GUIDANCE ON THE CHARACTERISATION OF MICROORGANISMS USED AS FEED ADDITIVES OR AS PRODUCTION ORGANISMS

EFSA meeting, 20 June 2023

Who are we?

AMFEP aims to represent, promote and defend the interests, safe use and regulatory framework of manufacturers and formulators of enzyme products, whilst communicating and exchanging information with stakeholders both in the EU and worldwide

EFFCA supports the growth and promotes the use of food cultures at global level. EFFCA's objective is to enhance public knowledge of the contribution the use of microbial cultures make within the food chain through accurate, fair and scientifically-based information; while discouraging any inappropriate promotion or misuse.

EuropaBio promotes an innovative and dynamic European biotechnology industry. It is committed to the socially responsible use of biotechnology to improve quality of life, to prevent, diagnose, treat and cure diseases, to improve the quality and quantity of food and feedstuffs and to move towards a bio-based and zero-waste economy.

FEFANA is the united voice of the specialty feed ingredients business in Europe. Our membership comprises manufacturers and traders of feed additives, functional feed ingredients, premixes and other mixtures of specialty ingredients that enter the food chain via feed. FEFANA promotes feed and food safety and a fair and competitive market

Revision of the guidance document – industry proposal

- General comments
- Scope
- Use of whole genome sequence data
- Bioinformatic analyses of whole genome sequence(s)
- Antimicrobial susceptibility
- Presence of recombinant DNA

General comments

- ✓ Our associations appreciate EFSA´s initiative to update the guidance document and the opportunity to contribute to this process
- ✓ Regulations and EFSA guidance documents must be proportionate and, whenever feasible, independent of specific technologies to ensure their applicability during longer periods
- ✓ Revisions should also consider streamlining processes and allowing for greater flexibility, when supported by new scientific knowledge
- ✓ It is important to ensure that dossiers prepared under the previous guidance are assessed without the requirement of repeating studies in accordance with a newly updated guidance
- ✓ The update of the guidance document requires consistency and harmonized provisions, considering all guidance documents applicable to microorganisms (feed additives, food enzymes, statement on WGS analysis)

Scope of the guidance document

Expanding the Scope: Including GMM Feed Additives in the Guidance Document

Existing regulatory framework permits the approval of GMM feed additives (Reg. (EC) No 1831/2003 as feed additive and Reg. (EC) No 1829/2003 as GMM)

Current guidance document:

- already acknowledges the applicability of the principles to GMM feed additives requiring authorisation (Regulation (EC) No 1829/2003)
- principles described in the guidance also extend to microorganisms used in feed materials (biomass) derived from GMMs

Amendment of scope and section 1:

- Feed additives containing viable **GM and/or non-GM** microorganisms (active agents)
- Feed additives produced by GM or non-GM microorganisms (production strains)

Use of whole genome sequence data

Providing WGS raw data and FASTA-files

If still considered by EFSA, submission of these data may be requested on a case-by-case basis. However, EFSA needs to duly justify why risk assessment cannot be reasonably done without submission of the FASTA files/whole-genome sequence data.

- FASTA files of the WGS is an essential part of the intellectual property/competitive position of producers → submission entails a potential risk of accidental dissemination to unauthorized parties
- Technical guidance documents should be a flexible tool to support applicants in the authorisation procedure → possibility of deviations if justified
- Risk assessment shall be based on submitted final analysis/reports and not on raw data

Bioinformatic analyses of whole genome sequence(s)

Validity of bioinformatic analyses

Bioinformatic analyses of the whole genome sequence are needed for i) the identification of the microorganism, ii) the search for the presence of known AMR genes iii) the search genes coding for known virulence factors and iv) confirmation of genetic modifications

Sequence comparisons should be performed against specific up-to-date databases

EFSA often requests re-analyses due to the lengthy risk assessment processes

Proposal to avoid re-analysis based on EFSA statement (EFSA Journal 2015;13(6):4129):

- "*Given that databases are regularly updated, analyses should be performed not earlier than one year prior to the submission of the application*." Analyses performed in full compliance with the guidance available at the time of submission should remain valid during the whole risk assessment process, even if guidance documents are updated in the meantime.
- It should be clarified that it is only the bioinformatic analyses which need to be done less than 12 months before submission (and notified) and that the sequencing does not need to be repeated.

Antimicrobial susceptibility

Definitions of intrinsic vs. acquired AMR

The guidance should provide clarity on the intrinsic and acquired antimicrobial resistance criterion and this should be aligned with EFSA's BIOHAZ ongoing work

We submitted comments to the public consultation on the BIOHAZ Draft Statement on how to interpret the QPS qualification on 'Acquired antimicrobial resistance genes':

- Requesting simple, clear criteria to distinguish between intrinsic and acquired AMR genes
- "*An AMR gene is considered 'intrinsic' if it is shared by the ~~vast~~ majority of wild type strains of the same species*"

Even if not present in "**the ~~vast~~ majority of"** strains of a species an AMR gene may still be considered intrinsic

- ✓ Gene loss is a natural phenomenon in any species
- ✓ In some cases clades/subgroups (branches in phylogenetic tree) within a species may not harbour the AMR gene due to natural evolution, while still being considered intrinsic.
- ✓ Public databases of microbial genomes may not always be accurate, and taxonomic classification is continuously evolving
- ✓ Quality of genome sequences in public databases depends on the DNA purification, sequencing and assembly method. Also, gene annotation may be insufficient or inaccurate

Antimicrobial susceptibility – Proposal to change the assessment procedure

We would prefer a phenotypic testing based on the outcome of the WGS search to select the group of antimicrobials for the MIC assay accordingly.

Two sets of data should be provided:

- A search of the WGS for the presence of known genes coding for resistance against critically or highly important antimicrobials according to the WHO list.
- Phenotypic testing based on determination of a minimum inhibitory concentration (MIC) for a selected group of critically or highly important antimicrobials. The selection of antimicrobials follows the outcome of the genotypic analyses.

Interpretation of the results: WGS search and phenotypic testing

Based on the proposed procedural change the current guidance text should be changed accordingly:

- If MIC ≤ (reference values), the likelihood of an acquired AMR gene to become active should be assessed (e.g. based on sequence comparison with active genes).
- If MIC > (reference values), this is considered as a hazard**. Information on whether the antimicrobial resistance genes are, transferable and functional shall be reported. The information provided shall be sufficient to perform an evaluation as to the risks for human and animal health due to a possible transfer of relevant acquired antimicrobial resistance genes.**

Presence of recombinant DNA

Recombinant DNA in fermentation products

The guidance requires assessing the presence of DNA from the production strain in all products obtained using GMMs and products obtained using non-GMMs carrying acquired AMR genes

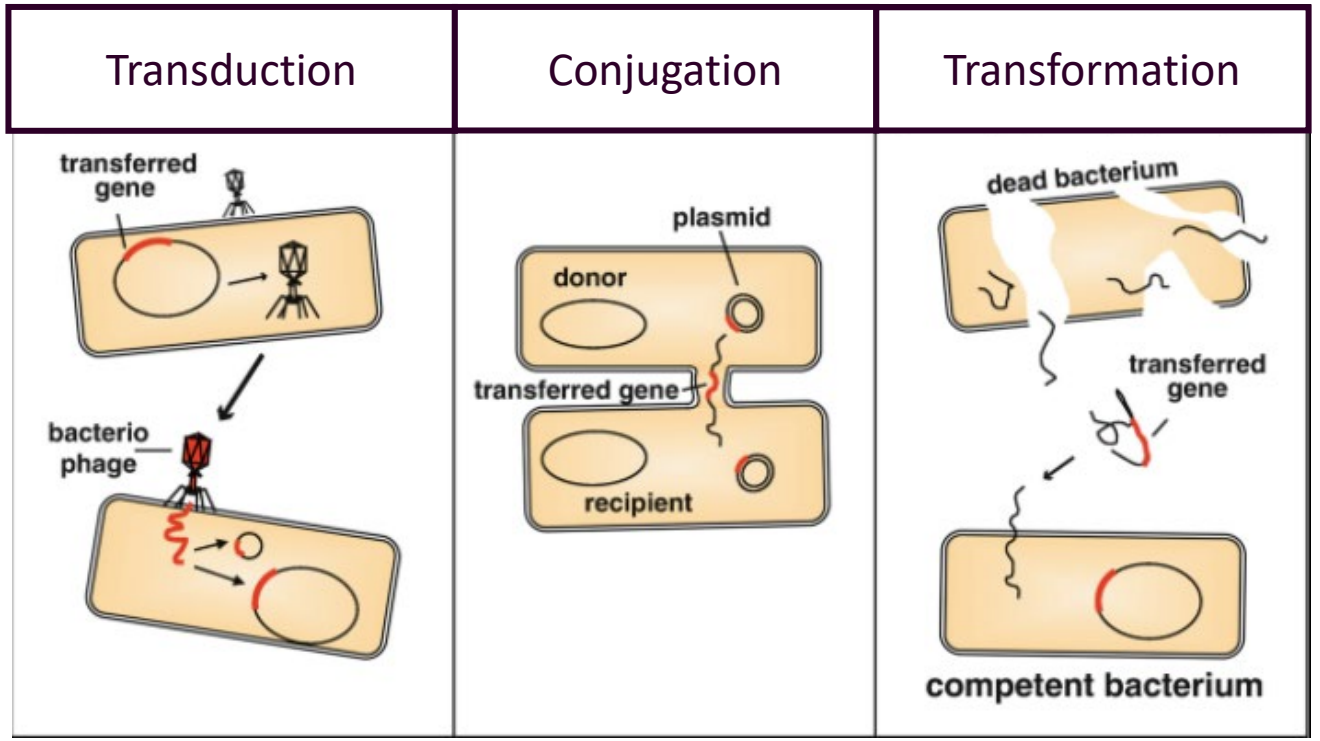
There are no legally binding provisions concerning the absence/presence of recombinant DNA in fermentation products

The request should be narrowed to GM and non-GM microorganisms (production strains) containing DNA sequences that, if transferred to other organisms, pose safety concerns (i.e., production strains carrying acquired AMR genes)

Horizontal gene transfer (HGT) - Facts

REQUIRED STEPS FOR TRANSFORMATION
UNDER NATURAL CONDITIONS:

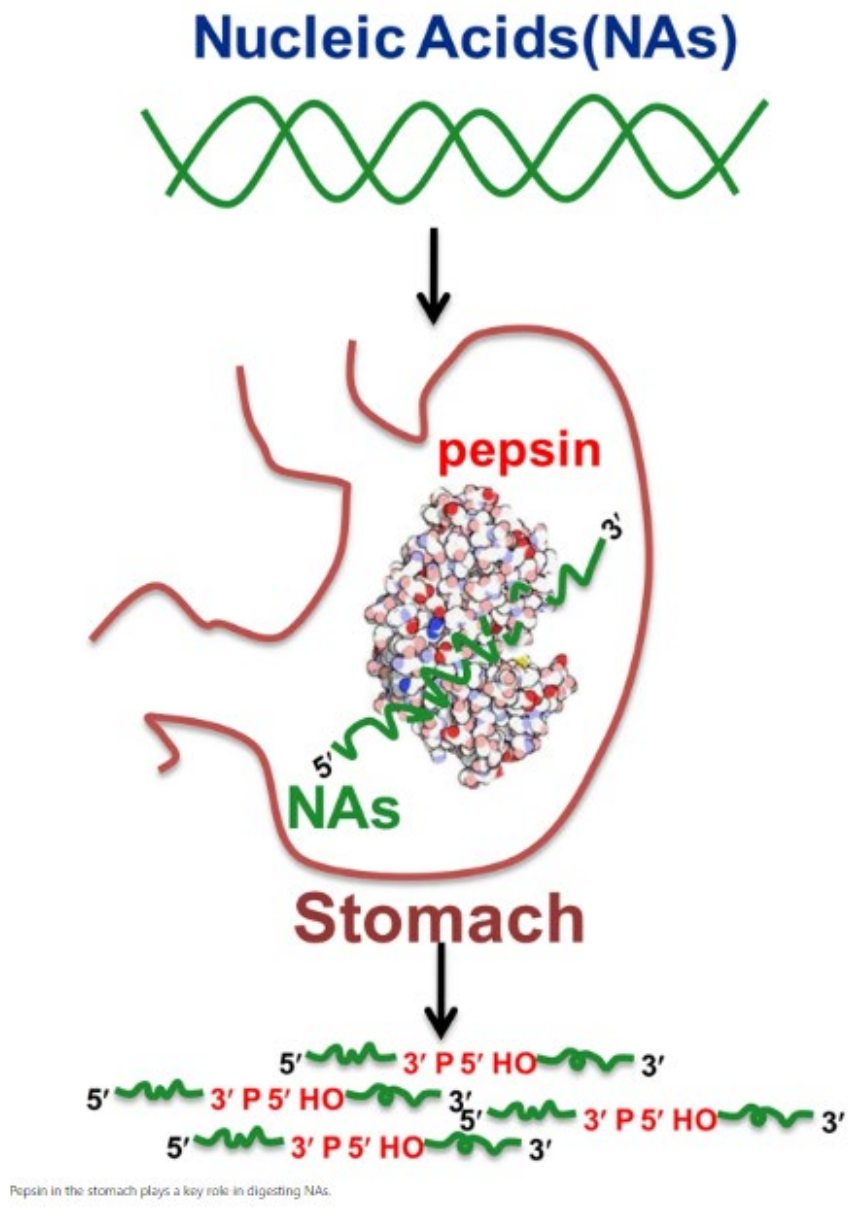
- Presence of DNA molecules in the microbial gut environment
- **Protection against DNases**
- Presence of genetically compatible bacterial genotypes
- **Appropriate biotic and abiotic conditions** (e.g., high cell density, nutritional limitation)
- Efficient adsorption of the DNA to the bacterial cell surface & uptake
- Chromosomal integration of the transforming DNA
- **Expression of a functional gene by the recipient bacterium**



rDNA fragments can only be acquired by **microbial gut flora via transformation**

How likely is residual DNA in fermentation products capable of Horizontal Gene Transfer (HGT)?

- **Residual DNA in fermentation products** - short linear DNA fragments in naked/free form, not organism associated
- **Foreign DNA (also rDNA) present in all food -** is broken down into nucleotides during feed/food processing and passage through the gastrointestinal tract – low **likelihood** of **intact genes present for HGT**
- rDNA fragments can only be taken up by **bacteria in the gut via transformation**
- **DNA** needs to **provide a selective advantage –** otherwise **lost in the population**
- The **acquisition of new genes**, such as antibiotic resistance genes from feed/food is – most likely – an **infinitesimally rare event**

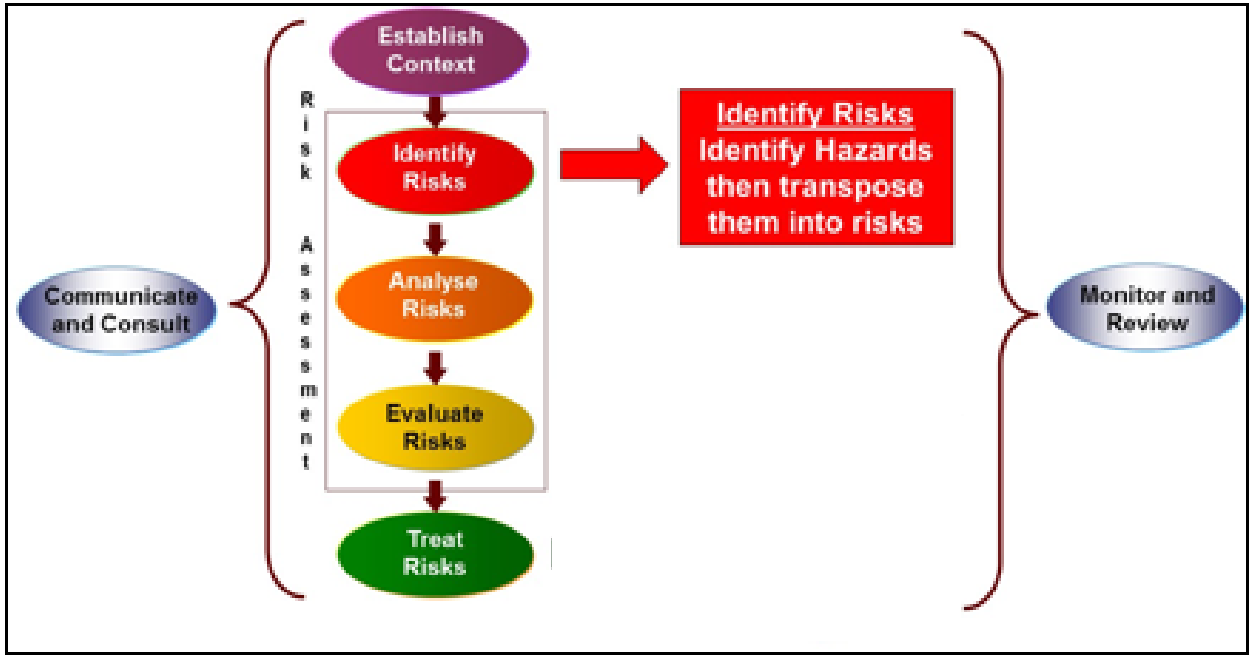


Digestion of Nucleic Acids Starts in the Stomach, Nature 11936 (2015)
Yu Liu, Yanfang Zhang, Ping Dong, Ran An, Changhu Xue, Yinlin Ge, Liangzhou Wei & Xingguo Liang
https://www.nature.com/articles/srep11936

Risk of Spread of Antibiotic Resistance

REGULATORY (INCL. SAFETY) REQUIREMENTS SHOULD BE PROPORTIONATE, i.e. PROPORTIONAL TO THE ACTUAL RISK (NOT HAZARD)

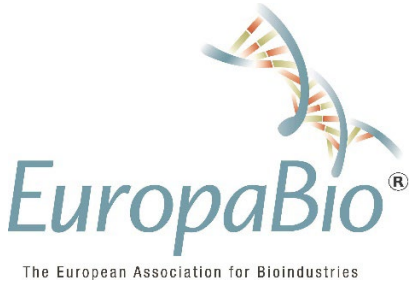
- What is the potential of naked DNA to be integrated into the genome of intestinal microbiota?
- What is the relative contribution of residual DNA from fermentation products, vs. the overuse of antibiotics in human and veterinary medicine, to the spread of antibiotic resistance?
- What is needed to quantify the risks?
- What can/should be EFSA's role in quantifying those risks?
- What does all this mean for defining proportionate risk measures?



https://paladinrisk.com.au/hazard-vs-risk/


amfep
association of manufacturers &
formulators of enzyme products


EFFCA
European Food & Fermentation Cultures Association


EuropaBio®
The European Association for Bioindustries


FEFANA
Specialty Feed Ingredients Industry

Thank you