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Combining prebiotics with probiotic bacteria can enhance bacterial growth and secretion of bacteriocins

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ABSTRACT

There is a growing interest in supporting human health by using prebiotics, such as oligosaccharides, and beneficial bacteria, also called probiotics. Combining these two components we can develop synbiotics. In order to create successful combination of synbiotic it is very important to evaluate the influence of prebiotic oligosaccharides to probiotic bacteria and their behavior, such as growth and secretion of health related biomolecules, including bacteriocins. In this study seven type strains of probiotic bacteria (five *Lactobacillus* sp. and two *Lactococcus* sp.) and two *Lactobacillus* sp. strains, isolated from probiotic yoghurt, were cultivated with various commercially available and extracted oligosaccharides (OS). The aim of this study was to evaluate the influence of these OS on type and isolated bacterial strains growth and antibacterial activity. Obtained results suggest that combination of certain OS with probiotic strains may considerably improve their growth and/or antibacterial activity. We also determined the antibacterial activity spectrum of investigated strains with combination of OS against common food borne pathogens. Results of this work show that prebiotic OS can be useful for modulating probiotic bacteria growth, antibacterial activity and even specificity of this activity.

1. Introduction

Prebiotics are described as non-digestible poli- or oligosaccharides (OS) that beneficially affect the host by selectively stimulating the growth and/or activity of one or a limited number of beneficial bacteria in the colon [1]. Probiotics (mainly bifidobacteria and lactobacilli) reside in human colon and there exert their action, modulating colon micro-flora, immunogenic responses or producing certain materials, which all together improves the health of the host. They may help to prevent infections, reduce cholesterol levels, promote vitamin and cytokine synthesis or even have anticancer effects [2–5]. In combination prebiotics and probiotic bacteria create synbiotic, which can provide even more benefits than probiotics and prebiotics alone.

To date the most studied prebiotics are fructooligoshaccarides (FOS) inulin and oligofructose [6,7]. Nevertheless, many other OS, like xylo-oligosaccharides (XOS), pectic oligosaccharides (POS), cyclodextrins, palatinose and OS from pullulan are also important prebiotic candidates [5,7–9]. Commercially available prebiotics, such as fructooligosaccharides (FOS), xylo-oligosaccharides (XOS), cyclodextrins, and palatinose used in this work are generally recognized as safe (GRAS) food additives [10,11]. Inulin is one of the most comprehensively studied OS [6,8]. It is a liner polymer consisting of β-(2 → 1)-fructosyl-fructose linkages and is mainly extracted from chicory roots [6,12]. Xylo-oligosaccharides (XOS) are sugars containing 2–7 xylose monomeric units linked through β-(1,4)-linkages. Depending on different sources used for XOS generation their structure varies in terms of polymerization degree and type of linkages present [13]. XOS appear naturally in vegetables, fruits, milk, honey and bamboo shoots or can be obtained by acid or enzymatic hydrolysis of xylan [5,13–15]. Palatinose is a reducing sugar, composed of glucose and fructose moieties, joined with α-1,6-glycozil bonds. Commercially palatinose is made from sucrose by enzymatically reorganizing glikozil bonds [11]. Pullulan is a linear polymer of maltotriose units (with two internal α-1,4-glucosidic linkages) that are joined by α-1,6-glucosidic bonds, and it is synthesized by the fungus *Aureobasidium pullulans* [16]. Cyclodextrins (CDs) are the circular oligosaccharides, composed of glucose residues linked by α-1,4-glikozil bonds. The hydrophilic part of the molecule is faced to the outside of the ring, and hydrophobic part is inside the ring [10,17]. Commercially cyclodextrins are produced from starch [17]. Although βCD is more often used industrially due to its lower price, but αCD is

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Table 1
OS used in this work, obtained in our and coworkers laboratories.

OS abreviation	Source	Polimerization degree (PD)	Monomers	Links
PL	Pullulan from *Aureobasidium pullulans* (Sigma-Aldrich)	≥3	Glucopyranose/maltodextrose (composed of three glucoses)	Between glucose: α-1,4 Between maltodextrose: α-1,6
XOS	Beech xylan	2, 3, 6	β-D-xylose	β−1,4
PG	Polygalacturonic acid	≥3	D-galacturonate	α−1,4
PK	Polygalacturonic acid metil ester (pectin)			
OS	**Name**	**PD**	**Monomer**	**Links**
S2	Xylobiose	2	D-xylose	β−1,4
S2OX	Oxidized xylobiose			
S4	Xylotetraose	4		
S4OX	Oxidized xylotetraose			

characterized by better solubility in water (140 mg/ml) than βCD (18 mg/ml, 25 °C) [18].

XOS, inulin, POS, palatinose and cyclodextrins are characterized by various physiologically important actions such as anti-oxidative effects; maintaining gastrointestinal health; improving the biological availability of calcium; stimulating immune system; reducing the amounts of pathogenic [6,19] or oral plaque bacteria [20]; reducing the risk of colon cancer; regulating of lipid and glucose metabolism with decreased glycemic response; reducing triglycerides, fatty acids and cholesterol amounts in the blood and so preventing cardiovascular diseases and decreasing the risk of obesity and type II diabetes; inhibiting starch retrogradation and improving the nutritional and sensory properties of food [2,8–10,13,21,22]. Although these OS also have sweetness, but due to their indigestibility they are characterized by low glycaemic index (for example, palatinose has about a half sweetness of sucrose, but its glycaemic index is only 32 [11]). This makes them even more favorable food additives for diabetic patients.

Like most prebiotics, FOS, cyclodextrins, POS and XOS are not digested in upper gastrointestinal tract (except palatinose), what makes them available and longer lasting carbon sources for beneficial colon bacteria, like bifidobacteria [6,22–24] and lactobacilli [25,26]. However, most investigations are focused on the influence of these OS on bifidobacteria and little is known about how they affect lactobacilli and other beneficial bacteria from other genera, like lactococci, which are widely used in the food industry [26–28]. Most of them produce various antibacterially active compounds and bacteriocins among them [3,8,25,29,30]. Not much research is done in this area, but there is evidence that bacteriocins may have various effects on the gut microbiota, e. g. facilitate the introduction of the producing strain into the gastrointestinal tract, inhibit the invasion of competing and pathogenic strains, modulate the composition of the gut microbiota and influence the host immune system [31]. While bacteriocin production is often growth-associated and is dependent on carbon availability, slow digestion of prebiotics is very important as they are much longer-lasting carbon sources and mostly fermented only in the colon [32]. In addition to this, it is important to clarify the effect of prebiotics to the growth and antibacterial activity of probiotic bacteria [29]. There is evidence that supplementation with FOS can increase antibacterial activity of certain *Lactobacillus* sp. strains [26,33] and inulin stimulates the secretion of bacteriocins by *Lactobacillus paracasei* CMGB16 strain [34] while it is widely recognized as bifidogenic prebiotic [6,8]. However, little is known about the influence of XOS, POS, αCD, palatinose and OS from pullulan on probiotic bacteria and their antibacterial activity, but it has been shown that αCD can maintain the growth of *Lactobacillus casei* and increase the amounts of bifidobacteria [35] while POS enhances the growth of both bifidobacteria and lactobacilli [9]. XOS are mainly bifidogenic [22,36], but there are some reports that they also maintains the growths of certain lactobacilli [36,37]. Pullulan is poorly metabolized and mainly only by bifidobacteria [16,37], but there is increasing evidence that pullulan may also promote the growth of fecal lactobacilli [38]. On the other hand, there are practically no data about probiotic bacteria ability to assimilate partially digested pullulan (OS from pullulan). However, studies have shown that ability of probiotic bacteria to ferment prebiotic OS is both strain and substrate specific [26,36].

In this work the possibilities of using prebiotic OS to increase not only the growth of probiotic bacteria, but also their antibacterial activity were investigated. Moreover, the ability of OS to modulate antibacterial activity spectrum of probiotic bacteria against common food borne pathogens was studied.

2. Materials and methods

2.1. Bacterial stains and growth conditions

Five *Lactobacillus* sp. and two *Lactococcus* sp. type strains were obtained from "German Collection of Microorganisms and Cell Cultures" (DSMZ, Braunschweig, Germany): *Lactobacillus acidophilus* DSM 20079 (LA), *Lactobacillus curvatus* DSM 20010 (LC), *Lactobacillus sakei* subsp. *sakei* DSM 20017(LS), *Lactococcus lactis* subsp. *lactis* DSM 20481 (LL) and *L. lactis* subsp. *lactis* DSM 20729 (LL2) (abbreviations of strains used further in the work are given in the brackets). Two strains (A11 and B13) isolated from probiotic yoghurts in previous study and most likely belonging to *Lactobacillus casei* and *Lactobacillus paracasei* species [39] were also used in this work. Isolated and type strains of *Lactobacillus* sp. were cultured in MRS broth (Merck, Kenilworth, NJ, USA) or basal MRS [40]. Type strains of *Lactococcus* sp. were grown in broth No. 92 (DSMZ culture medium list), which consisted of Tryptic Soy Agar (TSA) (Merck, Kenilworth, NJ, USA), supplemented with 0.3% Yeast Extract (YE) (DB Difco, Franklin Lakes, NJ, USA).

Isolated strains were cultured aerobically or anaerobically, with (100 rpm) or without agitation, at 30 °C. Type strain of *Lactobacillus acidophillus* was grown under anaerobic conditions, at 37 °C. Other type strains were cultured aerobically, at 30 °C. Strains were cultured in 50 ml flasks with 20 ml of liquid media or in 48 well plates ("CELLSTAR", Greiner Bio-One, Austria). The volume of cultivation media in the well was 1 ml. OD (Optical Density) changes were estimated using photoelectric colorimeter or "Varioscan Flash" plate reader (Thermo Fisher Scientific, Waltham, MA, USA).

2.2. Oligosaccharide substrates

Three different commercially available oligosaccharides were used in this study: inulin (Inl/I) (Alfa Aesar, Ward Hill, MA, USA), palatinose hydrate (Pal/P) (TCI) and α-cyclodextrin (Ctd/CD) (Merck, Kenilworth, NJ, USA). Eight OS obtained in our and cowork-