GUIDANCE ON MICROORGANISMS USED IN THE FOOD CHAIN

EUROPEAN FOOD SAFETY AUTHORITY

Why

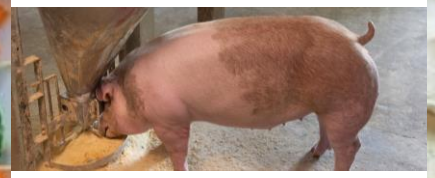
MICROORGANISMS IN THE FOOD CHAIN

REGULATED PRODUCTS

Feed additives

Food additives

Food enzymes



Food flavourings

Health claims

Novel foods

Plant protection products

Genetically modified microorganisms food/feed

Content and scope

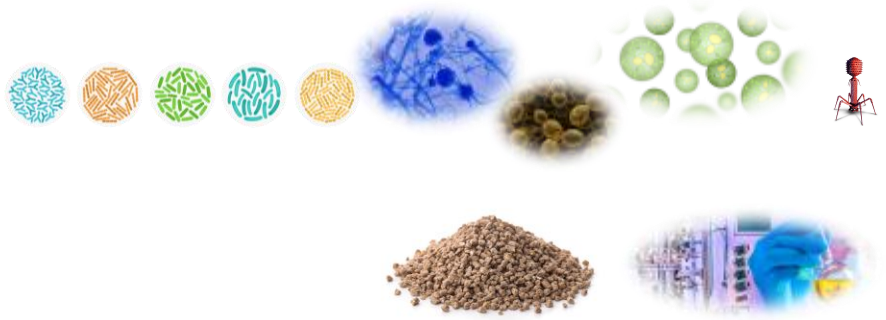
GUIDANCE - TABLE OF CONTENTS

Scope

- Taxonomic identification
- Antimicrobial resistance and production
- Toxigenicity and pathogenicity
- Genetic modification
- Presence of cells and/or DNA
- ERA and impact on gut and food/feed microbiome

Outcomes section

- What addresses and types of products at stake
- Data requirements – what and how



- Predictability of the risk assessment

SCOPE OF THE GUIDANCE

Scope

- Characterisation of the microorganisms (and products) – impact on the receiving environment
- It provides the basis for the risk assessment of microorganisms
- Microorganisms used as such (alive) or used to obtain products of interest - GM or not -

  Active agents, biomasses, and production organisms
- Focus on bacteria, yeasts, filamentous fungi, **microalgae, and viruses**
- Reference document for the risk assessments across EFSA

Each section specifies the products under scope

Taxonomic identification

TAXONOMIC IDENTIFICATION

- Internationally recognised codes of **nomenclature** for the different microorganisms
- For **bacteria, yeasts, filamentous fungi and viruses**: based on whole genome sequence (WGS) data analyses
- **Microalgae/protists**: by combining morphological and DNA sequencing information of selected genetic markers

- Unambiguous identification at the species level, for the strain under assessment
- WGS required (bacteria, viruses, fungi and yeasts),
- Microalgae /protists DNA

Adopted: 28 June 2024

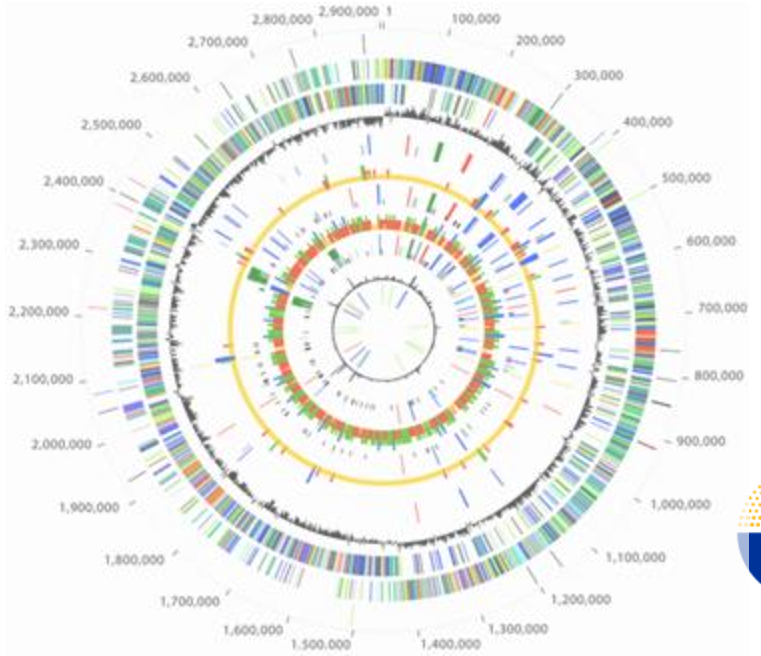
DOI: 10.2903/j.efsa.2024.8912

STATEMENT

efsa JOURNAL

EFSA statement on the requirements for whole genome sequence analysis of microorganisms intentionally used in the food chain

European Food Safety Authority (EFSA)

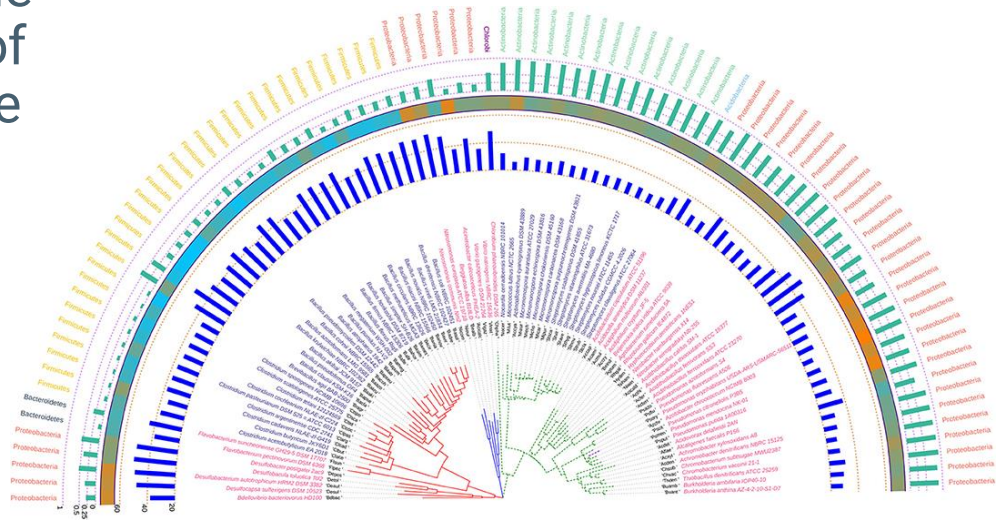


TAXONOMIC IDENTIFICATION

- If the strain cannot be assigned to any validly published microbial species, **phylogenetic position** with respect to the closest described species should be provided

- For microorganisms obtained by **synthetic biology**, the identification of the cellular **host** used as a recipient of engineered biological systems (i.e. chassis) should be provided

- Phylogenetic analysis if strain not described
- Genome taxonomy databases should be used

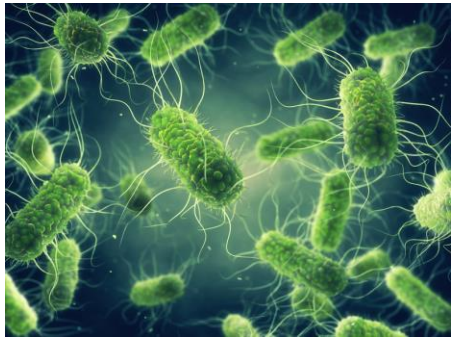


Toxigenicity and pathogenicity

TOXIGENICITY AND PATHOGENICITY - BACTERIA

- Based on WGS data analysis using at least one curated database
- Exceptions:
  - ***For Enterococcus faecium* and *Enterococcus lactis*:**
    - Based on discrimination between the species by computational approach for taxonomic assignment
    - In case of *E. faecium*: susceptibility to ampicillin and search for virulence markers by WGS data analysis
  - ***For Bacillus spp.* and related species included in the QPS list:** cytotoxicity test following recommended protocol or equivalent

Species listed in the QPS list are excluded except when a qualification exists

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European Food Safety Authority (EFSA)

TOXIGENICITY AND PATHOGENICITY - FUNGI

- Based on body of knowledge (literature search) and WGS data analysis using at least one curated database
- If a potential is identified:
  - ***For active agents:*** phenotypic tests under conditions relevant to the production or use of the product
  - ***For production strains and biomasses*:** quantitative analyses of the metabolites in the final product

The potential pathogenicity or ability to produce harmful metabolites should be established

TOXIGENICITY AND PATHOGENICITY - VIRUS

- For **viruses**:
  - **Host range/infectivity**
  - **Infectivity and the absence of adverse effects** for **non-intended species** on a representative set of species
  - For **bacteriophages**:
    - **Host range** on a representative set of strains belonging to the target and closely related bacterial species
    - **Nature** by WGS based analysis, in particular for:
      - genes coding for toxins and other virulence factors
      - genes coding for lysogeny
      - genetic elements known to be involved in transduction


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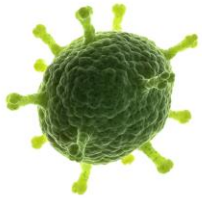
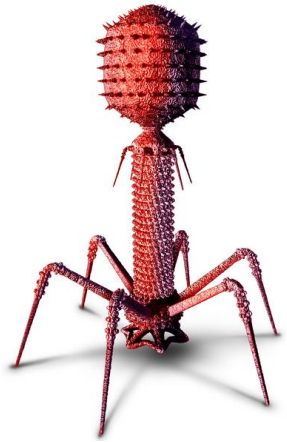
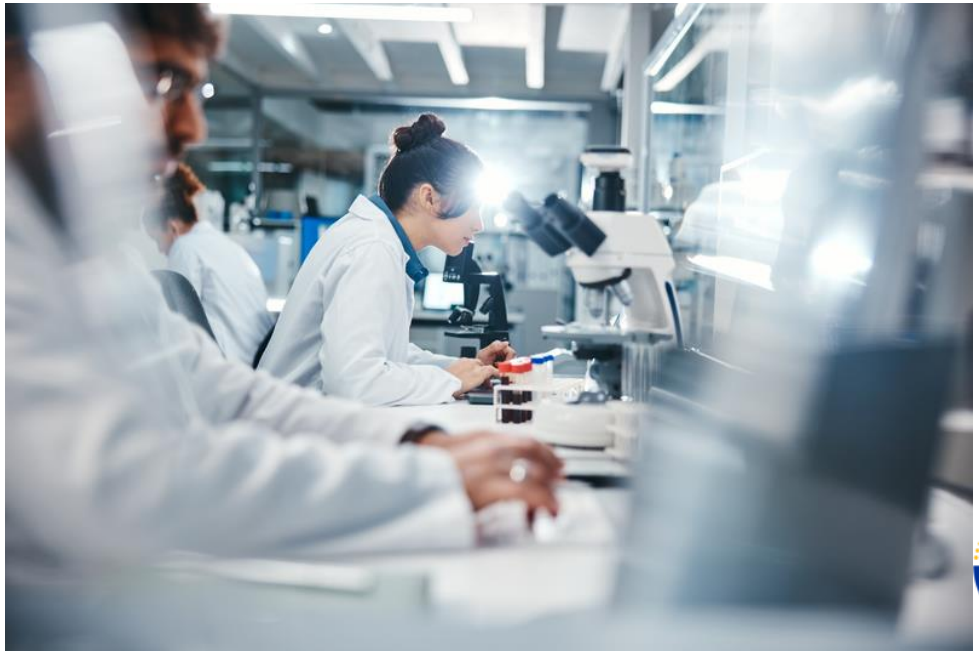




TOXIGENICITY AND PATHOGENICITY – MICROALGAE AND PROTISTS

- Based on body of knowledge (literature search) covering the **specific species and close-relates species**
- If a potential is identified, **phenotypic tests** are needed: quantitative analyses of the relevant metabolites on the cell biomass and in the cell culture supernatants.
- **Toxicological studies** may still be needed.

The potential pathogenicity or ability to produce harmful metabolites should be established



Genetic modification

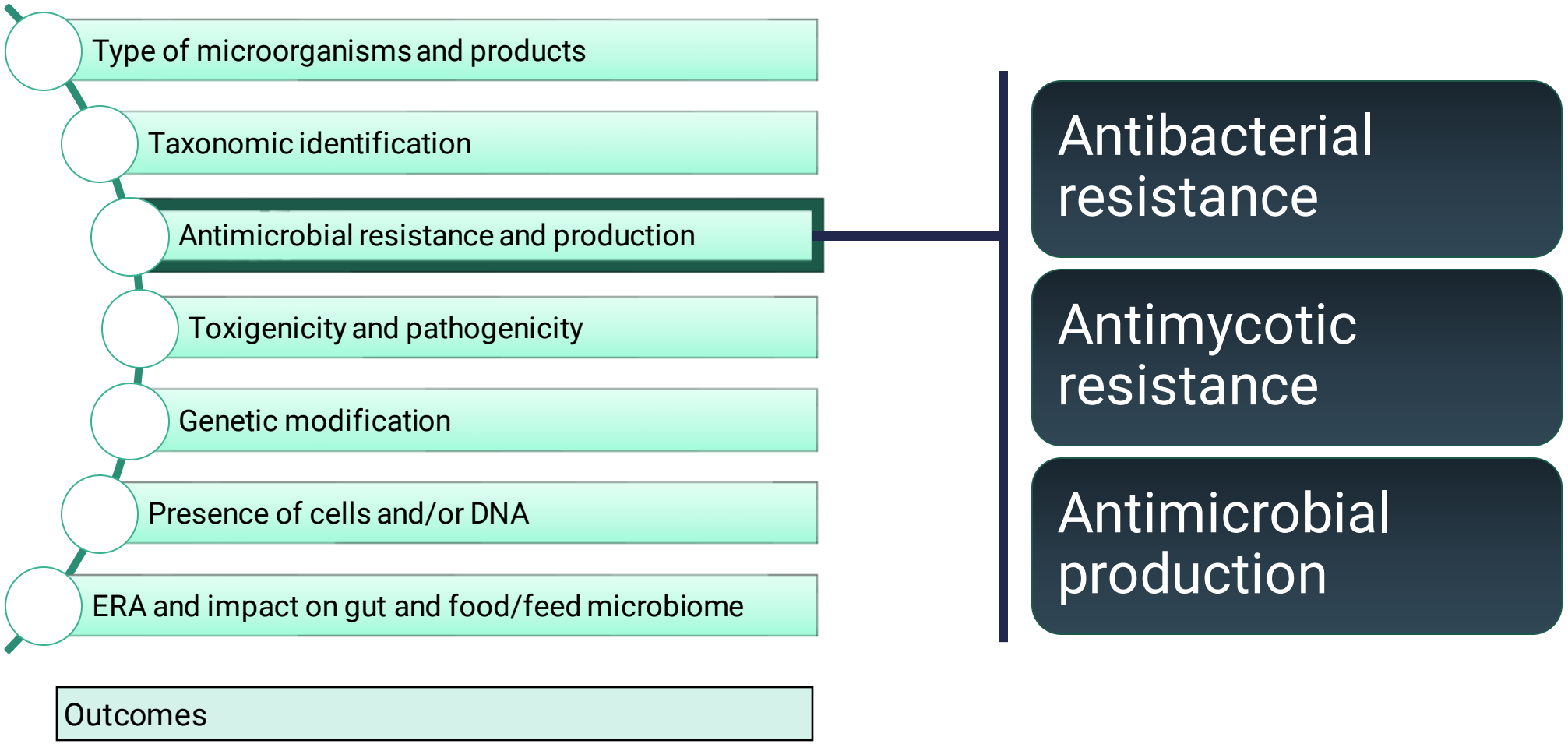
GENETIC MODIFICATION

- Genetic modification and purpose should be described to determine the safety of the modification and of the resulting phenotypic traits
- Inserted/deleted sequences and other mutations, donors/recipients strains, use of synthetic genes, vectors and structure of the genetic modification should be provided.

No changes with respect to current practices

QPS approach may still apply to GM strains belonging to species included in the QPS list

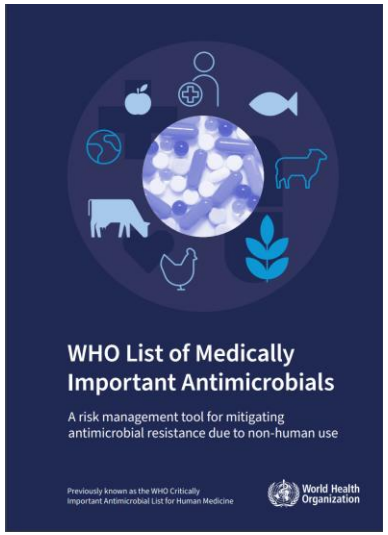
GUIDANCE ON MICROORGANISMS - CONTENT



Antibacterial resistance

GENERAL PRINCIPLES

Products intentionally added to the food chain should not contribute to the pool of antimicrobial resistance (AMR) in the receiving environment(s)

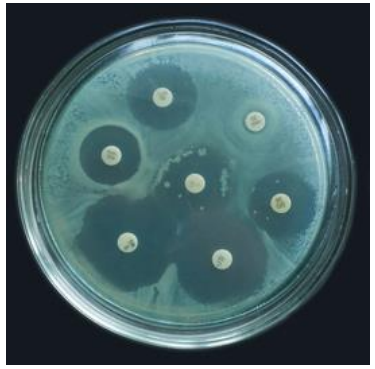


- Applies to bacteria and bacteriophages including host strain
- Relevant substances: medically important antimicrobials and veterinary critically important, highly important and important antimicrobial agents
  - WHO, 2024 (https://cdn.who.int/media/docs/default-source/gcp/who-mia-list-2024-lv.pdf?sfvrsn=3320dd3d_2)
  - WOAH, 2021 (https://www.woah.org/app/uploads/2021/06/a-oie-list-antimicrobials-june2021.pdf)

- Hazard: Acquired AMR genes according to EFSA BIOHAZ, 2023 (https://www.efsa.europa.eu/en/efsajournal/pub/8323)

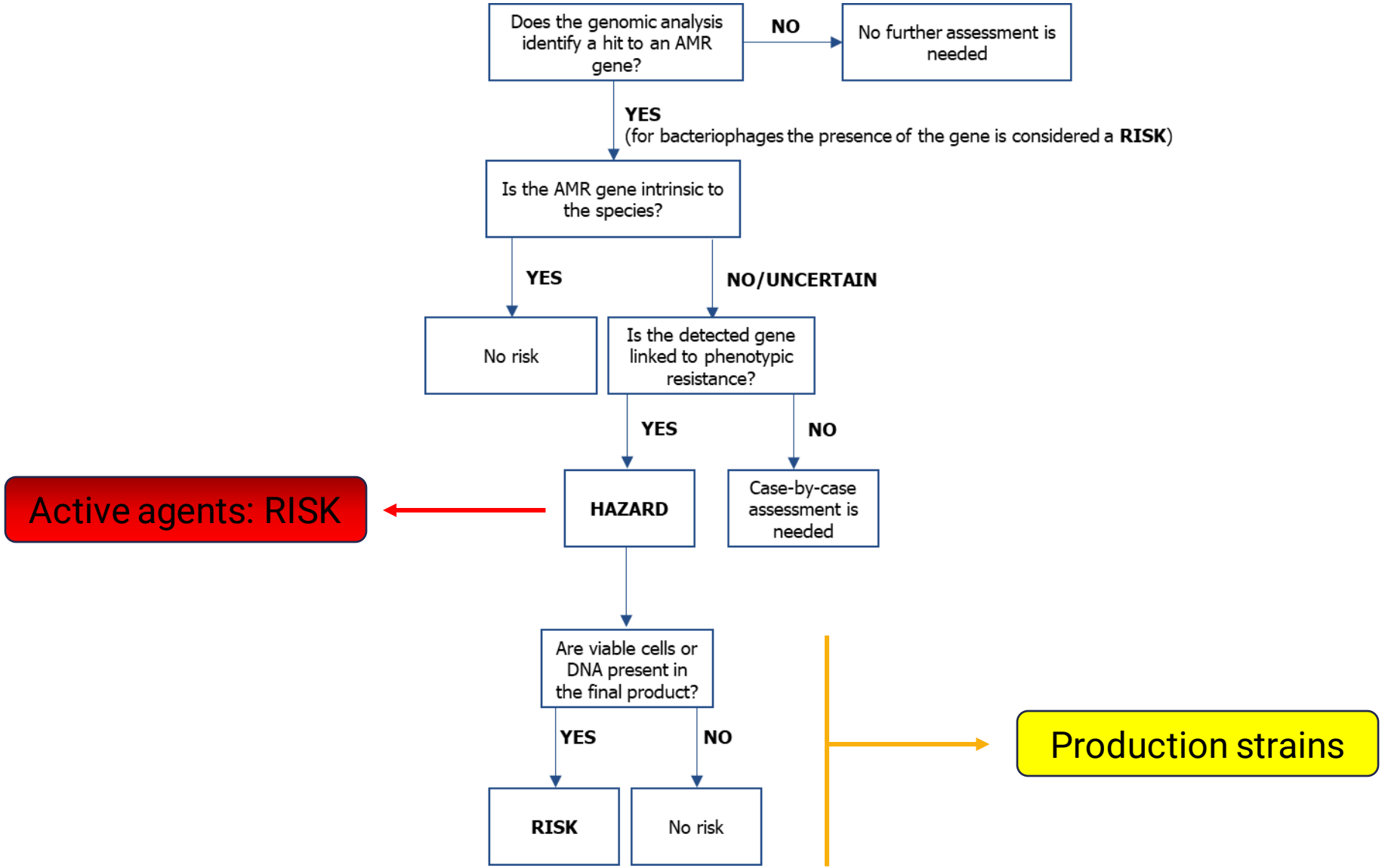
GENOTYPIC AND PHENOTYPIC TESTS

- Genomic analysis: WGS interrogation against at least two up-to-date curated databases
- Phenotypic analysis: Antimicrobial susceptibility testing (MIC)



	Antimicrobial compound												
	Amp	Vanc	Gent	Kan	Strepto	Erythro	Clin	Tet	Chlr	Tylo	Cipr	Colis	Fos
Lactobacillaceae obligate homofermentative[a]	2	2	16	64	16	1	4	4	4	-	-	-	-
Lactobacillaceae obligate heterofermentative[b]	2	-	16	64	64	1	4	8[c]	4	-	-	-	-
Limosilactobacillus reuteri	2	-	8	64	64	1	4	32	4	-	-	-	-
Lactobacillaceae facultative heterofermentative[d]	4	-	16	64	64	1	4	8	4	-	-	-	-
Lactiplantibacillus plantarum/Lpb. pentosus	2	-	16	64	-	1	4	32	8	-	-	-	-
Lacticaseibacillus rhamnosus	4	-	16	64	32	4	4	8	4	-	-	-	-
Lacticaseibacillus casei/Lcb. paracasei	4	-	32	64	64	1	4	4	4	-	-	-	-
Bifidobacterium	2	2	64	-	128	1	1	8	4	-	-	-	-
Pediococcus	4	-	16	64	64	1	1	8	4	-	-	-	-
Leuconostoc	2	-	16	16	64	1	1	8	4	-	-	-	-
Lactococcus lactis	2	4	32	64	32	1	1	4	8	-	-	-	-
Streptococcus thermophilus	2	4	32	-	64	2	2	4	4	-	-	-	-
Bacillus and related species	-	4	4	8	8[e]	4[f]	4[g]	8	8	-	-	-	-
Propionibacterium	2	4	64	64	64	0.5	0.25	2	2	-	-	-	-
Enterococcus faecium/E. lactis	2	4	32	-	128	4	4	4	16	4	-	-	-
Corynebacterium and Other Gram +	1	4	4	16	8	1	4	2	4	-	-	-	-
Enterobacteriaceae	8	-	2	8	16	-	-	8	-	-	0.06	2	8

WORFLOW


Does the genomic analysis identify a hit to an AMR gene?
NO
No further assessment is needed
YES
(for bacteriophages the presence of the gene is considered a RISK)
Is the AMR gene intrinsic to the species?
YES
NO/UNCERTAIN
No risk
Is the detected gene linked to phenotypic resistance?
YES
NO
Active agents: RISK
HAZARD
Case-by-case assessment is needed
Are viable cells or DNA present in the final product?
YES
NO
RISK
No risk
Production strains

Antimycotic resistance

GENERAL PRINCIPLES

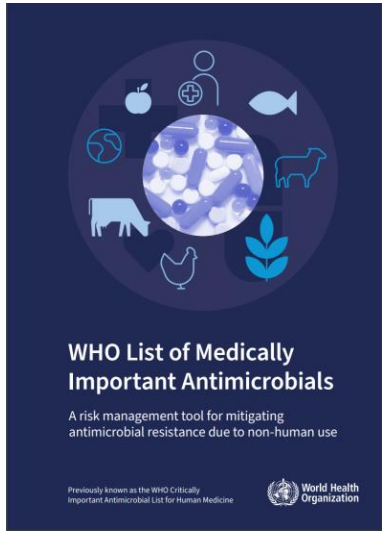
- Antimycotic resistance is not considered to be acquired by HGT
  - No need of genomic analysis
  - Applicable to **yeasts and fungi as active agents**

	Antimicrobial compound*								
	Nucleotide analog	**Polyene**		**Imidazole**	**Triazole 1st generation**		**Triazole 2nd generation**		
	Flucytosine	Amphotericin B	Nystatin	Ketoconazole	Fluconazole	Itraconazole	Voriconazole	Posaconazole	Isavuconazole
Saccharomyces cerevisiae	1	4	64	1	32	4	1	2	0.125
Aspergillus flavus / oryzae	-	8	128	-	IR	4	2	1	8
Debaryomyces hansenii	-	-	-	-	8	2	0.125	-	-
Kluyveromyces marxianus	2	4	-	-	2	0.5	0.0625	0.25	-
Pichia kudriavzevii	64	4	-	-	IR	2	2	2	2
Clavispora lusitaniae	2	2	-	-	4	0.5	0.0625	0.125	-

(*): categorised by classes.
(-): cut-off value not set by EFSA.
(IR): Intrinsically Resistant. Phenotypic resistance to this antifungal is considered inherent to the species in question

Antimicrobial production

GENERAL PRINCIPLES

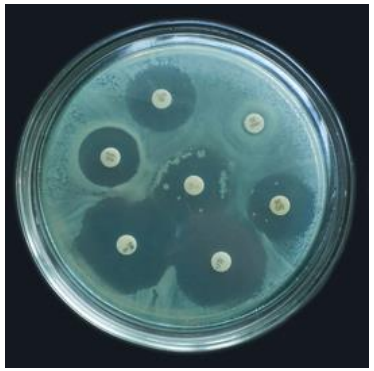


- Applies to active agents (excluding bacteriophages), biomasses and production strains (including host strains):
  - not qualifying for QPS approach, or
  - known to produce relevant antimicrobials, or
  - included in the QPS list with a qualification regarding antimicrobial production.

- Relevant substances: medically important antimicrobials and veterinary critically important, highly important and important antimicrobial agents
  - WHO, 2024 (https://cdn.who.int/media/docs/default-source/gcp/who-mia-list-2024-lv.pdf?sfvrsn=3320dd3d_2)
  - WOAH, 2021 (https://www.woah.org/app/uploads/2021/06/a-oie-list-antimicrobials-june2021.pdf)

GENOTYPIC AND PHENOTYPIC TESTS

Genomic analysis:
WGS interrogation against one up-to-date curated database



Phenotypic analysis:
Inhibition of growth of a battery of reference strains

Active agents = RISK

Production strains = Relevant antimicrobials to be analysed in the product

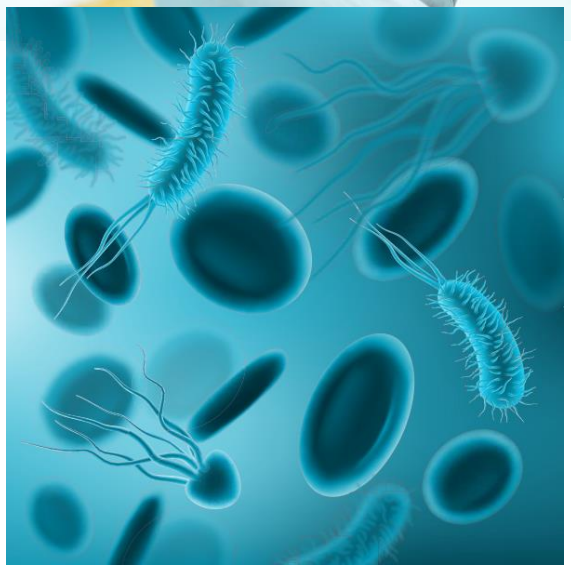
Presence of viable cells and DNA

VIABLE CELLS

- Minimum **amount** of sample and **number** of samples and batches to be tested
- **Type of samples** – Ideally the final product. If not, justify and explain well the relationship with the final product.
- Recovery of **stressed cells**, **spores germination**, etc.
- **Positive controls** with samples spiked with (a minimum concentration of) viable cells of the strain

By means of a culture-based method

No changes with respect to current practices



DNA

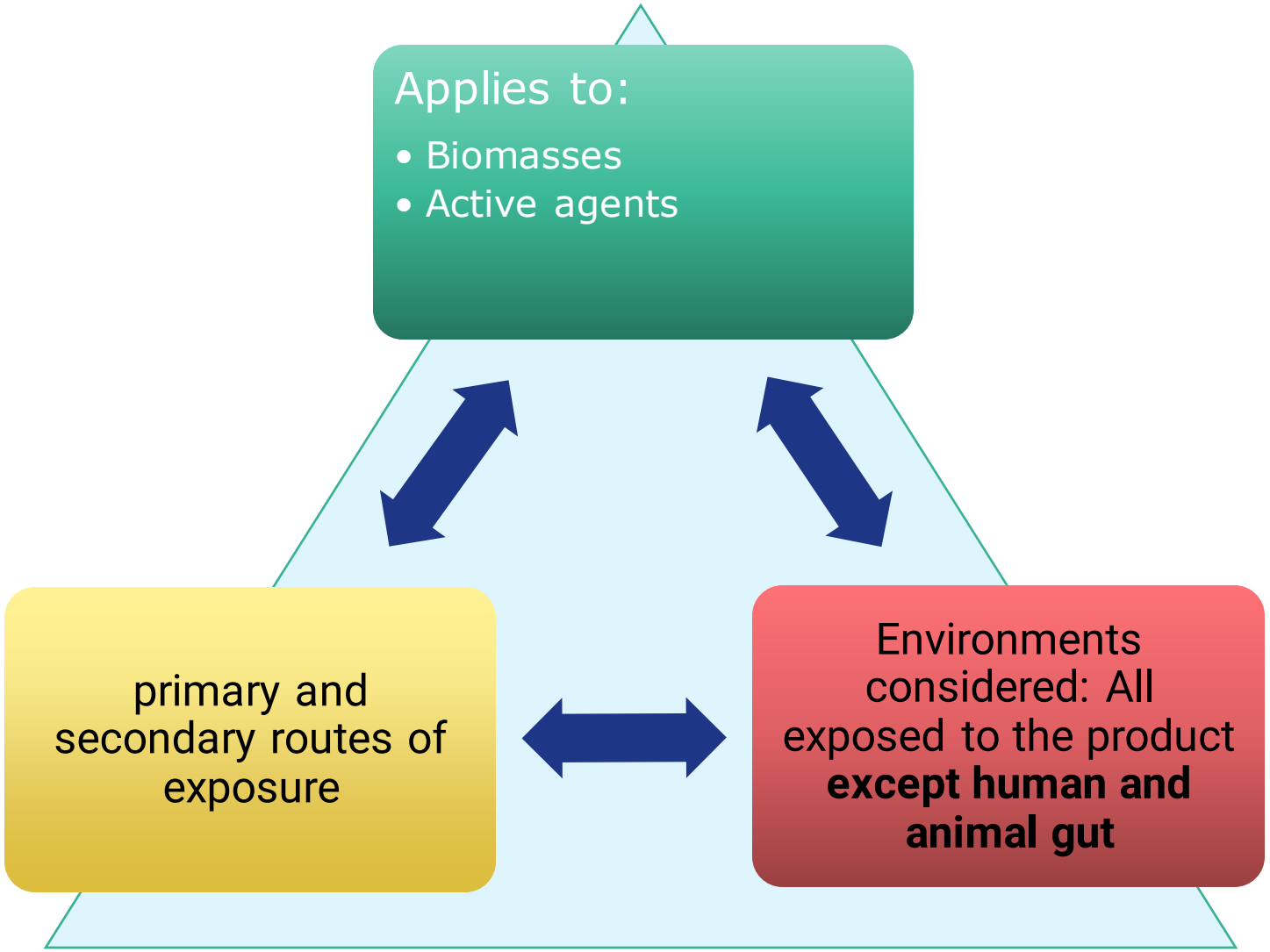
- Minimum **amount** of sample and **number** of samples and batches to be tested.
- **Type of samples** – Ideally the final product. If not, justify and explain well the relationship with the final product
- Guarantee the **recovery of DNA** during extraction
- **Target gene** to be amplified:
  - not exceeding the size of the smallest GoC and covering a maximum of 1 kb for **strains harbouring GoC**
  - covering a maximum of 1 kb for **GM strains not harbouring GoC**
- **Inclusion of controls and sensitivity tests**

By polymerase chain reaction allowing detection of at **least 10 ng of total DNA per gram or mL of product**

No changes with respect to current practices

Environmental risk assessment (ERA)

GENERAL PRINCIPLES



AREAS OF RISK

- As defined in Commission Directive (EU) 2018/350 on the ERA of GMOs (Annex II Section D.1)

Persistence and invasiveness	Horizontal gene transfer	Effects on target organisms	Effects on non-target organisms	Effects on humans or animals	Effects on biogeochemical processes	Effects of management techniques
						

BIOMASSES

ACTIVE AGENTS

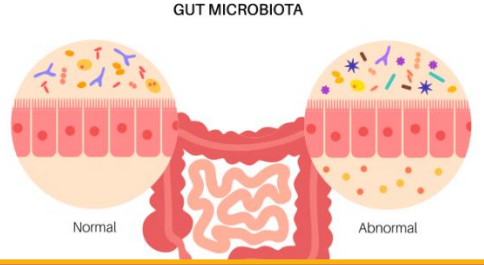
EXCEPTIONS

- General (e.g.):
  - Non-GM plant protection products
  - Non-GM QPS active agents
  - Non-GM active agents which are common in the receiving environment(s)
  - Biomasses (GM and non-GM) not containing genes of concern

- Specific per area of risk (e.g.):
  - Persistence and invasiveness- GM active agents which modification results in traits already present in microorganisms of the same taxonomic in the receiving environmental microbiome(s).
  - Effects on non-target organisms- GM active agent interacts solely with the target organism.

Impact on the gut and food/feed microbiomes

GENERAL PRINCIPLES



Active agents and biomasses on the gut microbiome in the context of its intended use

GM active agents or GM biomasses on the food/feed microbiome when added to food and feed

Persistence and invasiveness

Horizontal gene transfer

Effects on humans or animals

BIOMASSES

ACTIVE AGENTS

EXCEPTIONS

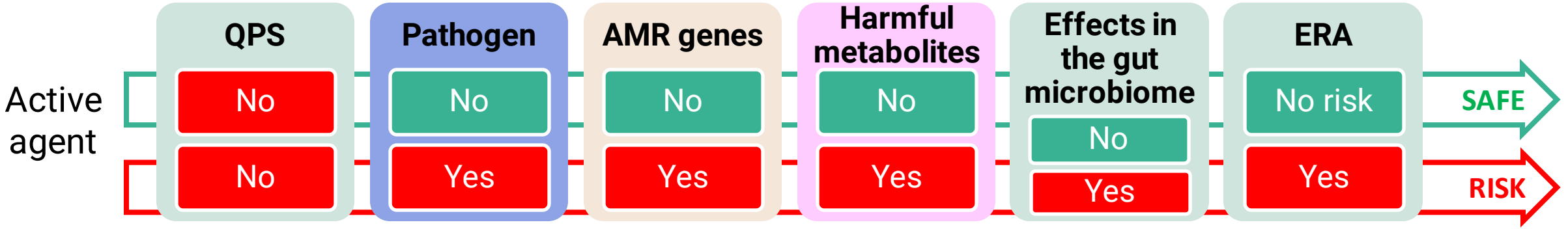
- General (e.g.):
  - Non-GM QPS active agents
  - Biomasses from QPS strains

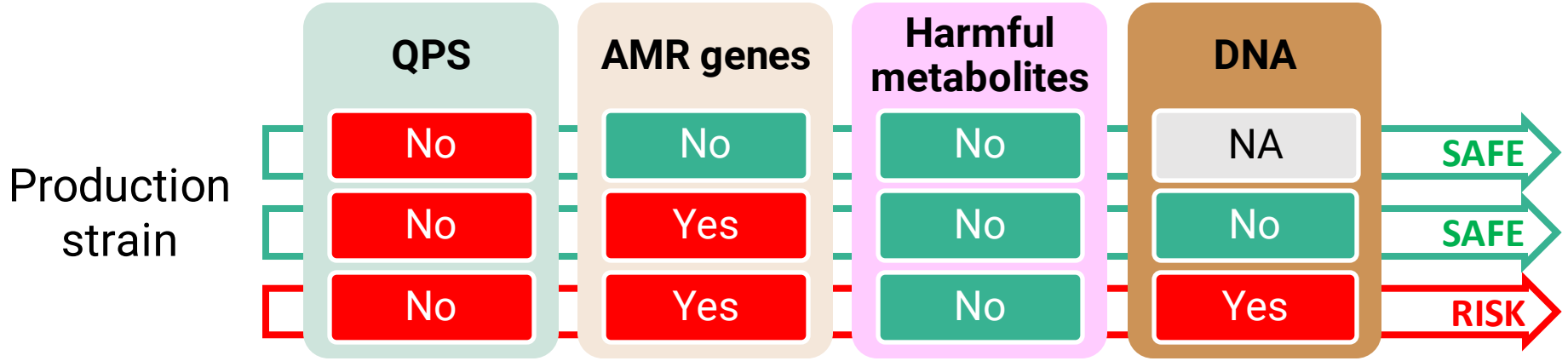
- Specific per area of risk (e.g.):
  - Persistence and invasiveness- GM active agents which modification results in traits already present in microorganisms of the same taxonomic in the receiving environmental microbiome(s).
  - Horizontal gene transfer- insertion conferring traits already present in the gut and/or food/feed microbiome.

Outcomes

GENERAL PRINCIPLES

- Aimed to provide predictability.
- Limited to concrete cases, e.g.:





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