WHOLE-GENOME SEQUENCING ANALYSIS OF FOOD ENZYME PRODUCTS REVEALS CONTAMINATION WITH GENETICALLY MODIFIED MICROORGANISM OF RELATED ORIGIN.

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INTRODUCTION

Genetically modified microorganisms (GMM) are frequently employed for the manufacture of microbial fermentation products, such as vitamins and enzymes. The presence of GMM, viable cells and/or their DNA, in the final product destined for consumption is regulated by European (EU) legislation, and is currently prohibited. Nevertheless, GMM contaminations have repeatedly been reported in diverse commercial microbial fermentation product types, which raises serious public health concerns, especially since GMM often carry antimicrobial resistance (AMR) genes.

This study focuses on commercial food enzyme (FE) products that were found to be contaminated with living Bacillus GMM material encoding a protease enzyme, which was reported at the EU level via the Rapid Alert System for Food and Feed (RASFF) notifications. The aim of this study was to perform an in-depth genomic characterisation and phylogenomic comparison of these GMM strains, isolated by classical microbiology, using a hybrid approach combining both short-read and long-read whole-genome sequencing (WGS).

METHODOLOGY

Following a positive qPCR result for the Bacillus protease encoding gene, culturing experiments were performed with FE products, which allowed us to obtain GM Bacillus isolates from products from four different brands. Genomic DNA extracts of 10 isolates were subjected to both short-read Illumina and long-read Oxford Nanopore Technology (ONT) WGS to employ a de novo hybrid assembly strategy and an additional bioinformatics analysis. To investigate the relationship between the GM isolates from different samples, short-read based Single Nucleotide Polymorphism (SNP)-phylogenomic and SNP typing analyses were conducted. Additionally, whole-genome alignments were performed to detect large-scale genomic rearrangements.

RESULTS

We found that the GMM, classified as Bacillus velezensis, primarily harbours the transgenic construct, with a single copy of the wild-type derived protease encoding gene, on a free high-copy pUB110-derived plasmid carrying AMR genes. Furthermore, the recombinant plasmid is partly present in the form of linear plasmid multimers, probably due to a disturbance of the normal plasmid replication mechanism, and it is likely only transiently integrated into the chromosome.

The SNP-phylogenetic analysis demonstrated that the isolates cluster together monophyletically, with bootstrap support of 100 %, and that they differ from each other by at most 21 SNPs. Taken together, these results indicate that the GM isolates are genetically almost identical, and probably originate from the same parental GMM strain.

DISCUSSION

To our knowledge, this study is the first to demonstrate the potential of an SNP-phylogenetic approach for GMM source-tracking. Additionally, our results show the added value of combining short- and long-read whole-genome sequencing in a hybrid assembly approach for resolving the complex genomic constitution of this GMM.

These findings raise serious food safety and public health concerns, which are emphasised by the fact that the AMR genes are harboured on a free plasmid, increasing the risk of AMR spreading via horizontal gene transfer. More stringent control measures, as well as the development of the necessary technical expertise, are required to ensure the quality and safety of microbial fermentation products.

The results also highlight the need for thorough genetic characterisation of GMM since the location of the transgenic construct, and associated AMR genes, is important information to consider in a safety evaluation. However, it is not always possible to obtain isolates for WGS. A potential alternative is a culture-independent approach such as shotgun metagenomics, although this method may make it difficult to associate a GMM plasmid with its host.