What have we learnt from the re-evaluation? EFSA's perspective

2 - Microorganisms and fermentation products

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SOME NUMBERS…

FERMENTATION PRODUCTS

	MANDATES	FINALISED	POSITIVE	INCONCLUSIVE	NEGATIVE
Amino acids	17	11	6	5	-
Enzymes	17	10	7	3	-
Vitamins	8	3	3	-	-

MICROORGANISMS

	MANDATES	FINALISED	POSITIVE	INCONCLUSIVE	NEGATIVE
Silage	43	41	25	16	3
Probiotics	22	15	13	2	1

CHARACTERISATION OF THE PRODUCTION STRAIN

Fermentation products (amino acids, enzymes, vitamins, coccidiostats)

- Identification of the production organism not provided
  - Production organism should be identified with up-to-date methodologies
- No information on its potential toxicity/pathogenicity
  - If the production organism belongs to a taxonomic group known not to be toxigenic or pathogenic, this should be documented
- Resistance to antibiotics not tested according to the GD on bacterial susceptibility to antimicrobials
  - Testing only one antibiotic concentration is not enough
  - If resistance is due to the presence of genes conferring it, their absence should be shown in the product

GENETICALLY MODIFIED MICROORGANISMS: MAIN ISSUES

- Declaration of GMM (Definition of GMM according to Directive 2001/18/ECC → self-clones are considered GMMs)
- Lack of information on the genetic modification:
  - Sequences introduced
  - Vectors
  - Techniques
  - Structure of the GM
  - Absence of the GMM in the product
  - Absence of recombinant DNA
- Information should be provided according to the Guidance on the risk assessment of genetically modified microorganisms and their products intended for food and feed use (2011)
- Safety aspects of the GMM must be assessed even when the product does not fall under Regulation (EC) No 1829/2003
- In general, information regarding products under Category 1 and 2 apply

GENETICALLY MODIFIED MICROORGANISMS: SPECIFIC ISSUES

ISSUE	FEEDAP Panel request
■ No information on the absence of recombinant DNA in the final product, or no appropriate controls included ■ No details on the experimental methodology, particularly on the steps guaranteeing the lysis of potentially remaining cells and the adequate recovery of DNA	■ Guidance document on GMMs provides detailed information on controls to be included, including those to check correct lysis of intact dead cells potentially remaining in the product

GENETICALLY MODIFIED MICROORGANISMS: SPECIFIC ISSUES

ISSUE	FEEDAP Panel request
■ Absence of recombinant DNA tested for one introduced gene, whereas absence of antibiotic resistance (AR) genes in the strain were not demonstrated	■ AR genes should be obligatorily targeted to show their absence in the final product
■ Fragments of AR gene found in the product; but the full genes were not targeted	■ The presence of the full gene should be tested. Presence of parts of a gene as such does not demonstrate full degradation of DNA
	■ If AR genes were used during the GM and then removed, their absence should be experimentally demonstrated

QUALIFIED PRESUMPTION OF SAFETY (QPS)

- QPS can only be considered if all conditions and/or qualifications are met
- Generic qualification for all bacterial taxonomic units is that the strains should not harbour any acquired antimicrobial resistance genes to clinically relevant antibiotics
- Other, specific qualifications, examples:
  - *Bacillus subtilis:* Absence of toxigenic activity
  - *Corynebacterium glutamicum*: QPS status applies only when the species is used for production purposes (no dead/live bacterial cells in the product)

SAFETY: MAIN ISSUES

- Antimicrobial resistance (AR): genetic basis not characterised
  - The absence of known genes coding for AR is not enough proof of non-transfer of the genetic trait, and thus is not proof of safety
  - For fermentation products, if origin of AR resistance is not known, absence of DNA of the strain should be demonstrated in the product. Experimental details and LOD should be provided
- Toxigenic potential
  - History of safe use cannot be considered as demonstration of safety per se
  - history of safe use can only be considered if appropriately documented with records showing no adverse effects

SAFETY: SPECIFIC ISSUES

Probiotics
Silage additives

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Antimicrobial susceptibility

- 5 strains (***Lactobacillus pentosus, Bacillus toyonensis, Pediococcus pentosaceus*** (2 strains) and ***Enterococcus faecium***) were found to be resistant to one or more antibiotics.
- From these, only in 1 case (***E. faecium***) the genetic basis of the resistance could be identified and the concern related to its potential transfer to other microbes dismissed.
- In the remaining cases the genetic basis of the resistance was not fully identified.

SAFETY: SPECIFIC ISSUES

Probiotics
Silage additives

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Toxigenic potential of *Bacillus* strains

- In two cases (***Bacillus toyonensis*** and ***Bacillus amyloliquefaciens***) the lack of toxigenic potential of the strain was not demonstrated.
- ***B. toyonensis***: the strain showed the capacity to produce functional toxins and thus, to pose a risk to humans exposed to the organism.
- ***B. amyloliquefaciens***: assessment performed according to principles of Guidance from 2011. Lack of cytotoxicity was not demonstrated.

FROM EXPERIENCE TO GUIDANCE

Antimicrobial susceptibility of bacteria

- Update of the Guidance in 2012
  - *Revision of cut-off values, species grouping, and antibiotics*
  - *Specific additional antibiotic for E. faecium (tylosine)*
  - *Criteria for Bacillus genus not modified*
  - *Will be updated when necessary*

FROM EXPERIENCE TO GUIDANCE

Antimicrobial susceptibility of bacteria

- First technical Guidance in 2011, whole genome sequence of B. cereus strains requested
- Update in 2014, criteria for assessment of non-B. cereus species changed from the detection of B. cereus-like toxins to the detection of cyclic lipopeptides able to cause measurable cell cytotoxicity.
- Criteria for Bacillus cereus group not modified

FROM EXPERIENCE TO GUIDANCE

Virulence of *Enterococcus faecium*

- Pioneer Guidance developed in 2012 setting clear criteria to distinguish between safe strains and those more likely to cause human infections

Genetically modified microorganisms

- Plan to develop a FEEDAP GD for fermentation products made with GMMs, based on experience and developments in risk assessment

All guidance documents were submitted to public consultation