Archives of Oral Biology 71 (2016) 97–103

Contents lists available at ScienceDirect

Archives of Oral Biology

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

Comparative in vitro investigation of the cariogenic potential of bifidobacteria

Remberto Marcelo Argandoña Valdez[a], Vanessa Rodrigues dos Santos[a], Karina Sampaio Caiaffa[b], Marcelle Danelon[a], Rodrigo Alex Arthur[c], Thais de Cássia Negrini[d], Alberto Carlos Botazzo Delbem[a], Cristiane Duque[a,*]

[a] UNESP - Univ. Estadual Paulista, Araçatuba Dental School, Department of Pediatric Dentistry and Public Health, Rua José Bonifácio, 1193, 16015050, Araçatuba, SP, Brazil
[b] UNESP - Univ. Estadual Paulista, Araçatuba Dental School, Department of Restorative Dentistry, Rua José Bonifácio, 1193, 16015050, Araçatuba, SP, Brazil
[c] UFRGS - Federal University of Rio Grande do Sul, Faculty of Dentistry, Department of Preventive Dentistry, Rua Ramiro Barcelos, 2492, Bairro Santa Cecília, 90035-003, Porto Alegre, RS, Brazil
[d] UFRGS - Federal University of Rio Grande do Sul, Faculty of Dentistry, Department of Conservative Dentistry, Rua Ramiro Barcelos, 2492, Bairro Santa Cecília, 90035-003, Porto Alegre, RS, Brazil

ARTICLE INFO

Article history:
Received 17 January 2016
Received in revised form 3 June 2016
Accepted 14 July 2016

Keywords:
Biofilms
Bifidobacterium
Lactobacillus
Streptococcus
Actinomyces
Dental caries

ABSTRACT

Objective: This study aimed to assess the in vitro cariogenic potential of some *Bifidobacterium* species in comparison with caries-associated bacteria.
Design: Bifidobacterium lactis, *Bifidobacterium longum*, *Bifidobacterium animalis*, *Bifidobacterium dentium*, *Lactobacillus acidophilus*, *Lactobacillus casei*, *Actinomyces israelii*, *Streptococcus sobrinus* and *Streptococcus mutans* were tested for acidogenicity and aciduricity by measuring the pH of the cultures after growth in glucose and bacterial growth after exposure to acid solutions. Biofilm biomass was determined for each species either alone or associated with *S. mutans* or *S. mutans*/*S. sobrinus*. Enamel hardness was analyzed before and after 7-days biofilm formation using bacterial combinations.
Results: B. animalis and *B. longum* were the most acidogenic and aciduric strains, comparable to caries-associated bacteria, such as *S. mutans* and *L. casei*. All species had a significantly increased biofilm when combined either with *S. mutans* or with *S. mutans*/*S. sobrinus*. The greatest enamel surface loss was produced when *B. longum* or *B. animalis* were inoculated with *S. mutans*, similar to *L. casei* and *S. sobrinus*. All strains induced similar enamel demineralization when combined with *S. mutans*/*S. sobrinus*, except by *B. lactis*.
Conclusion: The ability to produce acidic environments and to enhance biofilm formation leading to increased demineralization may mean that *Bifidobacterium* species, especially *B. animalis* and *B. longum*, are potentially cariogenic.

© 2016 Published by Elsevier Ltd.

1. Introduction

Dental caries is a biofilm-associated disease highly associated with frequent intake of dietary sugars. Fermentation of these sugars by biofilm microorganisms leads to acid production, which disrupts biofilm microbial homeostasis and can causes dissolution of tooth minerals (Marsh, 2003). The bacteria considered the most cariogenic are the mutans streptococci, especially *Streptococcus mutans* (van Houte, Sansone, Joshipura, & Kent, 1991; Mattos-Graner, Correa, Latorre, Peres, & Mayer, 2001). Other acidogenic and aciduric bacterial species, including *Actinomyces* spp. and *Lactobacillus* spp., have been observed in dental caries lesion development (Sansone, van Houte, Joshipura, Kent, & Margolis, 1993; van Houte, Lopman, & Kent, 1996). Additionally, *Lactobacillus* and *Bifidobacterium* species have been identified as part of the dental biofilm on white spot lesions (Van Ruyven, Lingstrom, van Houte, & Kent, 2000).

The *Bifidobacteriaceae* family consists of seven genera: *Bifidobacterium, Aeriscardovia, Falcivibrio, Gardnerella, Parascardovia, Scardovia* and *Alloscardovia*, which have been isolated from both animals and humans. These bacteria are anaerobic, gram-positive, rod-shaped, and principally colonize the gastrointestinal tract. Species in the genus *Bifidobacterium* have recently received

* Corresponding author.
E-mail addresses: cristianeduque@yahoo.com.br, cduque@foa.unesp.br (C. Duque).

http://dx.doi.org/10.1016/j.archoralbio.2016.07.005
0003-9969/© 2016 Published by Elsevier Ltd.

significant attention in the consumer food industry due to their beneficial roles in human health. They have been shown to have a role in increasing the adaptive immune response, in treating or preventing respiratory and urogenital infections, and in the prevention of allergies and atopic diseases during childhood (Saxelin, Tynkkynen, Mattila-Sandholm, & de Vos, 2005). Several studies suggest that the consumption of products containing probiotics, such as bifidobacteria, could suppress *S. mutans* levels in saliva (Caglar et al., 2005, 2007, 2008; Cildir et al., 2009). However, recent clinical randomized studies have shown that the administration of probiotics did not affect the mutans streptococci levels or the occurrence of dental caries in children (Taipale, Pienihäkkinen, Salminen, Jokela, & Söderling, 2012; Taipale, Pienihäkkinen, Alanen, Jokela, & Söderling, 2013; Nozari, Motamedifar, Seifi, Hatamizargaran, & Ranjbar, 2015). A confounding issue is that levels of caries-related microorganisms are not always related to the severity of dental caries (Mattos-Graner, Zelante, Line, & Mayer, 1998), making the beneficial effects of probiotics on the control of caries difficult to elucidate.

Bifidobacterium species have been isolated from dental plaque, saliva and dentinal caries (Modesto, Biavati, & Mattarelli, 2006; Beighton et al., 2008; Mantzourani et al., 2009). *B. dentium*, *B. longum* and *B. breve* were only detected in the oral cavities of children with occlusal caries and not in those of caries-free children (Mantzourani et al., 2009). Those authors suggested that these bacteria might have a role in the progression of occlusal caries lesions. These findings suggest that this family of bacteria may be related to caries development, possibly due to their acidogenicity and acid-tolerance (van Houte, Lopman, & Kent, 1996; Nakajo, Takahashi, & Beighton, 2010). Tanner et al. (2011) reported a high prevalence of the *Bifidobacterium* spp. in addition to *Streptococcus* in children with advanced dental caries and severe early childhood caries. Thus, this study aimed to determine the cariogenic potential of *Bifidobacterium* species in comparison with currently recognized caries-associated bacteria. The null hypotheses formulated was that the cariogenic potential of *Bifidobacterium* species, represented by acidogenicity, aciduricity, and the ability to form biofilms and to induce enamel demineralization, is not different compared with cariogenic potential of caries-associated bacteria.

2. Materials and methods

2.1. Bacterial strains and growth conditions

Assays were performed using the following *Bifidobacterium* species: *B. animalis* (from ACTIVIA®), *B. longum* (ATCC 15707), *B. lactis* (LMG 18905) and *B. dentium* (ATCC 27678); *Lactobacillus* species: *L. acidophilus* (ATCC 4356), *L. casei* (ATCC 393); *Streptococcus* species: *S. mutans* (ATCC 25175 and 3VF2), *Streptococcus sobrinus* (ATCC 27607); *Actinomyces* species: *A. israelii* (ATCC 12102). All ATCC strains were obtained from Oswaldo Cruz Foundation (FIOCRUZ, Rio de Janeiro, RJ, Brazil) or André Tosello Foundation (Campinas, SP, Brazil). *B. animalis* was isolated from the yogurt ACTIVIA® in Transgalactosylated Olygosaccharides-Propionate agar with supplement lithium-muporicina (50 mg/L) (TOS-MUP agar; Merck Millipore, Darmstadt, Germany). *S. mutans* 3VF2 is highly acidogenic clinical strain kindly provided by Dr. Renata de Oliveira Mattos-Graner (FOP-UNICAMP) (Mattos-Graner, Napimoga, Fukushima, Duncan, & Smith, 2004). Reactivation of strains on selective media was done as follow: *S. mutans* and *S. sobrinus* on Mitis Salivarius agar with bacitracin (0.2 U/mL) (Difco), *L. casei* and *L. acidophilus* on Rogosa agar (Difco); *B. animalis*, *B. longum*, *B. lactis* and *B. dentium* on TOS-MUP agar (Merck) and *A. israelii* on *Actinomyces* Garrod medium (HiMedia Laboratories, Mumbai, India). Plates were incubated anaerobically with Anaerocult system (Merck).

2.2. Acidogenicity tests

Up to 5 colonies of each species were individually transferred from agar plates to Brain Heart Infusion broth (BHI, Difco) and incubated at 37 °C for 24 h. Bacterial cultures were diluted 1:20 in BHI and the bacterial growth was monitored by turbidimetry. When the optical density reached 0.5 (approximately 10^8–10^9 CFU/mL) at 550 nm, tubes were centrifuged and the pellet was resuspended in a solution of 50 mM KCl and 1 mM $MgCl_2$. The suspension was centrifuged and the pellet was resuspended in 20 mM phosphate buffer and incubated at 37 °C for 60 min for starvation. After centrifugation, the pellet was resuspended in a solution of 50 mM KCl and 1 mM $MgCl_2$, the pH of the solution was adjusted to 7.0 and glucose was added to a final concentration of 55.5 mM. The pH of the suspension was evaluated immediately and 5, 15, 30, 60, 120, 180 and 240 min after the addition of glucose using a glass electrode (Orion 720 A+; Orion Research Inc.) previously calibrated with pH standards of pH 4.0 and 7.0. The area under the curve (AUC) was calculated of pH drop, considering pH 2.8 as a cut-off point, using UTHSCSA ImageTool software, version 3.0. The acidogenicity was expressed as the AUC (cm^2). Each analysis was performed in three distinct experiments (Arthur et al., 2011; Belli & Marquis, 1991).

2.3. Aciduricity tests

Similar to acidogenicity tests, 24 h broth cultures were diluted and grew until optical density at 550 nm of 0.5. 3 mL of each culture were individually distributed into tubes, which were centrifuged and resuspended in 0.1 M glycine buffer with a pH of 7.0, pH 5.0 or 2.8. Immediately after resuspension (Time 0) and after 60 min (Time 60), 100 μL of each sample was serially diluted and plated on BHI agar plates which were incubated for 24 h at 37 °C. Colony Forming Units/mL (CFU/mL) were then counted (Arthur et al., 2011; Duarte et al., 2008).

2.4. In vitro biofilm formation

After growing in BHI containing 1% sucrose and reaching an optical density of 0.5 at 550 nm, aliquots of 200 μL of each strain were individually transferred to the wells of 96-well plates (single-species). Additionally, equal volumes of each bacterial culture were also combined (at the same optical density) with *S. mutans* (3VF2) (dual-species) or *S. mutans* (3VF2)/*S. sobrinus* (multi-species) to complete a total of 200 μL per well. Triplicate of samples were incubated at 37 °C for 48 h. Wells containing only the culture medium were used as negative controls. After incubation, the plates were washed by immersion in distilled water three times to remove the non-adherent cells. After a brief drying, 150 μL of aqueous 1% crystal violet was added to each well, and the plates were incubated at room temperature for 30 min. The crystal violet solution was then removed and the plates washed again 3 times. The plates were inverted on paper towels and remained for 2 h at room temperature to dry. The crystal violet dye that stained the biofilm was then solubilized by incubating with 200 μL of ethanol per well for 30 min. Then, 100 μL of the dye in ethanol was transferred to wells of a new plate, and read at absorbance of 575 nm in a microplate spectrophotometer (BioTek Instruments. USA) to quantify the biomass of the biofilm (Mattos-Graner, Jin et al., 2001).

Download English Version:

https://daneshyari.com/en/article/3120572

Download Persian Version:

https://daneshyari.com/article/3120572

Daneshyari.com