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In hibitory effect of Lactobacillus reuteri against Streptococcus mutans; ain vitro study

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ABSTRACT: Dental caries is an oral health problem in public health worldwide. Probiotics are live microorganisms that provide benefits to general health. In recent years, Lactobacillus reuteri probiotic has distinguished itself by being able to control the oral microbiota, impacting dental caries. This study aimed to evaluate a commercial product containing the strain of Lactobacillus reuteri such as Biogaiadrops and one toothpaste with flour and a combination of both.

Streptococcus mutans samples were used as test organisms using the Kirby Bauer technique with modified wells. The effect of the products and the strain isolated from the commercial product BIOGAIA was evaluated. The Petri dishes with these cultures showed an inhibition halo (measured in mm) that demonstrated that the paste is more effective in conjunction with the drops containing the strain of Lactobacillus reuteri. This study suggests a synergy between both products, which shows the effect of the probiotic against pathogenic strains of dental caries.

I. INTRODUCTION

Dental caries is a highly prevalent disease in the global population that affects individuals of all ages and is considered an infectious, chronic disease characterized by the progressive destruction of the hardtissues of the tooth, a consequence of dysbiosis, which alters the balance and proportion between the different species of microorganisms that make up the oral microbiota. Its mechanism of action involves the presence of acids, which are a product of the metabolism of carbohydrates, such as simple sugars from the diet. The accumulation of biofilm on the tooth surface and the lack of control of the risk factors associated with this disease also contribute to its development (1). Prevention and control of dental caries requires a comprehensive approach, and there are multiple options, including conventional restorative treatments, minimally invasive dentistry approaches, and preventive treatments. For this reason, new alternatives are necessary to promote oral health (2).

For the prevention and control of cavities, we need a balanced diet with controlled carbohydrates and simple sugars, together with the appropriate use of hygiene aids. Hygiene aids can be toothbrushes, dental floss, and toothpaste. These last with different ingredients (3). The choice of toothpaste will depend on the patient's age and specific diagnosis. For pediatric patients, the toothpaste should contain at least 1000 ppm of total soluble fluoride to prevent carious lesions. Due to the potential adverse health effects associated with some ingredients in traditional toothpaste, many consumers search for products containing natural ingredients such as propolis, chitosan, xylitol, and probiotics. However, toothpastes with probiotic content are currently uncommon on the market and unavailable for all ages (2-3).

In recent years, probiotics have gained relevance in the health field. World Health Organization (WHO) describes probiotics as live microorganisms administered in adequate amounts, that confer health benefits on the host. Due to the increase in their consumption,numerous investigations have been carried out on probiotics and their benefits, since they can help prevent and act as adjuvants in the treatment of various diseases, such as gastrointestinal, autoimmune, allergic, and even mental disorders such as anxiety and depression (4).

Probiotic microorganisms, especially those of the Lactobacillus genus, have shown promising results in the treatment of oral diseases. Lactobacillus reuteri is a probiotic bacteria studied for its oral healthbenefits, colonizing diverse niches in the human body, such as the oral cavity, skin, gastrointestinal tract, and genitourinary tract. Whether alone or in combination, thanks to its antioxidant and antimicrobial properties, it can

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inhibit the colonization of pathogenic microbes and change microbial communities in the host. This work aims to evaluate the antimicrobial activity of Lactobacillus spp. strains on dental caries (5-6).

II. MATERIAL AND METHODS

2.1. Study Design and Material

This is an in vitro experimental design. Two commercial products were evaluated, see Table 1. Additionally, an antibiogram was performed against Streptococcus mutans, and clindamycin was selected for these assays. Furthermore,a combination of toothpaste and drops and isolate of L.reuteri from dropswere used.

2.2 Preparation and reactivation of microorganisms

Bacterial samples of Streptococcus mutans 35688 ATCC were used as test organisms. Initially, it was grown under anaerobic conditions at 37ºC for 24 h in BHI agar (Brain, heart infusion agar) and subsequently, it was grown aerobically at 37ºC. This strain was growth 0.5 Mc Farland for assays.

2.3 Preparation of Lactobacillus reuteri strain and commercial products

In this study, we used two commercial products: the BIOGAIA food supplement in drops presentation and the Oral B Stages fluoride toothpaste for children, bubble gum flavor. The drops contain the Lactobacillus reuteri strain. Table 1 shows the content of each product. The Lactobacillus reuteri strain was isolated from the BIOGAIA using an MRS agar medium. Once the strain was isolated, it was grown in MRS broth until it reached 0.5 McFarland and was grown for 48 hours.

2.3 Agar disc diffusion disc modified Wells

The method used was the Kirby Bauer test with modified wells to determine the sensitivity of the inhibition zone found around the wells. For this reason, Petri dishes were prepared with Mueller Hinton agar and made wells. These culture media were inoculated with the bacteria by the exhaustive method to obtain a uniform distribution. Subsequently, the samples were placed in wells of Mueller-Hinton agar.

For the case of the toothpaste alone and combined with the drops, a typical sample of peas of approximately 0.25 g was used. In the case of the positive control, the antibiotic used was clindamycin with a concentration of 0.2 ug/ml.

We were made three repetitions per plate independently, for each sample

2.4 Statistical analyses

For the analysis of the data, a one-way ANOVA (analysis of variance) were applied in IBM SPSS. P values <0.05 were statistically significant.

III. RESULTS

In this study, it was evaluated chosen toothpaste and drops products, and another samples against Streptococcus mutans cultivated in Mueller Hinton (Table 3).

Table 1. Products evaluated in the study, ingredients and manufacturer.

Product	Ingredients	Manufacturer
Oral B Stagestoothpaste	Sodiumfluoride (1100 ppm) sorbitol, aqua, hydratedsilica, sodiumlauryl sulfate, cellulosegum, trisodiumphosphate, sodiumphosphate, aroman, sodiumsacharinne, carbomer, limonene, benzyl alcohol, ci 454100	Mexico
Biogaiadrops	Proteins (0.0012 g) lipids (0.17g) carhohydrates (0.005g) sugar (0.002 g) fiber 0g) Sodium (0g) 100 millones DSM *L. reuteri*	Sweden

According to the means of the different groups are different, showing that the greatest inhibitory effect is found with the combination of paste and drops, showing that at 24 h there is an inhibition halo of 27.89 mm, very close to the positive control and a standard deviation of 1.58 about the average. All assays show that inhibition halos were present in all samples, where the halo of the isolate of the strain was smaller than in other samples. However, this is very close to the one presented by the drops presentation. Concerning toothpaste, this shows an inhibitory halo of 19 mm, two units upper of drops or strain. This was followed by the Lactobacillus reuteri strain isolated from drops with a 16 mm diameter inhibition zone. On the other hand, clindamycin exhibited the highest effect with a 30 mm diameter inhibition zone (Figure 1).

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Table 2 shows the mean values and standard deviations of the inhibition zones. Analysis of the average data from three separate experiments revealed that there was a significant impact on Streptococcus mutans. The negative control, consisting of sterile distilled water, showed no effect against both types of bacteria, as detailed in Figure 1.

Table 2. Average and standard deviation of samples.

	Drops	*L. reuteri*	Toothpaste	Toothpaste+drops
Average (mm)	17	16	19	27
Standard deviation (mm)	±1.48	±2.07	±1.30	±1.58
Upper level (mm)	20	14	20	30
Lower level (mm)	15	19	17	26

Table 3. Asaays of inhibition growth

Sample	Volume
Drops	80 µl
Toothpaste	1 pea
Drops+ toohtpaste	5 drops + 1 pea
StrainLactobacillus reuteri	80 µl
Clindamycin	2 µg
Steriledistilledwater	80 µl

IV. DISCUSSION

Overgrowth of pathogenic oral bacteria such as Streptococcus mutanscan lead to dysbiosis, associated with oral diseases such as dental caries. Recently, studies have shown that probiotics can affect the oral microbiome. There are compilations of commercial products with probiotics for oral health between capsules, pills and gels, among others (7,8). In this work, the antibacterial effectiveness of two commercial products against Streptococcus mutanswas evaluated. One is Oral B Stages toothpaste, and another is a dietary supplement in drops from BioGaia, as well as the strain isolated from this product and the combination of the two commercial products.

According to the results, the Lactobacillus reuteri strain isolated from the drops shows a halo of inhibition, similar to another study that reports the best inhibition against Streptococcus mutans was with the Lactobacillus reuteri strain at a concentration of 100% and at 48 h and a halo of 17.50 mm. Although the results were similar, the study has several differences such as a clinical isolate of Streptococcus mutans and, 0.5 McFarland of Lactobacillus reuteri strain (9).

Another study reports that the presence of Lactobacillus reuteri ATCC PTA 5289 can inhibit the development of acid tolerance response in the early stages of biofilm formation in common oral bacteria, therefore, probiotic strains of Lactobacillus could play a role in preventing caries by inhibiting the development of biofilm microbiota of acid-tolerant bacteria such as Streptococcus mutans. (10)

Many types of bacteria are involved in the development of dental caries, however, Streptococcus mutans is considered the main pathogen of the disease. Noda et al. In 2021 (11) concluded that Lactobacillus reuteri BM53-1 produces a substance that causes the disappearance of glucan viscosity by unbalancing the expression levels of three gtf genes of Streptococcus mutans, which may result in an inhibitory effect on the growth and development of Streptococcus.

Due to the adverse health effects of some ingredients in traditional toothpaste, people select toothpaste with other ingredients such as probiotics (12). One study showed that a toothpaste containing the strain Lactobacillus paracasei did not exhibit antimicrobial activity, but additional ingredients in the toothpaste should be considered and perhaps their metabolites are different than another strains (13)

In this experimental study, we examined the antimicrobial effectiveness of two commercial products and a combination, whose findings highlight the synergy of toothpaste with Lactobacillus reuteri strain.

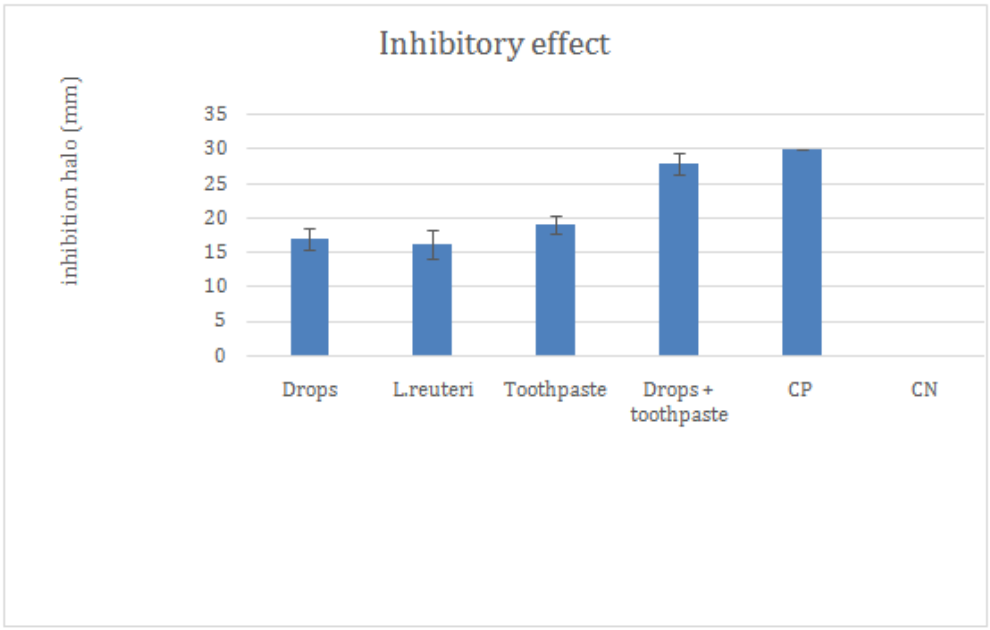

Figure 1. Effect of commercial products and Lactobacillus reuteri on S. mutans. CP: positive control Clindamycin, CN: negative control sterilewater.

V. CONCLUSION

Studies on probiotic bacteria show promising results for the prevention of biofilm-mediated diseases such as dental caries. However, the mechanisms are not currently understood.

Therefore, in the present study, it was of great interest to evaluate the inhibitory effect that Lactobacillus reuteri strains have on Streptococcus mutans by testing different alternatives such as mixing Biogaia drops (Lactobacillus) with children's fluoride toothpaste (Stages, Oral B, 1,100 ppm fluoride), obtaining favorable results. These findings demonstrate the effectiveness of the Lactobacillus reuteri on Streptococcus mutans.

In a future perspective, probiotic supplementation could be an alternative or adjuvant therapy of oral disease such as dental caries

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