Journal of Oral Biosciences ■ (■■■■) ■■■–■■■

Contents lists available at ScienceDirect

Journal of Oral Biosciences

journal homepage: www.elsevier.com/locate/job

Combining prebiotics and probiotics to develop novel synbiotics that suppress oral pathogens

Yukako Kojima [a], Tomoko Ohshima [b,*], Chaminda Jayampath Seneviratne [c], Nobuko Maeda [b]

[a] Department of Oral Microbiology, Graduate School of Dental Medicine, Tsurumi University, 2-1-3 Tsurumi, Tsurumi-ku, Kanagawa 230-8501, Japan
[b] Department of Oral Microbiology, School of Dental Medicine, Tsurumi University, 2-1-3 Tsurumi, Tsurumi-ku, Kanagawa 230-8501, Japan
[c] Oral Sciences, Faculty of Dentistry, National University of Singapore, Singapore

ARTICLE INFO

Article history:
Received 2 April 2015
Received in revised form
9 August 2015
Accepted 17 August 2015

Keywords:
Synbiotics
Probiotics
Prebiotics
Oral pathogens

ABSTRACT

Objective: Synbiotics, resulting from a synergistic combination of prebiotics and probiotics, is a new concept in clinical studies that is known to promote intestinal health. However, the beneficial effects of synbiotics on oral health have not been investigated. The present study attempted to develop new synbiotics against oral pathogens (bacterial and fungal).
Methods: Prebiotic screening was carried out by sugar assimilation tests using 12 saccharides. About 40 strains of lactobacilli were used for probiotic screening. Standard in vitro assays were performed against oral pathogens, such as *Candida albicans*, *Streptococcus mutans*, and *Porphyromonas gingivalis*. Growth inhibition and biofilm formation assays were conducted for *C. albicans* using lactobacilli in co-culture or with the culture supernatant (-CS). Subsequently, the disc diffusion assay was used as a growth inhibitory test against *P. gingivalis* and the amount of insoluble glucan produced by *S. mutans* was determined by phenol-sulfate staining.
Results: The results showed that arabinose, xylose, and xylitol are the saccharides with a strong potential to be used as prebiotics and five lactobacilli strains isolated from the oral cavity have the potential to be used as probiotics. These strains inhibited the growth of *C. albicans* and *P. gingivalis*, and had an inhibitory effect on the production of insoluble glucan by *S. mutans*. Lactobacilli strains isolated from dairy foods did not show a significant effect on human oral microbiota.
Conclusion: The present study indicates that prebiotics and probiotics can potentially be developed into novel synbiotics against oral pathogens in future.

1. Introduction

Major oral diseases such as dental caries, periodontal disease, and oral candidiasis are infectious in nature and these oral diseases are caused by oral pathogens *Streptococcus mutans, Porphyromonas gingivalis,* and *Candida albicans,* respectively. Oral diseases affect millions of people worldwide and the incidence of oral diseases is expected to rise in the next decade due to an increase in the number of elderly people and immunocompromised patients.

Prebiotics and probiotics are well known for their beneficial effects in promoting the health of the human gastrointestinal tract. The term "Probiotics" was coined by Kollath in the 1950s and subsequently Lilly and Stillwell employed the term in 1965 [1]. In 1989, Fuller redefined a probiotic as "A live microbial feed supplement which beneficially affects the host animal by improving its intestinal microbial balance" [2]. However, later it was later shown that probiotics have benefits beyond promoting intestinal health. Therefore, Salminen et al. defined a probiotic as "A viable microbial food supplement which beneficially influences the health of the host" [3] while, FAO/WHO define probiotics as "live microorganisms when administered in adequate amounts confer a health benefit on the host" [4]. In addition, the health benefits of oral probiotics have been a focus of several clinical trials [23].

In 1995, Gibson and Roberfroid defined "Prebiotics" as "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" [5]. However, there are only limited studies on oral prebiotics. Gibson and Roberfroid also introduced the concept of "synbiotics" in the same report and defined synbiotics as "a mixture of probiotics and prebiotics that beneficially affects the host by improving the survival and implantation of live microbial dietary supplements in the gastrointestinal tract, by selectively stimulating the growth and/or by activating the metabolism of one or a limited number of health-promoting bacteria, and thus improving host welfare" [1].

* Corresponding author. Tel.: +81 45 580 8441; fax: +81 45 572 3516.
E-mail address: ohshima-t@fs.tsurumi-u.ac.jp (T. Ohshima).

http://dx.doi.org/10.1016/j.job.2015.08.004
1349-0079/

The activities of probiotics and synbiotics have been studied extensively [24] however, little is known about the mechanism of their action on oral health. Therefore, in the present study we have attempted to discover probiotic and prebiotic candidates that can be used to develop a novel synbiotics against oral bacterial and fungal pathogens.

2. Material and methods

2.1. Saccharides

A total of 12 saccharides were screened to identify suitable prebiotic candidates (Table 1).

2.2. Bacterial strains and culture conditions

We used three oral pathogenic microbes: *Candida albicans* (ATCC18804), *Streptococcus mutans* (ATCC25175), and *Porphyromonas gingivalis* (ATCC33277), stored at −80 °C at the Department of Oral Microbiology, Tsurumi University School of Dental Medicine.

C. albicans was cultured on Sabouraud dextrose agar (Nissui, Tokyo, Japan) at 30 °C for 2 days under aerobic conditions, then one inoculation loop-full of the colony was subcultured in tryptic soy broth (Becton Dickinson and Company, Sparks, MD, USA) supplemented with 5% dextrose anhydrous (TSBD), and incubated under aerobic conditions at 30 °C overnight (14–16 h) with constant shaking to obtain a late logarithmic growth phase culture (the turbidity was 2.4 and the OD was measured at 620 nm (Ultrospec 4000 UV/vis spectrophotometer, Pharmacia Biotech Inc., NJ, USA). The pH was 5.0).

S. mutans was defrosted and inoculated in brain heart infusion broth (BHI) (Becton Dickinson and Company) and incubated under anaerobic conditions at 37 °C overnight (14–16 h), then subcultured in fresh BHI under aerobic conditions with constant shaking for 6 h. This subculture was incubated overnight (14–16 h) to obtain a late logarithmic growth phase culture (the turbidity was 1.2 and the OD was measured at 620 nm. The pH was 5.4).

P. gingivalis was defrosted and inoculated in BHI supplemented with 0.5% yeast extract, 0.05% cysteine–HCl, 5 μg/mL hemin, and 100 μg/mL Vitamin K under anaerobic conditions at 37 °C for 2 days. It was then subcultured in fresh medium and incubated for another 2 days to obtain a late logarithmic growth phase culture (the turbidity was 1.1 and OD was measured at 620 nm. The pH was 7.7).

A total of 40 strains of lactobacilli were used to identify potential candidates for use as probiotics (Table 2). This included seven reference strains, 22 strains isolated from the human oral cavity [13] and 11 strains isolated from dairy foods by selective cultivation on BBL™ LBS Agar (Becton Dickinson and company) supplemented with glacial acetic acid under anaerobic conditions at 37 °C for 2 days.

Table 1
12 Saccharides used in this study.

Saccharides	
Glucose[a]	Maltose[a]
Galactose[a]	Lactose[b]
Xylose[a]	Treharose[a]
Xylitol[a]	Arabinose[a]
Cellobiose[a]	Melezitose[c]
Sucrose[a]	Raffinose[a]

[a] Wako Pure Chemical Industries, Ltd. Japan.
[b] E. Merck, Darmstadt Germany.
[c] DIFCO LABORATORIES Detroit Michigan USA.

Table 2
40 Lactobacilli strains for this study.

Reference strains	
Lactobacillus animalis ATCC35046	
L. salivarius LS1	
L. reuteri PTA	
L. reuteri DSM	
L. brevis (Labostrain, Tsurumi Univ. Dental Med. Dept. Oral Microbiol.)	
L. murinus ATCC35020	
L. casei ATCC393	
Isolated from dairy foods	
11. Lactobacillus spp.	Yogurt
13L. *L. casei* YIT9029-L	Yogurt
13S. *L. casei* YIT9029-S	Yogurt
14. Lactobacillus spp.	Mozzarella cheese
17L. Lactobacillus spp.	Red Cheddar cheese
17S. Lactobacillus spp.	Red Cheddar cheese
19L. Lactobacillus spp.	Red Cheddar cheese
19S. Lactobacillus spp.	Red Cheddar cheese
20. Lactobacillus spp.	Gouda cheese
23. Lactobacillus spp.	Brie Hermitage cheese
24. Lactobacillus spp.	Foume d'Ambert cheese
Isolated from oral flora	
101. *Lactobacillus casei*	
102. *L. gasseri*	
103. *L. fermentum*	
104. *L. crispatus*	
105. *L. salivalius*	
106. *L. gasseri*	
107. *L. crispatus*	
108. *L. plantarum*	
109. *L. salivalius*	
110. *L. casei*	
111. *L. fermentum*	
112. *L. paracasei*	
113. Lactobacillus spp.	
114. Lactobacillus spp.	
115. Lactobacillus spp.	
116. Lactobacillus spp.	
117. *L. paracasei*	
118. Lactobacillus spp.	
119. Lactobacillus spp.	
120. Lactobacillus spp.	
121. Lactobacillus spp.	
122. *L. plantarum*	

Reference strains and clinical strains from the human oral cavity were stored at −80 °C at the Department of Oral Microbiology, Tsurumi University School of Dental Medicine until used.

All lactobacilli strains were subcultured in MRS Broth (Becton Dickinson and company Sparks, MD, USA) at 37 °C under anaerobic conditions for 2 days.

2.3. Saccharide assimilation tests of lactobacilli: C. albicans or S. mutans

Saccharide assimilation tests were performed on lactobacilli and oral pathogens *C. albicans* and *S. mutans* to identify candidates that can be used as prebiotics. Microbial suspensions of lactobacilli (1×10^4–10^6 CFU/10 μL), *C. albicans* (1×10^4–10^5 CFU/10 μL), and *S. mutans* ($\times 10^4$ CFU/10 μL) were each added to 190 μL of Tryptic soy broth (TSB) without dextrose supplemented with 4% of one of the mono- or oligo-saccharides shown in Table 1. The turbidities of culture solutions were determined as OD at 620 nm at 24, 48 and 72 h by using a microplate reader (Multiskan® Multisoft Labsystems).

2.4. Co-culture test for detecting growth inhibitory effect of lactobacilli on C. albicans

Lactobacilli cultured for 24 h in TSBD under anaerobic conditions were used for growth inhibitory assays. 190 μL of lactobacilli

Please cite this article as: Kojima Y, et al. Combining prebiotics and probiotics to develop novel synbiotics that suppress oral pathogens. J Oral Biosci (2015), http://dx.doi.org/10.1016/j.job.2015.08.004