



## **EXECUTIVE SUMMARY:**

DuPont Industrial Biosciences (IB) is seeking approval for a lactase ( $\beta$ -D-galactoside galactohydrolase, EC 3.2.1.23) for use as a processing aid in dairy processing and in the production of galacto-oligosaccharide (GOS). The enzyme is designated as "CB108 Lactase" throughout the dossier.

CB108 Lactase enzyme preparation is produced by submerged fermentation of *Bacillus subtilis* carrying the lactase gene from *Bifidobacterium bifidum* encoding the wild-type truncated lactase enzyme.

CB108 Lactase enzyme is intended for use in dairy processing for production of lactose reduced dairy products including but not limited to milk, yogurt, cheese, and the production of galacto-oligosaccharides (GOS). The CB108 Lactase is also considered a  $\beta$ -galactosidase because it is involved in the hydrolysis of lactose to galactose and glucose. In dairy processing, the enzyme will convert lactose into GOS and glucose. CB108 Lactase will be able to generate GOS in situ in raw milk or whey even with low lactose content (roughly 5% lactose content in milk), with as benefit to provide a low lactose/lactose free dairy product with reduced total sugars and caloric content in the final dairy product and enable dairy products to contain GOS prebiotic material. The enzyme will also be used in the production of purified GOS.

In all of these applications, CB108 Lactase will be used as a processing aid where the enzyme is either not present in the final food or present in insignificant quantities having no technical function in the final food.

To assess the safety of the CB108 Lactase for use in these applications, Dupont IB vigorously applied the criteria identified in the guidelines as laid down by Food Standards Australia New Zealand (FSANZ) and U.S. Food and Drug Administration (FDA) utilizing enzyme toxicology/safety data, the safe history of use of enzyme preparations from *B. subtilis* and of other lactase enzymes in food, the history of safe use of the *B. subtilis* production organism for the production of enzymes used in food, an allergenicity evaluation, and a comprehensive survey of the scientific literature.

The safety of the food enzyme from *B. subtilis* has been assessed using toxicology studies conducted on earlier strains of the DuPont *B. subtilis* Safe Strain Lineage. The most suitable standard package of toxicological tests from the Safe Strain Lineage was identified to support the safety of the food enzyme object of the current dossier. The toxicological tests showed the following results:

- Acute oral toxicity test: not acute toxic under the test condition. The oral LD<sub>50</sub> for in female rats was greater than 5000 mg/kg bw/day
- Ames test: no mutagenic activity under the given test conditions
- Chromosomal aberrations: no clastogenic activity under the given test conditions
- 90-day oral toxicity on rats: The No Observed Adverse Effect Level (NOAEL) is 1000 mg total protein/kg bw/day, which is the high dose in the study. This NOAEL is equivalent to 1,416.4 mg total organic solids (TOS)/kg body weight/day.

Based on a conservative assumption and a highly exaggerated value consumption data, the NOAEL still offers a 630 fold Margin of Safety.

Processing Aid Application  
Lactase



Based on the results of safety studies and other evidence, CB108 Lactase has been demonstrated as safe for its intended applications and at the proposed usage levels. Approval of this application would provide manufacturers and/or consumers with benefits of lowering the manufacturing cost and improving quality of final foods.

Approval of this application would provide manufacturers and/or consumers with benefits of facilitating dairy processing and GOS production, potentially lowering the manufacturing cost, and improving quality of final foods.