Safe strain lineage concept for risk assessment – proof of principle with 2 lineages

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INTRODUCTION

The safety of new microbially-derived food enzymes must be carefully assessed before their introduction on the market. The safety of microbial enzyme preparations is evaluated on a case-by-case basis by checking the following points: 1) the intrinsic safety of the enzyme, 2) the safety of the production process, and 3) the safety of the production organism. The safety of the production strain is the key element of the assessment. The term 'safe strain lineage' refers to a group of related strains derived from a single parental strain, where the safety of the derived products was shown via systemic and genetic toxicity studies. Once the safe strain lineage has been established, additional well-characterised genetic modifications that do not give rise to safety concerns can be applied without the need for additional toxicological studies. Two strain lineages are discussed here, both starting from the isolate of a well-documented, non-pathogenic and non-toxigenic microorganism – either Bacillus subtilis (B. subtilis) or Kluyveromyces lactis (K. lactis), followed by strain improvement via a combination of random mutagenesis and targeted genetic modifications.

METHODOLOGY

DSM developed a B. subtilis and a K. lactis lineage from well-documented non-pathogenic and non-toxigenic isolates and performed strain improvement via a combination of random mutagenesis and targeted genetic modifications. All production strains were thoroughly characterised by whole genome sequencing. The safety of several strains of the DSM strain lineages was investigated by performing packages of toxicity studies with three food enzymes produced by strains within the DSM B. subtilis lineage and four food enzymes produced by strains within the DSM K. lactis lineage. Each package consisted of two in vitro genotoxicity studies and a 90-day oral toxicity study in rats. In addition, the potential toxigenic activity of DSM's B. subtilis strains was assessed by performing cytotoxicity studies with culture supernatants of the strain fermentation broth.

RESULTS

Whole genome sequencing and subsequent bioinformatics data analysis of the production strains confirmed the expression cassettes' integrity and the correct integration and deletion of genes, the absence of plasmid DNA and the absence of antibiotic selection markers used during strain construction. As demonstrated in cytotoxicity studies, the introduction of strain modifications via either classical mutagenesis or genetic engineering did not lead to toxicity of the DSM B. subtilis strain supernatants towards CHO epithelial cells, suggesting that none of the introduced mutations lead to the formation of toxic peptides with cytotoxic properties. For all tested enzyme preparations, the NOAEL in the rat 90-day oral toxicity study was at the highest dose level tested, indicating the enzyme preparations produced by the two strain lineages were non-toxic. In vitro genotoxicity assays for all enzyme preparations indicated that the products were non-genotoxic.

DISCUSSION

Through the data presented here, DSM has established two safe strain lineages of food enzyme production strains. The use of the safe strain lineage approach enables waiving of toxicity studies on known food enzymes produced with strains from this lineage, as long as the modifications introduced are well characterised and do not give rise to safety concerns. In contrast to targeted genetic modification, classical mutagenesis introduces mutations in a random manner and most of the acquired mutations are single-nucleotide substitutions. Classical mutagenesis will only modify what is already present in the organism. It cannot introduce new genes and is not known to spontaneously create sequences of concern in a safe organism. In addition, whole genome sequence analysis has seen rapid developments in recent years, allowing for a detailed characterisation of both random and targeted changes in the genome. Based on the establishment of DSM's B. subtilis and K. lactis safe strain lineages, future strain modifications – whether performed by classical mutagenesis and screening or by targeted genetic engineering – do not warrant new toxicity studies, consequently saving animals and resources.