IMPACT OF ATMOSPHERIC PH AND INCUBATION TIME ON IN VITRO BIOFILMS OF LIMOSILACTOBACILLUS REUTERI AND LACTICASEIBACILLUS RHAMNOSUS

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SUMMARY:

Participation of the microbiome in the evolution of chronic infectious and non-infectious processes has been described by numerous authors. One of the ways to modulate it is through the use of probiotics (PB). It is important that these PB have the ability to form biofilm (BP) in different mucous membranes of the body. The microorganisms most frequently used as probiotics are Lactobacillus spp and related microorganisms. Lactobacilli are strictly aerotolerant or anaerobic gram-positive bacilli that are part of the human gastrointestinal and genitourinary microbiota. There are studies that show that probiotic properties are closely linked to the species and strain 1 . There are many probiotics with proven efficiency in the gastrointestinal tract but the same does not occur with probiotics at the urogenital level. In addition to the characteristics that are always listed such as the production of hydrogen peroxide, lactic acid, hemolytic activity, presence of resistance genes capable of being transferred [2,3,4]
It is necessary to establish characteristics in relation to the habitat such as tolerance to different pH, presence of oxygen and capacity to form biofilms (BP) that ensure their persistence over time. It must be taken into account that these parameters are very different at the intestinal level vs. the vaginal level. Therefore, it cannot be assumed a priori that the probiotics used to modulate the microbiota of a mucosa are the same to do so in another different mucosa.

OBJECTIVE:

To analyze the impact of pH, atmosphere, and incubation time on biofilms (BP) of Limosilactobacillus reuteri M0733(LRE) and Lacticaseibacillus rhamnosus 104410(LRH) .

MATERIALS AND METHODS:

Strains: LRE and LRH . Culture media . Two culture media were used (Man Rogosa Sharpe broth and agar -CMRS/AMRS- and tomato juice broth and agar -"made in house" CJT/AJT-).
Biofilms: Both liquid and solid culture media were used. They were developed from both strains on glass devices (GDD) that were introduced into 6 CMRS and 6 CJT. They were incubated at 36.5°C in aerobiosis and anaerobiosis for 15 days.
BP reading: each GDD was extracted at 24, 48, 72, 96 hours and after 15 days, they were colored with 0.5% crystal violet. In each reading there
was a control without GDD. They were observed with an optical microscope (1000x) and were interpreted according to a score previously established by us (0, absence of BP, up to 5, complete BP) [5] . From the broths where the BP were developed, subcultures were carried out on solid media to check the purity of the isolates.

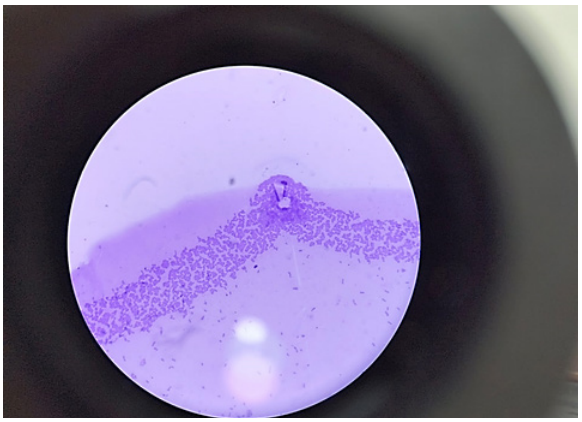
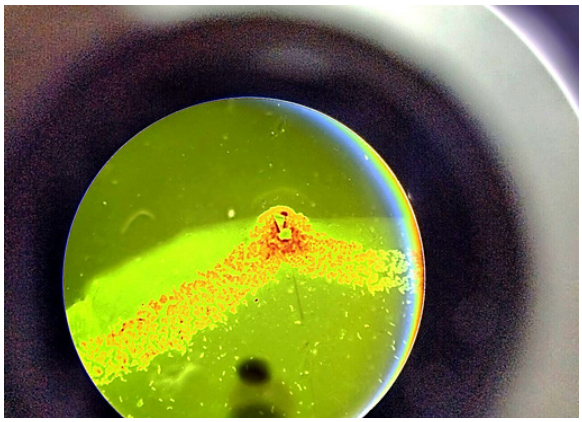
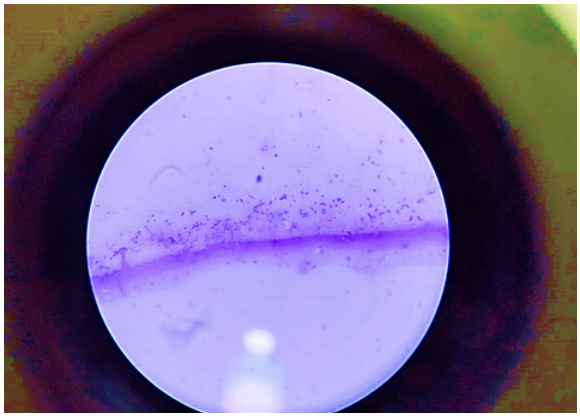
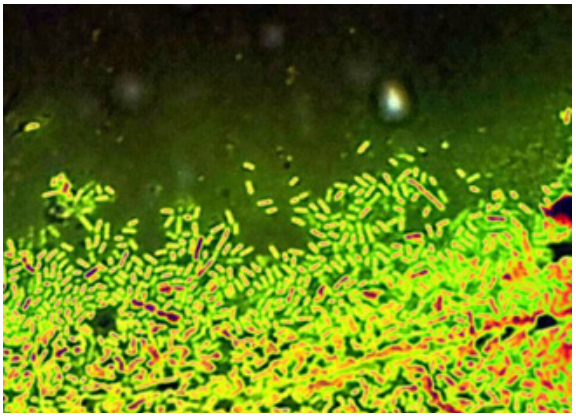
RESULTS:

Both strains formed BP in the media studied at pH 5.5 and pH 8.0, in aero and anaerobiosis, as observed in Figures 1, 2, 3 and 4.

CONCLUSIONS:

Although this is a preliminary work, we highlight that *L.*

reuteri and *L. rhamnosus* seem to be suitable as probiotics for the modulation of the intestinal and vaginal microbiota, due to their ability to form BP at different pH and atmospheres.

Limosilactobacillus reuteri: BP en CJT a las 24-48 horas en aerobiosis (cristal violeta)	*Limosilactobacillus reuteri:* BP en CJT a las 24-48 horas en aerobiosis . Se observa claramente a presencia de exopolímero (Efecto solarización)
	
Lacticaseibacillus rhamnosus en CJT a las 24-48 horas (cristal violeta)	*Lacticaseibacillus rhamnosus* en CJT a las 96 horas con efecto solarización
	

FIGURES 1, 2, 3 AND 4. BP OF L. REUTERI AND L. RHAMNOSUS IN CJT STAINED WITH CRYSTAL VIOLET AND WITH SOLARIZATION EFFECT.

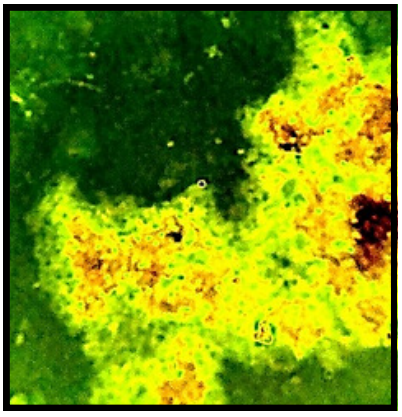


FIGURE 5. EXOPOLYMER IN A BP OF L. REUTERI AT 96 HOURS. THE ARROW INDICATES THE EXOPOLYMER.