Effects of Probiotic Lactobacilli on *C. albicans*: Implications for Early Childhood Caries

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ABSTRACT

Purpose: Probiotic *Lactobacillus species* have shown to have beneficial effects against early childhood caries (ECC). However, some research suggests that the wide use of probiotic *Lactobacillus spp.* in dairy food underlines the global increase in ECC prevalence. Interestingly, in the last two decades, *Candida albicans* have also been associated with ECC and is believed to be a risk factor for ECC. The objective of this study was to determine the interactions between lactobacilli and *C.albicans* in growth competition, biofilm formation and acid production.

Methods: Growth competition between *C. albicans* SC5314 (CA) and probiotic *L. rhamnosus* GG (L-GG) or caries-associated *L. rhamnosus* M72-26 (L-M72) were assessed on Lactobacillus MRS (LMRS) agar and Todd Hewitt-Yeast (THY) agar. Biofilm formation was evaluated by culturing lactobacilli and CA together or separately in transwell chambers to only allow metabolite exchange. Acid production by lactobacilli and CA single- and dual-species biofilm at different time intervals was analyzed using Orion pH meter.

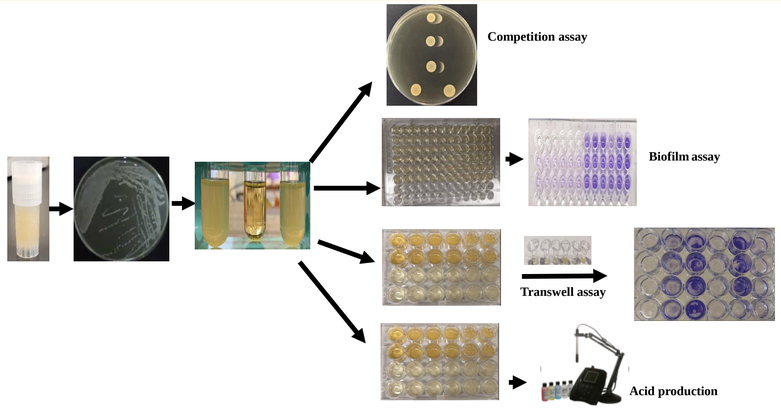
Results: L-GG and L-M72 inhibited the growth of CA on LMRS agar but not on THY agar when lactobacilli were grown 24 hours earlier. L-GG resulted in more biofilm formation than CA in THY medium. However, in MRS medium, CA resulted in significantly higher biofilm formation. L-M72+CA dual-species culture resulted in lesser biofilm than CA mono-species culture in LMRS and THY medium with or without physical contact. L-GG+CA dual-species culture showed significantly lesser biofilm formation than CA mono-species culture in LMRS medium, while the dual-species resulted in more biofilm than mono-species CA culture in THY medium. These results were noted only when organisms were in physical contact with each other. The lactobacilli and CA dual-species biofilm showed similar levels of acid production as lactobacilli single-species biofilm, but the levels were significantly higher than CA single-species biofilm.

Conclusions: Probiotic lactobacilli may prevent ECC development by inhibiting the growth and biofilm formation of *C. albicans* only under favorable conditions. It may, in fact, accelerate ECC progression by increasing acid production.

INTRODUCTION

Streptococcus mutans, Lactobacillus species and intriguingly, in the last two decades, *Candida albicans* are evidenced in the microbiome associated with ECC. Studies have demonstrated that metabolic and glucan-dependent synergism exist between these cariogenic microorganisms, contributing to an enhanced pathogenesis. The probiotic potential of *Lactobacillus spp.* is another established concept. Some researchers mention the wide use of probiotic *Lactobacillus spp.* in dairy food is responsible for the global increase in ECC prevalence, while other state the beneficial effects of these organisms against ECC. This contradiction makes the mechanism of action of Lactobacilli in ECC undefined.

MATERIALS AND METHODS



RESULTS

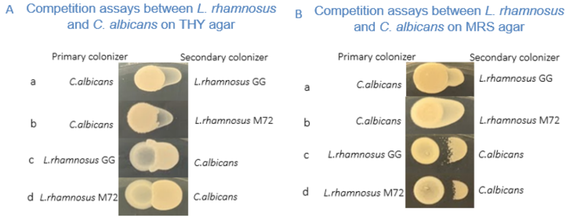


Figure 1. Growth competition between lactobacilli and *C. albicans*. 5µl of overnight cultures of *C. albicans* and lactobacilli (OD OD_{600nm} = 0.5) were plated on THY agar (A) or on MRS agar (B) and incubated for 24 hours. The next day, 5µl of overnight cultures were inoculated adjacent to the first inoculated organisms and incubated for another 24 hours.

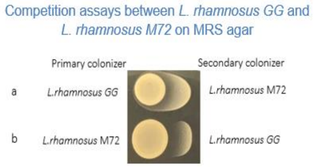


Figure 2. Growth competition between *L. rhamnosus GG* and *L. rhamnosus M72*. 5µl of overnight cultures of *L. rhamnosus* (OD OD_{600nm} = 0.5) were plated on MRS agar and incubated for 24 hours. The next day, 5µl of overnight cultures were inoculated adjacent to the first inoculated organisms and incubated for another 24 hours.

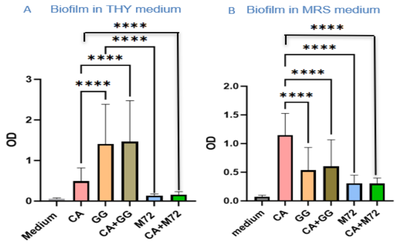


Figure 3 Lactobacilli and *C. albicans* single- and dual species biofilm formation. *C. albicans*, L-GG, and L-M72 were cultured in THY medium (A) or LMRS medium (B) containing 1% sucrose for 24 hours. Biofilm formation was evaluated by crystal violet staining and OD measurement at 562 nm. Data are expressed as mean ± SD (n = 4-6).

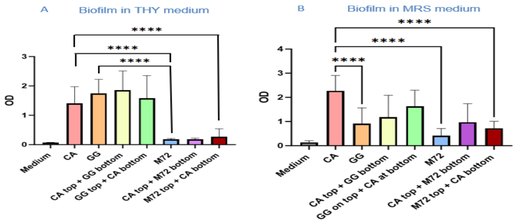


Figure 4. Effects of Lactobacilli on *C. albicans* biofilm formation in a separate culture system. *C. albicans*, L-GG, and L-M72 were cultured separately in a transwell system in THY medium (A) or LMRS medium (B) containing 1% sucrose for 24 hours. Biofilm formation was evaluated by crystal violet staining and OD measurement at 562 nm. Data are expressed as mean ± SD (n = 4-6).

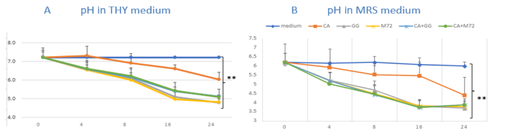


Figure 5. Acid production by Lactobacilli and *C. albicans* single- and dual-species biofilms. *C. albicans*, L-GG, and L-M72 were cultured in THY medium (A) or LMRS medium (B) containing 1% sucrose for 24 hours. Acid production was measured using an Orion pH meter. Data are expressed as mean ± SD (n = 3).

CONCLUSIONS

- Environment and time of colonization plays a crucial role for probiotic *L. rhamnosus* to show its beneficial effects against the growth of *C. albicans* and against biofilm formation
- Early colonization of caries-associated *L. rhamnosus M72* may inhibit the probiotic *L. rhamnosus GG* from displaying its beneficial effects
- Physical contact may be needed for probiotic *L. rhamnosus* to display its beneficial effects against *C. albicans*
- Presence of *L. rhamnosus* can result in increased acidity of the environment.
- Probiotic lactobacilli may prevent ECC development by inhibiting the growth and biofilm formation of *C. albicans* only in an optimal condition. It may, in fact, accelerate ECC progression by increasing acid production.