EFFCA
European Food & Fermentation Cultures Association

Update February 2026

Safety Assessment and Characterisation of Food Cultures

1. Scope and Disclaimer

This document is meant as a set of recommended guidelines agreed upon by EFFCA members for the safety assessment and characterisation of food cultures (FC). However, a case-by-case assessment may remain relevant.[1]

2. FC Definition

Food cultures are safe live bacteria, yeasts, or filamentous fungi (moulds) used in food production, which are in themselves a food ingredient.

FC preparations are formulations, consisting of concentrates (> 10^8CFU/g or ml for bacteria and yeasts and > 10^7CFU/g for filamentous fungi) containing one or more live and active microbial strains of one or more microbial species, including unavoidable metabolites and media components carried over from the fermentation and components (e.g., carbohydrates, organic acids, minerals, vitamins) which are necessary for their survival, storage and to facilitate their application in the food.

FC includes, but is not limited to, the terms: starter cultures, dairy starter, yoghurt starters, ripening cultures, meat cultures, sausage starter, wine cultures, plant-based starters, malolactic cultures, sourdough starter, probiotics, lactic acid bacteria, etc. For further explanations, see EFFCA's paper on the definition of food cultures (2023).

3. Safety Assessment: References

The 2025 EFSA guidance on the characterisation of microorganisms in support of the risk assessment of products used in the food chain was considered as a basis to develop the criteria for the safety assessment and characterisation of FC. A decision tree for the suggested steps in the safety assessment is shown in Fig. 1 with the criteria described below. In addition, the safety of traditional multi-strain food cultures can also be supported by the history of safe use (EFSA 2005, Pariza et al., 2015).

4. Criteria

- **Species identification**

For **single strains**, species identification is carried out preferably by using whole genome sequencing (WGS, e.g., according to EFSA 2025) and/or other up-to-date methods. For **multi-**

[1] EFFCA's Industry Guidance for the Quality, Safety, Effectiveness, and Labelling of food cultures (preparations) will be published soon.

strain FC, components constituting more than 1% of the culture should be described at the species level.

When WGS is applied to multi-strain food cultures, the collected data should be used to unequivocally identify the individual strains (present at above 1%) at the species level. In case of filamentous fungi and yeasts, hybrid sequencing is suggested. Phylogenetic tree-based species identification, performed against a curated database, is also accepted (EFSA 2025).

- **Antimicrobial susceptibility**

For **bacterial strains** is performed and assessed according to EFSA 2025 and 2021 guidelines.

For **yeast and filamentous fungi**, susceptibility to at least two commonly used antifungal compounds of clinical relevance should be shown. Susceptibility testing must be performed using internationally standardized methods, such as those issued by the European Committee on Antimicrobial Susceptibility Testing (EUCAST), the Clinical and Laboratory Standards Institute (CLSI), the International Organization for Standardization (ISO), or other recognised standards.

For **multi-strain FC**, the species composition of the product should be taken into consideration in the methods and data interpretation. The composition of some multi-strain FC may challenge the interpretation of phenotypic susceptibility testing.

The minimum inhibitory concentration (MIC) values should be compared with established cut-off values or with MIC distribution data retrieved from an accepted and authoritative literature (e.g., peer-reviewed publications) or generated in-house.

For the interpretation of MIC values for yeasts and filamentous fungi (mg/L), we refer to Annex I based on EFFCA's own literature review, including Appendix D (Cut-off values (mg/L) for fungal species most commonly notified to EFSA) of EFSA 2025.

- **QPS status**

Included in the latest QPS list (EFSA QPS list). For strains belonging to a QPS species, qualifications mentioned in the latest QPS list must be fulfilled.

- **Antimicrobial production**

For strains not belonging to a QPS species, except those already known not to produce relevant antimicrobials. Tests should be made to assess the antimicrobial production potential, such as the inhibitory activity of culture against reference strains known to be susceptible to a range of antibiotics, and/or genomic screening for biosynthetic gene clusters involved in the production of antimicrobial compounds (see Annex II) by using up-to-date databases and/or analytical tests for the presence of antimicrobial compounds.

Annex II implies that only antibiotics (HIAs and CIAs) are in scope, which is covered by the WGS analysis by searching for sequences of concern. If an indication of antimicrobial production, analyses should be made to exclude the presence of antimicrobial compounds or demonstrate that their concentration is of no safety concern in the intended applications.

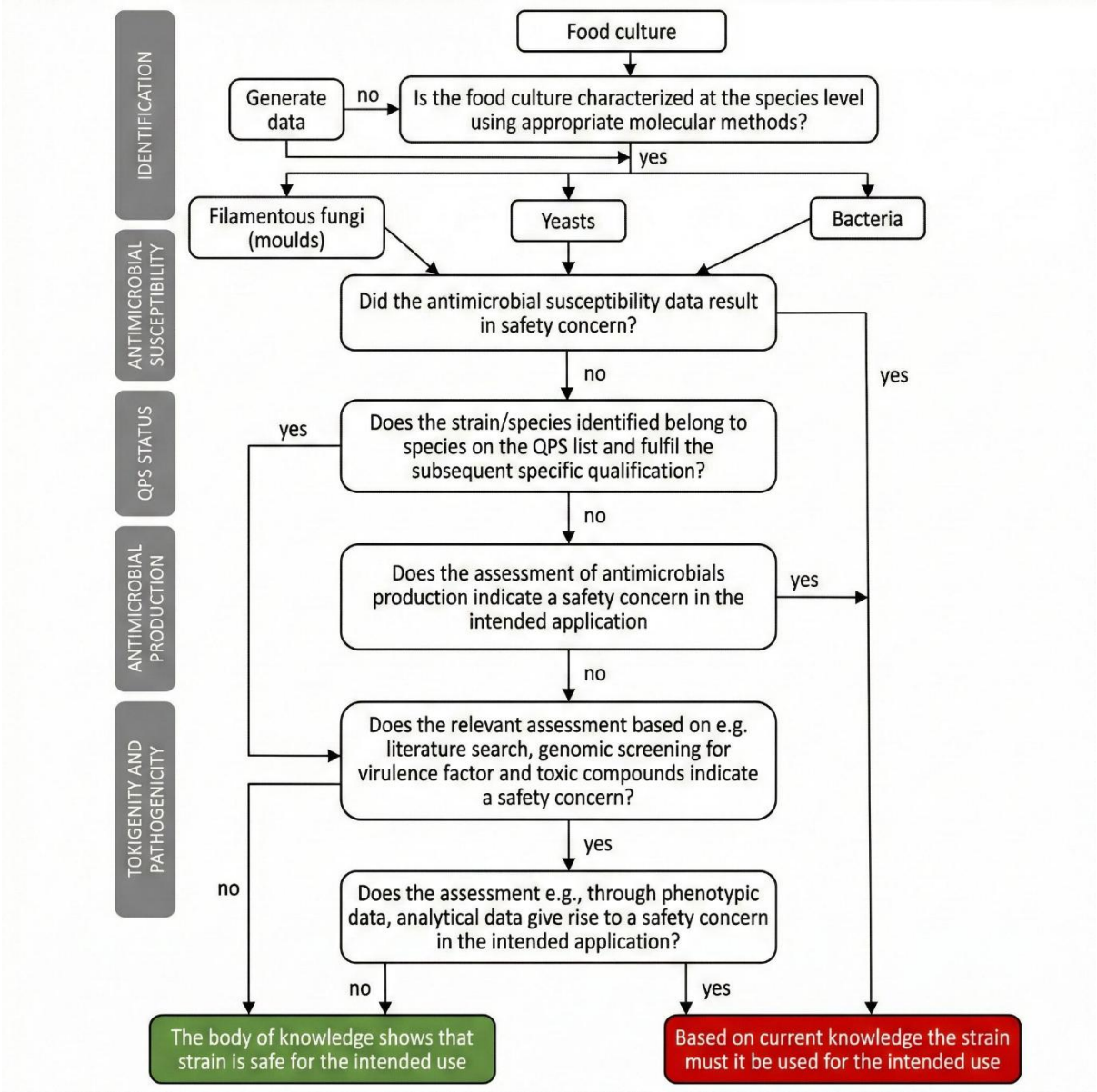
- **Toxigenicity and pathogenicity**

For strains belonging to a QPS species, qualifications mentioned in the latest QPS list must be fulfilled. For non-QPS species, up-to-date literature searches are done to collect information relating to toxigenicity and virulence for humans, animals, and the environment. This is achieved also by including the history of use in the intended application of the species, strain, or any close relative.

Targeted genomic similarity searches are performed for genes of concern identified in literature and for non-QPS bacterial species, also by interrogation of WGS against relevant databases (EFSA 2025). For bacterial strains belonging to *Enterococcus faecium* or *Bacillus* spp., other tests are required according to EFSA 2025. If there is an indication of toxic compound production, analyses should be made to exclude the presence of these compounds or demonstrate that their concentration are of no safety concern in the intended applications.[2]

[2] Disclaimer: each company remains responsible to determine the safety of a strain and its products.

Figure 1. Decision Tree on How to Assess the Safety of Strains Used in Food Cultures



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Annex I: AMR Assessment of Filamentous Fungi and Yeasts

The ECOFFs for filamentous fungi and yeasts were established based on a literature review and data from EFSA 2025. Inclusion criteria required the availability of data for at least 30 isolates and that MIC testing was conducted using EUCAST or CLSI methodologies. Depending on data availability, the reported ECOFF values were derived either from a bell-shaped MIC distribution or from MIC90 values.

	Antimicrobial compound (mg/L)										
Species	Amphotericin B	Caspofungin	Flucytosine	Fluconazole	Itraconazole	Micafungin	Nystatin	Voriconazole	Ketoconazole	Posaconazole	Isavuconazole
Pichia cactophila	0.5		32	32	0.5			0.5		0.125	
Clavispora lusitaniae	2		2	4	0.5			0.0625		0.125	
Candida orthopsilosis	2	0.25		1		0.5		0.12		0.12	
Meyerozyma guilliermondii		1	0.25	64						0.5	
Wickerharmonyces anomalus	0.5			4				0.25		0.5	
Debaryomyces hansenii					2			0.125			
Kluyveromyces marxianus	4	0.08	2	2	0.5	0.03		0.0625		0.25	
Saccharomyces cerevisiae	4		1	32	4		64	1	1	2	0.125
Candida krusei	4	0.5	64		2	0.125		2		2	2
Yarrowia lipolytica	1			4				0.125			
Geotrichum candidum				64				1		1	
Aspergillus flavus	8			IR	4		128	2		1	8

IR: Intrinsic Resistance

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Annex II: Antimicrobial Production – explanations

EFSA has not established its own definition of antimicrobials but is instead referring to the WHO definitions, as outlined below.

- **Antimicrobial**

An agent or substance, derived from any source (microorganisms, plants, animals, synthetic or semisynthetic) that acts against any type of microorganism: bacteria (antibacterial), mycobacteria (antimycobacterial), fungi (antifungal), parasite (antiparasitic), and viruses (antiviral). All antibiotics are antimicrobials, but not all antimicrobials are antibiotics. The scope of this report is limited to the antibacterial antimicrobials.

- **Antibiotic**

An agent or substance that is produced from microorganisms that can act against another living microorganism. Antimicrobial substances that are synthetic, semisynthetic, or those derived from plants or animals, are therefore, by strict definition, not considered antibiotics.

When considering "**antimicrobial production**", EFSA intends those compounds that could be produced by microorganisms, and that are used for treating human/animals diseases/infections. A WHO revision document includes tables identifying the compounds that fall within this category.

Therefore, when doing a safety evaluation of FC/bacteria, the idea would be to make sure that the strain is not able to produce any of the listed compounds. Only then would we be able to claim that the strain "does not produce antimicrobials".

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