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In vitro screening of probiotic lactic acid bacteria and prebiotic glucooligosaccharides to select effective synbiotics

Julien Grimoud [a,b,*], Henri Durand [b], Céline Courtin [a,b], Pierre Monsan [c], Françoise Ouarné [d], Vassilia Theodorou [e], Christine Roques [a]

[a] *Laboratoire de Bactériologie, Virologie et Microbiologie Industrielle, Faculté des Sciences Pharmaceutiques – Université Paul Sabatier, 35 chemin des Maraîchers, 31062 Toulouse Cedex 09, France*
[b] *Département Nutrition Humaine, Lallemand SAS 19 rue des Briquetiers, BP 59, 31072 Blagnac Cedex, France*
[c] *UMR 792 Laboratoire d'Ingénierie des Systèmes Biologiques et des Procédés, INSA INRA CNRS, 165 avenue de Rangueil, 31077 Toulouse Cedex 4, France*
[d] *CRITT Bio Industries, INSA INRA CNRS, 165 avenue de Rangueil, 31077 Toulouse Cedex 4, France*
[e] *Laboratoire de Neurogastroentérologie et Nutrition, INRA ESAP UPS, 180 chemin de Tournefeuille, St-Martin-du-Touch, BP 93173, 31027 Toulouse Cedex 03, France*

A B S T R A C T

Probiotics and prebiotics have been demonstrated to positively modulate the intestinal microflora and could promote host health. Although some studies have been performed on combinations of probiotics and prebiotics, constituting synbiotics, results on the synergistic effects tend to be discordant in the published works. The first aim of our study was to screen some lactic acid bacteria on the basis of probiotic characteristics (resistance to intestinal conditions, inhibition of pathogenic strains). *Bifidobacterium* was the most resistant genus whereas *Lactobacillus farciminis* was strongly inhibited. The inhibitory effect on pathogen growth was strain dependent but lactobacilli were the most effective, especially *L. farciminis*. The second aim of the work was to select glucooligosaccharides for their ability to support the growth of the probiotics tested. We demonstrated the selective fermentability of oligodextran and oligoalternan by probiotic bacteria, especially the bifidobacteria, for shorter degrees of polymerisation and absence of metabolism by pathogenic bacteria. Thus, the observed characteristics confer potential prebiotic properties on these glucooligosaccharides, to be further confirmed *in vivo*, and suggest some possible applications in synbiotic combinations with the selected probiotics. Furthermore, the distinctive patterns of the different genera suggest a combination of lactobacilli and bifidobacteria with complementary probiotic effects in addition to the prebiotic ones. These associations should be further evaluated for their synbiotic effects through *in vitro* and *in vivo* models.

1. Introduction

The gut microbiota is made up of diverse and complex microbial communities including bacteria, fungi and protozoa. This microbiota plays a key role in the host's overall health [1] through its metabolic activities and physiological regulation such as promotion of nutrient absorption, synthesis of bioactive compounds, improvement of intestinal barrier function, motility, resistance to pathogens or modulation of the immune system. Alteration of the microbiota may cause some direct or indirect digestive pathologies like infectious diseases and chronic inflammation [2,3], metabolic disorders [4] or atopic diseases [5]. This has led to the development of strategies aiming to restore or to maintain the intestinal ecosystem through probiotics and prebiotics. Probiotics were defined by the FAO/WHO in 2001 as "live microorganisms which, when administered in adequate amounts, confer a health benefit on the host" [6]. Prebiotics were defined as non-digestible food ingredients that beneficially affect the host by selectively stimulating the growth and/or the activity of one or a limited number of bacteria in the colon, and thus improve host health [7,8]. Most of the probiotic strains belong to the *Lactobacillus* and *Bifidobacterium* genera, which are health promoting bacteria forming part of the balanced intestinal microbiota [9]. The main prebiotics used are fibres and carbohydrates, such as resistant starch, wheat bran, inulin or oligosaccharide, which are short polymers of glycosidic residues such as fructose in fructooligosaccharides or galactose in galactooligosaccharides and have been demonstrated to selectively

* Corresponding author. Département Nutrition Humaine, Lallemand SAS, 19 rue des Briquetiers, BP 59, 31072 Blagnac Cedex, France. Tel.: +33 5 62 25 68 60; fax: +33 5 61 25 95 72.
E-mail address: julien.grimoud@orange.fr (J. Grimoud).

increase bifidobacteria and lactobacilli among the colic microflora [10]. Glucooligosaccharides, with glucose residues linked by specific glycosidic bonds are more recently described oligosaccharides but have shown some prebiotic properties concerning physiological effects on glucose metabolism or vaginal microflora [11,12]. A novel approach combines probiotics and prebiotics in an association defined as synbiotic [13,14] in an attempt to obtain synergistic effects of the two compounds by an improvement of the probiotic colonisation or metabolic effect. More recently, synbiotics have been shown to be more effective than probiotics or prebiotics alone in improving the quality of life in patients suffering from ulcerative colitis [15], in colorectal cancer prevention [16] or in very general positive regulation of the microbiota [17].

Therefore, in the present study, we aimed to i) investigate *in vitro* probiotic properties for some bacterial strains: their resistance to artificial gastric and intestinal juices and their ability to inhibit intestinal pathogenic strains and ii) select some potential prebiotic glucooligosaccharides, synthesised in biofermenters by maltose acceptor reaction providing short polymers, which can support the growth of some probiotic strains, and to demonstrate their specificity through their resistance to metabolisation by intestinal pathogenic strains in an attempt to select some pertinent synbiotics for potential further use in intestinal applications. Thus, we aimed to propose some synbiotics on the basis of *in vitro* probiotic and prebiotic properties.

2. Materials and methods

2.1. Strains and culture conditions

All the strains tested are listed in Table 1. *Lactobacillus farciminis* CIP 103136 was obtained from Institut Pasteur Collection (Paris, France). *Bifidobacterium bifidum* 02, 20 and *Bifidobacterium pseudocatenulatum* 14 were previously described new-born faeces clinical isolates [18]. Other probiotic strains were obtained from the Lallemand Company collection. The strains were stored at −80 °C in Eugon broth (AES, Rennes, France) with 20% (v/v) glycerol (Fluka, Butch, Switzerland) and reactivated at 37 °C under anaerobic conditions (Anoxomat System WS 80; Mart Microbiology, Drachten, the Netherlands) on agar plates as indicated: (a) on Reinforcement Clostridial Medium for the bifidobacteria (Oxoid, Cambridge, UK), (b) on MRS (AES, Rennes, France) for the lactobacilli, *Lactococcus lactis* R1058 and *Pediococcus acidilactici* R1001 and (c) on M17 (AES, Rennes, France) for *Streptococcus thermophilus* R0083. Before the experiments, overnight cultures were prepared in MRS broth (AES, Rennes, France) at 37 °C under anaerobic conditions.

Table 1
Strains tested.

Species	Strains	Origins
Probiotics		
Bifidobacterium bifidum	02	new-born faeces
Bifidobacterium bifidum	20	new-born faeces
Bifidobacterium breve	R0070	Lallemand
Bifidobacterium longum	R0175	Lallemand
Bifidobacterium pseudocatenulatum	14	new-born faeces
Lactobacillus acidophilus	R0240	Lallemand
Lactobacillus buchneri	R1102	Lallemand
Lactobacillus farciminis	CIP103136	Institut Pasteur Collection
Lactobacillus helveticus	R0052	Lallemand
Lactobacillus plantarum	R1012	Lallemand
Lactobacillus rhamnosus	R1102	Lallemand
Lactococcus lactis	R1058	Lallemand
Pediococcus acidilactici	R1001	Lallemand
Streptococcus thermophilus	R0083	Lallemand
Enteric pathogens		
Escherichia coli serotype O157:H7	CIP 103571	Institut Pasteur Collection
Salmonella enterica ssp. *enterica* serotype typhimurium	CIP 60.62T	Institut Pasteur Collection
Campylobacter jejuni	CIP 70.2T	Institut Pasteur Collection
Clostridium difficile	CIP 104282	Institut Pasteur Collection
Enterococcus faecalis	CIP 106996	Institut Pasteur Collection
Listeria monocytogenes	CIP 82.110T	Institut Pasteur Collection
Candida albicans	UMIP 884.65	Institut Pasteur Collection

Potential or opportunistic pathogenic strains were obtained from Institut Pasteur Collection (Paris, France). The strains were stored at −80 °C in Eugon broth (AES, Rennes, France) with 20% (v/v) glycerol (Fluka, Butch, Switzerland) and reactivated at 37 °C on agar plates as indicated: (i) on trypcase soy (Biomérieux, Marcy l'Etoile, France) under aerobic conditions for *Escherichia coli* serotype O157:H7 CIP 103571, *Salmonella enterica* subsp. *enterica* serotype tiphymurium CIP 60.62T and *Enterococcus faecalis* CIP 106996, (ii) on Columbia with 5% (v/v) sheep blood (Biomérieux, Marcy l'Etoile, France) under aerobic conditions for *Listeria monocytogenes* CIP 82.110T or (iii) under anaerobic conditions for *Clostridium difficile* CIP 104282 and (iv) under microaerophilic conditions (GENbox Microaer; Biomérieux, Marcy l'Etoile, France) for *Campylobacter jejuni* CIP 70.2T, (v) on Sabouraud (Biomérieux, Marcy l'Etoile, France) under aerobic conditions for *Candida albicans* UMIP 884.65.

2.2. Glucooligosaccharides

The glucooligosaccharides tested and synthesised by CRITT-Bio Industries (Toulouse, France) were: oligoalternan (OA), which consists of alternate α-(1,3)/α-(1,6)-linked glucosyl residues (DP 3 to DP 6) and is synthesised by maltose acceptor reaction catalysed by *Leuconostoc mesenteroides* NRRL B-1355 alternansucrase [19], oligodextran (OD), which consists of α-(1,6)-linked glucosyl residues (DP3 to DP 9) and is synthesised by maltose acceptor reaction catalysed by *L. mesenteroides* NRRL B-512F dextransucrase [20], resistant oligodextran (rOD), which consists of α-(1,6)-linked glucosyl residues (DP3 to DP 9) with a α-(1,2)-linked glucosyl residue located at the non-reducing end or constituting a branching point of each oligodextran and is synthesised by maltose acceptor reaction catalysed by *L. mesenteroides* NRRL B-1299 dextransucrase[21].

2.3. Investigation for probiotic properties

2.3.1. Resistance to simulated gastric and intestinal fluids

The simulated gastric and intestinal fluids used have been described previously [22]. Simulated gastric juice (NaCl 125 mM, KCl, 7 mM, $NaHCO_3$, 45 mM, pepsin, 3 g/L) at a final pH adjusted to 2.5 was inoculated with probiotic strain overnight cultures at a final concentration of 1×10^7 CFU/mL. Suspensions were incubated at 37 °C with stirring at 200 rpm. After 3 h of incubation, bacteria were washed with PBS (Lonza) by centrifugation at 3800 rpm for 10 min and the pellets were resuspended in simulated intestinal fluid (pancreatin 0.1% w/v, bovine bile salts 0.15% w/v) at a final pH adjusted to 8.0. Then, suspensions were incubated at 37 °C for 3 h with stirring at 200 rpm. Cell viability was assayed by plating samples collected after incubation in simulated gastric fluid and after incubation in simulated intestinal fluid. The percentage of bacterial survival was calculated as follows:

$$\frac{CFU_{assay}}{CFU_{control}} \times 100$$

where CFU_{assay} represents CFU after incubation in simulated gastric or intestinal fluids and $CFU_{control}$ the CFU after incubation in PBS as a control. Separate experiments were performed three times.

2.3.2. Growth inhibition of pathogenic strains

Growth inhibition of pathogenic strains by probiotics was evaluated using an agar spot test [23]. Briefly, 8 μL of each probiotic suspension at 1×10^8 CFU/mL was spotted onto the surface of MRS agar plates and incubated anaerobically at 37 °C for 48 h. Pathogenic strains were then inoculated in 5 mL of soft agar (containing 0.7% agar) in an appropriate medium, described previously, at a final concentration of 1×10^7 CFU/mL, and poured onto MRS agar with probiotic spots. The plates were incubated in conditions suitable for each pathogen for 24–48 h. Growth inhibition was then measured. Considering the diameter of inhibition in mm, significant inhibition was defined for inhibition areas having a diameter of at least 50 mm, intermediate inhibition when the diameter was between 20 mm and 50 mm, and inability to inhibit the pathogen for diameters of less than 20 mm. Separate experiments were performed three times.

2.4. Evaluation of glucooligosaccharides for use as carbon source

2.4.1. Probiotic growth on glucooligosaccharides

Glucooligosaccharide consumption by probiotics was evaluated in microtitre plates. MRS broth was reconstituted without glucose according to the composition previously described [24] and completed by filter-sterilised (0.2 μm) glucooligosaccharide solution or glucose solution as positive control at a final concentration of 10 g/L. Each well was inoculated with overnight cultures at a final concentration of 1×10^7 CFU/mL. Chemically induced anaerobic conditions were obtained with ascorbate at a final concentration of 1 mg/mL. The microtitre plates were sealed with a plastic film in sterile conditions. Growth parameters were kinetically monitored using a POLARstar Galaxy automated microplate reader (BMG Labtech, Offenburg, Germany). Turbidity was assayed at 640 nm every 15 min during 36 h of incubation at 37 °C pH values were monitored at the beginning and end of the cultures. The growth parameters were calculated by standard methods. Separate experiments were performed three times.

2.4.2. High performance liquid chromatography analyses

As the glucooligosaccharides tested were mixtures of different degrees of polymerisation (DP), high performance liquid chromatography analyses were performed to quantify the preferentially consumed DP of each glucooligosaccharide when the probiotic culture reached the stationary phase. Samples were prepared as follows: supernatants from probiotic strain growth assays on glucooligosaccharides were collected by centrifugation (3600 g/10 min) and filtered (0.2 μm) for residue removal. Standards were glucose, fructose and saccharose. The solutions were then analysed by HPLC (Ultimate 3000; Dionex, Sunnyvale, USA) on a C18 Synergi 4 μm hydro-RP 80A column (Phenomenex, Torrance, USA). Elution was performed with ultrapure water at a flow rate of 1 mL/min at 50 °C.

Glucooligosaccharide content was determined by refractive index detection (RI-101; Shodex, Kawasaki, Japan). The results were expressed as percentages of consumption of each DP of the glucooligosaccharides, corresponding to the following ratio:

$$\frac{[C_S]_0 - [C_S]_t}{[C_S]_0} \times 100$$

where $[C_S]_t$ represents the concentration of each DP at time t of culture and $[C_S]_0$ the concentration measured at time 0. Peaks related to media components were monitored and subtracted from the area of the corresponding DP. Separate experiments were performed three times.

2.4.3. Pathogen growth on glucooligosaccharides

The growth of pathogenic strains on oligosaccharide carbon source was assayed in appropriate reconstituted broth without glucose and completed with filter-sterilised (0.2 μm) glucooligosaccharide solution or glucose solution, as the positive control, at a final concentration of 10 g/L. Five mL of each medium was inoculated with overnight cultures at a final concentration of 1×10^7 CFU/mL. Strains were cultivated at 37 °C for 48 h as follows: (i) in reconstituted trypcase soy broth under aerobic conditions with stirring for *E. coli* serotype O157:H7 CIP 103571, *S. enterica* subsp. *enterica* serotype tiphymurium CIP 60.62T and *E. faecalis* CIP 106996, (ii) in reconstituted Schaedler broth under aerobic conditions for *L. monocytogenes* CIP 82.110T or (iii) under anaerobic conditions for *C. difficile* CIP 104282 and (iv) under microaerophilic conditions for *C. jejuni* CIP 70.2T, (v) on Sabouraud under aerobic conditions with stirring for *C. albicans* UMIP 884.65. Turbidity at 640 nm was determined at the end of the log phase. The growth parameters were calculated by standard methods. Separate experiments were performed three times.

3. Results

3.1. Investigation for probiotic properties

3.1.1. Resistance to simulated gastric and intestinal fluids

Probiotic survival rates are presented in Fig. 1. The bifidobacteria had high survival rates in both simulated gastric and intestinal conditions except for *Bifidobacterium longum*, which showed a loss of viability >50% after treatment by intestinal fluid. *Lactobacillus helveticus* and *Lactobacillus plantarum* were also strongly resistant to the various conditions. *Lactobacillus rhamnosus*, *L. lactis*, *P. acidilactici* and *S. thermophilus* were resistant to the artificial gastric juice but were inhibited to a greater extent by simulated intestinal fluid. *P. acidilactici* was the least sensitive among the latter strains, while the two strains of *Lactobacillus acidophilus*, *Lactobacillus buchneri* and *S. thermophilus* where much less resistant. *L. farciminis* had very poor resistance properties in both gastric and intestinal conditions.

3.1.2. Growth inhibition of pathogenic strains

The inhibitory activity exerted by probiotics against pathogens is presented in Table 2. Among the bifidobacteria, only *Bifidobacterium breve* and *B. bifidum* 02 presented a high inhibitory effect against Gram positive bacteria, respectively *C. difficile* and *E. faecalis*, whereas *B. bifidum* 20 significantly inhibited *E. coli* serotype O157:H7 as well as *S. thermophilus*. *L. farciminis* was the most effective strain for inhibiting pathogen growth with strong effects against the Gram negative and Gram positive bacteria: *S. typhi*, *C. jejuni* and *L. monocytogenes*. *L. heleveticus* was also able to inhibit growth of *C. jejuni* and *C. difficile*, and *L. buchneri* had a strong activity against *C. jejuni*. Concerning the other genera, *P. acidilactici* exerted strong inhibitory activities against *C. jejuni* and *C. difficile* and *S. thermophilus* had antimicrobial effects against *E. coli* O157:H7. The other strains exerted only intermediate inhibition effects. None of the strains was able to significantly inhibit the growth of *C. albicans*.

3.2. Evaluation of glucooligosaccharides for use as a carbon source

3.2.1. Probiotic growth on glucooligosaccharides

Kinetic analyses were used to monitor consumption of glucooligosaccharides by probiotics. The experimental conditions were validated by checking with positive and negative control experiments performed respectively with modified MRS broth complemented with glucose or without any carbon source. Table 3 presents the growth parameters for all the strains tested in presence of the different carbohydrates.

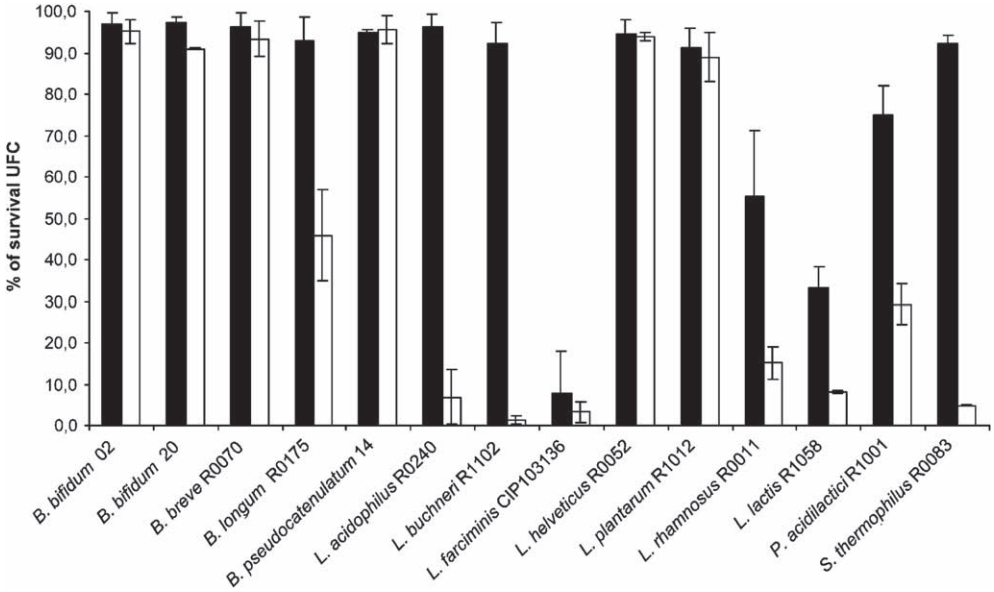

Fig. 1. Survival rates of probiotic strains after 3 h of exposure to simulated gastric fluid (black bars) and after a consecutive 3 h of exposure to simulated intestinal fluid (white bars). Results are given as the average value of three different experiments ± standard deviation.

Turbidity, generation time and pH values showed significant growth on OA for all the six bifidobacteria strains and no significant growth rates for lactobacilli. All the bifidobacteria were also able to grow on OD whereas only three of the lactobacilli and *L. lactis* used this carbohydrate. Microbial development on resistant rOD did not reach significant levels in any strain.

3.2.2. High performance liquid chromatography analyses

Supernatants from cultures supporting a significant growth in presence of OD and OA were collected at the stationary phase of culture and subjected to HPLC analyses in order to determine the degradation rate of each oligosaccharide DP during bacterial growth. The results are listed in Table 4.

Concerning OD, all the strains tested consumed most of the DP3 and DP4 (≥80%) except *B. bifidum* 02, *L. helveticus* and *L. rhamnosus* for DP4. DP5 were strongly consumed by *B. bifidum* 01, *B. breve*, *B. longum* and *L. farciminis* whereas *L. lactis* was the only strain able to consume DP6 at a similar rate. DP7 and DP8 were poorly hydrolysed in our conditions. Concerning OA, DP3 were consumed by the six bifidobacteria at some intermediate rates (between 50% and 65%). DP ≥ 3 were poorly metabolised.

These results suggest the potential use of OD and, to a lesser degree, OA in synbiotic combination with the selected probiotic strains. These carbohydrates were investigated to determine their resistance to pathogenic strains.

3.2.3. Pathogen growth on glucooligosaccharides

OD and OA were subjected to assays with intestinal pathogenic bacteria. Positive and negative controls were conducted in reconstituted media with glucose or no carbon source. Fig. 2 presents the turbidity reached by each strain at the stationary phase of culture. For all pathogenic microorganisms, growth on glucooligosaccharides did not exceed the negative control average value and remained markedly below the glucose control. These data demonstrate that the intestinal pathogens tested were unable to metabolise OD and OA and suggest a potential use of glucooligosaccharides as safe prebiotics or, combined with probiotics, in synbiotics.

Table 2
Pathogen growth inhibition by probiotics expressed in diameter of inhibition expressed in mm. ND means no significant results.

Probiotics	Pathogenic strains						
	Gram negative bacteria			Gram positive bacteria			Yeast
	E. coli serotype O157:H7 CIP 103571	*S. enterica* ssp. *enterica* serotype typhimurium CIP 60.62T	*C. jejuni* CIP 70.2T	*C. difficile* CIP 104282	*E. faecalis* CIP 106996	*L. monocytogenes* CIP 82.110T	*C. albicans* UMIP 884.65
B. bifidum 02	27 (±4)	31 (±2)	46 (±4)	40 (±4)	70 (±8)[a]	25 (±2)	ND
B. bifidum 20	53 (±4)[a]	38 (±5)	33 (±2)	ND	22 (±3)	33 (±3)	ND
B. breve R0070	29 (±2)	23 (±2)	35 (±1)	58 (±1)[a]	24 (±1)	26 (±3)	19 (±1)
B. longum R0175	27 (±3)	36 (±3)	37 (±3)	35 (±3)	25 (±4)	29 (±4)	17 (±2)
B. pseudocatenulatum 14	30 (±4)	35 (±3)	45 (±1)	36 (±1)	28 (±2)	35 (±4)	ND
L. acidophilus R0240	35 (±3)	35 (±3)	40 (±2)	41 (±2)	21 (±3)	46 (±4)	16 (±2)
L. buchneri R1102	31 (±2)	33 (±4)	56 (±4)[a]	46 (±1)	19 (±2)	31 (±3)	17 (±1)
L. farciminis CIP 1031136	36 (±4)	58 (±7)[a]	50 (±1)[a]	39 (±1)	26 (±4)	53 (±6)[a]	16 (±1)
L. helveticus R0052	44 (±4)	22 (±2)	53 (±1)[a]	52 (±2)[a]	32 (±2)	22 (±2)	26 (±2)
L. plantarum R1012	34 (±4)	35 (±4)	17 (±1)	29 (±4)	31 (±3)	21 (±3)	21 (±1)
L. rhamnosus R0011	44 (±5)	22 (±4)	50 (±1)	41 (±2)	31 (±4)	38 (±3)	19 (±3)
L. lactis R0058	28 (±3)	25 (±2)	36 (±1)	35 (±1)	ND	21 (±3)	ND
P. acidilactici R1001	39 (±4)	38 (±4)	50 (±4)[a]	53 (±2)[a]	35 (±3)	28 (±4)	21 (±2)
S. thermophilus R0083	50 (±5)[a]	26 (±2)	32 (±3)	32 (±6)	23 (±3)	29 (±2)	15 (±2)

[a] Means inhibitions considered as significant (diameter > 50 mm).

Table 3
Growth parameters of probiotic cultures on selected oligosaccharides or glucose as positive control after 48 h of incubation. Results are given as average values of three samples ± standard deviation. ND means no significant results.

Tested strains	Growth parameters								
	Δ_{max} T[a]			G[b]			Δ_{max} pH[c]		
	Glucose	OA	OD	Glucose	OA	OD	Glucose	OA	OD
B. bifidum 02	1.37 (±0.20)	0.71 (±0.38)	0.77 (±0.03)	05h20 (±01h47)	10h40 (±00h24)	05h02 (±01h59)	1.7 (±0.2)	0.3 (±0.1)	1.2 (±0.4)
B. bifidum 20	1.28 (±0.08)	0.58 (±0.09)	0.72 (±0.10)	03h39 (±00h40)	08h07 (±01h35)	05h52 (±01h18)	1.6 (±0.3)	0.4 (±0.1)	1.6 (±0.1)
B. breve R0070	1.39 (±0.13)	0.67 (±0.04)	0.95 (±0.07)	03h14 (±00h28)	11h36 (±03h54)	06h24 (±01h33)	1.8 (±0.1)	1.1 (±0.2)	1.6 (±0.1)
B. longum R0175	1.31 (±0.04)	0.86 (±0.15)	0.71 (±0.12)	03h03 (±00h39)	08h38 (±01h58)	03h22 (±00h46)	1.7 (±0.4)	1.4 (±0.2)	1.9 (±0.1)
B. pseudocatenulatum 14	1.11 (±0.09)	0.57 (±0.24)	0.75 (±0.11)	06h08 (±01h03)	06h40 (±01h47)	04h52 (±01h31)	1.7 (±0.2)	0.3 (±0.1)	1.1 (±0.2)
L. acidophilus R0240	0.64 (±0.01)	ND	ND	03h16 (±01h00)	ND	ND	1.5 (±0.4)	ND	ND
L. buchneri R1102	1.09 (±0.13)	ND	ND	05h39 (±01h33)	ND	ND	1.7 (±0.2)	ND	ND
L. farciminis CIP 103136	1.20 (±0.04)	ND	0.95 (±0.19)	02h03 (±00h36)	ND	05h27 (±01h55)	1.7 (±0.2)	ND	1.6 (±0.6)
L. helveticus R0052	1.28 (±0.27)	ND	0.79 (±0.08)	04h18 (±00h27)	ND	05h26 (±01h22)	1.7 (±0.3)	ND	1.3 (±0.8)
L. plantarum R1012	1.31 (±0.03)	ND	ND	04h46 (±00h08)	ND	ND	1.8 (±0.2)	ND	ND
L. rhamnosus R0011	1.34 (±0.09)	ND	0.67 (±0.22)	02h32 (±00h02)	ND	06h41 (±00h46)	1.8 (±0.1)	ND	1.2 (±0.5)
L. lactis R1058	0.55 (±0.07)	ND	0.59 (±0.23)	01h39 (±00h04)	ND	02h32 (±00h48)	1.6 (±0.2)	ND	1.7 (±0.4)
P. acidilactici R1001	0.99 (±0.11)	ND	ND	02h03 (±00h36)	ND	ND	1.8 (±0.5)	ND	ND
S. thermophilus R0083	0.92 (±0.20)	ND	ND	01h59 (±00h41)	ND	ND	0.7 (±0.3)	ND	ND

[a] Δ_{max} T = maximal variation of turbidity at 640 nm compared to the initial value.
[b] G = generation time (h:min).
[c] Δ_{max} pH = maximal variation of pH compared to the initial value.

4. Discussion

In this study, we first aimed to evaluate some probiotic characteristics of a panel of lactic acid bacteria. To be considered as probiotic, microorganisms had to meet some selection criteria. Among all the *in vitro* parameters defined (human origin for human use, survival in gastrointestinal conditions, pathogen inhibition, adhesion to intestinal epithelial cells, etc.) we chose to test resistance to acidic and bile salt conditions along the digestive tract and antimicrobial activity as defined previously [25]. Exposure to gastric and intestinal fluids along the digestive tract is the main stress that could decrease the viability of ingested probiotics [26]. Optimum delivery of viable microorganisms to the distal gut is critical for intestinal probiotic effects [27] and acid resistance is required for food applications. Thus we investigated the probiotics' resistance through a protocol simulating gastric and intestinal conditions as already described [22]. Most of the strains were resistant to gastric conditions but had more variable resistance profiles to artificial intestinal fluid. This decrease in viability after the transition to intestinal fluid from gastric fluid has been described previously and may be due to the rapid pH shift from acidic to basic conditions [28]. The observed variations of such resistance could be explained by strain dependent responses as reported previously [29] and provide us with a first-level relevant selection criterion, highlighting the strongest resistance effects of the bifidobacteria in the present study. Pathogen inhibition is also a major probiotic selection criterion and this mechanism is involved in the restoration of gut microbiota balance [30]. It thus has significant positive effects in various physiological functions and in the reduction of pathologies such as inflammatory bowel disease or colorectal cancer [31–33]. Here, the antimicrobial effects exerted by the lactic acid bacteria tested were strain-specific. Three strains among the Gram positive and Gram negative intestinal pathogens were inhibited significantly by three of the six bifidobacteria. Lactobacilli showed the most effective antimicrobial properties overall, with a noteworthy effect of *L. farciminis* and a lesser but significant effect of *L. helveticus*. *P. acidilactici* also showed significant antimicrobial properties. *C. albicans* resistance to antagonist activities exerted by probiotics has been described previously [12] and explained by yeast resistance to acidic conditions, oxidative stress or bacteriocins, which are among the main mechanisms involved in probiotic antibacterial activities [34]. These variations could be used as a second screening criterion. Some of the strains displaying interesting antimicrobial properties were active against only one or a few pathogenic strains. A broader antibacterial activity could be obtained by combining probiotics,

Table 4
Percentage of consumption by probiotics of each degree of polymerisation (DP) of selected oligosaccharides after 48 h of incubation. Results are given as average values of three samples ± standard deviation. ND means no significant results.

Tested strains	Carbohydrates							
	OD						OA	
	DP3	DP4	DP5	DP6	DP7	DP8	DP3	DP > 3
B. bifidum 02	97 (±2)	86 (±5)	71 (±10)	55 (±25)	30 (±4)	15 (±6)	49 (±11)	15 (±7)
B. bifidum 20	96 (±1)	38 (±6)	39 (±3)	21 (±4)	18 (±3)	18 (±1)	61 (±4)	21 (±7)
B. breve R0070	95 (±2)	94 (±1)	84 (±3)	35 (±25)	19 (±10)	12 (±8)	66 (±3)	22 (±1)
B. longum R0175	97 (±3)	96 (±3)	86 (±1)	48 (±4)	44 (±3)	2 (±1)	65 (±1)	40 (±3)
B. pseudocatenulatum 14	98 (±2)	78 (±9)	48 (±10)	39 (±11)	31 (±16)	23 (±1)	50 (±8)	9 (±7)
L. acidophilus R0240	ND	ND	ND	ND	ND	ND	ND	ND
L. buchneri R1102	ND	ND	ND	ND	ND	ND	ND	ND
L. farciminis CIP 103136	98 (±1)	83 (±14)	84 (±7)	62 (±23)	22 (±4)	20 (±7)	ND	ND
L. helveticus R0052	97 (±3)	39 (±25)	32 (±12)	29 (±15)	23 (±3)	13 (±8)	ND	ND
L. plantarum R1012	ND	ND	ND	ND	ND	ND	ND	ND
L. rhamnosus R0011	75 (±11)	33 (±27)	26 (±16)	25 (±22)	19 (±13)	16 (±7)	ND	ND
L. lactis R1058	94 (±5)	85 (±2)	60 (±6)	81 (±7)	38 (±9)	14 (±9)	ND	ND
P. acidilactici R1001	ND	ND	ND	ND	ND	ND	ND	ND
S. thermophilus R0083	ND	ND	ND	ND	ND	ND	ND	ND

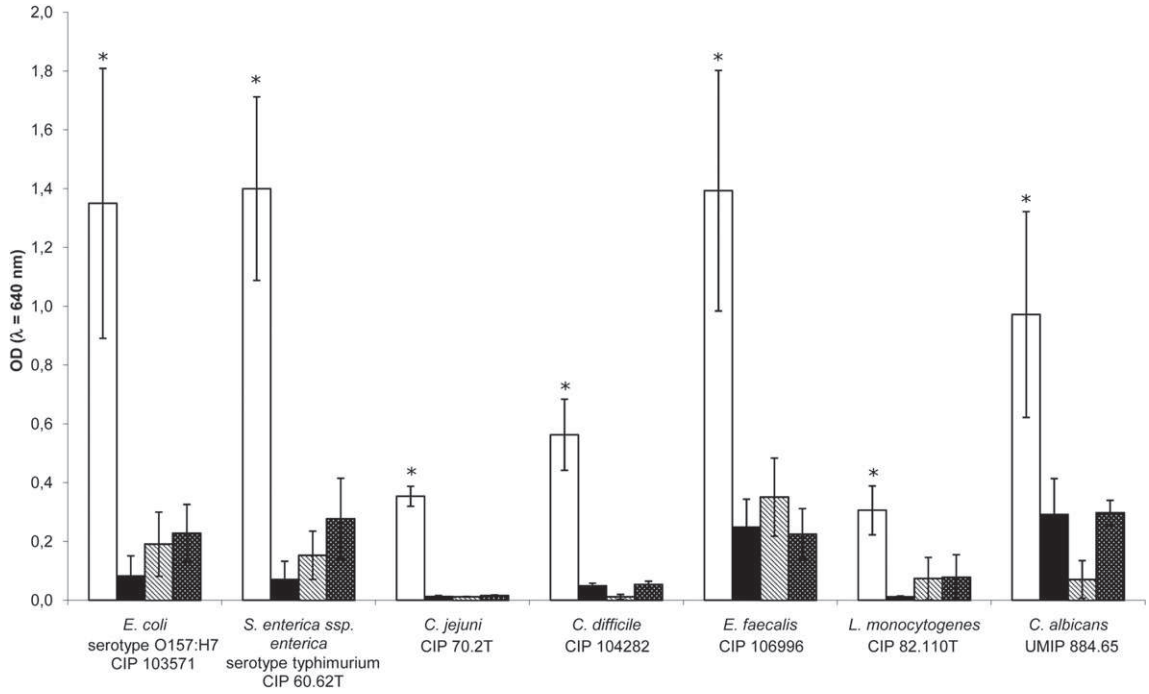

Fig. 2. Maximal variation of turbidity at 640 nm of pathogenic cultures on glucose as positive control (white bars), without carbohydrates as negative control (black bars) or selected oligosaccharides, oligoalternan (hatched bars) and oligodextran (dotted bars) after 48 h of incubation. Results are given as the average value of three different experiments ± standard deviation. * Different from negative control with no carbohydrates, $P < 0.05$.

especially among the lactobacilli tested strains that were the most effective against the different pathogens.

The second aim of this study was to select some probiotic strains able to grow on glucooligosaccharides, which are potential prebiotic carbohydrates, in order to propose a rational selection of original synbiotic combinations *in vitro*. Some works have previously demonstrated glucooligosaccharide fermentability characteristics with an induction of bifidobacteria and lactobacilli populations of human colonic microflora [35] or their potential prebiotic effects on vaginal lactobacilli [12]. The microoganisms tested did not use rOD. The absence of significant growth on rOD may be due to the chemical structure of this carbohydrate including an α-1,2 glycosidic bond at the non-reducing end or at branch point [21], which is very uncommon. This glucooligosaccharide was demonstrated to be more resistant than dextran with only α-1,6 glycosidic bonds in pH-controlled fermenters and was more significantly hydrolysed in *in vivo* conditions [36]. This suggests that the lack of growth on rOD could also be due to our experimental conditions, which were characterised by batch conditions, with no pH control and no renewal of nutrients in the culture medium. This is very different from an intestinal ecosystem with a great diversity of microorganisms, where more favourable conditions could lead to the expression of some enzymes involved in specific glycosidic bond hydrolyses. In consistency with probiotic bacteria growth parameters, we confirmed some fermentability characteristics here for two glucooligosaccharides, displaying some potential synbiotic uses: OD and OA. OD was metabolised by a larger number of lactic acid bacterial strains and promoted bacterial growth more efficiently than OA. *Bifidobacterium* was more effective than the other genus in metabolising glucooligosaccharides as a carbon source. Bifidobacteria possess a wide range of genes involved in carbohydrate catabolism [37] and have been described for their ability to grow on several short-chain oligosaccharides, such as fructooligosaccahrides and xylooligosaccahrides [38,39]. Among these short-chain carbohydrates, bifidobacteria are able to metabolise OD in batch cultures [40,41] and to hydrolyse OA [42]. OA can also support specific intestinal bacterial growth [43]. Moreover, the absence of significant growth of intestinal pathogens on OD and OA showed the specificity of the metabolisation by probiotics and thus conferred potential prebiotic characteristics on them. This finding is important as some potential prebiotics have been demonstrated to induce the development of pathogenic bacteria in animal models [44]. These properties need to be confirmed in gut models *in vitro* or in assays *in vivo* in order to assess the selective growth effects of the tested glucooligosaccharides on positive bacteria in the context of a diversified microbial community.

OD were more effective than OA to promote development of some lactic acid bacteria when generation time and turbidity at stationary phase culture were considered. This suggests a faster metabolisation of OD than OA by bifidobacteria.

Five of the six bifidobacteria that grew on OD and OA were resistant to the artificial gastric and intestinal conditions but only three of the four lactobacilli using OD were resistant. Some of these glucooligosaccharide-fermenting strains were also able to inhibit intestinal pathogenic bacteria, especially *L. farciminis*, which had antimicrobial characteristics against one Gram positive and two Gram negative pathogens. However, this tested strain was the most affected by the digestive tract conditions. Considering the good probiotic characteristics displayed *in vitro* by *L. farciminis* CIP 103136 in this study and its anti-inflammatory and anti-nociceptive effects [45,46], this strain should be assayed in formulations enhancing protection against damage induced by gastrointestinal transit, such as microencapsulation in gel beads [47] or ingestion with milk, soymilk or dairy products which buffer the pH variation that can occur along the digestive tract [48,49]. The same techniques could be used to protect other interesting strains before an *in vivo* evaluation of probiotic or synbiotic properties. HPLC analyses showed a preferential consumption of shorter OD DP and only OA DP3 were significantly consumed. Short DP preferential metabolisation could be explained by a faster consumption of these polymers or could be induced by batch conditions with a lack of some nutriments after

a certain period of bacterial growth. Extended time of analysis or continuous conditions could help to check whether bacteria (lactobacilli, *Pediococcus* and *Streptococcus*) are able to metabolise larger DP or not, or if it is possible to improve bifidobacteria growth on OA. It would be interesting to test these two glucooligosaccharides enriched in preferentially consumed DP in an attempt to optimise lactic acid bacteria growth. Bifidobacteria were the most effective in using glucooligosaccharides as a carbon source but displayed low antimicrobial characteristics, whereas lactobacilli, *P. acidilactici* and *S. thermophilus* significantly inhibited pathogenic strains but were generally less resistant to digestive tract conditions and were unable to grow on glucooligosaccharides. These observations suggest the use of associations of strains with complementary properties. Some studies have already highlighted the interest of combinations of different probiotic strains, especially bifidobacteria and lactobacilli, to improve their intrinsic properties [50,51].

In conclusion, some of the strains tested displayed interesting probiotic characteristics and two potential prebiotic glucooligosaccharides, OD and OA, were selected for their ability to support growth of specific probiotic bacteria. These carbohydrates need to be assayed in models *in vivo* to demonstrate their potential prebiotic properties before proceeding to clinical trial. Bifidobacteria were the most resistant to the gastrointestinal conditions and the most effective in metabolising glucooligosaccharides, and lactobacilli displayed the strongest inhibitory effects on enteric pathogens. A combination of a glucooligosaccharide-fermenting bacterium with a non-fermenting one could also improve the glucooligosaccharides metabolisation for the latter and extend the antibacterial spectrum. Thus this study has identified some potential synbiotics to be further evaluated for beneficial effects through interaction with human cells in assays *in vitro* and to extend the screening to *in vivo* models before potential use in human applications.

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