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STATEMENT

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Update of the list of QPS-recommended biological agents intentionally added to food or feed as notified to EFSA 13: suitability of taxonomic units notified to EFSA until September 2020

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Abstract

The qualified presumption of safety (QPS) approach was developed to provide a regularly updated generic pre-evaluation of the safety of biological agents, intended for addition to food or feed, to support the work of EFSA's Scientific Panels. It is based on an assessment of published data for each agent, with respect to its taxonomic identity, the body of knowledge, safety concerns and antimicrobial resistance. Safety concerns identified for a taxonomic unit (TU) are, where possible, confirmed at strain or product level, and reflected by 'qualifications'. In the period covered by this statement, no new information was found that would change the status of previously recommended QPS TUs. Of the 36 microorganisms notified to EFSA between April and September 2020, 33 were excluded; seven filamentous fungi (including *Aureobasidium pullulans* based on recent taxonomic insights), one *Clostridium butyricum*, one *Enterococcus faecium*, three *Escherichia coli*, one *Streptomyces* spp. and 20 TUs that had been previously evaluated. Three TUs were evaluated; *Methylorubrum extorquens* and *Mycobacterium aurum* for the first time and *Bacillus circulans* was re-assessed because an update was requested in relation to a new mandate. *M. extorquens* and *M. aurum* are not recommended for QPS status due to the lack of a body of knowledge in relation to use in the food or feed chain and *M. aurum,* due to uncertainty concerning its pathogenicity potential. *B. circulans* was recommended for QPS status with the qualifications for 'production purposes only' and 'absence of cytotoxic activity'.

Keywords: safety, QPS, bacteria, yeast, *Bacillus circulans*, *Methylorubrum extorquens*, *Mycobacterium aurum*

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Amendment: The link mentioned on page 3 on the list of biological agents notified to EFSA has been updated.

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Summary

The European Food Safety Authority (EFSA) asked the 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 biological agents, intentionally added to food and feed, having the 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 generic pre-evaluation procedure to support safety risk assessments of biological agents performed by EFSA's scientific Panels and Units. This process assesses the taxonomic identity, body of knowledge and safety of biological agents. 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 Panel Statement. The Panel Statement also includes the evaluation of microbiological agents newly notified to EFSA within the previous 6-month period.

The first ToR requires ongoing updates of the list of biological agents notified to EFSA, in the context of a technical dossier for safety assessment. The overall list (https://doi.org/10.5281/zenodo.4498901) was updated with the notifications received since the latest review in March 2020. Within this period, 36 notifications were received by EFSA, of which 22 were proposed for evaluation in Feed, 4 for use as Food Enzymes, Food Additives and Flavourings, 7 as Novel Foods, 2 as Plant Protection Products and 1 as a Genetically Modified Organism. The new notifications received between April and September 2020 are included in the current Statement (see Appendix F).

The second ToR concerns the revision of the TUs previously recommended for the QPS list and their qualifications. For this revision, articles published from January until June 2020 (for protists/algae from January 2019 to June 2020) 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.4428668) and the search strategies in Appendix C (see https://doi.org/10.5281/zenodo.4428691). No new information was found that would affect the QPS status of those TUs or their qualifications.

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.4428353, Appendix E). The current Statement focuses on the assessments of the TUs that were notified to EFSA between April and September 2020.

Three of the 36 notifications received, corresponding to three TUs, were evaluated for possible QPS status; two of these (*Methylorubrum extorquens* (previously known and notified as *Methylobacterium extorquens)* and *Mycobacterium aurum*) for the first time. *Bacillus circulans* was re-assessed because an update was requested in relation to the current mandate.

- *Methylorubrum extorquens* is not recommended for the QPS status due to a lack of body of knowledge in relation to its use in the food or feed chain;
- *Mycobacterium aurum* is not recommended for the QPS list due to a lack of body of knowledge and uncertainty concerning its pathogenicity potential;
- *Bacillus circulans* is recommended for the QPS status with the qualifications for 'production purposes only' and 'absence of cytotoxic activity'.

The remaining 33 notifications were excluded from QPS evaluation for the following reasons: 13 notifications were related to microorganisms that are generally excluded from QPS evaluation (seven were notifications of filamentous fungi (including *Aureobasidium pullulans*); one of *Clostridium butyricum*, one of *Enterococcus faecium*, three of *Escherichia coli*, one of *Streptomyces* spp.) and 20 were related to TUs that already had QPS status and did not require further evaluation.

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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 chain, in support of the respective Scientific Panels and Units in the frame of market authorisations, requiring an EFSA safety assessment (EFSA, 2007). 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 papers related to the TUs with QPS status. They also contain the assessment of newly arrived TUs to the EFSA Units on Feed, Food Ingredients and Packaging (FIP), Nutrition, Pesticides and Genetically Modified Organisms (GMO). 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 EFSA

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

EFSA's work on QPS activities started 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 the 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 chain. Its introduction was intended to improve transparency and ensure consistency in the approach used across the EFSA Panels. Applications involving a taxonomic unit belonging to a species that falls within a QPS group do not require a full safety assessment.

Several taxonomic units (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 a QPS approach should provide a generic concept to prioritise and to harmonise safety risk assessment of microorganisms intentionally introduced into the food chain, in support of the respective Scientific Panels and EFSA Units in the frame of the market authorisations. 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 taxonomic units 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 taxonomic units started 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://zenodo.org/record/1146566) and the list of notifications to EFSA

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

(https://zenodo.org/record/3607183) are constantly updated, independent of the QPS opinion and available at the Knowledge Junction in Zenodo. The most recent QPS opinion (EFSA BIOHAZ Panel, 2020a) 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 microbiological agents notified to EFSA requested by the Feed Unit, the Food Ingredients and Packaging (FIP) Unit, the Nutrition Unit, the Pesticides Unit and the Genetically Modified Organisms (GMO) Unit, 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 an Extensive Literature Search (ELS) performed every 6 months concerning possible new safety concerns related to the TUs already included in the QPS list. The data identified are used to decide 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 four pillars: [1] the taxonomic grouping for which QPS is sought ('*taxonomic identification*'); [2] whether sufficient information is available about the proposed group of organisms to conclude on human/animal exposure by food/feed ('*body of knowledge*'); [3] whether the grouping proposed contains known pathogens ('*safety*') 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. 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 proved if the yeasts are to be used as viable organisms in the food 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 provides a generic safety pre-assessment approach for use within EFSA that covers safety concerns for humans, animals and the environment. In the QPS concept, a safety assessment of a defined taxonomic unit 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 connected 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 (GMM) for which the species of the recipient strain qualifies for the QPS status, and for which the genetically modified state 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, if there is science-based evidence for microbial species it is reported. 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* and recently also *Clostridium butyricum* (EFSA BIOHAZ Panel, 2020b) are excluded from the QPS assessments based on an ambiguous taxonomic position or the possession of potentially harmful traits.

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

ToR 1: Keep updated the list of biological agents being notified in the context of a technical dossier to EFSA Units such as Feed, Pesticides, Food Ingredients and Packaging (FIP) and Nutrition, for intentional use directly or as sources of food and feed additives, food enzymes and plant protection products 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 a review of the updated literature aiming at verifying if 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 efficiently exclude safety concerns.

ToR 3: (Re) assess the suitability of new taxonomic units notified to EFSA for their inclusion in the QPS list. These microbiological agents are notified to EFSA and requested by the Feed Unit, the FIP Unit, the Nutrition Unit or by the Pesticides Unit.

2. Data and methodologies

2.1. Data

In reply to ToR 3, (re)assessment of the suitability of TUs notified within the time period covered by this Statement (from April to September 2020) is carried out. The literature review considered the identification, the body of knowledge, the potential safety concerns and the knowledge on acquired antimicrobial resistance (AMR). Relevant databases, such as PubMed, Web of Science, CAB Abstracts or Food Science Technology Abstracts (FSTA) and Scopus, were searched. More details on the search strategy, search keys and approach are described in Appendix A.

Only valid TUs covered by the relevant international committees on the nomenclature for microorganisms are considered for the QPS assessment.

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 biological agents being notified to EFSA. A total of 36 notifications were received between March and September 2020, of which 22 were for evaluation in Feed, four for use as Food Enzymes, Food Additives and Flavourings, seven as Novel Foods, two as Plant Protection Products and 1 as a Genetically Modified Organism (Table 1). *Aureobasidium pullulans* was notified twice, once as a yeast and once as a filamentous fungus, but only counted as one TU (filamentous fungus).

In response to ToR 3, three of the 36 notifications, corresponding to three TUs, were evaluated for possible QPS status, two of these for the first time, i.e. *Methylorubrum extorquens* (previously known and notified as *Methylobacterium extorquens*) and *Mycobacterium aurum*. The third, *Bacillus circulans*, was re-assessed because an update was requested in the current mandate. The remaining 33 notifications were excluded from QPS evaluation for the following reasons: 13 notifications were related to microorganisms that are generally excluded from QPS evaluation (seven were notifications of filamentous fungi (including *Aureobasidium pullulans*); one of *Clostridium butyricum*, one of *Enterococcus faecium*, three of *Escherichia coli*, one of *Streptomyces* spp.), and 20 were related to TUs that already have QPS status and do not require further evaluation in this mandate.

Table 1: Notifications received by EFSA, per risk assessment area and by biological group, from April to September 2020

Risk assessment area	Not evaluated in this Statement		Evaluated in this Statement[(b)]	Total
Biological group	**Already QPS**	**Excluded in QPS[(a)]**		
Feed	**15**	**7**	**0**	**22**
Bacteria	11	6	0	17
Filamentous fungi	0	1	0	1
Yeasts	4	0	0	4
Novel foods	**2**	**3**	**2**	**7**
Bacteria	1	0	2	3
Filamentous fungi	0	3	0	3
Protists/Algae	0	0	0	0
Yeasts	1	0	0	1
Plant protection products	**0**	**2**	**0**	**2**
Bacteria	0	0	0	0
Filamentous fungi	0	2	0	2
Viruses	0	0	0	0

Risk assessment area	Not evaluated in this Statement		Evaluated in this Statement[b]	Total
Biological group	Already QPS	Excluded in QPS[a]		
Food enzymes, food additives and flavourings	**3**	**1**	**0**	**4**
Bacteria	1	0	0	1
Filamentous fungi	0	1	0	1
Yeasts	2	0	0	2
Genetically modified organism	**0**	**0**	**1**	**1**
Bacteria	0	0	1	1
Total	**20**	**13**	**3**	**36**

QPS: qualified presumption of safety.
(a): The number includes seven notifications of filamentous fungi, one of *Clostridium butyricum* (bacterium), one of *Enterococcus faecium* (bacterium), three of *Escherichia coli* (bacterium) and one of *Streptomyces spp.* (bacterium), all excluded from QPS evaluation.
(b): Three notifications corresponding to three TUs, one was last evaluated in 2017 (*Bacillus circulans*) and two were evaluated for the first time (*Methylorubrum extorquens* (previously known and notified as *Methylobacterium extorquens*), *Mycobacterium aurum*).

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.4428668, and in Appendix C Search strategies – see https://doi.org/10.5281/zenodo.4428691, respectively.

The Artificial Intelligence (AI) function was used for pre-screening of papers for *Bifidobacterium* spp., *Carnobacterium divergens*, lactobacilli, *Lactococcus lactis*, bacilli and yeasts, followed by a second screening of those articles retrieved by AI which was carried out by two experts.

The aim of the ELS was to identify any publicly available studies reporting on safety concerns for humans, animals or the environment, caused by QPS organisms since the previous QPS review (i.e. publications from January to June 2020), and for protists/algae from January 2019 to June 2020.

For case reports of human infections or intoxications, important additional information includes whether any negative impacts are confined to affected persons with conditions favouring opportunistic infections, e.g. immunosuppression, and whether transmission occurred through food or other routes, when described (e.g. medical devices). 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 favouring endocarditis, people in the extreme lower or upper age spectrum, or with other conditions which can lead to impairment of the immunological 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 mucosal impairment can also be a predisposing factor for infection. Previous use of the microorganisms being assessed as food supplements for humans was reported in many of these cases. A living microorganism used as a food supplement does not fall under the remit of the QPS assessment. Nevertheless, the QPS assessment takes into consideration these reports, extracting relevant information whenever justified. For a detailed protocol of the process and search strategies, refer to Appendices B and C.

After removal of duplicates, 1,723 records were submitted to the title screening step, which led to the exclusion of 1,609 of them. The remaining 114 records were found eligible for the title and abstract screening step, which led to the exclusion of 57 of these. Of the 57 articles that finally reached the article evaluation step (full text), 21 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 papers for QPS is shown in Table 2.

Table 2: Flow of records by search strategy step

Species	Title screening step	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)	**1,099**	**54**	**25**	**11**
Bacilli[(a)]	332	18	9	3
Bifidobacterium[(a)]	69	7	4	1
Carnobacterium divergens[(a)]	11	0	0	0
Corynebacterium glutamicum	23	1	0	0
Gluconobacter oxydans/ Xanthomonas campestris	144	1	0	0
Lactobacilli[(a)]	179	17	6	5
Lactococcus lactis[(a)]	18	3	3	2
Leuconostoc	53	4	2	0
Microbacterium imperiale	0	0		0
Oenococcus	34	1	0	0
Pasteuria nishizawae		0		
Pediococci	164	1	1	0
Propionibacterium	22	0	0	0
Streptococcus thermophilus	50	1	0	0
Viruses (total)	**67**	**2**	**0**	**0**
Alphaflexiviridae/Potyviridae	24	2	0	0
Baculoviridae	43	0	0	0
Yeasts[(a)]	**355**	**52**	**27**	**10**
Protists/Algae	**202**	**6**	**5**	**0**
Total	**1,723**	**114**	**57**	**21**
Excluded	**1,609**	**57**	**36**	

(a): Number of references prescreened by AI: *Bifidobacterium spp.* (199)/*Carnobacterium divergens* (133); lactobacilli (387); *Lactococcus lactis* (128), bacilli (407), yeasts (660).

3. Assessment

The search strategy (key words, literature databases, number of papers found) followed for the assessment of the suitability of TUs notified to EFSA for their inclusion in the updated QPS list (reply to ToR 3) can be found in Appendix A.

3.1. Taxonomic units evaluated during the previous QPS mandate and re-evaluated in the current Statement

3.1.1. Bacteria

Bacillus circulans

Identity

Bacillus circulans is a valid species name with standing in nomenclature (Skerman et al., 1980), belonging to the genus *Bacillus*. It is a facultative anaerobe, motile, Gram-positive, endospore forming, rod shaped bacterium.

Body of knowledge

The vast majority of publications concerned enzymes for production or modification of polysaccharides that could be used in foods, such as glycosidase, galactosidase, mannase and chitinase (Walia et al., 2017; Tanaka et al., 2018, Itoh and Kimoto, 2019; Yano et al., 2019). One

publication reported the use of *B. circulans* strains as a probiotic in fish (Singh et al., 2019) and two articles report the use of its spores to control gastrointestinal nematodes in sheep and cattle (Sinott et al., 2016; Pinto et al., 2017).

Safety concerns

Alebouyeh et al. (2011) reported a case of fatal sepsis caused by a *B. circulans* strain in an immunocompromised man with a history of urinary tract infections. Sanyal et al. (2015) described a non-diabetic foot infection in a 60-year-old malnourished man caused by a haemolytic-positive *B. circulans* strain. The taxonomical identification of the isolates in both papers was made using phenotypic tests and the analysis of the 16S rRNA gene sequence.

Conclusion on a recommendation for the QPS list

B. circulans has been widely studied as an enzyme producer with potential application for food additives and is recommended for the QPS list with the qualifications 'for production purposes only' and 'absence of cytotoxic activity'.

3.1.2. Yeasts

Aureobasidium pullulans

Aureobasidium pullulans was evaluated for QPS (as a yeast) in 2009 and considered not suitable for inclusion in the QPS list. This species is excluded from further QPS assessment based on recent taxonomic insights (see further details in Section 3.4).

3.2. Taxonomic units to be evaluated for the first time

3.2.1. Bacteria

Methylorubrum extorquens

Identity

Methylorubrum extorquens is a TU with standing in nomenclature. It has been described by Green and Ardley (2018) as a novel species name for *Methylobacterium extorquens*, described by Bousfield and Green (1985) and Kato et al. (2005). Strains belonging to the *Methylorubrum* genus have a Gram-negative cell wall and grow as deep pink/red orange colonies (due to the pigment oxo-carotenoid) on media with methanol as the sole carbon and energy source. The G+C content of the DNA is 65.8–71.8 mol%. The genus *Methylorubrum* shares many common features with the genus *Methylobacterium.*

Body of knowledge

M. extorquens is isolated from environmental sources (soil, water etc.) can produce a variety of value-added products from methanol and is a well-studied microorganism for biotechnological applications but with limited information on its use in the food and feed chain (reviewed by Ochsner et al., 2015). The studies reviewed reported the production of bulk chemicals such as polyhydroxyalkanoates and amino acids, fine chemicals such as acids derived from the ethylmalonyl-CoA pathway and proteins as single cell proteins (biomass), insecticides, bacteriocins and green fluorescent protein.

Safety concerns

Case reports of infections with *M. extorquens* were reported in immunocompromised patients (Kaye et al., 1992; Engler and Norton, 2001; Kovaleva et al., 2014). Nosocomial infections are favoured by its ability to form biofilms and colonise medical devices (Kovaleva et al., 2014).

Antimicrobial resistance aspects

M. extorquens strains are reported to be intrinsically resistant to quinolones (Kim et al., 2005).

Conclusion on a recommendation for the QPS list

M. extorquens is not recommended for QPS status due to a lack of body of knowledge in relation to its use in the food or feed chains.

Mycobacterium aurum

Identity

Mycobacterium aurum is a taxonomic name with standing in nomenclature (Nouioui et al., 2018), within the order *Actinomycetales*, suborder *Corynebacterineae* and family *Mycobacteriaceae*. The species has the valid synonym *Mycolicibacterium aurum* (Gupta et al., 2018).

Body of knowledge

The information available mainly deals with the use of this species as a non-infectious surrogate model for *Mycobacterium tuberculosis.* Limited information is available on ecological aspects of this species and no data on the presence of this organism in the food chain is available. Due to its relatedness to *Mycobacterium tuberculosis* and its more rapid growth, this species has been used for screening the effects of new anti-tuberculosis compounds.

Safety concerns

The species has been associated with infections in immunocompromised patients (Esteban et al., 1998; Koranyi and Ranalli, 2003; Martín-Aspas et al., 2008) and with keratitis (Honarvar et al., 2012) in reports using bacterial identification methods currently recognised to be prone to misidentifications of *Mycobacterium* species (Tortoli, 2014). No food-borne origin could be established.

Conclusion on a recommendation for the QPS list

Mycobacterium aurum cannot be recommended for the QPS list due to a lack of body of knowledge and uncertainty with regard to its pathogenicity potential.

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. articles published between January and June 2020, and for protists/algae from January 2019 to June 2020) 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 E), are presented below.

3.3.1. Gram-positive non-sporulating bacteria

Bifidobacterium spp.

A search for papers potentially relevant for *Bifidobacterium spp.* provided 199 references. The AI analysis left 69. Title screening left seven references for abstract inspection, then four for a full article appraisal. This last step identified one article (Sirichoat et al., 2020) that might deal with safety concerns but the article was not considered relevant because it described the antimicrobial resistance of *Bifidobacterium* among other lactic acid bacteria from human vagina, a feature covered by the general qualification for bacteria 'absence of antimicrobial activity'.

Based on the available evidence, the QPS status of *Bifidobacterium* spp. is not changed.

Carnobacterium divergens

A search for papers potentially relevant for *Carnobacterium divergens* provided 14 references. The AI analysis left 11. No article was considered relevant at the level of title screening for this TU. Consequently, the QPS status of *C. divergens* is not changed.

Corynebacterium glutamicum

A search for papers potentially relevant to the QPS evaluation of *Corynebacterium glutamicum* provided 23 references. One paper reached the level of title and abstract screening but did not reach full text evaluation. Therefore, no new safety concerns were identified and the QPS status of *C. glutamicum* is not changed.

Lactobacilli

The genus *Lactobacillus* was recently subdivided into 25 genera (Zheng et al., 2020); the 37 species which have QPS status granted have been allocated to 13 of these genera. The search for papers that might raise safety concerns was done using both the previous and the new names and provided a total of 566 references. The AI analysis left 179 articles. Title screening of these provided 17 references for abstract inspection, which further reduced their number to 5 describing a possible safety concern. Full paper review identified three with reliable microorganism identification procedures. All three dealt with *Lacticaseibacillus rhamnosus* infections. Two of the papers described bacteraemia cases (Chiang et al., 2020; Sendil et al., 2020) while the third was on endocarditis (Campagne et al., 2020). In all three cases, predisposing conditions for opportunistic infections were recorded. Chiang et al. (2020) described the case of an extremely premature girl (born through Caesarean section at 26 weeks of pregnancy), who had a central catheter inserted and was administered *L. rhamnosus* GG (5×10^8 cfu/day) for necrotising enterocolitis prevention (the blood cultures recovered a strain identical to the probiotic). Sendil et al. (2020) described bacteraemia in a 75-year-old man who had received a renal transplant several years before which was showing signs of rejection despite extensive immunosuppressive therapy. Associated comorbidities included type 2 diabetes, hypertension, stroke and coronary insufficiency. Finally, Campagne et al. (2020) described endocarditis in a middle-aged man who suffered previous interatrial communication surgery and presented with extensive dental caries.

Based on the available evidence as described above, the QPS status of the species previously included within the genus *Lactobacillus spp.* and now belonging to any of the derived genera is not changed.

Lactococcus lactis

A search for papers potentially relevant for the QPS status of *Lactococcus lactis,* provided 146 references. The AI analysis left 18 papers. Title and abstract screenings of these reduced their number to 2, describing a possible safety concern. Both were excluded because the identification procedures were not considered to be reliable.

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

Leuconostoc spp.

A search for papers potentially relevant for the QPS evaluation of QPS *Leuconostoc* species provided 53 references. The analysis of their titles left four articles for title/abstract screening. Two articles reached full text evaluation, but neither dealt with safety concerns. Consequently, the QPS status of *Leuconostoc* spp. is not changed.

Microbacterium imperiale

A search for papers potentially relevant for the QPS evaluation of QPS *Microbacterium imperiale* provided no reference. Consequently, the QPS status of *M. imperiale* is not changed.

Oenococcus oeni

A search for papers potentially relevant for the QPS evaluation of *Oenococcus oeni* provided 34 references. The analysis of their titles left one article for title/abstract screening. This article was not considered further. Consequently, the QPS status of *O. oeni* is not changed.

Pediococcus spp.

A search for papers potentially relevant for the QPS evaluation of *Pediococcus* spp. provided 164 references. The analysis of their title/abstracts left one article for the evaluation phase, but it did not refer to safety concerns. No article reached the full text evaluation stage. Consequently, the QPS status of *Pediococcus* spp. is not changed.

Propionibacterium

A search for papers potentially relevant for the QPS evaluation of *Propionibacterium* spp. provided 22 references. Following the analysis of their titles, no articles were selected for title/abstract screening or the full article evaluation phase; thus, no new safety concerns were identified. Consequently, the QPS status of *Propionibacterium* spp. is not changed.

Streptococcus thermophilus

A search for papers potentially relevant for the QPS evaluation of *Streptococcus thermophilus* provided 50 references. The analysis of their titles left one article for title and abstract screening that did not deal with safety concerns. Therefore, no article reached the evaluation phase, and the QPS status of *S. thermophilus* is not changed.

3.3.2. Gram-positive spore-forming bacteria

Bacilli

A search for papers potentially relevant for bacilli provided 739 references. The AI analysis left 332 articles. The analysis of their titles by two experts left 18 articles for the title/abstracts phase and, from these, nine articles passed to the full text phase for further analysis. Six papers did not deal with safety concerns. The paper of Bog et al. (2020) described the results of an *in vitro* pathogenicity test of a *B. licheniformis* strain. The paper had methodological problems in relation to strain identification and used an unvalidated *in vitro* pathogenicity test with very high bacterial cell density. The paper of Farhan et al. (2020) reported the isolation of a *B. subtilis* strain in relation to mastitis. The paper had methodological problems in relation to strain identification and did not document source attribution. The paper of Mohkam et al. (2020) had methodological concerns in relation to weak and not reproducible PCR results. Weak haemolysis and cytotoxic effect of a *B. subtilis* strain were reported, a possible concern which is covered by the QPS qualification for bacilli of 'not showing cytotoxic activity'.

The ELS did not identify any information that would change the status of bacilli included in the QPS list and confirmed the qualification 'absence of cytotoxicity'.

Geobacillus stearothermophilus

A search for papers potentially relevant for *Geobacillus stearothermophilus* provided 407 references. The AI analysis left 332 articles. The analysis of their titles by two experts left 18 articles and for 9 of these the full text was analysed. None dealt with this species. Consequently, the QPS status *G. stearothermophilus* is not changed.

Pasteuria nishizawae

A search for papers potentially relevant for the QPS evaluation of *Pasteuria nishizawae* provided 0 references. Consequently, the QPS status of *P. nishizawae* is not changed.

3.3.3. Gram-negative bacteria

Gluconobacter oxydans

A search for papers potentially relevant to the QPS evaluation of *Gluconobacter oxidans* and *Xanthomonas campestris* provided 144 references.

The analysis of their titles left one article, which was excluded following the title and abstract screening. No paper reached the final selection phase for this TU. Consequently, the QPS status of *G. oxydans* is not changed.

Xanthomonas campestris

As previously mentioned, the search for papers potentially relevant for the QPS evaluation of *Gluconobacter oxidans* and *Xanthomonas campestris* provided 144 references. The analysis of their titles left one article, which was excluded following the title and abstract screening. No paper reached the evaluation phase for this TU. Consequently, the QPS status of *X. campestris* is not changed.

3.3.4. Yeasts

The ELS searches for potentially relevant studies on the yeasts with QPS status provided 1,015 references. The AI analysis left 355 articles. After title screening by two experts, 52 studies remained for the title/abstract phase, and from these 27 articles passed to the full article appraisal. Out of these, 14 were relevant for the QPS evaluation, of which 10 reported a possible safety concern.

Four studies did not directly report safety concerns. Two looked at potential factors for virulence in yeasts (Alves et al., 2020; Chakraborty et al., 2020), but not at relationships between these factors and actual virulence. Zhai et al. (2020) included the QPS species *D. hansenii*, *K. marxianus* and *Y. lipolytica* when calculating the relative abundance of potentially pathogenic fungi from

gastro-intestinal metagenomics data but reported no information on whether these species contributed to infections. Stavrou et al. (2020) reported new data on minimum inhibitory concentrations of antifungal drugs for opportunistic yeasts, among them the QPS species *D. hansenii*, *K. marxianus*, *W. anomalus* and *Y. lipolytica*. These data can be used for testing the qualification 'absence of antimycotic resistance' of QPS yeast species used as viable cells.

Ten studies discussed potentially relevant safety concerns for QPS yeast species, which are discussed below.

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

Debaryomyces hansenii

The anamorph name of *D. hansenii* is *Candida famata*.

Esteves et al. (2020) obtained 75 yeast isolates from the oral cavity of patients diagnosed with eating disorders at a clinic in Sao Paulo, Brazil. Out of the isolates, 43 were identified by conventional mycological methods and MALDI-TOF. Two were identified as *C. famata*. The source attribution in the paper was not documented and the identification by MALDI-TOF was not further confirmed by molecular methodology.

In conclusion, the literature update did not identify any information that would change the QPS status of *D. hansenii*.

Kluyveromyces marxianus

The anamorph name of *K. marxianus* is *Candida kefyr*.

Aboualigalehdari et al. (2020) characterised yeast isolates from the oropharyngeal tract of 201 HIV-infected patients in Iran. Out of the 127 isolates obtained from 88 of the patients, three were identified as *K. marxianus*. It is not clear, however, whether the patients had been diagnosed with candidiasis. In another study (Kord et al., 2020), a total of 137 yeast blood isolates from 107 patients in two tertiary care training hospitals in Tehran were retrospectively investigated. The patients had underlying diseases (pulmonary diseases, solid tumours or diabetes). Two of the strains were identified as *K. marxianus*. Mirhendi et al. (2020) obtained a total of 235 yeast strains from various body fluids of children admitted to a paediatric intensive care unit in a hospital in Iran, having suspected or documented invasive candidiasis. All children were prematurely born and/or had underlying diseases. Three of the isolates were identified as *K. marxianus*. Susceptibility to amphotericin B, fluconazole, voriconazole, micafungin and anidulafungin was determined. Finally, Pote et al. (2020) reported that three out of their 176 clinical yeast isolates from hospitals in India were *K. marxianus*. However, the study contains no clinical information for the isolates. They compared DNA sequencing of the 28S rRNA gene with growth on chromogenic media and MALDI-TOF for yeast identifications. Using the sequencing as reference, MALDI-TOF was able to identify the *K. marxianus* isolates, whereas growth on chromogenic media was not.

In conclusion, the literature update mentioned only the isolation of *K. marxianus* from patients who are immunocompromised and/or have underlying disease. Also, methodological problems concerning identification and source attribution were noted. So, the papers did not identify any information that would change the QPS status of *K. marxianus*.

Saccharomyces cerevisiae

The anamorph form of *S. cerevisiae* is not described. A synonym of this species is *Saccharomyces boulardii*.

Three references reported safety concerns for humans. Pote et al. (2020) compared three methods for yeast identifications, growth on chromogenic media, DNA sequencing of the 26S rRNA gene and protein profiles using MALDI-TOF. The conclusion is that MALDI-TOF is able to identify common species, but its effectiveness is limited for uncommon species of *Candida,* or members of uncommon genera. The preferred methodology is DNA sequencing. The other two papers which described clinical cases of *S. cerevisiae* concerned a 4-year-old boy with acute lymphoblastic leukaemia presenting with hepatosplenic *S. cerevisiae* infection (Davies et al., 2020) and *S. cerevisiae* fungaemia in a patient with *Clostridium difficile*-associated diarrhoea (Landaburu et al., 2020). The latter emphasised that probiotic

treatment of patients with serious gastrointestinal disorders has to be carefully considered and followed up.

These new reports of *S. cerevisiae* did not add any new information that would change the QPS status of this species.

Wickerhamomyces anomalus

The anamorph name of *W. anomalus* is *Candida pelliculosa*.

Two references related to possible concerns for human safety were identified.

Bhaskaran et al. (2020) characterised 80 clinical strains, from patients with diagnosed *Candida* infection, from a tertiary hospital in India. Identification was by traditional growth tests and multiplex PCR, which is not the usual technique used for yeast identification, and four of the isolates were reported as *W. anomalus*. In a clinical case report, infection with *W. anomalus* was responsible for fungal arthritis and led to irreversible joint destruction owing to delayed diagnosis and treatment. The case was not food related; the patient was a 75-year-old woman with a 3-year history of knee problems and surgery 32 months earlier without antifungal treatment (Song et al., 2020).

The literature update did not identify any information that would change the QPS status of *W. anomalus*.

3.3.5. Protists/Algae

ELS was performed for all three species together, as indicated below.

Aurantiochytrium limacinum

Aurantiochytrium limacinum is a member of the *Thraustochytriacea* and is considered to be a protist.

A search for papers potentially relevant for the QPS evaluation of protists/algae provided 202 references. Following the analysis of their titles, 6 and of their title/abstracts, 5 articles were selected for the full article evaluation phase, of which 1 was on a different microorganism and 1 on *A. limacinum.* No article identified a possible safety concern. Therefore, the QPS status of *A. limacinum* is not changed.

Euglena gracilis

A search for papers potentially relevant for the QPS evaluation of protists/algae provided 202 references. Following the analysis of their titles, 6 and of their title/abstracts, 5 articles were selected for the full article evaluation phase of which one was on a different microorganism and two were on *Euglena gracilis.* No article identified a possible safety concern. Therefore, the QPS status of *E. gracilis* is not changed.

Tetraselmis chuii

A search for papers potentially relevant for the QPS evaluation of protists/algae provided 202 references. Following the analysis of their titles, 6 and of their title/abstracts, 5 articles were selected for the full article evaluation phase of which one was on a different microorganism and one was on *Tetraselmis chuii*. No article identified a possible safety concern. Therefore, the QPS status of *T. chuii* is not changed.

3.3.6. Viruses used for plant protection

Alphaflexiviridae and Potyviridae

A search for papers potentially relevant for the QPS evaluation of viruses of the *Alphaflexiviridae* and *Potyviridae* provided 24 references. After title screening, two papers reached the title/abstract screening stage. No paper reached the final selection phase, thus no new safety concern was identified. Therefore, the current QPS status remains unchanged.

Baculoviridae

A search for papers potentially relevant for the QPS evaluation of *Baculoviridae* provided 43 references. No article dealing with *Baculoviridae* passed the title screening, thus no new safety concern was identified. Therefore, the current QPS status remains unchanged.

3.4. Distinction between yeasts and filamentous fungi

The distinction between a filamentous fungus and a yeast, and thus whether an organism would be eligible for QPS evaluation and status (yeast), or not (filamentous fungi) is sometimes not clear-cut.

In their taxonomic study, Kurtzman et al. (2011) define yeasts as follows: 'In summary, yeasts, whether ascomycetes or basidiomycetes, are generally characterised by budding or fission as the primary means of asexual reproduction and have sexual states that are not enclosed in fruiting bodies'. This definition implies that yeasts are not expected to belong to any other fungal phylum than ascomycetes and basidiomycetes. However, they consider that of the three lineages within the Ascomycota, only two contain yeasts, whereas the third (Pezizomycotina) does not.

The decision whether a species should be considered to be a yeast or a filamentous fungus for QPS purposes is taken on a case-by-case basis, but applying the following general rules and limitations: A fungus may be subject to evaluation if it i) belongs to the phyla Ascomycota (excluding the Pezizomycotina) or Basidiomycota and ii) is treated as a yeast by taxonomic literature. As supporting information, the taxonomy applied by internationally recognised microbial culture collections is considered (e.g. Fungal Biodiversity Centre (CBS) - Fungi strains; https://wi.knaw.nl/page/fungal_table with the yeast page https://theyeasts.org/).

Aureobasidium pullulans is a relatively well-studied fungal species since it is of interest within biotechnology and biological control of plant diseases. It is dimorphic and belongs to the Pezizomycotina lineage (and the order Dothidiales) of the ascomycetes and is sometimes referred to as a yeast or as 'yeast-like'. As documented above, Kurtzman et al. (2011) do not consider the genus *Aureobasidium* as yeasts and there is no chapter on the genus in their taxonomy.

In line with the exclusion of *Aureobasidium* by Kurtzman et al. (2011), a recent review (Naranjo-Ortiz and Gabaldón, 2019) reported that: 'The basic body plan of this subphylum (Pezizomycotina) is filamentous and anastomosed', and these authors do not refer to any fungi within Pezizomycotina as 'yeasts', or 'yeast-like' either. They also note that typically, members of Pezizomycotina contain a high abundance of enzymes for secondary metabolism, which is generally in contrast to yeasts but similar to filamentous fungi.

Conclusions

ToR 1: ***Keep updated the list of biological agents being notified, in the context of a technical dossier to EFSA Units (such as Feed, Food Ingredients and Packaging, Nutrition, Pesticides, Genetically Modified Microorganisms), for intentional use in feed and/or food or as sources of food and feed additives, enzymes, plant protection products for safety assessment*****:**

- Between April and September 2020 (for protists/algae from January 2019 to June 2020), the QPS list was updated with 36 notifications that were received by EFSA, of which 22 were proposed for evaluation in Feed, four for use as Food Enzymes, Food Additives and Flavourings, seven as Novel Foods, two as Plant Protection Products and one as a Genetically Modified Organism.

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 related to the QPS list, there were no results to justify removal of any TUs from the QPS list or changes in their respective qualifications.

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 36 notifications received, 20 were related to TUs that already had QPS status and did not require further evaluation.
- Of the remaining 16 notifications, 13 were related to TUs excluded from QPS evaluation: seven were notifications of filamentous fungi, (including *Aureobasidium pullulans* based on recent taxonomic insights); one of *Clostridium butyricum*, one of *Enterococcus faecium,* three of *Escherichia coli,* one of *Streptomyces* spp.

- Three notifications, corresponding to three TUs, were evaluated for possible QPS status, two of these (*Methylorubrum extorquens* (previously known as *Methylobacterium extorquens*) and *Mycobacterium aurum*) for the first time. *Bacillus circulans* was re-assessed because an update was requested in relation to the current mandate.
    - *Methylorubrum extorquens* is not recommended for QPS status due to a lack of body of knowledge in relation to its use in the food or feed chain;
    - *Mycobacterium aurum* is not recommended for the QPS list due to lack of body of knowledge and uncertainty concerning its pathogenicity potential;
    - *Bacillus circulans* is recommended for QPS status with the qualifications of 'for production purposes only' and 'absence of cytotoxic activity'.

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Zheng J, Wittouck S, Salvetti E, Franz CM, Harris HM, Mattarelli P, O'Toole PW, Pot B, Vandamme P, Walter J and Watanabe K, 2020. A taxonomic note on the genus Lactobacillus: description of 23 novel genera, emended description of the genus Lactobacillus Beijerinck 1901, and union of Lactobacillaceae and Leuconostocaceae. International Journal of Systematic and Evolutionary Microbiology, 70, 2782–2858.

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
FIP	EFSA Food ingredients and packaging Unit
FSTA	Food Science Technology Abstracts
GMM	genetically modified microorganism
MALDI-TOF	matrix-assisted laser desorption/ionisation (MALDI), time-of-flight (TOF)
QPS	qualified presumption of safety
ToR	Terms of Reference
TU	taxonomic unit
WG	Working Group

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)

A.1. *Bacillus circulans*

A search for the body of knowledge on *B. circulans* was done in the databases mentioned above, considering all years available in these databases, using a range of search terms in relation to food and feed, combined with '*Bacillus circulans*'. Seventy-one were screened.

Strings:

(food* OR feed* OR beverage* OR meat OR fish OR dairy OR milk OR vegetable* OR fruit* OR cereal* OR wheat OR barley OR rice OR rey OR grain* OR legum* OR bean* OR probiotic* OR fermented OR dish* OR condiment* OR pure* OR meal* OR enzyme* OR chitinas* OR polysacch*) AND Bacillus circulans

71 hits -last 5 years

toxi* OR disease* OR infection* OR virulence OR abortion* OR mastitis* OR bacteremi* OR poisoning OR hepatitis OR necrosis OR necrotizing OR pneumonia OR endophthalmitis OR gangrene OR endocarditis OR 'urinary tract' OR meningitis OR encephalopathy OR parodontitis OR liver OR hepatotoxi* OR abscess OR death OR mortal* Or morbid*OR sepsis OR colitis OR Pathogen* OR patholog* AND Bacillus circulans

22 hits -last 5 years

A.2. *Methylorubrum extorquens*

Methylorubrum/Methylobacterium extorquens; Pub Med: 577 hits,
With review: 26 hits
With safety 1 hit
With case report: 1 hit
With antimicrobial resistance: 8 papers

A.3. *Mycobacterium aurum*

"Mycobacterium aurum" AND infect* OR diseas* OR intox* OR toxin*; PubMed: 42 hits

Appendix B – Protocol for Extensive literature search (ELS), relevance screening and article evaluation for the maintenance and update of list of QPS-recommended biological agents (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 biological agents intentionally added to the food or feed (EFSA-Q-2020-00078) is available on the EFSA Knowledge Junction community on Zenodo, at: https://doi.org/10.5281/zenodo.4428668

Appendix C – Search strategies for the maintenance and update of list of QPS-recommended biological agents (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.4428691

Appendix D – References selected from the ELS exercise with potential safety concerns for searches January–June 2020 (reply to ToR 2) and January 2019 to June 2020 for protists/algae

Gram-Positive Non-Sporulating Bacteria

Bifidobacterium

Sirichoat A, Belen Florez A, Vazquez L, Buppasiri P, Panya M, Lulitanond V and Mayo B, 2020. Antibiotic susceptibility profiles of lactic acid bacteria from the human vagina and genetic basis of acquired resistances. International Journal of Molecular Sciences, 21.

Carnobacterium divergens

None.

Corynebacterium glutamicum

None.

Lactobacilli

Campagne J, Guichard JF, Moulhade MC, Kawski H and Maurier F, 2020. *Lactobacillus* endocarditis: a case report in France and literature review. IDCases, 21, e00811.

Chiang M-C, Chen C-L, Feng Y, Chen C-C, Lien R and Chiu C-H, 2020. *Lactobacillus rhamnosus* sepsis associated with probiotic therapy in an extremely preterm infant: Pathogenesis and a review for clinicians. Journal of Microbiology, Immunology, and Infection.

Ozer M, Goksu SY, Shahverdiani A and Mustafa M, 2020. *Lactobacillus acidophilus*-induced endocarditis and associated splenic abscess. Case Reports in Infectious Diseases, 2020.

Sendil S, Shrimanker I, Mansoora Q, Goldman J and Nookala VK, 2020. *Lactobacillus rhamnosus* Bacteremia in an Immunocompromised Renal Transplant Patient. Cureus, 12.

Tavernese A, Stelitano M, Mauceri A, Mollace R, Uccello G, Romeo F and Cammalleri V, 2020. Progression of *Lactobacillus plantarum* prosthetic valve endocarditis followed by transesophageal echocardiogram. International Journal of Infectious Diseases: IJID : official publication of the International Society for Infectious Diseases, 97, 160–161.

Lactococcus lactis

Daneshamouz S, Haghi F and Zeighami H, 2020. Detection and identification of bacterial pathogens in rainbow trout (*Oncorhynchus mykiss*) samples from fish farms in Iran. Thalassas, 36, 133–141.

Smith JCS, Moroni P, Santisteban CG, Rauch BJ, Ospina PA and Nydam DV, 2020. Distribution of *Lactococcus* spp. in New York State dairy farms and the association of somatic cell count resolution and bacteriological cure in clinical mastitis samples. Journal of Dairy Science, 103, 1785–1794.

Leuconostoc

None.

Microbacterium imperiale

None.

Oenococcus oeni

None.

Pediococci

None.

Propionibacterium

None.

Streptococcus thermophilus

None.

Gram-Positive Spore-forming Bacteria

Bacilli

Bog ES, Erturk O and Yaman M, 2020. Pathogenicity of aerobic bacteria isolated from honeybees (*Apis mellifera*) in Ordu Province. Turkish Journal of Veterinary & Animal Sciences, 44, 714–719.

Farhan MG, Abd El-Hamid MI and Hassan MN, 2020. Propidium monoazide conventional PCR and DNA sequencing: detection of negative culture bacterial pathogens causing subclinical mastitis. Journal of Applied Microbiology, 128, 1595–1605.

Mohkam M, Nezafat N, Berenjian A, Zamani M, Dabbagh F, Bigharaz R and Ghasemi Y, 2020. Multifaceted toxin profile of *Bacillus* probiotic in newly isolated *Bacillus* spp. from soil rhizosphere. Biologia, 75, 309–315.

Geobacillus stearothermophilus

None.

Pasteuria nishizawae

None.

Gram-negative bacteria

Gluconobacter oxydans

None.

Xanthomonas campestris

None.

Yeasts

Aboualigalehdari E, Tahmasebi Birgani M, Fatahinia M and Hosseinzadeh M, 2020. Oral Colonization by *Candida* Species and Associated Factors among HIV-infected Patients in Ahvaz, Southwest Iran. Epidemiology and health, e2020033.

Bhaskaran R, Valsan C and Sathiavathy KA, 2020. Molecular speciation and antifungal susceptibility profile of *Candida* Species in a Tertiary Care Centre in Central Kerala. Journal of Evolution of Medical and Dental Sciences-Jemds, 9, 357–362.

Davies E, Shipp A, Hawkes R and Wynn RF, 2020. Successful management of hepatosplenic infection due to *saccharomyces cerevisiae* in a child with acute lymphoblastic leukemia. Journal of Pediatric Hematology Oncology, 42, E117-E120.

Esteves CV, Freitas RS, e Campos WG, Shimabukuro N, Thomaz DY, Cordas T, Benard G, Witzel AL and Lemos CA, 2020. Oral yeast colonization in patients with eating disorders: commensal acquisition or due to purgative habits? Revista Do Instituto De Medicina Tropical De Sao Paulo, 62.

Fernanda Landaburu M, Lopez Daneri GA, Relloso S, Jorge Zarlenga L, Alejandra Vinante M and Teresa Mujica M, 2020. Fungemia following *Saccharomyces cerevisiae var. boulardii* probiotic treatment in an elderly patient. Revista Argentina De Microbiologia, 52, 27–30.

Gkentzi D, Marangos M, Karatza A, Spiliopoulou A, Varvarigou A and Dimitriou G, 2020. *Saccharomyces cerevisiae* fungaemia in an immunocompetent toddler. Journal of Paediatrics and Child Health, 56, 182.

Kord M, Salehi M, Khodavaisy S, Hashemi SJ, Ghazvini RD, Rezaei S, Maleki A, Elmimoghaddam A, Alijani N, Abdollahi A, Doomanlou M, Ahmadikia K, Rashidi N, Pan W, Boekhout T and Arastehfar A, 2020. Epidemiology of yeast species causing bloodstream infection in Tehran, Iran (2015-2017); superiority of 21-plex PCR over the Vitek 2 system for yeast identification. Journal of Medical Microbiology, 69, 712–720.

Mirhendi H, Charsizadeh A, Eshaghi H, Nikmanesh B and Arendrup MC, 2020. Species distribution and antifungal susceptibility profile of *Candida* isolates from blood and other normally sterile foci from pediatric ICU patients in Tehran, Iran. Medical Mycology, 58, 201–206.

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Song KY, Park C, Byun J-H, Chun H-S, Choi J-H, Han EH, Lee SO, Jeong Y, Kim YJ and Kim S-H, 2020. Fungal arthritis with adjacent osteomyelitis caused by *Candida pelliculosa*: a case report. Bmc Infectious Diseases, 20, 438.

Protists/algae

For protists/algae, the time period searched was from January 2019 to June 2020.
None

Viruses used for plant protection

Alphaflexiviridae

None.

Potyviridae

None.

Baculoviridae

None.

Appendix E – The 2020 updated list of QPS Status recommended biological agents in support of EFSA risk assessments

The list of QPS status recommended biological agents (EFSA BIOHAZ Panel, 2020a) is being maintained in accordance with the mandate of the BIOHAZ Panel (2020–2022), extended for the following years. Possible additions to this list are included around every 6 months, with the last Panel Statement 12 adopted in June 2020 (EFSA BIOHAZ Panel, 2020b). These additions are published as updates to the Scientific Opinion (EFSA BIOHAZ Panel, 2020a); the updated QPS list is available at https://doi.org//10.2903/j.efsa.2021.6377 and, as of January 2018, also as supporting information linked to every Panel Statement available on the Knowledge Junction at https://doi.org/10.5281/zenodo.4428353.

Appendix F – Microbial species as notified to EFSA, received between April and September 2020 (reply to ToR 1)

Species	Strain	EFSA risk assessment area	Category Regulated product	Intended usage	EFSA Question No[(a)] and EFSA webpage link[(b)]	Previous QPS status of the respective TU[(c)]	Assessed in this Statement? Yes or no
Bacteria							
Bacillus amyloliquefaciens	CECT 5490	Feed additives		Ecobiol, Ecobiol 500, Ecobiol Plus (Bacillus amyloliquefaciens CECT 5940)	EFSA-Q-2020-00452	Y	No (already QPS)
Bacillus amyloliquefaciens	TOA5001	Feed additives	Zootechnical additives	Gut microbiota stabiliser	EFSA-Q-2020-00496	Y	No (already QPS)
Bacillus circulans		Novel foods		Production of enzyme used in production process of Galacto-oligosaccharide	EFSA-Q-2020-00466	N	Yes
Bacillus coagulans	DSM 32789	Feed additives	Technological additives	Technological additive: preservatives	EFSA-Q-2020-00281 (FAD-2019-0092)	Y	No (already QPS)
Bacillus subtilis	TO-A	Feed additives	Zootechnical additives	Viable spores of *Bacillus subtilis* TO-A, *Enterococcus faecium* T-110, *Clostridium butyricum* TO-A used as gut microbiota stabilisers for chickens for fattening, chickens reared for laying/ breeding,	EFSA-Q-2020-00556	Y	No (already QPS)
Bacillus subtilis	CGMCC 13326	Feed additives	Nutritional additives	Production of Vitamin B2	EFSA-Q-2020-00637	Y	No (already QPS)
Bacillus subtilis	ROM	Food enzymes, food additives and flavourings		Production of glucan 1,4- -maltohydrolase	EFSA-Q-2020-00583	Y	No (already QPS)
Bifidobacterium bifidum		Novel foods		Production of enzyme used in production process of Galacto-oligosaccharide	EFSA-Q-2020-00466	Y	No (already QPS)

Species	Strain	EFSA risk assessment area	Category Regulated product	Intended usage	EFSA Question No[(a)] and EFSA webpage link[(b)]	Previous QPS status of the respective TU[(c)]	Assessed in this Statement? Yes or no
Clostridium butyricum	TO-A	Feed additives	Zootechnical additives	Viable spores of Bacillus subtilis TO-A, Enterococcus faecium T-110, Clostridium butyricum TO-A used as gut microbiota stabilisers for chickens for fattening, chickens reared for laying/ breeding,	EFSA-Q-2020-00556	N	No (excluded)
Corynebacterium glutamicum	KCTC 12307BP (C123) (GMO)	Feed additives	Nutritional additives	Production of amino acid, their salts and analogues	EFSA-Q-2020-00326 (FAD-2020-0024)	Y	No (already QPS)
Corynebacterium glutamicum	KCTC 12307 BP (C123)	Feed additives	Zootechnical additives	L-lysine monohydrochloride, concentrated liquid L-lysine (base)	EFSA-Q-2020-00326 (FAD-2020-0024)	Y	No (already QPS)
Corynebacterium glutamicum	CGMCC 7.366		Zootechnical additives	L-valine for all animal species	EFSA-Q-2020-00375 (FAD-2020-0033)	Y	No (already QPS)
Corynebacterium glutamicum	2256, NITE BP-01681	Feed additives	Technological additives	Used for the production of L-glutamic acid and monosodium glutamate	EFSA-Q-2020-00502	Y	No (already QPS)
Enterococcus faecium	T-110	Feed additives	Zootechnical additives	Viable spores of *Bacillus subtilis* TO-A, *Enterococcus faecium* T-110, *Clostridium butyricum* TO-A used as gut microbiota stabilisers for chickens for fattening, chickens reared for laying/ breeding,	EFSA-Q-2020-00556	N	No (excluded)
Escherichia coli	CGMCC 13325 (GMO)	Feed additives	Nutritional additives	Production of amino acid, their salts and analogues	EFSA-Q-2020-00273 (FAD-2020-0017)	N	No (excluded)
Escherichia coli	KCCM 80210 (W008)	Feed additives	Nutritional additives	L-tryptophan production through fermentation.	EFSA-Q-2020-00499	N	No (excluded)

Species	Strain	EFSA risk assessment area	Category Regulated product	Intended usage	EFSA Question No[(a)] and EFSA webpage link[(b)]	Previous QPS status of the respective TU[(c)]	Assessed in this Statement? Yes or no
Escherichia coli	NITE SD 00268	Feed additives	Nutritional additives	Production of L-histidine monohydrochloride (HCl) monohydrate to permit use as nutritional and sensory additive	EFSA-Q-2020-00604	N	No (excluded)
Lactobacillus plantarum	DSM26571	Feed additives	Technological additives	Technical additive: silage	EFSA-Q-2020-00279 (FAD-2019-0091)	Y	No (already QPS)
Lactobacillus plantarum	DSM 21762	Feed additives	Technological additives	*Lactobacillus plantarum* DSM 21762 is added to the forage, used for ensiling process. The obtained silage can be fed to all animal species/categories.	EFSA-Q-2020-00602	Y	No (already QPS)
Methylobacterium extorquens	KB203	GMO	Zootechnical additives	Dried killed genetically modified bacterial biomass from GM *Methylobacterium extorquens* KB203 for use in feed	EFSA-Q-2020-00397	N	Yes
Mycobacterium aurum		Novel foods		Novel Food	EFSA-Q-2020-00566		Yes
Streptomyces albus		Coccidiostats and histomonostats	Coccidiostats and histomonostats	Coccidiostat	EFSA-Q-2020-00282 (FAD-2019-0093)	N	No (excluded)
Filamentous Fungi							
Aspergillus flavus	MUCL54911	Plant protection products		Plant protection product	EFSA-Q-2020-00506	N	No (excluded)
Aspergillus oryzae		Novel foods		Modification of the conditions of use of an already authorised novel food	EFSA-Q-2020-00466	N	No (excluded)
Aureobasidium pullulans	SM-2001	Novel foods	Novel Food	Production of beta-glucan	EFSA-Q-2020-00264 (NF 2018/0741)	N	No (excluded)
Aureobasidium pullulans		Food enzymes, food additives and flavourings		Production of an extra cellular polysaccharide by *Aureobasidium pullulans*	EFSA Q 2020-00517	N	No (excluded)

Species	Strain	EFSA risk assessment area	Category Regulated product	Intended usage	EFSA Question No[(a)] and EFSA webpage link[(b)]	Previous QPS status of the respective TU[(c)]	Assessed in this Statement? Yes or no
Eremothecium ashbyii	CCTCCM 2019833	Feed additives		Vitamin B2 (Riboflavin) for all animal species	EFSA-Q-2020-00323 (FAD-2020-0027)	N	No (excluded)
Rhizomucor pusillus	CBS 143028	Novel foods	Novel Food	Production of fungal protein-fibre-rich biomass by fermentation of carbon sources	EFSA-Q-2020-00476	N	No (excluded)
Trichoderma harzianum	T78 - The strain T78 is a natural and wild type and it is not genetically modified. It is deposited as CECT 20714.	Plant protection products		Plant protection product	EFSA-Q-2020-00616	N	No (excluded)
Yeasts							
Komagataella pastoris		Food enzymes, food additives and flavourings		Production of UDP-glucosyltransferase and sucrose synthase	EFSA Q 2020-00520	Y	No (already QPS)
Saccharomyces cerevisiae	MUCL 39885	Feed additives		Biosprint® for piglets (weaned)	EFSA-Q-2020-00313 (FAD-2020-0025)	Y	No (already QPS)
Saccharomyces cerevisiae	CNCMI-3060	Feed additives	Nutritional additives	Organic form of Selenium produced by *Saccharomyces cerevisiae* CNCM I-3060.	EFSA-Q-2020-00495	Y	No (already QPS)
Saccharomyces cerevisiae	M17906	Food enzymes, food additives and flavourings		Production of the food enzyme maltogenic α-amylase	EFSA-Q-2020-00456	Y	No (already QPS)
Saccharomyces cerevisiae	BCCM/MUCL 39885	Feed additives	Zootechnical additives	Viable spores of *Saccharomyces cerevisiae* BCCM/MUCL 39885 used as gut microbiota stabilisers in all pigs (other than sows and weaned piglets) and all minor porcine species	EFSA-Q-2020-00600	Y	No (already QPS)

Species	Strain	EFSA risk assessment area	Category Regulated product	Intended usage	EFSA Question No[(a)] and EFSA webpage link[(b)]	Previous QPS status of the respective TU[(c)]	Assessed in this Statement? Yes or no
Saccharomyces cerevisiae	BCCM/MUCL 39885	Feed additives	Zootechnical additives	Viable spores of *Saccharomyces cerevisiae* BCCM/MUCL 39885 used as gut microbiota stabilisers in pets (cats and dogs) not used for food-producing animals	EFSA-Q-2020-00603	Y	No (already QPS)
Yarrowia lipolytica	A-101	Novel foods	Novel Food	Production of inactivated yeast biomass as novel food	EFSA-Q-2020-00491	Y	No (already QPS)