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# NEW PREBIOTICS FOR FUNCTIONAL FOOD*

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A technology of fructan syrup production from sucrose using bacteria *Zymomonas mobilis* 113 "S" has been developed. The obtained fructan syrup contained 64% of total carbohydrates and 45–48% of fructans (fructooligosaccharides and levan) from total carbohydrates. The product has a reduced energetic value and excellent honey-like taste. Fructan syrup additive of 4 to 11% was used for fat-free milk and oat mash to study the influence on *Bifidobacterium lactis* 12 growth during 24 h. High cell count of *Bifidobacterium* was achieved after the 6 h of fermentation.

**Keywords:** *Zymomonas mobilis,* fructan syrup, fructooligosaccharides, functional food products

The term "functional food" is understood as a product which, as well as providing the normal attributes expected of food, also confers a specific health benefit (WOOD, 1997). Several components of food are known to be particularly beneficial to human health, e.g. various living lactic acid bacteria, the dietary fibre, polyunsaturated fatty acids, oligosaccharides and antioxidants (GOLDBERG, 1994; PSZCZOLA, 1996). Functional foods are one of the fastest-growing segments of the food industry and different kinds of functional food products have been designed and supplied to the market (KNORR, 1998; SANDERS, 1998). In Japan, England and some other countries, functional foods have already become part of the dietary landscape. Especially popular have become dairy based products – yogurts or milk drinks, containing live, active probiotic bacteria belonging to genera *Lactobacillus* and *Bifidobacterium*. Probiotics are defined as a microbial dietary additive that beneficially influences the host physiology by modulating mucosal and systematic immunity, as well as improving the nutritional and microbial balance in the intestinal tract (DE VUYST, 1998; NAIDU et al., 1999). Specific foodstuffs – prebiotics are used to stimulate the development of probiotic bacteria in the intestinal tract. Prebiotics practically are non-digestible food ingredients and did not supply the organism with energy. In gut prebiotics can metabolize several bacteria including *Bifidobacterium*, which reproduce and produce metabolites – lower fatty acids, further lowering the cholesterol level in blood (GIBSON & ROBERFROID, 1995).

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In many respects the activity of prebiotics complements that of probiotics. This has led to the concept of "synbiotics" which combines live probiotic bacteria and an appropriate prebiotic in the same product (O'BRIEN, 1998). Prebiotics are mainly oligo-form carbohydrates. Several companies in the world, and especially in Japan, produce prebiotics as sweeteners and food-additives (O'BRIEN, 1998). It is known that fructooligosaccharides (FOS) are used as prebiotics in yogurts. The Meiji Seika Company (Japan) produces several oligo yogurts applying FOS as active ingredients (O'BRIEN, 1998). The dosage of prebiotics in yogurts usually is 2–5 g/100 g (ROBERFROID & DELZENNE, 1998) and the accepted daily intake of FOS in Japan is about 0.8 g per 1 kg body weight (YUN, 1996).

FOS are fructans containing 2 to 9 fructose units and are naturally found not only in plants, but also in bacteria and fungi, probably serving very different functions. Most bacterial fructans are high molecular weight polymers of the levan type, i.e. they are composed of β-(2,6)-fructosyl-fructose linked molecules and side chains (MARX et al., 1999). Fructans act as low-calorie sweeteners, prebiotic dietary fibres, and nutraceuticals with cholesterol regulating effects, enhancers of calcium intake and fat replacers. Fructan polymers levans are part of the exopolysaccharide that protects the cells from desiccation, helps in surface attachment, and in some plants pathogenic species is involved in preventing the invading bacteria from being recognized by the host defense system (KASAPIS et al., 1994; HETTWER et al., 1995). Levan is extracellulary produced by different bacteria *Bacillus subtilis*, *Erwinia herbicola* (COTE & AHLGREN, 1993) and *Zymomonas mobilis* (VIIKARI, 1984). *Z. mobilis* is a unique bacterium among the microbial evolutionary world and its taxonomic position has not been fully established. In a sucrose-based medium this gram-negative, ethanol-producing bacterium produces various by-products: levan of high molecular mass (BEKERS et al., 1993), sorbitol, gluconic acid and FOS (VIIKARI, 1988). Levan is viscous, biologically active, non-toxic and can be used as thickener or stabilizer in the food, pharmaceutical and cosmetic industries. It acts as immunomodulator, blood plasma substitute, prolongator of medicine, and a cholesterol lowering agent (YAMAMOTO et al., 1999).

Fructan syrup and levan (BEKERS et al., 2000) were obtained from *Z. mobilis* sucrose fermentation. Both products are especially interesting as food and functional food ingredients because of their beneficial influence on intestinal flora, functionality, and reduced caloric value.

The objectives of this investigation were to evaluate fructan syrup and levan produced by *Z. mobilis* as prebiotics.

## 1. Materials and methods

The levan-producing strain *Zymomonas mobilis* 113 "S" and a two-stage fermentation process were used as described previously (BEKERS et al., 1990). After the second stage of fermentation, the culture liquid was centrifuged at 28,600 ×*g*. The cell-free

supernatant was treated with ethanol (65 V%) (1:2.5) to obtain crude levan precipitate. After hydrolysis of polysaccharide the content of levan was determined as fructose (VIIKARI, 1984).

Fructan syrup was produced in conformity with the patented method (BEKERS et al., 2000). Fructan syrup was prepared from sucrose syrup (65%) using as biocatalyst 5 g/100 g levan-levansucrase sediment at incubation temperature of 45 °C for 48 h. In accordance with the technical guidance, fructan syrup was qualified as a food product and a raw material for the food industry. Both products – levan and FOS, are practically nondigesting compounds: FOS is sweet, and levan has no taste.

The sugar content was calculated as reducing sugars (glucose, fructose), considering the levan content as a fructose source. The concentrations of glucose, fructose and sucrose were determined by HPLC (the column Pinacle Amino 5 μm, 250×4.6 with a mobile phase of acetonitryle:water 75:25, refractive index detector). Reducing sugars (RS) were determined using the Lane-Eynon method (VELIKAJA et al., 1964).

*Bifidobacterium lactis* 12 (Chr. Hansens Applied Technological Laboratory, Denmark), 0.0014 g/100 g was grown in a fat-free milk media with additives of glucose or fructan syrup at 37 °C for 24 h. Oat mash medium prepared from oat flacks and water (100 g/1000 ml) was partly hydrolyzed by α-amylase enzyme preparation "Fungamyl" (Nova Nordisk) and 0.0017 g/100 g of *Bifidobacterium lactis* 12 was inoculated. The titre of bacteria was determined by dilution method (BANNIKOVA et al., 1987).

## 2. Results

Levan is a major by-product produced by *Z. mobilis* in sucrose fermentation. The enzyme levansucrase (EC 2.4.1.10) is responsible for sucrose hydrolysis, levan formation and oligosaccharide production. Being incubated in glucose medium at 30 °C and fermented in sucrose medium, Z. *mobilis* 113 "S" produces 30–50 l $g^{-1}$ of levan during batch or continuous fermentation (BEKERS et al., 1990; BEKERS et al., 1993). Being incubated in sucrose medium at 30 °C, during subsequent batch fermentation in sucrose medium at 25 °C, *Z. mobilis* 113 "S" produces 20–30 l $g^{-1}$ of levan and 40–60 l $g^{-1}$ of ethanol. The biomass concentration in the culture liquid reached 1.5–2.2 l $g^{-1}$ after the second fermentation stage (BEKERS et al., 1999). After centrifugation of the biomass, levan was precipitated by ethanol (VIIKARI, 1984). The molecular mass of levan was up to $2\times10^6$ Da. Obviously, in the fermentation medium, extracellular levansucrase forms a complex with levan, and is simultaneously a product of the enzymatic reaction and substrate (REECE & AVIGAD, 1966; CRITTENDEN & DOELLE, 1994). Levansucrase extracted from culture liquid was applied as biocatalyst to convert sucrose into levan or FOS. In a medium with 10–15% of sucrose at 25 °C levansucrase forms levan, but at 45–55 °C in a medium with 50–65% forms FOS. The obtained fructan syrup contains 64% of total carbohydrates and 45–48% of fructans

(FOS and levan) from carbohydrates. Fructan syrup has a pleasant honey-like taste and like levan sediment could be applied as a prebiotics. Figure 1 shows the flow chart of fructan syrup production by *Z. mobilis*. The chemical composition of obtained fructan syrup is shown in Table 1. It must be noted that the energetic value of 100 g fructan syrup is 186 kcal or 776 kJ.

*Table 1.* The chemical composition of fructan syrup

| Component | Concentration (%) |
|---|---|
| Carbohydrates, total | 65 |
| FOS | 27 |
| Levan | 7 |
| Sucrose | 6 |
| Reducing sugars (glucose + fructose) | 22 |
| Water | 35 |

The obtained fructan syrup has been investigated as a food additive on mice and rats at the Latvian Academy of Medicine. Investigations of the acute toxicity on mice have shown that fructan syrup in quantities up to 2000 mg per 1 kg body weight is a

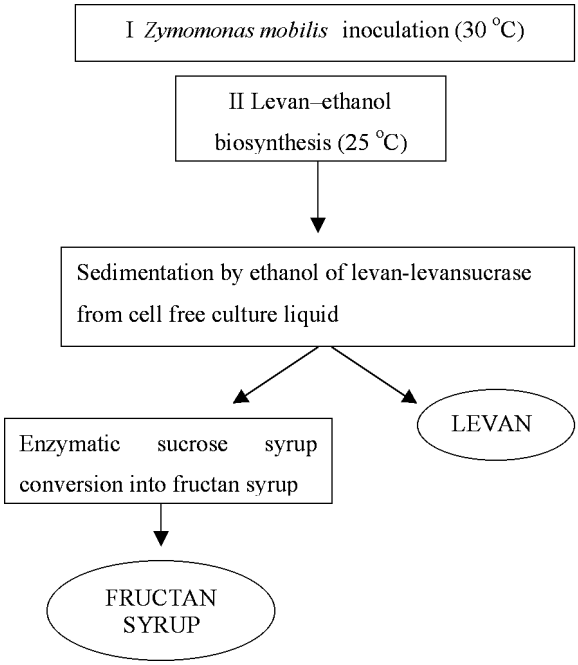


*Fig. 1.* Flow chart of levan and fructan syrup production

non-toxic product. Fructan syrup used as a feed additive (1 g per kg body weight per day) has been shown to decrease the total cholesterol level and increase the high-density lipoprotein cholesterol fraction in blood serum of rats. Obviously, fructan syrup is a promising product for prevention of cardiovascular and gastrointestinal diseases (SPRUDZA et al., 2002).

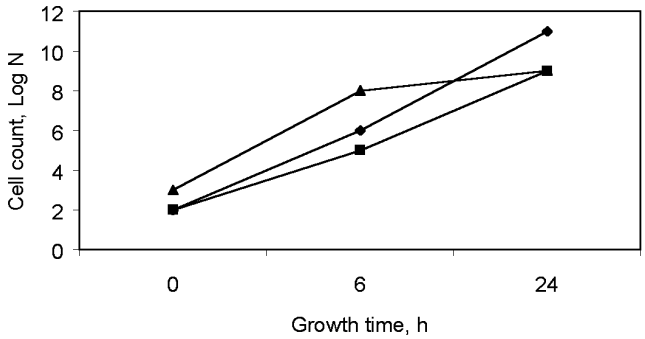


*Fig. 2.* The development of *Bifidobacterium lactis* 12 in fat-free milk medium enriched with fructan syrup. ♦: Milk, ■: milk and 5.0% fructan syrup; ▲: milk and 10% fructan syrup

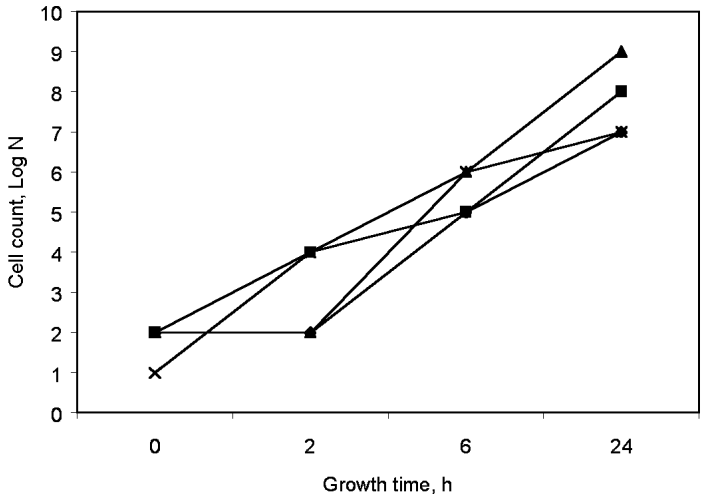


*Fig. 3.* The development of *Bifidobacterium lactis* 12 in oat mash medium enriched with fructan syrup. ♦: Oat mash; ■: oat mash and 4% fructan syrup; ▲: oat mash and 8% fructan syrup; **x**: oat mash and 11% fructan syrup

Fructan syrup as prebiotics could be used as a food-additive in dairy and milk-based products. Taking into account the practice of applying similar prebiotics in the USA and the European countries, the influence of 5–10% fructan syrup additive was under investigation. Fat-free milk and oat mash are used as components for yogurt production and therefore the influence of fructan syrup on the development of *Bifidobacterium* in these media was studied. The total cell counts during the bacterial growth in fat-free milk and oat mash medium with fructan syrup additives for 24 h are shown in Figs 2 and 3. The taste properties of yogurt enriched with fructan syrup were pleasant – sweet and sour. Fermented by amylolytic enzyme, oat mash provided good development of *Bifidobacterium* in medium enriched with 10% fructan syrup already after 6 h of growth. Using 10% fructan syrup as an additive to yogurt, 2–3 g of fructans were added to 100 g of the product.

## 3. Conclusions

The two-stage *Zymomonas mobilis* fermentation allows obtaining two new prebiotics – levan and fructan syrup. Fructan syrup obtained from *Z. mobilis* is composed not only of FOS but one more fructose polymer levan. In concentrations from 5 to 10% fructan syrup could be applied as a food-additive, preferably for dairy products.

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