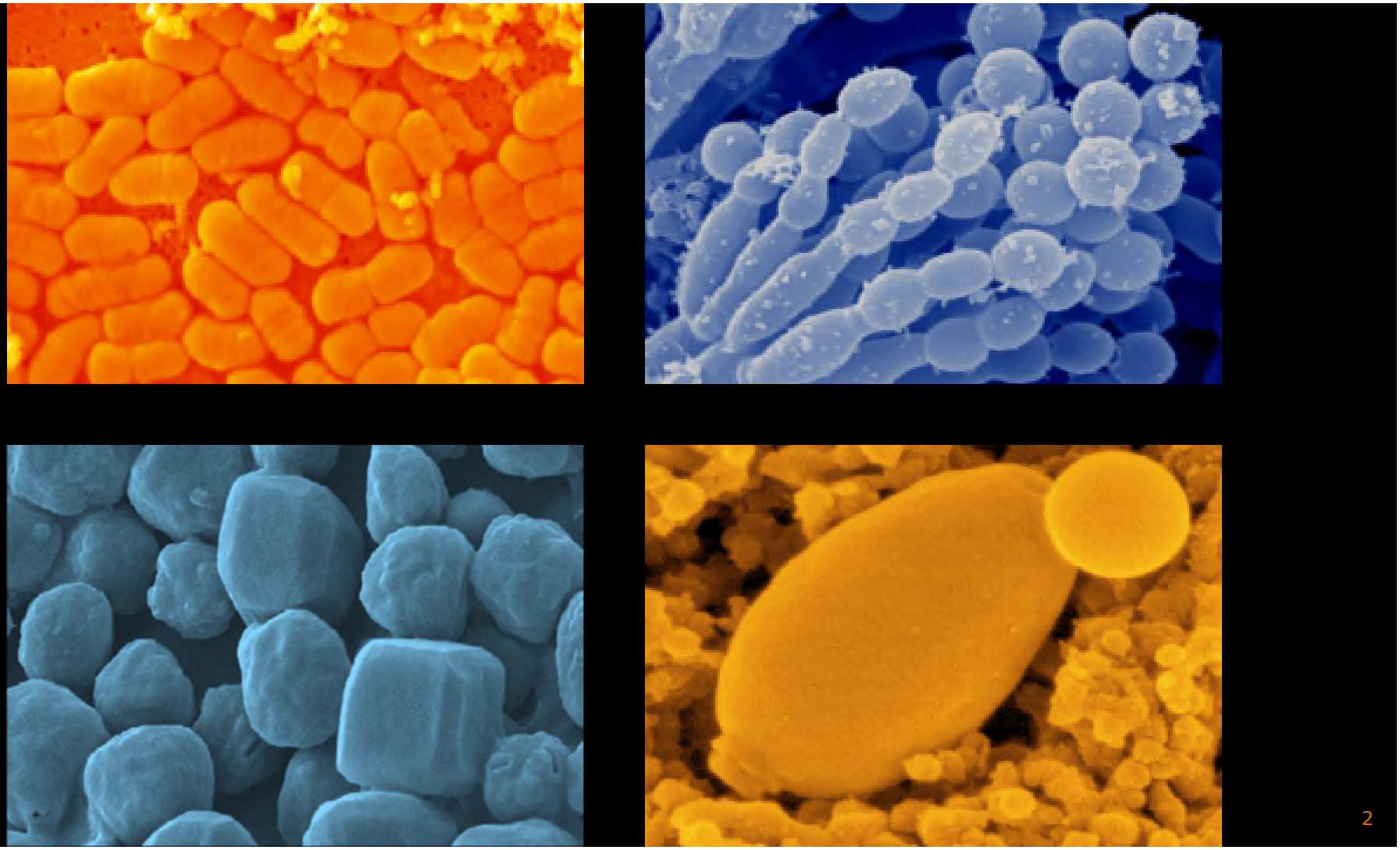
Characterisation of production strains of additives obtained by microbial fermentation

Pier Sandro Cocconcelli– EFSA FEEDAP Panel



MICROORGANISMS INTENTIONALLY USED IN THE FOOD CHAIN

- **More than 100 bacterial species**

  Arthrobacter globiformis ..... Streptococcus thermophilus

- **Filamentous fungi**

  Fusarium solani ..... Trichothecium domesticum

- **Yeasts**

  Candida famata .....Williopsis mrakii

- **Virus**

  Bacteriophages – Baculovirus -Potovirus

OUTLINE

Characterisation of production strains of additives obtained by microbial fermentation:

- options for change
- future challenges
- Opportunities

- General principles
  - Regulatory Frameworks
  - Focus on risks
  - Most updated Risk Assessment approaches

OPTIONS FOR CHANGE - 1

Taxonomical identification

- **Key aspect of the safety assessment**
  - QPS or non-QPS
  - Virulence/Toxigenic potential
- **Accurate and update identification**
  - Molecular Taxonomy (e.g. 16S rRNA gene – ITS)
  - Species dependent approach
  - Whole Genome Sequence (phylogenomics)
- **Re-classifications, synonymous and new names**

OPTIONS FOR CHANGE - 2

Strain Identification

- **Unique identification of microbial products**
  - Viable strains
  - Additive producers
- **Essential for the safety assessment**
- **Critical for a safe lineage assessment (e.g. *E. coli* K12 derivatives)**
- **DNA based methods**
  - PFGE - PCR based (e.g. RAPD, REP, ERIC, AFLP)
  - Sequenced based (e.g. MLST)
  - Whole Genome Sequence (e.g. SNPs)

OPTIONS FOR CHANGE - 3

Toxigenic potential/Virulence

- **QPS:**
  - waive for tox studies for consumer safety
  - no tolerance studies with target animal species
- **Qualifications**
  - Absence of toxigenic potential
  - Lack of acquired AMR genes
  - End use (viable vs production purposes)

- **QPS + genetic modification:**
  - Genetic modification = no safety concern
  - Alignment with food enzymes

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OPTIONS FOR CHANGE - 3

Non-QPS:

- History of safe use:
  - Limitations
  - Scientific substantiation
- Criteria
  - Known toxigenic potential
  - Literature searches
  - Whole Genome Sequence
    - Annotation
    - Analysis

Metabolite	Organism
Aspergillomarasmine	*A. oryzae*
Cyclopiazonic acid	*A. oryzae*
Kojic acid	*A. oryzae*
Malformins	*A. niger*
Malformin A	
Malformin C	
Maltoryzine	*A. oryzae*
Naphtho-r-pyrones	*A. niger*
Aurasperone D	
Nigerazine B	*A. niger*
Nigragillin	*A. niger*
3-Nitropropionic acid	*A. oryzae*
Ochratoxin A	*A. niger*
Oxalic acid	*A. niger*
Trichodermin	*T. reesei*
Violacetin	*A. oryzae*

Blumenthal, 2004. ***Reg. Toxicol. Pharmacol***. 39: 214-228

OPTIONS FOR CHANGE - 3

Safe Strain Lineage

- On-going procurement on safe strain lineages
  - 36 Bacterial species
  - 42 fungal species
- Extended Literature Review
- Genomic Data

283318-2015: Italy-Parma: OC/EFSA/FEED/2015/01 — Database on the taxonomical identification and potential toxigenic capacities of non-QPS production strains of industrially produced food and feed additives			
Publication date:	12-08-2015	**Deadline:**	15-10-2015
Document:	Contract notice		
Authority name:	European Food Safety Authority (EFSA)		

Antimicrobial susceptibility

OPTIONS FOR CHANGE - 4

Antimicrobial resistance

European Food Safety Authority

EFSA Journal 2012;10(6):2740

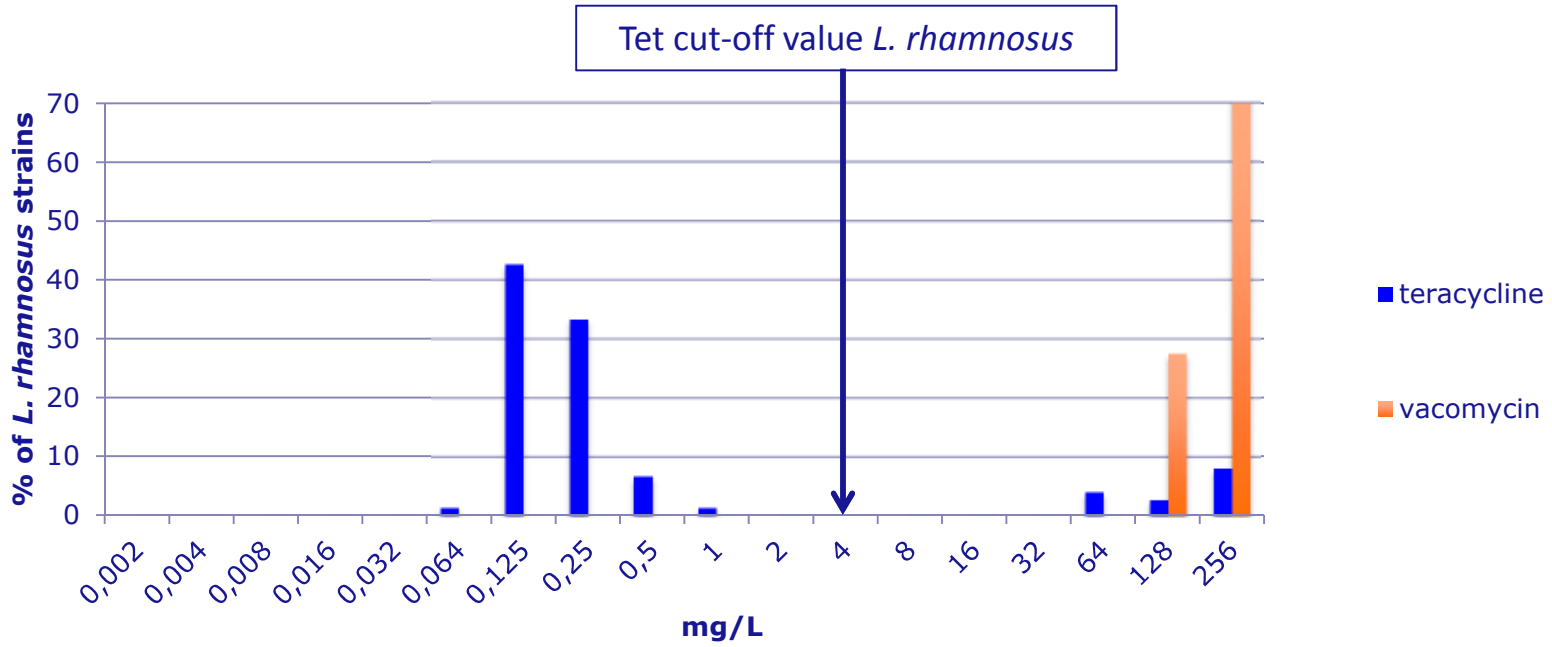
SCIENTIFIC OPINION[1]

Guidance on the assessment of bacterial susceptibility to antimicrobials of human and veterinary importance[2]

EFSA Panel on Additives and Products or Substances used in Animal Feed (FEEDAP)[3,4]

European Food Safety Authority (EFSA), Parma, Italy

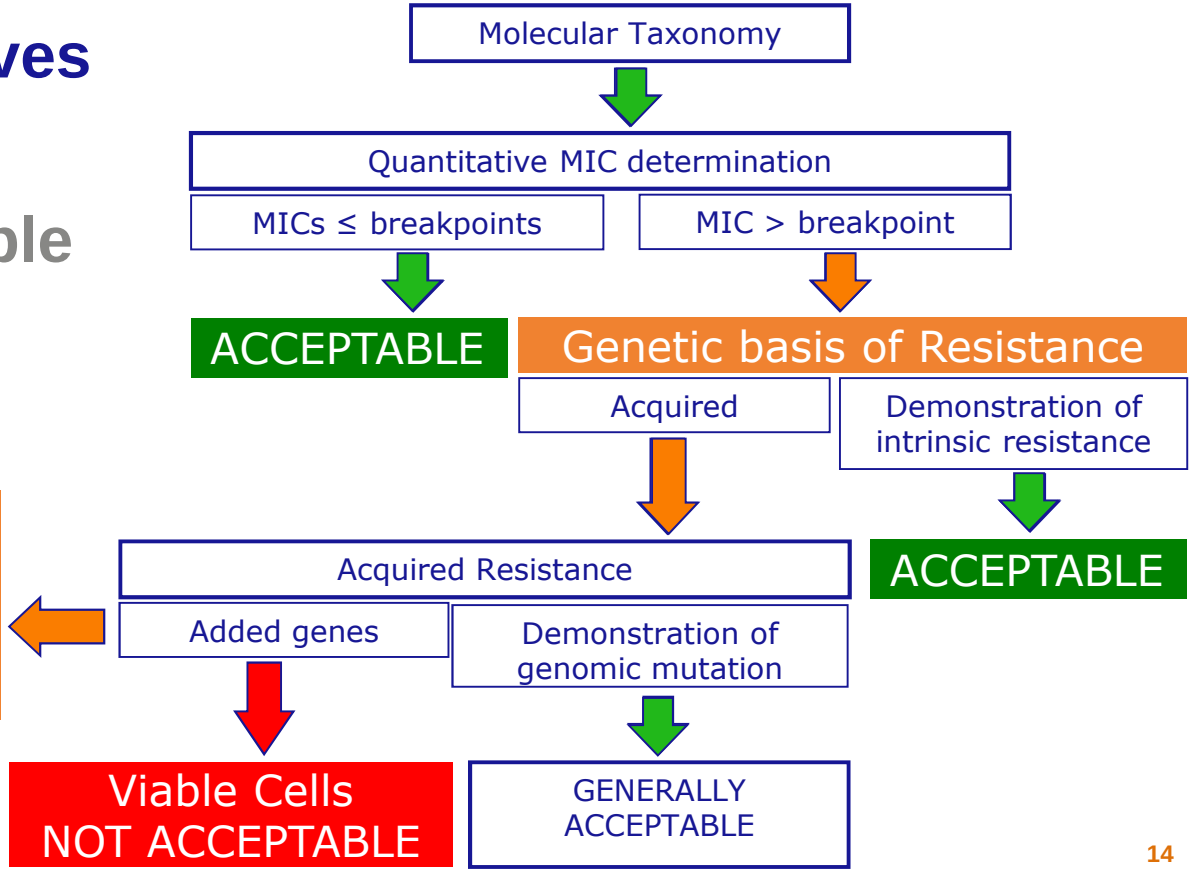
INTRINSIC vs ACQUIRED



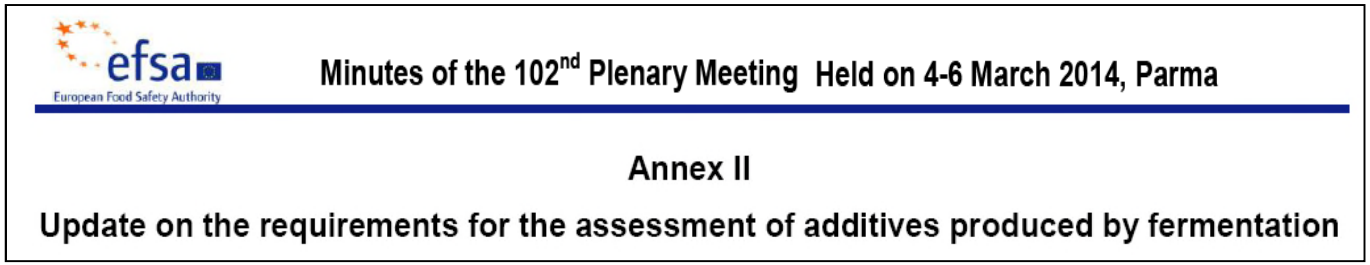
- **Where all strains within a given taxonomic group show phenotypic resistance to an antimicrobial, such resistance can be considered intrinsic to the taxonomic group**
- **Molecular basis of intrinsic resistance are generally well described .**

OPTIONS FOR CHANGE - 4

- **Microbial Additives**
  - Viable cells
  - Absence of viable cells



OPTION FOR CHANGE - 4



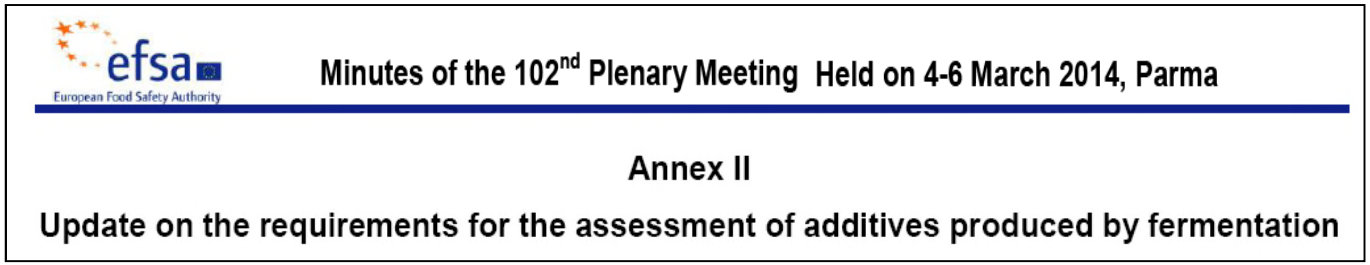
Minutes of the 102nd Plenary Meeting Held on 4-6 March 2014, Parma

Annex II

Update on the requirements for the assessment of additives produced by fermentation

- If atypically antibiotic resistance is detected, it should be demonstrated that the DNA from the production strain is not present in the final product.
- This can be done by PCR targeting known DNA sequences coding for the resistance or other sequences specific to the production strain.
- The specificity of the target sequence should be demonstrated

OPTION FOR CHANGE - 4



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Update on the requirements for the assessment of additives produced by fermentation

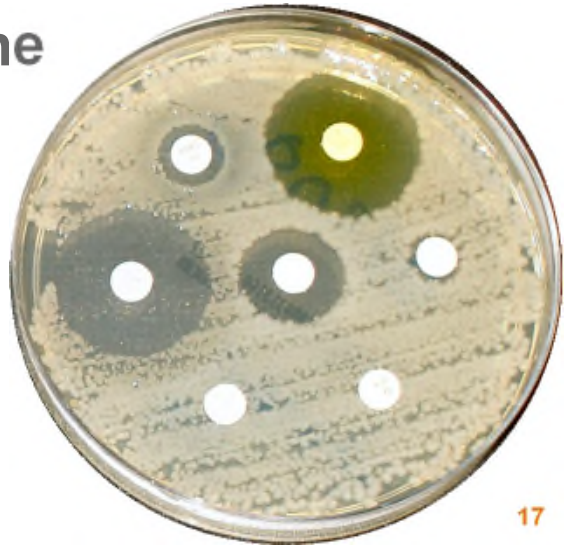
Demonstration of the absence of DNA by PCR

- Sequence to target (specificity, length)
- Methods (PCR – qPCR)
- Controls (for PCR, for DNA extraction)
- Limits of detection / thresholds

OPTION FOR CHANGE - 4

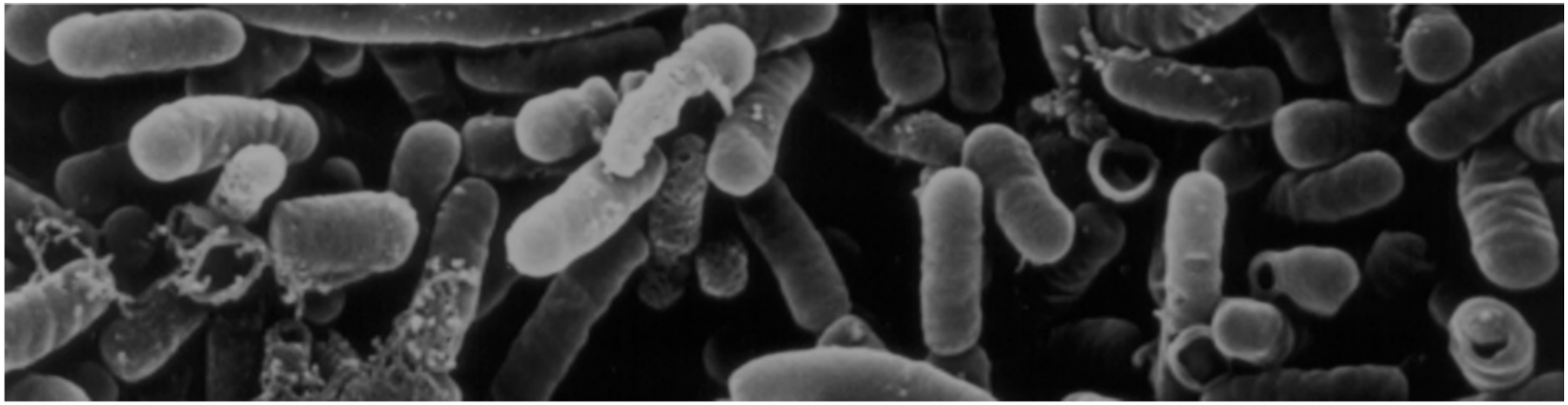
Susceptibility testing

- Phenotypic analysis
- Inclusion of new species, including production species (e.g. *C. glutamicum*)
- Revisit lists of antibiotics (e.g. colistine and ciprofloxacin for *E. coli*)
- Updated cut-off values
- Whole Genomic Sequence data for resistance mechanisms
- Presence/absence of DNA



OPTIONS FOR CHANGE - 5

VIABLE vs DEAD cells



- Critical point for the Risk assessment of GMM and non-GMM
- Definition the amount of product to be tested
- Resuscitation approaches
- Background/contaminant microbiota

OPTIONS FOR CHANGE - 6

Genetic modifications

- Most common cause of inconclusive opinions
- New options and methodologies not well covered in the current Guidance Document
- Reiterated questions on aspects not well clear
- New assessment criteria, based on experience

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OPTIONS FOR CHANGE - 6

Current GMM Guidance Document:

- From the GMO Panel
- GM food and feed
- GM fermentation products
  - Food enzymes
  - Food additives
  - Feed additives

New Guidance Document for GM fermentation products:

- From the FEEDAP Panel
- Based on experience
- Harmonised among Panels (FEEDAP, CEF)