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# Development of birch (*Betula pendula* Roth.) sap based probiotic fermented beverage

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**Abstract**

Birch sap is traditionally used as a refreshing beverage in the springtime in northern Europe. The aim of this study was to determine the suitability of birch sap for the growth of potentially probiotic *Lactobacillus reuteri* strains in order to develop a non-dairy functional beverage. All *L. reuteri* strains used in the study grew well in birch sap. pH values fell from an initial pH 6 to pH 4.20–3.18 characteristic for fermented products. Total acidity up to 36 T° and sufficient cell count was reached (6.79 cfu/ml). Glucose and fructose supplementation as well as their combination at a concentration of 0.5–1% did not significantly improve the growth of *L. reuteri.* Supplementation with 0.5–2% sucrose and a 2% glucose-fructose combination had a notable effect, although the latter had less effect than the former. Given that the viable cell count is the most important parameter of probiotic products, supplementation with sucrose was chosen as the best way to improve the substrate. The addition of sucrose stimulated biomass formation and improved acidification power, with the best results for sucrose 0.5–2%. Several other food grade supplements were evaluated to improve the growth of *L. reuteri* strains in 1% sucrose-supplemented birch sap. The best results were achieved using peppermint and malt extract supplements, which clearly indicate that *L. reuteri* growth in birch sap is limited not only by the availability of carbon but also by the availability of other growth factors present in the supplements used.



## Introduction

Despite the wide popularity of dairy-based probiotic/functional products there are some limitations to their consumption. Large numbers of individuals are lactose intolerant or have milk protein allergy or are on cholesterol-restricted diets. In addition vegetarianism and veganism are becoming more and more widespread. Thus the development of non-dairy-based functional/probiotic products has been in the spotlight during recent years. Tree and particularly birch sap has traditionally been used as a source of nutrients and as a refreshing drink in the springtime in boreal and hemiboreal regions of the northern hemisphere such as Scandinavia, the Baltic countries, Slovakia Romania etc (Svanberg *et al*., 2012). Nowadays Estonia, Latvia, Lithuania, Russia, Ukraine and Belarus are the only countries in which the gathering of birch and to a lesser extent maple sap has remained popular. However given the potential for boosting the market of eco/healthy/functional products the potential of birch sap should not be underestimated. Tree sap has also been used for several other purposes such as beer, wine and syrup making. Fermentation by lactic acid bacteria could increase the functional value of the product and extend its shelf life (De Vuyst, 2000; Matilla-Sandholm *et al*., 2002).

In the past birch sap was used as a medicine. It has been reported that birch sap can be used against anaemia, arthritis, kidney and liver stones, gout, rheumatism and colds (Svanberg *et al*., 2012). Birch sap was also used as a cosmetic product for hair and skin care (Svanberg *et al*., 2012). It was assumed that birch sap could be used as a diuretic, and as an anti-infectious, anti-rheumatic and anti-inflammatory remedy when administered at a rate of 200–300 ml/day (Peev *et al*., 2010). However, there is almost no clinical evidence on the health effects of birch sap. For instance, despite the detectable anti-inflammatory, antipyretic and phagocytosis-inhibiting effect of birch sap no therapeutic activity of importance compared with classical and modern antipyretics/analgesics has been demonstrated (Klinger *et al.*, 1989).

Birch sap contains up to 5–8 g/l of glucose and fructose (Kallio and Ahtonen, 1987b; Jeong *et al.*, 2013) and small amounts of sucrose (0.07 g/l) and galactose (0.01–0.03 g/l) (Jeong et al., 2013). The macro- and microelements reported to be found in birch sap are: K, Ca, Mg, Mn, Cu and Fe. Crude

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ash content can vary from 0.01% to 0.04% (Jeong *et al*., 2013). The dominant amino acids found in birch sap are glutamine, citrulline, glutamic acid, isoleucine, valine and asparagine. During the flow season total amino acid content varied widely from 100–500 mg/l (Kallio and Ahtonen, 1989). Oligosaccharides in the birch sap were identified as fructosylsucrose, glucosylsucrose, gentiobiose, melibiose, manninotriose and verbascotetraose (Haq and Adams, 1962). Birch sap also contains several organic and inorganic acids including malic acid, citric acid, succinic acid and phosphoric acid; the concentration of these acids as well as the sugar: acid ratio varies during the flow season (Kallio and Ahtonen, 1987a ; Jeong *et al.*, 2013).

There are almost no data on birch sap fermentation by lactic acid bacteria (LAB). It was reported that *L. acidophilus*, *L. brevis*, *L. mesenteroides*, *Leuconostoc lactis*, *L. lactis. P. pentosaceus*, *P. dextrinicus* and *S. thermophilus* were used as starter cultures for *Betula platyphylla* sap fermentation. All the tested bacteria except *P. dextrinicum* grew (up to $10^6$ – $10^7$ cfu/ml) and lowered pH down to about pH 4 within 48 hours. Due to lactic acid fermentation it was possible to extend the product's shelf life (Kim *et al.*, 2009).

Like birch sap, maple sap is traditionally used as a refreshing drink during springtime, particularly in North America and Eastern Europe. However a major difference between birch sap and maple sap is its sucrose content, which can reach up to 30 g/l in maple sap (Cochu *et al.*, 2008). Maple sap was used as a carbon source for the growth of *L. acidophilus*, *L. helveticus*, *L. casei* and *L. rhamnosus.* Compared with a sucrose-based medium, maple-sap–based media produced four- to seven-fold higher viable cell counts in two lactobacilli strains out of five (Cochu *et al.*, 2008). To develop new non-dairy–based probiotic products *B. lactis* Bb12 and *L. rhamnosus* GG were inoculated into maple sap and the viability of cells during storage was evaluated. The viability of both strains was maintained during storage for 28 days, with the same order of $10^7$ to $10^8$ CFU/ml as the initial cell count (Khalf *et al*., 2010).

*L. reuteri* is a symbiotic *Lactobacillus* species reported to inhabit the gastrointestinal tract of all vertebrates and mammals, ranging from birds to humans (Casas and Dobrogosz, 2000). Probiotic administration of certain *L. reuteri* strains has been shown to confer protection against various diseases in a broad spectrum of hosts, including protection from certain viral, bacteria, fungal and protozoal diseases (Casas and Dobrogosz, 2000). The main beneficial effects attributed to *L. reuteri* are the prevention of lactose maldigestion (DSM 17938) (Ojetti *et al*., 2010), diarrhoea (MM53 and ATCC SD2112) (Wolf *et al*., 1995; Shornikova *et al*., 1997) and hypercholesterolaemia (NCIMB 30242) (Jones *et al.*, 2012). During recent years, *L. reuteri* has been widely used as a probiotic supplement in dairy-based functional foods (Casas and Dobrogosz, 2000; Hernandez-Mendoza *et al*., 2007). The aim of this study was to determine the suitability of birch sap for the growth of probiotic *L. reuteri* strains in order to develop a non-dairy-based probiotic beverage.

## Material and Methods

*Strains*

The strains of *Lactobacillus reuteri* used in the study were obtained from the Collection of Microorganisms of the Institute of Microbiology and Biotechnology, University of Latvia.

*Media and growth conditions*

MRS growth medium (De Man *et al.*, 1960) was used for the maintenance and propagation of the cultures: 10.0 g/l peptone, 8.0 g/l beef extract, 5.0 sodium acetate, 4.0 g/l yeast extract, 2.0 g/l ammonium citrate, 2.0 g/l $KH_2PO_4$, 1.0 g/l Tween-80, 0.1 g/l Mg $SO_4x7H_2O$, 0.05 g/l $MnSO_4x5H_2O$ and 20.0 g/l glucose (pH 6.0). Birch sap was collected during spring 2013 in a local forest and immediately frozen. The sap was pasteurized at 60°C for 20 min. The sap contained 0.03% ash, 3.32 g/l glucose, 4.67 g/l fructose and 0.13 g/l sucrose. Fermentation was performed in 250 ml flasks at 37°C for 48 h. Two percent of the overnight culture (0.7–0.8 optical density (OD) grown in the MRS medium was used as an inoculum. Birch sap was supplemented with sucrose, glucose, fructose and food-grade supplements (lemon, quince syrup, lime, raisins, malt extract, brown sugar, peppermint, ginger) when appropriate.

*Analytical measurements*

The growth of *L. reuteri* strains was monitored by OD spectrophotometric measurement at 550 nm (Helios Gamma, Thermo Scientific, UK). Total acidity was determined by alkaline titration (0.1 mol/l NaOH) of the samples, using phenolphthalein as the indicator, and was expressed in Thörner degrees (ºT) (Scott *et al.*, 1998). The concentrations of organic acids were quantified by HPLC (Agilent 1100, HP, USA) with a diode array detector, column Shidex SH 1011, column temperature 50°C, mobile phase 0.01 N $H_2SO_4$, and flow 0.6 ml/min. The concentrations of sugars and ethanol were quantified by HPLC (Agilent 1100, HP, USA) with a refraction detector, column Shidex SH 1011, column temperature 50°C, mobile

phase 0.01 N $H_2SO_4$, and flow 0.6 ml/min.

*Sensory evaluation*

The overall pleasantness (OP) of taste and flavour were assessed using 100 mm graphical non-structured line segments with specified end-points, and was expressed as a percentage of the scale.

*Statistical analyses*

The data presented are from at least three independent cultivations. All analytical measurements were repeated five times. The Student's t-test was employed to check the differences between means at a significance level < 0.05.

## Results and Discussion

All eight *L. reuteri* strains used in the study grew in birch sap (Table 1). pH values dropped down from an initial pH 6 to pH 4.20–3.18 characteristic for fermented products. Total acidity reached up to 36 °T. All *L. reuteri* strains produced a considerable amount of biomass (OD). However strain-specific trends were observed for all analysed parameters, indicating a strain-specific response towards the substrate. The growth rate of almost all strains was highest during the first 24 h of fermentation. For example pH and °T values barely changed between 24 h and 48 h for *L. reuteri* 25 but *L. reuteri* 19 grew until 48 h, which could indicate lower acid tolerance and higher demand for growth factors for *L. reuteri* 25 compared to *L. reuteri* 19. In terms of the organoleptic qualities of birch sap samples fermented by different *L. reuteri* strains, the highest quality was achieved by *L. reuteri* 42, and thus this strain was used in further experiments (Table 1).

Non-fermented birch sap contains organic acids including oxalic, citric, malic, succinic and formic acid in minor concentrations that also varied non-significantly during sap fermentation by *L. reuteri* strains (Table 2). The consumption of fructose was very low during fermentation but glucose was fully consumed and lactic acid, acetic acid and ethanol were synthesized, as is characteristic for heterofermentative LAB. Lactic acid, acetic acid and ethanol were not detected in fresh birch sap. The concentrations of synthesized products were strain specific. Fructose was consumed comparatively more during spontaneous fermentation than in single starter fermentations, thus indicating that microorganisms preferring fructose as a carbon source, for example, yeasts, were taking part in spontaneous fermentation. Notably the lowest ethanol concentration was found in the spontaneous fermentation sample, indicating

Table 1. Changes in pH, total acidity and biomass concentration during birch sap fermentation by *Lactobacillus reuteri* strains

| | 24 h | | | 48 h | | | OP*, % of scale |
|---|---|---|---|---|---|---|---|
| | pH | Total acidity, °T | OD | pH | Total acidity, °T | OD | |
| Birch sap (control) | 6.10 ± 0.28 | 2 ± 1.0 | 0.04 ± 0.00 | 6.10 ± 0.30 | 2 ± 1.0 | 0.04 ± 0.00 | |
| *L. reuteri* 25 | 4.05 ± 0.20 | 10 ± 1.0 | 0.53 ± 0.02 | 4.05 ± 0.18 | 12 ± 1.0 | 0.53 ± 0.03 | 70 |
| *L. reuteri* 42 | 4.26 ± 0.20 | 12 ± 1.0 | 0.43 ± 0.02 | 4.04 ± 0.18 | 14 ± 1.0 | 0.50 ± 0.03 | 100 |
| *L. reuteri* 43 | 4.15 ± 0.21 | 13 ± 1.0 | 0.45 ± 0.02 | 3.78 ± 0.19 | 16 ± 1.0 | 0.50 ± 0.03 | 60 |
| *L. reuteri* 44 | 3.33 ± 0.18 | 24 ± 2.0 | 0.85 ± 0.05 | 3.18 ± 0.16 | 36 ± 2.0 | 0.88 ± 0.05 | 90 |
| *L. reuteri* 45 | 4.01 ± 0.20 | 13 ± 1.0 | 0.61 ± 0.04 | 3.95 ± 0.22 | 15 ± 1.0 | 0.65 ± 0.03 | 30 |
| *L. reuteri* 12 | 3.99 ± 0.22 | 18 ± 1.0 | 0.42 ± 0.03 | 3.99 ± 0.20 | 20 ± 1.0 | 0.44 ± 0.02 | 50 |
| *L. reuteri* 16 | 4.20 ± 0.21 | 11 ± 1.6 | 0.45 ± 0.02 | 3.88 ± 0.22 | 17 ± 1.0 | 0.60 ± 0.04 | 90 |
| *L. reuteri* 19 | 3.20 ± 0.19 | 14 ± 1.0 | 0.50 ± 0.03 | 3.27 ± 0.17 | 22 ± 1.0 | 0.68 ± 0.04 | 50 |

* Sensory properties expressed as the overall pleasantness (OP) of birch sap samples fermented by different Lactobacillus reuteri strains (48 h) were assessed using 100 mm graphical non-structured line segments with specified end-points, and was expressed as a percentage of the scale.

LAB prevalence over the yeasts, as subsequently confirmed by microscopic examination of the sample.

Several LAB were able to grow in birch sap (Kim *et al.*, 2009), however the lowering of pH was not satisfactory. The addition of xylitol to the sap before fermentation accelerated growth of LAB. This indicates that to improve the acidification power and viable cell count, which are the main characteristics of effective probiotic products (Minelli and Benini, 2008), growth supplements could be useful. Moreover it is known that LAB have limited metabolic capacity, requiring a nutrient-rich media for growth (Felis and Dellaglio, 2007). Therefore the main reasons why LAB develop poorly in birch sap could be 1) low carbon source content (as shown with xylitol supplementation by Kim *et al.* (2009); and/or 2) shortage of other growth factors, especially nitrogen. It was reported that acidification was rapid (16 h) during maple sap fermentation by several *Lactobacillus* strains and that the viable cell count reached $10^9$ CFU/ml (Cochu *et al*., 2008); interestingly, maple sap is known for its high sucrose content (up to 30 g/l). Therefore birch sap was supplemented with several carbon sources and their combinations (glucose, fructose, sucrose) at concentrations ranging from 0.5 to 2% (Table 3). It was shown that glucose and fructose supplementation as well as a glucose-fructose combination at a concentration of 0.5–1% did not significantly improve the growth of *L. reuteri.* Supplementation with 0.5–2% sucrose and a 2% glucose-fructose combination had a notable effect, although the latter had less effect than the former. However, it should be noted that the highest ºT value was reached following supplementation with 2% glucose or 0.5–2% glucose-fructose. At the same time the lowest pH value was reached following supplementation with 0.5–2% glucose, although there was no concentration-dependent effect. Thus it appears that both sugar concentration and growth factors limit the growth of *Lactobacilli* in birch sap. Given that the viable cell count is the most important parameter in probiotic products, supplementation

Table 2. Concentration (g/l) of organic acids, ethanol and sugars in birch sap samples fermented by *Lactobacillus reuteri* strains (48 h)

| | Lactic acid | Acetic acid | Citric acid | Gluconic acid | Malic acid | Succinic acid | Formic acid | Ethanol | Glucose | Fructose |
|---|---|---|---|---|---|---|---|---|---|---|
| Control* | traces | 0.15 ± 0.01 | 0.061 ± 0.003 | 0.069 ± 0.003 | 0.18 ± 0.01 | 0.061 ± 0.003 | 0.046 ± 0.002 | traces | 3.32 ± 0.17 | 4.67 ± 0.25 |
| Control** | 1.12 ± 0.05 | 0.17 ± 0.01 | 0.031 ± 0.002 | 0.058 ± 0.003 | 0.10 ± 0.01 | 0.021 ± 0.001 | traces | 0.02 ± 0.00 | 0.70 ± 0.04 | 0.86 ± 0.05 |
| *L. reuteri* 25 | 2.72 ± 0.14 | 0.37 ± 0.02 | 0.031 ± 0.015 | 0.039 ± 0.002 | 0.12 ± 0.01 | 0.087 ± 0.003 | 0.062 ± 0.002 | 0.26 ± 0.01 | traces | 1.84 ± 0.09 |
| *L. reuteri* 42 | 1.12 ± 0.06 | 0.14 ± 0.07 | 0.031 ± 0.002 | 0.043 ± 0.002 | 0.25 ± 0.02 | 0.063 ± 0.002 | 0.100 ± 0.004 | 0.24 ± 0.01 | traces | 2.10 ± 0.11 |
| *L. reuteri* 43 | 1.40 ± 0.07 | 0.19 ± 0.01 | 0.037 ± 0.002 | 0.043 ± 0.001 | traces | traces | traces | 0.16 ± 0.01 | traces | traces |
| *L. reuteri* 44 | 2.32 ± 0.12 | 0.32 ± 0.02 | 0.044 ± 0.002 | 0.078 ± 0.004 | 0.15 ± 0.01 | 0.033 ± 0.002 | 0.056 ± 0.004 | 0.29 ± 0.01 | traces | 1.47 ± 0.08 |
| *L. reuteri* 45 | 1.19 ± 0.06 | 0.15 ± 0.03 | 0.029 ± 0.001 | 0.084 ± 0.003 | 0.15 ± 0.01 | 0.054 ± 0.004 | 0.056 ± 0.003 | 0.34 ± 0.02 | traces | 1.55 ± 0.08 |
| *L. reuteri* 12 | 2.74 ± 0.14 | 0.38 ± 0.02 | 0.112 ± 0.005 | 0.064 ± 0.003 | 0.15 ± 0.01 | 0.040 ± 0.002 | 0.061 ± 0.004 | 0.38 ± 0.02 | traces | 1.72 ± 0.09 |
| *L. reuteri* 16 | 1.66 ± 0.09 | 0.24 ± 0.01 | 0.044 ± 0.002 | 0.062 ± 0.002 | 0.05 ± 0.00 | 0.017 ± 0.002 | 0.057 ± 0.003 | 1.22 ± 0.06 | traces | traces |
| *L. reuteri* 19 | 2.94 ± 0.15 | 0.40 ± 0.02 | 0.171 ± 0.009 | 0.099 ± 0.005 | 0.15 ± 0.01 | 0.025 ± 0.001 | 0.070 ± 0.004 | 0.53 ± 0.03 | traces | 1.58 ± 0.08 |

*at the beginning of fermentation
**spontaneous fermentation without starter culture addition (48 h)

Table 3. Influence of glucose, fructose and sucrose supplementation on growth and organic acid synthesis during birch sap fermentation by *Lactobacillus reuteri* 42 (48 h)

| | pH | Total acidity, °T | OD | Lactic acid | Acetic acid | Citric acid | Gluconic acid | Malic acid | Succinic acid |
|---|---|---|---|---|---|---|---|---|---|
| Control | 3.72 ± 0.19 | 18 ± 1.0 | 0.47 ± 0.03 | 2.62 ± 0.13 | 0.37 ± 0.02 | 0.01 ± 0.001 | 0.09 ± 0.00 | 0.20 ± 0.01 | 0.05 ± 0.002 |
| +0.5% glucose | 3.57 ± 0.18 | 21 ± 1.0 | 0.47 ± 0.02 | 3.21 ± 0.16 | 0.37 ± 0.02 | 0.02 ± 0.001 | 0.15 ± 0.01 | 0.26 ± 0.01 | 0.06 ± 0.003 |
| +1% glucose | 3.57 ± 0.18 | 22 ± 1.0 | 0.47 ± 0.03 | 3.06 ± 0.15 | 0.34 ± 0.02 | 0.02 ± 0.001 | 0.14 ± 0.01 | 0.23 ± 0.01 | 0.09 ± 0.004 |
| +2% glucose | 3.55 ± 0.20 | 26 ± 1.0 | 0.48 ± 0.02 | 3.31 ± 0.16 | 0.49 ± 0.02 | 0.02 ± 0.001 | 0.18 ± 0.01 | 0.25 ± 0.01 | 0.06 ± 0.003 |
| +0.5% fructose | 3.70 ± 0.19 | 18 ± 1.0 | 0.47 ± 0.02 | 2.56 ± 0.13 | 0.40 ± 0.02 | 0.02 ± 0.001 | 0.08 ± 0.00 | 0.52 ± 0.03 | 0.07 ± 0.004 |
| +1% fructose | 3.68 ± 0.20 | 19 ± 1.0 | 0.47 ± 0.03 | 2.93 ± 0.15 | 0.39 ± 0.02 | 0.02 ± 0.001 | 0.07 ± 0.00 | 0.52 ± 0.03 | 0.08 ± 0.005 |
| +2% fructose | 3.66 ± 0.17 | 21 ± 1.0 | 0.47 ± 0.03 | 2.99 ± 0.15 | 0.42 ± 0.02 | 0.02 ± 0.001 | Traces | 0.52 ± 0.03 | 0.07 ± 0.004 |
| +0.5% sucrose | 3.65 ± 0.16 | 22 ± 1.0 | 0.67 ± 0.04 | 2.88 ± 0.15 | 0.59 ± 0.03 | 0.07 ± 0.005 | 0.18 ± 0.01 | 0.32 ± 0.02 | 0.08 ± 0.005 |
| +1% sucrose | 3.66 ± 0.18 | 22 ± 1.0 | 0.74 ± 0.04 | 2.76 ± 0.14 | 0.52 ± 0.03 | 0.08 ± 0.003 | 0.16 ± 0.01 | 0.30 ± 0.02 | 0.08 ± 0.004 |
| +2% sucrose | 3.64 ± 0.18 | 22 ± 1.0 | 0.73 ± 0.04 | 2.19 ± 0.12 | 0.56 ± 0.03 | 0.09 ± 0.004 | 0.12 ± 0.01 | 0.32 ± 0.02 | 0.09 ± 0.005 |
| +0.5% glucose/fructose* | 3.62 ± 0.20 | 23 ± 2.0 | 0.48 ± 0.02 | 3.05 ± 0.16 | 0.39 ± 0.02 | 0.02 ± 0.001 | 0.13 ± 0.01 | 0.42 ± 0.02 | 0.07 ± 0.003 |
| +1% glucose/fructose* | 3.61 ± 0.18 | 24 ± 2.0 | 0.49 ± 0.02 | 2.57 ± 0.13 | 0.38 ± 0.02 | 0.02 ± 0.001 | 0.13 ± 0.01 | 0.50 ± 0.03 | 0.06 ± 0.003 |
| +2% glucose/fructose* | 3.59 ± 0.19 | 24 ± 2.0 | 0.51 ± 0.03 | 3.04 ± 0.16 | 0.48 ± 0.03 | 0.02 ± 0.001 | 0.15 ± 0.01 | 0.86 ± 0.05 | 0.07 ± 0.005 |

*glucose and fructose were supplemented in equal ratio

Table 4. Changes in pH, total acidity and biomass concentration in birch sap fermented by *Lactobacillus reuteri* 42 and supplemented with sucrose

| | 24 h | | | 48 h | | |
|---|---|---|---|---|---|---|
| | pH | Total acidity, °T | OD | pH | Total acidity, °T | OD |
| Control | 3.82 ± 0.20 | 18 ± 1.0 | 0.68 ± 0.03 | 3.66 ± 0.20 | 28 ± 2.0 | 0.68 ± 0.04 |
| +0.5% sucrose | 4.10 ± 0.21 | 18 ± 1.0 | 0.75 ± 0.04 | 3.75 ± 0.20 | 34 ± 2.0 | 0.87 ± 0.04 |
| +1% sucrose | 4.09 ± 0.22 | 18 ± 1.0 | 0.79 ± 0.04 | 3.73 ± 0.18 | 33 ± 2.0 | 0.96 ± 0.05 |
| +2% sucrose | 4.24 ± 0.18 | 18 ± 1.0 | 0.80 ± 0.05 | 3.73 ± 0.17 | 32 ± 2.0 | 0.99 ± 0.05 |
| +4% sucrose | 4.25 ± 0.18 | 18 ± 1.0 | 0.83 ± 0.05 | 3.80 ± 0.19 | 31 ± 2.0 | 0.99 ± 0.05 |
| +8% sucrose | 4.35 ± 0.20 | 18 ± 1.0 | 0.84 ± 0.04 | 3.82 ± 0.19 | 31 ± 2.0 | 0.98 ± 0.05 |

Table 5. Influence of various food-grade supplements on growth of *Lactobacillus reuteri* 42 during birch sap fermentation*

| | pH** | | Total acidity, °T | | OD | | logCFU/ml |
|---|---|---|---|---|---|---|---|
| | 24 h | 48 h | 24 h | 48 h | 24 h | 48 h | 48 h |
| Control | 3.85 ± 0.19 | 3.73 ± 0.16 | 24 ± 1.0 | 30 ± 1.0 | 0.66 ± 0.03 | 0.72 ± 0.04 | 6.79 ± 0.32 |
| + lemon | 3.92 ± 0.20 | 3.75 ± 0.18 | 23 ± 1.0 | 35 ± 2.0 | 0.46 ± 0.03 | 0.69 ± 0.03 | 6.77 ± 0.33 |
| + quince syrup | 3.80 ± 0.20 | 3.50 ± 0.18 | 27 ± 1.0 | 32 ± 2.0 | 0.48 ± 0.03 | 0.63 ± 0.03 | 6.72 ± 0.34 |
| + raisins | 3.86 ± 0.19 | 3.78 ± 0.19 | 32 ± 2.0 | 40 ± 2.0 | 0.70 ± 0.04 | 0.81 ± 0.03 | 6.83 ± 0.34 |
| + peppermint | 3.92 ± 0.20 | 3.65 ± 0.20 | 63 ± 3.0 | 91 ± 4.0 | 1.60 ± 0.08 | 1.75 ± 0.08 | 7.17 ± 0.36 |
| + malt extract | 3.55 ± 0.15 | 3.37 ± 0.17 | 56 ± 3.0 | 74 ± 4.0 | 1.33 ± 0.07 | 1.36 ± 0.07 | 7.06 ± 0.35 |
| + brown sugar | 3.94 ± 0.20 | 3.85 ± 0.15 | 22 ± 1.0 | 24 ± 1.0 | 0.68 ± 0.04 | 0.71 ± 0.05 | 6.78 ± 0.34 |
| + ginger | 3.88 ± 0.20 | 3.86 ± 0.19 | 23 ± 1.0 | 27 ± 1.0 | 0.69 ± 0.03 | 0.77 ± 0.50 | 6.81 ± 0.28 |
| + lime | 3.88 ± 0.19 | 3.85 ± 0.20 | 24 ± 1.0 | 23 ± 1.0 | 0.40 ± 0.02 | 0.71 ± 0.04 | 6.78 ± 0.31 |

* All samples were supplemented by 1% of sucrose; food-grade supplements were added in concentration of 2%
** Initial pH after food-grade supplement addition was adjusted to pH 6.0 ± 0.2

with sucrose was chosen as the best way to improve the substrate (Table 4). Furthermore, samples supplemented with sucrose achieved a higher score during sensory evaluation (data not shown) and, as shown in Table 4, the addition of sucrose stimulated biomass growth and improved acidification power, with the best results for sucrose 0.5–2%. However, a concentration-dependent effect was not observed.

The concentration of malic acid increased significantly in samples supplemented with fructose 0.5–2%, sucrose, and glucose-fructose 0.5–2%, but not in samples supplemented with glucose. The addition of sucrose caused a significant increase in acetic acid concentration whereas the addition of glucose caused an increase in lactic acid concentration.

Several other food grade supplements were evaluated to improve the growth of potentially probiotic *L. reuteri* strains in 1% sucrose-supplemented birch sap with the aim of developing a functional non-dairy–based beverage (Table 5). The best results were achieved using peppermint and malt extract supplements, which clearly indicates that LAB growth in birch sap is restricted not only by the availability of carbon but also by the availability of other growth factors present in the supplements used. Malt extract and peppermint supplementation accelerated growth much more significantly than supplementation only by sugars. Malt extract has also been used as a growth factor to increase the growth of *Lactobacillus acidophilus* and *Bifidobacterium bifidum* in milk and yoghurt (Marhamatizadeh *et al*., 2011).

## Conclusions

It was shown that during birch sap fermentation it is possible to achieve a high biomass of potentially probiotic *L. reuteri* strains by the enrichment of birch sap with sugars and co-substrates of food origin that are rich in favourable growth factors. Thus the development of birch sap-based functional fermented beverages could be a promising way to broaden choice of non-dairy functional foods.

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## References

Ahtonen, S. and Kallio, H. 1989. Identification and

seasonal variations of amino acids in birch sap used for syrup production. Food Chemistry 33: 125-132.

Casas, I.A. and Dobrogosz, W.J. 2000. Validation of the probiotic concept: *Lactobacillus reuteri* confers broad-spectrum protection against disease in humans and animals. Microbial Ecology in Health and Disease 12(4): 247-285.

Cochu, A., Fourmier, D., Halasz, A. and Hawari, J. 2008. Maple sap as a rich medium to grow probiotic lactobacilli and to produce lactic acid. Letters in Applied Microbiology 47(6): 500-507.

De Man, J.C., Rogosa, M. and Sharpe, M.E. 1960. A medium for the cultivation of Lactobacilli. Journal of Applied Microbiology 23(1): 130-135.

De Vuyst, L. 2000. Technology aspects related to the application of functional starter cultures. Food Technology and Biotechnology 38(2): 105-112.

Felis, G.E. and Dellaglio, F. 2007. Taxonomy of Lactobacilli and Bifidobacteria. Current Issues in Intestinal Microbiology 8: 44-61.

Haq, S. and Adams, G.A. 1962. Oligosaccharides of birch sap. Canadian Journal of Biochemistry and Physiology 40(8): 989-97.

Hernandez-Mendoza, A., Robles, V.J., Angulus, J.O., De La Cruz, J. and Garcia, H.S. 2007. Preparation of a whey-based probiotic product with *Lactobacillus reuteri* and *Bifidobacterium bifidum.* Food Technology and Biotechnology 45(1): 27-31.

Jeong, S.J., Jeong, H.S., Woo, S.H. and Shin, C.H. 2013. Consequences of ultrafiltration and ultraviolet on the quality of white birch (*Betula platyphylla* var. *japonica*) sap during storage. Australian Journal of Crop Science 7(8): 1072-1077.

Jones, M.L., Martoni, C.J., Parent, M. and Prakash, S. 2012. Cholesterol-lowering efficacy of a microencapsulated bile salt hydrolase-active *Lactobacillus reuteri* NCIMB 30242 yogurt formulation in hypercholesterolaemic adults. British Journal of Nutrition 107(10): 1505-1513.

Kallio, H. and Ahtonen, S. 1987a. Seasonal variations of the acids in birch sap. Food Chemistry 25(4): 285-292.

Kallio, H. and Ahtonen, S. 1987b. Seasonal variations of the sugars in birch sap. Food Chemistry 25(4): 293-304.

Khalf, M., Dabour, N., Kheadr, E. and Fliss, I. 2010. Viability of probiotic bacteria in maple sap products under storage and gastrointestinal conditions. Bioresource Technology 101(20): 7966-7972.

Kim, J.H., Lee, W.J., Cho, Y.W. and Kim, K.Y. 2009. Storage life and palatability extension of Betula platyphylla sap using lactic acid bacteria fermentation. Journal of the Korean Society of Food Science and Nutrition 38(6): 787-794.

Klinger, W., Hirschelmann, R. and Suss, J. 1989. Birch sap and birch leaves extract - screening for antimicrobial, phagocytosis influencing, antiphlogistic and antipyretic activity. Die Pharmazie 44(8):558-560.

Marhamatizadeh, M.H., Karmand, M., Farokhi, A. R., Rafatjou, R. and Rezazadeh, S. 2011. The effects of malt extract on the increasing growth of probiotic bacteria *Lactobacillus acidophilus* and *Bifidobacterium bifidum* in probiotic milk and youghurt. Journal of Food Technology and Nutrition 8(2(30)): 78-84.

Mattila-Sandholm, T., Myllrinen, P., Crittenden, R., Mogensen, G., Fonden, R. and Saarela, M. 2002. Technological challenges for future probiotic foods. International Dairy Journal 12(2-3): 173-182.

Minelli, E.B. and Benini, A. 2008. Relationship between number of bacteria and their probiotic effects. Microbial Ecology in Health and Disease 20: 180-183.

Ojetti, V., Gigante, G., Gabrielli, M., Ainora, M.E., Mannocci, A., Lauritano, E.C., Gasbarrini, G. and Gasbarrini, A. 2010. The effect of oral supplementation with *Lactobacillus reuteri* or tilactase in lactose intolerant patients: randomized trial. European Review for Medical and Pharmacological Sciences 14(3): 163-170.

Peev, C., Dehelean, C., Mogosanu, C., Feflea, S. and Corina, T. 2010. Spring drugs of Betula Pendula roth.: biologic and pharmacognostic evaluation. Studia Universitatis "Vasile Goldis", Seria Stiintele Vietii 20(3): 41-43.

Scott, R., Robinson, R. K. and Wilbey, R.A. 1998. Cheesemaking practice 3rd edn., Kluwer Academic.

Shornikova, A.V., Casas, I., Isolauri, E., Mykkanen, H. and Vesikari, T. 1997. Lactobacillus reuteri as a therapeutic agent in acute diarrhea in young children. Journal of Pediatric Gastroenterology and Nutrition 24(4): 399-404.

Svanberg, I., Soukand, R., Luczaj, L., Kalle, R., Zyryanova, O., Denes, A., Papp, N., Nedelcheva, A., Šeškauskaite, D., Kolodziejska-Degorska, I. and Kolosova, V. 2012. Use of tree saps in northen and eastern parts of Europe. Acta Societatis Botanicorum Poloniae 81(4): 343-357.

Wolf, B.W., Garleb, D.G. and Casas, I. 1995. Safety and tolerance of *Lactobacillus reuteri* in healthy adult male subjects. Microbial Ecology in Health Disease 8(2): 41-50.