A SHOTGUN METAGENOMICS APPROACH TO DETECTING AND CHARACTERISING UNAUTHORISED GENETICALLY MODIFIED MICROORGANISMS IN MICROBIAL FERMENTATION PRODUCTS

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INTRODUCTION

The presence of a genetically modified microorganism (GMM) or its DNA, often harbouring antimicrobial resistance (AMR) genes, in microbial fermentation products on the market is prohibited by European regulations. GMMs are currently screened for through several specific real-time polymerase chain reaction (qPCR) assays, each targeting an AMR gene or expression vector. When the presence of a GMM is suspected, this is confirmed by targeting the specific GM constructs/events. However, this is only feasible when the GMM has been previously characterised, and many GM constructs are still unreported. Such characterisation by whole-genome sequencing requires that an isolate be obtained, which is not straightforward, especially due to the modifications in the genome that can often impact culturing conditions. Moreover, qPCR assays used for the first screening have not yet been developed for all AMR genes or expression vectors potentially present in GMMs. Culture-independent open approaches could offer a solution to these current bottlenecks in detecting and identifying GMMs in microbial fermentation products.

METHODOLOGY

We developed a strategy based on shotgun metagenomics to be used for the detection and characterisation of GMMs in microbial fermentation products. This method is a culture-independent and open approach based on the sequencing of all genetic material in the sample, without prior knowledge, potentially revealing all information in one test. It could deliver proof of the presence of a GMM in a microbial fermentation product by detecting AMR genes and vectors in the sequenced DNA, species from which the DNA originates, and unnatural associations present in the GMM genome. To deliver a proof of concept, we selected three samples, representative of scenarios that could potentially occur in a routine setting, i.e. a previously analysed sample containing a GMM Bacillus subtilis overproducing vitamin B2, isolated and fully characterised at that time (RASFF 2014.1249), a sample positive for some qPCR markers but for which no isolate could be obtained, and a sample with no GMM contamination. The short and long-read sequencing technologies were compared for their performances (Illumina, MinION, Flongle). An appropriate data analysis workflow was considered, depending on the sequencing technology.

RESULTS

Our results deliver a proof-of-concept for a shotgun metagenomics approach as a viable alternative method for detecting and characterising a GMM present in a microbial fermentation product without the need for isolation or enrichment. In our workflow, the prediction of the presence of a GMM was based on the simultaneous detection of AMR genes or vectors in species previously described as common GMM producers, and the encountering of unnatural associations in the genome. For both GMM-contaminated samples, we were even able to detect more AMR genes than detected with the currently developed qPCR assays. We were also able to identify the main species (B. subtilis) and detect unnatural associations in the genome, confirming that indeed a GMM was present in the fermentation product. The results obtained with Illumina and MinION sequencing were equally satisfying, leading to the detection of all genes of interest and unnatural associations. Flongle sequencing allowed species identification and detection of the genes of interest. Due to the degraded nature of the DNA extracts of our samples, it has not yet been possible to demonstrate the full potential of the long reads; this requires further optimisation.

DISCUSSION

This proof-of-concept study delivered a novel method of detecting GMMs in food/feed products using shotgun metagenomics, by uncovering unnatural associations linked to the presence of typically used AMR genes, as well as identifying the species. Altogether, our method was able to achieve the same information as obtained with the currently used standard methods. However, all these analyses were performed at once, thereby saving time. Our method even extends the characterisation of the GMM, such as detecting the presence of AMR genes for which no qPCR methods have yet been developed, and enabling identification to species level, possibly even when multiple species are present. This method also enables description of previously unknown unnatural associations that could lead to the development of new event-specific qPCR methods. Therefore, this approach would fit within the workflow used by enforcement laboratories when the detection of DNA and qPCR screening prompts the suspicion of the presence of an unknown GMM, when no isolate could be obtained. However, currently and until appropriately validated, it would instead be used as an orientation step, requiring confirmation of the findings by PCR and/or Sanger sequencing.