EFSA guidance on the characterization of microorganisms used as feed additives or as production organisms, 2018

EFSA/Industry meeting, Brussels

20 Nov. 2018

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

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 (EU Association of Specialty Feed Ingredients and their Mixtures) 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

What do we do?

We produce:

- Amino-acids
- Enzymes
- Vitamins
- Micro-organisms (e.g. food & feed cultures)

These are used as:

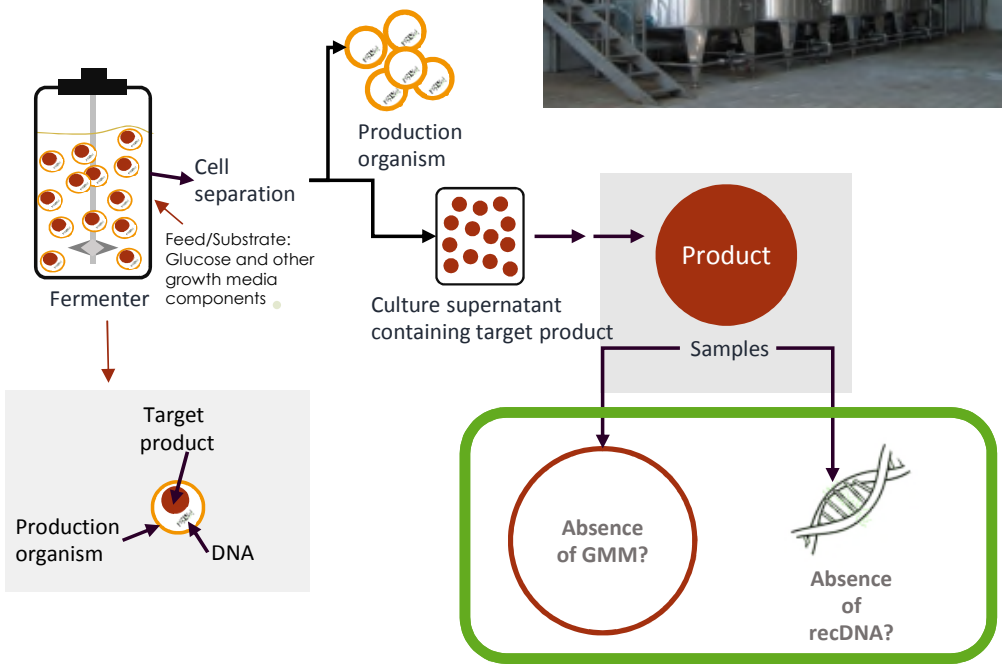
- Food ingredients
- Food additives
- Food enzymes
- Feed additives (incl. enzymes)

How are specialty ingredients and enzymes produced?

They are manufactured by fermentation using genetically modified microorganisms (GMM).

The microorganism is genetically modified to produce the desired product.

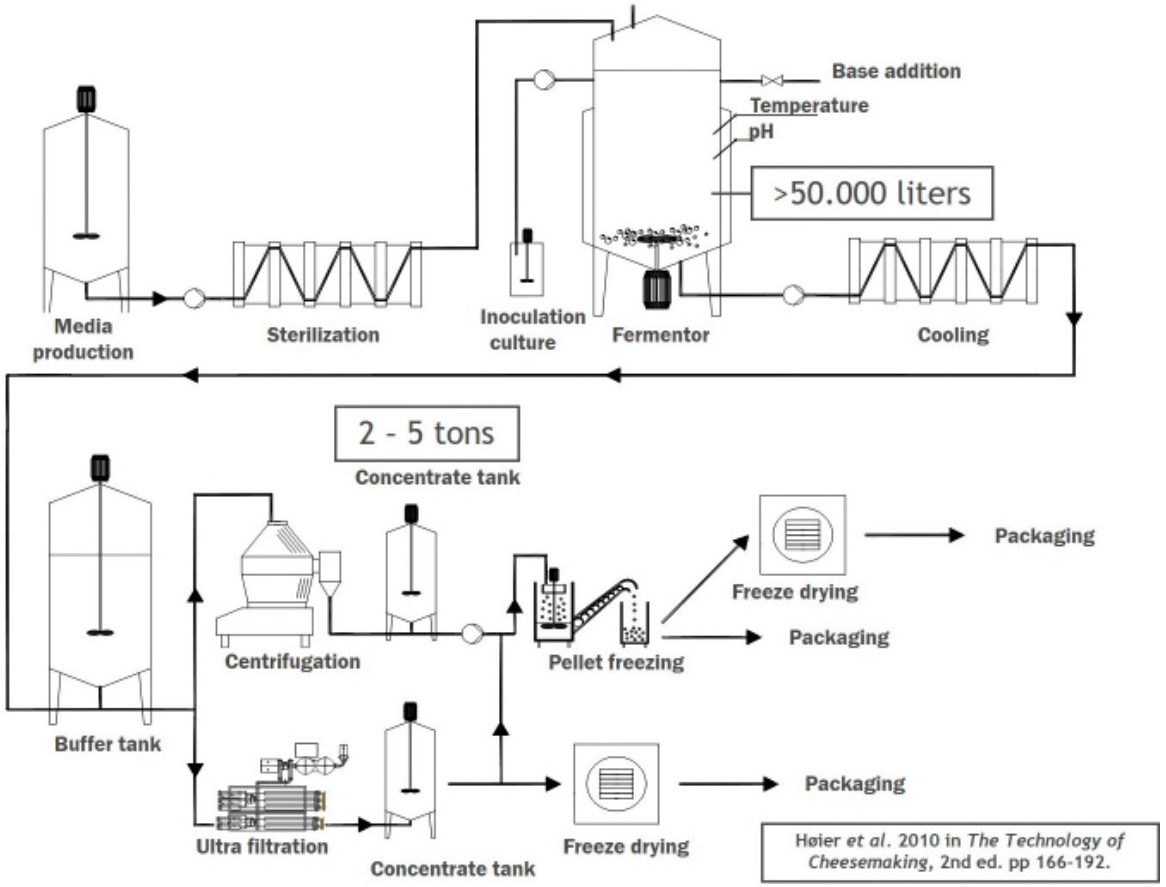
The product is made with the help of a GMM in “contained use”: the GMM is physically separated from the product.



How are microorganisms produced?

They are manufactured by fermentation using non-genetically modified microorganisms (non-GMM).

The product is the microorganism itself.



General views of industry

We appreciate EFSA's constant effort to update and streamline the guidance documents

We suggest that the EFSA guidance 2018 applies to both Food and Feed (instead of Feed only)

We are concerned by the impact of the new guidance on the competitiveness of the EU-based biotech industry

(also in light of the EC legislative proposal on transparency and sustainability in risk assessment in the Food Chain)

New EFSA guidance:
3 aspects of special impact for industry

Characterization of the genome sequence of a microorganism

Establishment and use of MIC threshold values

Detailed requirements for the demonstration of absence of DNA

Characterization of the whole genome sequence (WGS) of a microorganism

2.1.1. Use of whole genome sequence for characterization of microorganisms

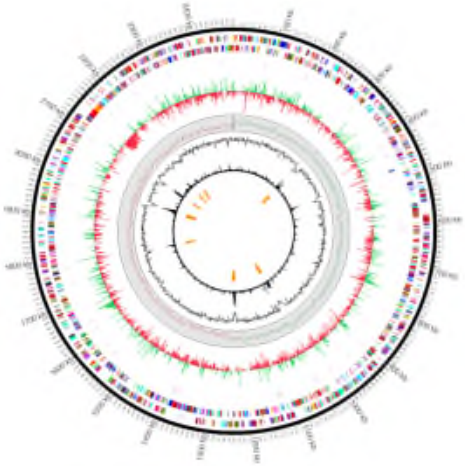
Genome sequencing – context

Whole-genome sequencing (WGS) is a powerful tool and will increasingly be an integral part of risk assessment of fermentation products

We welcome and support guidance by EFSA on the use of genome sequencing in risk assessment

Still, the following aspects require further consideration:

- The requirement to submit FASTA files of the WGS data
- The current lack of best practices how to appropriately use WGS data in risk assessment (e.g., re. virulence factors)



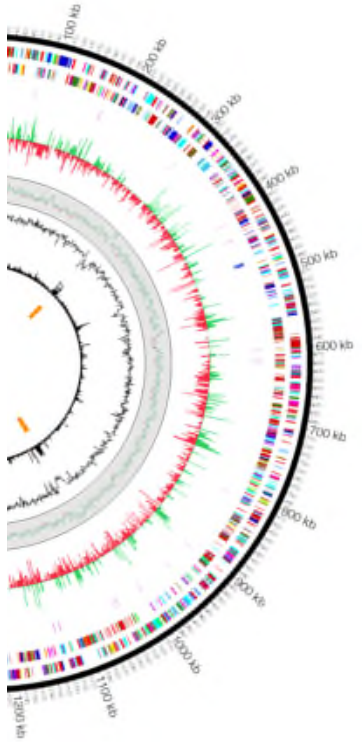
Requirement to submit complete WGS data set

Introduced after public consultation → no opportunity for industry to address associated risks, and to discuss modalities with EFSA

Acceptable for live or inactivated microorganisms (dairy cultures, food & feed probiotics, silage microorganisms, spent biomass), since genomic DNA is an integral part of the product and can be sequenced by anybody accessing the product (*caveat*: Nagoya Protocol/ABS restrictions!)

Major issue for contained-use products, because:

- The specific characteristics of the production strain are a **crucial asset** of the producer
- With current molecular biology tools, reconstruction of a strain becomes more and more simple
- Misappropriation by a competitor **cannot be proven** in the commercial product



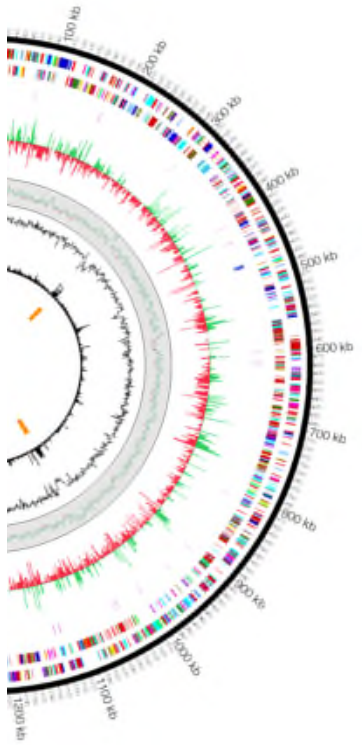
Use of WGS data to confirm strain safety

WGS data just provide a linear order of nucleotides; they do not *per se* provide reliable information on their functional expression

Current challenges:

- Functional annotation of a genome sequence (prediction of genes and their encoded functions) is still **not a mature science**, requires a lot of manual curation, and is error-prone. In addition, many genes still have "unknown functions".
- **Lack** of high-quality, updated, well-curated **reference databases** makes the interpretation of results very challenging (e.g., for virulence factors)
- **Lack** of best practices to define **cut-off values** to determine **meaningful hits**, to reduce/eliminate false positives, and to confirm/dismiss sequences of concern

Better mutual understanding is necessary on the proportionate and meaningful use of WGS data for risk assessment



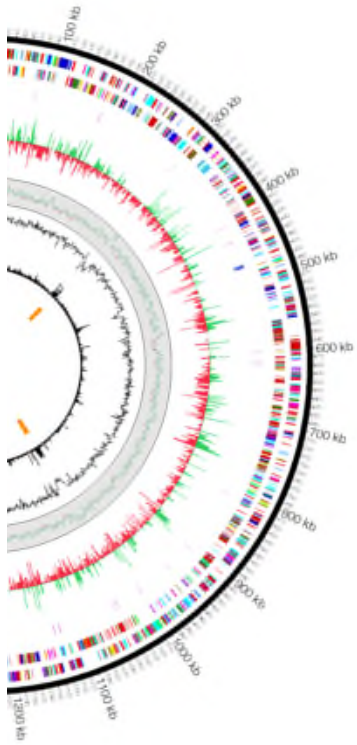
Industry recommendations on the requirements for WGS

For strains for contained use, only relevant parts of the genome sequence (modified loci) should be required to be submitted (i.e. the specific genetic modifications and any genes of concern).

- Clarification of confidentiality status of FASTA files (if required) in view of the Commission's proposal on transparency.

WGS may raise potential safety concerns based on annotation, homology etc. but the safety of the strains is unchanged.

Proven methodology to characterize genome modifications (e.g., Southern blot) should still be acceptable.



Establishment and use of MIC cut-off values

2.2. Antimicrobial susceptibility

Challenges for the use of Minimal Inhibitory Concentrations (MIC) cut-off values

MIC cut-off values are species-specific. Setting limits at the genus level has considerable consequences.

Role of genes with homology to amR genes in databases may be difficult to verify due to lack of methodology and interpretation criteria.

It can be difficult to distinguish between **intrinsic** and **acquired** resistance.

Location (rather than the presence) of acquired antibiotic resistance markers (ARMs) is important for the safety assessment. Transmissible antibiotic resistance is considered to pose a much greater risk than non-transmissible resistance.

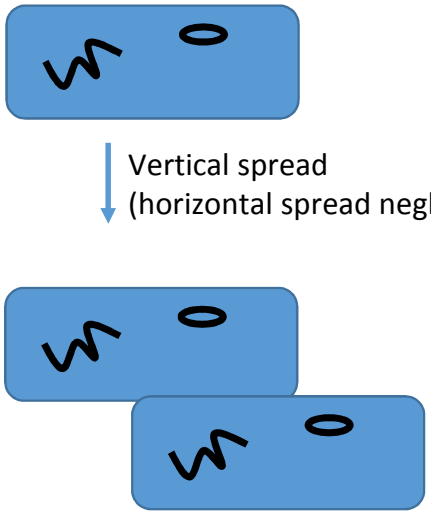
Minimal inhibitory concentrations (MIC) distributions follow the *Bacillus* species for several antibiotics

Antimicrobial agent	Species	Distribution (%) of MIC's															Tentative ECOFF	MIC50	MIC90
		0.016	0.03	0.06	0.12	0.25	0.5	1	2	4	8	16	32	64	128	256	>256			
Ampicillin	*B. paralicheniformis*				4		21	46	18	11									1	4
	B. licheniformis				6	14	37	31	11										0.5	2
	B. megaterium		3	3	45	45	3												0.12	0.25
	B. velezensis		46	46	8														0.06	0.06
	B. amyloliquefaciens		33	67															0.06	0.06
Gentamicin	*B. paralicheniformis*						93	7										1	0.5	0.5
	B. licheniformis						74	26										1	0.5	1
	B. megaterium						100											0.5	0.5	0.5
	B. velezensis						100											0.5	0.5	0.5
	B. amyloliquefaciens						100											0.5	0.5	0.5
Kanamycin	*B. paralicheniformis*								50	50								4	2	4
	B. licheniformis								43	49	9							8	4	4
	B. megaterium								100									2	2	2
	B. velezensis								100									2	2	2
	B. amyloliquefaciens								100									2	2	2
Streptomycin	*B. paralicheniformis*									11	36	39	14					32	16	32
	B. licheniformis									6	31	51	11					32	16	32
	B. megaterium						45	52	3									2	1	1
	B. velezensis						4	35	46	12	4							8	2	4
	B. amyloliquefaciens								67	17	17							8	2	8
Tetracycline	*B. paralicheniformis*								4	93	4							8	4	4
	B. licheniformis					17	66	11		3	3							8	0.5	1
	B. megaterium					45	45	10										1	0.5	0.5
	B. velezensis					4	8	4	4	15	54	12						16	8	16
	B. amyloliquefaciens					17				17	50	17						16	8	16
Erythromycin	*B. paralicheniformis*											100						NR	>16	>16
	B. licheniformis					20	34	23				23						1	0.5	>16
	B. megaterium			45	21	28	3	3										1	0.12	0.25
	B. velezensis			46	42	8	4											0.5	0.12	0.25
	B. amyloliquefaciens			17	67	17												0.5	0.12	0.25
Clindamycin	*B. paralicheniformis*											32	68					NR	>32	>32
	B. licheniformis											3	97					NR	>32	>32
	B. megaterium									3	17	14	66					NR	>32	>32
	B. velezensis				8	19	50	23										1	0.5	1
	B. amyloliquefaciens					33	67											1	0.5	0.5
Chloramphenicol	*B. paralicheniformis*										54	39	7					32	8	16
	B. licheniformis									23	9	60	9					32	16	16
	B. megaterium								59	38		3						4	2	4
	B. velezensis							4	81	15								4	2	4
	B. amyloliquefaciens								50	50								4	2	4
Vancomycin	*B. paralicheniformis*						43	57										1	1	1
	B. licheniformis						69	31										1	0.5	1
	B. megaterium					97	3											0.5	0.25	0.25
	B. velezensis						88	12										1	0.5	1
	B. amyloliquefaciens						67	33										1	0.5	1

(Agersø et al, 2018, Appl. Environ. Microbiol.) MIC distribution for nine antimicrobial agents. *B. paralicheniformis* (n = 28), and *B. licheniformis* (n = 35), *B. megaterium* (n = 29), *B. velezensis* (n = 26), *B. amyloliquefaciens* (n = 6). The vertical solid lines indicate the EFSA ECOFF for the genus *Bacillus*. For ampicillin, the EFSA does not define an ECOFF and no other interpretation criteria exist.

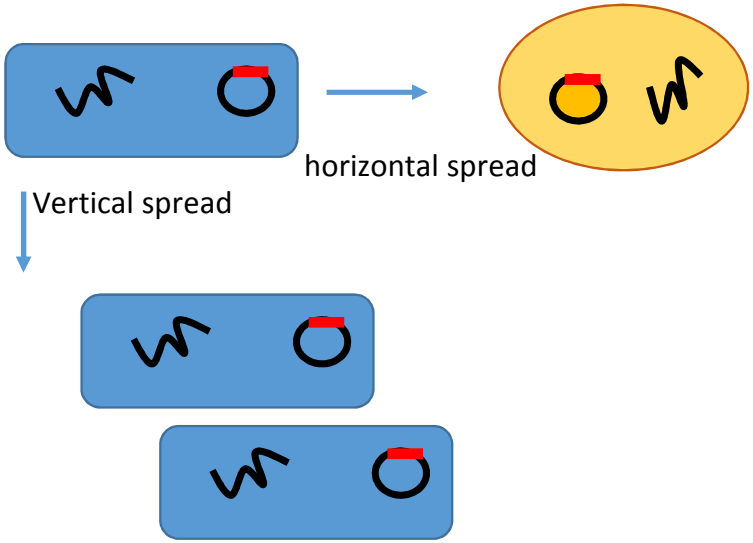
Spread of antimicrobial resistance

Intrinsic: Common trait of a phylogenetic group, (e.g. a species or related species)



Mechanism: missing target, lack of uptake of the antimicrobial
presence of an intrinsic gene

Acquired resistance: Only some strains within a species



Mechanism: AMR gene on mobile element (e.g. a plasmid, conjugative transposon)

Industry recommendations on the requirements for the use of MIC values

MIC cutoff values should be species-specific.

We need guidance on when and how the cutoff values will be updated. What data is required and how frequent will the updates be? We recommend EU funded projects on determining cut-off values for relevant species.

Guidance is required on interpretation of *in silico* findings (spurious homology).

We propose antibiotic resistance should only be considered a risk if it is due to the presence of a clearly defined acquired antibiotic resistance gene (2.2.3) and the genetic context suggests a risk of further transfer.

Presence of DNA

3.2. Presence of DNA from the production strain

Technical challenges for the presence of DNA from the production strain

DNA is not a safety issue.

A single, harmonized limit of detection (LOD) is difficult to work with

- A ”high” LOD may be due to e.g. PCR inhibition, matrix effects or presence of nuclease.

A ”high” LOD does not *per se* indicate presence of DNA.

How will industry deal with the LOD for DNA

Investments in method development and set up of PCR analysis on that sensitivity level need to be done.

For cases where the LOD cannot be met for technical reasons, industry will have to invest in creating documentation.

Customers will have increased focus on DNA and enzyme producers will have to provide additional data.

Industry recommendations on the requirements for presence of DNA

We acknowledge the need of EFSA to have a target LOD.

In line with the guidance document, we recommend taking the presence of PCR inhibitors and nucleases into consideration.

This may affect the LOD achievable for the individual product.