Development of a Fermented Nutritional Probiotic Drink for the Elderly Population

A Scientific Product Development Project

Fanny Lagerström

Independent project • 30 credits
Swedish University of Agricultural Sciences, SLU
Department of Molecular Sciences
NJ Faculty
Agronomy – Food Science
Molecular Sciences, 2025:32
Uppsala, 2025

Development of a Fermented, Nutritional Probiotic Drink for the Elderly Population. A Scientific Product-development Project

Utvecklingen av en fermenterad, näringsrik probiotisk dryck för den äldre populationen. Ett vetenskapligt produktutvecklingsprojekt

Fanny Lagerström

Supervisor:	**Stefan Roos, Swedish University of Agricultural Sciences, Department of Molecular Sciences**
Assistant supervisor:	Ludwig Ermann Lundberg, Swedish University of Agricultural Sciences, Department of Molecular Sciences
Examiner:	Su-Lin Hedén, Swedish University of Agricultural Science, Department of Molecular Sciences

Credits:	30 credits
Level:	Second cycle, A2E
Course title:	Independent project in Food Science Agriculture
Course code:	EX1028
Programme/education:	Food Agronomist programme
Course coordinating dept:	Department of Molecular Sciences
Place of publication:	Uppsala
Year of publication:	2025
Title of series:	Molecular Sciences
Part number:	2025:32

Keywords:	Prebiotics, Probiotics, Postbiotics, Synbiotics, Psychobiotics, Dietary Fibre, Arabinoxylan-oligosaccharides (AXOS), Gastro-intestinal tract, Nutrition, Fermented dairy, *Bifidobacterium longum*, *Lactococcus lactis*, *Limosilactobacillus reuteri*

Swedish University of Agricultural Sciences
NJ Faculty
Department of Molecular Sciences
Probiotics Unit

Abstract

The aim of this study was to develop a fermented, nutritional probiotic drink with the elderly population being the main target group, because of common health issues related to aging, such as gastrointestinal health and nutritional deficiencies. The probiotic drink was formulated with the addition of the probiotic bacterial strains *Bifidobacterium longum* subsp. *longum* BG-L47, *Limosilactobacillus reuteri* ATCC PTA 6475 and *Lactococcus lactis* 10399. These were co-cultivated in milk-based substrates with and without the addition of arabinoxylan-oligosaccharides (AXOS) as a prebiotic fibre. The strains were evaluated for their individual growth in co-cultivation, viability, ability to reduce pH, metabolite production, bile tolerance, and 5'nucleotidase activity, as well as the products' sensory taste acceptability. The results from co-cultivations suggested synergistic interactions between *B. longum* BG-L47 and *L. reuteri* ATCC PTA 6475, where they showed an improved cell viability in milk-based media with AXOS supplementation. The pH decrease suggested an active fermentation and sensory tests highlighted the potential of this product as well as improvement areas in terms of flavour and acceptability. HPLC analysis showcased the production of short-chain fatty acids, where the content of lactate and acetate were particularly notable, contributing with functional benefits of the product. A risk assessment was performed to confirm microbial, physical and chemical safety during the production until storage stage of the product development. This risk assessment concluded the main concerns of this product is the potential contamination of *Enterobacteriaceae, B. cereus* and *S. aureus* during the production steps. These hazards were controlled with multiple heat treatments as well as acidic environments and cold storage, and hygienic handling of the product. Prior to the sensory analysis a contamination analysis for *Enterobacteriaceae* was performed and no contamination was observed. Overall, this study suggests that the product formulation that is proposed in this report could be a promising foundation for a future fermented, nutritional probiotic drink contributing to improved health in the elderly population.

Keywords: Prebiotics, Probiotics, Postbiotics, Synbiotics, Psychobiotics, Arabinoxylan-oligosaccharides (AXOS), Gastro-intestinal tract, Fermented Dairy product, *Bifidobacterium longum*, *Lactococcus lactis*, *Limosilactobacillus reuteri*

Table of contents

List of tables

List of figures

Abbreviations

Abbreviation	Description
mMRS	Modified Man-Rogosa-Sharpe growth medium
GIT	Gastrointestinal tract
AXOS	Arabinoxylan-oligosaccharides
AX	Arabinoxylan
5’NT	5’nucleotidase
CFU	Colony forming units
SCFA	Short chain fatty acid

Introduction

Background

There has been a growing understanding of the importance of the GIT microbiota for health and disease (Sarita et al., 2025) and how probiotics can play a role in the homeostasis of the microbiota of the GIT. Probiotics have been associated with the prevention of various diseases such as allergies, lactose intolerance, diarrhoea, irritable bowel syndrome, inflammatory bowel disease, hypercholesterolemia and even cancer (Latif et al., 2023). These conditions could be influenced with the help of lifestyle and diet choices. The prevention of chronic diseases is connected to a healthy diet, sleep and regular exercise practices (Oster & Chaves, 2023).
There is also a growing interest in treating symptoms caused by GIT disturbances using prebiotics, probiotics and postbiotics. These types of biotics serve different purposes, but they all have in common that together they can serve in the prevention and treatment of varying diseases (Ji et al., 2023). Prebiotics typically consist of nondigestible dietary fibres that can aid the growth of beneficial bacteria present in the GIT. The prebiotics are prone to fermentation by beneficial gut bacteria while they are hardly influenced by hydrolyzation by enzymes or affected by acids, keeping their properties available for the good bacteria present in the GIT (Sarita et al., 2025). Probiotics are defined as "Live microorganisms that, when administered in adequate amounts, confer a health benefit on the host." (ISAPP, 2018)
During the cultivation of the probiotic bacterial strains, the probiotics produce beneficial active substances, that may classify within the concept of postbiotics (Ji et al., 2023). The postbiotics are not studied on the same level as prebiotics and probiotics, but are often referred to as biogenics, metabolites or metabiotics, which refers to metabolites that are produced or secreted by probiotic bacteria (Aguilar-Toalá et al., 2018). Furthermore, there is a type of biotic called synbiotics, that refers to the technique of combining prebiotics with probiotic bacteria to increase the viability of the bacteria as well as increasing their stability. The synbiotics also contribute to a stimulated growth of the bacteria that are native in the GIT. Another purpose of synbiotics is creating an inhibition of unwanted pathogenic bacteria (Sarita et al., 2025).

There has been an increasing interest in using gut bacteria in the psychological aspect of human health. The interest lies in the impact on human behaviour of the microbiota-gut-brain axis, that is itself governed by several factors, where dietary factors are considered to have a large influence because of metabolites produced by the gut microbiota (Balasubramanian et al., 2024). Bifidobacteria have been connected to an increase in tryptophan levels after treatment in mice models. Tryptophan is a precursor to serotonin (5-hydroxytryptamine) and is an important actor in depression, acting as an antidepressant neurochemical. The tryptophan pathways are connected to emotional activities and endocrine regulation (Altaib et al., 2021).

Introduction to Bacterial Strains

Bifidobacterium longum subsp. *longum* BG-L47

Bifidobacteria are the most prevalent gut bacteria in the intestine of healthy breastfed infants. The abundance of bifidobacteria decreases with the aging of the individual (Arboleya 2016), and this abundance is an important part in regulating the intestinal microbiota, which plays an important part in the human health. It is also important in regulating glycolipid metabolism. One major functionality of the bifidobacteria is their ability to utilize non-digestible fibres as a carbon source, making it possible for them to work together as synbiotics to promote intestinal health (Wang et al., 2022). Bifidobacteria play a part in stimulation of the immune system, as well as being able to produce metabolites such as short chain fatty acids (SCFA). These factors contribute to the homeostasis of the immunological system and the intestinal tract (Lundberg et al., 2024). The strain *Bifidobacterium longum* subsp. *longum* BG-L47 has been shown to have a greater tolerance of bile acid, as well as better mucus adhesion than the commercial strain BB536 of Bifidobacteria. It has also been shown that subsp. BG-L47 has a wide ability to metabolize different macronutrients such as carbohydrates and sugar. Moreover, BG-L47 can stimulate 5'nucleotidase (5'NT) activity in membrane vesicles (MV) from *L. reuteri* DSM 17938. 5'NT is an important enzyme that works in converting AMP into the anti-inflammatory signal molecule adenosine (Lundberg et al., 2024).

Limosilactobacillus reuteri ATCC PTA 6475

Limosilactobacillus reuteri ATCC PTA 6475 has a wide range of applications and benefits. It has been shown in *in-vitro* animal models that the strain has clear anti-inflammatory potential as well as overall gut homeostasis supportive effects (Cruchet et al., 2024). The strain has been shown to be tolerant to bile acid for over 180 minutes without any viability decrease. *L. reuteri* ATCC PTA 6475 evidently supports reactions in gut-brain signalling by inducing oxytocin, especially in animal models for autism spectrum disorders (Cruchet et al., 2024).

Lactococcus lactis 10399

Lactococcus lactis is a lactic acid producing bacteria, which decreases the pH of its surroundings. This makes *L. lactis* a fitting bacterium for preserving food, together with its ability to produce hydrogen peroxide, diacetyl, and organic acids, among others. Another important property of *L. lactis* is its production of exopolysaccharides, that contribute to texture and consistency. Aroma could also be improved by the production of alcohols, aldehydes and ketones (Khelissa et al., 2020). These properties are why *L. lactis* is a common bacterial strain used in fermented dairy product production in the food industry (Chiara et al., 2024). Furthermore, *L. lactis* contributes with a proteolytic activity that aids in the breakdown of milk proteins into peptides that can later become amino acids, which are important for bacterial growth (Liu et al., 2010). The strain *L. lactis* 10399 that

is used in this project is a previously non-described strain of *L. lactis* that was isolated from Swedish traditional "långfil", which is a type of sour milk.

Arabinoxylan-oligosaccharides

Arabinoxylan-oligosaccharides (AXOS) are oligosaccharides that are a hydrolyzed product of arabinoxylans (AX) (Rudjito et al., 2023). Different types of AX are sourced from cereals such as rice and wheat and represent a type of non-digestable fibre (Schupfer et al., 2021). More specifically, AX is sourced from the cell wall material in both the endosperm and the bran parts of the cereals (Kaur & Sharma, 2019). Both AX and AXOS are a source of dietary fibre and act as a prebiotic for the probiotic bacteria. The fermentation of AXOS occurs in different parts of the GIT, which depends on the structural properties of the oligosaccharides (Schupfer et al., 2021).

Ingestion of AXOS have several purposes in combination with probiotic bacterial strains. As mentioned in the background, non-digestible fibre plays an important role in gastrointestinal health, acts as a nutrient source for the colonic bacteria and aids their growth (Norman et al., 2021). It has been shown that AX(OS) play a part in the increase of bifidobacteria abundance (Rivière et al., 2014). *B. longum* subsp. *longum* has been shown to metabolize AX(OS) to a greater extent than *B. longum* subsp. *infantis* because of the requirement of complex enzymes such as glycoside hydrolazes (GH). These enzymes play a part in breaking glycosidic bonds in order to create products that are more metabolizable. This enzyme is considered to have a higher expression in *B. longum* subsp. *longum* than is in *B. longum* subsp. *infantis*, which is more specialized to metabolise breast milk oligosaccharides (Calvete-Torre et al., 2023).

Application in Deficiencies Among the Elderly and General Health Declines

It is found that the proportion of bifidobacteria decreases with age during childhood, and stabilizes in adulthood. However, when aging, the bacterial population starts to decline again (Arboleya et al., 2016). The elderly population has been recognized to suffer from several issues regarding nutritional deficiencies and declining health. These factors contribute to poorer quality of life and ultimately increased mortality in the elderly population. The aging process contributes to e.g. an increase in colonic transit time, changes in the intestinal microbiota and an altering in gastrointestinal hormones such as ghrelin (Norman et al., 2021), that is partly responsible for appetite signalling in the human body (Müller et al., 2015). Furthermore, it is well known that different metabolic and cognitive disorders are in higher prevalence among the elderly.

Probiotics has been evaluated for treatment of e.g. obesity, diabetes, digestive disorders, inflammatory and cardiovascular diseases (Sarita et al., 2025). *B. longum* has been evaluated for amelioration of irritable bowel syndrome (IBS), where it has shown effectiveness in relieving its symptoms including abdominal pain, diarrhoea, distress and bloating. Type-2 diabetes is a common metabolic disease among the elderly and individuals suffering from obesity and a poor diet. Probiotics can potentially act as modulators of gut hormones and play a role in gut homeostasis. These regulatory abilities can potentially aid in the resistance to insulin which is the most prevalent cause for type-2 diabetes (Sarita et al., 2025). In connection to diabetes, obesity is also commonly discussed in the subject of gut microbiota changes. Studies have shown that a lower level of bifidobacteria is associated with a higher prevalence of enterobacteria and *Staphylococcus* species in children suffering from obesity. Correlating to this, it has also been shown that mothers that has gained a significant amount of weight during pregnancy had a decrease in bifidobacteria in their babies (Arboleya et al., 2016).

There is some research addressing how probiotics could act in recovery of muscles, which is believed to have a connection to release of SCFA and their role in supporting glycogen levels. Glycogen aids in protein absorption, which could potentially help in the recovery and repair of muscles. Furthermore, there are studies showing that combining a fibre rich diet and probiotics reduces recovery times after an intense workout, as well as alleviating gastrointestinal issues and psychological stress (Shirkoohi et al., 2025).

Objectives of the project

The objective of this project was to develop a fermented and nutritional drink, characterize features of potential importance for its probiotic activity and functionality in term of bacterial growth, as well as evaluate the sensory profile of the product. The project ultimately aimed to primarily support the elderly population in fibre degradation.

Methods

Strain Selection

B. longum BG-L47 was selected as the strain of bifidobacterium as it is a proprietary strain of BioGaia and has been shown to have a symbiotic/synergistic relationship with *L. reuteri*. From this former research, the strain *L. reuteri* ATCC PTA 6475 was chosen as an interesting strain for its properties and potential synergy with *B. longum* BG-L47.

L. lactis 10399 was chosen for its proteolytic activity and ability to produce lactic acid as well as for being a potent exopolysaccharide producer, important for improving texture of the product. The ultimate strain selection was made after a co-cultivation experiment together with *L. reuteri* ATCC PTA 6475, where this strain showed the most satisfactory results in repeated analysis of bacterial density.
From this point onward the bacterial strains will be referred to as *B. longum*, *L. reuteri* and *L. lactis*, if not otherwise stated.

Propagation of Bacterial Strains and Culture Conditions

The bacterial strains were propagated from frozen glycerol stock samples into fresh Man-Rogosa-Sharpe (MRS) broth (Oxoid). *B. longum* was incubated anaerobically for 48h and reinoculated in new MRS broth for 24h. The *L. lactis* and *L. reuteri* strains were incubated anaerobically for 24 hours and re-inoculated for 24 hours. The bacterial cultivations were centrifuged at 4000 rpm for 10 min to separate bacteria from the supernatant. The supernatant was removed and 1 mL of sterilized glycerol (15% w/w) stock was added to the bacterial pellet and stirred using a pipette (see full glycerol stock recipe in appendix 4). The solution was transferred to a freezer vial and placed in a -70℃ freezer.

Preparation of Growth Media

MRS and M17 Substrate

For the propagation and cultivation of *B. longum* and *L. reuteri*, MRS broth was prepared according to the manufacturer's instructions and autoclaved at 121℃ for 15 minutes. Likewise, M17 broth was used for the propagation and cultivation of *L. lactis*.

Modified MRS Substrate

The modified Man-Rogosa-Sharpe (mMRS) media used for the co-cultivation experiments of the bacterial strains was optimized for the growth of *B. longum*, *L. reuteri* and *L. lactis*. The media was modified to utilize arabinoxylan oligosaccharides (AXOS) as the main carbon source, replacing the glucose in the original MRS formulation. The peptone and meat extract that is present in regular MRS substrate was replaced with yeast peptone and yeast extract and lecithin was added as well for the addition of phospholipids. The pH was set to 5.8 using HCl (5M). See appendix 1 for full media recipe.

mMRS Food Grade

For preparation of cultivations planned for the sensory analyses, a food grade substrate was designed to create a fully safe and edible product. To create a food grade substrate, the standard MRS recipe was modified by using special food grade ingredients***; where when necessary, some ingredients were substituted on a molar mass basis to meet the food grade criteria. The pH was adjusted to 6.3 for *B. longum* and *L. reuteri* and 7.17 for *L. lactis* to create favourable growth conditions. The adjustment of pH was performed using distilled vinegar (12%). L-cysteine HCl was added to benefit the growth of *B. longum*. The full recipe of the mMRS food grade substrate can be found in appendix 2.

Milk- and Buttermilk Substrates

Two of the co-cultivation experiments used various versions of milk- and buttermilk substrates. The buttermilk was autoclaved at 121℃ for 15 minutes and the milk substrates were pasteurized at 90℃ for 20 minutes to avoid protein denaturation and the milk from burning. The milk peptone and AXOS were added in the form of 10 % solutions to sterilize them separately before addition into the milk and buttermilk. The AXOS solution was prepared by dissolving 5 g AXOS powder into 50 ml of distilled water. The milk peptone solution was prepared in the same way to create a 10 % (w)v) solution. These were stirred with a magnetic stirrer. Once dissolved, the two solutions were placed inside an autoclave and were autoclaved at 121℃ for 15 minutes. The solutions were added to relevant substrates to create a 1% concentration of milk peptone and a 2 % concentration of AXOS. Five different substrates were formulated with a base of either milk or buttermilk. The formulation of each substrate type is described in table 1.

Table 1. *Milk- and buttermilk substrates used in co-cultivation experiments. Milk (3% fat) (M), Milk+AXOS (M+Ax), Buttermilk (B), Buttermilk+Peptone (B+P) and Buttermilk+Peptone+AXOS (B+P+Ax)*

Substrate	**M**	**M+Ax**	**B**	**B+P**	**B+P+Ax**
Ingredient formulation	Milk 3%	Milk 3% fat, AXOS (1% or 2%)	Buttermilk	Buttermilk and milk peptone (1%)	Buttermilk, milk peptone (1%) and AXOS (2%)

Cultivation Methods

Before inoculation into the final substrate for cultivation, the strains were propagated in regular MRS media. The *B. longum* was inoculated and incubated anaerobically at 37°C for 48 hours and re-inoculated at the same conditions for 24 hours in new MRS media. The *L. reuteri* and *L. lactis* were inoculated into MRS as well and incubated anaerobically for 24 hours at 37°C. They were re-inoculated into new MRS and incubated for 24 hours again in the same conditions. From these cultivations the strains were inoculated into the relevant substrate for further analysis.

Co-cultivations

Co-cultivation 1

Into each of the five milk- and buttermilk-based substrates described in Table 1, the three bacterial strains were co-cultivated in volumetric proportions of 5:1:1 and 5:1, as outlined in table 2. In all samples, *B. longum* was added at a five-fold higher proportion compared to *L. reuteri* and *L. lactis.* These proportions corresponded to inoculating 500 µL *B. longum* together with 100 µL of *L. lactis* and 100 µL of *L. reuteri* in the 5:1:1 combination, or 500 µL *B. longum* with 100 µL of either *L. lactis* or *L. reuteri* in the 5:1 combination. These volumetric bacterial proportions were added into 10 mL of each serum. The samples were incubated anaerobically at 37°C for 24 hours.

Table 2. *The bacterial strain combinations tested in each milk- and buttermilk substrate and the proportions of each bacterial strain added.*

Combination 1	**Combination 2**	**Combination 3**
B. longum: *L. reuteri*: *L. lactis* (5:1:1)	*B. longum*: *L. reuteri* (5:1)	*B. longum*: *L. lactis* (5:1)

Co-cultivation 2

After the first milk- and buttermilk co-cultivation the same combinations and ratios were tested in this experiment, with the addition of control samples, where the bacterial strains were added separately into each substrate. This created 6 samples in total. All samples were incubated at 37°C at anaerobic conditions for 24 hours.

Co-cultivation 3

After analyses of the previous experiments of both the milk and buttermilk co-cultivations, the substrates with the milk-base were chosen for further analysis. A new experiment was prepared where the samples with only milk (Milk) as well as

milk with added AXOS (Milk+AXOS) was selected for a repeated experiment. The amount of AXOS added to the milk was adjusted to 1% in this experiment. All samples for the experiment are presented in table 3. The Milk samples were made in four replicates, and the Milk+AXOS samples were made in two replicates where a 10% AXOS solution was added into the samples before the incubation. All samples were incubated at 37°C under anaerobic conditions for 24 hours. After the fermentation process, AXOS was added into two of the Milk samples at a concentration of 1 and 2% to try and create flavour and aroma characteristics. These samples were named Milk (+AXOS 1%) and Milk (+AXOS 2%), respectively, where the numbers symbolise the concentration of AXOS added. After the incubation period, a sensory analysis test was conducted within the work group to make selections for the next experiment.

***Table 3**. Complied formulations of all substrates used in the milk co-cultivation.*

substrate	**Milk**	**Milk+AXOS**
Ingredient formulation	Milk (3% fat)	Milk (3% fat), AXOS (1 and 2%)
Strain combination	*B. longum*: *L. reuteri*: *L. lactis*	*B. longum*: *L. reuteri*: *L. lactis*
Proportions	5:1:1	5:1:1

This milk co-cultivation experiment was repeated in a bigger scale with the purpose of applying flavours to the separate samples after the fermentation process. Each substrate co-cultivation was produced in batches of 200 mL to allow for several flavour additions following the fermentation process. The flavours that were added to each substrate type were: vanilla in the form of vanilla extract; strawberry and blueberry in the form of homemade syrups; as well as AXOS in a 20 % solution format.

The AXOS was added after the fermentation as a flavour component at 1% and 2% final concentrations in the different substrates. The strawberry and blueberry flavourings were made from frozen berries with an addition of water and golden syrup. The recipe for the flavourings is shown in table 4. The flavourings were made by cooking the berries with the water and syrup for 30 minutes or until the berries had disintegrated. The mixtures were then strained and put in air-tight containers.

***Table 4.** Recipes of the blueberry and strawberry flavourings added to the milk co-cultivation 3*

Berries	**Water**	**Syrup**
200 grams	100 mL	50 mL

Analytical Methods

pH Analysis

The milk- and buttermilk and milk co-cultivation experiments were analysed by measuring the pH values before and after the fermentation. Measurements were performed using a pH-meter (Mettler Toledo) which was calibrated within the pH range 4-7. In between each measurement, the electrode was washed with distilled water and dried.

Sensory Taste Analysis

Sensory Analysis 1

A sensory evaluation was performed to select which samples would generate the most satisfactory taste characteristics from co-cultivation 3. This analysis was conducted on an untrained panel consisting of five people from the work group. The samples that were evaluated were Milk, Milk+AXOS, Milk (+AXOS 1%) and Milk (+AXOS 2%) from the third co-cultivation. The flavour attributes that were evaluated on a hedonic scale of one to nine were: fermented, sweet and funky, where the sweet attribute corresponded to baked goods, the fermented attribute to yoghurt, and the funky attribute to aged cheese as reference. The evaluation was performed solely on taste attributes of the samples. The formulations for each sample and flavour are shown in table 5.

Table 5. *Sample formulations and flavour additions for sensory analysis 1*

Sample	**Formulation**
Milk	Milk, *B. longum*, *L. reuteri* and *L. lactis*
Milk+AXOS	Milk, AXOS 1% (before fermentation), *B. longum*, *L. reuteri* and *L. lactis*
Milk (+AXOS 1%)	Milk, *B. longum*, *L. reuteri* and *L. lactis*, AXOS 1% (after fermentation)
Milk (+AXOS 2%)	Milk, *B. longum*, *L. reuteri* and *L. lactis*, AXOS 2% (after fermentation)

Sensory Analysis 2

For the second sensory analysis, the same type of sensory test was performed. The same substrates were used where the differing factor was the flavour additions in the samples. All of the flavourings were added after the fermentation process, where each flavouring was added into both types of substrates. The vanilla flavour was added in a 1% concentration, while the blueberry and strawberry flavours were added in 5%. In addition, AXOS was added in two different ways where in the Milk substrate the AXOS was added in a 1% concentration after the fermentation,

creating a total concentration of 1% of AXOS. Into the Milk+AXOS substrate, AXOS was added in the same concentration both pre-fermentation and post-fermentation, creating a total AXOS concentration of 2%. The flavour additions are showcased in table 6, describing the order of each flavour addition to the substrates. This sensory analysis was also performed on the internal work group.

Table 6. Composition of samples used in sensory analysis 2

Substrate	**Pre-fermentation additions**	**Post-fermentation additions**	**Final composition**
Milk	-	Vanilla 1%	Vanilla 1%
Milk+AXOS	AXOS 1%	Vanilla 1%	Vanilla 1%, AXOS 1%
Milk	-	Blueberry 5%	Blueberry 5%
Milk+AXOS	AXOS 1%	Blueberry 5%	Blueberry 5%, AXOS 1%
Milk	-	Strawberry 5%	Strawberry 5%
Milk+AXOS	AXOS 1%	Strawberry 5%	Strawberry 5%, AXOS 1%
Milk	-	AXOS 1%	AXOS 1%
Milk+AXOS	AXOS 1%	AXOS 1%	AXOS 2%

Sensory Analysis 3

As a finishing sensory evaluation, an acceptance test was performed on 13 individuals between the ages of 23-31 where the taste, consistency and overall liking was evaluated on a hedonic scale (1-9). The samples used in this evaluation was selected from the results of sensory analysis 2. Each sample was evaluated separately. Questions were formulated to collect general data and generate context concerning the participants' history with similar products as well as the likelihood of them buying a product like this. A ranking test was performed as well, where the participants were asked to rank the samples from 1-3 where 1 was most liked and 3 was least liked out of the three samples. The samples that were evaluated in this sensory analysis were Milk (vanilla+AXOS), Milk+AXOS (strawberry) and Milk+AXOS (blueberry). The samples were coded as 3, 7 and 12. Table 7 shows each code with their respective samples.

Table 7. *Sample codes and formulations used in sensory analysis 3. Flavourings added after fermentation shown in parentheses.*

Code	**Sample formulation**
3	Milk (AXOS 1% + vanilla 1%)
7	Milk+AXOS (strawberry 5%)
12	Milk+AXOS (blueberry 5%)

The results data was normally distributed (Shapiro-Wilk test). For evaluation of taste, consistency and general liking, an ANOVA with Tukey's multiple comparison test was performed.

Viable Cell Count

To analyse the viability of the bacteria, a viable cell count was performed by serially diluting the samples and plating them on appropriate agar plates. For each bacterial strain a specific agar media was used to cultivate the bacteria selectively and count each bacterial strain in the co-cultivations. The agar plates were incubated at 37 °C under anaerobic conditions for 24 to 72 hours (depending on strain and agar type) after which visible colonies were counted. For the selective enumeration of *B. longum*, selective agars BSM (Bifidobacterium Selective Medium) agar, MRS+Mupirocin agar and Hichrome Bifidobacterium agar were used. To selectively enumerate *L. reuteri* and *L. lactis*, Hichrome Lactobacillus agar and M17 agar were used, respectively.

Optical Density Measurement

The optical density was measured using a Bioscreen instrument to follow the growth of *B. longum*, *L. reuteri* and *L. lactis* separately and in different combinations. The bacterial strains were cultivated in MRS media for 24 at 37 °C anaerobically (?) and re-inoculated into mMRS media for the Bioscreen analysis. The samples evaluated were control samples of each strain where 20 µL of the bacteria was added to 280 µL of mMRS media in triplicates into the honeycomb microplate. For the other samples, the combinations analyzed were *B. longum* with *L. reuteri*, *B. longum* with *L. lactis* and all three bacterial strains in combination. The proportions were 1:1 and 1:1:1, meaning that 10 µL of each bacterial strain was added to all combinations. The OD600 of each cultivation was measured over 24 hours at 37°C and shaking was applied before each measurement every 15 minutes. The samples that were analysed in the first Bioscreen experiment are summarized in table 8.

Table 8. *Proportions of strains inoculated in the first bioscreen experiment*

Proportions	**Strain(s)**	**Sample nr.**
Control (20µL)	*B. longum*	1
Control (20µL)	*L. reuteri*	2
Control (20µL)	*L. lactis*	3
1:1 (10µL:10µL)	*B. longum*: *L. reuteri*	4
1:1 (10µL:10µL)	*B. longum*: *L. lactis*	5
1:1:1 (10µL:10µL:10µL)	*B. longum*: *L. reuteri L. lactis*	6

The second Bioscreen experiment was performed with the same bacterial strain combinations, with a change in proportions of *B. longum* to increase the final numbers of that strain. The proportions that were tested were 5:1:1, 3:1:1, 2:1:1 and 1:1:1 for the combination with all three bacterial strains. For the combinations of *B. longum* with either *L. reuteri* or *L. lactis*, the proportions tested were 5:1, 2:1 and

1:1. The control samples contained the inoculum amounts of 30, 20 and 10 µL of *B. longum* and 10 µL for *L. reuteri* and *L. lactis*.

High-Performance Liquid Chromatography Analysis

To create clear sample solutions that would not clog the HPLC column, the substrates were centrifuged at 11,000 rpm for 15 minutes, and the supernatant was collected. The supernatant was then filtered through a 0.2 µl sterile filter two times before the addition of 50 µl of H_2SO_4 (5M). The samples were frozen overnight to allow precipitation to form. After thawing, the samples were centrifuged at 11,000 rpm for 10 minutes and the supernatant was collected. The supernatant was filtered through a 0.2 µm sterile filter and 500 µl of sample was transferred into HPLC vials and was ready for analysis.

The metabolites that were analysed were the sugars arabinose, xylose and lactose, as well as the organic acids of butyric acid, lactic acid, acetic acid and propionic acid. The organic acids had pre-made reference solutions while the saccharide references were prepared beforehand. The saccharide solutions were prepared by mixing sugar with distilled water to create a 10 g/L solution. This solution was filtered through a 0.2 µm sterile filter and was later diluted to the concentrations of 10, 5, 1 and 0.1 g/L.

Volatile fatty acids and saccharides were analysed by high-performance liquid chromatography (HPLC) on an Agilent 1100 series with a refractive index detector and an ion exclusion column (Rezex ROA - Organic Acid H+, 300x7,80 mm, Phenomenex). The mobile phase that was used was 5 mM H_2SO_4 with a flow rate of 0.6 ml min^{-1}.

Contaminant Analyses

According to the Swedish Food Agency's documentation on required contaminant analyses on fermented milk products, an analysis of *Enterobacteriaceae* was necessary to conduct a sensory analysis. To detect potential contamination with *Enterobacteriaceae*, Violet Red Bile Dextrose (VRBD) agar was used. Each milk co-cultivation (M and M+Ax) was serially diluted four times (to 10^{-4}), and 1 mL aliquots were mixed with the agar and poured into petri dishes to solidify. After solidification, an overlay of soft VRBD-agar was poured over and solidified in room temperature. The solidified plates were incubated at 37°C for 24 h aerobically.

Bile Tolerance Test

An analysis on the bile tolerance of the added *B. longum*, *L. reuteri* and *L. lactis* was performed on the milk co-cultivation samples as well as control samples where the same substrates were inoculated with each probiotic strains separately, to track their separate viability when exposed to 0.3% bovine bile.

Regular MRS broth was mixed with 0.3% bile bovine and autoclaved. Into 10mL of the MRS + 0.3% bile substrate, 100 µL of a fresh cultivation of each sample was added. The samples were incubated aerobically for 0, 30 and 90 minutes, respectively, to analyse the bile tolerance. At each time point, each sample was serially diluted (1/10) six times and plated on MRS agar. The plates were incubated anaerobically at 37°C for 48 hours.

5'Nucleotidase Analysis

An enzymatic test was performed on the co-cultivations using a 5'-Nucleotidase kit (Crystal Chem, Inc) for the quantitative determination of 5'-Nucleotidase activity. The samples that were analysed was both Milk and Milk+AXOS substrates, with all three bacterial strains added as well as the same substrates where each bacterial strain was cultivated separately as controls.

To evaluate the activity of the different phases of the samples, the samples were made as supernatants, cells (pellet from centrifugation) as well as whole fermentate. All samples were made in replicates of three. Both the cells and whole fermentate was diluted 10 times before analysis, and the supernatants were analysed without dilution. The preparation of the samples was performed according to the kit manufacturer's instructions. 180 µl of reagent CC1 was added to each sample well. Each well was then loaded with 10 µl of each sample in duplicates, as well as calibrator, saline solution (0.9%). Two reference samples were added of the un-inoculated substrates as well. This assay started by addition of 90 µl CC2 reagent to each well, and measurements were performed at 3, 5, 9 and 13 minutes.

Product Risk Assessment

A minor risk assessment was conducted on the product from the handling of raw materials to storage of the post-fermentation product. The risk assessment followed all the stages in the product development where each point in the workflow was considered a control point. The stages that were considered were the handling of raw materials such as milk, AXOS and peptone; bacterial addition and cultivations; fermentation; flavouring additions; and the storage of the final product. The assessment used a base of existing literature as well as guidelines from the Swedish food agency in the existing commissions regulations 2073/2005 related to pasteurized milk products.

Results

Co-cultivation Outcomes

Viable Cell Count and Growth Measurements

In the cell viability results, the CFU/mL were converted into a logarithmic scale for ease of interpretation.

Growth Performance of B. longum in mMRS Compared to MRS Media

In a test of the viability and growth of *B. longum* in mMRS media compared to MRS media, both cultivations were plated on regular MRS agar and counted. The analysis of *B. longum* in mMRS media with the added AXOS showed a count of 5.0×10^8. The MRS cultivation generated a value of log 2.0×10^8. This indicates that the AXOS added into the mMRS had a slight positive effect on the cell count of *B. longum* and potentially stimulates the growth of the strain.

Cell Viability in Co-cultivation 1

The first co-cultivation showed that the viability of *B. longum* was the highest in the substrates with buttermilk and peptone, and with buttermilk, peptone and AXOS added. The CFU/mL showed values of 1.1×10^9 and 1.0×10^9 respectively. *L reuteri* showed the highest viability in the substrates with AXOS added to them, where they had a CFU/mL of 3.0×10^8 in the Milk+AXOS substrate and 2.8×10^8 in the buttermilk with added peptone and AXOS. *L. lactis* showed the highest viability in both the milk substrates of 2.3×10^8. The results are summarized in figure 1.

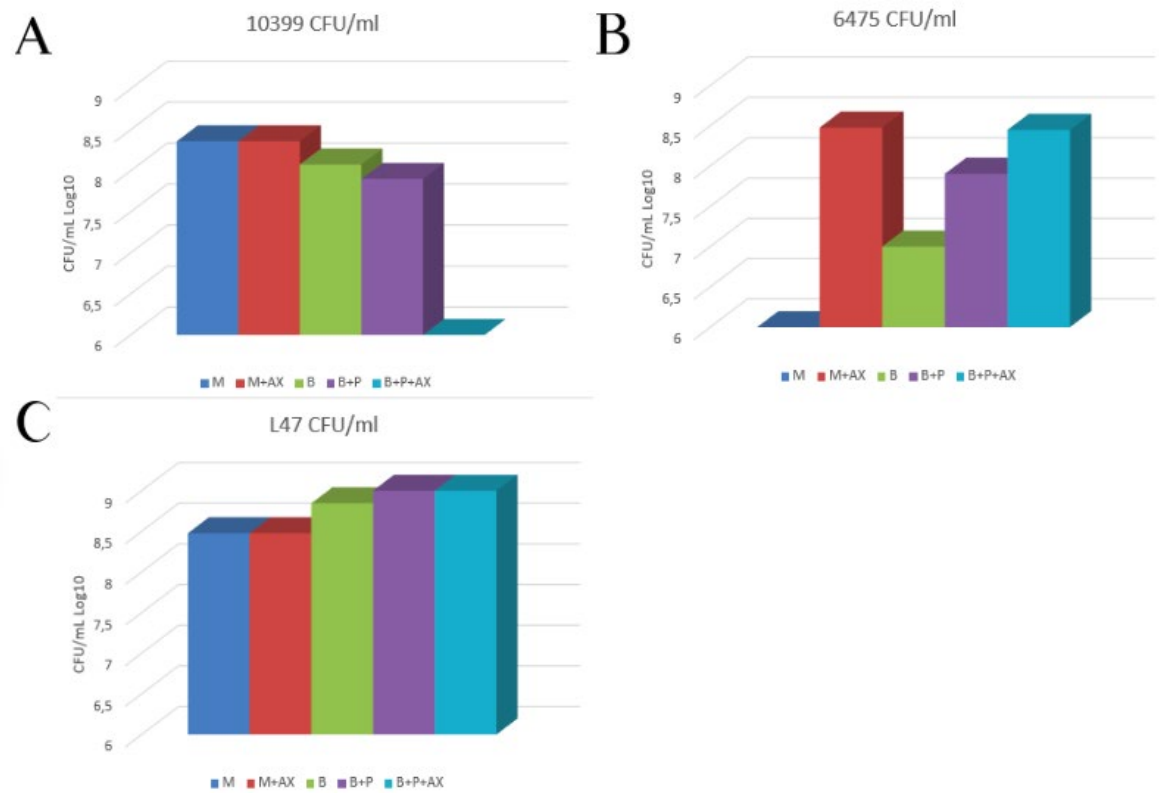


Figure 1. *CFU/mL count of each substrate and bacterial strain presented in a bacterial count (log CFU/mL) scale, where A shows L. lactis, B shows L. reuteri and C shows B. longum. (Abbreviations M, AX, B, and P represent Milk, AXOS, Buttermilk and Peptone respectively)*

Cell Viability in Co-cultivation 2

The second co-cultivation showed *B. longum* had the highest CFU/mL value in the Buttermilk+Peptone substrate where it was added singularly, with a CFU/mL of 2.3×10^9. When comparing which substrate that *B. longum* had the highest viability in combination with *L. reuteri* and *L. lactis*, it was shown that the substrate with only milk and all three strains had the highest CFU/mL count. *L. reuteri* showed the highest viability in the Milk+AXOS substrate, not only in combination with *B. longum* alone, but also in combination with all three bacterial strains with a value of 1.0×10^9. In the same substrate, but with *L. reuteri* added alone, the viability was counted to 1.3×10^8. This indicates that co-cultivation of the strains *B. longum* and *L. reuteri* had a positive effect on their viability in that environment. The *L. lactis* showed the highest viability in the Milk+AXOS substrate as well with a value of 5.9×10^8 but only when grown as a single strain.

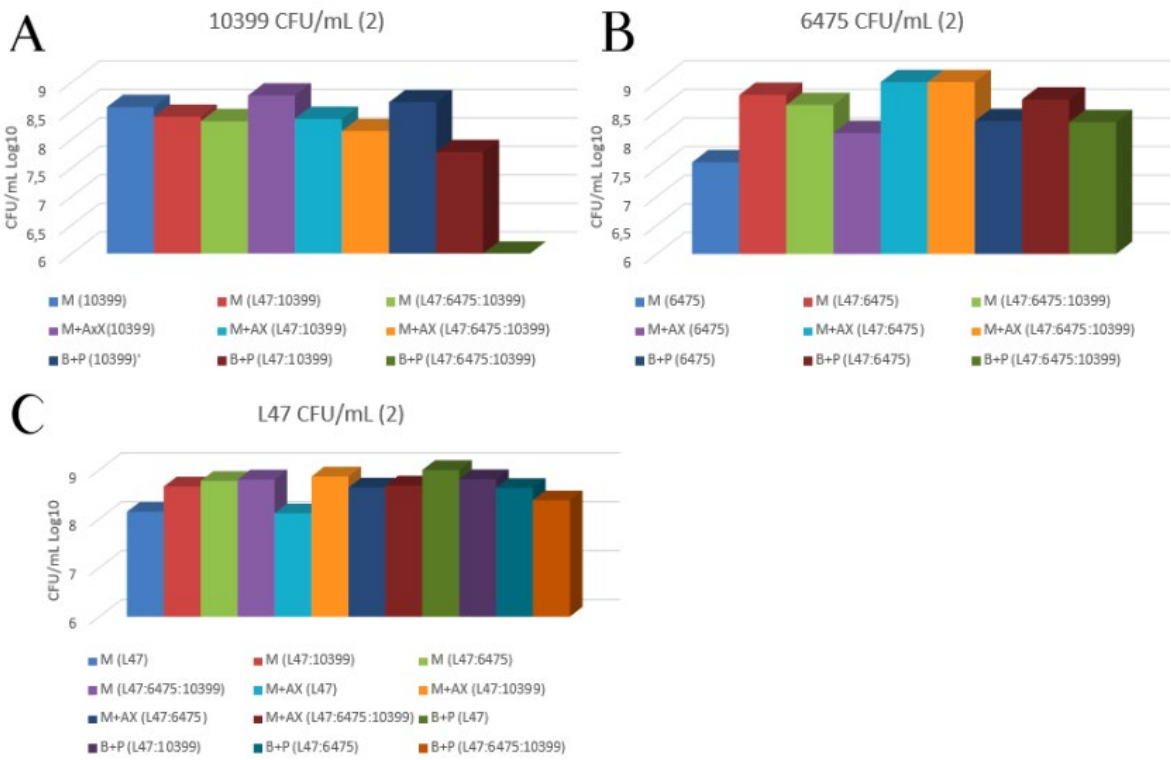


Figure 2. *CFU/mL count of each substrate and bacterial strain presented in a bacterial count (log CFU/mL) scale, where A shows L. lactis, B shows L. reuteri and C shows B. longum. (Abbreviations M, AX, B, and P represent Milk, AXOS, Buttermilk and Peptone respectively)*

Cell Viability in Co-cultivation 3

Co-cultivation 3 consisted of two repeated co-cultivations in the same substrates. *B. longum* generated the highest CFU/mL count in the Milk+AXOS substrate for both co-cultivations with the values 1.6×10^9 and 2×10^9. The *L. reuteri* also portrayed a higher growth in the Milk+AXOS substrate in both co-cultivations where the first co-cultivation showed a value of 1.2×10^8 and the second co-cultivation showed a CFU/mL value of 7.9×10^8. The *L. lactis* was shown to grow equally well in both substrates in both co-cultivation experiments, indicating that addition of AXOS does not noticeably affect cell viability.

Optical Density Measurements

MRS substrate was used in the first bioscreen experiment. All samples, except for the sample with *B. longum* and *L. reuteri* in co-cultivation, showcased a typical bacterial growth curve with an initial lag phase, which was followed by exponential growth and a plateau phase. The sample with *L. reuteri* alone and the sample with all the bacterial strains added in co-cultivation had the highest OD600 values with a peak of 1.543 and 1.52, whereas the sample with *B. longum* alone and *B. longum* in combination with *L. lactis* with a peak of 1.35 and 1.311 followed a similar growth curve; the values suggested that they had moderate growth. The sample with

L. lactis alone had the lowest growth curve with a plateau value around 0.73. This suggests that the conditions in this sample were suboptimal. The sample with *B. longum* and *L. reuteri* showed values of 0 over the entire measuring period in one of the triplicate wells which suggests an error during the measurement. In contrast to this, the two remaining wells generated relevant OD values of just under 1.1. The plotted results for the first bioscreen experiment are shown in figure 3.

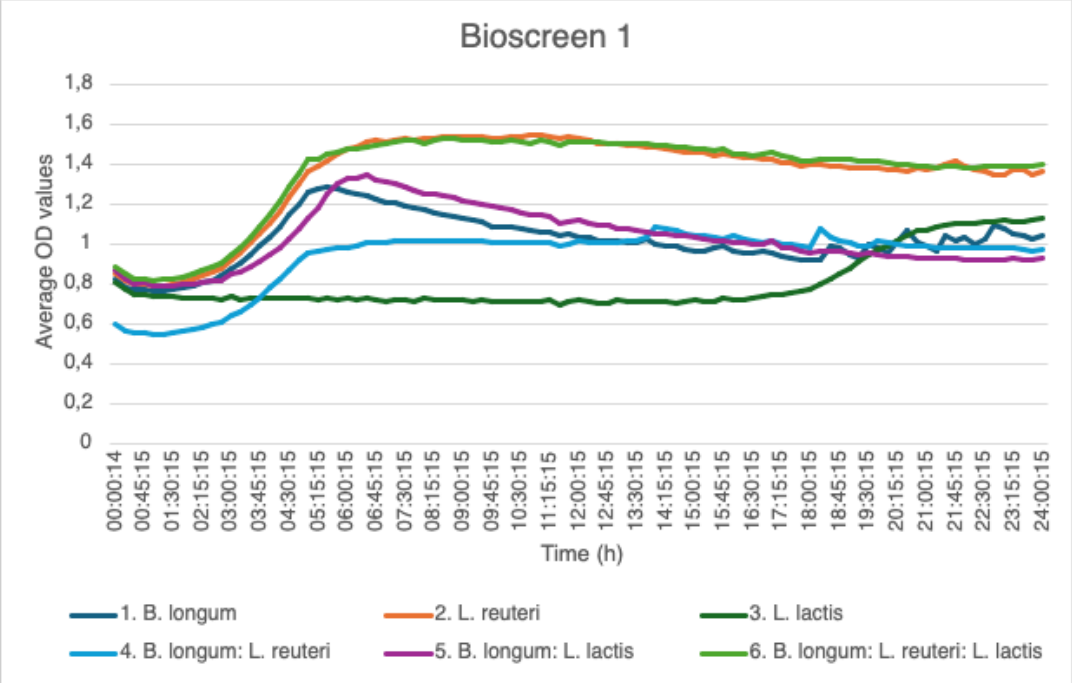


Figure 3. *Bioscreen experiment 1 result presented in OD values that were the average of triplicate wells, except for B. longum and L. reuteri where the mean of duplicates is shown*

In the second Bioscreen experiment the mMRS substrate was used and the results showed varied growth, where several of the samples displayed typical bacterial growth curves. The sample with all three bacterial strains in co-cultivation and the proportion of 5:1:1 exhibited the highest growth with the final OD of 1.59, which indicated a rapid proliferation. The growth curve showed a short lag phase and was followed by a sharp growth curve, plateauing after around 9 hours. The samples with all three strains and the proportion 1:1:1, 2:1:1 and 3:1:1 also showcased strong results with their OD values peaking between 1.47 to 1.52. This indicates that these samples also had a high growth and stabilized at around 8-10 hours. The sample with *L. lactis* added alone showed little to no growth, indicating an error somewhere in the experiment process. Figure 4 shows the plotted average OD values from Bioscreen experiment 2.

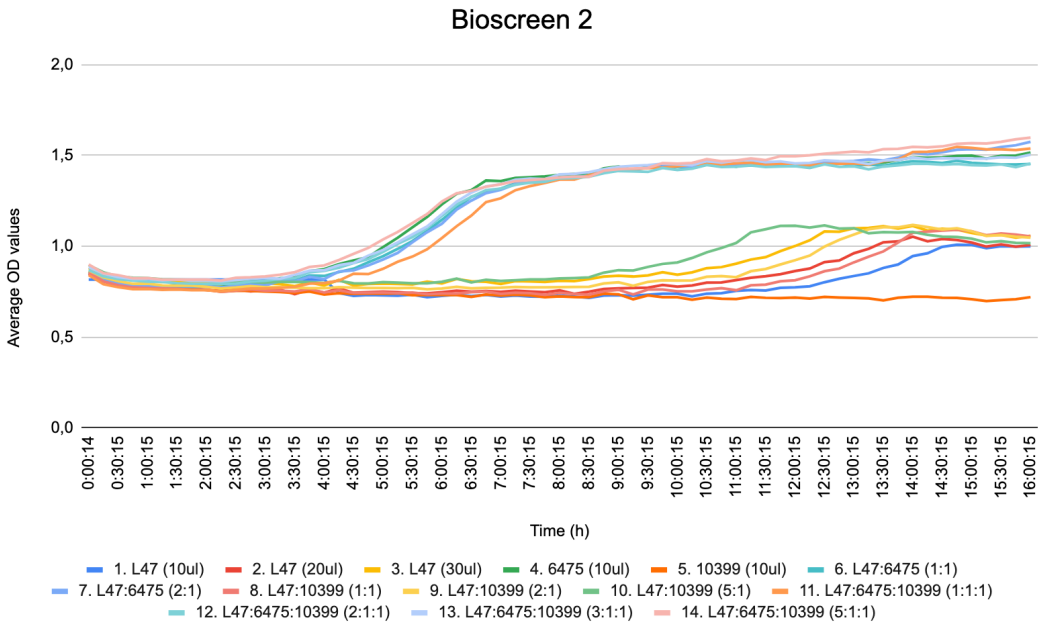


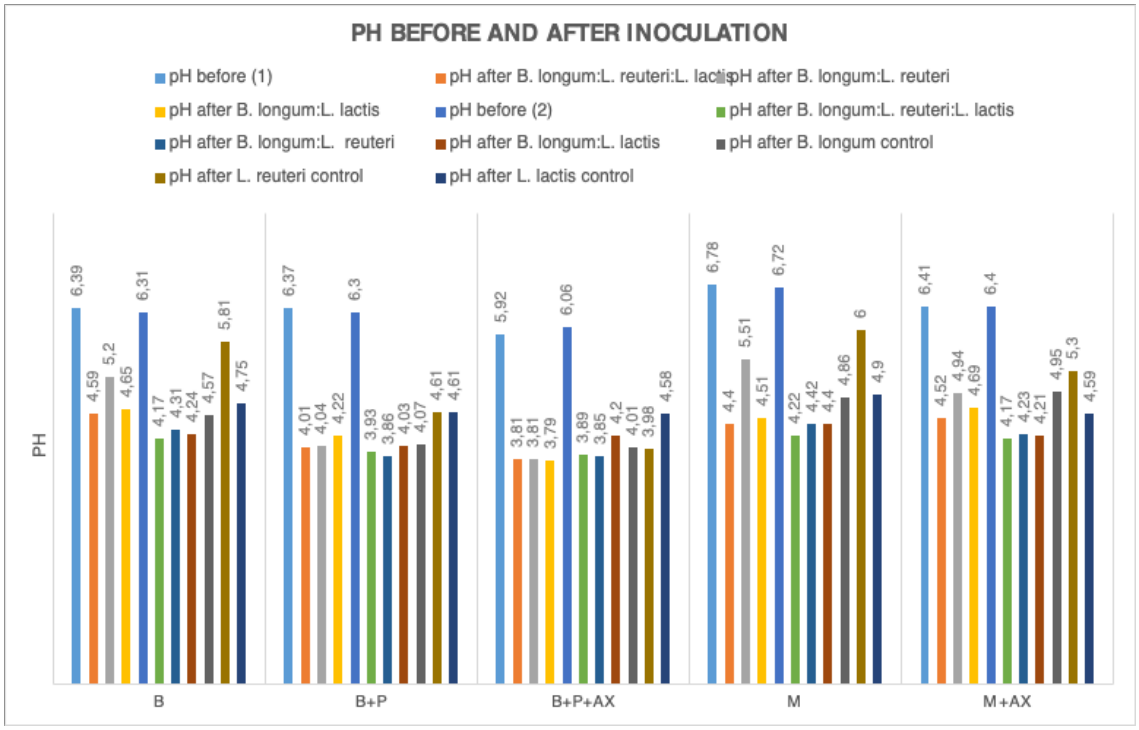
Figure 4. *Bioscreen experiment 2 result presented in OD values that were the average of triplicate wells*

pH Measurements

Co-cultivations 1& 2

The pH values from the first co-cultivation showed that all samples with all three bacterial strains generally showed the largest decrease in pH after the fermentations compared to the other combinations of bacteria. The combinations with *B. longum* and *L. reuteri* showed the lowest decrease in pH in all substrate types, except the substrates with peptone added. The pH change of these substrates was comparable to the substrates with all three bacterial strains added. The second co-cultivation showed similar results to the first one. It is evident that the samples with all three bacterial strains added gave the largest pH drop in all types of substrates. The substrate that showed the largest pH change out of all was the Milk substrate with no additives and all three strains added, with a pH decrease of -2.38 in the first co-cultivation and -2.50 decrease in the second co-cultivation. Figure 5 shows a summary of all pH values before and after the fermentation.

Figure 5. *Summary of pH values before and after the fermentation from both milk-and buttermilk co-cultivations, labelled 1 and 2 for the first and second co-*



cultivation. Abbreviations M, AX, B, and P represent Milk, AXOS, Buttermilk and Peptone respectively)

Co-cultivations 3

In the first milk co-cultivation, the pH before and after fermentation with the three bacterial strains showed a pH of 6.75 for the Milk substrate before and a pH of 4.14 after the fermentation process. The Milk+AXOS substrate showed a starting pH of 6.72 and a pH of 4.11 after fermentation. This generates a drop in pH of -2.61 for both the Milk substrate and the Milk+AXOS substrate. Both substrates had very similar decreases in pH. The second milk co-cultivation showed a similar drop in pH to the first milk co-cultivation where Milk had a drop of -2.52 and Milk+AXOS has a drop of -2.47. These results suggests that the addition of AXOS does not have a notable effect on the pH drop in the milk co-cultivations with all three bacterial strains added.

Sensory Taste Analyses

Sensory Analysis 1

The first taste evaluation was performed on people within the internal work group. The results were normally distributed and plotted with a mixed effect as shown in figure 6A. The samples where AXOS was added in different concentrations after the fermentation process showed a higher score in the general liking category of the sensory analysis. These were the samples with the highest score in sweetness as well. The sample where 2% of AXOS was added had the highest general liking out of all the samples as well as the highest score in sweetness

and funkiness. The samples M and Milk+AXOS showed the highest in fermented flavour. There was a statistical significance observed between the Milk sample and Milk+AXOS (2% after fermentation) in the sweetness attribute as well.

Sensory Analysis 2

For the second milk co-cultivation, there were two milk substrates used for inoculation which were flavoured after the fermentation process and evaluated by taste and smell attributes separately. This analysis was also performed by the internal work group. The plotted results for the taste evaluation are shown in figure 6B. The results showed that sample with Milk (blueberry 5% + AXOS 1% before fermentation) scored the highest in general liking of 8.3 and the sample with Milk (blueberry 5% + AXOS 1% after fermentation) had a slightly lower score of 8.0. Both these samples (Nr 7 and Nr 3) had the flavour addition of blueberry, where the difference was that sample 3 had added AXOS in a total concentration of 1% after the fermentation process, whereas sample 7 had AXOS added in the same concentration before the fermentation the substrate. Sample 7 with Milk (blueberry 5% + AXOS 1% before fermentation) also showed the highest score in sweetness. The highest scores for the fermented attribute were observed in the sample containing only 1% AXOS added after fermentation, the samples containing AXOS added both before and after fermentation (2% total) as well as the sample containing vanilla combined with 1% AXOS before fermentation. There was no statistical significance observed in this analysis.

From these results the samples that were selected for the next sensory evaluation were Milk (blueberry 5% + AXOS 1% before fermentation), Milk (strawberry 5% + AXOS 1% before fermentation) and Milk (vanilla 1% + AXOS 1% after fermentation). These samples showed some of the highest rankings in their flavour category.

Sensory Analysis 3

The third and last sensory analysis using the larger group of 13 panellists showed varying results in both the acceptance test as well as the ranking test. There was an overall agreement among the participants that the samples were too sour in the taste attribute and a few also mentioned that the flavour was too pungent and astringent. The data was normally distributed (Shapiro-Wilk test). For evaluation of taste, consistency and general liking, an ANOVA with Tukey's multiple comparison test was performed. A statistical significance was observed between sample 3 (Milk (vanilla 1% + AXOS 1% after fermentation)) and 12 (Milk (Blueberry 5% + AXOS 1% before fermentation)) in the taste attribute, where the sample with Blueberry and AXOS before fermentation generally had higher scores than the sample with vanilla and AXOS. Sample 7 (Milk (strawberry 5% + AXOS 1% before fermentation)) showed the highest ranking in taste with a score of 8.0 on a hedonic scale of 1-9 from two people. In the general liking attribute, a statistical significance was observed between the sample with vanilla and AXOS added and the sample with blueberry and AXOS added, where more people rated the sample with blueberry and AXOS added the highest. In the consistency attribute, no statistical significance was observed. The plotted results are shown in figure 6C.

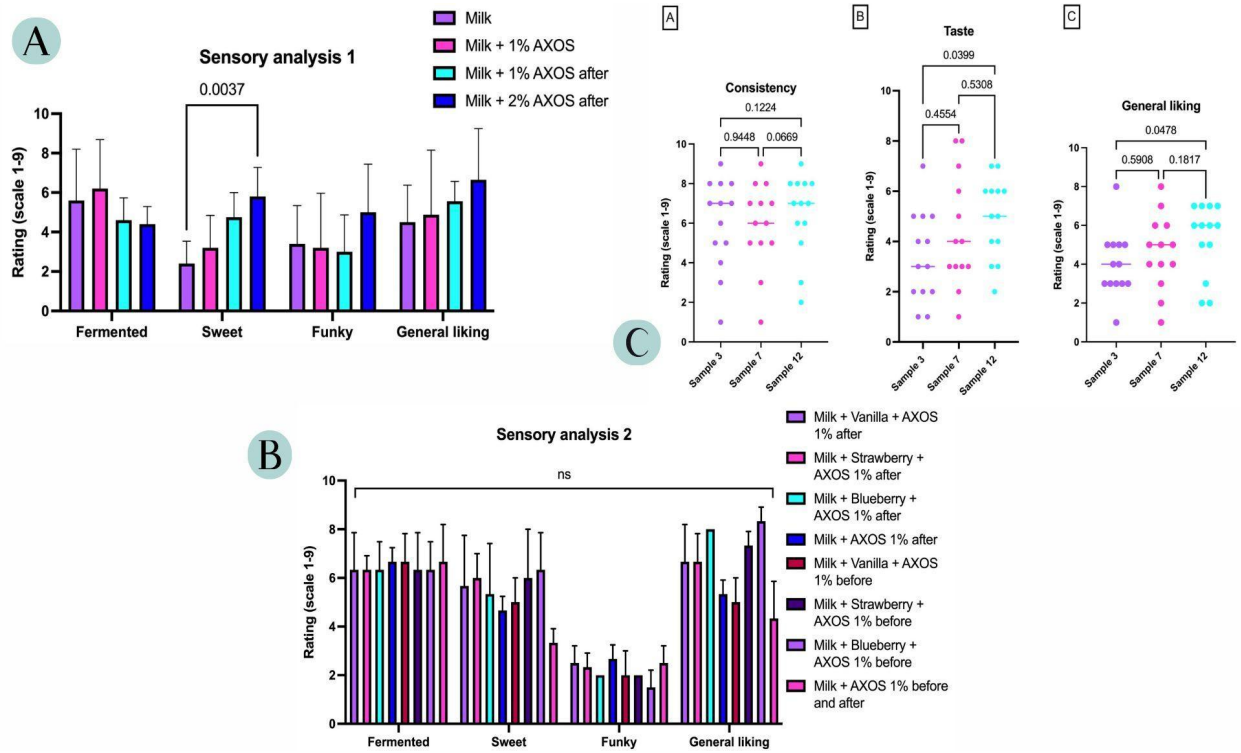


Figure 6. *Summarizing figure of the results from sensory analysis 1, 2 and 3, where A represents sensory analysis 1, B represents sensory analysis 2 and C represents sensory analysis 3 (in figure C, A represents consistency, B represents taste and C represents general liking). In figure C the samples 3, 7 and 12 consists respectively of Milk (vanilla 1% + AXOS 1% after fermentation), Milk (strawberry 5% + AXOS 1% before fermentation) and Milk (Blueberry 5% + AXOS 1% before fermentation)*

Metabolite Analysis

The HPLC results for the volatile fatty acid (VFA) content showed that all the samples that contained *B. longum* showed a presence of propionate whereas the samples with only *L. lactis* showed no content of propionate. Lactate was present in all samples, reaching a value of 7.3 g/L in Milk+AXOS with all bacterial strains. Acetate was present in all samples, where the samples with *B. longum* had a notably higher content than the samples with only *L. lactis*. The samples with *B. longum* in combination with the other two strains in the Milk substrate showed an acetate content of 2.8 g/L, whereas the same bacterial combination in the Milk+AXOS substrate showed an acetate content of 4.5 g/L. The samples with only *B. longum* added showed an acetate content of 2.7 g/L in the Milk substrate and 4.4 g/L in the Milk+AXOS substrate. The samples with *L. lactis* added alone had an acetate content of 0.09 g/L in the Milk substrate and 0.15 g/L in the Milk+AXOS substrate.

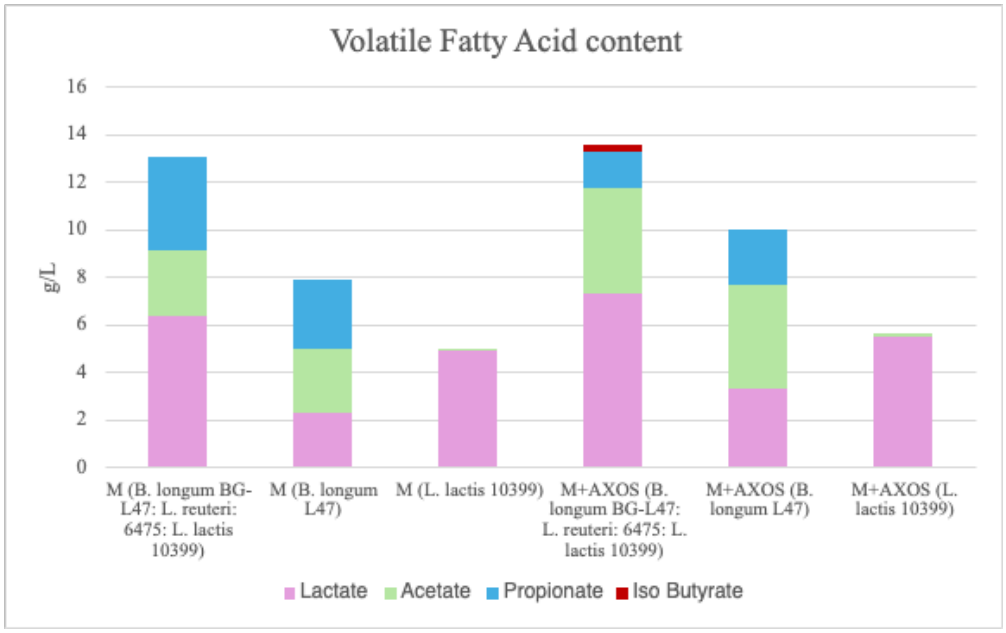


Figure 7. *Volatile fatty acid content (g/L) in Milk (M) and Milk + 1% AXOS (M+AXOS) fermented with combinations of bacterial strains.*

The analysis of sugar content showed that lactose was the most abundant sugar and was also present in all samples. Xylose was also detected in all the samples, where the highest content of xylose was documented in the milk sample with *B. longum* alone at a value of 4.3 g/L. Arabinose was absent in all the samples except for the Milk+AXOS and only *L. lactis* added. Notably, samples without AXOS addition should not have contained any detectable levels of xylose or arabinose in the HPLC analysis, since these monosaccharides are derived from AXOS after degradation.

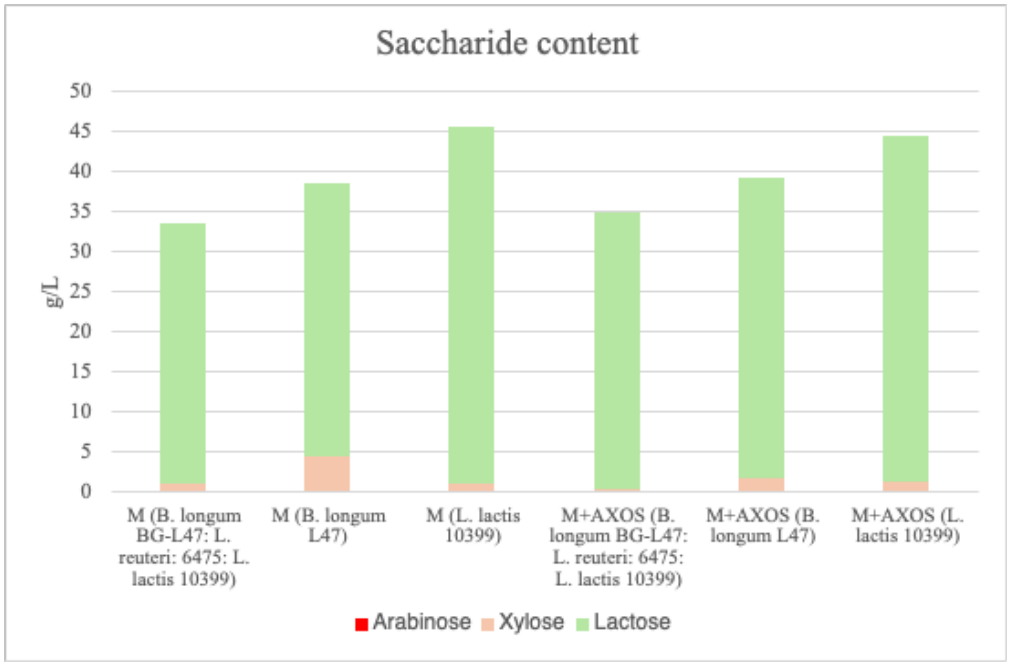


Figure 8. *Saccharide content in Milk (M) and Milk + 1% AXOS (M+AXOS) fermented with combinations of bacterial strains*

Contaminant Analysis

The contaminant analysis for *Enterobacteriaceae* showed no signs of contamination from inspection of the VRBD agar plates. The *Enterobacteriaceae* was expected to show up as red/violet colonies after a 24-hour incubation at 37°C under aerobic conditions. The plates showed no colonies and there was therefore no indication of a contamination with *Enterobacteriaceae* in the samples.

Bile Tolerance Test

It was shown that the viability reduction followed a time-dependent decrease as the viability at 0 minutes was 6.3×10^7 CFU/mL in the Milk substrate and 5.0×10^7 CFU/mL in the Milk+AXOS substrate. At 30 minutes of bile exposure, both substrates showed a CFU/mL of 5.0×10^6. At 90 minutes of exposure to bile, a decline was observed in the viability of *B. longum* in the samples with co-cultivations. In the Milk substrate the viability showcased a decrease to 2.5×10^6 CFU/mL, and the Milk+AXOS substrate had decreased to 6.3×10^5 CFU/mL.

The gradual decrease portrayed in the results suggests a loss of *B. longum* viability over time in co-cultivation with both *L. reuteri* and *L. lactis* when exposed to bile. The control sample of *B. longum* in the Milk+AXOS substrate displayed consistent viability over time, with 1.5×10^7, 1.7×10^7, and 1.7×10^7 CFU/mL at 0, 30, and 90 minutes, respectively. This indicates that *B. longum* on its own in this substrate is more tolerant to bile than when in co-cultivation with the other two strains.

Due to morphological similarities in the colonies of *L. lactis* and *L. reuteri* it was not possible to distinguish these from each other; therefore, their bile tolerance was assessed with the help of the control samples. *L. reuteri* showed a stable cell viability over time in both the Milk substrate and Milk+AXOS substrate. In the Milk substrate the values at 0, 30 and 90 minutes were 7.4×10^5, 5.1×10^5 CFU/mL, and 5.9×10^5 CFU/mL, respectively, and in the Milk+AXOS substrate the cell counts were 1.7×10^6, 2.2×10^6 CFU/mL and 2.6×10^6 CFU/mL. The *L. lactis* control on the Milk+AXOS substrate also remained stable during bile exposure times, namely, 3.2×10^4, 4.6×10^4 CFU/mL and 3.9×10^4 CFU/mL at 0, 30 and 90 minutes, respectively.

Data are not available on viability of *B. longum* on its own in Milk, and *L. lactis* on its own in Milk, due to an error in inoculation. This error was noticed when plates with the *L. lactis* control sample showed no visible growth and the plates with the *B. longum* control showed two different colony types.

5’NT Analysis

The numerical data from the 5’NT analysis showed unreliable results where the absorbance readouts did not change over time. The numerical data was therefore excluded from the results and not considered for the discussion in this project. The results that will be taken into consideration is the visible colour changes of the samples after the incubation period of the analysis, which reflect the 5’nucleotidase activity. Figure 9 shows the colour variation of the samples as well as a reference of the sample placements.

Figure 9. *5’NT test plate showing the samples and references to the sample placements. Sample 1-4 are milk based, where sample 1 contains all three bacterial strains, 2, 3 and 4 contains B. longum, L. lactis and L. reuteri respectively. Samples 5-8 are milk based with added AXOS where sample 5 contains all three bacterial strains and samples 6, 7 and 8 contains B. longum, L. lactis and L. reuteri respectively. These samples are analysed as supernatant (S), cells (C) and whole fermentate (W) in duplicates. Ref 1 and 2 is Milk and Milk+AXOS substrate respectively without bacterial strains added. Sal is 0.9% saline solution (blank calibrator) and Cal is calibrator. (Sample S4 (supernatant, B. longum) are excluded because of pipetting error)*

In order to track the 5´NT enzymatic activity in each sample, the colour changes were compared to the calibration sample (Cal), where the darker purple colour shows the 5´NT enzymatic activity in the samples. Samples S1 and S5 (supernatant) both showed a substantial colour change somewhat similar to the calibration well

after the incubation period, indicating that there is an active 5'NT enzyme present in these samples. These samples contained a combination of all three bacterial strains in both the Milk substrate and Milk+AXOS substrate. The samples with supernatant and the bacteria added singularly showed little or no activity after inspection of colour changes compared to the calibration well. None of the samples with whole fermentate or cells showed any activity after inspection of colour changes, indicating a potential inhibition of the 5'NT enzymatic activity.

Product Risk Assessment

The risk assessment identified some potential hazards of this product after an evaluation of the production steps. The most critical hazards were associated with the use of raw materials, fermentation, inoculation and post-fermentation handling.

The raw materials were assessed to pose a risk of *Enterobacteriaceae, Bacillus cereus* and *Staphylococcus aureus* in the milk. These hazards are mitigated by the first pasteurization of the milk in commercial facilities, as well as the second pasteurization (90℃ for 20 minutes) before bacterial inoculation in the production steps. There is a potential risk of spore forming *B. cereus* surviving the double pasteurization of the milk. The spores could impose a risk if germination takes place, which could occur even during cold storage of the product. This hazard is minimized by the low pH of the product at around 4.2-4.1 which inhibits the germination of spores (Soni et al., 2018). The usage of berries in the falvourings could impose a risk for mycotoxins from moulds in contaminated berries, which is monitored by supplier control, visual inspection, and cold storage. The berries were added in form of a heat-treated syrup with the addition of sugar syrup for taste applications in the post-fermentation stage of the product. The heat treatment acts as a hazard control to decrease the risk of mould and yeast growth in product, in combination with cold storage which inhibits their growth (Tournas & Katsoudas, 2005). The same hazards could be identified from the addition of AXOS, which could carry mould spores, and this hazard is also controlled by heat treatment in the form of autoclaving at 121℃ before the addition into the pasteurized milk.

In the inoculation and fermentation steps of the production there were also a few risks identified. In the inoculation step, there are risks of contamination if handling is not performed in a controlled and hygienic manor. There are risks of contamination of *S. aureus* from human skin contact, as well as faecal contamination from *Enterobacteriaceae.* This is controlled by maintaining an aseptic environment and strict hygiene practices in the handling stages of the product. Achieving a rapid acidification to a pH that is sufficient within the time frame of the fermentation process is crucial. This is because the low pH prevents the growth of potential contaminant bacteria that might be present in the product. Because of these factors, the pH is carefully monitored during fermentation to ensure a rapid acidification.

Overall, the main concerns in terms of risk assessment of this product were the potential contamination of *Enterobacteriaceae*, *B. cereus,* and *S. aureus* during the production steps. These hazards were controlled by repetitive heat treatments as well as acidic environments and cold storage and hygienic handling practices. The product also went through a contaminant analysis for *Enterobacteriaceae* prior to the sensory analysis. In practice, these hazards can therefore be controlled through strict hazard protocols monitoring temperature and time, and pH drops within specified timeframes, as critical control points according to the principles of HACCP.

To control further contamination during the storage step, the product was packaged in air-tight containers and stored in refrigerated conditions at around 4°C. It is also crucial to have re-sealable packaging after opening of the product as well as cold storage to delay mould contamination causing spoilage of the product. In the post-fermentation stage, the monitoring of pH serves primarily as a quality control in order to ensure that the desired taste and consistency is maintained in the final product.

Discussion

The aim of this project was to develop a fermented nutritional probiotic drink that is mainly targeted towards the elderly population. The project focused on bacterial growth performance, functional activity as well as sensory acceptability. The results showed overall promising results regarding the cell viability of the selected probiotic strains, as well as the ability of AXOS acting as a prebiotic in the product. The potential of the product concept generally shows promise and opens for more research of mainly the functional properties of the product. The sensory properties of the product are open for improvement and modifications to create a palatable and overall pleasant product.

Cell Viability and Bacterial Growth

Results from the cell viability counts and bioscreen experiments of the different co-cultivations indicate that the bacterial strains selected for this project are capable of co-existing as well as maintaining high viability in a milk-based media. The milk-based media showed an increase in growth of *B. longum* when combined with both *L. reuteri* and *L. lactis*, indicating a synergistic relationship between the strains. The results suggests that the usage of the two complementary strains increases the growth of *B. longum* and decreases the fermentation time of the product. The fact that *B. longum* demonstrates higher growth in combination with *L. reuteri* is probably due to cross-feeding between the strains, where they work in a synergistic manor, producing favourable metabolites and promoting each other's growth.

In the third co-cultivation experiment, *B. longum* reached 2.0×10^9 cfu/ml in the Milk+AXOS substrate, and 1.3×10^9 in the Milk substrate with all three bacterial strains in combination, suggesting that the AXOS addition had little to no effect as a prebiotic additive in co-cultivations with all three bacterial strains. *L. reuteri* showed an improved cell count in the AXOS enhanced substrate, with a cell count of 7.9×10^8 in the Milk+AXOS substrate and 5.0×10^7 in the regular Milk substrate. This suggests that the *L. reuteri* is supported by an AXOS addition. This could further be explained by cross-feeding or nutrient enhancement between the strains, which has been shown on other strains in earlier studies on *B. longum* and *L. reuteri* DSM 17938. In those experiments, it was shown that *B. longum* produced and provided *L. reuteri* 17938 with acetate as an electron acceptor (Lundberg et al., 2024).

The *L. lactis* showed a high cell count in both regular Milk substrate and the Milk+AXOS substrate, which indicates they have an ability to adapt and that their growth performance is not affected by AXOS addition. The growth patterns observed in the Bioscreen experiments showed that a higher proportion of *B. longum* (5:1:1) generated a notable increase in the OD values, indicating the bacterial ratio was beneficial for the general bacterial population. These results

indicates that the three bacterial strains work in a synergistic manner and could provide a base for further research on their compatibility.

pH Changes and Fermentation

Observations showed that there was an active fermentation taking place in most substrate types and bacterial combinations. The only bacterial strain that did not do well alone in the milk or buttermilk substrates was *L. reuteri*, which is probably due to the lack of proteolytic activity of this strain. The *L. lactis* provides support through their proteolytic activity that is lacking in *L. reuteri* in milk-based substrates (Meijer et al., 1996). It can also be observed from the cell viability experiments that *L. reuteri* grew better on milk-based substrates with added milk peptone. There was an especially large pH drop in the substrates with all three bacterial strains added. For instance, a decrease of approximately 2.6 pH units was observed, showing that the fermentation process was effective. The AXOS also seemed to aid in the lowering of pH, which is another indicator that the addition of AXOS supports the bacterial growth. The samples containing only *L. reuteri* and *B. longum* in combination, showed a smaller pH decrease than the combination of all three, especially in the substrates with no added peptone or AXOS. Bifidobacteria produce lactate and acetate, as shown in the HPLC analysis, which contribute to some pH drop. Furthermore, the decrease in pH was not as great in the samples where *B. longum* was added solely, compared to the same sample but with AXOS addition. This suggests that the AXOS aids the production of organic acids and a greater drop in pH.

Sensory Characteristics and Consumer Feedback

The sensory analysis showed that the sample that was most liked among the participants in sensory analysis 3 was the Milk+AXOS (blueberry) sample with the AXOS added before fermentation. The fact that there was a statistical significance observed between this sample and the sample with vanilla flavour and AXOS addition after the fermentation showed that there could be a preference for the flavour where the AXOS is added before the fermentation. This could possibility be linked to a change in flavour caused by the AXOS post-fermentation, where the complexity in flavour profile will likely be enhanced because of the production of metabolites from the added bacteria. This, in turn, is probably connected to the results where the growth of the bacteria is enhanced by the addition of AXOS pre-fermentation.

The feedback showed that most of the participants perceived the flavour to be slightly too sour and astringent, which was the major downside to the perception of this product. The high acidity is an advantage in terms of product stability and in terms of contamination risks, where the low pH contributes to a safe product. The flavour could be improved by either decreasing the fermentation time to limit the

acidification, or by the addition of more flavour compounds such as sweeteners. The addition of honey could be an option in terms of health-related factors, since honey has a lower glycaemic index compared to refined sugars. This is a good option when considering that the target group often suffer from type-2 diabetes or other metabolic disorders. It is also a good option when considering weight management or heart health. Furthermore, honey has prebiotic properties, supporting gut balance and gut health, which makes it even more appealing as a sweetener (G. Sharaf El-Din et al., 2025). The addition of honey could therefore be performed both at the pre-fermentation and post-fermentation stages, depending on how much prebiotic activity is desired for the product. In this case, it would probably be preferable to add it at the post-fermentation stage, considering the high prebiotic activity recorded from the AXOS addition in this product.

Apart from the flavour profile, the consistency of all the three samples of the product was considered very good by most of the participants. The samples demonstrated some issues with homogenization, where there were some clumps left after the homogenization stage. In a potential industrial application, a more effective or vigorous homogenization would be needed to create a completely smooth product.

Functional Properties and Bioactivity

A bile tolerance test showed that both *B. longum* and *L. reuteri* remained stable in the control samples over the course of the 90 minutes exposure. The co-cultivation samples with all three strains showed a decrease of *B. longum* over time. This suggests that the combination of the strains weaken the bile tolerance of *B. longum*. It is known that bifidobacteria in general are sensitive to acid exposure with some variation between species (Lundberg et al., 2024). The pH of the co-cultivation with all three strains varied between 4.1-4.2 in the Milk and Milk+AXOS substrates, whereas the control samples with *B. longum* showed higher pH, from 4.2-5.5 in both the Milk and Milk+AXOS substrates. This would benefit from further investigation and experimental procedures to confirm these results and diagnose the root cause of the bile tolerance differences.

The 5’NT analysis showed a colour change in the samples with supernatant and all three bacterial strains added, in contrast to the rest of the samples with supernatant and the singular bacterial strain additions. This could indicate that the expression of the 5’NT enzyme was stimulated in the samples where all three strains were present. As mentioned in the results section, the absorbance data from the analysis was unreliable, which means that this experiment would need to be repeated to collect relevant data to support this statement further.

In the HPLC analysis of organic acids and sugars, propionate was surprisingly detected in the samples containing *B. longum*. The presence of propionate was not expected since bifidobacteria are not a documented producers of this fatty acid in larger amounts. Some strains could produce propionate in minor quantities (Louis

& Flint 2016). The presence of propionate in these larger quantities is therefore implausible. The results could be explained by a lack of reference samples for other fatty acids that potentially could be produced by *B. longum*, which could generate a similar peak to propionate in an HPLC profile. One example of a potentially co-eluting fatty acid is succinate. This experiment either must be repeated, or other tests could be performed like NMR or LC-MS analysis, in order to identify what fatty acid the peaks could represent. Apart from this, the HPLC showed that the samples with all three bacterial strains present and the addition of AXOS showed a higher presence of both lactic acid and acetic acid. These acids are important contributors to both flavour characteristics as well as the acidification of the final product. The saccharide analysis showed a high presence of lactose, which was expected considering the substrate was based on milk. There was xylose detected in all samples in small amounts, which was not expected for the Milk substrates considering they did not contain any addition of AXOS. This could be due to some type of contamination during sample preparation, or it could be a similar error as for the fatty acids analysis, where the xylose co-elutes with another unknown saccharide in the samples. The samples with AXOS added showed small amounts of xylose and no content of arabinose in the samples except for the Milk+AXOS with *L. lactis* added. These results were surprising in the sense that the presence of both arabinose and xylose was expected in the samples with AXOS added. This would mean that the AXOS had been metabolised into the oligo-saccharides fragments. These experiments would benefit from further testing and an inclusion of additional controls such as galactose and glucose.

Product Safety

The product risk assessment of this product showed that the product is safe if hazard prevention protocols are followed. The low pH of the product is key for a safe product. To reduce hazards of the product in the current development stage that it is now, further modifications of ingredients could be made to reduce mould hazards from the berry addition for example. It would be favourable to use the berry concentrates that has been sterilized at higher temperatures, as well as strained several times before the addition into the milk fermentate. In general, the safety hazards of this product are well monitored, and the production steps contain several control points that ensures a safe product.

Limitations and Future Perspectives

The limitations of this product development project were mainly the limited time for laboratory studies. Time was not sufficient to repeat experiments and generate more results, particularly in the analyses of functional properties of the product. Most of the project time was spent on screening practices to find a bacterial combination that yielded satisfactory growth dynamics and viability results. Some

limitations appeared in the sensory analysis of the product, where it would be preferable to test the product on the main target group (elderly).

In the future, it would be interesting to include clinical testing on individuals with certain metabolic disturbances, such as type-2 diabetes or irritable bowel syndrome, where the test persons regularly ingest the product and go through regular screenings of, for example, blood sugar levels, well as interviews covering their perception of their wellbeing over time. This could be done by screening two groups, where one of the groups receives a placebo product and the other group receives the described probiotic drink. Furthermore, considering that bifidobacteria have been studied as a psychobiotic, connected to tryptophan levels increase, it would be interesting to investigate the increase in tryptophan and its catabolites related to the specific strain *B. longum*, and its potential applications as a treatment for psychological stress and depression.

References

Aguilar-Toalá, J. E., Garcia-Varela, R., Garcia, H. S., Mata-Haro, V., González-Córdova, A. F., Vallejo-Cordoba, B., & Hernández-Mendoza, A., (2018). Postbiotics: An evolving term within the functional foods field. *Trends in Food Science & Technology*, *75*, 105–114. https://doi.org/10.1016/j.tifs.2018.03.009

Altaib, H., Badr, Y., & Suzuki, T. (2021). Bifidobacteria and psychobiotic therapy: Current evidence and future prospects. *Reviews in Agricultural Science*, *9*, 74–91. https://doi.org/10.7831/ras.9.0_74

Arboleya, S., Watkins, C., Stanton, C., & Ross, R. P. (2016). Gut bifidobacteria populations in human health and aging. *Frontiers in Microbiology*, *7*. https://doi.org/10.3389/fmicb.2016.01204

Balasubramanian, R., Schneider, E., Gunnigle, E., Cotter, P. D., & Cryan, J. F. (2024). Fermented foods: Harnessing their potential to modulate the microbiota-gut-brain axis for mental health. *Neuroscience & Biobehavioral Reviews*, *158*, 105562. https://doi.org/10.1016/j.neubiorev.2024.105562

Calvete-Torre, I., Sabater, C., Delgado, S., Ruas-Madiedo, P., Rupérez-García, A., Montilla, A., Javier Moreno, F., Margolles, A., & Ruiz, L. (2023). Arabinoxylan-based substrate preferences and predicted metabolic properties of Bifidobacterium longum subspecies as a basis to design differential media. *Food Research International*, *167*, 112711. https://doi.org/10.1016/j.foodres.2023.112711

Chiara, I. D., Marasco, R., Gala, M. D., Fusco, A., Donnarumma, G., & Muscariello, L. (2024). Probiotic Properties of Lactococcus lactis Strains Isolated from Natural Whey Starter Cultures. *Foods*, *13*(6). https://doi.org/10.3390/foods13060957

Cruchet, S., Hirsch, S., Villa-López, D., Moreno-Portillo, M., Palomo, J. C., Abreu-Abreu, A. T., Abdo-Francis, J. M., Jiménez-Gutiérrez, C., Rojano, M., López-Velázquez, G., & Gutiérrez-Castrellón, P. (2024). Limosilactobacillus reuteri DSM 17938 and ATCC PTA 6475 for the treatment of moderate to severe irritable bowel syndrome in adults: A randomized controlled trial. *Frontiers in Gastroenterology*, *2*. https://doi.org/10.3389/fgstr.2023.1296048

G. Sharaf El-Din, M., F.S. Farrag, A., Wu, L., Huang, Y., & Wang, K. (2025). Health benefits of honey: A critical review on the homology of medicine and food in traditional and modern contexts. *Journal of Traditional Chinese Medical Sciences*, *12*(2), 147–164. https://doi.org/10.1016/j.jtcms.2025.03.015

ISAPP. (2018). *Probiotics - International Scientific Association for Probiotics*

and Prebiotics (ISAPP). International Scientific Association for Probiotics and Prebiotics. https://isappscience.org/for-scientists/resources/probiotics/

Ji, ng J., Jin, W., Liu, S., Jiao, Z., & Li, X. (2023). Probiotics, prebiotics, and postbiotics in health and disease - PMC. *MedComm*, *4*(6). https://doi.org/10.1002/mco2.420

Kaur, R., & Sharma, M. (2019). Cereal polysaccharides as sources of functional ingredient for reformulation of meat products: A review. *Journal of Functional Foods*, *62*, 103527. https://doi.org/10.1016/j.jff.2019.103527

Khelissa, S., Chihib, N.-E., & Gharsallaoui, A. (2020). Conditions of nisin production by Lactococcus lactis subsp. lactis and its main uses as a food preservative. *Archives of Microbiology*, *203*(2), 465–480. https://doi.org/10.1007/s00203-020-02054-z

Latif, A., Shehzad, A., Niazi, S., Zahid, A., Ashraf, W., Iqbal, M. W., Rehman, A., Riaz, T., Aadil, R. M., Khan, I. M., Özogul, F., Rocha, J. M., Esatbeyoglu, T., & Korma, S. A. (2023). Probiotics: Mechanism of action, health benefits and their application in food industries. *Frontiers in Microbiology*, *14*. https://doi.org/10.3389/fmicb.2023.1216674

Liu, M., Bayjanov, J. R., Renckens, B., Nauta, A., & Siezen, R. J. (2010). The proteolytic system of lactic acid bacteria revisited: A genomic comparison. *BMC Genomics*, *11*(1), 1–15. https://doi.org/10.1186/1471-2164-11-36

LIVSMEDELSVERKET. (2001, January 1). EG-förordning 2073/2005. *Kontrollwiki*. https://kontrollwiki.livsmedelsverket.se/lagstiftning/213/eg-forordning-2073-2005

Louis, P. and Flint, H.J. (2017), Formation of propionate and butyrate by the human colonic microbiota. Environ Microbiol, 19: 29-41. https://doi.org/10.1111/1462-2920.13589

Lundberg, L. E., Pallabi Mishra, P., Liu, P., Mata Forsberg, M. M. F., Sverremark-Ekström, E., Grompone, G., Håkansson, S., Linninge, C., & Roos, S. (2024). Bifidobacterium longum subsp. longum BG-L47 boosts growth and activity of Limosilactobacillus reuteri DSM 17938 and its extracellular membrane vesicles. *Applied and Environmental Microbiology*.

Meijer, W., Marugg, J. D., & Hugenholtz, J. (1996). Regulation of Proteolytic Enzyme Activity in Lactococcus lactis - PMC. *Applied and Environmental Microbiology*, *62*(1). https://doi.org/10.1128/aem.62.1.156-161.1996

Oster, H., & Chaves, I. (2023). Effects of healthy lifestyles on chronic diseases: Diet, sleep and exercise - PMC. *Nutrients*, *15*(21). https://doi.org/10.3390/nu15214627

Prasanna, P. H. P., Grandison, A. S., & Charalampopoulos, D. (2014). Bifidobacteria in milk products: An overview of physiological and biochemical properties, exopolysaccharide production, selection criteria of milk products and health benefits. *Food Research International*, *55*, 247–

262. https://doi.org/10.1016/j.foodres.2013.11.013
Rattanaprasert, M., van Pijkeren, J.-P., E Ramer-Tait, A., Quintero, M., Reen Kok, C. R. K., Walter, J., & W Hutkins, R. (2019). Genes Involved in Galactooligosaccharide Metabolism in Lactobacillus reuteri and Their Ecological Role in the Gastrointestinal Tract - PubMed. *Applied and Environmental Microbiology*, *85*(22). https://doi.org/10.1128/AEM.01788-19
Rivière, A., Moens, F., Selak, M., Maes, D., Weckx, S., & Vuyst, L. D. (2014). The ability of bifidobacteria to degrade arabinoxylan oligosaccharide constituents and derived oligosaccharides is strain dependent - PMC. *Applied and Environmental Microbiology*, *80*(1). https://doi.org/10.1128/AEM.02853-13
Rudjito, R. C., Jiménez-Quero, A., Muñoz, M. D. C. C., Kuil, T., Olsson, L., Stringer, M. A., Krogh, K. B. R. M., Eklöf, J., & Vilaplana, F. (2023). Arabinoxylan source and xylanase specificity influence the production of oligosaccharides with prebiotic potential. *Carbohydrate Polymers*, *320*, 121233. https://doi.org/10.1016/j.carbpol.2023.121233
Sánchez, B., Champomier-Vergès, M.-C., Collado, M. del C., Anglade, P., Baraige, F., Sanz, Y., Reyes-Gavilán, C. G. de los, Margolles, A., & Zagorec, M. (2007). Low-pH Adaptation and the Acid Tolerance Response of Bifidobacterium longum Biotype longum - PMC. *Applied and Environmental Microbiology*, *73*(20). https://doi.org/10.1128/AEM.00886-07
Sarita, B., Samadhan, D., Hassan, M. Z., & Kovaleva, E. G. (2025). A comprehensive review of probiotics and human health-current prospective and applications. *Frontiers in Microbiology*, *15*. https://doi.org/10.3389/fmicb.2024.1487641
Schupfer, E., Pak, S. C., Wang, S., Micalos, P. S., Jeffries, T., Ooi, S. L., Golombick, T., Harris, G., & El-Omar, E. (2021). The effects and benefits of arabinoxylans on human gut microbiota – A narrative review. *Food Bioscience*, *43*, 101267. https://doi.org/10.1016/j.fbio.2021.101267
Shirkoohi, N. M., Mohammadi, H., Gallaly, D. Q., & Djafarian, K. (2025). The effects of probiotic supplementation on body composition, recovery following exercise-induced muscle damage, and exercise performance: A systematic review and meta-analysis of clinical trials - PMC. *Physiological Reports*, *13*(8). https://doi.org/10.14814/phy2.70288
Soni, A., Oey, I., Silcock, P., & Bremer, P. J. (2018). Impact of temperature, nutrients, pH and cold storage on the germination, growth and resistance of Bacillus cereus spores in egg white. *Food Research International*, *106*, 394–403. https://doi.org/10.1016/j.foodres.2018.01.006
Tournas, V. H., & Katsoudas, E. (2005). Mould and yeast flora in fresh berries, grapes and citrus fruits. *International Journal of Food Microbiology*,

105(1), 11–17. https://doi.org/10.1016/j.ijfoodmicro.2005.05.002

Wang, H., Huang, X., Tan, H., Chen, X., Chen, C., & Nie, S. (2022). Interaction between dietary fiber and bifidobacteria in promoting intestinal health. *Food Chemistry*, *393*, 133407. https://doi.org/10.1016/j.foodchem.2022.133407

Popular science summary

Aging is often associated with health challenges like poor digestion, decreased appetite, and a loss of beneficial gut bacteria. In this product development project, a new type of probiotic drink was developed to support digestive health and bring other health benefits to elderly individuals especially. The drink contains three types of bacteria that have different properties - *Bifidobacterium longum, Limosilactobacillus reuteri* and *Lactococcus lactis*. These three bacterial strains work together to improve gut health. A certain dietary fibre called arabinoxylan-oligosaccharides (AXOS) was added to aid the growth of the bacterial strains and to create more health benefits.
The drink was tested for its bacterial growth, how much they lowered the pH and therefore acidity of the product, what types of nutrients that they produced, and how people perceived the taste of the drink. The results showed that the drinks with both AXOS and the addition of blueberry flavour was most liked among the people trying the product. The bacteria showed a high survival rate, particularly in the drinks where AXOS was added. The drinks were found to be safe to consume and had no signs of unwanted pathogenic bacteria. It was also found that the drink had a production of beneficial nutrients, that contributed to the drink's flavour profile as well. Some adjustments are still needed to reduce sourness of the product, even though the general feedback showed positive results.
This fermented drink could become an option in the future for the support in digestion and other health related issues among elderly people.

Appendix

Appendix 1

Modified MRS (mMRS) substrate

1% yeast peptone
0.5% yeast extract
0.2% dipotassium phosphate
0.5% sodium acetate
0.2% ammonium citrate
0.02% magnesium sulfate
0.005% manganese sulfate
0.1% Lecithin
0.05% cysteine
1% AXOS

Appendix 2

mMRS food grade

Yeast peptone
Yeast extract
Sucrose
di-Potassium phosphate
Sodium acetate
Magnesium sulfate
tri-Sodium citrate
Manganese gluconate
Lecithin
Cysteine HCl

pH of substrate for *B. longum* BG-L47 and *L. reuteri* ATCC PTA 6475: 5.5-6.0
pH of *L. lactis* 10399 substrate: 6.5-7.0

***The measurements were re-calculated using the molecular masses of each substituted ingredient from the mMRS in order to create an adequate addition amount.

Appendix 3

Sensory analysis 3 questionnaire answers

Deltagare	Age	Gender	How often do you consume probiotic products?	How often do you consume dairy products?	When did you have your last meal?	How many meals in a day?	Would you consider buying a product like this if it was on the market?	Would you consider using this product as an after workout recovery?	Comments
Deltagare 1	24	female	rarely	daily	10 min ago	3-4	Yes	Yes	3 tastes burnt
Deltagare 2	23	male	rarely	2-3 times/ week	6 h ago	3	If 7 was less watery, yes	No	3 has a very strong smell and taste that I don't like
Deltagare 3	27	female	few times a week	daily	4 h ago	3 meals, 1-2 snacks	No	No	3: tastes like caramel but also very sour. 7: The smell made me rank lower on overall. Smells like canned tuna, and tastes very sour. 12: Also smells like tuna, tastes sour.
Deltagare 4	25	female	daily	daily	4 h ago	3-4	Maybe	No	Very sour and quite astingent. However, nr. 3 was milder and had a

									smoother mouthfeel. The smell could be improved, nr. 7 and 12 smell fermented.
Deltagare 5	27	female	few times a week	daily	3 h ago	3	Yes	No	The taste is rather sharp, which is off-putting. The consistency is overall nice as well as the appearence.
Deltagare 6	24	female	one time/week	daily	4 h ago	3	yes maybe, I dont consume probiotic products regularly	yes	Tyckte 7 och 12 var lite för sura men goda. 3 var lite sötare vilket var gott!
Deltagare 7	23	female	almost every day (A-fil)	almost every day	30 min ago	3-4	Maybe 12	If it was very effective	The taste was a bit strong and especially the smell was strong. If I had it multiple times maybe I would have started to like it even more but for now I didn't like the samples.
Deltagare 8	29	female	never	daily	10 min ago	5	Maybe	No	
Deltagare 9	31	male	daily	daily	30 min ago	3	Yes	No	
Deltagare 10	23	female	never	daily	1 h ago	3	No	No	All except 12 tasted very

									bad. 12 was not tasty enough to buy.
Deltagare 11	25	female	daily	daily	20 min ago	4	yes	As a snack, absolutely	12 and 3 was much better than 7.
Deltagare 12	25	male	rarely	daily	20 min ago	4	No	No	Too sour for me, and weird by-taste. Weird taste could stem from last meal.
Deltagare 13	29	female	A few times a month	Sometimes	yesterday	2-3	Yes	Yes	

Appendix 4

Glycerol stock recipe

0.82 g K_2HPO_4
0.18 g KH_2PO_4
0.59 g Na-citrate
0.25 g $MgSO_4$ x 7 H_2O
172 ml glycerol (87%) Þ 15% final concentration
water to 1000 ml

Publishing and archiving

Approved students' theses at SLU can be published online. As a student you own the copyright to your work and in such cases, you need to approve the publication. In connection with your approval of publication, SLU will process your personal data (name) to make the work searchable on the internet. You can revoke your consent at any time by contacting the library.

Even if you choose not to publish the work or if you revoke your approval, the thesis will be archived digitally according to archive legislation.

You will find links to SLU's publication agreement and SLU's processing of personal data and your rights on this page:

- https://libanswers.slu.se/en/faq/228318

X YES, I, Fanny Lagerström, have read and agree to the agreement for publication and the personal data processing that takes place in connection with this

NO, I/we do not give my/our permission to publish the full text of this work. However, the work will be uploaded for archiving and the metadata and summary will be visible and searchable.