GMM DETECTION STRATEGY AND ITS IMPLEMENTATION ON THE EUROPEAN FOOD AND FEED MARKET

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INTRODUCTION

In the food/feed chain, the presence of genetically modified organisms (GMOs), including microorganisms (GMMs), is strictly regulated by European legislation (EC/1830/2003). No GMMs are currently authorised for human/animal consumption. However, unexpected GMM contaminations, both viable cells and DNA, were reported in commercial microbial fermentation products (additives, enzymes, flavourings and supplements). Public health concerns were also raised because GMMs commonly used by the industry to produce microbial fermentation products frequently harbour antimicrobial resistance (AMR) genes as selection markers. Given the potential horizontal transfer of AMR genes to other microorganisms, including pathogens, present in gut microbiota and the environment, GMM contaminations represent a potential risk of future treatment failure. To guarantee food/feed safety and traceability, the competent Belgian authorities asked for tools to be developed to control and monitor GMMs in commercial microbial fermentation products. However, as opposed to GMOs authorised for human/animal consumption, key genetic sequences from GMMs are confidential and no method specific to GMMs is provided to enforcement laboratories.

METHODOLOGY

Publicly available patents related to genetically modified (GM) bacteria reported by the European Commission as being commonly used to produce microbial fermentation products were collected and analysed to identify key targets frequently found in GMMs. On this basis, qPCR methods specific to these key targets (B. subtilis group, AMR genes, pUB110 shuttle vector), as well as conventional PCR methods followed by Sanger sequencing to assess the presence of the full-length AMR genes, were developed. The characterisation of unnatural sequence associations between the pUB110 shuttle vector and Bacillus sp. from currently known GMMs was performed by either whole-genome sequencing (if possible, microbial isolation of the GMM strain) or DNA walking. On this basis, qPCR methods specific to currently known GMMs were developed. The performance of all these PCR methods was assessed as specific and sensitive, complying with the

Minimum Performance Requirements for Analytical Methods of GMO Testing of the European Network of GMO Laboratories. These methods were applied to 70 microbial fermentation products collected from the European food/feed market, comprising different brands, forms and sectors.

RESULTS

A GMM detection strategy was proposed. First, the potential presence of GMMs is screened using qPCR methods targeting key targets frequently found in GM bacteria. These key targets include a 16S-23S region specific to the B. subtilis group, the pUB110 shuttle vector and 3 AMR genes conferring resistance to chloramphenicol (cat), kanamycin (aadD) or tetracycline (tet-l). In addition, given associated public health concerns, the presence of full-length AMR genes detected by qPCR is assessed by conventional PCR followed by Sanger sequencing. If a positive signal for at least one key target specific to the vector and AMR genes is observed, the presence of GMMs is suspected. To prove it, qPCR methods specific to the 4 currently known GMMs are used.
To monitor GMM contaminations on the European food/feed market, these methods were applied on 70 commercial microbial fermentation products. Among the 70 samples, GMM contaminations were observed in 31 samples, including 4 samples with viable GMMs, leading to 15 RASFF notifications. Moreover, the presence of unknown GMMs was suspected for 5 samples, as signals from screened key targets were not explained by the GMM-specific qPCR methods.

DISCUSSION

A detection strategy targeting GM bacteria was successfully developed to determine their potential presence and to identify specific GMMs. Numerous GMM contaminations were observed using this strategy, highlighting the importance of controlling the presence of GMMs in the food/feed chain. In the event signals from the screened key targets are not explained by known GMMs, the presence of unknown GMMs is suspected. To prove this, additional analysis is needed. If the GMM strain is isolated by classical microbiology, whole-genome sequencing is applicable (e.g. GM B. subtilis producing vitamin B2, GM B. velezensis producing protease). Microbial isolation can however be challenging, especially with unknown GMMs. To overcome such bottlenecks, a culture-independent approach is possible, such as DNA walking if a minimum of prior knowledge is available (e.g. GM B. amyloliquefaciens producing alpha-amylase, B. amyloliquefaciens producing protease). Otherwise, shotgun metagenomics is a promising alternative. In the context of enforcement, several developments/optimisations are however needed to detect GMMs in the food/feed chain.

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