Evaluation of xylitol as an agent that controls the growth of skin microbes: *Staphylococcus aureus*, *Staphylococcus epidermidis*, and *Cutibacterium acnes*

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Xylitol is a natural sugar alcohol that is often utilized in personal care products for hydration purposes. The growth of skin-associated bacteria, *Staphylococcus aureus*, *Staphylococcus epidermidis*, and *Cutibacterium acnes* was examined in the presence or absence of 1% (w/v) and 5% (w/v) xylitol. Xylitol inhibited the growth of *S. aureus* and *C. acnes* at the 5% level, whereas the growth of *S. epidermidis* was promoted by the 1% xylitol, but not by 5% xylitol. These results indicate that xylitol has specific antimicrobial and growth-promoting effects on skin-associated bacteria, and can be used in personal care products to control the growth of these bacteria.

Keywords: *Cutibacterium acnes*, *Staphylococcus aureus*, *Staphylococcus epidermidis*, skin-associated bacteria, xylitol

Xylitol is a naturally occurring pentose sugar alcohol, that can be found in fruits and vegetables, such as plums, strawberries, cauliflower, and pumpkin (Ur-Rehman *et al.*, 2015). Commercially, xylitol is produced by the hydrogenation of xylose. Because xylitol has a similar sweetness to sucrose, it has been applied in the food industry, in low-calorie products, confectioneries, and chewing gum, and in the pharmaceutical industry, as an excipient or sweetener; however, xylitol also acts against otitis media and upper respiratory infections (Ur-Rehman *et al.*, 2015). Xylitol has beneficial odontological effects, due to its anticariogenicity, tooth rehardening, and remineralization properties. Xylitol also reduces *Streptococcus mutans* colonization, growth, and biofilm

formation (Salli *et al.*, 2016, 2017). When ingested, approximately 50% of xylitol enters the colon, where it is fermented by the colonic microbiota, increasing the production of short-chain fatty acids and promoting the growth of butyrate-producing bacteria, thus having prebiotic function (Livesey, 2003; Lenhart and Chey, 2017; Sato *et al.*, 2017).

In cosmetic products, xylitol has been used primarily for its skin-hydrating properties (Leite e Silva *et al.*, 2009); however, xylitol has been suggested to protect the skin barrier, due to its ability to regulate the mRNA expression of filaggrin, loricrin, involucrin, and occludin (Payer *et al.*, 2018), and to reduce transepidermal water loss, when applied in combination with glycerol (Korponyai *et al.*, 2011; Szel *et al.*, 2015). Interest in skin-associated microbiota research continues to evolve rapidly, because many aspects of skin health and disease can be affected by the regulation of skin-associated microbial populations and because certain conditions, such as acne and atopic dermatitis, have been associated with disruptions in the skin-associated microbial balance (Byrd *et al.*, 2018). The prevailing hypothesis suggests that skin health can be enhanced by promoting the growth and survival of beneficial skin-associated bacteria. The effects of xylitol on skin-associated microbial growth, particularly *Staphylococcus aureus*, *Staphylococcus epidermidis*, and *Cutibacterium acnes* (formerly known as *Propionibacterium acnes* [Scholz and Kilian, 2016]), in pure cultures were examined in this study.

S. aureus ATCC29213, *S. epidermis* ATCC14990, *C. acnes* ATCC6919, and *C. acnes* ATCC11827 were purchased from

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American Type Culture Collection (ATCC). *S. aureus* ATCC29213, and *S. epidermis* ATCC14990 were cultured in tryptic soy broth (TSB) (BD Biosciences), whereas *C. acnes* ATCC6919 and *C. acnes* ATCC11827 were cultured in Reinforced Clostridial Medium (RCM) (Lab M Limited). Xylitol (DuPont) was prepared as 10% or 50% aqueous stock solutions that were sterile-filtered (0.2 μm Minisart, Sartorius AG) prior to being used in the bacterial growth assays.

The growth of bacterial strains in the presence of xylitol was measured with the automatic Bioscreen® C system (Labsystems), which recorded kinetic changes in the absorbance (600 nm) of liquid samples in a 100-well plate, and the growth rates were determined from these data. The Bioscreen® system was placed in an anaerobic hood (80% N_2, 10% CO_2, and 10% H_2), and all bacterial strains were cultured under anaerobic conditions with anaerobic media. The test substrate solution (10% or 50%) was added to each well of the plate (20 μl), and a cell suspension (180 μl) that contained a single type of microbe (1% v/v) was

added. Thus, the final concentration of the carbohydrate substrate in each well was 1% (w/v) or 5% (w/v). For control wells, containing medium without added carbohydrates, 20 μl water was added, and wells were filled with 180 μl of the microbial suspensions. The strains were incubated at 37°C for 24 h, and the optical density was measured at 600 nm every 30 min. Plates were mixed for 10 sec before measurements were made. Three replicates were performed for each strain/ xylitol combination.

When the growth curves were examined (Fig. 1A–D), *S. aureus* ATCC29213 grew the most rapidly under these experimental conditions, with a steep slope during the exponential growth phase, and the lag-phase was reached approximately 6 h from the start of the culture (Fig. 1A). For *S. epidermidis* ATCC 14990, the exponential growth phase had a shallow slope, and the lag phase was reached approximately 10 h from the start of the culture (Fig. 1B). The *C. acnes* strains, ATCC6919 and ATCC11827, showed the slowest exponential growth phases, with a shallow slopes, and neither reached the lag phase during
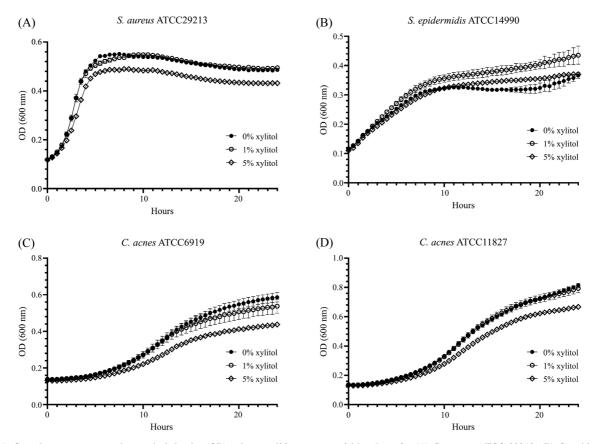

Fig. 1. Growth curves, expressed as optical density (OD) values at 600 nm, over a 24-h culture for (A) *S. aureus* ATCC 29213, (B) *S. epidermidis* ATCC14490, (C) *C. acnes* ATCC6919, and (D) *C. acnes* ATCC11827, in the presence of 0% (w/v) (Control), 1% (w/v), and 5% (w/v) xylitol.

the experimental period (Fig. 1C and D). Visual inspection of the growth curves showed that xylitol affected all of the tested skin-associated bacterial strains, and 5% xylitol clearly inhibited the growth of *S. aureus* ATCC29213 (Fig. 1A) and the *C. acnes* strains, ATCC6919 (Fig. 1C) and ATCC11827 (Fig. 1D). In the case of *S. epidermidis* ATCC14990 (Fig. 1B), 1% xylitol increased the growth rate, but growth in the presence of 5% xylitol was largely similar to that of the control wells without xylitol.

To calculate the statistical significances of changes in growth curves, bacterial growth was evaluated from the area under the growth curve (OD600 × min) (AUC), obtained from the Bioscreen® data, and growth in the blank control medium (TSB or RCM) without added carbohydrates, which represented baseline growth, was subtracted from the growth results in the presence of added carbohydrates (Jaskari *et al.*, 1998). Evaluating the AUC facilitates comparisons between conditions. In addition, the speed of bacterial growth and the accumulation of bacterial mass can influence the absorbance results; therefore, absorbance values that are obtained using different substrates at the end of the growth assay can be difficult to compare. One-way, two-tailed analysis of variance (ANOVA), followed by Dunnett's post hoc test, was used to calculate the significant differences between groups, using GraphPad Prism, version 8.0.1 (GraphPad Software, Inc.). Differences were considered to be significant when $p < 0.05$.

Xylitol decreased the growth of *S. aureus* ATCC29213 ($p < 0.001$) (Fig. 2A), *C. acnes* ATCC6919 ($p < 0.005$) (Fig. 2C), and *C. acnes* ATCC11827 ($p < 0.001$) (Fig. 2D) at 5% (w/v) but not 1% (w/v). In contrast, the growth rate of *S. epidermidis* ATCC14990 (Fig. 2B) increased significantly in the presence 1% (w/v) xylitol ($p < 0.001$) and 5% (w/v) xylitol ($p < 0.05$), but the effect that was elicited by 1% (w/v) xylitol was significantly stronger than that by 5% (w/v) xylitol ($p < 0.005$), indicating that the growth of *S. epidermidis* is affected by xylitol at higher concentrations.

Xylitol has been suggested to possess selective antimicrobial properties, primarily by affecting the adhesion and growth of responsive bacteria (Salli *et al.*, 2019). For example, anti-
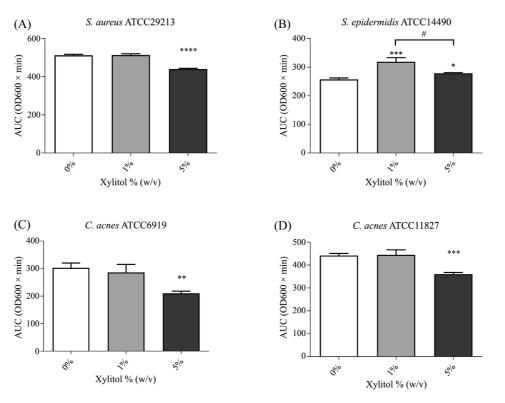

Fig. 2. Effects of 0% (w/v) (Control), 1% (w/v), and 5% (w/v) xylitol on the growth of skin-associated bacteria, expressed as the area under the growth curve (AUC) (OD600 × min), for (A) *S. aureus* **ATCC 29213, (B)** *S. epidermidis* **ATCC14490, (C)** *C. acnes* **ATCC6919, and (D)** *C. acnes* **ATCC11827.** Results are expressed as the mean ± Standard deviation (SD), and statistical analyses were performed by one-way ANOVA; * $p < 0.05$, ** $p < 0.005$, *** $p < 0.001$ compared with the 0% xylitol control; # $p < 0.005$ when 1% (w/v) xylitol was compared with 5% (w/v) xylitol.

adherence activity has been observed against *Escherichia coli* (da Silva *et al.*, 2011), *Clostridioides difficile* (formerly *Clostridium difficile*) (Naaber *et al.*, 1996), *Pseudomonas aeruginosa*, and various *Streptococcus* strains (Söderling and Hietala-Lenkkeri, 2010). Xylitol inhibits the growth of microbes (Söderling and Hietala-Lenkkeri, 2010). Previously, xylitol has been shown to function against *S. aureus* through its anti-adherence property (Ferreira *et al.*, 2009, 2015). In wound care applications, xylitol impedes the growth of *P. aeruginosa*, *S. aureus*, and *Enterococcus faecalis* in *in vitro* wound biofilm models (Dowd *et al.*, 2009; Ammons *et al.*, 2011). In addition, 5% xylitol, in combination with 0.2% farnesol, decreased *S. aureus* levels *in vivo*, and improved skin hydration when applied to volunteers with atopic dermatitis (Katsuyama *et al.*, 2005).

In this study, we used two concentrations of xylitol, 1% (w/v) and 5% (w/v), to study effects of xylitol on the growth rates of skin resident bacteria. In our preliminary experiment (data not shown), we used 1% (w/v) xylitol, which has been shown to be effective in inhibiting the growth of oral pathogen *S. mutans* (Söderling *et al.*, 2008). The 1% (w/v) xylitol did not however, show any marked growth-inhibiting effects against skin-associated bacteria, and therefore, in this study, we included the 5% xylitol concentration, which has been shown to inhibit growth of *S. aureus* previously (Katsuyama *et al.*, 2005). We confirmed that xylitol acts as an antimicrobial agent against *S. aureus*, by reducing its growth. *S. aureus* is a prominent pathogen on the skin of atopic dermatitis patients, and produces various toxins and enzymes that can injure the skin, causing inflammatory responses (Ikezawa *et al.*, 2010). *C. acnes* and *S. epidermidis* are predominant skin-associated microbes, that are often regarded as commensals with beneficial effects but can also act as opportunistic pathogens (Christensen and Bruggemann, 2014). Xylitol acted selectively against these bacteria, inhibiting the growth of *C. acnes* but not that of *S. epidermidis*. *C. acnes* is a major cause of acne, involved in acne inflammation and lesion formation (Kanwar *et al.*, 2018), whereas *S. epidermidis* might have beneficial effects, such as the production of anti-microbial components, depending on the strain (Christensen and Bruggemann, 2014). Bacteriocin production by *S. epidermidis* has been suggested to improve the health of human skin, perhaps by restricting the colonization of pathogenic organisms (Christensen and Bruggemann, 2014). Further, commensal *S. epidermidis* elicits a specific T cell response, promoting the innate immune functions of the skin and limiting pathogenic invasion (Gallo, 2015; Naik *et al.*, 2015). The ability of xylitol to modulate the growth of *S. aureus*, *C. acnes*, and *S. epidermidis* is notable, suggesting that xylitol can be used to regulate skin-associated microbial populations, necessitating further studies to examine the effects of xylitol on the entire microbiota on the skin and the related immune responses. Future studies should also include additional concentrations of xylitol to determine the minimum effective dose.

In conclusion, in pure cultivations and at concentrations that are used in skin care products, xylitol inhibits the growth of potentially pathogenic *S. aureus* and *C. acnes* but not *S. epidermidis*. Instead, the growth of potentially beneficial *S. epidermis* increased at the low xylitol concentration, indicating that xylitol could be applied to balance the microbiota in the skin. Further studies on the entire skin-associated microbiota are needed to determine how the effects of xylitol are translated into the immune system functions (Salli *et al.*, 2019).

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