

Application to amend the Australia New Zealand Food Standards Code to permit a new genetically modified source organism - *E. coli* K12 strain E997 – for the production of 2'-Fucosyllactose (2'-O-fucosyllactose, 2'-FL).

Executive Summary

Date: 1 June 2021

This application is made by FrieslandCampina Nederland B.V. seeking an amendment to Schedule 26 of the Food Standard Code (The Code) to permit an alternative genetically modified source organism - *Escherichia coli* K12 strain E997 - for the production of 2'-fucosyllactose (2'-O-fucosyllactose, 2'-FL) by fermentation and recognise the applicable specification for 2'-FL published by the European Union (EU).

Schedule S29-5 permits the addition of 2'-O-fucosyllactose (2'-fucosyllactose), as a nutritive substance, to infant formula products. FrieslandCampina produces 2'-FL by microbial fermentation, using the non-pathogenic *E. coli* K12 strain E997, through the coupling of fucose to the galactose moiety of lactose in an α -1,2-linkage. This organism is not currently listed in Schedule 26 as a permitted source organism for 2'-FL.

There are no costs to consumer, industry or governments from this application. Consumers may benefit from the availability of foods containing 2'-FL, a beneficial human milk oligosaccharide, and food manufacturers may benefit from the opportunity to offer such products.

This application will align Australian and New Zealand with the USA and The European Union and has the potential to enhance international trade in respect of both the import and export of fortified food products.

The application describes how the production strain *E. coli* K12 E997 is derived from the non-pathogenic bacterium *E. coli* K12, a host organism widely used to produce many oral and injectable human drugs. All genetic modifications are well documented in filed patents. Extensive purification steps remove viable cells, cell debris, and protein and peptide particles. Extensive qPCR testing demonstrated that no residual DNA from the genetically modified *E. coli* production strain was present in the final 2'-FL product.

The donor plasmid is based on the pUC-plasmid series, and contains, aside from *E. coli* K-12 host derived genes *thyA* and *rcsA*, the *futN* gene encoding for the 1,2-fucosyltransferase of *Bacteroides vulgatus*, a human gut commensal. The host organism is derived from *E. coli* strain K12. It has a defective cell envelope that renders it incapable of colonizing or surviving in the human gut. There is no known pathogenicity, toxicity or allergenicity of relevance to the food. *E. coli* are bacteria that normally inhabit the intestinal tract of humans and other animals. *E. coli* K12 contains no known pathogenic genes (either colonization factors or toxin genes) and is universally recognized as a safe, commercial manufacturing host. *E. coli* K12 is used globally in the commercial manufacturing of products ranging from amino acids and vitamins for foodstuff applications, to recombinant human proteins used in pharmaceutical applications, including protein products used as injectables.

There are no Codex assessments or standards that expressly name the use of 2'-FL as an ingredient in foods.

FrieslandCampina's 2'-FL, produced using *E. coli* K12 strain E997, has received EU novel food approval and achieved GRAS status from the Food and Drug Administration in the U.S. (Glycosyn, LLC and FrieslandCampina Domo B.V. (2017) GRN No. 735: 2'-Fucosyllactose).

2'-FL is already approved for use as in the Code. This application is limited to the approval a new source organism for its production, consequently, the general procedure, level 1 or 2, is the appropriate procedure to be adopted in assessing this application.

The application does not contain confidential commercial information or other information for which confidential treatment is requested.

FrieslandCampina is not the sole manufacturer of 2'-FL. Accordingly, approval of FrieslandCampina's 2'-FL as a food ingredient would not confer an exclusive capturable commercial benefit, *per se*.

However, exclusivity for FrieslandCampina's 2'-FL prepared by microbial fermentation using a genetically modified *Escherichia coli* K-12 containing the gene for alpha-1,2-fucosyltransferase from *Bacteroides vulgatus* is also sought, thereby conferring an Exclusive Commercial Capturable Benefit.