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Anaerobe 13 (2007) 185–192

Anaerobe

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Ecology/Environmental Microbiology

Selection and optimization procedure of synbiotic for cholesterol removal

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Received 7 January 2007; received in revised form 1 June 2007; accepted 7 June 2007
Available online 13 June 2007

Abstract

A selection and optimization procedure for the synbiotic combination of probiotic and prebiotics was established to optimize its cholesterol removal *in vitro*. In light of fermentability, prebiotics utilization by probiotics was highly variable and interspecies differences existed. Based on the results of fermentability, *L. plantarum* LS12, Ls31, LP529 and *L. ruminis* La3 could be the better candidates for symbiotic research. The bile tolerance of all the tested strains could be improved by the strain-specific prebiotics comparing to the control carbon source (glucose). The strain LS12 was finally selected to form the symbiotic according to its better ability to ferment prebiotics and bile tolerance, while the five prebiotics (FOS, stachyose, GOS, IMO and mannitol) were selected to make their synbiotic combination because of their better enhancement of bile tolerance and growth support to LS12. The synbiotic combination for cholesterol removal was optimized by use of response surface methodology. The first-order model showed that the selected prebiotics mannitol and GOS were significant factors. Then through the second-order polynomial regression model, the optimum conditions of the two factors for cholesterol removal by the synbiotic were suggested.

Keywords: Cholesterol removal; Probiotics; Prebiotics; Synbiotic; Bile tolerance; Response surface methodology

1. Introduction

Studies have shown that a small reduction in serum cholesterol of 1% may reduce the risk of coronary heart disease by 2–3% [1]. There is currently much interest in the concept of actively managing the colonic microflora with the aim to reduce serum cholesterol. It is traditionally attempted by the consumption of live microbial food supplements, known as ''probiotics'', ''a live microbial feed supplement which beneficially affects the host animal by improving its intestinal microbial balance [2]''. *In vitro* studies have shown that probiotics can remove cholesterol from culture media [3–5], while in humans studies have reported that probiotics can lower total cholesterol and low-density lipoprotein cholesterol levels [6].

On the other hand, it is possible that the beneficial effect of probiotics after ingestion may be compromised by adverse conditions *in vivo* [7]. A synbiotic (combination of prebiotic and probiotic) approach offers an alternative. An *in vivo* report [8] has showed that the concentrations of serum total lipids, triacylglycerol and total cholesterol were significantly reduced in rats fed the symbiotic in comparison to those only on the probiotic or prebiotic diet. Here, the probiotic effect could be potentiated by specific prebiotics, ''a non-digestible food ingredient that beneficially affects the host by selectively stimulating the growth and/or activity of one or a limited number of bacteria in the colon, and thus improves host health'' [9]. Moreover, *in vivo* experiments [6] have reported that prebiotics themselves also can lower total cholesterol and low-density lipoprotein cholesterol levels.

However, most of synbiotics studies [8,10] in light of reducing serum cholesterol were set *in vivo* situation, in which the prebiotic support and benefit for probiotic is poorly understood, therefore there is need for basic *in vitro* research to make clear the interaction between probiotic and prebiotic. Although the optimization of cholesterol

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doi:10.1016/j.anaerobe.2007.06.001

removal by the combination of probiotic and prebiotics has been effectively studied before [11,12], finding synbiotic pairs before the optimization is not a simple task [13]. Therefore, the purpose of the present study was to investigate the support and benefit of prebiotics for probiotics *in vitro*, which was also used as the selection standards of probiotic and prebiotics for synbiotic, and then to conduct optimization procedure for synbiotic of cholesterol removal *in vitro* and confirm whether the procedure would work or not.

2. Materials and methods

2.1. Strains and medium preparation

Seven strains of lactobacilli were isolated in our laboratory from the gastrointestinal tract of healthy infants (three strains) and adults (four strains). The strains were classified as the following species: *L. salivarius* strains Lma1 (Accession no.: AY766420) and La5 (AY766421); *L. helveticus* strain Zl51 (AB125907); *L. ruminis* strain La3 (AF335899); *L. plantarum* strains LS12 (DQ235651), Ls31 (AY851751) and LP529 (DQ235650).

The tested strains were all cultured under anaerobic condition (Oxoid Anaerobic Gas Generating Kit) provided by an anaerobic indicator (Oxoid) with a 1% inoculum. The organisms were grown in sterile de Mann, Rogosa, Sharpe (MRS) broth with a 24-h incubation at 37 °C and was transferred successively three times in MRS broth prior to use.

Seven commercially available prebiotic oligosaccharides were investigated in this study: fructooligosaccharide (FOS; Meiji Seika Kaisha Ltd. Co., Tokyo, Japan, 95% oligosaccharides), inulin (Orafti Pty. Ltd., Tienen, Belgium, 95% inulin), mannitol (Man; Sinopharm Chemical Reagent Co. Ltd., 100% mannitol), isomaltooligosaccharide (IMO; New Francisco Biotechnology Corporation, 90% oligosaccharides), xylooligosaccharide (XOS; New Francisco Biotechnology Corporation, 95% XOS), galactooligosaccharide (GOS; Xi'an Dapeng Biotechnology, 55% min GOS), stachyose (Sta; Xi'an Dapeng Biotechnology, 85% stachyose, the majority of the remaining is rafinose). Two nonprebiotics (glucose and maltodextrin (Mal; New Francisco Biotechnology Corporation, a dextrose equivalent value in the range of 10–18)) were used as controls too.

2.2. Fermentability experiment

Fermentability of the prebiotics and nonprebiotic controls was compared by the measurement of growth capability of the strains cultured in the medium containing these carbohydrates. The strains were cultured anaerobically in 5-ml portions of MRSC medium (MRS containing 1% each of the seven prebiotics or nonprebiotic controls as the only carbon source in MRS) with a 1% inoculum at 37 °C for up to 24 h. All experiments were replicated three times. Bacterial growth capability was determined in terms of maximum optical density at 620 nm (OD_{620}) in culture.

2.3. Bile tolerance

Bile tolerance of the strains was compared in terms of their growth capability in 5-ml portions of MRSC medium containing 0% (as the control), 0.2%, 0.3% and 0.4% oxgall (Sigma) with a 1% inoculum at 37 °C, respectively. Growth capability was determined by the calculation of the lag time (LT)—the delay time required for the OD_{620} to increase by 0.3 U in the MRSC medium with bile comparing to the control medium (without bile).

The time required for the OD_{620} increase of 0.3 U was calculated by the growth curve, which was constructed by plotting the hourly increases in OD_{620} against incubation time for 12 h. All the presented results were mean values from three separate experiments.

2.4. Measurement of cholesterol removal

In addition with the prebiotics described in Table 1, MRS media contained 65–85 μg/ml water-soluble filter-sterilized cholesterol (polyoxyethanyl-cholesteryl sebacate, Sigma) and 0.3% oxgall were incubated with 1% inoculum anaerobially at 37 °C for 24 h. The concentration of cholesterol removed was measured as described by Gilliland et al. [3].

Table 1
Treatment combinations and responses for screening experiments

Run	Block	*X*1 FOS	*X*2 IMO	*X*3 Man	*X*4 GOS	*X*5 Sta	Cholesterol removed (μg/ml)
1	1	1	−1	−1	1	1	32.71
2	1	−1	1	1	1	−1	35.40
3	1	−1	−1	−1	−1	−1	27.33
4	1	1	1	1	−1	1	28.32
5	1	0	0	0	0	0	32.99
6	2	1	−1	1	−1	−1	31.46
7	2	0	0	0	0	0	32.51
8	2	−1	−1	1	1	1	36.89
9	2	−1	1	−1	−1	1	33.26
10	2	1	1	−1	1	−1	33.41
11	3	1	−1	1	1	−1	35.09
12	3	0	0	0	0	0	34.80
13	3	1	1	−1	−1	−1	34.66
14	3	−1	1	−1	1	1	33.80
15	3	−1	−1	1	−1	1	35.67
16	4	1	1	1	1	1	33.48
17	4	−1	1	1	−1	−1	37.13
18	4	0	0	0	0	0	37.72
19	4	−1	−1	−1	1	−1	31.43
20	4	1	−1	−1	−1	1	33.04

FOS: 0.50–1.50% (w/v); IMO: 0.50–1.50% (w/v); Man: 0.50–1.50% (w/v); GOS: 0.50–1.50% (w/v); Sta: 0.50–1.50% (w/v).