Appendix B to:

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 following protocol for extensive literature search (ELS) will be 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-2020-00079).

1. Description of the process

An ELS of studies related to safety concerns for humans, animals, plants and/or the environment of microorganisms recommended for the Qualified Presumption of Safety (QPS) 2020-2022 list will be performed.

The process will be performed according to the following main steps:

- ELS for potentially relevant citations;
- Relevance screening to select the citations identified by the literature search, based on titles and abstract and then full text;
- Evaluation of articles according to pre-specified categories of possible safety concerns;
- Discussion between experts to come to collective expert evaluation of the outcome, reflected in the QPS Opinion and Panel Statements.

Considering the purpose of the QPS approach, a broad search will be performed.

The review questions will be broken down into key elements using the PECO conceptual model:

- Population of interest (P)
- Exposure of interest (E)
- Comparator (C)
- Outcomes of interest (O).

1.1. Objective

The aim is to identify any publicly available studies reporting on safety concerns for humans, animals or the environment caused by microorganisms on the QPS recommended list (see Appendix E).

1.2. Target population

The populations of interest are humans, animals, plants and the environment.

1.3. Exposure

Citations must report on at least one species included in one of the six groups of named species specified in the EFSA QPS recommended list of the QPS 2020 update (see Table A.1 in Appendix A to (EFSA BIOHAZ Panel, 2020):

a) Gram-positive non-spore-forming bacteria;
b) Gram-positive spore-forming bacteria;
c) Gram-negative bacteria;
d) Yeasts
e) Protists/algae
f) Viruses used for plant protection;

In more detail:

a) Gram-positive non-spore forming bacteria:

Bifidobacterium adolescentis, Bifidobacterium animalis, Bifidobacterium bifidum, Bifidobacterium breve, Bifidobacterium longum, Carnobacterium divergens, Corynebacterium glutamicum, Brevibacterium lactofermentum, Corynebacterium ammoniagenes, Lactobacillus acidophilus, Lactobacillus amylolyticus, Lactobacillus amylovorus, Lactobacillus alimentarius, Lactobacillus animalis, Lactobacillus aviaries, Lactobacillus brevis, Lactobacillus buchneri, Lactobacillus casei, Lactobacillus zeae, Lactobacillus cellobiosus, Lactobacillus coryniformis, Lactobacillus crispatus, Lactobacillus curvatus, Lactobacillus delbrueckii, Lactobacillus farciminis, Lactobacillus fermentum, Lactobacillus gallinarum, Lactobacillus gasseri, Lactobacillus helveticus, Lactobacillus hilgardii, Lactobacillus johnsonii, Lactobacillus kefiranofaciens, Lactobacillus kefiri, Lactobacillus mucosae, Lactobacillus panis, Lactobacillus collinoides, Lactobacillus paracasei, Lactobacillus parafarraginis, Lactobacillus paraplantarum, Lactobacillus pentosus, Lactobacillus plantarum, Lactobacillus pontis, Lactobacillus reuteri, Lactobacillus rhamnosus, Lactobacillus sakei, Lactobacillus salivarius, Lactobacillus sanfranciscensis Lactococcus lactis, Leuconostoc citreum, Leuconostoc lactis, Leuconostoc mesenteroides, Leuconostoc pseudomesenteroides, Microbacterium imperiale, Oenococcus oeni, Pasteuria nishizawae, Pediococcus acidilactici, Pediococcus dextrinicus, Pediococcus parvulus, Pediococcus pentosaceus, Propionibacterium freudenreichii, Propionibacterium acidopropionici, Streptococcus thermophilus;

b) Gram-positive spore-forming bacteria:

Bacillus amyloliquefaciens, Bacillus atrophaeus, Bacillus clausii, Bacillus coagulans, Bacillus flexus, Bacillus fusiformis, Bacillus lentus, Bacillus licheniformis, Bacillus megaterium, Bacillus mojavensis, Bacillus pumilus, Bacillus smithii, Bacillus subtilis, Bacillus vallismortis, Bacillus velezensis, Geobacillus stearothermophilus, Paenibacillus illinoisensis, Parageobacillus thermoglucosidasius;

c) Gram-negative bacteria:

Cupriavidus necator, Gluconobacter oxydans; Komagataeibacter sucrofermentans, Xanthomonas campestris

d) Yeasts:

Candida cylindracea, Debaryomyces hansenii, Hanseniaspora uvarum, Kluyveromyces lactis, Kluyveromyces marxianus, Komagatella phaffii, Komagataella pastoris, Lindnera jadinii, Ogataea angusta, Saccharomyces bayanus, Saccharomyces cerevisiae, Saccharomyces pastorianus, Schizosaccharomyces pombe, Wickerhamomyces anomalus, Xanthophyllomyces dendrorhous, Yarrowia lipolytica, Zygosaccharomyces rouxii.

For the yeast species, as previously, the name of the teleomorphic form is used in the list of QPS species, when available. Important synonyms and older names were also included in the searches. For instance, names of the anamorphic growth forms were included, when such a form is known:

- *Debaryomyces hansenii*: anamorph *Candida famata*;
- *Hanseniaspora uvarum*: anamorph *Kloeckera apiculata*;
- *Kluyveromyces lactis*: anamorph *Candida spherica*;
- *Kluyveromyces marxianus*: anamorph *Candida kefyr*;
- *Komagataella pastoris*: synonym *Pichia pastoris*;

- *Lindnera jadinii*: synonyms *Pichia jadinii, Hansenula jadinii, Torulopsis utilis*, anamorph *Candida utilis*;
- *Ogataea angusta*: synonym *Pichia angusta*;
- *Saccharomyces cerevisiae*: synonym *Saccharomyces boulardii*;
- *Saccharomyces pastorianus*: synonym *Saccharomyces carlsbergensis*;
- *Wickerhamomyces anomalus*: synonyms *Hansenula anomala, Pichia anomala, Saccharomyces anomalus*, anamorph *Candida pelliculosa*;
- *Xanthophyllomyces dendrorhous*: anamorph *Phaffia rhodozyma*

  e) Protists/algae:

Euglena gracilis; Auranthiochytrium limacinum, synonym Schizochytrium limacinum; Tetraselmis chuii, synonym Carteria chuii

  f) Viruses used for plant protection:

Plant viruses (Family): *Alphaflexiviridae, Potyviridae*
Insect viruses (Family): *Baculoviridae*

1.4. Comparator

It is expected that the prevalent study designs will be case reports or case series and studies based on surveys or isolate collections. The remaining study designs may include: studies using laboratory isolates; randomised controlled trials, field trials or experimental designs in the laboratory; experimental designs in live animals with a deliberate disease challenge; observational study designs; animal or insect models; investigations to identify or to understand the causes of safety concerns (e.g. identification, characterisation of toxic factors, virulence mechanisms); studies to demonstrate beneficial effects but with reporting of unwanted side-effects.

Since it is expected that, in the majority of the study designs relevant for the review question, the comparator will not be available, the latter will not be included as a key element in the search strategy.

1.5. Outcomes of interest

The outcomes of interest to this ELS are:

Question 1:

- potential harms
- safety issues
- virulence or infectivity
- intoxication.

Question 2:

- (acquired/intrinsic) antimicrobial resistance (AMR) covering phenotypic and genotypic aspects.

The QPS concept does not address hazards linked to the formulation or processing of the products based on biological agents added into the food or feed chain. Neither the safety of users handling the product, nor the genetic modifications are taken into account.

1.6. Identification of the review questions

The following research questions will be addressed:

- Is there evidence of any safety concerns, including virulence features and toxin production, for humans, animals, plants and/or the environment associated with microbial species currently recommended for the QPS list since the previous QPS review?

- Is there evidence related to the presence or absence of antimicrobial resistance or antimicrobial resistance genes for the same microbial species published during the same time period?

2. Eligibility criteria for study selection

The selection of studies relevant to questions 1 and 2 will be performed applying the eligibility criteria described in Table 1 below.

Table 1: Eligibility criteria for questions 1 and 2

	Criteria
Study design	No specific type of study design will be used to include/exclude relevant studies, although it is expected that the prevalent study designs will be case reports or case series and studies based on surveys or isolate collections
Study characteristics:	No exclusion will be based on study characteristics
Population	Humans, animals, plants, environment
Exposure	Studies must report on at least one TU as identified in Section 1.3
Outcome of interest	Outcomes as listed in Section 1.5
Language	English
Time	From January to June 2020, and for protists/algae from January 2019 to June 2020
Publication type	Primary research studies and secondary studies reporting previously unpublished primary studies

3. Literature searches

Searches will be conducted in a range of relevant information sources to identify any evidence of safety concerns and AMR regarding the target microbial species.

Considering the results of the previous QPS exercise, to handle the high number of studies identified in each group, 22 search strategies were prepared: 3 for yeasts, 1 for insect viruses, 1 for plant viruses, 13 for Gram-positive bacteria and 4 for Gram-negative bacteria according to named species specified by EFSA in the QPS recommended list of the QPS 2020 update (see Table A.1 in Appendix A to EFSA BIOHAZ Panel, 2020).

The 22 subgroups of target microbial species will be searched separately.

Each search strategy will comprise two elements: the search terms (Section 3.1) and the information sources (Section 3.2) to be searched.

3.1. Search terms

The search strategies used to identify studies are described in Appendix C.

Each strategy will comprise two key elements:

- Target microbial species as described in Section 1.3 ('Exposure')
- Safety issues as described in Section 1.5 ('Outcomes').

In order to maximise the sensitivity of the search for the species for which the number of overall publications in the relevant time period is expected to be low, the search strategy will not include outcome-related terms.

The population of interest (humans, animals, plants or the environment) will not be included as a key element in the search strategies, as it is often not explicitly described within a title or abstract. It would also have been difficult to describe adequately such a broad population using title/abstract words and/or subject headings. Population information will be captured at the time of evaluating the articles (see Section 1 above).

Search terms for safety issues were identified in close collaboration with the information specialist; example of such terms, are the following: 'toxin*', 'disease*', 'infection*', 'clinical*', 'virulen*', 'antimicrobial resistan*', 'endocarditis'.

The 22 subgroups of target microbial species will be entered on separate search lines. The search line for each group will be combined with the safety terms individually.

The searches will not be limited by language or study design.

The review period will be from January to June 2020, and for protists/algae from January 2019 to June 2020

3.2. Information sources searched

The same information sources used for the previous QPS exercise (EFSA BIOHAZ Panel, 2020) will be searched for studies reporting safety concerns regarding the target microbial species (see Table 2 below).

Table 2: Information sources to be searched to identify relevant studies

Information source	Interface
Web of Science Core Collection	Web of Science, Thomson Reuters 2018
CAB Abstracts	Web of Science, Thomson Reuters 2018
BIOSIS Citation Index	Web of Science, Thomson Reuters 2018
MEDLINE	Web of Science, Thomson Reuters 2018
Food Science Technology Abstracts (FSTA)	Web of Science, Thomson Reuters 2018

Search results will be downloaded from the information sources and imported into EndNote® X9 bibliographic management software. For each of the 22 species groups, within-group removal of duplicate entries will be done in EndNote® X9. Following uploading of the species groups into the DistillerSR[1] online software, removal of duplicates will again be undertaken, using the Duplicate Detection feature.

4. Study selection and article evaluation

To identify potentially relevant studies to be included in the review the studies will be selected by a three -step procedure using the DistillerSR online software.

The results of the different phases of the study selection process will be reported in a flowchart.

4.1. Screening for potential relevance at title level

Articles will initially be screened at title level in parallel by two Working Group (WG) expert reviewers and, if needed, EFSA staff.

If the information in the title is not relevant for the research objectives, the article will not proceed to the next step (Section 4.2).

Articles that are excluded during screening at this step will be stored in Distiller SR.

In case of doubts or divergences between the reviewers, the paper will proceed to step 2.

4.2. Screening for potential relevance at title and abstract level

The articles passing the first step will undergo a screening at abstract level in parallel by two experts.

If the information in title and abstract is not relevant for the research objectives, the article will not proceed to the next step (Section 4.3).

Articles that are excluded during screening at this step will be stored in Distiller SR.

In case of doubts or divergences between the reviewers, the paper will proceed to step 3.

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[1] DistillerSR, Evidence Partners, Ottawa, Canada. https://www.evidencepartners.com/products/distillersr-systematic-review-software/

4.3. Article evaluation

The aim of this step will be to confirm that the article is relevant for the QPS project and, if it is, to evaluate it. This will be carried out at full text level.

The articles passing the second step will undergo a validation procedure carried out by two experts. One reviewer will initially be tasked with the evaluation of the paper. The evaluation will be then forwarded to another reviewer for the validation of the appraisal received.

In case of disagreement with the initial appraisal, the second reviewer will write down their comments. The reviewers will initially try to solve the disagreement. If this is not possible, the conflicting information will be presented for collective expert evaluation of the ELS outcome (see Section 5).

If the information contained in the article is not relevant for the research objectives, the article will not be evaluated. Articles that are not considered relevant will be stored in Distiller SR.

4.3.1. Questions for study selection and article evaluation

STEP 1 (Screening for potential relevance):

Question 1: Is the full-text available, in English and dealing with safety concerns?

Option	Result
1. Yes	Include and continue to Article evaluation form
2. Full text not available	Exclude
3. Full text not in English	Exclude
4. Full text in English but not dealing with safety concerns	Exclude
5. Full text in English but article not pertaining to this TU	Exclude
6. Full text in English, article not dealing with safety concerns but relevant for QPS assessment	Exclude

When option 6 is selected a dedicated free text box will appear to describe why the paper is relevant for QPS.

STEP 2 (Article evaluation):

Question 1: Identification of the microorganisms

- The article will be characterised in terms of the microorganisms involved
  Single choice question: the Experts will identify the microorganism/s described in the article. If more than one microorganism is described in the paper, the form will be repeated for each microorganism.

Question 2: Is there any "methodological" problem identified in the paper under consideration?

- No problems identified
- Yes some problems were identified.

Question 3: Which "methodological" problems were identified in the paper under consideration?
(this question will appear if, in question 2, the option "Yes some problems were identified" is selected)

- Methodology used for identity confirmation of the microorganism
- Reliability of the source attribution
- Misuse of the microorganism (e.g. parenteral exposure)
- Predisposing factors in the exposed subjects
- Others.

When one of the above options is selected a dedicated free text box will appear to describe the problem identified.

Question 4: Is there any safety concern identified?

- No safety concerns identified
- Yes some safety concerns were identified.

Question 5: Which safety concerns were identified? (this question will appear if, in question 5, the option "Yes some safety concerns were identified" is selected)

- On human health
- On animal health
- On the environment
- On AMR
- On other aspects.

When one of the above options is selected a dedicated free text box will appear to describe the safety concern identified.

Question 6: Overall, is there any information that could potentially lead to a change in the QPS status of the microorganism? (this question will appear if, in question 5, the option "Yes some safety concerns were identified" is selected)

- No
- Yes.

If the option "Yes" is selected, a dedicated free-text box will appear to describe the information that could potentially lead to a change in the QPS status of the microorganism.

Question 7: Does this paper have to be discussed with the WG? (this question will appear if, in question 5, the option "Yes some safety concerns were identified" is selected)

- No
- Yes.

5.   Collective expert evaluation of the ELS outcome and presentation in the QPS opinion

The overall results of the searches and evaluations of individual articles will be presented in tabular format for each group/subgroup and species. These results will be further evaluated collectively by the working group and the outcome will be reflected in the QPS opinion.

6.   Update of the process

The literature search, study selection and collective expert evaluation will be repeated every 6 months.

References

EFSA BIOHAZ Panel (EFSA Panel on Biological Hazards), Koutsoumanis K, Allende A, Alvarez-Ordóñez A, Bolton D, Bover-Cid S, Chemaly M, Davies R, De Cesare A, Hilbert F, Lindqvist R, Nauta M, Peixe L, Ru G, Simmons M, Skandamis P, Suffredini E, Cocconcelli PS, Fernández Escámez PS, Maradona MP, Querol A, Suarez JE, Sundh I, Vlak J, Barizzone F, Correia S and Herman L, 2020. Scientific Opinion on the update of the list of QPS-recommended biological agents intentionally added to food or feed as notified to EFSA (2017–2019). EFSA Journal 2020;18(2):5966, 56 pp. https://doi.org/10.2903/j.efsa.2020.5966.

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