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IN VITRO AND ANIMAL STUDIES

In vitro study of the prebiotic xylooligosaccharide (XOS) on the growth of *Bifidobacterium* spp and *Lactobacillus* spp

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Abstract

We recently demonstrated that XOS increased the counts of *Bifidobacterium in vivo* without increasing *Lactobacillus* in healthy adults. In the current study, we evaluated the effect of XOS on the growth of 35 *Bifidobacterium* and 29 *Lactobacillus* strains in *in vitro* conditions. Bacteria were identified by 16S rRNA sequence analysis. The growth stimulation was determined by agar dilution technique on plates containing two-fold serial dilutions of XOS (100–0.1 mg/ml). The growth of 86% of *Bifidobacterium* strains was stimulated at 1.56 mg/ml XOS and 100% at 6.25 mg/ml XOS. The growth of 38% of *Lactobacillus* strains was stimulated at 1.56 mg/ml XOS and 62% at 6.25 mg/ml XOS; 31% of *Lactobacillus* were not stimulated by XOS. Our results further suggest that XOS may be beneficial in stimulating intestinal *Bifidobacterium* without having much effect on *Lactobacillus*. The potential role for XOS in managing obesity should be investigated further.

Keywords

Bifidobacterium, *Lactobacillus*, prebiotic, xylooligosaccharide

History

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Introduction

Prebiotics are highly effective and important for many applications in medicine. They are not absorbed and do not contribute to human nourishment, but rather exert a profound effect on the human bowel flora. The principal effect of prebiotics on the human bowel flora is to stimulate the growth of *Bifidobacterium* and *Lactobacillus* species (Slavin, 2013). These are benign organisms in that they are seldom involved in infections or other pathological processes. However, numerous health promoting benefits of these bacterial genera have been noted, with effects on infectious and non-infectious disease (Delzenne et al., 2013; Slavin, 2013). Studies on prebiotic intake and stimulation of bifidobacteria and other intestinal bacteria reveal that they play a very significant role in many important areas such as immune function and inflammation (Slavin, 2013).

Studies have shown a decreased number of *Bifidobacterium* in the stools of obese subjects (Collado et al., 2008; Kalliomaki et al., 2008; Schwiertz et al., 2010), and that administration of a *Bifidobacterium breve* to mice with high-fat diet-induced obesity led to a significant weight decrease (Kondo et al., 2010). Recent human studies have also suggested that obesity-associated gut microbiota has increased numbers of *Lactobacillus* (Armougom et al., 2009; Million et al., 2012) and an increased *Firmicutes/Bacteroidetes* ratio (Ley et al., 2006; Turnbaugh et al., 2009). The potential for prebiotics to be effective in the treatment of metabolic disorders and obesity is an area of potentially great significance. In our recent study, we demonstrated that XOS increased the counts of *Bifidobacterium in vivo* without increasing the counts of *Lactobacillus* in healthy adults (Finegold et al., 2014). Our findings suggested that XOS supplementation may be useful in the management of obesity. In the current study, we further evaluated the effect of XOS on the growth of *Bifidobacterium* and *Lactobacillus* strains in *in vitro* conditions to establish the concentration(s) where XOS had no effect on the growth, where XOS stimulated the growth, and where the growth stimulation by XOS reached a plateau for each test strain.

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Material and methods

Phytochemical preparations

The XOS was obtained from LifeBridge International Corp., Riverside, CA (xylo-oligosaccharide 70P). XOS stock solutions (0.2 g/ml) were prepared by dissolving XOS in water. The solutions were filter-sterilized (MillexGP, PES membrane filter 0.22 µm, EMD Millipore, Billerica, MA) and refrigerated. The solutions were used within 24 h of preparation.

Bacterial strains

The bacteria included in this study were clinical isolates from patients in the Greater Los Angeles VA Healthcare Center (Table 1). Altogether 35 strains of *Bifidobacterium* species consisting of 24 strains representing eight different species recovered from human intestinal contents and 11 American Type Culture Collection (ATCC) *Bifidobacterium* type strains were studied. Similarly, 29 strains of *Lactobacillus* species consisting of 22 strains recovered from human intestinal contents

Table 1. Growth of *Bifidobacterium* and *Lactobacillus* strains on various XOS concentrations. 0, no growth; 1, weak growth with translucent colonies; 2, moderate growth, semi-opaque, grey colonies; 3, strong growth with opaque, white colonies. No highlight, no growth stimulation; light shade, weak growth stimulation; dark shaded, strong growth stimulation by XOS.

			XOS mg/ml											
Source	Strain #	Species	0	0.1	0.2	0.39	0.78	1.56	3.125	6.25	12.5	25	50	100
Bifidobacterium														
ATCC	15703	*adolescentis*	0	0	0	1	1	2	3	3	3	3	3	3
ATCC	15706	*adolescentis*	0	0	1	1	1	2	2	3	3	3	3	3
Stool	19896	*adolescentis*	1	1	2	2	2	2	3	3	3	3	3	3
Stool	19933	*adolescentis*	1	1	1	1	2	2	3	3	3	3	3	3
Stool	19909	*animalis*	0	0	1	2	2	3	3	3	3	3	3	3
Stool	18691	*animalis*	0	1	1	1	1	1	1	1	1	1	1	0
Stool	19814	*animalis*	0	1	1	1	2	3	3	3	3	3	3	3
Stool	19915	*animalis*	0	0	1	1	2	3	3	3	3	3	3	3
ATCC	15696	*bifidum*	0	0	0	0	0	0	1	1	2	3	3	3
Stool	19877	*bifidum*	1	1	1	1	1	1	1	2	2	2	2	2
Stool	19886	*bifidum*	0	0	0	0	0	1	1	2	2	2	2	2
Stool	19851	*bifidum*	0	0	0	0	0	0	1	1	1	2	3	3
Stool	19895	*bifidum*	0	0	0	0	0	1	1	2	2	2	3	2
ATCC	15701	*breve*	0	0	0	1	1	2	3	3	3	3	3	3
ATCC	15700	*breve*	1	1	1	2	2	2	2	2	3	3	3	3
Stool	9597	*breve*	1	1	2	2	2	3	3	3	3	3	3	3
Stool	9535	*breve*	1	2	2	2	3	3	3	3	3	3	3	3
Stool	19855	*catenulatum*	1	1	1	2	3	3	3	3	3	3	3	2
Stool	19861	*catenulatum*	1	1	1	2	3	3	3	3	3	3	3	3
ATCC	15697	*infantis*	1	1	1	1	2	2	2	3	3	3	3	3
Stool	14599	*infantis*	0	0	0	0	0	1	2	2	3	3	3	3
Stool	14760	*infantis*	0	0	0	0	0	1	2	2	3	3	3	3
Stool	19815	*infantis*	0	1	1	1	2	3	3	3	3	3	3	3
ATCC	15707	*longum*	0	0	0	0	0	0	1	3	3	3	3	3
ATCC	15708	*longum*	0	0	0	1	1	1	2	3	3	3	3	3
Stool	6965	*longum*	0	0	0	0	1	1	2	3	3	3	3	3
Stool	19887	*longum*	0	0	0	0	0	1	1	1	3	3	3	3
Stool	19907	*longum*	0	0	0	1	2	3	3	3	3	3	3	3
Stool	19860	*longum*	0	0	1	2	2	3	3	3	3	3	3	3
Stool	19891	*longum*	0	0	1	1	2	2	3	3	3	3	3	3
ATCC	27919	*pseudocatenulatum*	2	2	3	3	3	3	3	3	3	3	3	3
ATCC	25526	*pseudocatenulatum*	2	2	2	2	3	3	3	3	3	3	3	3
Stool	16455	*pseudocatenulatum*	1	1	1	2	3	3	3	3	3	3	3	3
Stool	19876	*pseudocatenulatum*	0	0	1	2	3	3	3	3	3	3	3	3
ATCC	25525	*thermophilum*	2	2	2	2	2	2	3	3	3	3	3	3
Lactobacillus														
ATCC	4356	*acidophilus*	0	0	0	0	0	0	0	0	0	0	0	0
Stool	19916	*acidophilus*	0	0	0	0	0	0	0	0	0	0	0	0
ATCC	14869	*brevis*	1	1	1	1	1	1	1	1	1	1	1	1
Stool	19925	*brevis*	2	2	2	2	2	2	2	3	3	3	3	2
Stool	19928	*brevis*	1	1	1	1	2	2	2	2	2	2	2	2
ATCC	9595	*casei*	1	1	1	1	1	1	3	3	3	3	3	3
Stool	19880	*casei*	1	1	1	1	1	1	1	2	2	1	1	1
Stool	19882	*casei*	1	1	1	1	1	2	2	3	3	2	2	2
Stool	19908	*casei*	1	1	1	1	1	1	2	2	2	2	3	2
Stool	19893	*crispatus*	1	1	1	1	1	1	2	2	2	2	2	2
Stool	7157	*fermentum*	1	1	1	2	2	2	2	3	3	2	1	1
ATCC	14931	*fermentum*	1	1	1	1	1	1	1	1	2	2	2	2
Stool	19935	*fermentum*	1	1	2	2	2	2	2	2	3	3	3	3
Stool	19939	*fermentum*	1	1	1	1	1	1	1	1	1	1	1	1
Stool	19879	*gasseri*	0	0	0	0	0	0	0	0	0	0	0	0
Stool	19897	*gasseri*	0	0	0	0	0	0	0	0	0	0	0	0
ATCC	53103	GG	1	1	1	1	1	2	2	3	3	3	3	3
Stool	19911	*johnsonii*	0	0	0	0	0	1	2	2	2	2	2	0
Stool	19921	*johnsonii*	0	0	0	0	1	1	1	2	2	2	2	1
Stool	19883	*lactis*	1	1	1	1	1	1	1	1	1	1	1	1
Stool	19878	*mucosae*	1	1	1	1	1	2	2	2	2	2	2	1
Stool	19881	*mucosae*	1	1	1	1	1	1	1	1	1	1	1	1
Stool	19884	*plantarum*	1	1	1	1	1	2	2	2	3	3	3	3
Stool	19918	*plantarum*	1	1	1	1	1	1	1	2	2	2	2	2
Stool	19888	*reuteri*	0	0	0	0	1	2	2	2	2	2	2	2
Stool	19920	*rhamnosus*	1	1	1	1	1	1	2	2	2	2	2	2
Stool	19922	*rhamnosus*	1	1	1	1	1	1	1	1	2	2	2	2
ATCC	11146	*sakei*	2	2	2	2	2	2	2	2	2	2	2	2
ATCC	393	*zeae*	1	1	1	1	1	2	2	3	3	3	3	2

and 7 ATCC *Lactobacillus* type strains were studied. Bacteria were identified by 16S rRNA sequence analysis.

Growth stimulation testing

The growth stimulation was determined by agar dilution technique (Jousimies-Somer et al., 2002). *Bifidobacterium* and *Lactobacillus* strains were grown on Brucella agar plates (BD BBL, Sparks, MD) under anaerobic conditions at 37 °C for 48 h and sub-cultured once. Anaerobic conditions consisted of a gas mixture of 5% CO_2, 5% H_2 and 90% N_2; the residual oxygen was removed by palladium catalysts. After incubation, inocula were prepared by making suspensions equal to MacFarland 0.5 standard [$\sim 1 \times 10^8$ colony forming units (CFU)/ml] in 1 ml of 0.9% sterile saline. The testing was done on Columbia agar [BD BBL; pancreatic digest of casein (10 g), meat peptic digest (5 g), yeast extract (5 g), heart pancreatic digest (3 g), corn starch (1 g), sodium chloride (5 g), agar (13.5 g) in 1 l dH_2O] plates supplemented with 0.001% riboflavin and 0.05% cysteine. The plates were prepared to contain two-fold serial dilutions of XOS (100–0.1 mg/ml). Control plates without XOS were also prepared. Using a Steers replicator, 10 µl volumes of the inocula suspensions were inoculated on XOS-containing plates, achieving a final inoculum of 10^5 CFU/spot. The plates were incubated anaerobic conditions for 48 h at 37 °C. After incubation, the plates were examined. The amount of growth and the concentrations of XOS resulting in change in the appearance of growth as compared to the control plate were recorded. No growth was recorded as ''0'', weak growth with translucent colonies was recorded as ''1'', moderate growth, semi-opaque, grey colonies as ''2'', and strong growth with opaque, white colonies as ''3''. Slight enhancement of growth (1 and 2), as compared to the control plate, was recorded as weak stimulation; marked change in the appearance of growth as compared to the control plate (3) was recorded as strong stimulation. The testing was done twice. We established for each test strain the concentration where XOS had no effect on the growth, where XOS stimulated the growth, and where the growth stimulation by XOS reached a plateau. The effect of XOS on the growth of *Bifidobacterium* and *Lactobacillus* strains was compared using Fisher's exact test. Significance was defined as $p \leq 0.05$.

Results

Altogether 64 bacterial strains were tested (Table 1). The results of the two separate testings were always identical or within one dilution, indicating reproducibility. The growth of all 35 *Bifidobacterium* strains tested was stimulated by XOS: 60% showed growth stimulation at 0.39 mg/ml XOS, 71% at 0.78 mg/ml XOS, 86% at 1.56 mg/ml XOS, 97% at 3.125 mg/ml XOS and 100% at 6.25 mg/ml XOS. Ninety-one percent of the *Bifidobacterium* strains showed strong stimulation by XOS supplementation. The growth of 69% (20/29) of *Lactobacillus* strains tested was stimulated by XOS, 31% of the *Lactobacillus* strains showed no growth stimulation by XOS. Of the *Lactobacillus*, 7% (two strains) showed growth stimulation at 0.39 mg/ml XOS, 17% at 0.78 mg/ml XOS, 38% at 1.56 mg/ml XOS, 52% at 3.125 mg/ml XOS and 62% at 6.25 mg/ml XOS. Twenty-eight percent of the *Lactobacillus* strains showed strong stimulation by XOS supplementation. Overall, the growth stimulation of *Bifidobacterium* by XOS was statistically more significant than the growth stimulation of *Lactobacillus* at all XOS concentrations of 0.2 mg/ml and above ($p < 0.002$).

Figure 1 illustrates the growth of 15 of *Bifidobacterium* strains and 17 *Lactobacillus* strains on control XOS plate (0 mg/ml XOS) and a test plate containing 1.56 mg/ml XOS. Growth stimulation was seen in 13/15 of the *Bifidobacterium* strains and 2/17 *Lactobacillus* strains pictured (Figure 1).

Discussion

The present study demonstrated that XOS stimulated the growth of both *Bifidobacterium* and *Lactobacillus* species, but *Bifidobacterium* exhibited statistically significantly stronger growth stimulation and at lower concentrations. The growth of all (100%) *Bifidobacterium* species was stimulated by XOS compared to 69% of *Lactobacillus* species. Up to 60% of *Bifidobacterium* species exhibited growth stimulation at very

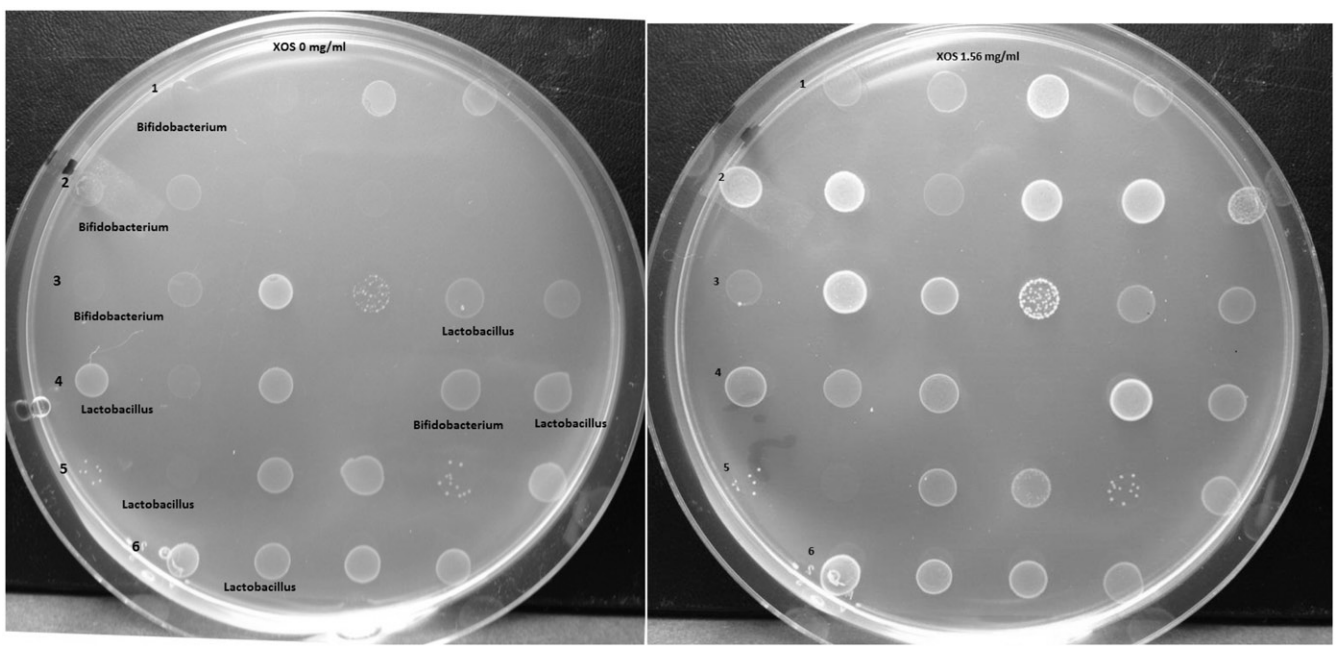

Figure 1. Control XOS plate (0 mg/ml XOS) and plate containing 1.56 mg/ml XOS. Rows 1 and 2: *Bifidobacterium* sp. Row 3: spots1–4 *Bifidobacterium* sp., 5–6 *Lactobacillus* sp. Row 4: 1–4 *Lactobacillus* sp., 5 *Bifidobacterium* sp., 6 *Lactobacillus* sp. Rows 5 and 6: *Lactobacillus* sp. Growth stimulation is seen in 13/15 of the *Bifidobacterium* strains and 2/17 *Lactobacillus* strains pictured.

low XOS concentration (0.39 mg/ml) whereas only 7% of *Lactobacillus* species were stimulated at this concentration. Altogether 91% of *Bifidobacterium* species and 28% of *Lactobacillus* species exhibited strong growth stimulation by XOS. *Bifidobacterium bifidum* species showed the weakest growth stimulation in general, compared to the other *Bifidobacterium* species.

The current *in vitro* study results correlated with the XOS *in vivo* study where XOS increased *Bifidobacterium* counts in a dose-response manner (Finegold et al., 2014). Assuming that human large intestine has ~4 l volume, the high dose XOS (2.8 g 70P) would deliver ~0.5 g/ml concentration and the low dose (1.4 g 70P) ~0.25 mg/ml concentration in the colon. In the present, *in vitro* study 60–71% of the *Bifidobacterium* strains were stimulated at ~0.5 mg/ml concentration and 37% at 0.2 mg/ml XOS. At a slightly higher concentration, 1.56 mg/ml XOS, the growth of 86% of *Bifidobacterium* strains was stimulated, half of these exhibiting strong growth stimulation, whereas only 38% of the *Lactobacillus* strains showed weak stimulation. This suggests that a higher dose of XOS, e.g. 4–8 g/day, may be more beneficial in stimulating *in vivo Bifidobacterium* counts without having much effect on intestinal *Lactobacillus* species.

The current *in vitro* study results similarly correlated with our XOS *in vivo* study where XOS did not increase *Lactobacillus* counts (Finegold et al., 2014). In this *in vitro* study only 7–20% of the *Lactobacillus* strains exhibited weak growth stimulation ~0.5 mg/ml XOS concentration, which is the estimated concentration in the colon obtained by the high dose XOS (2.8 g 70P) supplementation. The most common *Lactobacillus* species recovered in our XOS *in vivo* intervention study were *Lactobacillus casei*, *L. gasseri* and *L. acidophilus* (Finegold et al., 2014). Similar species distribution has been described by another culture-based study (Lonnermark et al., 2012), whereas a non-culture based study of a Japanese population found *L. fermentum* as the most predominant *Lactobacillus* species (Matsuda et al., 2009). In the present *in vitro* study, the growth of *L. gasseri* and *L. acidophilus* was not stimulated by XOS and three of four *L. fermentum* strains exhibited growth stimulation by XOS. The species specificity appears to further correlate with our *in vivo* study results, where the level of *Lactobacillus* was not increased by XOS consumption (Finegold et al., 2014).

In conclusion, XOS stimulated the growth of *Bifidobacterium* and, to a lesser degree, *Lactobacillus* species. An XOS dose of 4–8 g/day may be beneficial in stimulating *Bifidobacterium in vivo* without having much effect on intestinal *Lactobacillus* species. The potential role for XOS and bifidobacteria in managing obesity should be investigated further.

Declaration of interest

The authors report no conflict of interest. This work was supported by Life Bridge International Corporation.

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