EFFCA
European Food & Fermentation Cultures Association

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Industry Guidance for the quality, safety, and labelling of food cultures

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1. Introduction

Food cultures (FC) are safe live bacteria, yeasts, or filamentous fungi (moulds) used as ingredients in foods. Fermented foods are produced by the interaction and growth of FC with raw materials, leading to characteristic changes in the product itself; for example, FC will convert milk into yoghurt.

FC can also be used in foods without such a clear characteristic change in the product matrix. In these cases, fermentation still takes place but is less visible and may be restricted to the surface of the product; for example, FC are added to smoked salmon to inhibit the growth of *Listeria monocytogenes*.

This guidance is in line with the main principle in the General Food Law Regulation (178/2002, Art. 17(1)) that the responsibility for ensuring food safety lies with the Food Business Operator (FBO), unless it cannot be duly managed at that level.

2. Scope and objective

The scope and objective of the guidance is to document best practices of the global FC industry on:

- Safety assessment of FC in the intended application.
- Quality parameters for FC preparations, including viable cell counts and/or activity and purity.
- Good labelling practices in Business-to-Business (B2B) and Business-to-Consumer (B2C) commerce of FC preparations and fermented foods.
- Documentation to inform authorities in inspections or audits of FC manufacturers/suppliers.

3. Terms and definitions

The following terms and definitions apply throughout this industry best practice document.

3.1 Food cultures

Food cultures are safe live bacteria, yeasts, or filamentous fungi (moulds) used in food production as food ingredients (EFFCA, 2023). Terms to describe FC include, but are not limited to: starter cultures, dairy starters, yoghurt starters, ripening cultures, meat cultures, sausage starters, wine cultures, plant-based starters, malolactic cultures, sourdough starters, probiotics, lactic acid bacteria, etc. A food culture can consist of one or more species and several strains of the same species, as defined in ISO 27205. The culture is characterised at species level.

3.2 Food culture preparations

Formulations, consisting of concentrates (> 10^8 CFU/g or ml for bacteria and > 10^7 CFU/g or ml for yeast and filamentous fungi) of 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 matrices.

FC preparations are used as food ingredients at one or more stages in the food manufacturing process to develop their desired metabolic activity. They contribute to one or multiple unique properties of food, such as flavour, colour, texture, wholesomeness, health and nutritional benefits, as well as microbial quality and food safety through protection and conservation. FC preparations are identified by their commercial name.

3.3 Fermentation

Fermentation is the process in which microorganisms such as bacteria, fungi, yeasts, and micro-algae are used to preserve and/or transform raw materials into, e.g., food, feed, chemicals, pharmaceuticals, fuel, biomass (Joint Industry Definitions of Fermentation and Precision Fermentation, 2025).

3.4 Fermented foods

Fermented foods have undergone a process of fermentation resulting in: an increased number of live microbial cells, acidification, maturation, ripening, flavouring, and/or preservation through desired microbial growth and enzymatic conversions of food components.

4. Safety of FC preparations

The safety assessment of the FC preparations relies on assessing the safety of the microorganisms in the intended application and the production process, including raw materials. Such an assessment should be performed according to existing regulatory and scientific frameworks (e.g., Regulation (EC) No. 178/2002, Regulations (EC) No. 2073/2005, 852/2004 and 853/2004, GMP, ISO 27205/IDF149, Pariza et al., 2015).

In addition to the isolation, identification, and selection of safe and suitable microorganisms, the safety of FC preparations is ensured by manufacturing the microbial strains under Good Manufacturing Practice (GMP) and Hazard Analysis Critical Control Point (HACCP) – see chapter 4.3.

GMP and HACCP include but are not limited to: seed culture preparation, seed culture storage and documentation, appropriate scale-up from seed stocks to the final fermentation volume under controlled conditions, use of safe and suitable food-grade raw materials at all stages of

manufacturing, equipment design, and post-harvest handling procedures that are designed to prevent contamination of the FC preparation with substances, foreign particles or other microorganisms that may render it unsafe or unsuitable for the intended application.

4.1 Safety of microorganisms

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).

- **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-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**, antimicrobial susceptibility should be 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 standardised methods, such as those issued by the European Committee on Antimicrobial Susceptibility Testing (EUCAST), the Clinical and Laboratory Standards Institute (CLSI), the International Organisation for Standardisation (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**

For the confirmation of the QPS status, please refer to 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 that do not belong to a QPS species, with the exception of those already demonstrated not to produce relevant antimicrobials, assessments should be conducted to determine their potential for therapeutic antimicrobial production (EFSA, 2025). Such assessments may include testing the inhibitory activity of cultures against reference strains known to be susceptible to a broad range of antibiotics, genomic screening for biosynthetic gene clusters associated with antimicrobial compound production (see Annex I) using up to date databases, and/or analytical testing for the presence of antimicrobial compounds.

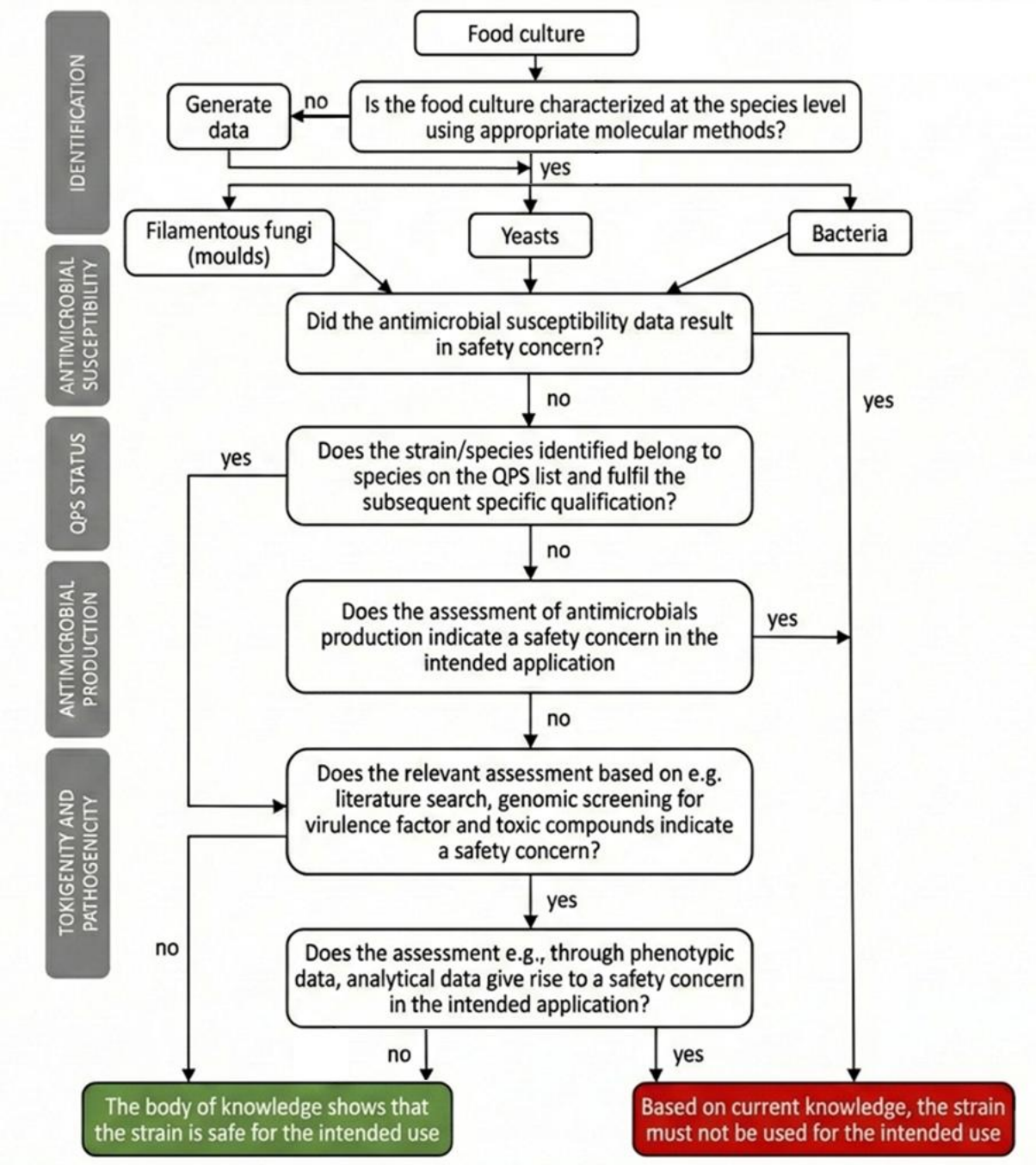
Only therapeutic antimicrobials are in scope, which is covered by the WGS analysis by searching for sequences of concern. If an indication of antimicrobial production is found, analyses should be made to exclude the presence of antimicrobial compounds or demonstrate that their concentration is of no 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 concentrations are of no safety concern in the intended applications.

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



4.2 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.

Species	***Antimicrobial compound (mg/L)***										
	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	
Wickerhamomyces 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

4.3 Production process

The manufacturing of FC (Fig. 2) consists of several consecutive steps in which the volume of fermentation broth is gradually increased from the first pre-culture fermentation to the main fermentation.

Fermentation nutrients supply the microorganisms with the necessary food-grade nutrients such as carbon, nitrogen, minerals, and vitamins. These raw materials are mostly consumed during fermentation, with any remaining amounts serving no purpose in the intended application. Post-fermentation substances, like cryoprotectants (e.g., sucrose), are used to protect cells during freezing and/or freeze-drying. Substances added post-fermentation to maintain viability and/or for processing purposes are classified as processing aids.

The culture concentrate may be packaged directly, frozen using liquid nitrogen, and/or dried using various technologies, including but not limited to freeze- and spray-drying.

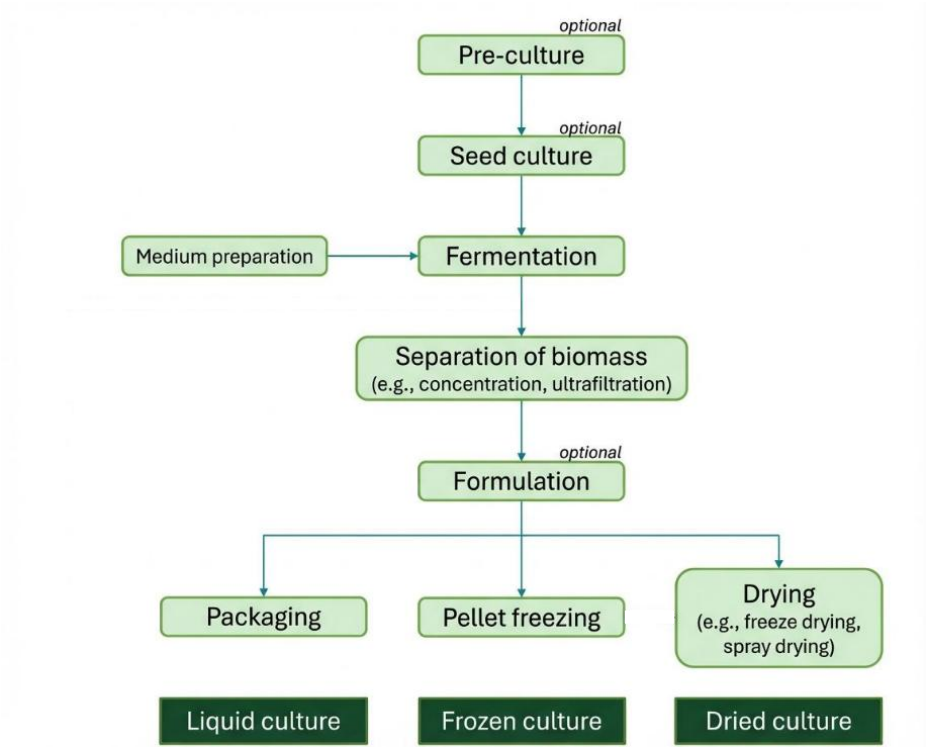


Figure 2. Production process of food culture preparations. Alternative technologies allow the manufacture of liquid, frozen, or dried cultures.

4.3.1. Quality and food safety management

To control the composition of FC preparations, the manufacturer shall put in place, implement, and maintain a quality management system, i.e., Good Manufacturing Practices (GMP, pre-requisite programs), based on Hazard Analysis Critical Control Point (HACCP) principles,

according to Regulations (EC) N°852/2004, 853/2004 and 178/2002 and/or GFSI recognised standards (e.g., FSSC 22000).

5. Composition and microbial purity of FC preparations

The following specifications are examples of current practices for FC preparations to be placed on the market for the duration of their shelf-life (ISO 27205). Recommended analytical methods are specified in Annex II.

5.1 Viable cell counts

The number of viable cells expressed as colony-forming unit (CFU) per gram, ml, or selling unit shall meet the minimum specification claimed by the FC manufacturer or supplier when using the manufacturer's methods for the duration of their shelf-life.

For certain applications, testing for acidification activity, texture, optical density, flow cytometry, or other alternative new technologies instead of viable cells may be more appropriate (ISO 26323:2009 | IDF 213:2009).

5.2 Microbial purity

Microbiological criteria of FC preparations **depend on the microorganism(s) included in the FC and the intended application.** The following tables propose microbial specifications in different applications. Local regulations should be consulted before marketing the product. Additional microbiological criteria or different specifications than those defined in the tables below may be applied, depending on the application of the FC preparations.

Table 1. Specifications for microbiological criteria for FC preparations used in dairy applications

Type of criteria	Contaminants[a]	Units	Liquid and frozen	Dry
Process hygiene	*Enterobacteriaceae*	CFU/g	< 1	< 10
	Coagulase-positive staphylococci[b, c]	CFU/g	< 1	< 10
	Yeasts and moulds (other than specified ones)	CFU/g	< 1	< 10
	Non-lactic acid bacteria[c]	CFU/g	< 5x10²	< 5x10²
Food safety	*Salmonella* spp.	Detected/not detected in 1 g	Not detected	Not detected
	Listeria monocytogenes	Detected/not detected in 1 g	Not detected	Not detected

[a] Contaminants can be tested in the process environment and in product samples. The setup of environmental samples compared to product samples shall be based on HACCP principles (ISO 22000) and justified against the specifications given here. Refer to Annex II for the list of recommended analytical methods.

[b] This contaminant is not valid for FC preparations containing *Staphylococcus* spp.

[c] This criterion is only relevant as a contaminant in cultures containing only lactic acid bacteria.

Table 2. Specifications for microbiological criteria for FC preparations used in meat applications

Type of criteria	Contaminants[a]	Units	Liquid and frozen	Dry
Process hygiene	*Enterobacteriaceae*	CFU/g or ml	< 10	< 10^2
	Coagulase-positive staphylococci[b, c]	CFU/g or ml	< 50	< 5x10^2
	Yeasts and moulds (other than specified ones)	CFU/g or ml	< 10^2	< 10^3
	Non-lactic acid bacteria[c]	CFU/g or ml	<10^2	<10^3
	Enterococcus	CFU/g or ml	<10^2	<10^3
	Anaerobic sulphite reducing bacteria	CFU/g or ml	<10	<10^2
Food safety	*Salmonella* spp.	Detected/not detected in 1 g or ml	Not detected	Not detected
	Listeria monocytogenes	Detected/not detected in 1 g or ml	Not detected	Not detected

[a] Contaminants can be tested in the process environment and in product samples. The setup of environmental samples compared to product samples shall be based on HACCP principles (ISO 22000) and justified against the specifications given here.

[b] This contaminant is not valid for FC preparations containing *Staphylococcus* spp.

[c] This criterion is only relevant as a contaminant in cultures containing only lactic acid bacteria.

Table 3. Specifications for microbiological criteria for FC preparations used in wine applications (based on the OIV monograph)

Type of criteria	Contaminants	Units	Yeasts	Bacteria	
			All types	Frozen or liquid	Dry
Process hygiene	Yeasts contaminant (other than specified ones)	CFU/g	< 5%	< 10^2	< 10^3
	Moulds	CFU/g	< 10^3	< 10^3	< 10^3
	Lactic acid bacteria	CFU/g	< 10^5	-	-
	Acetic acid bacteria	CFU/g	< 10^4	< 10^3	< 10^4
	Acetobacter + *Gluconobacter*	CFU/g	-	< 10^3	< 10^4
	Coagulase positive staphylococci	Detected/ not detected in 1g	-	Not detected	Not detected
	Coliforms	CFU/g	< 10^2	< 10^2	< 10^2

Food safety	*Salmonella* spp.	Detected/ not detected in 25g	Not detected	Not detected	Not detected
	E. coli	Detected/ not detected in 1g	Not detected	Not detected	Not detected

Table 4. Specifications for microbiological criteria for FC preparations used in baking applications

Type of criteria	Contaminant	Units			
			All types	**Liquid**	**Dry**
Process hygiene	Coliforms	CFU/g	$< 10^3$		
	Coagulase positive staphylococci (incl. *S. aureus*)	CFU/g	< 10		
Food safety	*L. monocytogenes*	CFU/g	$< 10^2$		
	Salmonella spp.	Detected/not detected in 25g	Not detected		
	E. coli	CFU/g		$< 10^2$	< 10

6. Product information on FC preparations

6.1 Business-to-Business (B2B) labelling of FC preparations, product documentation, or accompanying documents

B2B labelling shall bear appropriate information in clear and easily understandable terms enabling the purchaser to comply with their labelling obligations to consumers (ISO 27205, 2010; Regulation (EU) No 1169/2011). In B2B, products are sold with accompanying documents which may provide more detailed information on the product composition.

The following particulars are recommended to be on the product label or accompanying documents:

1. Commercial name of the FC preparation.
2. Species name(s) in accordance with international scientific nomenclature and local legislation.
3. Type of product (e.g., liquid, frozen or freeze-dried).
4. Net content indicated in one of the following units: g, ml, units, doses (in accordance with applicable law, if any).
5. Name and address of the manufacturer, distributor, importer, exporter, or vendor.
6. Code or lot identification.
7. Best before date (month and year).
8. Storage conditions.
9. Additional technical information, such as:

- Application areas of use;
- Instructions for use;

- Recommended dose level.

Specific labelling rules may exist on FC depending on their intended use. Local regulations may also apply and should always be consulted.

6.2 Business-to-Consumer (B2C) labelling of food culture preparations

When used as food ingredients, FC must be listed on the ingredients list of the final food in accordance with Article 17 of Regulation (EU) No 1169/2011. An exemption may apply following Article 19 or 20 of the same regulation. FC could be mentioned under a generally understood category name, such as “food cultures”.

7. Methods of analysis

Methods of analysis depend heavily on the FC species composition and the food application. Some examples of recommended analytical methods are given in Annex II.

In general, compendia methods recommended for cell counts and contaminants have not been validated for FC preparations, but for final food products. When appropriate, the use of such methods should be verified by the manufacturer of the relevant FC preparations. Other methods can be used when validated, and therefore Annex II shall be seen as indicative rather than mandatory.

8. References (including references for Annex II)

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Annex I: 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 to treat human or animal diseases or infections. A WHO revision document includes tables identifying the compounds that fall within this category.

Therefore, when carrying out 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".

Annex II: Recommended analytical methods

General
For dated references, only the edition cited applies. For undated references, the latest edition of the referenced document (including any amendments) applies.

Preparation of samples
The sample to be tested shall be homogeneous and truly representative of the batch of the product to be tested. For preparation of the sample, the rules specified in ISO 6887-1 and -2 are recommended. Diluents listed in ISO 6887-1 and ISO 6887-2 or other equivalent diluents can be used when preparing initial suspensions from samples and further dilutions.

Methods for cell counts
An internationally recognised enumeration method whose scope is applicable to FC incorporated in food products only exists today for lactic acid bacteria. In the absence of a method acknowledged by legislation, standardised or subject to a ring test for the enumeration of *Staphylococcus*, yeasts and moulds, FC manufacturers may use internal methods validated in-house to guarantee the compliance of the FC product with declared specifications.

General international methods for microbiological analysis

General

Standard	Purpose
ISO 6887-1	Microbiology of food and animal feeding stuffs - Preparation of test samples, initial suspension and decimal dilutions for microbiological examination - Part 1: General rules for the preparation of the initial suspension and decimal dilutions
ISO 16140-1	Microbiology of the food chain - Method validation - Part 1: Vocabulary
ISO 16140-2	Microbiology of the food chain - Method validation - Part 2: Protocol for the validation of alternative (proprietary) methods against a reference method
ISO 3534-1	Statistics - Vocabulary and symbols - Part 1: General statistical terms and terms used in probability

Enumeration

Standard	Microorganism	Purpose
ISO 15214	Lactic acid bacteria	Microbiology of food and animal feeding stuffs - Horizontal method for the enumeration of mesophilic lactic acid bacteria - Colony-count technique at 30 degrees C

OIV Resolution OIV-OENO 632-2021	Lactic acid bacteria	Enumeration of viable lactic acid bacteria on modified MRS (Man, Rogosa and Sharpe)
OIV Resolution OIV-OENO 632-2021	Acetic bacteria	Enumeration of acetic bacteria
ISO 6888-1	*Staphylococcus*	See ISO 6888-1:2000 For distinction between *Staphylococcus* and *Kocuria*, the use of agar containing furazolidone is recommended
ISO 6611 - IDF 94	Yeast and moulds	Enumeration of colony-forming units of yeasts and/or moulds - colony-count technique at 25 degrees C
ISO 21527-1:2008	Yeast and moulds	Microbiology of food and animal feeding stuffs — Horizontal method for the enumeration of yeasts and moulds Part 1: Colony count technique in products with water activity greater than 0,95
ISO 21527-2:2008	Yeast and moulds	Part 2: Colony count technique in products with water activity less than or equal to 0,95
OIV Resolution OIV-OENO 632-2021	Yeast	Enumeration of total yeasts on YM agar medium (Malt Wickerham) Enumeration of non-Saccharomyces yeasts on Lysine medium or on YPD medium with addition of cycloheximide
OIV Resolution OIV-OENO 632-2021	Mould	Enumeration of mould

Microbial contaminants

The listed methods might be adapted and evaluated on a company level to fulfil specific requirements and incorporate experience. Note that when using the following standards, FC may lower the pH to an extent that may inhibit the contaminants (target organisms) and therefore may need neutralisation. This is seen as part of the validation of methods for relevant products.

Contaminant	**Literature**
Enterobacteriaceae	ISO 21528-1 and ISO 21528-2
	NF V08-054
Coliforms	OIV Bacteriological Control COEI-2-CONBAC: 2009
	ISO 4832
Enterococcus	ISO 7899
	NMKL 68

	Slanetz and Bartley, 1957
	Vanderzant and Splittstoesser, 1992
	BVL L 06.00-32
Anaerobic sulphite-reducing bacteria	NMKL 56
	ISO 15213
	NF V08-061
	OIV Bacteriological ControlCOEI-2-CONBAC: 2009
Coagulase-positive staphylococci	NMKL 66
	NF V08-057-1
	ISO 6888-1
	ISO 6888-2 and ISO 6888-3
Other than specified yeasts and moulds	ISO 6611 - IDF 94
	ISO 21527-1 and ISO 21527-2
	NF V08-059
	NMKL 98
Acetobacter	OIV Bacteriological ControlCOEI-2-CONBAC: 2009
Gluconobacter	OIV Bacteriological ControlCOEI-2-CONBAC: 2009
Salmonella spp.	ISO 6579
	ISO 27205
	Amtliche Sammlung (BVL L 00.00-20)
	NMKL 71
	NF V06-052-1
	AOAC Official Method 2004.03
	OIV Bacteriological ControlCOEI-2-CONBAC: 2009
Listeria monocytogenes	ISO 11290-1
	ISO 27205
	Amtliche Sammlung (BVL L 00.00-32)
	NMKL 136
	NF V08-028-1
	NF V08-055
	AOAC Official Method 993.12
	AOAC Official Method 2004.06
Mesophilic microorganisms other than specified ones	ISO 4833-1 Horizontal method for the enumeration of microorganisms — Part 1: Colony count at 30°C by the pour plate technique
Non-lactic acid bacteria	ISO 13559
	OIV Resolution OIV-OENO 632-2021