Be a QPS or not to be, that is the Quirky Paradox of Safety

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The role of microorganisms in food production

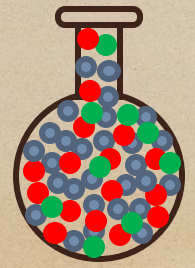
- Fermentation and Preservation
  - **Extended shelf life**: by producing compounds that inhibit the growth of spoilage and pathogenic microorganisms
  - **Safety**: the acidic environment created by fermentation makes it difficult for harmful pathogens to survive
- Aroma and Texture Enhancement
  - **Flavour profiles**: contribute to the development of complex flavors and aromas in products
  - **Texture modification**: exopolysaccharides production affecting the texture
- Production of Bioactive Compounds
  - **Vitamins and aminoacids**: enhancing the nutritional profile
  - **Antioxidants and antimicrobial compounds**: can contribute to overall health and disease prevention
- Nutritional Improvements
  - **Enhanced digestibility**: breaks down complex molecules, making nutrients more accessible and easier to digest
  - **Probiotics**: fermented products can contain live beneficial bacteria which can improve gut health
- Cultural and Economic Significance
  - **Culinary traditions**: fermented foods are integral to many cultural cuisines and traditional diets around the world, contributing to culinary diversity and heritage
  - **Economic value**: the fermentation industry is significant economically, with large-scale production of foods and contributing to the global food market
- Sustainability
  - **Reduction of food waste**: fermentation can help in utilizing surplus produce and reducing food waste

Biodiversity Loss and Natural Starters

The increased use of stringent hygiene practices in food manufacturing has led to a biodiversity loss in raw matrices and production environments. To counteract this, starter cultures, mainly LAB, are added to the raw material to facilitate and safely carry out the fermentation

Selected starters

- Made up of two or a few well known selected strains, associated in the laboratory
- Replace the native microbiota with simpler microbiota
- Reduce the microbial biodiversity in products and processing environments causing genetic erosion in the processing environments



Natural biodiverse starters

- Made up of different microbial species represented by an undefined number of strains (even uncharacterised), in natural balance
- Contribute to the conservation of microbial biodiversity and uniqueness of traditional and PDO products
- Strengthen the link between product and production area (autochthony)

Starters in PDO productions

According to the PDO (Protected Designation of Origin) regulation, for typical cheesemaking in Sardinia different methods can be used to ensure the authenticity of the product by preserving the traditional practices and specific characteristics of the production area

The PDO regulation allows a relative flexibility:

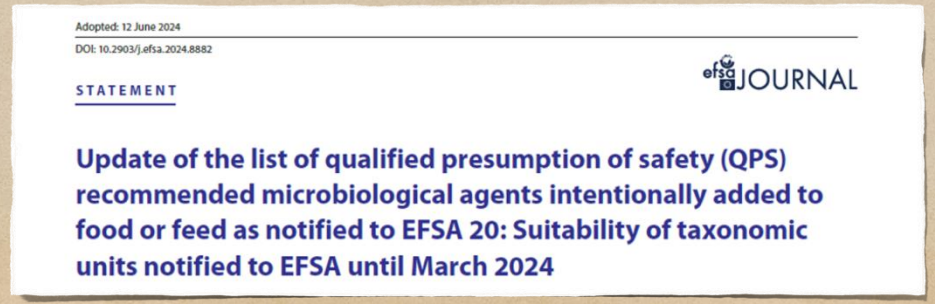
- Pecorino Sardo: use of thermised/pasteurised milk and, if needed, inoculated with selected starters isolated from the production area
- Pecorino Romano: use of thermised milk and a natural starter culture (*scotta-innesto*), possibly supplemented with selected starters isolated from the production area
- Fiore Sardo: use of only raw milk and, if needed, inoculated with natural starters like *scotta-innesto* or *latto-innesto*

QPS in the starters

The EFSA introduced, in 2007, the definition of Qualified Presumption of Safety (QPS) for microorganisms that can be intentionally added into the food and feed chain. The QPS list is revised, with an overall assessment carried out by the BIOHAZ Panel, every 3 years and updated every 6 months, and is based on these main points:

- Taxonomic identification
- Body of knowledge
- Safety

The taxonomic units (TUs) of microorganisms that are not well identified, and for which it is not possible to conclude whether they pose a safety concern, are not considered suitable for QPS status and some must undergo a full safety assessment



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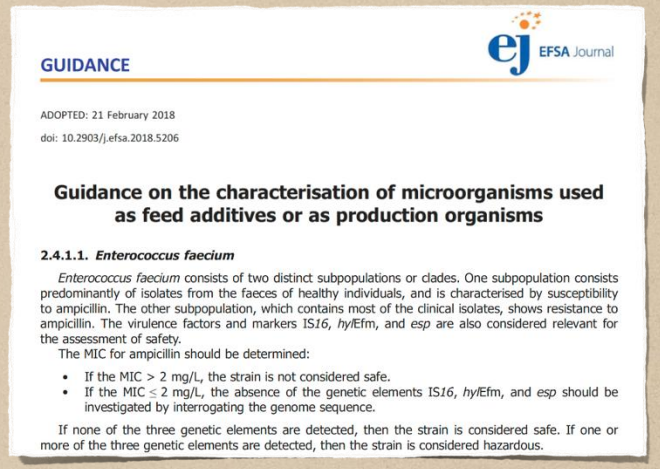
STATEMENT

efsa JOURNAL

Update of the list of qualified presumption of safety (QPS) recommended microbiological agents intentionally added to food or feed as notified to EFSA 20: Suitability of taxonomic units notified to EFSA until March 2024

Can non-QPS be food starters?

Among the LAB, *Enterococcus faecium* is currently excluded from the QPS list due to its ambiguous hospital (Clade A, isolated from clinical patients) or non-hospital (Clade B, from healthy individuals) origin, and prior to be used as food/feed additive must be tested for safety



EFSA Journal

GUIDANCE

ADOPTED: 21 February 2018

doi: 10.2903/j.efsa.2018.5206

Guidance on the characterisation of microorganisms used as feed additives or as production organisms

2.4.1.1. *Enterococcus faecium*

Enterococcus faecium consists of two distinct subpopulations or clades. One subpopulation consists predominantly of isolates from the faeces of healthy individuals, and is characterised by susceptibility to ampicillin. The other subpopulation, which contains most of the clinical isolates, shows resistance to ampicillin. The virulence factors and markers IS*16*, *hyl*Efm, and *esp* are also considered relevant for the assessment of safety.

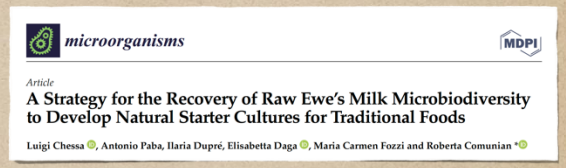
The MIC for ampicillin should be determined:

- If the MIC > 2 mg/L, the strain is not considered safe.
- If the MIC ≤ 2 mg/L, the absence of the genetic elements IS*16*, *hyl*Efm, and *esp* should be investigated by interrogating the genome sequence.

If none of the three genetic elements are detected, then the strain is considered safe. If one or more of the three genetic elements are detected, then the strain is considered hazardous.

A natural starter biodiverse culture (23 strains) obtained from raw ewe milk, without heat treatment or selection of pro-technological microorganisms, included strains belonging to non-QPS species. Following the current EFSA guidelines, the natural starter culture should not be used for food production

- *Enterococcus faecium*
- *Enterococcus faecalis*
- *Enterococcus durans*
- *Lacticaseibacillus paracasei*
- *Streptococcus gallolyticus* subsp. *macedonicus*
- *Streptococcus equinus*
- *Streptococcus lutetiensis*
- *Streptococcus oralis*
- *Streptococcus salivarius*



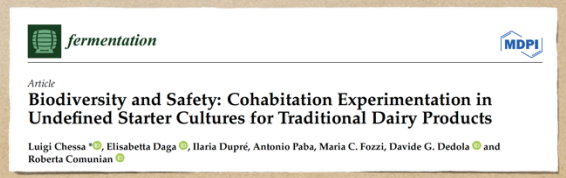
microorganisms

MDPI

Article

A Strategy for the Recovery of Raw Ewe's Milk Microbiodiversity to Develop Natural Starter Cultures for Traditional Foods

Luigi Chessa, Antonio Paba, Ilaria Dupré, Elisabetta Daga, Maria Carmen Fozzi and Roberta Comunian *



fermentation

MDPI

Article

Biodiversity and Safety: Cohabitation Experimentation in Undefined Starter Cultures for Traditional Dairy Products

Luigi Chessa *, Elisabetta Daga, Ilaria Dupré, Antonio Paba, Maria C. Fozzi, Davide G. Dedola and Roberta Comunian

Can non-QPS be food starters?

All the *Enterococcus* and *Streptococcus* strains found in the natural starter culture revealed antibiotic sensitivity below the breakpoints indicated by EFSA, EUCAST, and CLSI, and absence of the genetic elements IS*16*, *hylEfm*, and *esp*

Bacteria Tested	Culture	Antibiotics Tested								
		Penicillins	Macrolides	Amphenicols	Oxazolidinones	Tetracyclines	Glycycyclines	Glycopeptides		Fluoroquinolones
		AMP	ERY	CHL	LZD	TET	TGC	VAN	TEI	CIP
E. faecium	NSC	S [1]	n.t.	n.t.	n.t.	n.t.	n.t.	n.t.	n.t.	n.t.
	LNSC	S [1]	n.t.	n.t.	n.t.	n.t.	n.t.	n.t.	n.t.	n.t.
E. faecalis	NSC	S [1]	I [2]	S [2]	S [1]	R [2]	S [1]	S [1]	S [1]	S [1]
	LNSC	S [1]	I [2]	S [2]	S [1]	R [2]	S [1]	S [1]	S [1]	S [1]
E. durans	NSC	abs.	abs.	abs.	abs.	abs.	abs.	abs.	abs.	abs.
	LNSC	S [1]	S [2]	S [2]	S [1]	S [2]	S [1]	S [1]	S [1]	S [1]

NSC, natural starter culture; LNSC, Lyophilized natural starter culture (LNSC). AMP, Ampicillin; PEN, Penicillin; AZI, Azithromycin; ERY, Erythromycin; FEP, Cefepime; FOT, Cefotaxime; AXO, Ceftriaxone; CHL, Chloramphenicol; CLI, Clindamycin; ETP, Ertapenem; MERO, Meropenem; LEVO, Levofloxacin; LZD, Linezolid; TET, Tetracycline; TGC, Tigecycline; VAN, Vancomycin; TEI, Teicoplanin; SYN, Quinupristin/Dalfopristin; CIP, Ciprofloxacin; S, sensitive; R, resistant; I, intermediate; n.t., not tested; abs., the species was absent in the culture. [1] Breakpoint by EUCAST 2023. [2] Breakpoint by CLSI 2020.

Bacteria Tested	Culture	Antibiotics Tested															
		Penicillins		Macrolides		Cephalosporins			Amphenicols	Lincosamides	Carbapenems		Fluoroquinolones	Oxazolidinones	Tetracyclines	Glycopeptides	Streptogramins
		AMP	PEN	AZI	ERY	FEP	FOT	AXO	CHL	CLI	EPT	MERO	LEVO	LZD	TET	VAN	SYN
S. gallolyticus macedonicus	NSC	n.t.	S [2] *	S [2]	S [2]	S [2]	S [2]	S [2]	S [2]	S [2]	S [2]	S [2]	S [2]	S [2]	S [2]	S [2]	n.t.
	LNSC	n.t.	S [2]	S [2]	S [2]	S [2]	S [2]	S [2]	S [2]	S [2]	S [2]	S [2]	S [2]	S [2]	S [2]	S [2]	n.t.
S. equinus	NSC	n.t.	S [2]	S [2]	S [2]	S [2]	S [2]	S [2]	S [2]	S [2]	S [2]	S [2]	S [2]	S [2]	S [2]	S [2]	n.t.
	LNSC	abs.	abs.	abs.	abs.	abs.	abs.	abs.	abs.	abs.	abs.	abs.	abs.	abs.	abs.	abs.	abs.
S. lutetiensis	NSC	n.t.	S [2]	S [2]	S [2]	S [2]	S [2]	S [2]	S [2]	S [2]	S [2]	S [2]	S [2]	S [2]	S [2]	S [2]	n.t.
	LNSC	n.t.	S [2]	S [2]	S [2]	S [2]	S [2]	S [2]	S [2]	S [2]	S [2]	S [2]	S [2]	S [2]	S [2]	S [2]	n.t.
S. oralis	NSC	S [1]	S [2]	R [2]	R [2]	S [2]	S [2]	S [2]	S [2]	S [2]	S [2]	S [2]	S [2]	S [2]	S [2]	S [2]	I [2]
	LNSC	n.t.	S [2]	R [2]	R [2]	S [2]	S [2]	S [2]	S [2]	S [2]	S [2]	S [2]	S [2]	S [2]	S [2]	S [2]	n.t.
S. salivarius	NSC	S [1]	I [2]	R [2]	R [2]	S [2]	S [2]	S [2]	S [2]	S [2]	S [2]	S [2]	S [2]	S [2]	S [2]	S [2]	S [2]
	LNSC	abs.	abs.	abs.	abs.	abs.	abs.	abs.	abs.	abs.	abs.	abs.	abs.	abs.	abs.	abs.	abs.

NSC, natural starter culture; LNSC, Lyophilized natural starter culture (LNSC). AMP, Ampicillin; PEN, Penicillin; AZI, Azithromycin; ERY, Erythromycin; FEP, Cefepime; FOT, Cefotaxime; AXO, Ceftriaxone; CHL, Chloramphenicol; CLI, Clindamycin; ETP, Ertapenem; MERO, Meropenem; LEVO, Levofloxacin; LZD, Linezolid; TET, Tetracycline; TGC, Tigecycline; VAN, Vancomycin; TEI, Teicoplanin; SYN, Quinupristin/Dalfopristin; CIP, Ciprofloxacin; S, sensitive; R, resistant; I, intermediate; n.t., not tested; abs., the species was absent in the culture. * one isolate was intermediate. [1] Breakpoint by EUCAST 2023. [2] Breakpoint by CLSI 2020.

... and food starters can be non-QPS?

Also other LAB can be used as starters, or can be found, naturally, in the raw matrices, production environments, natural starter, and maybe in products

BIOHAZ statement on QPS: suitability of taxonomic units notified until September 2022

efsa JOURNAL

Streptococcus oralis

Identity

Streptococcus oralis is a bacterial species with Standing in Nomenclature (Bridge and Sneath, 1982). Based on whole genome sequence analysis three subspecies are recognised: *S. oralis* subsp. *dentisani*, *S. oralis* subsp. *tigurinus* and *S. oralis* subsp. *oralis* (Jensen et al., 2016; Oren and Garrity, 2017).

Body of knowledge

S. oralis is part of the normal microbiota of the oropharyngeal, nasal, gastrointestinal and genitourinary tracts and has a 'probiotic' effect providing protection against invading pathogens in the oral cavity (reviewed by Okahashi et al., 2022a; Bidossi et al., 2018). Strains of this species are used as oral 'probiotics', but strains of the same species are responsible for human infections.

Safety concerns

S. oralis may cause various diseases such as meningitis, endocarditis and bloodstream infections in which streptococcal surface proteins and other virulence factors might be involved (Basaranoglu et al., 2019; Cruz Cardoso et al., 2021; Nakamura et al., 2021; Okahashi et al., 2022b).

Conclusion on a recommendation for QPS status

S. oralis is not recommended for QPS status due to safety concerns.

The Paradox of non-QPS in natural Consortia

In traditional and PDO productions the challenge faced by artisanal manufacturers is to use their own natural starter cultures to obtain a food with peculiar sensory characteristics and more related to the territory of production (autochthony), also preserving traditional technological methods and microbial diversity

The Paradox:

The presence of non-QPS microbial species in natural starter cultures obtained from raw matrices, which are inherently part of the traditional production processes, creates a paradox. Artisanal manufacturers may not be allowed to produce or use their own natural starter cultures, as they likely include non-QPS species, but they must comply with the regulations

An answer to this paradox is needed to allow the use of these natural microbial consortia in food production while ensuring food safety

Addressing the Paradox

The use of microorganisms in food production is a critical aspect of ensuring food safety and quality. The QPS list provides a valuable framework for evaluating the safety of microorganisms intentionally added to food or feed

For some of the species not recommended for QPS status, additional testing is required to ensure the absence of antibiotic resistance genes and virulence factors. While this is a prudent measure to safeguard public health, the lack of specific guidelines for other LAB highlights the need for more comprehensive and flexible regulations

The paradox emerges when the current guidelines prevent the use of these natural starters, as they do not comply with the QPS list requirements. Artisanal manufacturers, who rely on these traditional production methods, may no longer be able to use their own natural starter cultures, jeopardizing the integrity and diversity (also microbial) of their products

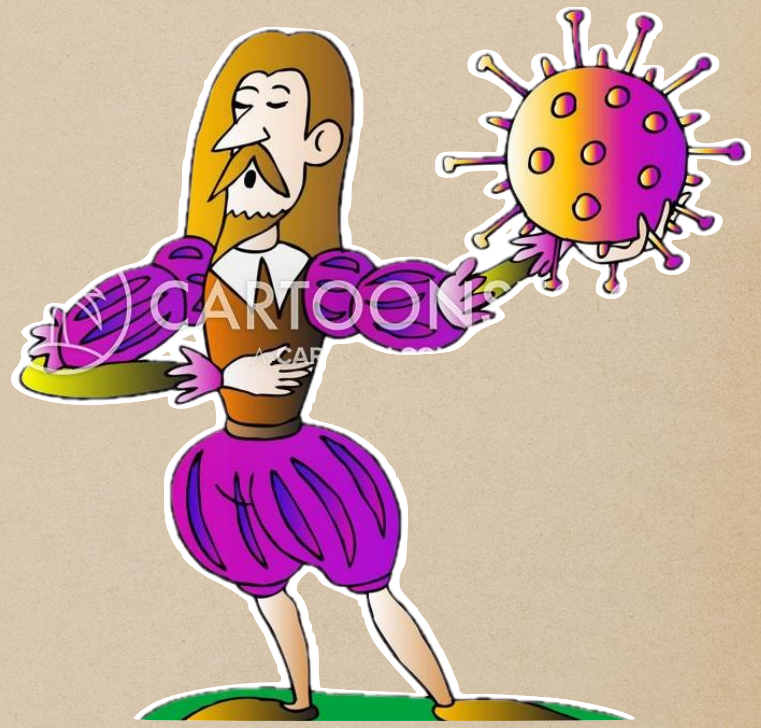
Which guidelines should be followed? The PDO regulation mandates the use of traditional techniques (i.e. natural cultures), while EFSA imposes restrictions for food safety reasons. This conflict highlights the challenge of balancing heritage with modern safety standards

Addressing the Paradox

In conclusion, while the QPS framework serves an important purpose in safeguarding food safety, it should evolve to accommodate the realities of traditional food productions and the inherent value of microbial diversity

By embracing the complexities of natural fermentation processes and the microbial communities involved, a revision of the criteria for the QPS status should be considered in order to find a way for a more inclusive and sustainable food system that honors both safety and tradition

Thanks for your attention



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