INTEREST OF QPS (QUALIFIED PRESUMPTION OF SAFETY) FOR PROBIOTIC DEVELOPMENTS

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Summary

- Introduction
- Feed additive EU regulation
- QPS
- How to assess strain safety?
  - Strain listed
  - Strain not listed
- Conclusion

Live microorganisms

- Food to produce
  - Fermented products as cheese, yoghourt, wine, beer, etc
  - Use as starter to produce cheese, novel food
- Feed to produce
  - Confined use: enzymes, vitamins, amino acids
  - Not confined, directly in feed: probiotics, silage, detoxifying agents
  - Conventional and GMM organisms

Safety concern

Microorganisms - not to produce

- Endo-exo toxin
- Antibiotics

Microorganisms – not generate

- Antibioresistance

Only concern microorganisms intentionally used

→ Regulation feed and food (novel food)

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Feed additive EU regulation

- Regulation (EC) No 1831/2003 of the EU Parliament (22 Sept. 2003) on additives for use in animal nutrition

- Regulation (EC) No 429/2008 of the Commission (25 April 2008) detailed rules for the implementation of Regulation (EC) No 1831/2003

Feed additive EU regulation

Regulation (EC) No 429/2008

- Detailed rules
  - For preparation and presentation of application
  - For assessment and the authorisation of feed additives
- Concerns different types of product
  - Chemically defined substances
  - Plant extracts
  - Dead microorganisms
  - **Live microorganisms, confined and not confined as probiotics, silage agents, detoxifying agents….**

Feed additive EU regulation

Feed additive application dossier content

- Section I: summaries of the dossier
- Section II: Identity, characterisation and conditions of use of the additive – Methods of analysis
- **Section III: Studies concerning the safety of the additive**
  - **Microbial studies**
- Section IV: Studies concerning the efficacy of the additive
- Section V: Post-monitoring plan

Feed additive EU regulation

Microbial requirements (CR No 429/2008)

[Confined and not confined live microorganisms]

- Name and taxonomic classification (latest international codex of nomenclature)
- Deposit in internationally recognised culture collection
- Culture collection provides
  - Certificate of deposition
  - Accession number under which the strain is hold
  - Morphology, physiology
- Molecular characteristics (for identification of the strain)
- History of modification
- Accession number is included in the Regulation autorisation
- Genotoxicity, mutagenicity studies
- Tolerance studies

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Safety consideration of microorganisms (QPS)

- Live microorganisms intentionally introduced into the feed chain
- Scientific Committee (EFSA 2007) set up the concept of Qualified Presumption of Safety (QPS) [not so far than GRAS (Generally Recognised As Safe) concept]
  - Assessment tool for safety
  - Generic concept to prioritize and harmonize
  - Assessment on case-by-case basis (always required)
- First list of microorganisms established in 2007
- Be reviewed annually by BIOHAZ
  - 2008 antimicrobial resistance introduced
  - 2009-2010-2011: qualification regarding absence of antimycotic resistance for yeast introduced

BIOHAZ: EFSA panel on biological hazards

QPS: Qualified Presumption of Safety

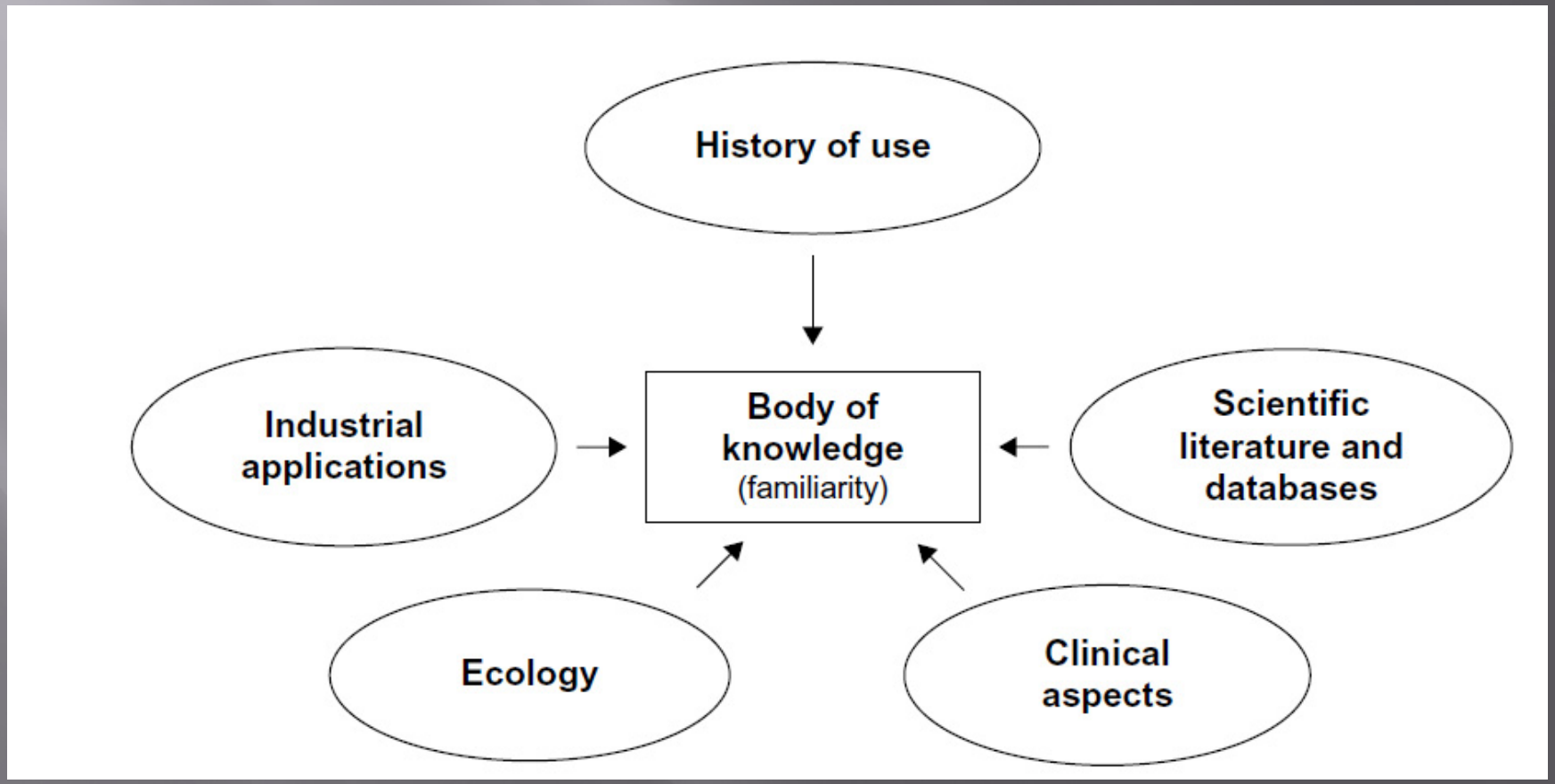
- Safety pre-assessment of defined taxonomic group (eg. generic)
- QPS based on 4 pillars
  - Taxonomy (establishing identity)
  - Familiarity (body of knowledge)
  - Possible pathogenicity (safety, antibioresistance)
  - Other qualifications
- **QPS would avoid to provide genotox, mutagenicity, tolerance studies**

QPS first pillar: taxonomy

- Species, sub-species
- Other grouping such as homofermentative, heterofermentative should be considered
- Bacteria (international code of nomenclature or international journal of systematic ISSEM)
- Filamentous fungi and yeast (ICBM – IMA)
- Viruses (ICTV)

QPS 2nd pillar: familiarity

- Concerns a defined taxonomy unit
- Assessment to conclude its safety



QPS 3rd pillar: possible pathogenicity

- Assessment of antimicrobial resistance
- Strain should not harbour any acquired any antimicrobial resistance to chemically relevant antibiotics
- Strain carrying acquired resistance should not be intentionally introduced into feed and food
- Not produce antibiotics relevant in animals and humans

QPS 4th pillar: Other qualifications

- *Bacillus*: some rare strain among *bacillus* have caused food-born intoxication
  - Technical specific guidance [EFSA Journal 2014;12(5):3665]
- *Enterococcus faecium*: assessment of this microorganism has been made at strain level, absence of putative virulence and acquired antibiotic resistance should be demonstrated [guidance, EFSA Journal 2012;10(5):2682]

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How to assess the strain safety?

- QPS: last published version [EFSA Journal 2013;11(11):3449]
- Check if the strain is listed
  - **If so**
  - If not

QPS listed microorganisms

QPS list

[EFSA Journal 2013;11(11):3449]

1. Check the strain in the list

Gram-Positive Non-Sporulating Bacteria			
Species			**Qualifications ***
Bifidobacterium adolescentis *Bifidobacterium animalis*	*Bifidobacterium bifidum* *Bifidobacterium breve*	*Bifidobacterium longum*	
*Corynebacterium glutamicum***			QPS only apply when the species is used for amino acid production
Lactobacillus acidophilus *Lactobacillus amylolyticus* *Lactobacillus amylovorus* *Lactobacillus alimentarius* *Lactobacillus aviaries* *Lactobacillus brevis* *Lactobacillus buchneri* *Lactobacillus casei* *** *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 paraplantarum* *Lactobacillus pentosus* *Lactobacillus plantarum* *Lactobacillus pontis* *Lactobacillus reuteri* *Lactobacillus rhamnosus* *Lactobacillus sakei* *Lactobacillus salivarius* *Lactobacillus sanfranciscensis*	
Lactococcus lactis			
Leuconostoc citreum *Leuconostoc pseudomesenteroides*	*Leuconostoc lactis*	*Leuconostoc mesenteroides*	
Oenococcus oeni			
Pediococcus acidilactici	*Pediococcus dextrinicus*	*Pediococcus pentosaceus*	
Propionibacterium freudenreichii	*Propionibacterium acidipropionici*		
Streptococcus thermophilus			
Bacillus			
Species			**Qualifications***
Bacillus amyloliquefaciens *Bacillus atrophaeus* *Bacillus clausii* *Bacillus coagulans*	*Bacillus fusiformis* *Bacillus lentus* *Bacillus licheniformis* *Bacillus megaterium*	*Bacillus mojavensis* *Bacillus pumilus* *Bacillus subtilis* *Bacillus vallismortis*	Absence of toxigenic activity.
Geobacillus stearothermophilus			Absence of toxigenic activity.
Gram-Negative Bacteria			
Species			
Gluconobacter oxydans			QPS only apply when the species is used for vitamin production

QPS listed microorganisms

Cont'd

Yeasts††			
Species			**Qualifications**
Debaryomyces hansenii			
Hanseniaspora uvarum			
Kluyveromyces lactis	*Kluyveromyces marxianus*		
Komagataella pastoris *Lindnera jadinii* *Ogataea angusta*			QPS only apply when the species is used for enzyme production
*Saccharomyces bayanus*****	*Saccharomyces cerevisiae*†****	*Saccharomyces pastorianus*****	
Schizosaccharomyces pombe			
*Wickerhamomyces anomalus*****			QPS only apply when the species is used for enzyme production
Xanthophyllomyces dendrorhous (imperfect form *Phaffia rhodozyma)*			
Virus			
Plant viruses			
Family			
Alphaflexiviridae	*Potyviridae*		
Insect viruses			
Family			
Baculoviridae			

QPS listed microorganisms

2. Ensure the purity of the strain

* PFGE (Pulsed Field Gel Electrophoresis)
* 16s rRNA

QPS listed microorganisms

3. **MIC (Microbial Inhibition Concentration)**

MIC=Microbiological cut-off (ISO 1932:2010 or similar)

Cut-off values obtained by studying the distribution of the chosen antimicrobial in bacterial population belonging to a single taxonomial unit

S = Susceptible (strain inhibited at [equal or lower] to the cut-off value [S ≤ x mg/l])

R = Resistant (strain not inhibited at [equal] to the cut-off value [R > x mg/l]

QPS listed microorganisms

Microbiological cut-off

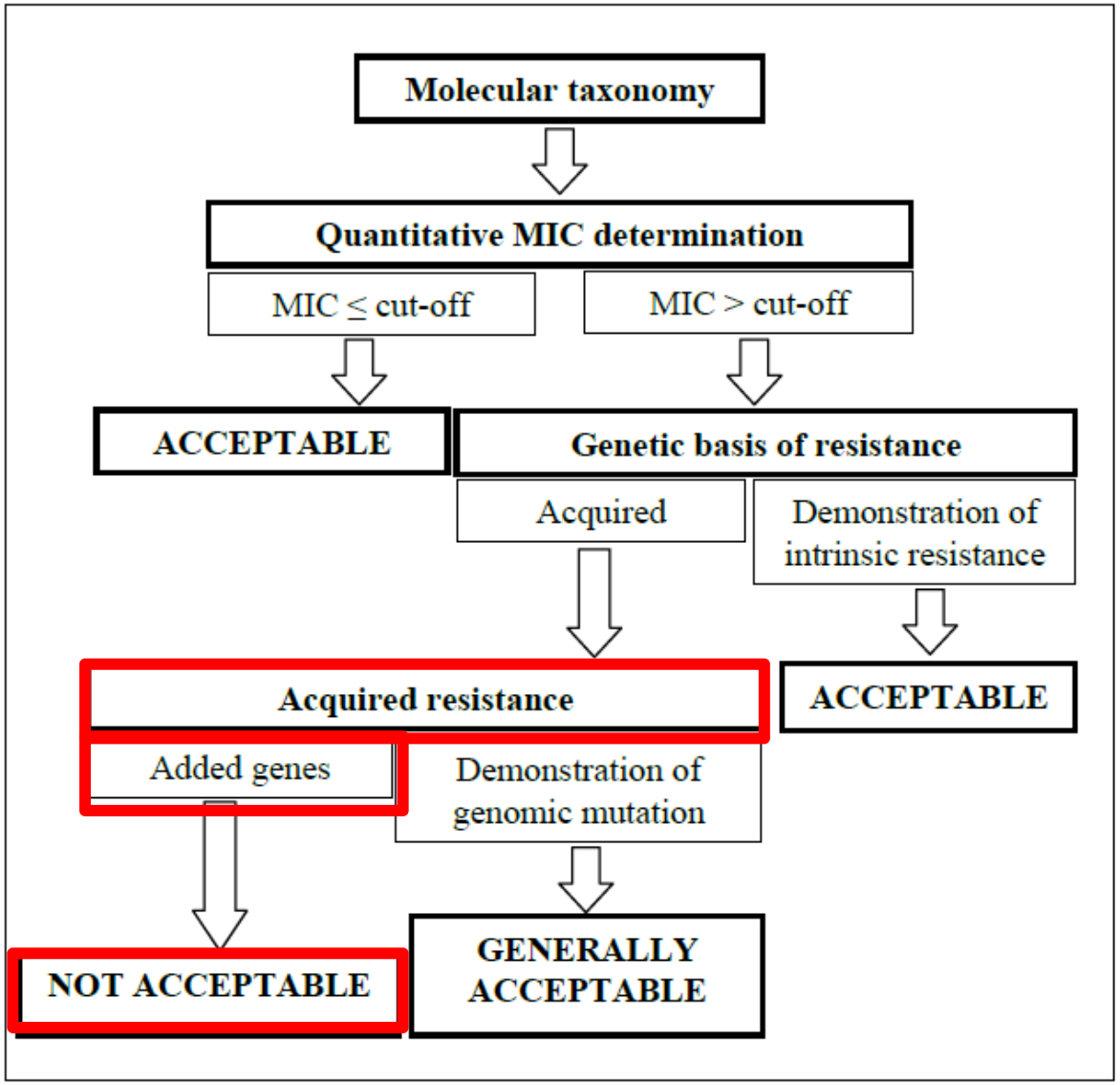
	ampicillin	vancomycin	gentamycin	kanamycin	streptomycin	erythromycin	clindamycin	tetracycline	chloramphenicol
Lactobacillus obligate homofermentative[a]	1	2	16	16	16	1	1	4	4
Lactobacillus acidophilus group	1	2	16	64	16	1	1	4	4
Lactobacillus obligate heterofermentative[b]	2	n.r.	16	32	64	1	1	8	4
Lactobacillus reuteri	2	n.r.	8	64	64	1	1	16	4
Lactobacillus facultative heterofermentative[c]	4	n.r.	16	64	64	1	1	8	4
Lactobacillus plantarum/pentosus	2	n.r.	16	64	n.r.	1	2	32	8
Lactobacillus rhamnosus	4	n.r.	16	64	32	1	1	8	4
Lactobacillus casei /paracasei	4	n.r.	32	64	64	1	1	4	4
Bifidobacterium	2	2	64	n.r.	128	1	1	8	4
Pediococcus	4	n.r.	16	64	64	1	1	8	4
Leuconostoc	2	n.r.	16	16	64	1	1	8	4
Lactococcus lactis	2	4	32	64	32	1	1	4	8
Streptococcus thermophilus	2	4	32	64	64	2	2	4	4
Bacillus spp	n.r.	4	4	8	8	4	4	8	8
Propionibacterium	2	4	64	64	64	0.5	0.25	2	2
Other Gram +	1	2	4	16	8	0.5	0.25	2	2

n.r. not required.
[a] including *L. delbrueckii, L. helveticus*
[b] including *L. fermentum*
[c] including the homofermentative species *L. salivarius*

	ampicillin	vancomycin	gentamycin	kanamycin	streptomycin	erythromycin	clindamycin	tylosine	tetracycline	chloramphenicol
Enterococcus faecium	2	4	32	1024	128	4	4	4	4	16

	ampicillin	gentamycin	kanamycin	streptomycin	tetracycline	chloramphenicol	nalidixic acid	sulfonamide	trimethoprim	apramycin
Escherichia coli	8	2	8	16	8	16	16	256	2	8

Proposed scheme for the antimicrobial resistance assessment of a bacterial strain used as a feed additive



EFSA Journal 2012;10(6):2740

QPS listed microorganisms

- Acquired resistance can be due
  - Acquired genes (bacteria via gain of exogenous DNA)
  - Mutation (of indigenous genes)
- Absence of known antimicrobial resistance gene not sufficient to explain detected resistance
- We have to provide the origin of this resistance !! The best is to stop

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How to assess the strain safety?

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- Check if the strain is listed
  - If so
  - **If not**

Strain not listed in QPS

(New strain to be used in the feed/food chain)

1. Establishing the cut-off
   - Verify taxonomy and purity
   - ATB test
2. Antibiotic / toxin production
3. Genotoxicity, mutagenicity studies
4. Tolerance studies

Strain not listed in QPS

1. **Establishing the cut-off**
    - Collect at least 10-50 corresponding strain issued from different international collection + control strain
    - Verify taxonomy and purity (16s rRNA + PFGE)
    - ATB test (EFSA + EUCAST lists)

Strain not listed in QPS

2. Antibiotic / toxin production

- Not relevant for use
- If species known to produce ATB, absence to be confirmed by analysis

Strain not listed in QPS

3. Genotoxicity, mutagenicity studies

- Stepwise approach
  - 1st step: 2 *in vitro* tests
    - bacterial reverse mutation (OECD TG 471) (mutagenicity)
    - *In vitro* mammalian cell micronucleus (OECD TG 487) (genotoxicity)
- Conclusion:
  - If negative, stop
  - If positive, continue

Strain not listed in QPS

3. Genotoxicity, mutagenicity studies (cont'd)

- Stepwise approach, if positive
  - Mammalian erythrocytes micronucleus test (OECD TG 474)
  - Transgenic rodent somatic and germ cell gene mutation assays (OECD TG 488)
  - *In vivo* Comet assay (no international protocol available)
- If negative test , no genotoxin

Strain not listed in QPS

4. Tolerance studies

- *In vivo* trial
  - 10X the recommended dose (biochemistry and hematology)
  - 100X the recommended dose (only animal performance)

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Live microorganisms

Conclusion

- QPS is qualified generic pre-assessment system
- Safety assessment for microorganisms
- Reduce the trial investment
- Annual revision of QPS list