EVALUACIÓN in vitro DE LA ACCIÓN DE *Lactobacillus plantarum* CON CARACTERÍSTICAS PROBIÓTICAS SOBRE *Yersinia pseudotuberculosis*

EVALUATION in vitro OF THE ACTION OF *Lactobacillus plantarum* WITH PROBIOTIC CHARACTERISTICS ON *Yersinia pseudotuberculosis*

AVALIAÇÃO in vitro DA AÇÃO DE *Lactobacillus plantarum* COM CARACTERÍSTICAS PROBIÓTICAS SOB *Yersinia pseudotuberculosis*

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RESUMEN

Con el objetivo comprobar el potencial probiótico de Lactobacillus plantarum sobre una de las enfermedades más frecuente en cuyes (Cavia porcellus) causada por Yersinia pseudotuberculosis, se efectuaron pruebas de inhibición y comparación frente a antibióticos, mostrando resultados favorables, al inhibir a la bacteria patógena. Se valoró por HPLC los posibles péptidos producidos por L. plantarum, evidenciándose la presencia de dos péptidos. Se realizaron pruebas de selección de la bacteria láctica la cual mostró el siguiente perfil: catalasa negativa, no productora de gas, resistente a sales biliares (0,5%, 1%, 2% y 3%), pH (2,5, 3,5 y 7,6)

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y temperatura de 38 a 45°C. Además, se determinó las características de la cinética de fermentación, utilizando dos medios, donde se evaluó UFC/mL, pH, consumo de azúcares totales (mg/L) y producción de ácido láctico (%); Lactobacillus plantarum alcanzó la fase exponencial de crecimiento en los medios MRS y Pro, a las 12 y 14:24 horas, con valores de 7,0x10[11] UFC/mL y 5,0x1013 UFC/mL, respectivamente. Se aplicó un diseño de bloqueas al azar con dos tratamientos y once bloques, el resultado permitió determinar que no existe diferencias estadísticas significativas (P>0,05) entre los medios propuestos, pero si entre las horas de evaluación (P<0,05).

ABSTRACT

With the objective to check the potential probiotic of Lactobacillus plantarum on one of themost frequent diseases in guinea pig (Cavia porcellus) caused by Yersinia pseudotuberculosis, tests were conducted of inhibition and comparisonin front of antibiotics, showing favorable results, the inhibitt othe pathogenic bacterium. It valued by HPLC the possible peptides produced by L. plantarum, characterizing an presence of two peptides. Tests were performed for the selection of lactic bacteria which showed the following profile: catalase-negative, not producer of gas, resistant to bile salts (0, 5%, 1%, 2% and 3%), pH (2,5, 3,5 and 7,6) and temperature of 38 at 45°C. Also determined the characteristics of the kinetics of fermentation, using two mediums, where it is evaluated CFU/mL, pH, consumption of total sugars (mg/L) and production of lactic acid (%); Lactobacillus plantarum reached the exponential phase of growth in the mediums MRS and Pro, at the 12 and 14:24 hours, with values of 7,0x10[11] UFC/mL and 5,0x10[13] UFC/mL, respectively. A design of blocks was applied at random with two treatments and eleven blocks, the result allowed to determine that it doesn't exist significant statistical differences (P>0,05) between the proposed means, but if between the hours of evaluation (P <0,05).

RESUMO

Com o objetivo comprovar o potential probiótico de Lactobacillus plantarumsobre uma das doenças mais freqüentes em cuyes (Cavia porcellus) causada por Yersinia pseudotuberculosis, efectuaram-se testes de inibição e comparação em frente a antibióticos, mostrando resultados favoráveis, ao inhibir à bactéria patogénica. Valorizou-se por HPLC os possíveis péptidos produzidos por L. plantarum, evidenciándose a presença de duas péptidos. Realizaram-se testes de seleção dabactérias láctica a qual mostrou o seguinte perfil: catalase negativo, não produtor de gás, resistente a sais biliares (0,5%, 1%, 2% e 3%), pH (2,5, 3,5 e 7,6) e temperatura de 38 a 45°C. Além disso, determinaram-se as características da cinética de fermentação, utilizando dois meios, onde se avaliou UFC/mL, pH, consumo de açúcares totais (mg/L) e produção de ácido láctico (%); Lactobacillus plantarum alcançaram a fase exponencial de crescimento nos meios MRS e Pro, às 12:00 e 14:24 horas, com valores de 7,0x10[11] UFC/mL e 5,0x10[13] UFC/mL, respectivamente. Aplicou-se um desenho de blocos ao acaso com dois tratamentos e onze blocos, o resultado permitiu determinar

PALABRAS CLAVE:
Probióticos, Bacteria patógena, Inhibición, Bacteria láctica, Cinética de fermentación.

KEYWORDS:
Probiotics, Bacteria pathogen, Inhibition, Lactic bacteria, Kinetics of fermentation.

PALAVRAS-CHAVE:
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que não exista diferenças estatísticas significativas (P>0,05) entre os meios propostos, mas se entre as horas de avaliação (P <0,05).

INTRODUCTION

At present, the use of probiotics takes importance to be considered as the cultivation of one or more living microorganisms, that to be supplied in the diet have an influence on the microflora already established, producing a positive effect very marked on the health and development of the host [1]. The benefits of the probióticos are multiple, between them is outlined the production of antibacterial substances that improve the defense against the invasion of other microorganisms; and they help to populate the intestinal flora that can turn altered by the administration of antibiotics [2], being considered therefore with therapeutic effects or preventive in the guest [3].

The use of bacteria of the kind *Lactobacillus* specially the species *L. plantarum*, has been used widely *in vivo* and *in vitro,* demonstrating his antimicrobial capacity against different bacteria gram positive and gram negative like *Listeria monocytogenes, Staphylococcus aureus, Salmonella tiphymurium, Escherichia coli, Klebsiella pneumoniae*, between others [4,5,6]; is capable of tolerating low pH and bile salts, has antagonistic activity against pathogenic intestinal such as the enterobacterias [7], and corresponds to the microorganisms GRAS, that is to say Generally Recognized As Safe [8]. These properties turn it into a promissory probiotic as food additive and therapeutic.

The main infectious agent within guinea pigs productions in the department of Nariño is *Yersinia pseudotuberculosis,* which causes mortalities between 7 and 60% of the population and almost 20% morbidity, causing great economic losses [9]. In the actuality do not report neither is offered by the biological commercial houses for the control of this illness [10], by which formulate medicines of other species without knowing his action or residual effects that can generate.

Therefore the objective of this research was to evaluate the action *in vitro* of *Lactobacillus plantarum* with probiotic characteristics on *Yersinia pseudotuberculosis*, as an alternative to control yersiniosis in guinea pigs.

METHOD

Study site

The present investigation realized in the Laboratory of Sciences Pecuarias of Zootecnia, section of Microbiology, of the University of Nariño, Pasto, Nariño, Colombia.

Microorganism's employees

Were used two strains of collection, the probiotic strain *Lactobacillus plantarum* ATCC® 8014 and pathogenic *Yersinia pseudotuberculosis* NCTC 8580. Both strains were reactivated in accordance to the instructions of the manufacturer.

Cultivation of the inoculum of *Lactobacillus plantarum*. Inoculum is a hoe of strain in an Erlenmeyer flask containing 40 mL of sterile commercial MRS broth, was incubated at 37°C/24 hours. Then there was a chime of 4 mL of the same to other 40 mL of MRS broth and was incubated in the above mentioned conditions. According to Crueger and Crueger [11], the percentage of inoculum must be adjusted to 10% v/v, to start the fermentation and one must calculate the number of bacteria per mL. When presented greater population of the established sterile broth was added taking into account the following mathematical calculation of proportionality according to Guerrero quoted by Montes *et al.* [12].

Is the value of X_1

M_1 ------M_2

M_2--------- X_1

$$X_1 = \frac{M_2 * V_1}{M_1}$$

It is then V_2

$V_2 = V_1 - X1$

Finally gets the value of X_2

V_1 ------V_2

V_3--------- X_2

$$X_2 = \frac{V_3 * V_2}{V_1}$$

M_1 = population or cell density that must be adjusted.

M_2 = 1,50 x 10^8 bact/mL. Density used for the first fermentation.

V_1 = 1 mL from the inoculum volume total to obtain the dilution 10^{-1}.

V_2 = what is added to 1 mL to adjust to 1,50 x 10^8 bact/mL.

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V_3= 100 mL total quantity of inoculums.X_2= amount of sterile commercial MRS broth that is added to V_3 for the population to adjust the value of M_2.

Inhibition tests and compared against pathogens to antibiotics

Test of *in vitro* inhibition of *Lactobacillus plantarum* against *Yersinia pseudotuberculosis*. The test was conducted using the technique of agar diffusion, was taken of agar discs impregnated with the lactic bacteria in different quantities and placed into the previously Mueller Hinton agar impregnated with the pathogenic bacteria; planted the petri dishes were incubated at 32°C/18 hours, after this time you proceeded to carry out the reading of halos formed. The critical size of the halo to determine if there is inhibition was considered to be equal or superior to 2 mm [13].

Inhibitory test for supernatants (bacteriocins). Was centrifuged 1,5 mL sample of the culture the lactic bacteria to 4°C/15 minutes and 1000 rpm, the supernatant was filtered in membrane of 0,45 μm. The bactericidal activity was assessed using two modified methods of diffusion: discs and plastic cylinders, on Mueller Hinton agar inoculated with the pathogenic strain. By the method with discs, was added 50 μl, 75 μl and 100 μl of the bacteriocin in discs of filter paper of 8mm diameter pre-sterilized for 15 minutes at 121°C and 15 psi. Later on the surface of the agar were placed the discs in the bacteriocin in study, the petri dishes were incubated at 35°C/18 hours. In the method with cylinders, used sterile tips cut from 8mm in diameter, which were placed in the petri dishes and on which is added the different amounts of the supernatant and carried to incubation time and described above. The inhibition was determined by the halo produced around sensidisco and cylinder; was measured distance from the end discs and the edge of cylinder halo.

Antimicrobial susceptibility testing of antibiotics against *Yersinia pseudotuberculosis* and *Lactobacillus plantarum.* Through the Kirby Bauer method was compared the inhibitory action of eight commercial antibiotics (Gentamicin Cn 10 μg, Penicillin GP 10 IU, Ciprofloxacin Cip 5 μg, Dicloxacillin Dcx 1 μg, Cefepime Fep 30 μg, Cephalotin Kf 30 μg, Enrofloxacin Enr 5 μg and Trimethoprim sulfamethoxasole Cot 25 μg) against the pathogen and the lactic bacteria. The inhibition was determined by the diameter of the halo occurred around the sensidiscos after 18 hours of growth in the middle where grew the pathogenic bacteria.

Identification of peptides from the supernatant by HPLC-DAD. The sample was prepared as follows: strain grown in MRS broth at 32°C/24 hours, then was adjusted spectrophotometric ally to an optical density of 0,125 at a 0,5 Mc Farland scale equivalent to a concentration of 1,5x 10^8 bacteria/mL; samples were transferred to eppendorf tubes and centrifuged to 4°C/30 minutes and 18.000 rpm; the supernatant was filtered using a 0,2 micron PVDF membrane and was read [14]. The analysis conditions were: Equipment: HPLC Waters Binary Pump 1525. Column: C18 300A. Detector: PDA 2998-214 and 280 nm, Scan (200-350nm). Injector: Rheodyne Loop of 20 μL. Composition: A (water: TFA 0.1%) B (water: acetonitrile: TFA, 0.1%).

Viability study versus concentration of bile salts. Using the method of Cai *et al* [15] and Cai *et al.* [16]. The strain was incubated in medium Pro during 48 hours testing concentrations of 0,5%, 1%, 2% and 3% of bile salts w/v, and was subsequently made the plate count.

Gas production. Following the methodology of Cai *et al.* [16], using MRS broth with 5% glucose and placing Durham tubes to verify the presence of gas.

Catalase activity. Using hydrogen peroxide over a colony of lactic bacteria [17].

Viability at different concentrations of pH. According to the methodology of Cai *et al.* [16], with pH 2,5, 3,5 and 7,6 .

Resistance to different temperature levels. According to the methodology of Cai *et al.* [15], at temperatures of 38 and 45°C.

Kinetics of fermentation

Two proposed culture media, commercial media MRS and Pro formulated from white sugar (10g/L), soy milk (15 g/L), milk powder (150 g/L) and wheat bran (15 g/L) [18]. The kinetics was performed in an Erlenmeyer flask with 600 mL of the medium and the probiotic bacteria set to an initial concentration of 1,50 x 10^8 bact/mL (540 mL of the medium and 60 mL of inoculum) carried to continuing unrest in incubator shaker at 100 rpm and 32°C.The variables evaluated during the kinetics were: production of biomass by counting of viable microorganisms in plate (CFU/mL), sugar consumption total by the anthrone method to 625 nm, determination of pH measured with pHmetro digital

and production of lactic acid by titration with sodium hydroxide 0,1 N. Sampling was carried out in duplicate from time zero and subsequently each 2:24 hours for 24 hours for a total of 11 times in order to determine the parameters of fermentation.

Experimental design

For counting of viable microorganisms in plaque CFU/mL obtained during fermentation kinetics adopted a randomized block design (DBA) given the number of data used, the means were considered as the two treatments and assessment hours corresponded to the blocks (11 times), the behavior was assessed from time 0 (zero hour), up to 11 time (24 hours), considered two replicas for each medium, and time.

The data obtained were analyzed S.A.S. Version 9.1.3 2007 and were subjected to analysis of variance ANOVA and Tukey's test of significance to compare changes that are statistically significant (P less than 0,05) [19].

Mathematical model for randomized block design

$$Y_{ij} = \mu + \alpha_i + \beta_j + \varepsilon_{ij}$$

Y_{ij} = score of subject *i* under the experimental condition or treatment *j.*

μ = the overall mean of all the experimental data.

α_i = effect of treatment i that is common to all individuals receiving this treatment.

β_j = effect of block j that is common to all treatments that were applied in that block.

ε_{ij} = the residual or random error (within).

To analyze pH, total sugar consumption (mg/L) % lactic acid and result of kinetic analysis was applied to each variable regression compared to CFU / mL formed. The mathematical model used was as follows:

$y = \beta_0 + \beta_1 x + \varepsilon$,

y = Dependent variable .

β_0 = Intercepted the origin.

β_1 = Slope of the line.

x = Independent variable.

ε = Error.

RESULTS

In testing the inhibitory action, *L. plantarum* presented inhibition against *Y. pseudotuberculosis,* the form of halos 2 and 4 mm to a quantity of 25 μl, 100 μl and 50 μl respectively (figure 1). Measures of greater magnitude (>8 mm) were reported using BAL against to *Yersinia enterocolitica* [20]. While other authors using *L. casei,* reported equal ranges for the inhibition against *Y. pseudotuberculosis* [21].

The employment of the supernatant using the two proposed methods revealed that a quantity of 75 μl and 100 μl if inhibition was presented in front of *Y. pseudotuberculosis*, the form of halos 2 and 3 mm, respectively these antibiotics, since these will inhibit its antimicrobial effect (figure 2).

These results indicate that very possibly this action is due to the bacteriocins [22]. However, must not be overlooked that there are several types of compounds that produce lactic acid bacteria and that exert inhibitory action [23], it is recommended to carry out tests of molecular identification to check what types of substances are acting.

Test the antimicrobial susceptibility of antibiotics against pathogenic bacteria (table 1), indicates that *Y.*

Figure 1. Inhibitory action of *Lactobacillus plantarum* against *Yersinia pseudotuberculosis.*

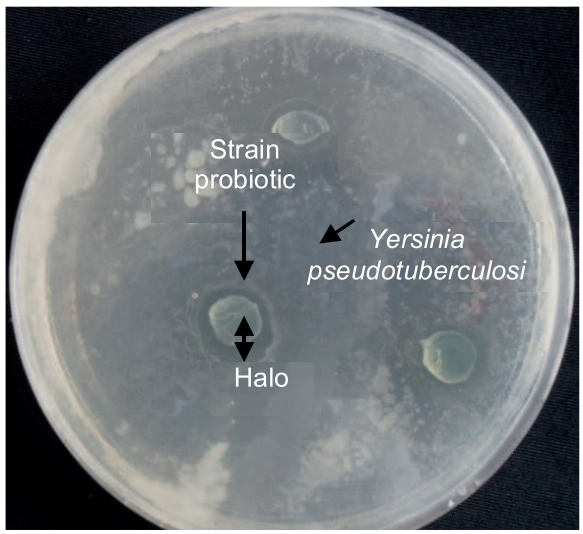

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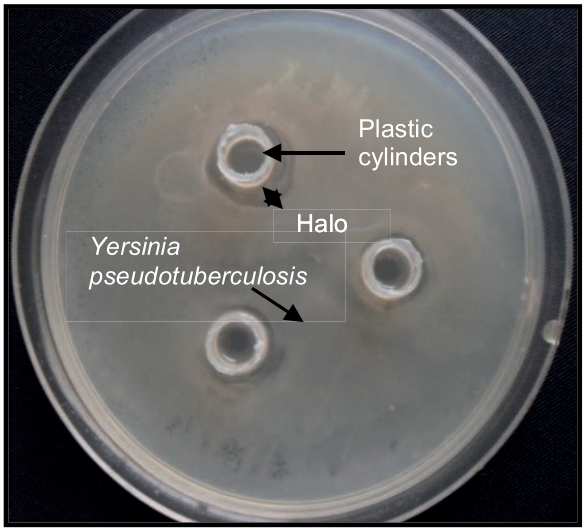
Figure 2. Inhibition halos of supernatant with plastic cylinders of *L. plantarum* against *Y. pseudotuberculosis.*



pseudotuberculosis showed resistance to Kf 30 μg, Fep 30 μg, Dcx 1 μg and G P 10 IU; sensitivity to Cip 5 μg, CN 10 μg, Enr 5 μg and Cot 25μg, being the last two antibiotics most commonly used by the producers of guinea pigs as curative and preventive measure against yersiniosis, although they are not drugs themselves for the species cuyícola. For its part, the lactic bacteria revealed only resistance to G P 10 IU, and

Table 1. Testing of antibiotics against *Y pseudotuberculosis* and *Lactobacillus plantarum.*

	Diameter of inhibition zone in mm			
Antibiótics	**Y. p NCTC 8580**	**S.I**	**L. p NTCC 8014**	**S.I**
KF 30 μg	12	- [24]	51	+[24]
FEP 30 μg	11	- [24, 25]	35	+ [24]
CIP 5 μg	30	+ [24, 25]	33	+[24, 25]
DCX 1 μg	9	- [24]	29	-[25]
Enr 5 μg	30	+ [24]	34	+[24]
CN 10 μg	23	+ [24, 25]	28	+[24, 25]
G P 10 IU	0	- [24]	24	-[25]
COT25μg	17	+ [24, 25]	30	+[24, 25]

Y.p: *Yersinia pseudotuberculosis,* ***L.p***: *Lactobacillus plantarum ATCC8014,* **S.I** Sensitivity level (+ Sensitive –Resistant)

sensitivity to other antibiotics, these values suggest that *L. plantarum* could not.

According to the UV spectra taken at 214 and 280 nm the sample revealed the presence of peptides (figure 3), which according to characteristics of chromatographic separation by RP-HPLC of the peptides are hydrophilic character. In the sample, the peak No 9 (tr = 12,1 min), has a similar time to the peak No. 2 retaining calibration standard, therefore possibly the peptide of the present sample an amino acid chain comprising TIR-VAL-VAL (MW = 379,5). The UV spectra show similar absorption maximum lengths. The No. 3 standard peak, which corresponds to peptide methionine enkephalin acetate composition (TIR-GLI-GLI-FA-MET) M6638 (MW = 573,7 for free base) has a similar retention time to peak sample 12. The maximum absorbance of the spectra show very close lengths absorption maximum (figure 4).

The result of the test of gas production to *L. plantarum* was negative, and this is a favorable property that must comply with the lactic acid bacteria used in animal feed, in order to prevent the animal manifest problems of bloat and in severe cases the death of the same.

The strain probiotic manifested a negative catalase activity by the absence of gas, general feature of the genus *Lactobacillus,* since species composition is strict or facultative anaerobic lacking this enzyme [6].

In the test at different pH values of 2,5, 3,5 and 7,6 is a high viability at time t0 (time 1), T1 (time 2) and T2 (time 3), introducing similar results of $3{,}0x10^{12}$, $5{,}1x10^{8}$ y $1{,}2x10^{11}$ UFC/mL, indicating that if the lactic bacteria is fed to guinea pigs, up to three hours, maintain a high viability, exercising its probiotic effects against the pathogenic bacteria. Also, the values found are of great importance, in so far as they enable know if strain probiotics to use is capable of surviving a pH values slightly acidic and basic, such as that found in the duodenum of the guinea pig, which reaches a value of 7,6.

L. plantarum showed that different concentrations of bile salts do not have a significant impact for its growth, noting that at concentrations of 2 and 3% in dilutions of 10^{9} and 10^{10} were found counts of $1{,}1x10^{11}$, $1{,}62x10^{12}$ and $2{,}26x10^{11}$ and $1{,}19\ x10^{12}$ CFU/mL. This is an important characteristic, because the resistance to bile salts is a critical condition inside the evaluation of stumps probiotics [26].

Figure 3. Peptides representing different wavelengths (280 nm black, blue 214 nm).

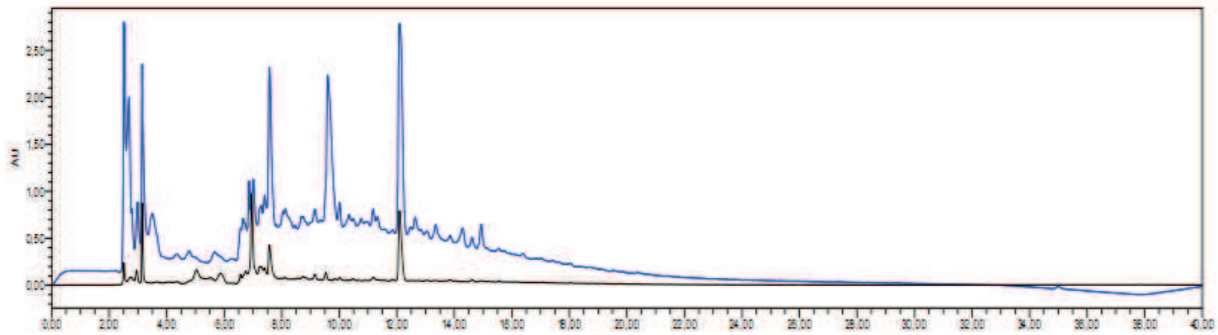
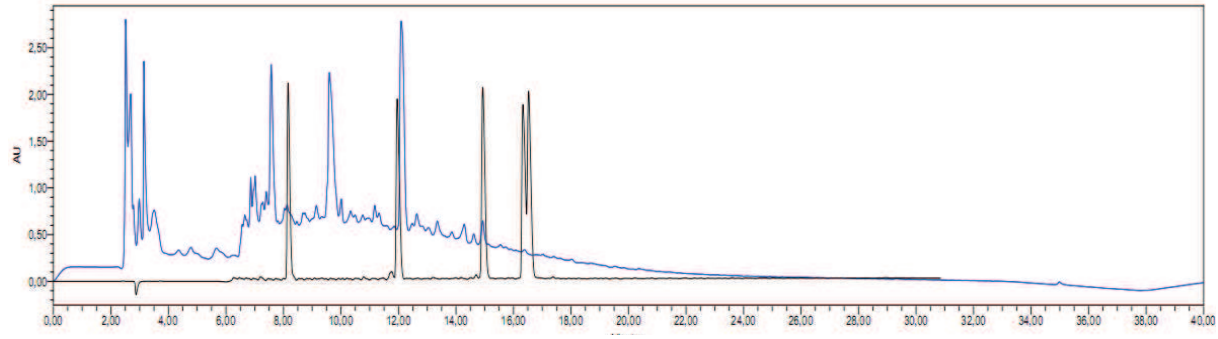


Figure 5. Chromatogram of the sample at 214 nm (blue) and the calibration standard peptide (black)



In the test of temperature to 38°C the lactic bacteria indicated a growth of 1,25x10^{8}, 1,0x10^{8}, 1,4x10^{9}, 3,4x10^{10} and 8,6x10^{11} CFU/mL. At 45°C there was a growth of 7,2x107, 1,6x10^{8}, 4x10^{8}, 1,48x10^{11} and 3x10^{10} CFU/mL. It can be inferred that in addition to the optimum temperature for growth (32°C) *L. plantarum* can be developed at higher temperatures as to 38 and 45°C.

Kinetics of fermentation

In figure 5 shows that *L. plantarum* in the media MRS and Pro reaches the exponential phase of growth, at 12 and 2:24 hours of kinetics with a values 7x10^{11} CFU/mL and 5x10^{13} CFU/mL, respectively. The values obtained coincide with those of other authors where *L. plantarum* obtained the maximum growth at 12 hours with values of 3,0x10^{12} CFU/mL and 2,0x10^{12} CFU/mL and 9,6x10^{11} CFU/mL and 8,2x10^{11} CFU/mL [18]. The statistical analysis reported no significant differences between the treatments ($P>0,05$) but if between the blocks ($P<0,05$) the above enables us to conclude that the only effect that produces a significant change in the CFU/mL are the hours of evaluation more not the effect that produces the environment where it is cultivated the lactic bacteria. The evolution of the pH in the MRS medium and Pro submitted values of 6,87 and 6,78 respectively at 12 and 2:24 hours initiated the kinetics (figure 6). Values of 6,41, 6,13 is reported for *L. plantarum* isolated from feces of children, in medium with milk [6]. It should be noted that although most generally develop *Lactobacillus* pH below 5, there are some that can grow LAB from pH 3,2 to 9,6 [27]. Regression analysis showed values of B_1, -0,63 in the MRS medium and B_1, -0,14 in the Pro medium, which means that for every unit of CFU/mL that increases, the pH decreases in -0,36 and -0,14 units of pH in each medium.

The total sugar for *L. plantarum* in MRS medium and Pro (figure 7) presented values of 13,76 mg/L and 37,66 mg/L respectively at 12 and 2:24 hours made the kinetics. The evidence as *L. plantarum* more efficiently utilizes the substrate in the medium Pro, consumption reflected in higher biomass production at the end of the exponential growth phase. These results may be due to the sufficient concentration of nutrients from the raw materials used in the preparation of the probiotic medium. The regression analysis showed values of B_1 -0,56 for the MRS medium and B_1 -1,16 for

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Figure 6. CFU/mL of the probiotic strain grown in the media MRS and Pro for 24 hours.

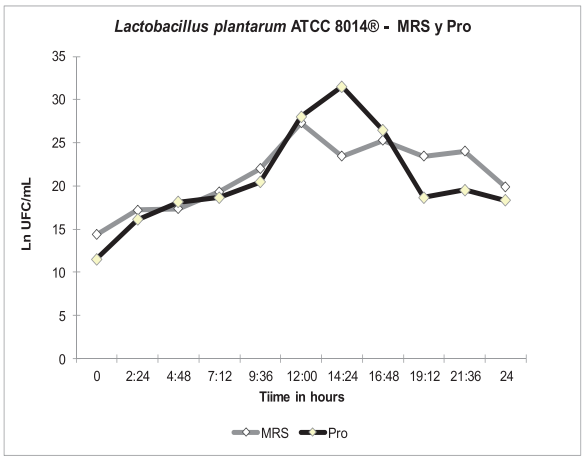
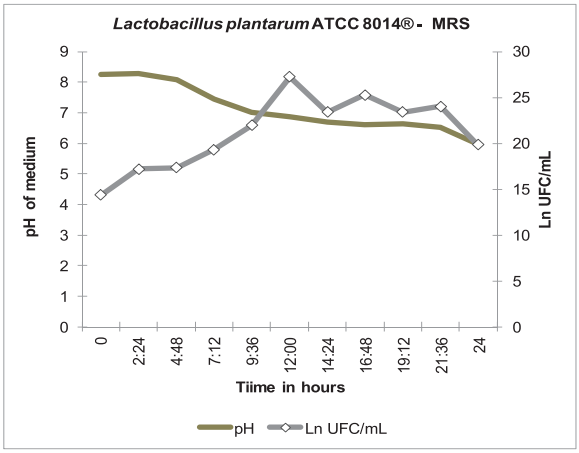


Figure 7. pH of MRS and Pro medium and CFU/mL of the probiotic strain cultivated for 24 hours.



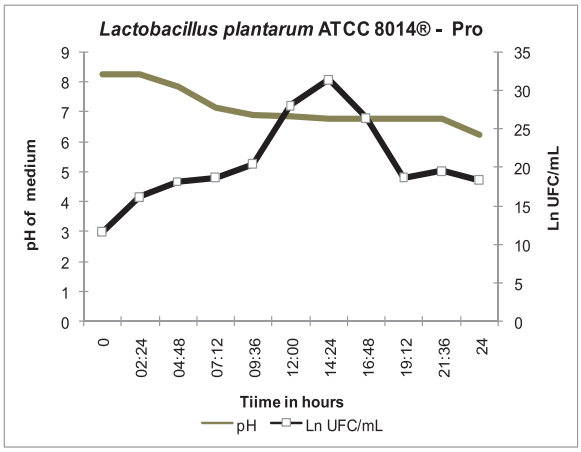
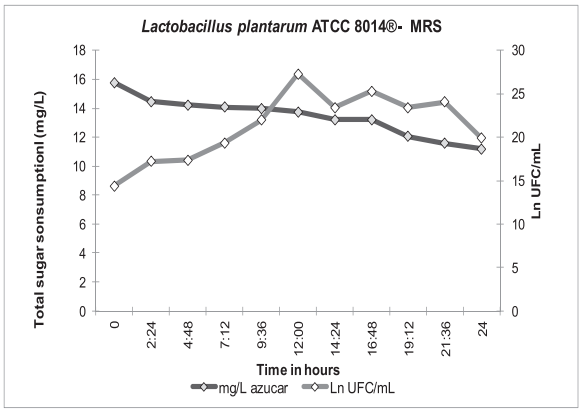
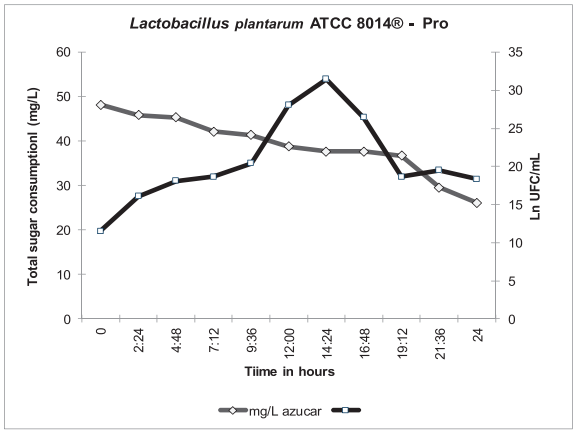


Figure 8. Total sugar consumption (mg/L) and growth CFU/mL medium MRS and Pro.



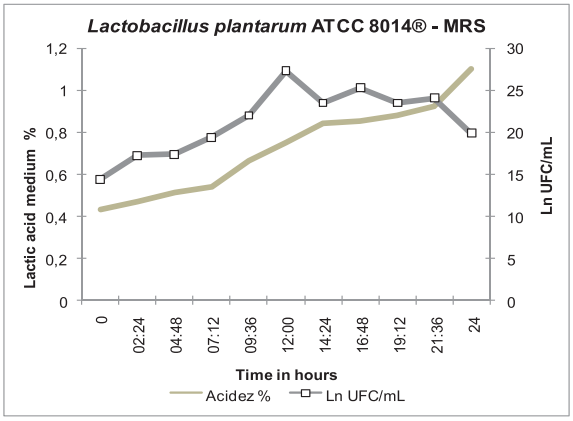


the medium Pro, which indicates that on average 0,56 mg/L and 1,16 mg/L consumed by each increase of one unit of CFU/mL in each medium.

The values obtained for the production of lactic acid in the MRS medium and Pro at 12 and 2:24 hours presented a value of 0,75% and 0,7% respectively (figure 8). In this way, the production of lactic acid by *L. plantarum* is an important factor, taking into account that its production decreases the pH creating unfavorable conditions for the growth of the pathogenic bacteria, which has the capacity to grow in ranges from 4 to 10 [28,29]. The regression analysis indicated that the percentage of lactic acid is increased more in the MRS medium that has a value of 0,086 %, for every increase in a unit of CFU/mL, compared with the medium Pro that for every increase in a unit of CFU/mL, the acidity increases by 0,037 %.

According to the data obtained during the kinetics (table 2), it was found that both media (MRS and Pro) are found to be suitable for the development of seeds, to

Figure 9. Acidity (%) of MRS and Pro medium and CFU/mL of strain probiotic cultivated for 24 hours.



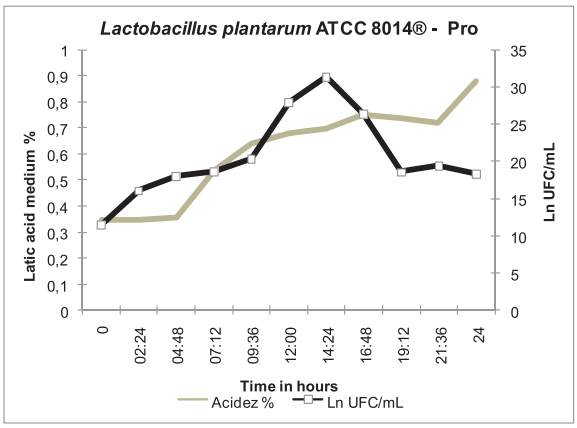


Table 2. Kinetic data of bacterial growth of *Lactobacillus plantarum* in two culture media.

Lactobacillus plantarum	Medium	
	MRS	Pro
Phase latency (lat)	0	0
Specific growth rate (μ h^{-1})	2,3079	3,073
End log phase(h)	12:00	14:24
Doubling time (min)	18,02	13,53
Increase cel. Total	4E+08	9E+07
Increase cel. End log phase	7E+11	5E+13
% Total sugars consumed	29,19	45,86
% Sugars consumed so log phase	16,33	21,88
R2	0,907	0,923

achieve a specific growth rate of 2,307 and 3,073 μ h^{-1}, values that favor a proper training of biomass at the end of the logarithmic phase. Although it should be noted that the medium Pro offers greater advantages with regard to MRS medium, highlighting a lower cellular duplication time which is reflected in having reached a higher formation of CFU/mL in the logarithmic phase, as well as production costs that are much more economical in the east that the commercial Pro.

CONCLUSIONS

The results of the tests of inhibition propose to *L. plantarum* as a strain with great potential probiotic, capable of acting against *Yersinia pseudotuberculosis,* and propose as a valuable alternative and safe in the replacement or supplementation of the antibiotic action in future investigations *in vivo*. Likewise the viability assays compared to bile salt concentration and pH, gas production, the catalase activity and resistance to different levels of temperature, corroborated his potential probiotic. The determination of the characteristics of the kinetic (pH, acidity, sugar consumption, growth medium, time), made it possible to verify and optimize the best conditions for the growth of probiotic bacteria, results that will ensure the increased production of biomass to be used in the elaboration of inoculants probiotics.

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