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Human milk oligosaccharide consumption by probiotic and human-associated bifidobacteria and lactobacilli

Taksawan Thongaram,*[1] Jennifer L. Hoeflinger,* JoMay Chow,† and Michael J. Miller*[2]
*Department of Food Science and Human Nutrition, University of Illinois, Urbana 61801
†Abbott Nutrition, Columbus, OH 43219

ABSTRACT

Human milk contains high concentrations of nondigestible complex oligosaccharides (human milk oligosaccharides; HMO) that reach the colon and are subsequently fermented by the infant gut microbiota. Using a high-throughput, low-volume growth determination, we evaluated the ability of 12 lactobacilli and 12 bifidobacteria strains, including several commercial probiotics, to ferment HMO and their constituent monomers. Of the 24 strains tested, only *Bifidobacterium longum* ssp. *infantis* ATCC 15697 and *Bifidobacterium infantis* M-63 were able to ferment 3′-sialyllactose, 6′-sialyllactose, 2′-fucosyllactose, and 3′-fucosyllactose. *Bifidobacterium infantis* M-63 degraded almost 90% of the 2′-fucosyllactose but left most of the fucose in the supernatant, as detected by HPLC. Among bifidobacteria, only the *B. infantis* strains and *Bifidobacterium breve* ATCC 15700 were able to ferment lacto-*N*-neotetraose (LNnT). Among lactobacilli, *Lactobacillus acidophilus* NCFM was found to be the most efficient at utilizing LNnT. The extracellular β-galactosidase (*lacL*, LBA1467) of *L. acidophilus* NCFM cleaves the terminal galactose of LNnT for growth, leaving lacto-*N*-triose II in the media as detected by HPLC. Inactivation of *lacL* abolishes growth of *L. acidophilus* NCFM on LNnT. These results contribute to our knowledge of HMO–microbe interactions and demonstrate the potential for synbiotic combinations of pre- and probiotics.

Key words: human milk oligosaccharide, *Bifidobacterium*, *Lactobacillus acidophilus* NCFM, probiotic

INTRODUCTION

Human milk contains essential nutrients required for infant growth and development. Human milk oligosaccharides (**HMO**), the third most abundant component of human milk (approximately 5–23 g/L; Kunz et al., 2000; Zivkovic et al., 2011), consist of more than 200 complex linear and branched polymers of glucose (**Glc**), galactose (**Gal**), *N*-acetylglucosamine (**GlcNAc**), fucose (**Fuc**), and *N*-acetylneuraminic acid (sialic acid, **SA**), with lactose (**Lac**) at the reducing end (Stahl et al., 1994; Ninonuevo et al., 2006). Preclinical data suggest that HMO confer multiple physiological benefits, including immunomodulation of the host (He et al., 2014), improved cognition (Vázquez et al., 2015), modulation of intestinal motility and perfusion (Bienenstock et al., 2013; Good et al., 2016), microbial production of short-chain fatty acids (Vester Boler et al., 2013) and other metabolites (Chow et al., 2014), and prevention of pathogen attachment (Kunz et al., 2000; Newburg et al., 2005). Moreover, HMO promote the growth of mucus-adapted and HMO-adapted microbes, such as bacteroides and bifidobacteria (Marcobal et al., 2010; Asakuma et al., 2011).

In addition to HMO, human milk contains bifidobacteria and lactobacilli (Fernández et al., 2013) that readily colonize the infant gastrointestinal tract (**GIT**; Sekirov et al., 2010). Formula-fed infants lack exposure to these potentially health-promoting oligosaccharides and microbes. Therefore, delivery of HMO and probiotic bifidobacteria and lactobacilli in infant formula or milk substitutes remains a priority. In vitro HMO utilization experiments revealed strain-dependent HMO fermentation among *Bifidobacterium* and *Lactobacillus* species. Specifically, *Bifidobacteria longum* ssp. *infantis* consumed fucosylated, sialylated, and Type I and II HMO (Ward et al., 2006; LoCascio et al., 2009; Asakuma et al., 2011; Yu et al., 2013; Garrido et al., 2015). *Bifidobacterium bifidum* and *Bifidobacterium breve* readily consume Type I and II HMO, whereas utilization of fucosylated and sialylated HMO is strain dependent (LoCascio et al., 2009; Asakuma et al., 2011; Ruiz-Moyano et al., 2013). On the other hand, *Lactobacillus delbrueckii* ssp. *lactis* moderately consumed fucosylated and sialylated HMO (Yu et al., 2013), and *Lactobacillus casei* BL23 utilized Type I HMO (Bidart et al., 2015). *Lactobacillus casei*, *Lactobacillus acidophilus*, and *Lactobacillus plantarum* possess enzymes

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[1]Current address: Department of Microbiology, Faculty of Science, Silpakorn University, Nakhonpathom 73000, Thailand.
[2]Corresponding author: mille216@illinois.edu

Table 1. Microorganisms used in this study

Bacteria	Source/strain	Abbreviation	Origin
Bifidobacteria			
Bifidobacterium adolescentis	ATCC 15703	BA_15703	Adult intestine
Bifidobacterium animalis ssp. *animalis*	ATCC 25527	BA_25527	Rat feces
Bifidobacterium animalis ssp. *lactis*	Bb-12	BL_Bb12	NA[1]
Bifidobacterium animalis ssp. *lactis*	Bf-6	BL_Bf6	Human feces
Bifidobacterium animalis ssp. *lactis*	DSM 10140	BL_10140	Yogurt
Bifidobacterium bifidum	ATCC 29521	BB_29521	Infant feces
Bifidobacterium breve	ATCC 15700	BB_15700	Infant intestine
Bifidobacterium breve	M-16V	BB_M16V	Infant feces
Bifidobacterium longum	BB536	BL_BB536	Infant feces
Bifidobacterium longum ssp. *infantis*	M-63	BI_M63	Human
Bifidobacterium longum ssp. *infantis* S12	ATCC 15697	BI_15697	Infant intestine
Lactobacilli			
Lactobacillus acidophilus	La-5	LA_La5	NA
Lactobacillus acidophilus	NCFM	LA_NCFM	Human
Lactobacillus gasseri	ATCC 33323	LG_33323	Human
Lactobacillus fermentum	CECT 5716	LF_5716	NA
Lactobacillus jensenii	ATCC 25258	LJ_25258	Adult vagina
Lactobacillus johnsonii	ATCC 11506	LJ_11506	NA
Lactobacillus johnsonii	ACD-1/La-1	LJ_La1	NA
Lactobacillus paracasei	LCV-1	LP_LCV1	NA
Lactobacillus plantarum	LP-66	LP_LP66	NA
Lactobacillus reuteri	DSM 17938	LR_17938	Probiotic[2]
Lactobacillus rhamnosus HN001	DR20	LR_DR20	NA
Lactobacillus rhamnosus GG	ATCC 53103	LR_53103	Human feces

[1]NA = source of origin not publicly available.

[2]Isolated from commercially available ProTectis drops (BioGaia, Stockholm, Sweden).

capable of hydrolyzing HMO, but growth is not always supported (Rodríguez-Díaz et al., 2011; Schwab and Gänzle, 2011). For example, purified α-L-fucosidases from *L. casei* BL23 liberate Fuc from 2′-fucosyllactose (**2′-FL**) in vitro (Rodríguez-Díaz et al., 2011), but it is unclear whether this occurs with whole cells. Additionally, *L. casei* BL23 coupled fermentation of fucosyl-α-1,3-GlcNAc with excretion of Fuc, suggesting that the active fucosidases act intracellularly (Rodríguez-Díaz et al., 2012).

Given that human milk, containing HMO, bifidobacteria, and lactobacilli, is ingested by infants, we determined the fermentation parameters for various probiotic *Bifidobacterium* and *Lactobacillus* strains on purified HMO and HMO constituent monomers. Furthermore, we investigated the degradation pathway of lacto-*N*-neotetraose (**LNnT**) by *L. acidophilus* NCFM. Results from this study add to our current understanding of HMO–microbe interactions and could potentially lead to the development of truly synergistic, synbiotic combinations of pre- and probiotics.

MATERIALS AND METHODS

Bacterial Strains and Growth Conditions

Commercial probiotic and human-isolated bifidobacteria (n = 12) and lactobacilli (n = 12) strains (Table 1) were purchased from the American Type Culture Collection (**ATCC**), isolated from a commercial probiotic product, or supplied from the culture collection of Abbott Nutrition (Columbus, OH). Bacterial strains were cultured in de Man, Rogosa and Sharpe broth (Difco, Franklin Lakes, NJ) and incubated anaerobically (90% N_2, 5% CO_2, and 5% H_2) at 37°C for 24 h. For bifidobacteria, all growth medium was supplemented with 0.05% (wt/vol) L-cysteine.

Carbohydrate Utilization Assays

Stationary phase cultures were twice subcultured in semidefined de Man, Rogosa and Sharpe (**sMRS**) broth (Barrangou et al., 2003) containing 1% (wt/vol) Glc. Cells were washed twice with PBS, harvested by centrifugation at 3,220 × *g* for 10 min, and resuspended in 10 mL of carbohydrate-free sMRS. Cells were inoculated (1%, vol/vol) into sMRS containing 1% (wt/vol) of Glc, Gal, Lac, Fuc, GlcNAc, SA, 3′-sialyllactose (**3′-SL**), 6′-sialyllactose (**6′-SL**), 2′-FL, 3′-fucosyllactose (**3′-FL**), LNnT, or no carbohydrate. All HMO were purchased from V-Labs Inc. (Covington, LA) except LNnT, which was provided by Abbott Nutrition. A honeycomb plate was prepared with 250 μL of bacterial inocula covered with 50 μL of mineral oil, held for 1 h, and incubated anaerobically at 37°C for up to 120 h. The change in optical density at 600 nm ($\mathbf{\Delta OD_{600}}$) was