

Contaminations of AMR genes linked to the presence of genetically modified microorganisms in the food and feed chain

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Introduction



Several genetically modified microorganism (GMM) contaminations, viable cells and DNA, notified in commercial fermentation products (e.g., additives, enzymes).





GMM, commonly used by the industry to produce such food/feed products, carried frequently antimicrobial resistance (AMR) genes as selection markers.

These GMM contaminations in the food/feed chain raised therefore **public health concerns** related to **potential horizontal transfer of AMR genes** to other microorganisms, including pathogens, present in gut microbiota and the environment.

Consequently, to guarantee the food/feed safety and traceability, **tools to control and monitor** GMM carrying AMR genes in commercial fermentation products were developed.

Methodology

Using publicly available patents related to genetically modified (GM) bacteria commonly used to produce fermentation products, PCR-based methods specific to **key targets allowing to screen and identify GMM carrying AMR genes** were developed.



(i) qPCR methods specific to screening genetic sequences frequently found in GM bacteria (16S-23S region specific to the *B. subtilis* group, AMR genes (*cat, aadD* and *tet-I*) and pUB110 shuttle vector)
 (ii) qPCR methods specific to unnatural associations between the pUB110 shuttle vector and *Bacillus* sp. from currently known GMM

(iii) PCR methods followed by Sanger sequencing to assess the full-length AMR genes

These methods were applied on **156 fermentation products** collected from the European food/feed market, comprising different brands, forms and sectors.

Results and conclusion

A GMM detection **strategy** was developed.

1st line qPCR screening step

Screening of key genetic sequences (16S-23S region, AMR genes*, pUB110 vector) to detect the potential presence of GMM



Monitoring of GMM contaminations



- Identification of DNA specific to known GMM carrying AMR genes in 70 products
- Isolation of viable GMM carrying AMR genes (aadD) in 4 products
- Detection of AMR genes (*cat, aadD and tet-I*) in
 40 products for which the presence of unknown

→ Suspicion of GMM contaminations if positive signal for at least one target

2nd line qPCR analysis step

Identification of specific GMM** carrying AMR genes



* Full-length of detected AMR genes is evaluated given potential public health concerns,
 ** Suspicion of new GMM if signals from the 1st line are not explained by the 2nd line.

GMM suspected

Based on these results, a **first overview** of AMR genes linked to GMM contaminations in the food/feed chain was established.

References

Fraiture et al., 2020 (Food Control); Fraiture et al., 2020 (Sci. Rep.); Fraiture et al., 2020 (Int. J. Food Microbiol.); Fraiture et al., 2020 (Food Anal. Methods); Fraiture et al., 2021 (Food Control); Fraiture et al., 2021 (Int. J. Food Microbiol.); Fraiture et al., 2021 (Food Anal. Methods); Deckers et al., 2021 (Food Control); Fraiture et al., 2022 (Fermentation).

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