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Antimicrobial Effect of *Lactobacillus reuteri* on Cariogenic Bacteria *Streptococcus gordonii*, *Streptococcus mutans*, and Periodontal Diseases *Actinomyces naeslundii* and *Tannerella forsythia*

Magda Lorena Baca-Castañón · Myriam Angélica De la Garza-Ramos · Andrea Guadalupe Alcázar-Pizaña · Yohann Grondin · Anahí Coronado-Mendoza · Rosa Isela Sánchez-Najera · Eloy Cárdenas-Estrada · Carlos Eduardo Medina-De la Garza · Erandi Escamilla-García

Abstract Lactic acid bacteria (LAB) are well known for their beneficial effects on human health in the intestine and immune system; however, there are few studies on the impact they can generate in oral health. The aim of this study was to test and compare in vitro antimicrobial activity of *L. reuteri* on pathogenic bacteria involved in the formation of dental caries: *S. mutans*, *S. gordonii*, and periodontal disease: *A. naeslundii* and *T. forsythia*. Also, we determined the growth kinetics of each bacterium involved in this study. Before determining the antimicrobial action of *L. reuteri* on cariogenic bacteria and periodontal disease, the behavior and cell development time of each pathogenic bacterium were studied. Once the conditions for good cell growth of each of selected pathogens were established according to their metabolic requirements, maximum exponential growth was determined, this being the reference point for analyzing the development or inhibition by LAB using the Kirby Bauer method. Chlorhexidine 0.12 % was positive control. *L. reuteri* was shown to have an inhibitory effect against *S. mutans*, followed by *T. forsythia* and *S. gordonii*, and a less significant effect against *A. naeslundii*. Regarding the effect shown by *L. reuteri* on the two major pathogens, we consider its potential use as a possible functional food in the prevention or treatment of oral diseases.

Keywords *Lactobacillus reuteri* · Probiotic · Cariopathogens · Periopathogens · Bacterial inhibition · Oral health

Magda Lorena Baca-Castañón and Myriam Angélica De la Garza-Ramos are first authors.

M. L. Baca-Castañón · M. A. De la Garza-Ramos · A. G. Alcázar-Pizaña · A. Coronado-Mendoza · E. Escamilla-García (✉)
Unidad de Odontología Integral y Especialidades, Centro de Investigación y Desarrollo en Ciencias de la Salud (CIDICS), Universidad Autónoma de Nuevo León (UANL), Ave. Carlos Canseco s/n con Ave. Gonzalitos, Mitras Centro, CP: 64460 Monterrey, Nuevo León, Mexico
e-mail: erandi.escamillagrc@uanl.edu.mx

M. L. Baca-Castañón · M. A. De la Garza-Ramos · A. G. Alcázar-Pizaña · R. I. Sánchez-Najera · E. Escamilla-García
Departamento de Microbiología, Facultad de Odontología, Universidad Autónoma de Nuevo León (UANL), Monterrey, Nuevo León, Mexico

Y. Grondin
Facultad de Ciencias Biológicas, Universidad Autónoma de Nuevo León, San Nicolás de los Garza, Nuevo León, Mexico

E. Cárdenas-Estrada
Unidad de Ensayos Clínicos, Centro de Investigación y Desarrollo en Ciencias de la Salud (CIDICS), Universidad Autónoma de Nuevo León (UANL), Ave. Carlos Canseco s/n con Ave. Gonzalitos, Mitras Centro, CP: 64460 Monterrey, Nuevo León, Mexico

C. E. Medina-De la Garza
Unidad de Inmunomoduladores, Centro de Investigación y Desarrollo en Ciencias de la Salud (CIDICS), Universidad Autónoma de Nuevo León (UANL), Ave. Carlos Canseco s/n con Ave. Gonzalitos, Mitras Centro, CP: 64460 Monterrey, Nuevo León, Mexico

C. E. Medina-De la Garza
Departamento de Inmunología, Facultad de Medicina, Universidad Autónoma de Nuevo León, Monterrey, Nuevo León, Mexico

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Introduction

The oral cavity microbiota is an ecosystem formed nearly by 700 different microbial species [1]. This complex system can be altered by diverse factors, including poor hygiene and diet, smoking, stress and systemic diseases [2]. All these perturbations favor the colonization by pathogenic bacteria and the formation of biofilms, which are both etiological factors of periodontal diseases. Identified pathogenic bacteria, including *Streptococcus gordonii* [3], *Actinomyces naeslundii* [4], and *Tannerella forsythia* [5, 6], contribute differently to the onset of periodontal diseases. For example, early colonization of *S. gordonii*, one of the most abundant microorganisms in dental plaques [7], is essential for further colonization by other bacterial strains and biofilm formation as it produces bacterial growth-enabling adherence substrates [8]. Other bacteria such as *A. naeslundii* affect the cervical areas of the teeth and supporting tissues [9] and have been specifically implicated in the development of caries on root surfaces [4]. Finally, *T. forsythia*, which is found at the active sites of infection, has been associated with advanced and recurrent periodontitis [10].

Controlling the colonization of the oral cavity by pathogenic bacteria is essential for the prevention of periodontal diseases. Studies in that respect have been focusing on a class of nonpathogenic microorganisms called probiotics [11] among which Gram-positive lactic acid bacteria (LAB) are most promising candidates. The potential health benefits of these LAB are multiple, from stimulating the immune system, maintaining the intestinal microflora balance, competing with pathogens, reducing childhood allergies to controlling hyperlipidemia and/or liver disease [12]. For example, a mix of *L. acidophilus* and *L. casei* enhances the effect of anticancer drug 5-fluorouracil by increasing apoptosis of colorectal cancer cell line LS513 in vitro [13, 14].

Most interesting for the control of periodontal diseases is LAB, with antimicrobial and anti-inflammatory properties such as *L. reuteri*, *L. fermentum,* and *L. casei Shirota* [15, 16]. Of these bacteria, *L. reuteri* is most interesting for the control of oral microbiota as it produces reuterin, a wide spectrum antimicrobial agent [17]. Reuterin inhibits growth of both Gram-positive and Gram-negative bacteria, including oral pathogens *S. mutans*, *Actinomyces actinomycetemcomitans*, *Prevotella intermedia*, *Fusobacterium nucleatum*, as well as yeasts like *Candida albicans*, some fungi and protozoa [18–20].

Some studies have shown promising results on the inhibition by *L. reuteri* isolated from humans and rats of both the growth of various oral pathogens, including *T. forsythia*, and biofilm formation [21]. Even more interesting is that antimicrobial property of *L. reuteri* appearing to be maintained when food products are used as a vector for their delivery. Çaglar et al. [22] reported in in vivo studies that levels of *S. mutans* in saliva were positively reduced without antagonistic effects in the presence of *L. reuteri* contained in tablets at a concentration of 1×10^9 CF U/mL. Another study [23] showed that consumption of yogurts containing *L. reuteri* at different concentrations reduced *S. mutans* in the oral cavity.

In this study, we determined the in vitro antimicrobial action of *L. reuteri* on pathogens associated with the formation of dental caries, *S. gordonii* and *S. mutans*, and with the development of periodontal diseases, *A. naeslundii* and *T. forsythia*.

Materials and Methods

Kinetics was assessed prior to the Kirby Bauer method, in particular, the determination of lag and exponential phase. The time required for both the LAB and the odontopathogenic bacteria to reach their maximum exponential growth was fundamental to testing the inhibitory effect of *Lactobacillus reuteri* ATCC55730 by the Kirby Bauer method.

Biological Material

Four strains of pathogenic oral bacteria were selected for this study: *S. gordonii* (ATCC10558), *Streptococcus mutans* UA130 (ATCC700611), *Tanarella forsythia* (ATCC43037), and *A. naeslundii* (ATCC51655). Culture and growth conditions for each bacterium were based on the technical sheets of the American Type Culture Collection (ATCC) (Table 1).

Table 1 Media and culture conditions for organisms used as established by the American Type Culture Collection (ATCC)

Bacterium	Pre-culture–Culture media (agar–broth)	Atmosphere	Incubation time at 37 °C (h)
Lactobacillus reuteri	MRS–MRS	Anaerobic	24–48
Streptococcus gordonii	TS–BHI	Aerobic	24
Streptococcus mutans	BHI–BHI	Aerobic	24–48
Tanerella forsythia	NAM–PY media with Horse serum and NAM	Anaerobic	24–48
Actinomyces naeslundii	CAMG medium pH 7.5 (±0.1)	Anaerobic	24–48

Growth Conditions

Specific pre-culture on agar in Petri dishes was performed for the bacteria for 24–48 h at 37 °C (Thermo Scientific Incubator Lab-Line, Marietta, OH); as indicated by ATCC. *L. reuteri* and two of the Gram-negative oral pathogens, *T. forsythia* and *A. naeslundii* are anaerobes and their handling requires a controlled atmosphere in an anaerobic chamber (Plas-Labs, Lansing/MI). This atmosphere was a mixture of H_2 (20.13 %), CO_2 (10.13 %), and N_2 (69.74 %) gas (Praxair/Mexico). All culture media as well as the material were pre-sterilized 15 min at 120 °C (All-American, Hillsville, USA). Broth culture were carried out in 250-mL flasks containing 200 mL of culture medium as appropriate for each microorganism (see Table 1) and inoculated to an optical density (OD) of 0.2 at 600 nm. Prior to inoculation, pre-culture was washed with normal saline (0.9 % w/v of NaCl).

The culture medium used for *L. reuteri* is based on requirements indicated by the ATCC with suitable growth conditions. The original strain was lyophilized and frozen at −80 °C. Activation was done by inoculating Man Rogosa and Sharpe (MRS) broth (BD-Difco, Becton–Dickinson and Company, Pont-de-Claix/France). For subsequent activations from a broth culture, 15 g/L of agar was added (Becton–Dickinson and Company).

Tannerella forsythia was previously grown on *N*-acetyl muramic acid agar [24] (Sigma-Aldrich, St. Louis/MO) that consists of 49 g tryptone soy agar (Becton–Dickinson and Company), and 10 mL of hemin (AppliChem GMbH, Darmstadt/Germany) suspended in 1 L of distilled water. It was subsequently grown in peptone-yeast (PY) comprised (g/L) of 5-peptone (Dibico/Mexico), 5-tryptone (BioBasic-Inc.), 10-yeast extract (Dibico), 4 mL of resazurin, 40 mL of water or normal saline, 10 mL of hemin, 0.2 mL of vitamin K, 0.5 L-cysteine (Sigma-Aldrich), and pH 7.0 (±0.1).

Actinomyces naeslundii also requires a specific medium, this being CAMG composed of (g/L) 5-tryptone, 5-yeast extract (Dibico), 5-K_2HPO_4, 2-glucose (Jalmek-Científica/Mexico), and 0.5 mL polysorbate 80, pH 7.5 (±0.1). The development of both microorganisms was carried out in a controlled gas mixture atmosphere at 37 °C for 24–48 h.

Streptococcus mutans required pre-culture in tryptone soy agar and cultured in Brain Heart Infusion broth (both from BD Bioxon, Becton–Dickinson and Company), while BHI was used for *S. gordonii*, in both inocula (Table 1). Incubation was performed at 37 °C for both strains for 24 h for *S. gordonii* and 24–48 h for *S. mutans*.

The morphology of the strains was confirmed by growth on agar (Petri dish) and by microscopy using differential staining (Gram; data not shown). An inverted fluorescence microscope (Axio Inverter Z.1, Carl Zeiss-AG/Germany) was used.

Growth Kinetics

To determine the doubling time and specific growth rate of each bacteria, we used a bacterial cell suspension with a McFarland standard 2.0 ($OD_{600\ nm} = 0.2–0.5$) determined with a Genesys 10 UV Scanning Spectrophotometer (Thermo-Scientific, Madison/WI). Three 250-mL flasks containing 200 mL of appropriate culture medium were inoculated (Table 1) and incubated at 37 °C for 3 min for further adaptation of cells to culture conditions. Then a first sample was taken, which represents time zero. Development was monitored by taking samples and measuring the OD (600 nm) and pH (Ulltrabasic-Potentiometer, Denver-Instruments, Arvada/CO) every 3 h during 72 h for *L. reuteri* culture and every hour during 25–30 h for *S. gordonii*, *S. mutans*, *T. forsythia*, and *A. naeslundii*. Growth kinetics was performed in duplicate for all bacterial strains.

Doubling time (h) and specific growth rate ($\mu = h^{-1}$) were calculated from the exponential growth phase for each bacterium.

Inhibitory Effect of *Lactobacillus reuteri*

Kirby Bauer Method

The Kirby Bauer method is performed to study the activity of antimicrobial agents against pathogenic microorganisms [25]; in this case, *L. reuteri* was the agent to show antimicrobial activity. This method is based on the formation of growth inhibition zones around disks made of filter paper impregnated with the antimicrobial agent. For this study, the Kirby Bauer method was made inside an anaerobic chamber because anaerobic conditions are required for *L. reuteri*. As previously described, the pathogenic bacteria are inoculated in a Petri dish that has the appropriate bacterial agar forming a monolayer. A disk is subsequently immersed in the culture that contains the LAB (*L. reuteri*) and is placed in the Petri dish. All pathogens selected for the study were previously activated, and each was taken in their exponential phase according to the results previously obtained in growth kinetics.

To determine the antimicrobial effect of *L. reuteri*, Chlorhexidine-digluconate 0.12 % (CHX, Consepsis Ultradent Products/Inc., South Jordan/UT), an antiseptic agent used in dentistry as a mouthwash or mouth rinse, was used as inhibition positive control and to establish a reference of the effect of *L. reuteri* on oral pathogens and sterile distilled water as a negative control. Both controls were handled in the same way as *L. reuteri,* and all the procedures were performed in triplicate. Finally, the Petri dishes containing the respective antibiograms were incubated at 37 °C for 24 h to determine the presence or absence of the halos formed around the disk.

Evaluation of the Kirby Bauer method was performed in a qualitative manner by formation of inhibition zones at the periphery of the disk, both for the sample of interest and its controls. Then a quantitative analysis is made by measuring the diameter of the inhibition halo formed for each condition. The result was an indicator of the efficiency of antimicrobial agent (*L. reuteri*) against selected pathogenic bacteria.

Statistical Analysis

Three independent replicates of each experiment were performed, and their results were expressed as mean diameter ± standard deviation (SD). We used data analysis, and the significances of the inhibitory effect of *L. reuteri* against CHX were evaluated with unequal variance analysis based on the *t test and the **Mann–Whitney ($P < 0.05$) test and calculated with statistical software, STATA-13, Serial-Nr. 1910531247

Results

Bacterial morphology and differentiation were confirmed for each oral pathogen selected in this study by standard stains and cultures (data not shown).

Growth Kinetics

The results presented correspond to the mean value of each experiment.

Lactobacillus reuteri showed the typical phases of microbial growth. The lag phase lasted 8 h with no change in $OD_{600\ nm}$: 0.12 ± 0.01 (Fig. 1) and a pH of 6.45 ± 0.10; at this time, exponential growth phase begins and lasts up to 18 h, where maximum growth occurs with an OD of 1.77 ± 0.02 and a pH 4.10 ± 0.10. After this time, stationary phase is established with an OD of 1.8 ± 0.10 and a pH 4.29 ± 0.13.

The Gram-positive, cariogenic bacteria are seen in Fig. 2a. *S. mutans*, with only 2 h from start of culture with a pH 7.14 ± 0.01 (Fig. 2a.1), presented exponential growth reaching its peak in 5 h with an OD of 1.15 ± 0.05 and a pH 5.83 ± 0.0. After this time, growth remained constant until about 17 h maintaining a pH 6.185 ± 0.13.

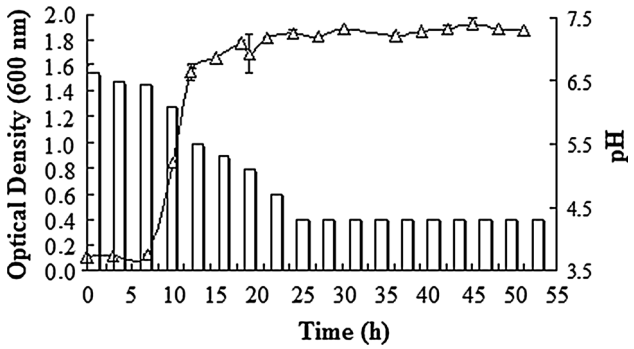

Fig. 1 Growth kinetics (*triangle*) and pH (*white bar*) of *L. reuteri*

Streptococcus gordonii has a different behavior than *S. mutans*. It has no lag phase at inoculation, and logarithmic growth reaches its maximum peak of development at 7 h with an OD of 1.4 ± 0.05 and a pH 4.99 ± 0.01 (Fig. 2a.1); the stationary phase is then established for a long period of time (over 20 h).

The Gram-negative, periodontopathogenic bacteria are seen in Fig. 2b. *A. naeslundii* grows slowly with an initial pH 6.7 ± 0.04 (Fig. 2b.1), and a lag phase of about 14 h; afterward, exponential growth starts with a duration of just 3 h, reaching its maximum OD of 0.5 and a pH 5.38 ± 0.03 at 17 h of incubation. Then the stationary phase remains constant up to 22 h, maintaining a pH 5.34 ± 0.03. *T. forsythia* grew very quickly, with a very short lag phase. The start of the logarithmic phase occurred almost immediately, reaching its peak in 5 h with an OD of 0.87 ± 0.1 and a pH 5.47 ± 0.01 (Fig. 2b.1). Subsequently, the stationary phase was established at about 19 h.

Doubling Time and Specific Growth Rate

The doubling time and specific growth rate (Fig. 3) were determined and analyzed for each microorganism prior to assessing the antimicrobial activity of *L. reuteri*. Of all bacteria, growth kinetics showed that the doubling time was the shortest for *S. mutans* at 0.86 ± 0.03 h and the longest for *S. gordonii* and *L. reuteri* at about 3 h. Remaining bacteria *A. naeslundii* and *T. forsythia* showed intermediate doubling times of 2 h.

In general, these values were confirmed with the determination of the specific growth rate (μ), this still being very high from *S. mutans* compared with other microorganisms 0.81 ± 0.03 h^{-1}. *L. reuteri* and *S. gordonii* presented very similar specific growth rate (slower compared with studied bacteria), with values of $\mu = 0.24 \pm 0.01\ h^{-1}$ and $\mu = 0.23 \pm 0.04\ h^{-1}$, respectively. On the other hand, *A. naeslundii* has a μ of $0.32 \pm 0.00\ h^{-1}$ and a value of $0.34 \pm 0.03\ h^{-1}$, from *T. forsythia*.

Inhibitory Effect of *L. reuteri*

The result of each kinetic, specifically the time required for both the LAB and the odontopathogenic bacteria to reach their maximum exponential growth, was utilized to determine the inhibitory effect of *L. reuteri* by the Kirby Bauer method.

The inhibitory halos around the disk impregnated with *L. reuteri* during their peak log-growth (16 h) were measured (mm) to determine the activity of *L. reuteri* against bacteria. Results of measurement of inhibition halos of

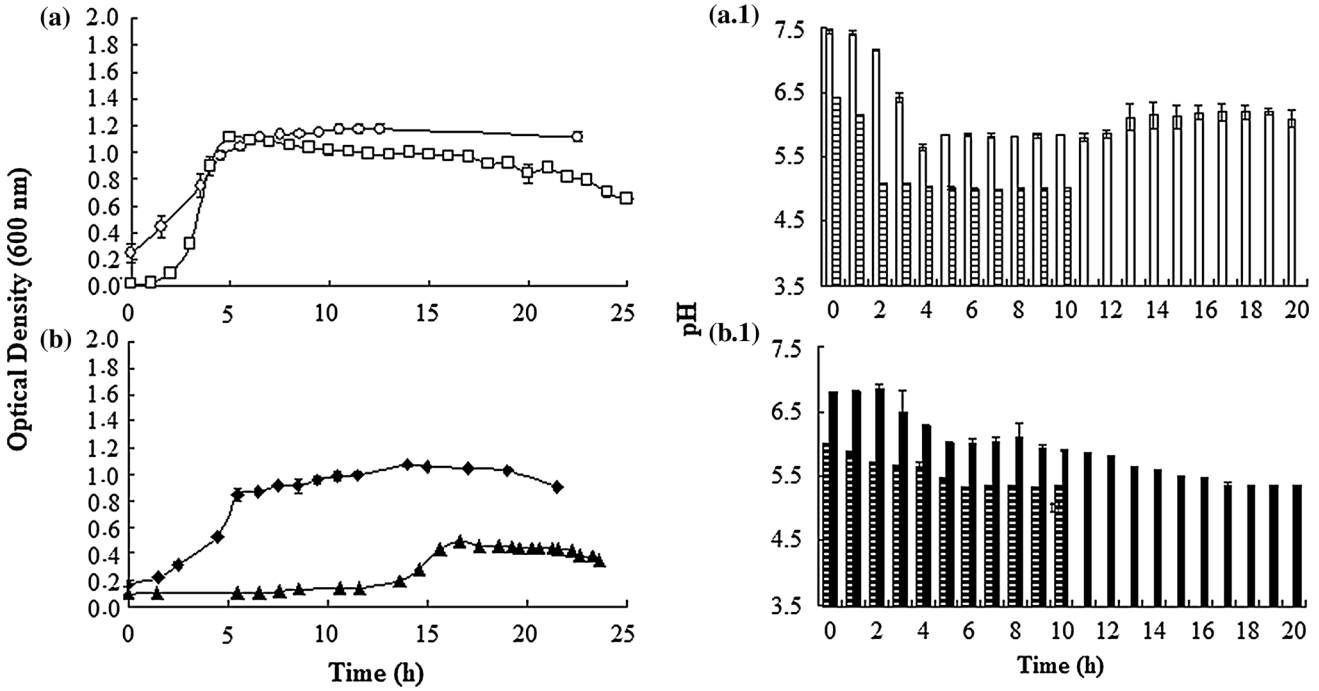

Fig. 2 Growth kinetics and pH values (represented in *bar*) of oral pathogenic bacteria in dental caries (**a**, **a.1**): *S. gordonii* (*open circle*, *striped bar*) and *S. mutans* (*open square*, *open bar*), and periodontal disease (**b**, **b.1**): *A. naeslundii* (*filled triangle*, *filled bar*) and *T. forsythia* (*filled diamond*, *filled striped bar*)

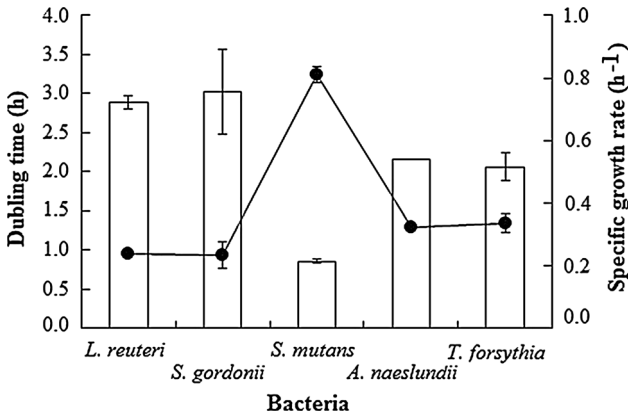

Fig. 3 Doubling time (histogram) and specific growth rate (*filled circle*) determined for each tested bacterial strain. Data are given as mean diameter ± standard deviation

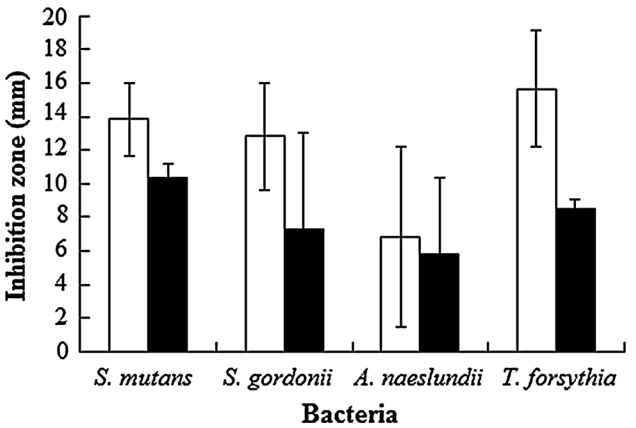

Fig. 4 Determination of bacterial susceptibility of *S. mutans* and *S. gordonii* (present in dental caries) and *A. naeslundii* and *T. forsythia* (in periodontal disease), to *L. reuteri* (*filled square*) and Chlorhexidine (*open square*), as antimicrobial compounds

both LAB and Chlorhexidine (CHX, as a positive control) are shown in Fig. 4. *L reuteri* had a growth inhibitory activity against three of the pathogenic bacteria used, showing different inhibition halos against each one. Thus, the larger halos were observed against *S. mutans*, *S. gordonii*, and *T. forsythia* (10.3 ± 0.81, 7.3 ± 5.68 and 8.5 ± 0.54 mm, respectively), while to *A. naeslundii,* the inhibition halo was small (5.8 ± 4.53 mm). With CHX, the halos were 13.83 ± 2.13, 12.83 ± 3.18, 15.66 ± 3.50, and 6.83 ± 5.38 mm, against *S. mutans*, *S. gordonii,* and *T. forsythia,* respectively. Again, the inhibition halo of *A. naeslundii* was smaller than other pathogens, thus showing resistance to CHX, as happened with *L. reuteri*.

Discussion

The trend in recent years is the search for new antimicrobial substances that can be used as an alternative to the existing ones and that are safe, in this respect, the LAB are

very promising candidates because of its well-known health effect. In the search for therapeutic options, *L. reuteri* could be a possible biological alternative for prevention or treatment of oral diseases. This study demonstrates the sensitivity of three major oral pathogens that cause caries or periodontal disease to the probiotic *L. reuteri*. Finally, analysis of bacterial growth is of great interest in dental and general health since it contributes to the understanding of microbial processes and their impact on oral health.

The first part of this study include the growth kinetics of each of the microorganisms involved, in determining the doubling time and specific growth rate of each bacterium, important parameters for analysis of the inhibitory effect of *L. reuteri* by the Kirby Bauer method, because it is important that microorganisms are more active metabolically.

Regarding pathogenic bacteria, growth kinetics were very different among them. It should be noted that some of these bacteria are specific for damaging hard structures, like teeth enamel, while others damage soft structures, such as those of the oral cavity. The biggest and best protective effect of a microorganism or aggression is caused when they are metabolically active, a phenomenon that occurs during maximum exponential growth. Knowledge of this phase in the kinetics of each pathogen lets us understanding better the time that each one needs to cause damage to soft tissues by degrading collagen present in periodontal ligament, altering pH conditions in oral cavity creating an ideal microenvironment for *A. naeslundii*, *T. forsythia* growth. However, in hard structures, *S. mutans* and *S. gordonii,* for example, produce organic acids as a result of their carbohydrate metabolism, causing enamel demineralization. These data give us more information on the behavior and growth of pathogen to implement more studies about a biological agent, which prevents their proliferation and maybe irreversible damage on some structures of the oral cavity. With respect to growth, the duration of the lag phase differed greatly between pathogenic bacteria, from a few hours or less for *T. forsythensis*, *S. gordonii,* and *S. mutans*, to 14 h for *A. naeslundii* (Fig. 2). The lag phase for *L. reuteri* is then considered as intermediate.

There are some reports on the growth of these bacteria, although strains and culture media may differ to a point where growth kinetics is altered [21]. For example, *L. reuteri* isolated from human feces to treat against pathogenic gastrointestinal bacteria presented a similar slow growth dynamics as the one reported here [26]. However, it is possible to achieve a much shorter lag phase and higher growth rate, as shown with *L. reuteri* PRO 137 strain suspended in glycerol solution [27]. Our data are consistent with those observed in both studies.

When *S. mutans* is grown in anaerobic conditions, there is an absence of a lag phase and the exponential phase is stable for 8 h [21]. Other authors report the behavior of the same bacteria during biofilm formation on the surface of female rat teeth. Two hours after inoculation of the bacteria, growth declined due to the presence of sucrose, and the logarithmic growth phase occurred reaching its maximum exponential point at 6 h [28].

On the other hand, there are no reports in the literature on the growth of cariogenic bacteria *S. gordonii*. Data exist only on biofilm formation and its adhesion to tooth surfaces with the addition of carbon sources (glucose/sucrose), in which different stages of adaptation, prevalence, and biofilm structure development appear [29].

About the Gram-negative periodontopathogenic bacteria, particularly with *A. naeslundii*, no previous reports on growth stages were found. On the other side, Kang et al. [21] used the same *T. forsythia* strain as in this study; however, the lag phase lasted 8 h with a slower rate of growth.

With these data, we can conclude that at least two of the cariogenic bacteria selected for the study are very different in their growth, which would explain the ability of *S. mutans* to form biofilm. Development of this biofilm requires that other microbial sources help it establish on the surface of teeth and cause damage. It may also contain genetic material and metabolites that are easier to synthesize for division and generation of two identical daughter cells. However, we must not forget that conditions of culture medium and incubation are important factors for early or slow development. Logarithmic phase of each bacterium was considered to test the inhibitory effect of *L. reuteri* because microorganisms are most active and begin the process of division in this stage. The log phase is generally quite rapid and puts the bacterial cells in a vulnerable position in the possibility to be more pathogen or effective as the case.

Antibiograms are widely used to test inhibition of microbial growth. Asikainen [30] used antibiograms to evaluate the effect of *L. reuteri* on pathogenic bacteria from periodontal tissue, such as *A. actinomycetemcomitans*, *P. intermedia*, and *F. nucleatum*, obtaining favorable results in the inhibition of these pathogens. In our study, a significative difference between the inhibition halo formed by *L. reuteri* and CHX for both *S. mutans* and *T. forsythia* ($*P = 0.004$ and $**P = 0.001$) was observed. On the other hand, no significant difference between the effect of LAB and CHX against *S. gordonii* and *A. naeslundii* was found ($*P = 0.065$ and $**P = 0.073$).

Çaglar et al. [22] reported in vivo studies where numbers of *S. mutans* in saliva were positively reduced without antagonistic effects in the presence of *L. reuteri*, administered in the form of chewing tablets at a concentration of 1×10^9 CFU/mL. Another study by Nikawa et al. [23] revealed that consumption of yogurt containing *L. reuteri*

in different concentrations reduced *S. mutans* in the oral cavity. They pointed out that *L. reuteri* was three times more effective than other lactobacilli and reported a mortality of 90 % for *S. mutans*. Other studies performed with the same LAB applied to various pathogens in the oral cavity (*C. albicans*, *S. mutans*, *Actinomyces actinomycentemcomitans*, *P. intermedia*, *F. nucleatum*, etc.) and/or the gastrointestinal tract (*E. coli*, *Salmonella*, *Shigella*, *Proteus*, *Pseudomonas*, *Clostridium*, *S. aureus*, yeast, fungi, protozoa, viruses) as Connolly showed [17].

Similar studies about reduction of *S. mutans* and periodontal pathogens, but with other LAB, concern different species of *Lactobacillus paracasei. L. paracasei* GMNL-33 is proposed as a strategy for caries prevention and management [31]. *L. paracasei* DSMZ16671 was used in a pilot study under the form of sugar-free candies, showing that their consumption reduces up to 86.6 % (±0.1) of *S. mutans* in human saliva [32]. Administration of *L. paracasei* in foods, oral hygiene, or oral treatment medicine can inhibit or reduce the number of pathogens of dental caries (*S. mutans*, *S. sobrinus*) and periodontal diseases (*P. gingivalis*, *P. intermedia*), in oral cavity of the user [33].

The inhibition assays in this study were performed only with the microorganism and not with the active substance, (reuterin). Therefore, we may expect the possibility of an increased antimicrobial effect against these dental plaque bacteria with the use of the active substance alone, and this possibility is currently on testing in our laboratory. Even the difference in sensitivity expressed by *S. mutans*, *S. gordonii,* and *T. forsythia* to the action of *L. reuteri* may represent a promising and interesting biological and natural alternative in the treatment and/or prevention of oral diseases. Thus, continuous study and testing of its antimicrobial effect at different concentrations and recognition of its activity spectrum represent an area for active and ongoing research. A major target is *S. gordonii*, a bacteria considered an initial colonizer in the oral cavity and responsible for the establishment of other microorganisms with more pathogenic potential. To date, there is little information on the mechanism of action of *L. reuteri* on other pathogenic microorganisms, but it appears that reuterin acts on thiol groups of proteins, causing oxidative stress [34]. Based on our results, we suggest an extended study of *L. reuteri* for a deeper knowledge on its antibacterial properties, to allow its proposal as a supplement in the agri-food area, as a probiotic contributing to maintain a good oral health and avoid caries or periodontal infections.

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Conflict of interest M. L. Baca-Castañón, M. A. De la Garza-Ramos, A. G. Alcázar-Pizaña, Y. Grondin, A. Coronado-Mendoza, R. I. Sánchez-Najera, E. Cárdenas-Estrada, C. E. Medina-De la Garza and E. Escamilla-García declare that they have no conflict of interests.

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