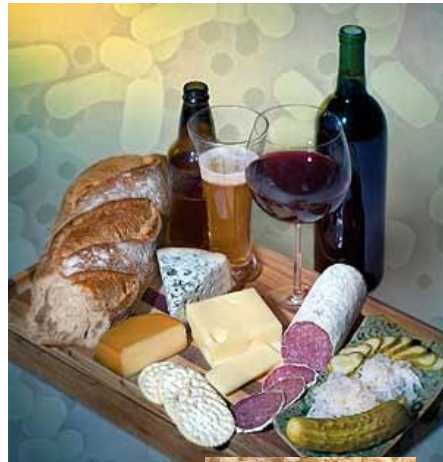


Microorganismos inocuos en la producción de alimentos: usos y costumbres con base científica

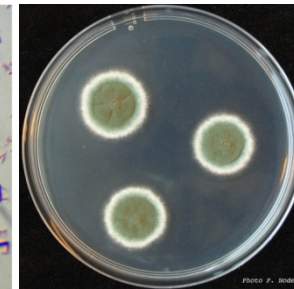
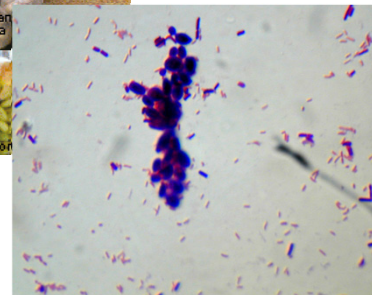
Dra. Lucrecia Delfederico

Laboratorio de Microbiología Molecular, DCyT, UNQ

Agentes biológicos en cadena alimenticia



Células viables
Enzimas, metabolitos
Biomasa no viable
Productos de protección vegetal



QPS (Qualified presumption of safety)

- 2007: la EFSA (*European Food Safety Authority*) adopta las pautas para la Calificación Presuntiva de Seguridad (QPS)



Herramienta genérica y armonizada para establecer la seguridad de los microorganismos introducidos deliberadamente en alimentos

- Evaluación de seguridad de los agentes biológicos propuestos
- Productos que requieren autorización para ser comercializados (nuevos alimentos, aditivos, GMO, etc)
- Comité de científicos

Comité de científicos

- Enfoque general
- Elaboración de la primera lista de agentes biológicos propuestos para el status QPS (EFSA, 2007; Leuschner et al., 2010)
- Hasta 2013 se actualizaba anualmente, desde entonces cada tres años
- El Panel de Riesgo Biológico (BIOHAZ) revisa y actualiza el listado (última actualización: Nov/2016)

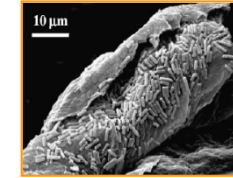
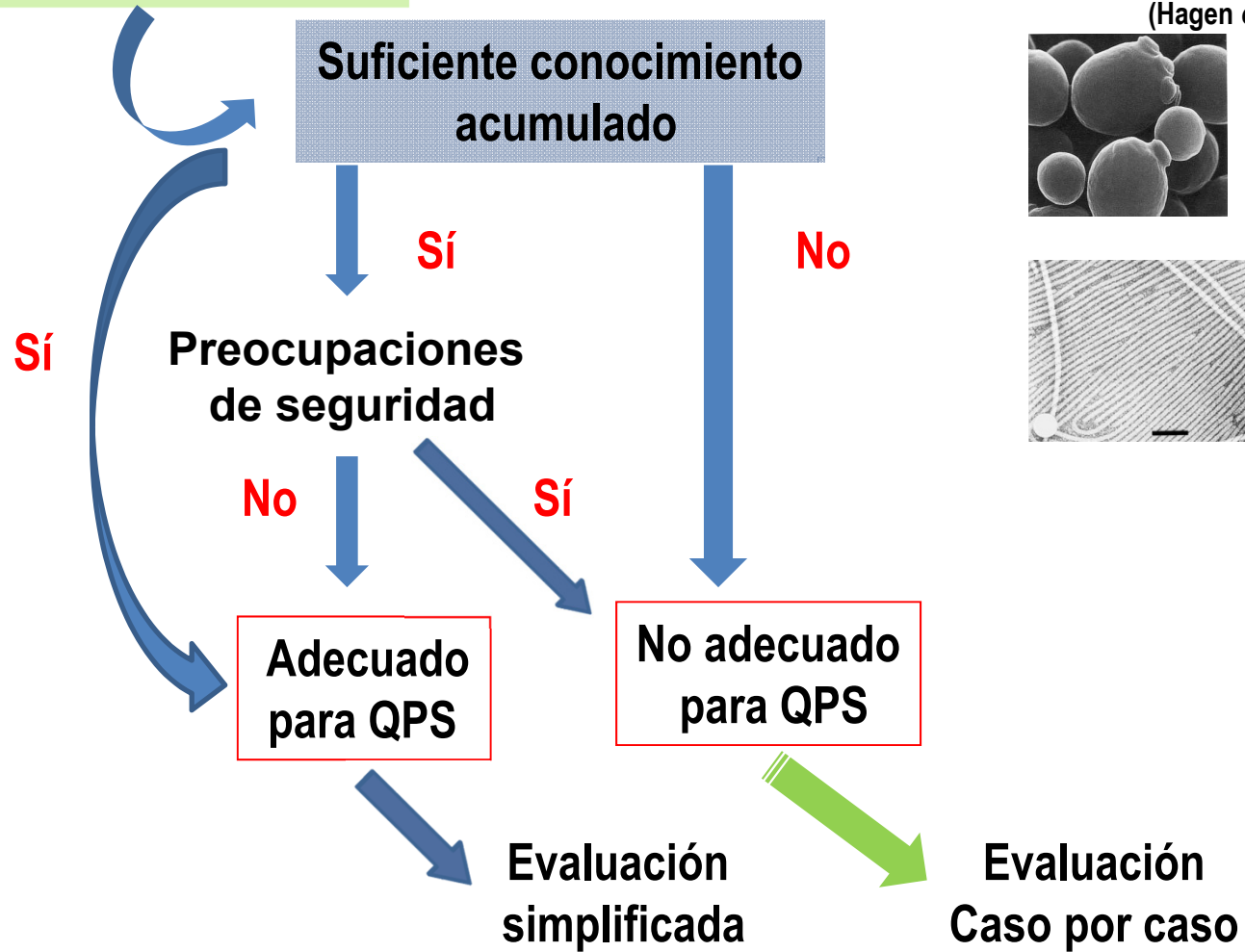
www.efsa.europa.eu/en/topics/topic/qualified-presumption-safety-qps

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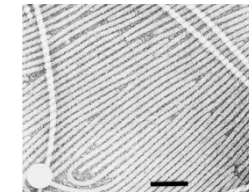
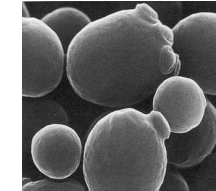
- La definición de la **Unidad Taxonómica** (identificación)
- *Body of knowledge* o conocimiento acumulado
- Posibles preocupaciones de seguridad (patogenicidad, resistencia a antibióticos, etc)
- Uso final previsto



Definición de la Unidad Taxonómica
Bacterias y levaduras: **especie y cepa**
Virus: familia



(Hagen et al., 2005)



Definición de la Unidad Taxonómica

Bacterias y levaduras: **especie y cepa**

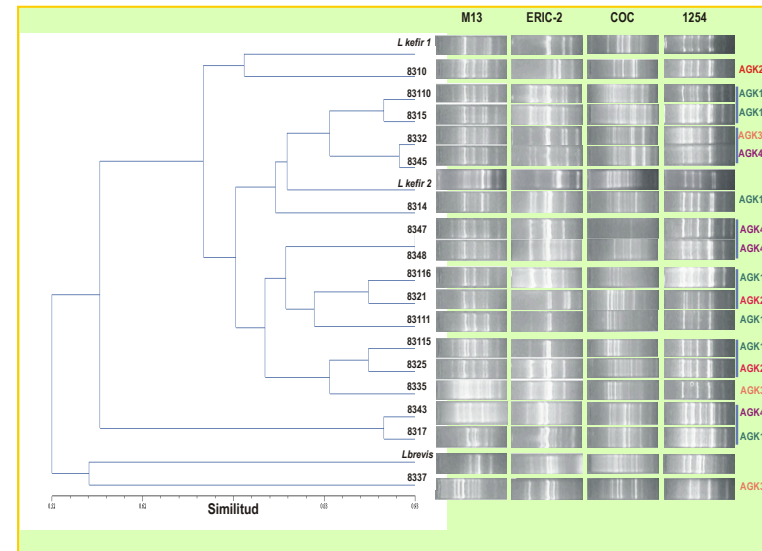
Virus: familia

Métodos basados en Reacción en Cadena de la Polimerasa (PCR)

Amplificación y secuencia de los genes del 16S RNA o el 26S RNA

RAPD

PFGE (*Pulse Field Gel Electrophoresis*)



Definición de la Unidad Taxonómica

Bacterias y levaduras: **especie y cepa**

Virus: familia





Nomenclatura utilizada en tipificación molecular

Aislamiento microbiano *monocultivo de una colonia primaria creciendo en un medio sólido*

Cepa: *aislamiento o grupo de aislamientos que muestran características sean fenotípicas, genéticas o ambas, que las separan de otros aislamientos pertenecientes a la misma especie.*

Clon o grupo clonal de aislamientos o cepas: *comprende organismos que descienden de un ancestro común debido a una cadena directa de replicación. La identificación de clones debe basarse en un análisis exhaustivo de varios marcadores moleculares de suficiente poder discriminatorio. Un clon genético comprende un grupo de cepas que son completamente idénticas a nivel de DNA*

Table 1: Differences between the GRAS guidelines (FDA, USA) and the QPS system (EFSA, EU) (amended from Wassenaar and Klein, 2008)

GRAS	QPS
 Applies to food additives including microorganisms Performed after a specific GRAS notification to the FDA	Applies to microorganisms only Performed for microorganisms used as a source of/contained in products assessed for the EU market authorisation
 Determination of a GRAS status by the FDA and/or external experts Open to all types of food additives	Determination of a QPS status by EFSA Restricted only to the microorganisms related to regulated food and feed products
GRAS	QPS
Applicants request a GRAS status	EFSA requests evaluation of new taxonomic units within the scope of an internal mandate
 Based on history of use, body of knowledge and the absence of adverse effects at the strain level Describes specific substance or microorganism at the strain level Case-by-case safety assessment at the strain level Based on specific Guidance ^(a)	Based on history of use, body of knowledge and the absence of adverse effects at the TU level Describes taxonomic unit (usually species level for bacteria and yeasts, families for viruses, not at strain level) General safety assessment at the TU level Support to the safety assessment required in the Founding EU Regulation ^(b)
 Open tool to all applicants	Internal tool only under the frame of dossiers for authorisation of regulated products by EFSA

FDA: US Food and drug Administration; GRAS: Generally Recognised as Safe; QPS: Qualified Presumption of Safety; TU: taxonomic unit.

(a): <http://www.fda.gov/Food/IngredientsPackagingLabeling/GRAS/>

(b): Regulation (EC) No 178/2002 of the European Parliament and of the Council of 28 January 2002 laying down the general principles and requirements of food law, establishing the European Food Safety Authority and laying down procedures in matters of food safety. OJ L 31, 1.2.2002, p. 1–24.

Table 2: Table summarising the areas of assessment for the QPS approach and for each EFSA food and feed safety risk assessment area when microorganisms are considered

QPS assessment remit	Feed area assessment remit ^(a)	Food Ingredients area assessment remit ^(b)	Pesticides area assessment remit ^(c)	Nutrition area assessment remit ^(d)
1. Identity: taxonomy identification parameters	Verification of species and strain Certificate of deposit in a culture collection	Verification of species and strain Certificate of deposit in a culture collection	Verification of species and strain Certificate of deposit in a culture collection	Verification of species and strain Certificate of deposit in a culture collection <i>(for Health Claims and Novel Foods (NF))</i>
2.1. Body of Knowledge: history of safe use in the food and feed chain	Other authorisations and uses Description of the genetic modifications Confirm genetic stability	Other authorisations of the same strain lineage Description of the genetic modifications Confirm genetic stability	Proposed uses Historical background Organisms genetically modified should comply to the GMO Regulation Confirm genetic stability	History of safe use <i>(for NF)</i>
2.2 Body of Knowledge: general ecology/ distribution in ecosystems	Origin to be declared Production of antimicrobial compounds	 Production of antimicrobial compounds	Origin (geographical and place in the ecosystem) and natural occurrence (if possible at strain level) Ability to colonise available niches Production of antimicrobial compounds	Out of the scope of the specific Regulation
3.1. Safety concerns: virulence/pathogenicity/toxicity for humans	Pathogenicity potential and virulence factors Production of toxins and toxic secondary metabolites	Pathogenicity potential and virulence factors Production of toxins and toxic secondary metabolites	Pathogenicity potential and virulence factors Possible toxicity of secondary metabolites	Pathogenicity potential and virulence factors <i>(for NF)</i>
3.2. Safety concerns: virulence/pathogenicity/toxicity for animals (domestic and wild)	Pathogenicity potential and virulence factors Production of toxins and toxic secondary metabolites	Out of the scope of the specific Regulation	Adverse effects of organism or metabolites on non-target animals (in the environment)	Out of the scope of the specific Regulation

QPS assessment remit	Feed area assessment remit^(a)	Food Ingredients area assessment remit^(b)	Pesticides area assessment remit^(c)	Nutrition area assessment remit^(d)
3.3. Safety concerns: virulence/pathogenicity for plants	Out of the scope of the specific Regulation	Out of the scope of the specific Regulation	Adverse effects of organism or metabolites on non-target vascular plants and algae (in the environment)	Out of the scope of the specific Regulation
3.4. Safety concerns: antimicrobial resistance	Verification of the absence of antimicrobial resistance associated with acquired genes	Verification the of absence of antimicrobial resistance associated with acquired genes	Verification of the absence of antimicrobial resistance and of possible transfer of genes coding for resistance	Verification of the absence of antimicrobial resistance associated with acquired genes <i>(for NF)</i>
3.5. Safety concerns: environmental safety	Genetically modified microorganisms (GMMs): deliberate release GMMs: the absence of viable cells GMMs: the absence of recombinant DNA Effect on water and soil	Not direct applicable within Food Enzymes legislation: Microorganisms used for production purposes, including GMMs: the absence of viable cells GMMs: the absence of recombinant DNA	Risk for non-target organisms (which are not vertebrate animals or plants), arising from exposure to the microorganism and its secondary metabolites remaining in or on plants or plant products, in soil water and air	Out of the scope of the specific Regulation
4.1. End use: intended exposure to viable cells of animals and consumers	Tolerance studies in target animals Toxicological studies <i>in vitro</i> and in laboratory animals	Not direct applicable within Food Enzymes legislation: Microorganisms used for production purposes, including GMMs: the absence of viable cells GMMs: the absence of recombinant DNA	End use is intended exposure of a target organism, i.e. organisms that are: 1) pathogenic to or damage/consume plants or plant commodities; or 2) unwanted plants (weeds). Efficacy investigations are required for these purposes	Intended exposure of viable cells to consumers <i>(for NF)</i>
4.2. End use: enzymes/metabolites producer: no or limited exposure to viable cells	Microorganisms used for production purposes, including GMMs: absence of viable cells GMMs: absence of recombinant DNA Tolerance studies in target animals Toxicological studies <i>in vitro</i> and in laboratory animals	Microorganisms used for production purposes, including GMMs: absence of viable cells GMMs: absence of recombinant DNA	When there are no viable cells in a plant protection product then it is regulated as a chemical (not a microorganism) even if it was produced by microbial fermentation	<i>(for NF)</i>

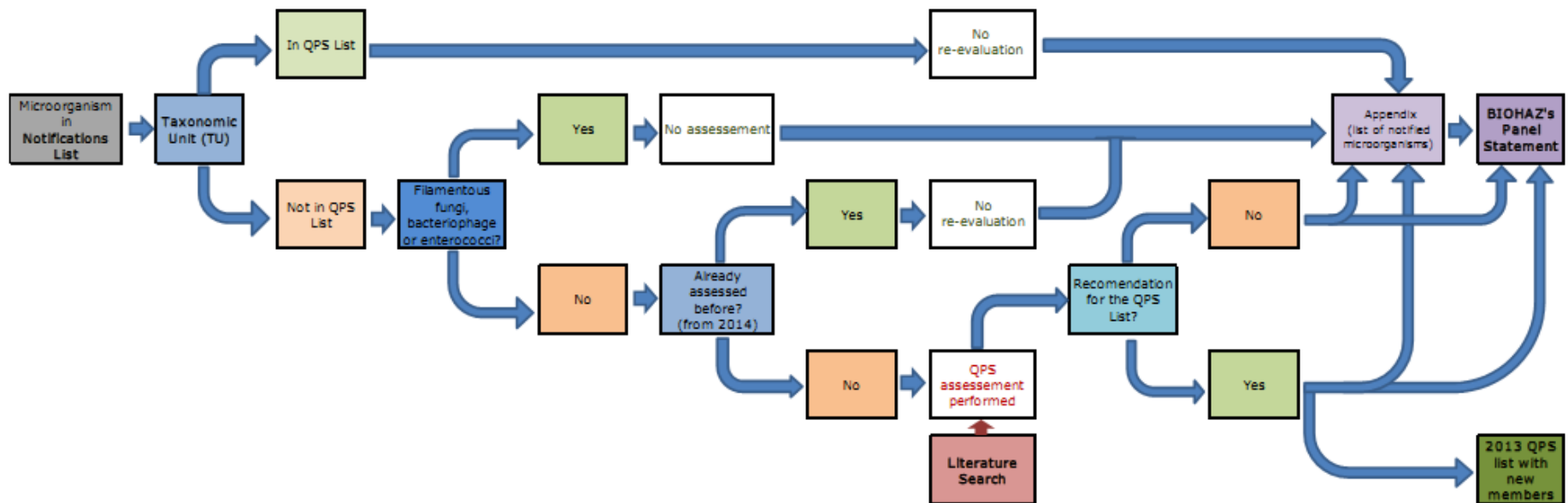
GMM: genetically modified microorganisms; NF: novel food.

(a): Based on the specific Feed Regulation (EC) No 1831/2003 and Regulation (EC) No 429/2008.

(b): Based on the specific FIP/GMMs Regulation: absence of recombinant DNA (under Reg. 1829/2003).

(c): Based on the specific Pesticides Regulation (EC) No 1107/2009 of the European Parliament and of the Council of 21 October 2009 concerning the placing of plant protection products on the market and repealing Council Directives 79/117/EEC and 91/414/EEC.

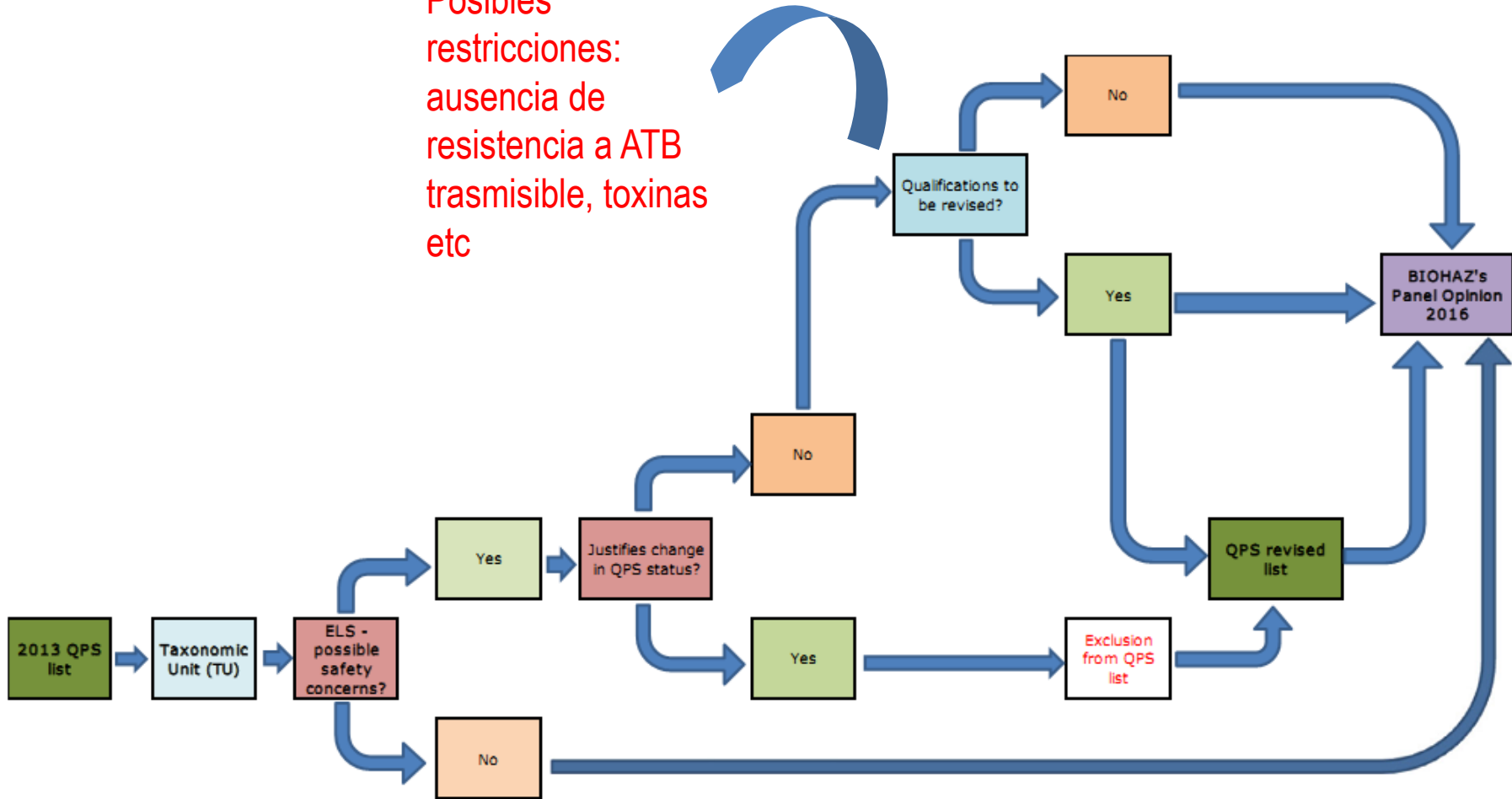
(d): Based on the specific Health Claims Regulation (EU) No 1924/2006 and Novel foods Regulation (EU) 2015/2283.



BIOHAZ: Biological Hazards Panel; QPS: Qualified Presumption of Safety.

Figure 2: Workflow diagram describing how QPS status is assessed for the TU related to the microorganism notified to the EFSA Units under the frame of applications for market authorisation – elaboration of the BIOHAZ QPS Panel Statements

Posibles
restricciones:
ausencia de
resistencia a ATB
transmisible, toxinas
etc



BIOHAZ: Biological Hazards Panel; QPS: Qualified Presumption of Safety.

Qué hay en la lista de QPS?

Los microorganismos se distribuyen en grupos:

Bacterias Gram positivas no esporulantes

Especies de *Bacillus*

Bacterias Gram negativas

Levaduras

Virus utilizados para la protección de plantas

Virus de insectos

La lista actualizada y el texto completo con las consideraciones esta disponible en:

<https://www.efsa.europa.eu/en/efsajournal/pub/4664>

Species			Qualifications
<i>Bifidobacterium adolescentis</i> <i>Bifidobacterium animalis</i>	<i>Bifidobacterium bifidum</i> <i>Bifidobacterium breve</i>	<i>Bifidobacterium longum</i>	
<i>Carnobacterium divergens</i> ^(b)			
<i>Corynebacterium glutamicum</i> ^(c)			QPS only applies when the species is used for amino acid production.
<i>Lactobacillus acidophilus</i> <i>Lactobacillus amylolyticus</i> <i>Lactobacillus amylovorus</i> <i>Lactobacillus alimentarius</i> <i>Lactobacillus aviaries</i> <i>Lactobacillus brevis</i> <i>Lactobacillus buchneri</i> <i>Lactobacillus casei</i> ^(d) <i>Lactobacillus cellobiosus</i> <i>Lactobacillus collinoides</i> <i>Lactobacillus coryniformis</i> <i>Lactobacillus crispatus</i> <i>Lactobacillus curvatus</i>	<i>Lactobacillus delbrueckii</i> <i>Lactobacillus diolivorans</i> ^(e) <i>Lactobacillus farciminis</i> <i>Lactobacillus fermentum</i> <i>Lactobacillus gallinarum</i> <i>Lactobacillus gasserii</i> <i>Lactobacillus helveticus</i> <i>Lactobacillus hilgardii</i> <i>Lactobacillus johnsonii</i> <i>Lactobacillus kefiranofaciens</i> <i>Lactobacillus kefirii</i> <i>Lactobacillus mucosae</i>	<i>Lactobacillus panis</i> <i>Lactobacillus paracasei</i> <i>Lactobacillus paraplantarum</i> <i>Lactobacillus pentosus</i> <i>Lactobacillus plantarum</i> <i>Lactobacillus pontis</i> <i>Lactobacillus reuteri</i> <i>Lactobacillus rhamnosus</i> <i>Lactobacillus sakei</i> <i>Lactobacillus salivarius</i> <i>Lactobacillus sanfranciscensis</i>	
<i>Lactococcus lactis</i>			
<i>Leuconostoc citreum</i> <i>Leuconostoc lactis</i>	<i>Leuconostoc mesenteroides</i>	<i>Leuconostoc pseudomesenteroides</i>	
<i>Microbacterium imperiale</i> ^(b)			QPS only applies when the species is used for enzyme production.
<i>Oenococcus oeni</i>			
<i>Pasteuria nishizawae</i> ^(f)			QPS only applies when used in pesticides to combat cyst nematodiasis.
<i>Pediococcus acidilactici</i> <i>Pediococcus dextrinicus</i>	<i>Pediococcus parvulus</i> ^(e)	<i>Pediococcus pentosaceus</i>	
<i>Propionibacterium freudenreichii</i>	<i>Propionibacterium acidipropionici</i>		
<i>Streptococcus thermophilus</i>			

Gram-positive spore-forming bacteria

Bacillus			Qualifications^(a)
Species			
<i>Bacillus amyloliquefaciens</i>	<i>Bacillus fusiformis</i>	<i>Bacillus mojavensis</i>	Absence of toxigenic activity.
<i>Bacillus atrophaeus</i>	<i>Paenibacillus lentus</i> ^(g)	<i>Bacillus pumilus</i>	
<i>Bacillus clausii</i>	<i>Bacillus licheniformis</i>	<i>Bacillus smithii</i> ^(h)	
<i>Bacillus coagulans</i>	<i>Bacillus megaterium</i>	<i>Bacillus subtilis</i>	
<i>Bacillus flexus</i> ^(e)		<i>Bacillus vallismortis</i>	
<i>Geobacillus stearothermophilus</i>			

Gram-negative bacteria

Species			Qualifications^(a)
<i>Gluconobacter oxydans</i>			
<i>Xanthomonas campestris</i> ⁽ⁱ⁾			QPS only applies when the species is used for the production of xanthan gum.

Viruses

Plant viruses

Family

Alphaflexiviridae	Potyviridae		
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Insect viruses

Family

Baculoviridae

Yeasts ^(j)			
Species			Qualifications
<i>Candida cylindracea</i> ^(b)			QPS only applies when the species is used for enzyme production and no viable cells are found.
<i>Debaryomyces hansenii</i>			
<i>Hanseniaspora uvarum</i>			
<i>Kluyveromyces lactis</i>	<i>Kluyveromyces marxianus</i>		
<i>Komagataella pastoris</i> <i>Lindnera jadinii</i> <i>Ogataea angusta</i>			QPS only applies when the species is used for enzyme production and no viable cells are found.
<i>Saccharomyces bayanus</i>	<i>Saccharomyces cerevisiae</i> ^(k)	<i>Saccharomyces pastorianus</i>	Absence of resistance to antimycotics used for medical treatment of yeast infections in cases where viable cells are added to the food or feed chain. In the case of <i>Saccharomyces cerevisiae</i> this qualification applies for yeast strains able to grow above 37°C.
<i>Schizosaccharomyces pombe</i>			
<i>Wickerhamomyces anomalus</i>			QPS only applies when the species is used for enzyme production and no viable cells are found. Absence of resistance to antimycotics used for medical treatment of yeast infections in cases where viable cells are added to the food or feed chain.
<i>Xanthophyllomyces dendrorhous</i>			

En 2009 se discutió la potencial aplicación de QPS para productos de protección vegetal. Se evaluaron virus por primera vez y se incluyeron en los listados QPS a los Baculovirus (virus de insectos) y a la familia *Potyviriidae* (virus del mosaico amarillo de zucchinis).

Los bacteriofagos no se consideraron apropiados para ser incluidos en la lista ya que se concluyó que deberían efectuarse evaluaciones caso por caso de la secuencia de ácidos nucleicos para establecer la imposibilidad de ciclo lisogénico y la ausencia de cualquier factor de virulencia potencial o de resistencia a antimicrobianos, así como el riesgo de transducción de genes bacterianos.

Se revisó la posible presencia de resistencia a antimicóticos de uso en humanos en cepas de levadura con carácter QPS.

Los hongos filamentosos se mantienen fuera del status QPS por la posible producción de metabolitos tóxicos (conocimiento acumulado insuficiente) y los inconvenientes para la determinación de la unidad taxonómica.

Obstáculos para la implementación del esquema de la EFSA

- There are no approved standards for phenotype and genotype evaluation of antibiotic resistance in food isolates.
- There is no optimal growth medium capable of growing the majority of *Lactobacillus* species.
- There are no approved standard MICs at which an organism is considered resistant or susceptible to an antibiotic, except for *Enterococcus* species.
- When the genetic basis for resistance is unknown, whether resistance is intrinsic or transferable is unable to be ascertained for many antibiotics.

Pasos para establecer el riesgo de la resistencia a antibióticos en una cepa

- identify the resistance gene;
- attempt to transfer resistance to normal gastrointestinal flora;
- characterize the biochemical mechanism of resistance;
- elucidate the genetic basis for resistance.