluconobacter oxydans/Xanthomonas campestris/Cupriavidus necator/Komagataeibacter sucrofermentans/Agrobacterium radiobacter synonym Rhizobium radiobacter.*

[b]*Gluconobacter oxydans* (44)/*Xanthomonas campestris* (158)/*Cupriavidus necator* (107)/*Komagataeibacter sucrofermentans* (7) / *Agrobacterium radiobacter* synonym *Rhizobium radiobacter (678).*

3 | ASSESSMENT

3.1 | Taxonomic units evaluated during the previous QPS mandate and re-evaluated in the current statement

3.1.1 | Bacteria

Bacillus thuringiensis

Identity

B. thuringiensis is a species with Standing in Nomenclature. *B. thuringiensis* belongs to *B. cereus* sensu lato (s.l.), also known as the *Bacillus cereus* group.

Currently, the *B. cereus* group consists of the following 20 species with Standing in Nomenclature: *Bacillus albus*, *B. anthracis*, *B. cereus* sensu stricto (s.s.), *B. cytotoxicus, B. gaemokensis, B. luti, B. manliponensis, B. mobilis, B. mycoides, B. nitratireducens, B. pacificus, B. paranthracis, B. paramycoides, B. proteolyticus, B. pseudomycoides, B. thuringiensis, B. toyonensis, B. tropicus, B. weihenstephanensis* and *B. wiedmannii*.

Members of this taxonomic group were classified based on specific phenotypic characteristics. They may contain plasmids that harbour genetic elements responsible for traits such as anthrax toxin production, insecticidal crystal proteins and cereulide synthetase proteins. Production of entomotoxin crystal proteins is a common trait of *B. thuringiensis* strains (Trunet et al., 2023). The *cry* genes are predominantly located on plasmids, characterised by a complex and modular structure. Often the plasmids contain multiple *cry* genes that confer specific insecticidal activities against various insect species (Cardoso et al., 2020).

B. thuringiensis strains have been identified through microscopic analysis of presence of insecticidal crystals. Additional identification approaches complement this phenotypic analysis by detecting genes coding for insecticidal Cry toxins (Chung et al., 2024). Based on immunological reactions to the bacterial flagellar antigen flagellin, *B. thuringiensis* strains have been allocated to serovars given names as subspecies that are still in use today (e.g. *kurstaki*, *aizawai, israelensis, tenebrionis, morrisoni)*. This subspecies division without Standing in Nomenclature, is only based on one characteristic of the species (flagellin amino acid sequence) and, therefore, has very little value in predicting other phenotypic characteristics (EFSA BIOHAZ Panel, 2016; Xu & Côté, 2008).

The taxonomy of *B. cereus* s.l. evolved in the last decade as results of the application of genome-based taxonomy using average nucleotide identity (ANI) values as threshold to separate different genomospecies. Using diverse thresholds, ranging from 92% to 96% ANI, novel genomospecies of *B. cereus* s.l. were described (Jiménez et al., 2013; Liu et al., 2017; Miller et al., 2016).

More recently two studies proposed a revised genomospecies circumscription. Torres Manno et al. (2020) using core-based phylogeny and all versus all ANI (96% threshold) on 2116 genomes of strains from the *B. cereus* group identified 57 genomospecies, 37 of which are newly described. These authors showed that *B. cereus* sensu stricto (s.s.) and *B. thuringiensis* form a common clade, and that *B. thuringiensis* is divided into two genomovars, gv. *thuringiensis* and gv. *cytolyticus*, as previously proposed by Baek et al. (2019). The entomotoxin-encoding *cry* genes were present in similar proportions within the genomes of both *B. cereus* sensu stricto (s.s.) and *B. thuringiensis*. Carroll et al. (2020) using ANI with the 92.5% threshold proposed a nomenclatural framework for the *B. cereus* group that recognised 12 genomospecies. Moreover, they proposed three biovars, namely Biovar Anthracis, Biovar Emeticus and Biovar Thuringiensis, to account for phenotypes, respectively related to the production of the anthrax toxin, cereulide and insecticidal toxins, which can be spread across various genomospecies and heterogeneous in their occurrence within specific lineages. The diffusion of the Biovar Thuringiensis within the *B. cereus* s.l. group was determined by Chung et al. (2024). The presence of insecticidal toxin genes was complemented with phenotypic toxin crystal detection by microscope-observations. The strains containing the genes for entomotoxins were detected across various *B. cereus* s.l. lineages and were mixed with non-Thuringiensis strains, indicating that phylogenetic analysis by itself is inadequate for identifying strains of biovar Thuringiensis. This observation is consistent with earlier studies (Biggel et al., 2022) supporting the hypothesis that Cry toxin genes have spread among *B. cereus* group strains via horizontal gene transfer (Méric et al., 2018).

Recognising that the molecular taxonomy approach based on genome comparison, such as ANI, is not capable of identifying *B. thuringiensis* (including the strains used as bioinsecticides), the following sections employ the presence of crystal proteins or the genes encoding them as the discriminating factor in the selection of scientific articles dealing with the body of knowledge and the safety of *B. thuringiensis* strains. For the assessment of the papers, the presence of crystal proteins, investigated by microscopical examination is considered as the standard method; the presence of the crystal toxin genes (*cry*) is considered useful as complementary information. When the identification was performed by alternative methods, this is mentioned specifically.

Body of knowledge

B. thuringiensis contain insecticidal proteins, the reason why *B. thuringiensis* is commercialised as a biopesticide. The *B. thuringiensis*-based formulations that have been registered in the market are containing a mixture of dried spores and toxin crystals. *B. thuringiensis*-based biopesticides are increasingly used and form an important part of the actual applied insecticides (Jalali et al., 2020). *B. thuringiensis* can be transferred to edible parts of the plants after biopesticide applications or from the soil, where they are naturally present. Their spores can survive dehydration and food processing and can end up in diverse food products where they can further proliferate under appropriate conditions (EFSA BIOHAZ Panel, 2016).

Data from recent literature, focusing on the prevalence, the level and the source of *B. thuringiensis* on a variety of food products, are summarised below and the data on fresh vegetables are reported in more detail.

B. thuringiensis has been isolated from dairy products (Chaves et al., 2017; Kovac et al., 2016); dried spices and dried grain products (Cufaoglu et al., 2022, Kindle et al., 2019; Park et al., 2022), vegetable-based puree (Bassi et al., 2016), pepper, paprika and parsley (Frentzel et al., 2018), and from edible processed insects (Fasolato et al., 2018). In many of these studies *B. thuringiensis* strains, first identified by microscopical examination of the presence of crystals, were further analysed for the presence of virulence genes, indicating that the *hbl* and *cytK* genes and the genes of the Nhe complex are neither inclusive nor exclusive for *B. cereus* s.s. or *B. thuringiensis* (Bassi et al., 2016; Fasolato et al., 2018; Kovac et al., 2016; Park et al., 2022). *B. thuringiensis* strains were not found positive for cereulide production and/or the presence of *ces* genes (Cufaoglu et al., 2022; Frentzel et al., 2018).

In a Korean study, *B. thuringiensis* was detected in 30 out of 39 tested organic vegetables with a mean value of 26 cfu/g, mainly on leafy vegetables (88.5%), flowered brassicaceae (66.7%) and fruiting vegetables (75.0%) and not on root and tuber vegetables (Kim et al., 2017). European studies found *B. thuringiensis* on bell peppers, tomatoes and lettuce (prevalence from 18% to 41% of tested samples) in concentrations ranging from 10^2 to 10^5 cfu/g (Biggel et al., 2022, Bonis et al., 2021; Frentzel et al., 2020).

The persistence of *B. thuringiensis* in vegetables is consistent with its capacity to colonise the endophytic niche in plants (Espinoza-Vergara et al., 2023) and, therefore, *B. thuringiensis* is expected to be present on harvested plants as a natural contaminant. In certain papers, the analysis of single nucleotide polymorphisms in the whole-genome sequence (wgSNP analysis) of *B. thuringiensis* strains showed that biopesticides application can also be a source of contamination (Bonis et al., 2021; Frentzel et al., 2000).

A concentration of presumptive *B. cereus* ranging from 1.95×10^4 cfu/g to 1.75×10^5 cfu/g was found for spinach samples which were treated with a *B. thuringiensis* biopesticide and ranged from 100 cfu/g till 850 cfu/g for the non-treated ones. In the treated samples, within presumptive colonies *B. thuringiensis* were identified but not in the untreated ones. Whole-genome sequencing (WGS) of the *B. thuringiensis* strains isolated from the biopesticide treated spinach samples confirmed that they were the biopesticide strains (pairwise wgSNP distance of 0–12). The biopesticide strains used showed no growth at refrigeration temperatures and a low or moderate biofilm-forming ability. The strains were shown to carry the *nhe, hbl, cytK-2* genes and were expressing Hbl enterotoxin in vitro (Zhao et al., 2022).

B. thuringiensis was isolated from tomatoes from the retail in Belgium in 56% of the 109 samples tested. The counts ranged from 1.3×10^2 to 1.3×10^5 cfu/g. Spores from commercial granule formulated *B. thuringiensis* biopesticide products showed easier wash-off properties than the unformulated lab-cultured *B. thuringiensis* spores of the same strains (Zhao et al., 2023).

De Bock et al. (2021) theoretically estimated, based on the maximum dose allowed to be sprayed, a concentration of *B. thuringiensis* spores on treated vegetables ranging from 10^5 to 10^6 cfu/g just after application of the biopesticide. Several studies reviewed by De Bock et al. (2021) showed a decrease in *B. thuringiensis* concentration during pre-harvest growth in open field conditions with about 1.5–2 log reduction in 5–15 days and seems to reach a constant level of contamination at approximately 10^2–10^3 cfu/g. It is not clear if the reduction seen in open field grown vegetables would be as prominent in greenhouse grown treated produce.

Safety concerns

1. Safety concerns related to foodborne disease:

   a) *Background information on the involvement of* Bacillus cereus s.l. *in foodborne disease*

B. cereus s.l. is a recognised causative agent of two primary types of foodborne illness: diarrheal syndrome and emetic syndrome. In some instances, mixed syndromes can occur and rarely, severe or atypical infections may develop.

- The diarrheal syndrome, which accounts for approximately 90% of *B. cereus* s.l.-associated foodborne outbreaks, is characterised by a delayed onset of symptoms (typically 6–15 h after ingestion) and a relatively short duration (12–24 h) (Bonis et al., 2021). This syndrome has been mainly associated with the production of heat-labile enterotoxins in the small intestine. Three chromosomally encoded enterotoxins are implicated: Hemolysin BL (Hbl), non-hemolytic enterotoxin (Nhe) and Cytotoxin K (CytK).
  Hbl is a tripartite toxin encoded by the *hblC*, *hblD* and *hblA* genes, responsible for hemolysis and fluid accumulation in the ileal loop model (Fagerlund et al., 2010). Nhe, another three-component toxin encoded by *nheA*, *nheB* and *nheC* genes, is recognised for its potent cytotoxic effects (Ehling-Schulz et al., 2004). CytK, a single-component toxin encoded by *cytk1* and *cytk2*, exhibits strong hemolytic activity and toxicity toward mammalian cells. While CytK2 is more prevalent, CytK1 has been linked to more severe forms of the disease (Fagerlund et al., 2010). While CytK2, Nhe and Hbl are central to the enteropathogenic potential, other toxins such as phospholipases, with synergistic interactions reported in hemolysis and cytotoxicity, and immune modulators like InhA also play significant roles in its pathogenicity (Beecher & Wong, 2000; Doll et al., 2013). The overall virulence depends on the combined effect of these factors, their expression and environmental conditions that affect their production.
- The emetic syndrome typically presents with a short incubation period (0.5–6 h) and can last from 6 to 24 h, occasionally extending to several days. This syndrome is caused by cereulide, an emetic toxin, preformed in the food due to *B. cereus* growth. The cereulide is a dodecadepsipeptide produced by non-ribosomal peptide synthetase complexes encoded by the *ces* gene cluster located on megaplasmids (Ehling-Schulz et al., 2006, 2015).
- In some cases, a mixed syndrome can occur when food is contaminated with strains of *B. cereus* s.l. capable of producing both diarrheal enterotoxins and emetic cereulide. This results in a combination of symptoms from both syndromes (Ehling-Schulz et al., 2004).
- Although rare, *B. cereus* s.l. can cause severe or atypical infections, particularly in immunocompromised individuals or neonates. These cases may include life-threatening conditions such as necrotising enteritis (Decousser et al., 2013; Dierick et al., 2005).

b) *Bacillus thuringiensis as possible source of foodborne disease*

B. thuringiensis strains belong to the *Bacillus cereus* s.l. group and their possible source of foodborne disease is assessed based on the available literature data. On one hand the toxigenic/pathogenic potential of *B. thuringiensis* strains was assessed (*i*) and on the other hand their possible involvement in foodborne outbreaks (*ii*).

(i) *Virulence potential of B. thuringiensis strains*

B. thuringiensis strains, including those used in biopesticides, possess a complex array of genes associated with both insecticidal activity and potential human pathogenicity. Genomic analyses have revealed significant enrichment of these genes across multiple *B. thuringiensis* isolates, highlighting the genetic diversity within the species and strain-specific variations in insecticidal and virulence gene profiles (Zhu et al., 2015).

The insecticidal activity of *B. thuringiensis* is primarily attributed to Cry and Cyt proteins (different from CytK), which accumulate in parasporal crystals during sporulation (Xu et al., 2014). Over 850 different Cry proteins have been described, and approximately 10 different Cyt proteins. Cry proteins are usually encoded in megaplasmids. *B. thuringiensis* strains can harbour varying numbers of *cry* genes, ranging from 1 up to 8 or more per strain. Additionally, vegetative insecticidal proteins (Vip) and secreted insecticidal proteins (Sip) contribute to *B. thuringiensis*' insecticidal properties. Some strains also produce Zwittermicin A, an antibiotic that enhances insecticidal activity (Broderick et al., 2000). Zhu et al. (2015) identified new pore-forming toxin genes, such as *nep1* and *pft*, expanding the repertoire of insecticidal proteins produced by *B. thuringiensis* beyond just the Cry and Cyt toxins. Genomic analyses of *B. thuringiensis* have revealed that highly virulent strains for insects contain a wider array of virulence-related genes compared to less toxic strains.

Genes associated with human pathogenicity in *B. thuringiensis* strains include those encoding for non-hemolytic enterotoxin (*nhe*), hemolysin BL (*hbl*) and cytotoxin K (*cytK*). These genes are similar to those found in *B. cereus* s.s., a well-known human pathogen. The *nhe* and *hbl* operons are widely distributed among *B. thuringiensis* strains, including those used in biopesticides (Bonis et al., 2021; Johler et al., 2018; Schwenk et al., 2020). The *cytK* gene, previously thought to be exclusive to *B. cereus* s.s. has also been identified in some *B. thuringiensis* strains. Notably, the cereulide synthetase (*ces*) gene, responsible for emetic toxin production, is generally absent in *B. thuringiensis* strains (Biggel et al., 2022, Cufaolglu et al., 2022, Frentzel et al., 2018).

B. thuringiensis also produces other virulence factors such as phospholipases (e.g. phospholipase C, sphingomyelinase) and enterolysins. Phospholipases assist *B. thuringiensis* in overcoming cellular defences, promoting bacterial spread and possibly enhancing the action of other virulence factors, such as toxins like Hbl and Nhe. Enterolysins, particularly Enterolysin A, are cell wall hydrolases that contribute to bacterial cell lysis facilitating the release of bacterial toxins. Alveolysin a member of the cholesterol-dependent cytolysin (CDC) family of toxins, and immune inhibitor A (InhA) may play a role in both insecticidal activity and potential human impact (Guillemet et al., 2010). This is not unique to *B. thuringiensis* but is a characteristic shared across many members of the *B. cereus* group, including *B. cereus* (Carroll et al., 2021).

Studying the enthomopathogenicity of *B. thuringiensis* strains, Zhu et al. (2015) noticed similar insecticide toxin profiles, while exhibiting varying levels of virulence, which suggests the presence of specific mechanisms driving virulence expression in different environments. It can be assumed that this differential gene expression could also have implications at human level. Moreover, comparative transcriptomic studies have shown that virulence-related genes are differentially expressed depending on the bacterial growth phase, suggesting a complex regulation of pathogenic potential (Zhu et al., 2015).

Expression of virulence factors in *B. thuringiensis* has been demonstrated through various assays. Bassi et al. (2016) showed expression of *nhe*, *hbl* and *cytK* genes in food models. Bonis et al. (2021) confirmed toxin production in culture supernatants using immunological tests for the most tested commercial *B. thuringiensis* strains. However, expression levels can vary significantly between strains and under different conditions.

Several assays such as cytotoxicity assays on cell lines and the *Drosophila melanogaster* model have been used to assess pathogenic potential. Although none of these tests are able to reproduce the human pathogenic potential, they may provide insights about some aspects of virulence. Cytotoxicity assays on cell lines have been employed to assess the overall toxin activity. Studies using Vero cells (Johler et al., 2018) and Caco-2 cells (Schwenk et al., 2020; Fichant et al., 2024) have shown that certain *B. thuringiensis* strains including biopesticide strains can induce cytotoxicity and pro-inflammatory responses, as evidenced by the release of IL-8 cytokine. Moreover, Fox et al. (2020) have shown that Hbl and Nhe can act synergistically to trigger inflammation. The *D. melanogaster* model has been used to study the pathogenic potential of *B. thuringiensis* strains in vivo (Fichant et al., 2024; Hachfi et al., 2024). Fichant et al. (2024) classified strains into four categories from low to high virulence, with 90% of *B. thuringiensis aizawai* and *kurstaki* biopesticide strains showing strong to medium virulence associated with loss of intestinal barrier integrity.

Recent studies on mammalian models have raised concerns about potential adverse effects of *B. thuringiensis* biopesticide formulations. Alves et al. (2021, 2023) reported that exposure of pregnant rats to *B. thuringiensis* formulations throughout pregnancy and lactation led to malformations, increased pup mortality, reproductive alterations and organ damage in offspring. However, these findings require further validation through rigorous study designs to confirm their relevance to human health.

(ii) *Possible involvement of* B. thuringiensis *strains in foodborne outbreaks*

In routine foodborne outbreak investigations, the diagnosis at human clinical and food level focuses on the identification of the causing agent as belonging to *B. cereus* s.l. with no further identification down to the level of *B. thuringiensis*. This limitation in routine testing has significant implications for our understanding of the role of *B. thuringiensis* in foodborne outbreaks. To address this gap, investigations summarised in Table 3 were carried out in retrospective studies using strains collected during former foodborne outbreak investigations where they were identified as *B. cereus* s.l. In these retrospective studies the *B. cereus* s.l. strains were tested for the production of crystal proteins. The strains producing these proteins were identified as *B. thuringiensis*. In several studies, these strains were further assessed for their relationship with biopesticide *B. thuringiensis* strains by using several typing methods (Table 4).

TABLE 3 *B. thuringiensis* strains from foodborne outbreaks.

B. Thuringiensis **isolates**[a]	**Typing method**[b]	**Result**	**Reference**
8 biopesticide strains, 24 food strains, 7 outbreak related strains linked to 3 outbreaks from which 2 were human faecal strains and 5 were food isolates (fruit salad, bell pepper, 3 from lettuce).	*pan*C typing and FTIR spectroscopy	Intermixed clustering of food and human outbreak strains with biopesticides *B. thuringiensis aizawai* and *B. thuringiensis kurstaki* strains; 1 food isolate clustered with *B. thuringiensis tenebrionis/ morrisoni*; no clustering with *B. thuringiensis israelensis*.	Johler et al. (2018)
13 biopesticide strains 18 food strains, 3 of them outbreak related and isolated from lettuce, 2 human faecal strains outbreak related, the 5 outbreak related strains (3 from food and 2 human faecal strains) are the same strains as in Johler et al. (2018)	wgSNPs and cgSNP	5 outbreak related strains (threefrom food and 2 human faecal strains) differed by 0–3 wgSNPs (0–2 cgSNPs) with the *B. thuringiensis aizawai* biopesticide strain ABTS-1857.	Biggel et al. (2022)
19 biopesticide strains, 143 food strains, linked to 49 outbreaks and from which 21 strains were from outbreaks where no other putative food pathogens were detected during the outbreak investigation; these 21 strains are isolated from raw vegetables (e.g. tomatoes, cucumber, lettuce, carrots); fresh apple, fresh pineapple; rice and pasta salads; tabbouleh; mixed salads e.g. with mozzarella, zucchini, corn, beef, fish.	cgSNPs[c]	Food strains linked to 47 outbreaks differed by 0 to 10 wgSNPs from *B. thuringiensis aizawai* and *B. thuringiensis kurstaki* biopesticide strains. The 47 outbreaks related to 18.8% of the *B. cereus* s.l. associated foodborne outbreaks. Levels in food products for which *B. thuringiensis* was the only pathogen, ranged from 10^2 to more than 10^7 cfu/g (median 9×10^2). *B. thuringiensis israelensis* and *B. thuringiensis tenebrionis/ morrisoni* biopesticide strains did not link with any food outbreak strains, 2 food strains linked to 2 outbreaks were not linked to any biopesticide strain.	Bonis et al. (2021)
7 strains isolated from (2) mackerel and mussels in the garlic butter sauce, and (5) from vomit sample of one patient.	All strains were identified as *B. thuringiensis*-based on a polyphasic approach including the microscopical detection of the toxin crystals; MLST for subtyping the isolates.	No link found between strains isolated from vomit and from food. 4 isolates from vomit samples belong to ST8, 1 to a new ST type (ST2805). The food isolates belong to ST15 and to a new ST type (ST2804).	Pheepakpraw et al. (2023)

[a]*B. thuringiensis* strains in these studies were identified based on microscopical detection of insecticide crystals in several studies complemented by detection of the *cry* genes by PCR.

[b]wgSNP: whole-genome single nucleotide polymorphism; cgSNP: core genome single nucleotide polymporphism; FTIR: Fourier Transform Infrared Spectroscopy; *pan*C typing: typing based on difference in the sequence of the *pan*C gene, encoding for pantothenate- β- alanine ligase.

[c]SNPS analysis using the iVARCall2 v1.0 workflow.

TABLE 4 Application of *B. thuringiensis* biopesticide strains and the presence of *cytK2, nhe, hbl* genes.

Strain	Application	Presence of *cytK2, nhe, hbl* genes[a]
B. thuringiensis subsp. *aizawai*		
GC-91	Fruit, vegetables, ornamentals	*cytK2, nhe, hbl*
ABTS-1857	Fruit, vegetables, ornamentals	*cytK2, nhe, hbl*
B. thuringiensis subsp. *kurstaki*		
ABTS-351	Fruit, vegetables, ornamentals, forestry	*cytK2, nhe, hbl*
SA-11	Fruit, vegetables, ornamentals, forestry	*cytK2, nhe, hbl*
SA-12	Fruit, vegetables, ornamentals	*cytK2, nhe, hbl*
PB-54	Fruit, vegetables, ornamentals	*cytK2, nhe, hbl*
EG2348	Fruit, vegetables, ornamentals	*cytK2, nhe, hbl*
B. thuringiensis subsp. *israelensis*		
AM65-52	Mosquito control (not agricultural use)[b]	*cytK2, nhe, hbl*
BMP144	Mosquito control (not agricultural use)	*cytK2, nhe, hbl*
B. thuringiensis subsp. *tenebrionis/ morrisoni*		
NB-176	Potato foliage[c]	*nhe*[d], *hbl*

[a]Johler et al. (2018) (PCR), Bonis et al. (2021) (PCR), Biggel et al. (2022) (PCR, WGS).
[b]Limited exposure is expected due to agricultural use when applied for mosquitoes (Brühl et al., 2020).
[c]Limited foodborne exposure is expected when used on potato foliage (Bonis et al., 2021).
[d]Of the *nhe* operon, the *nheA* gene has a disrupted promotor (Biggel et al., 2022).

There are few studies that have investigated the role of *B. thuringiensis* subspecies in foodborne outbreaks. The outbreak investigations indicate that a substantial part of the foodborne outbreaks with *B. cereus* s.l. are correlated with the presence of *B. thuringiensis* biopesticide strains, mainly of the subspecies *aizawai* and *kurstaki* (Table 3).

The involvement of *B. thuringiensis* subsp. *israelensis* and *tenebrionis/morrisoni* biopesticide strains in foodborne outbreaks has not been detected. This could be due to the applications target of these biopesticides (Table 4). The strains of *B. thuringiensis* subsp. *israelensis* are not used in agricultural applications. They are applied to control mosquitos to reduce nuisance in several European regions. They are also used in vector control programs to reduce mosquito populations to combat diseases such as malaria, Dengue and West Nile Virus (Brühl et al., 2020). *B. thuringiensis* subsp. *tenebrionis/morrisoni* strain NB-176 is used in formulations being sprayed on potato foliage (the non-edible/aerial part of the potato plant) and have minimal direct contact with the food supply. Their use in agriculture may result in minimal exposure through residues on crops. *B. thuringiensis* subsp. *tenebrionis/morrisoni* strain NB-176 has also a disrupted promotor in *nheA* (Biggel et al., 2022) and no *cytK2* gene (Table 4) (Bonis et al., 2021), thus could also have a different pathogenic potential compared to the other biopesticide strains.

2) Safety concerns not related to foodborne disease:

B. cereus s.l. is increasingly recognised as an opportunistic pathogen capable of causing severe infections in vulnerable populations, such as premature neonates, elderly and immunocompromised (Lotte et al., 2022; Veysseyre et al., 2015). Several articles described the role of different *B cereus* s.l. genes in the host-bacteria interaction and in the severity of experimental infections (Ghelardi et al., 2007; Mursalin et al., 2020).

B. thuringiensis strains were reported to cause a wide clinical spectrum of extra-digestive human infections, localised or systemic e.g. wound and eye infections – endophthalmitis, and systemic bloodstream infections (Borgman, 2018; Bianco et al., 2021; Yuan et al., 2019) affecting in some cases immunocompromised individuals (Butcher et al., 2021). In all these articles methodological problems were present which precluded an unambiguous taxonomic assignment. Therefore, these articles were not further considered in the assessment.

Conclusions on a recommendation for the QPS list

Bacillus thuringiensis is not recommended for the QPS list due to safety concerns.

Enterococcus lactis synonym Enterococcus xinjiangensis

E. lactis has not been recommended for the QPS status due to potential safety concerns in the previous QPS assessment (EFSA BIOHAZ Panel, 2022). New information published since then is assessed in this statement.

Identity

E. lactis strains are, based on WGS analysis, separately clustered from *E. faecium* strains (Belloso Daza et al., 2021) and are not displaying a separate clustering based on their isolation source from foods, humans, animals or environment (Choi et al., 2024).

Body of knowledge

E. lactis strains are commonly found in human and animal guts and in fermented foods and are reported for their probiotic potential (Ahmed et al., 2023; Almeida-Santos et al., 2024; Alsaud et al., 2023; Fu et al., 2022). The antibacterial activity of several strains was documented and attributed to bacteriocins (Kotakonda & Marappan, 2024; Oliveira et al., 2024). Distinct bacteriocin producing genes were discovered in *E. lactis* compared to *E. faecium* strains (Tedim et al., 2024).

Safety concerns

Roer et al. (2024) published an enhanced database for the detection of putative virulence markers in the WGS of *E. faecium* and *E. lactis* strains. Several papers report that *E. lactis* are only carrying a subset of commonly reported antibiotic resistance and virulence genes present in *E. faecium* strains (Lu et al., 2023; Olanrewaju et al., 2024; Tedim et al., 2024). Ocejo et al. (2024) reported that, although most *E. lactis* strains isolated from dairy cattle lack virulence factors and resistance genes, one isolate carried a plasmid with eight antimicrobial resistance genes.

Analysis of 164 hospital enterococcal isolates, revealed that 8 isolates were identified by genomic analysis as *E. lactis* (Fujii et al., 2024).

At the moment there are still insufficient data on the ability to cause infections in non-susceptible humans and the correlation with virulence genes.

Conclusion

Enterococcus lactis is not recommended for the QPS status due to insufficient information on safety.

3.2 | Taxonomic units evaluated for the first time

3.2.1 | Bacteria

Bacillus nakamurai

Identity

B. nakamurai is a species with Standing in Nomenclature. The species was described by Dunlap et al. (2016) based on two strains isolated from soil. A phylogenomic analysis on the core genome of these two strains and all members of the *Bacillus subtilis* group revealed these two strains formed a distinct monophyletic clade with the nearest neighbour *Bacillus amyloliquefaciens*. In silico DNA–DNA hybridizations showed a value far below the species threshold of 70% (31.4% with the type strain of *B. amyloliquefaciens* and 30.9% with the type strain of *B. velezensis*).

Body of knowledge

B. nakamurai has been described as a novel potential biocontrol agent based on its antimicrobial activities against a range of bacterial and fungal phytopathogens e.g. against *Erwinia amylovora* causing fire blight in the Rosaceae family (Leathers et al., 2020), against Fusarium head blight in barley (Zanon et al., 2024), against early blight and northern leaf blight caused by *Alternaria solani* and *Exserohilum turcicum*, respectively in tomato and maize plants (Nimbeshaho et al., 2024). Genome mining coupled with metabolomics revealed that the *B. nakamurai* strain BDI-IS1 produces multiple non-ribosomal secondary metabolites including surfactin, iturin A, bacillaene, bacillibactin and bacilysin, together with some ribosomally-synthesised and post-translationally modified peptides (RiPPs) such as plantazolicin and potentially amylocyclicin, bacinapeptin and LCI. It reveals that synthesis of the non-ribosomal compounds surfactin, iturin A, bacillaene, bacilysin and bacillibactin is conserved across the *B. nakamurai* strains (Nimbeshaho et al., 2024). *B. nakamurai* has also been reported for its ability to produce transglutaminase (Sorde & Ananthanarayan, 2019).

Safety concerns

No reports on safety concerns were retrieved.

Conclusions on a recommendation for the QPS list

Bacillus nakamurai cannot be recommended for the QPS list due to a lack of body of knowledge for its use in the food and feed chain.

Lacticaseibacillus huelsenbergensis

Identity

L. huelsenbergensis is a valid species according to the List of Prokaryotic Names with Standing in Nomenclature. The two described strains of the organism were isolated from grass and corn silage respectively (Grabner et al., 2023).

Body of knowledge

Search in Pub Med of the term *L. huelsenbergensis* rendered two matches. One (Grabner et al., 2023) was the paper in which the new species is described. The other one (Grabner et al., 2024) is the description of two new *Lacticaseibacillus* species, also from silage, whose respective genomes appeared to be related to that of *L. huelsenbergensis*. Although no more papers on this species exist, there is plenty of information on *L. casei, L. zeae* and *L. paracasei*, three QPS species very closely related to it. In addition, the complete genome of a strain isolated from fermented plants and named *Lacticaseibacillus* sp. BCRC 81376, is almost identical to that of *L. huelsenbergensis* type strain. This indicates that the species is common and widely distributed in silage and that has been regarded as either one or the other of the QPS species cited above until molecular taxonomy was applied to the organism.

Safety concerns

The close genomic relatedness of *L. huelsenbergensis* to several *Lacticaseibacillus* QPS species, supports the safety of the species.

Furthermore, its health-harming potential was challenged through analysis of its genome by the QPS working group, in search of virulence determinants[5] with negative results.

Conclusions on a recommendation for the QPS list

Lactocaseibacillus huelsenbergensis can be granted the QPS status based on its close relatedness to several other QPS *Lacticaseibacillus* species.

Serratia plymuthica

Identity

S. plymuthica is a bacterial species belonging to the *Enterobacterales* order and *Yersiniaceae* family with Standing in Nomenclature (Breed et al., 1948). The species consists of Gram-negative, rod shaped, facultative anaerobic bacteria.

Body of knowledge

Strains of *S. plymuthica* are known as typical rhizobacteria, present in soil, growing in plants as endophytes and having plant growth promoting activity (Nordstedt & Jones, 2021). The majority of strains produce the red pigment prodigiosin which was shown to have antifungal and antitumoral activities (Woodhams et al., 2018). Also other metabolites (Levenfors et al., 2004) and lytic enzymes (Kamensky et al., 2003) were reported to inhibit fungal development. Several isolates are proposed as potential biocontrol agents against fungal plant pathogens (Bustamante et al., 2022; Campos et al., 2024; Sun et al., 2022). However, certain strains are reported to also exert plant-pathogenic activity (Kim & Kim, 2021).

Safety concerns

Sporadic cases of *S. plymuthica* infections are reported related to bacteraemia, often in patients with underlying disease (Carrero et al., 1995; Domingo et al., 1994; Horowitz et al., 1987; Martínez & Carrascosa, 1997; Ramos et al., 1995; Reina et al., 1992), osteomyelitis (Zbinden & Blass, 1988), exudates after surgery (Carrero et al., 1995), septic pseudoarthrosis (Mostafa et al., 2008), peritonitis (Nouh & Bhandari, 2000). In all these cases the identification was only done based on phenotypic/biochemical tests leading to uncertainty on the identity of the causing agent. The red pigment prodigiosin has been shown to have an immunosuppressant activity (Woodhams et al., 2018).

[5] https://mopsportal.efsa.europa.eu/.

Conclusion on a recommendation for QPS status

Serratia plymuthica is not recommended for the QPS status due to safety concerns.

3.3 | Monitoring of new safety concerns related to organisms on the QPS list

The summaries of the evaluation of the possible safety concerns for humans, animals or the environment described and published since the previous ELS exercise (i.e. scientific articles published between January to June 2024) as described in Appendices B and C with reference to the articles selected as potentially relevant for the QPS exercise (Appendix D) for each of the TUs or groups of TUs that are part of the QPS list (Appendix F), are presented below.

3.3.1 | Gram-positive non-sporulating bacteria

***Bifidobacterium* spp.**

A search for scientific articles potentially relevant for QPS-listed *Bifidobacterium* spp. (*B. adolescentis, B. animalis, B. bifidum, B. breve* and *B. longum*) provided 575 references. None of these articles were considered relevant at the level of title and abstract; consequently, the QPS status of *Bifidobacterium* spp. is not changed.

Carnobacterium divergens

A search for potentially relevant scientific articles on *C. divergens* provided seven references. None of these articles were considered relevant at the level of title and abstract; consequently, the QPS status of *C. divergens* is not changed.

Corynebacterium glutamicum

A search for scientific articles potentially relevant to the QPS evaluation of *C. glutamicum* provided 135 references. One of these articles was considered relevant at the level of title and abstract screening (Kuroda et al., 2024) but the article concerns the *Corynebacterium* genus and not the species *C. glutamicum* which is included in the QPS list. No new safety concerns were identified and the QPS status of *C. glutamicum* is not changed.

Lactobacilli

A search of papers referring to any of the QPS species, formerly belonging to the genus *Lactobacillus* and in 2020 split into 13 new genera, provided 1122 references. After title and abstract screening, three were selected for the full text phase evaluation. In two cases (Eze et al., 2024; Nagayama et al., 2024) no indication on the methods used for identification of isolates of *Lacticaseibacillus rhamnosus* and *Ligilactobacillus salivarius* respectively, were provided. In the third paper (Fukuda et al., 2024) describing a central catheter persistent infection by *Lacticaseibacillus paracasei*, a competent identification procedure, through the use of MADI-TOF-MS plus 16S RNA gene sequencing was performed. However, the case described was suffering from comorbidities that made the patient extremely susceptible to infection even by non-pathogenic microorganisms.

Based on the available evidence as described above, the status of any of the QPS species included in the group of lactobacilli is not changed.

Lactococcus lactis

The search for papers dealing with *L. lactis* associated to safety concerns provided 174 references. After title and abstract screening, two articles were selected for the full text phase evaluation but only one was considered as the other was related to other species (Xie et al., 2023). The paper (Sahoo et al., 2024) analysed the udder microbiota of mastitis suffering lactating cows. Among the isolates, 4.69% were classified as *L. lactis*. Given that the samples also contained recognised pathogens, it is plausible that the *L. lactis* isolates were not the cause of the infections.

Based on the available evidence as described above, the QPS status of *L. lactis* is not changed.

***Leuconostoc* spp.**

A search for scientific articles potentially relevant for the QPS evaluation of *Leuconostoc* QPS species (*L. citreum, L. lactis, L. mesenteroides, L. pseudomesenteroides*) provided 152 references. The analysis of their titles and abstracts left three articles for full text evaluation. One of them was not reporting safety concerns (Anvarifard et al., 2024) but the other two were considered relevant for the exercise. Bush and Williams (2023) reported an infection in a patient with acute myeloid leukaemia, receiving chemotherapy, but the identification methodology was not included. Tripathy et al. (2024) reviewed 14 cases of *L. lactis* bacteraemia in patients from a tertiary care centre in northern India, with underlying conditions leading

to immunosuppression (e.g. carcinoma, chronic kidney disease…). In both cases, identification was achieved using MALDI-TOF MS. The information from the ELS did not lead to a change in the status of QPS-listed *Leuconostoc* species.

Microbacterium imperiale

A search for scientific articles potentially relevant for the QPS evaluation of *M. imperiale* provided one reference which was not considered relevant at the level of title and abstract screening. Consequently, the QPS status of *M. imperiale* is not changed.

Oenococcus oeni

A search for scientific articles potentially relevant for the QPS evaluation of *O. oeni* provided 23 references. The title/abstract screening left no articles for the full text phase. Consequently, the QPS status of *O. oeni* is not changed.

***Pediococcus* spp.**

A search for scientific articles potentially relevant for the QPS evaluation of *Pediococcus* spp. (*P. acidilactici*, *P. parvulus*, *P. pentosaceus*) provided 295 references. The analysis of their title/abstract left one article for the full text evaluation stage (Mantzios, 2024). This article has some limitation related to the identification method used and reported a case of endocarditis after a transcatheter aortic valve implantation in a patient with underlying comorbidity. The articles reviewed did not identify any information that would change the status of QPS-listed *Pediococcus* spp.

***Propionibacterium* spp.**

A search for scientific articles potentially relevant for the QPS evaluation of *Propionibacterium* spp. (*Acidipropionibacterium acidipropionici, Propionibacterium freudenreichii*) provided 40 references. Following the analysis of their titles and abstracts, no articles passed to the full article evaluation phase. Consequently, the status of QPS-listed *Propionibacterium* spp. is not changed.

Streptococcus thermophilus

A search for scientific articles potentially relevant for the QPS evaluation of *S. thermophilus* provided 169 references. Following the analysis of their titles and abstracts, no articles passed to the full article evaluation phase. Consequently, the status of QPS-listed *S. thermophilus* is not changed.

3.3.2 | Gram-positive spore-forming bacteria

A search for scientific articles potentially relevant for *Bacillus* spp., related species and *Geobacillus stearothermophilus* provided 1172 references.

***Bacillus* spp. and related species**

1172 articles were found for *Bacillus* spp. and related species. Of the seven scientific articles that passed to the full text phase for further analysis, five were reporting a possible safety concern. One article had a methodological problem identifying the causing agent of an endophthalmitis as *B. pumilus* or *B. safensis* (Etheridge et al., 2024). Two articles linked a bacteraemia due to *B. subtilis* var. natto to the consumption of fermented food by a patient with an underlying disease (Ishikawa et al., 2024) or with a bleeding anal fissure (Amemiya et al., 2024). In both articles, the clinical strain and the strain present in the fermented food were not confirmed to be identical. A bacteraemia caused by *Shouchella clausii* (synonym *B.* claussii) was reported in two immunocompromised patients and was linked to the treatment with *S. clausii* spores as probiotic (Corredor-Rengifo et al., 2024). The clinical and the probiotic strain were not confirmed to be identical. One article reported the detection of the antibiotic bacitracin the fermentation broth of a *B. paralicheniformis* enzyme production strain (EFSA CEP Panel, 2024). This safety concern is covered by the qualification 'absence of genetic information to synthesise bacitracin' for *B. paralicheniformis*, included in the QPS list.

Through the ELS, no information was identified that would change the status of members of *Bacillus* spp. included in the QPS list.

Geobacillus stearothermophilus

None of the 7 scientific articles that passed to the full text phase (see above) for further analysis dealt with this species. Consequently, the QPS status of *G. stearothermophilus* is not changed.

Pasteuria nishizawae

A search for scientific articles potentially relevant for *P. nishizawae* provided one reference. Following the analysis of their titles and abstracts, this article did not pass to the full article evaluation phase. Consequently, the QPS status of *P. nishizawae* is not changed.

Clostridium tyrobutyricum

A search for scientific articles potentially relevant for *C. tyrobutyricum* provided 33 references. Following the analysis of its title and abstract, none was selected for the full text analysis phase. Consequently, the QPS status of *C. tyrobutyricum* is not changed.

3.3.3 | Gram-negative bacteria

A search for scientific articles potentially relevant to the QPS evaluation of *Gluconobacter oxidans*, *Xanthomonas campestris, Cupriavidus necator, Komagataeibacter sucrofermentans* and *Agrobacterium radiobacter* synonym *Rhizobium radiobacter* provided in total 994 references. The analysis of the titles left 2 articles to be checked at full text level.

Cupriavidus necator

A search for scientific articles potentially relevant for *C. necator* provided 107 references. Following the analysis of their titles and abstract, none was selected for the full text analysis phase. Consequently, the QPS status of *C. necator* is not changed.

Gluconobacter oxydans

A search for scientific articles potentially relevant for *G. oxydans* provided 44 references. Following the analysis of their titles and abstracts, none was selected for the full text phase. Consequently, the QPS status of *G. oxydans* is not changed.

Komagataeibacter sucrofermentans

A search for scientific articles potentially relevant for *K. sucrofermentans* provided seven references. Following the analysis of their titles and abstracts, none was selected for the full text phase. Consequently, the QPS status of *K. sucrofermentans* is not changed.

Xanthomonas campestris

A search for scientific articles potentially relevant for *X. campestris* provided 158 references. Following the analysis of their titles and abstracts, none was selected for the full text phase. Consequently, the QPS status of *X. campestris* is not changed.

Agrobacterium radiobacter* synonym *Rhizobium radiobacter

A search for scientific articles potentially relevant for *R. radiobacter* provided 678 references. Following the analysis of their titles and abstracts, two were selected for the full text phase. One (Wang and An, 2022) was not dealing with safety concerns and the other described two cases of *R.radiobacter* infections in two immunocompromised, debilitated patients (Hartman et al., 2023). Consequently, the QPS status of *X. campestris R. radiobacter* is not changed.

3.3.4 | Yeasts

The ELS searches for potentially relevant scientific articles on the yeasts with QPS status provided 3640 references. After the title/abstract screening phase, 62 articles passed to the full article appraisal phase. Out of these, 22 are not related to safety concerns, 4 are not related to the QPS yeast group, 1 not available and 1 not in English, therefore, only 34 reported a possible safety concern. The 34 articles are discussed below.

For the species ***Hanseniaspora uvarum, Kluyveromyces lactis, Komagataella pastoris***, ***Komagataella phaffi***, ***Limtongozyma cylindracea***, ***Ogataea angusta, Ogataea polymorpha***, ***Saccharomyces bayanus***, ***Saccharomyces pastorianus***, ***Schizosaccharomyces pombe*** and ***Zygosaccharomyces rouxii*** no safety concerns were reported. Consequently, the QPS status does not change for these species.

Cyberlindnera jadinii

The anamorph name of *C. jadinii* is *Candida utilis*. Synonyms of this species are *Hansenula jadinii, Pichia jadinii and Lindnera jadinii*.

Three publications were related to *C. jadinii*. Shoukat et al. (2023) compared the species composition of culturable yeasts in the human faeces of obese with a control group. However, the data are qualitative and only weakly connected with safety concerns. Al-Janabi et al. (2023) have identification problems; the species was only identified using traditional morphological and biochemical growth tests. The authors also tested the susceptibility to tamoxifen, an anticancer drug with some antifungal activity. However, its antifungal action is not sufficiently strong enough to recommend its use during the treatment. Umamaheshwari et al. (2023) performed a retrospective study characterising clinical yeast isolates from a hospital in India over 4 years. Three of their yeast isolates (0.4%) were *C. jadinii*, however species identification was only by traditional (not molecular) methods. The patients likely have predisposing factors, but no details are given.

The studies on *C. jadinii* did not add any new information that would change the current QPS status of this species.

Debaryomyces hansenii

The anamorph name of *D. hansenii* is *Candida famata*. Synonyms of this species are *Debaryozyma hansenii, Pichia hansenii, Torulaspora hansenii, Debaryomyces hansenii var. hansenii, Debaryomyces tyrocola var. hansenii*.

From the six publications related to *D. hansenii*, one (Mulinganya et al., 2024) investigated relationships between vaginal '*Candida* colonisation' and different risk factors. It is not possible from the data to evaluate any relationship between the presence of a specific yeast and clinical signs. Four publications have species identification problems or are associated with patients with risk factors (Badiee et al., 2024; Bilgi et al., 2023; Sigei et al., 2023; Umamaheshwari et al., 2023). Badiee et al. (2024) performed a prospective study in Iran. The isolates were from high-risk patients, solid organ transplantation, haematology, paediatric oncology and intensive care unit (ICU) wards; 8.3% of the isolates were identified as *C. famata*. The authors performed an antimycotic resistance study, but no information about *C. famata* was included. Bilgi et al. (2023) found one *C. famata* isolate (4.5%) from 22 yeast species isolated from HIV-infected children. Sigei et al. (2023) report the presence of *D. hansenii* in clinical samples, but there are shortcomings related to methods for species identification and information on clinical conditions. Umamaheshwari et al. (2023) performed a retrospective study characterising clinical yeast isolates from a hospital in India over 4 years. Seven of their yeast isolates (1%) were *D. hansenii*. The patients likely have predisposing factors, but no details are given. Yazdanpanah et al. (2024) characterise the presence of *D. hanesii* isolates (3%) from infected nails (onychomycosis) of humans in Iran. Nail infections are comparatively superficial and cause no severe morbidity. The authors also tested the susceptibility to eight antimycotics; the three isolates were susceptible to the antimycotics tested.

The studies on *D. hansenii* did not add any new information that would change the current QPS status of this species.

Kluyveromyces marxianus

The anamorph name of *K. marxianus* is *Candida kefyr*. Synonyms of this species are *Dekkeromyces marxianus, Guilliermondella marxiana, Zygofabospora marxiana, Zygorenospora marxiana, Zygosaccharomyces marxianus*.

New studies confirm that in rare cases, *K. marxianus* can cause opportunistic or superficial infections. Three studies from Algeria, Iran and Turkey reported a low proportion of *K. marxianus* among yeasts isolated from women with vulvovaginal candidiasis (Benhadj, 2023; Jannati, 2024; Kilbas, 2024), however only Jannati et al. confirmed species identification with molecular methods. Benhadj et al. also reported antimycotic susceptibility of the isolates. In a prospective study from a hospital in Ecuador (Acosta-Mosquera et al., 2024), one yeast isolate (0.5%) was identified to *K. marxianus*, however no details were given about clinical background and predisposing factors in the patients. Umamaheshwari et al. (2024) reported that four of their yeast isolates (0.5%) from a hospital in India were *K. marxianus*, however species identification was only by traditional (not molecular) methods. Noruaei et al. (2024) interestingly found no differences in putative virulence factors in clinical, vaginal/oral and environmental strains of *K. marxianus* (22 strains in each group). However, there was no information about which (molecular) methods were used for species determination.

Raheel et al. (2023a Refid 38,776) isolated *K. marxianus* in four out of 53 cases of mycotic mastitis. They also determined antimycotic susceptibility of the strains (Raheel et al. 2023b). However, species identification was only by traditional methods and thus uncertain.

The articles did not identify any information that would change the QPS status of *K. marxianus*.

Phaffia rhodozyma

The teleomorph name of *P. rhodozyma* is *Xanthophyllomyces dendrorhous*. A synonym of this species is *Cryptococcus rhodozymus*.

Vysoka et al. (2023) investigated potential cytotoxicity of one extract of *P. rhodozyma* with the MTT assay. There was no cytotoxic effect up to 20% extract (limit of cytotoxicity in the assay is 50% inhibition) but a slight effect at 28% extract, the highest concentration tested. They concluded that their results show that the strain is safe.

The update did not identify any information that would change the QPS status of *P. rhodozyma*.

Saccharomyces cerevisiae

The anamorph form of *S. cerevisiae* is not described. An exceptional synonym of this species is *Saccharomyces boulardii*. Other synonyms are *Mycokluyveria cerevisiae, Eutorulopsis cerevisiae, Eutorula cerevisiae, Kloeckera cerevisiae*.

Eleven publications refer to *S. cerevisiae*. No safety concerns were identified for one of them (Mulinganya et al., 2024). The authors investigated relationships between vaginal 'Candida colonisation' and clinical correlates, risk factors and pregnancy outcomes in pregnant women in the Democratic Republic of Congo. A low fraction (3.2%) of the isolates were *S. cerevisiae*. However, it is impossible from the data to evaluate any relationship between the presence of a specific yeast and clinical signs. Yazdanpanah et al. (2024) characterise the presence of *S. cerevisiae* isolates (2%) from infected nails (onychomycosis) of humans in Iran. Nail infections are comparatively superficial and cause no severe morbidity. The rest of the publications have identification problems or yeasts were isolated from patients with risk factors, except Morard et al. (2024). In this study, the authors demonstrate, using whole-genome analysis and phenotypic characterisation, that the food environment could be the origin of infections with *S. cerevisiae*, such as bread and probiotic supplements. Also, the authors observed that host adaptation to infection could drive important phenotypic and genomic changes in these strains, which could be good markers for determining the source of infection.

Regarding the articles with some problems, three use MALDI-TOF to identify the isolates. Benhadj et al. (2023), from 22 yeasts isolated from non-pregnant women in Algeria with vulvovaginal candidiasis, 3.5% was identified as *S. cerevisiae*. Diop et al. (2024) and Flores-Delgado et al. (2024) also describe cases with patients having predisposition factors. Diop et al. (2024) is a retrospective study of infections with *S. cerevisiae* reported from a hospital in Belgium, and Flores-Delgado et al. (2024) describe catheter-related bloodstream infection with *S. cerevisiae* in two patients undergoing cancer treatment. Spiliopoulou et al. (2023) is a review of cases of non-*Candida* species infections in a hospital in Greece. Of 16 cases with non-Candida yeasts, three isolates (18.7%) were identified as *S. cerevisiae*; one corresponds to a 2-year-old child who was given probiotics after diarrhoea, and the other two to patients serious underlying disease, one was long-term hospitalised (tetraplegia, gastrostomy and suprapubic catheter) and the second had chronic heart failure, hypertension, dyslipidaemia, obesity and respiratory insufficiency under biphasic positive airway pressure.

Three papers did not describe the method used to identify yeasts. Furuya et al. (2023) described a case in which a 73-year-old man from Japan was hospitalised after an operation for pancreatic cancer, and Kloub et al. (2024) described a 64-year-old female with a history of the human immunodeficiency virus (HIV) who developed *S. cerevisiae* peritonitis following PEG tube insertion. Finally, Vinayagamoorthy et al. (2023) is a systematic review that addresses the underlying diseases and risk factors in *Saccharomyces* fungemia patients, along with the treatment and outcome of the disease from June 2005 to March 2022. This review identified 117 *Saccharomyces* fungemia cases; 108 were included in the analysis. *Saccharomyces* fungemia is commonly seen in patients treated with *S. boulardii* probiotics ($n = 73$, 67.6%), and 35 (32.4%) patients did not receive probiotic therapy. The underlying disease and risk factors significantly associated with *S. boulardii* probiotic-associated fungemia were intensive care unit stay ($n = 34$, 31.5%), total parenteral nutrition or enteral feeding ($n = 32$, 29.6%), patients with gastrointestinal symptoms such as diarrhoea ($n = 23$, 21.3%) and diabetes mellitus ($n = 14$, 13.0%). In patients without probiotic therapy, immunosuppression ($n = 14$, 13.0%), gastrointestinal surgery ($n = 5$, 4.6%) and intravenous drug use ($n = 5$, 4.6%) were the significant risk factors for *Saccharomyces* fungemia. The all-cause mortality rate of the total cohort is 36.1%. No significant variation in the mortality rate is observed between *S. boulardii* probiotic-treated patients ($n = 29$, 26.9%) and patients without probiotic therapy ($n = 10$, 9.3%). The authors concluded that *S. boulardii* probiotic administration in patients on prolonged intensive care unit stay, total parenteral nutrition or enteral feeding and pre-existing gastrointestinal illness such as diarrhoea should be monitored carefully, as these groups of patients are at high risk of acquiring *Saccharomyces* fungemia.

The literature update did not identify any information that would change the current QPS status of *S. cerevisiae*.

Wickerhamomyces anomalus

The anamorph name of *W. anomalus* is *Candida pelliculosa*. Synonyms of this species are *Endomyces anomalus, Pichia anomala, Willia anomala, Hansenula anomala*.

W. anomalus is known in rare cases to be able to cause opportunistic infections in patients with underlying disease. Thus, three papers (Ioannou et al. 2024; Kosmeri et al. 2024; Sakai et al. 2024) reviewed reports of *W. anomalus* infections, demonstrating that common risk factors are prolonged hospital stay with intensive care (particularly neonates and children), immunosuppression, use of central venous catheter, use of parenteral nutrition and treatment with broad-spectrum antibiotics. Aboutalebian et al. (2024) reported a nosocomial bloodstream infection with *W. anomalus* in a critically ill (Griscelli syndrome with hemo-phagocytic syndrome) and immunodeficient 5-year-old boy in paediatric intensive care in a hospital in Iran. An 84-year-old immunocompromised man received antimicrobial therapy for acute cholangitis but remained febrile (Sakai et al. 2024). Subsequently, *W. anomalus* (identified with MALDI-TOF MS) was isolated from his blood. He was given fluconazole which cured the infection. Umamaheshwari et al. (2024) reported that eight of their yeast isolates (1%) from a hospital in India were *W. anomalus*, however species identification was only by traditional (not molecular) methods.

Duggan et al. (2024) describes septic fungal arthritis by *W. anomalus* in a horse. The horse had problems with its hindlimb and had previously been subject to intra-articular medication in a joint of the limb. The paper lacks information on the methods used for species identification of the yeast.

There was no new information that would change the QPS status of *W. anomalus*.

Yarrowia lipolytica

The anamorph form of *Y. lipolytica* is *Candida lipolytica* and *Candida oleophila*. A synonym of this species is *Saccharomycopsis lipolytica*.

Simonetti et al. (2023) reported *Y. lipolytica* bloodstream infection in a 53-year-old man admitted for alcohol withdrawal syndrome and mild COVID-19, and it was discovered that he also had cancer. He was treated with broad-spectrum antibiotics against otitis (ear inflammation). Umamaheshwari et al. (2024) reported that six of their yeast isolates (1%) from a hospital in India were *Y. lipolytica*, however species identification was only by traditional (not molecular) methods.

Lavergne et al. (2023) investigated the genetic background to decreased susceptibility to fluconazole in a clinical isolate of *Y. lipolytica*. It was shown that the strain has a substitution in ERG11, which had previously been described in fluconazole-resistant *Candida* isolates.

There was no new information that would change the QPS status of *Y. lipolytica*.

3.3.5 | Protists

Aurantiochytrium limacinum (Schizochytrium limacinum)

A search for scientific articles potentially relevant for *A. limacinum* provided 20 articles. Following the analysis of their titles and abstract, none was selected for the full text phase. Therefore, the current QPS status of *A. limacinum* is not changed.

3.3.6 | Algae

A search for scientific articles potentially relevant for algae provided 605 articles. Following the analysis of their titles and abstract, none were selected for the full text phase.

Euglena gracilis

No scientific articles dealt with potential safety concerns for *E. gracilis*. Therefore, the current QPS status of *E. gracilis* is not changed.

Haematococcus lacustris* synonym *Haematococcus pluvialis

No scientific articles dealt with potential safety concerns for *H. lacustris*. Therefore, the current QPS status of *H. lacustris* is not changed.

Tetraselmis chuii

No scientific articles dealt with potential safety concerns for *T. chuii*. Therefore, the current QPS status of *T. chuii* is not changed.

3.3.7 | Viruses used for plant protection

Alphaflexiviridae* and *Potyviridae

A search for scientific articles potentially relevant for the QPS evaluation of viruses of the *Alphaflexiviridae* and *Potyviridae* families provided 140 references. Following the analysis of their titles and abstract, none were selected for the full text phase. Therefore, the current QPS status remains unchanged.

Baculoviridae

A search for scientific articles potentially relevant for the QPS evaluation of the *Baculoviridae* family provided 131 references. Following the analysis of their titles and abstract, none were selected for the full text phase. Therefore, the current QPS status remains unchanged.

CONCLUSIONS

ToR 1: ***Keep updated the list of microorganisms being notified, in the context of a technical dossier to EFSA Units (Feed and Contaminants (FEEDCO), Pesticides Peer Review (PREV), Food Ingredients and Packaging (FIP) and Nutrition and***

Food Innovation (NIF)[6]), for intentional use in feed and/or food or as sources of food and feed additives, enzymes, plant protection products and as novel foods for safety assessment

- Between April to September 2024 (inclusive) the list of notifications was updated with 54 notifications that were received by EFSA, of which 33 were proposed for evaluation as feed additives, 17 for use as food enzymes, food additives and flavourings and 4 as novel foods.

ToR 2: ***Review taxonomic units previously recommended for the QPS list and their qualifications when new information has become available***

- In relation to the results of the monitoring of possible new safety concerns relevant for the QPS list, there were no results that would affect the QPS status or the qualifications for the TUs on the QPS list.
- A new procedure has been established to ensure the TUs are kept up to date in relation to recent taxonomical insights. Every 6 months, the QPS TUs of bacteria, yeast, algae, protists and viruses are verified against their respective authoritative databases to ensure the accuracy for each Panel Statement. This ELS cycle to review the QPS list TUs included the updated names/synonyms as keywords.

ToR 3: ***(Re)assess the suitability of taxonomic units notified to EFSA not present in the current QPS list for their inclusion in that list***

- Out of the 54 notifications received between April to September 2024, 29 were related to TUs that already had QPS status and therefore did not require further evaluation.
- Of the remaining 25 notifications, 21 notifications were related to microorganisms that are generally excluded from QPS evaluation (12 were notifications of filamentous fungi, 1 of *Enterococcus faecium* (bacterium) and 8 of *Escherichia coli* (bacterium)).
- Two of the other four notifications, corresponding to two TUs, had already been evaluated for a possible QPS status in a previous Panel Statement: *Ensifer adhaerens* (EFSA BIOHAZ Panel, 2024b), which will not be reassessed now, and *Enterococcus lactis* (EFSA BIOHAZ Panel, 2022), which is assessed in this document, as the previous assessment was done within the previous 3-years QPS cycle.
- The other two notifications belonging to two TUs were notified for the first time and, therefore, were assessed for a possible QPS status in this Panel Statement: *Serratia plymuthica* and *Lacticaseibacillus huelsenbergensis*.
- *Bacillus thuringiensis* has been reassessed for a possible QPS status in response to an internal request.
- *Bacillus nakamurai* has also been included in response to another internal ad-hoc request.

The following conclusions were drawn:

- *Bacillus thuringiensis* is not recommended for the QPS status due to safety concerns.
- *Enterococcus lactis* is not recommended for the QPS status due to insufficient information on safety.
- *Bacillus nakamurai* cannot be recommended for the QPS list due to a lack of body of knowledge for its use in the food and feed chain.
- *Lacticaseibacillus huelsenbergensis* can be granted the QPS status based on its close relatedness to several other QPS *Lacticaseibacillus* species.
- *Serratia plymuthica* is not recommended for the QPS status due to safety concerns.

GLOSSARY

Anamorph name	Valid name of a fungus based on the asexual reproductive state (morphologically)
Antimicrobial compounds	Antibiotics, bacteriocins and/or small peptides with antimicrobial activity
Basonym name	the earliest validly published name of a taxon
Synonymous name/ Homotypic synonym	have the same type (specimen) and the same taxonomic rank.
Teleomorph name	Valid name of a fungus based on the sexual reproductive state (morphologically)

ABBREVIATIONS

AI	artificial intelligence
AMR	antimicrobial resistance
BIOHAZ	EFSA Panel on Biological Hazards
ELS	extensive literature search
FEEDAP	EFSA Panel on Additives and Products or Substances used in Animal Feed

[6]Units as in December 2022.

FIP	EFSA Food ingredients and Packaging Unit
FSTA	Food Science Technology Abstracts
GMM	genetically modified microorganism
GMO	EFSA Unit on Genetically Modified Organisms
MALDI-TOF MS	matrix-assisted laser desorption/ionisation (MALDI), time-of-flight (TOF) mass spectrometry (MS)
QPS	qualified presumption of safety
PPR	Pesticide Peer Review Unit
ToR	Term(s) of reference
TU	taxonomic unit
WG	working group

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PANEL MEMBERS

Ana Allende, Avelino Alvarez-Ordóñez, Valeria Bortolaia, Sara Bover-Cid, Alessandra De Cesare, Wietske Dohmen, Laurent Guillier, Lieve Herman, Liesbeth Jacxsens, Lapo Mughini-Gras, Maarten Nauta, Jakob Ottoson, Luisa Peixe, Fernando Perez-Rodriguez, Panagiotis Skandamis, and Elisabetta Suffredini.

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APPENDIX A

Search strategy followed for the (re)assessment of the suitability of TUs notified to EFSA not present in the current QPS list for their inclusion in the updated list (reply to ToR 3)

Relevant databases, such as PubMed, Web of Science, CAB Abstracts or Food Science Technology Abstracts (FSTA) and Scopus, were searched, based on the judgement of the experts. Details on the search strategy, search keys and approach for each of the assessments of the TUs evaluated in the statement may be found below.

A.1 | *Bacillus thuringiensis*

String for species: "Bacillus thuringiensis," "B thuringiensis."

Outcome	String
1. Antimicrobial/antibiotic/antimycotic	"antimicrobial resistan*" OR "antibiotic resistan*" OR "antimicrobial susceptibil*"
2. Infection/bacteremia/fungemia/sepsis	infection* OR abscess* OR sepsis* or septic* OR bacteremia OR bacteraemia OR toxin*
3. Type of disease	endocarditis OR abscess OR meningitis
4. Mortality/morbidity	Not applied
5. Disease risk	opportunistic OR virulen*
6. Genotoxicity	"ames assay*" OR "ames test*" OR aneugen* OR apoptos* OR Aneuploid* OR clastogen* OR chromatid OR chromosom* OR "comet assay*" OR "comet test*" OR ((dna) NEA"ames assay*" OR "ames test*" OR aneugen* OR apoptos* OR Aneuploid* OR clastogen* OR chromatid OR chromosom* OR "comet assay*" OR "comet test*" OR ((dna) NEAR/5 (adduct* OR binding OR break* OR deletion* OR damage* OR fragmentation* OR impair* OR inhibition* OR injur* OR lesion* OR polymorphism* OR repair*)) OR ((gene*) NEAR/5 (adduct* OR binding OR break* OR deletion* OR damage OR fragmentation* OR impair* OR inhibition* OR injur* OR lesion* OR polymorphism* OR repair*)) OR (genetic NEAR/5 toxicity) OR "genomic instability" OR genotox* OR homeosta* OR micronucl* OR mutagen* OR mutagenicity OR mutation* OR "oxidative stress" OR "sister chromatid exchange" OR "strand break*")R/5 (adduct* OR binding OR break* OR deletion* OR damage* OR fragmentation* OR impair* OR inhibition* OR injur* OR lesion* OR polymorphism* OR repair*)) OR ((gene*) NEAR/5 (adduct* OR binding OR break* OR deletion* OR damage OR fragmentation* OR impair* OR inhibition* OR injur* OR lesion* OR polymorphism* OR repair*)) OR (genetic NEAR/5 toxicity) OR "genomic instability" OR genotox* OR homeosta* OR micronucl* OR mutagen* OR mutagenicity OR mutation* OR "oxidative stress" OR "sister chromatid exchange" OR "strand break*"
7. Special keywords for this search	taxonom* OR biopesticide* OR "bio pesticide*" OR "detected" OR "detection" OR prevalen* OR "identification" OR "identified"

A.2 | *Enterococcus lactis* synonym *Enterococcus xinjiangensis*

The search on Pubmed for the following terms led to the number of hits indicated below:

- "Enterococcus lactis" and 2024: 20 hits,
- "Enterococcus lactis" and 2023: 18 hits,
- "Enterococcus lactis" and 2022: 16 hits.

A.3 | *Bacillus nakamurai*

The search on Pubmed for the following terms led to the number of hits indicated below:
- "Bacillus nakamurai": seven hits, all checked.

A.4 | *Lacticaseibacillus huelsenbergensis*

- The search on Pubmed for the following terms led to the number of hits indicated below:
- "Lacticaseibacillus huelsenbergensis", two results, both checked.

A.5 | *Serratia plymuthica*

The search on Pubmed for the following terms led to the number of hits indicated below:

- "Serratia plymuthica" and "pathogen", 68 results, all checked.
- "Serratia plymuthica" and "disease", 49 results, all checked.

APPENDIX B

Protocol for extensive literature search (ELS), relevance screening and article evaluation for the maintenance and update of the list of QPS-recommended microorganisms (reply to ToR 2)

The protocol for extensive literature search (ELS) used in the context of the EFSA mandate on the list of QPS-recommended microorganisms intentionally added to the food or feed is available on the EFSA Knowledge Junction community on Zenodo, at: https://doi.org/10.5281/zenodo.3607188

APPENDIX C

Search strategies for the maintenance and update of the list of QPS-recommended microorganisms (reply to ToR 2)

The search strategies for each taxonomic unit (TU), i.e. the string for each TU and the search outcome, are available on the EFSA Knowledge Junction community on Zenodo at: https://doi.org/10.5281/zenodo.3607192

APPENDIX D

References selected from the ELS exercise with potential safety concerns for searches done from January to June 2024 (reply to ToR 2)

Gram-Positive Non-Sporulating Bacteria

Bifidobacterium spp.

None.

Carnobacterium divergens

None.

Corynebacterium glutamicum

Kuroda, Y., Yang, L., Shibata, T., Hayashi, M., Araki, Y., Nishida, M., Namiki, T., Makino, T., Shimizu, T., Suzuki, T., Sayo, T., Takahashi, Y., Tsuruta, D., & Katayama, I. (2024). High α-diversity of skin microbiome and mycobiome in Japanese patients with vitiligo. *Journal of Dermatological Science, 114*(1), 34–43. https://doi.org/10.1016/j.jdermsci.2024.02.008

Lactobacilli

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Lactococcus lactis

Sahoo, S., Behera, M. R., Mishra, B., Kar, S., Sahoo, P., Sahoo, N., & Biswal, S. (2024). Microbial diversity and resistome in milk of cows with subclinical mastitis in a coastal district of Odisha, India. *Indian Journal of Microbiology, 64*(4), 1627–1636. https://doi.org/10.1007/s12088-024-01198-6

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Leuconostoc spp.

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Tripathy, S., Jamwal, A., Varghese, G., Sarawat, D., Patel, S. S., Tejan, N., & Sahu, C. (2024). Characterisation of Leuconostoc lactis Bacteremia during a 2-year study at a tertiary care center in north india-an observational analysis. *The American Journal of Tropical Medicine and Hygiene, 111*(1), 129–131. https://doi.org/10.4269/ajtmh.23-0678

Microbacterium imperiale

None.

Oenococcus oeni

None.

Pediococci spp.

Mantzios, P. G., Spyropoulou, P., Hatzianastasiou, S., Efthymiou, D., Filippopoulos, E., Mamarelis, C., Potsios, C., Filioti, K., & Letsas, C. A. (2024). Pediococcus pentosaceus endocarditis in a patient with recent transcatheter aortic valve implantation and liver cirrhosis: A case report and review of the literature. *Cureus, 16*(4), e57509. https://doi.org/10.7759/cureus.57509

Propionibacterium spp.

None.

Streptococcus thermophilus

None.

Gram-Positive Spore-forming Bacteria

Bacilli

Amemiya, T., Ohkusu, K., Murayama, M., Yamamoto, T., & Itoh, N. (2024). A rare case of Bacillus subtilis variant natto-induced persistent bacteremia with liver and splenic abscesses in an immunocompetent patient. *IDCases, 35,* e01925. https://doi.org/10.1016/j.idcr.2024.e01925

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Geobacillus stearothermophilus

None.

Pasteuria nishizawae

None.

Gram-negative bacteria

Cupriavidus necator

None.

Gluconobacter oxydans

None.

Komagataeibacter sucrofermentans

None.

Xanthomonas campestris

None.

Agrobacterium radiobacter synonym *Rhizobium radiobacter*

Hartman, R. E., Freyer, C. W., Athans, V., McCurdy, S. R., & Frey, N. V. (2023). Central line-associated Rhizobium radiobacter bloodstream infection in two allogeneic hematopoietic cell transplant recipients. *Journal of Oncology Pharmacy Practice: Official Publication of the International Society of Oncology Pharmacy Practitioners*, 10781552231161826. https://doi.org/10.1177/10781552231161826

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Protists

None.

Algae

None.

Viruses used for plant protection

Alphaflexiviridae

None.

Potyviridae

None.

Baculoviridae

None.

APPENDIX E

References selected from the ELS exercise for *Bacillus thuringiensis* (reply to ToR 3)

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APPENDIX F

Updated list of QPS Status recommended microorganisms in support of EFSA risk assessments

The list of QPS status recommended microorganisms (EFSA BIOHAZ Panel, 2023) is being maintained in accordance with the mandate of the BIOHAZ Panel. Possible additions to this list are included approximately every 6 months, with this Panel Statement (21) adopted in December 2024. These additions are published as updates to the Scientific Opinion (EFSA BIOHAZ Panel, 2023); the updated QPS list is available at https://doi.org/10.5281/zenodo.1146566 (the link opens at the latest version of the QPS list, and also shows the versions associated to each Panel Statement).

APPENDIX G

Microbial species as notified to EFSA, received from April to September 2024 (reply to ToR 1)

The overall list of microorganisms being notified to EFSA in the context of a technical dossier to EFSA Units (for intentional use directly or as sources of food and feed additives, food enzymes and plant protection products for safety assessment), is kept updated in accordance with the mandate of the BIOHAZ Panel and can be found in https://doi.org/10.5281/zenodo.3607183.

The list was updated with the notifications received from April to September 2024, listed in the Table below.

Species	EFSA risk assessment area	Category regulated product	Intended usage	EFSA question no[a]	Previous QPS status of the respective TU[b]	Assessed in this statement? Yes or no
Bacteria						
Bacillus licheniformis	Feed additives	Zootechnical additives	Gut flora stabiliser. Non GMM	EFSA-Q-2024-00035	Yes	No
Bacillus licheniformis	Food enzymes, food additives and flavourings	Food enzyme	Production of the food enzyme xylanase. GMM	EFSA-Q-2024-00453	Yes	No
Bacillus licheniformis	Food enzymes, food additives and flavourings	Food enzyme	Production of the food enzyme alpha-amylase. GMM	EFSA-Q-2024-00524	Yes	No
Bacillus subtilis	Food enzymes, food additives and flavourings	Food enzyme	Production of food enzyme dextransucrase. GMM	EFSA-Q-2024-00208	Yes	No
Bacillus subtilis	Food enzymes, food additives and flavourings	Food enzyme	Production of food enzyme glucosyltransferase (mutansucrase). GMM	EFSA-Q-2024-00264	Yes	No
Bacillus subtilis	Food enzymes, food additives and flavourings	Food enzyme	Production of the food enzyme maltogenic amylase. GMM	EFSA-Q-2024-00448	Yes	No
Bacillus subtilis	Feed additives	Zootechnical additives	Production of endo-1,4-beta-xylanase as digestibility enhancer. GMM	EFSA-Q-2024-00263	Yes	No
Bacillus velezensis	Feed additives	Zootechnical additives	Gut flora stabilisers. Non GMM	EFSA-Q-2024-00395	Yes	No
Bacillus paralicheniformis	Food enzymes, food additives and flavourings	Food enzyme	Production of the food enzyme subtilisin. GMM	EFSA-Q-2024-00552	Yes	No
Bifidobacterium animalis	Feed additives	Zootechnical additives	Gut flora stabiliser and other zootechnical additive. Non GMM	EFSA-Q-2023-00451	Yes	No
Corynebacterium glutamicum	Feed additives	Nutritional additives	Production of L-isoleucine. Non GMM	EFSA-Q-2024-00316	Yes	No
Corynebacterium glutamicum	Feed additives	Nutritional additives	Production of L-isoleucine. Non GMM	EFSA-Q-2024-00499	Yes	No
Corynebacterium glutamicum	Feed additives	Nutritional additives	Productions of L-argynine. Non GMM	EFSA-Q-2024-00423	Yes	No
Ensifer adhaerens	Feed additives	Nutritional additives	Vitamins, pro-vitamins and chemically well-defined substances having a similar effect. Production of vitamin B12 (cyanocobalamin). Non GMM	EFSA-Q-2024-00521	No	No
Enterococcus faecium	Feed additives	Zootechnical additives	Digestibility enhancer. Non GMM	EFSA-Q-2024-00476	No	No

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Species	EFSA risk assessment area	Category regulated product	Intended usage	EFSA question no[a]	Previous QPS status of the respective TU[b]	Assessed in this statement? Yes or no
Enterococcus lactis	Feed additives	Technological additives	Silage additive. Non GMM	EFSA-Q-2024-00329	No	Yes
Escherichia coli	Novel foods	Novel foods	Production of lacto-N-tetraose (LNT). GMM	EFSA-Q-2024-00281	No	No
Escherichia coli	Food enzymes, food additives and flavourings	Food additive	Production of rebaudioside M (E960c). GMM	EFSA-Q-2024-00293	No	No
Escherichia coli	Food enzymes, food additives and flavourings	Food enzyme	Production of amidase. GMM	EFSA-Q-2024-00307	No	No
Escherichia coli	Food enzymes, food additives and flavourings	Food enzyme	Production of the food enzyme D-psicose 3-epimerase. GMM	EFSA-Q-2024-00310	No	No
Escherichia coli	Feed additives	Nutritional additives	Production of L-valine. GMM	EFSA-Q-2024-00486	No	No
Escherichia coli	Feed additives	Nutritional additives	Production of L-isoleucine. GMM	EFSA-Q-2024-00506	No	No
Escherichia coli	Feed additives	Nutritional additives	Production of L-arginine. GMM	EFSA-Q-2024-00507	No	No
Escherichia coli	Novel foods	Novel foods	Production of the enzymes ribose kinase, 5-phosphoribosyl-1-pyrophosphate synthetase, Nampt, AMP kinase and polyphosphate kinase used for the synthesis of the novel food β-nicotinamide mononucleotide (NMN). GMM	EFSA-Q-2024-00420	No	No
Lacticaseibacillus huelsenbergensis	Feed additives	Technological additives	Silage additive. Non GMM	EFSA-Q-2024-00510	No	Yes
Lacticaseibacillus rhamnosus	Feed additives	Zootechnical additives	Gut flora stabiliser and other zootechnical additive. Non GMM	EFSA-Q-2023-00451	Yes	No
Lactiplantibacillus plantarum	Feed additives	Zootechnical additives	Gut flora stabiliser and other zootechnical additive. Non GMM	EFSA-Q-2023-00451	Yes	No
Lactobacillus acidophilus	Feed additives	Zootechnical additives	Gut flora stabiliser. Non GMM	EFSA-Q-2024-00259	Yes	No
Lactobacillus acidophilus	Feed additives	Zootechnical additives	Gut flora stabiliser and other zootechnical additive. Non GMM	EFSA-Q-2023-00451	Yes	No
Ligilactobacillus salivarius	Feed additives	Zootechnical additives	Gut flora stabiliser and other zootechnical additive. Non GMM	EFSA-Q-2023-00451	Yes	No
Pediococcus pentosaceus	Feed additives	Technological additives	Silage additive. Non GMM	EFSA-Q-2024-00222	Yes	No
Serratia plymuthica	Novel foods	Novel foods	Production of the enzyme isomaltulose synthase (sucrose glucosylmutase) used for the synthesis of the novel food 'Isomeric sucrose (Vitalose)'. GMM	EFSA-Q-2024-00437	No	Yes

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Species	EFSA risk assessment area	Category regulated product	Intended usage	EFSA question no[a]	Previous QPS status of the respective TU[b]	Assessed in this statement? Yes or no
Xanthomonas campestris[c]	Feed additives	Technological additives	Stabiliser and thickeners. Production of xanthan gum. GMM	EFSA-Q-2024-00509	Yes	No
Xanthomonas campestris[c]	Feed additives	Technological additives	Stabiliser and thickeners. Production of xanthan gum. Non GMM	EFSA-Q-2024-00509	Yes	No
Xanthomonas campestris[c]	Feed additives	Technological additives	Stabiliser and thickeners. Production of xanthan gum. Non GMM	EFSA-Q-2024-00509	Yes	No
Xanthomonas campestris[c]	Feed additives	Technological additives	Stabiliser and thickeners. Production of xanthan gum. Non GMM	EFSA-Q-2024-00509	Yes	No
Xanthomonas campestris[c]	Feed additives	Technological additives	Stabiliser and thickeners. Production of xanthan gum. Non GMM	EFSA-Q-2024-00509	Yes	No
Xanthomonas campestris	Feed additives	Technological additives	Stabiliser and thickeners. Production of xanthan gum. Non GMM	EFSA-Q-2024-00594	Yes	No
Filamentous fungi						
Aspergillus niger	Food enzymes, food additives and flavourings	Food enzyme	Production of the food enzyme triacylglycerol lipase. GMM	EFSA-Q-2024-00206	No	No
Aspergillus niger	Food enzymes, food additives and flavourings	Food enzyme	Production of the food enzyme glucoamylase. GMM	EFSA-Q-2024-00220	No	No
Aspergillus niger	Food enzymes, food additives and flavourings	Food enzyme	Production of the food enzyme glucoamylase. GMM	EFSA-Q-2024-00221	No	No
Aspergillus niger	Food enzymes, food additives and flavourings	Food enzyme	Production of the food enzyme pectine lyase. GMM	EFSA-Q-2024-00525	No	No
Aspergillus oryzae	Food enzymes, food additives and flavourings	Food enzyme	Production of the food enzyme aspergillopepsin I. Non GMM	EFSA-Q-2024-00205	No	No
Aspergillus oryzae	Food enzymes, food additives and flavourings	Food enzyme	Production of the food enzyme fructosyltransferase. Non GMM	EFSA-Q-2024-00323	No	No
Aspergillus oryzae	Food enzymes, food additives and flavourings	Food enzyme	Production of the food enzyme alpha-amylase. Non GMM	EFSA-Q-2024-00451	No	No
Aspergillus tubingensis	Feed additives	Zootechnical additives	Digestibility enhancer (CAPSOZYME SB PLUS). Production of alpha-galactosidase. Non GMM	EFSA-Q-2024-00262	No	No
Talaromyces versatilis[c]	Feed additives	Zootechnical additives	Digestibility enhancers (ROVABIO® ADVANCE). Production of the feed enzymes endo-1,4-beta-xylanase and endo-1,3(4)- beta-glucanase. Non GMM	EFSA-Q-2024-00301	No	No

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Species	EFSA risk assessment area	Category regulated product	Intended usage	EFSA question no[a]	Previous QPS status of the respective TU[b]	Assessed in this statement? Yes or no
Talaromyces versatilis[c]	Feed additives	Zootechnical additives	Digestibility enhancers (ROVABIO® ADVANCE). Production of the feed enzymes endo-1,4-beta-xylanase and endo-1,3(4)- beta-glucanase. GMM	EFSA-Q-2024-00301	No	No
Trichoderma longibrachiatum	Feed additives	Zootechnical additives	Digestibility enhancer (CAPSOZYME SB PLUS). Production of endo-1,4-beta-xylanase. Non GMM	EFSA-Q-2024-00262	No	No
Trichoderma reesei	Feed additives	Zootechnical additives	Digestibility enhancers. Production of the feed enzyme endo-1,4-beta-xylanase. GMM	EFSA-Q-2024-00418	No	No
Yeasts						
Komagataella phaffii	Food enzymes, food additives and flavourings	Food enzyme	Production of food enzyme triacylglycerol lipase. GMM	EFSA-Q-2024-00201	Yes	No
Saccharomyces cerevisiae	Novel foods	Novel foods	As an active agent. Iron-containing yeast. Non GMM	EFSA-Q-2024-00523	Yes	No
Saccharomyces cerevisiae	Feed additives	Zootechnical additives	Gut flora stabilisers. Non GMM	EFSA-Q-2024-00260	Yes	No
Saccharomyces cerevisiae	Feed additives	Nutritional additives	Vitamins, pro-vitamins and chemically well-defined substances having similar effect. Production of 25-hydroxycholecalciferol. GMM	EFSA-Q-2024-00273	Yes	No

[a]To find more details on specific applications please access the EFSA website – OpenEFSA at https://open.efsa.europa.eu/questions.

[b]Included in the QPS list as adopted in December 2022 (EFSA BIOHAZ Panel, 2023).

[c]Different strains from same species in the same application.