Traceability of antibiotic resistance markers in the industrial production of food enzymes

Aleix Vázquez Pereyra
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Introduction

The aim of this bibliographic review resides in finding the antibiotic resistance genes used as selection markers in industrial production of food enzymes, present and revise the detection methods available, and assess if a real problematic exist regarding accidental food contamination and if they suppose a health risk for the consumer.

A total of 40 references were used in the final version of this review.

Alerts

Table 1. Summary of food and feed alerts on GMM contamination, indicating microorganism, their use and years of the alerts [1] [2].

Microorganism	Years	Use
Bacillus subtilis	2014, 2018, 2019	Vitamin B_2 production
Bacillus amyloliquefaciens	2019, 2020	Alpha-amylase production
Bacillus velezensis	2019, 2020	Protease production
B. amyloliquefaciens	2021	Protease production

Resistance Markers

Table 2. Most used resistance markers according to 241 of *Bacillus* spp. evaluated [3].

Resistance	Gene
KanR-1	*aadD*
KanR-2	Kanamycin phosphotransferase
CmR-1	*cat*
CmR-2	*cat*
TetR-1	*tetL*
TetR-2	*tetC*
AmpR-1	*bla*
AmpR-2	*bla*
EryR-1	Erythromycin ribosome methylation B
EryR-2	Erythromycin ribosome methylation C
BleoR	*ble*
SpecR	Spectinomycin resistance gene
ThioR	Thiostrepton resistance gene

References:
[1] Paracchini V, Petrillo M, Reiting R, Angers-Loustau A, Wahler D, Stolz A, Schönig B, Matthies A, Bendiek J, Meinel DM, et al. 2017. Molecular characterization of an unauthorized genetically modified Bacillus subtilis production strain identified in a vitamin B2 feed additive. Food Chem. 230:681–689.
[2] Fraiture MA, Gobbo A, Papazova N, Roosens NHC. 2022. Development of a Taxon-Specific Real-Time PCR Method Targeting the Bacillus subtilis Group to Strengthen the Control of Genetically Modified Bacteria in Fermentation Products. Fermentation. 8(2):78.
[3] Fraiture MA, Deckers M, Papazova N, Roosens NHC. 2020. Are antimicrobial resistance genes key targets to detect genetically modified microorganisms in fermentation products? Int J Food Microbiol. 331.

Detection Methods

Table 3. Summary of the detection methods evaluated

Method
PCR
qPCR
Multiplex PCR
DNA Walking
Shotgun metagenomics high-throughput sequencing
DNA Microarray

Conclusions

- GMM food contamination is a rising problem that should be adressed.
- Use of antibiotic resistance genes as selection markers should be avoided, research must be focused in finding better ways to produce this products.
- Most of the food enzyme information are behind confidenciality, difficulting research.
- Actual methods are in early adoption, needing more time to fully develop.