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Optimization of Culture Conditions for The Probiotic Bacterium *Pediococcus Pentosaceus* using a Factorial Design

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ABSTRACT

Pediococcus pentosaceus is a probiotic-bacterium able to confer a health benefit on the host because this bacterium can prevent the pathogen adhesion to intestinal cells as well as could tolerate bile salts, gastric and intestinal conditions. In particular, there are evidence that P. pentosaceus attenuates the severity of colitis and improve the intestinal permeability. By this reason is important stablishing the adequate conditions for the growth of probiotic-bacterium P. pentosaceus. The aim of the present study was evaluated the effect of temperature in shaken flasks, as well as the effect of pH and stirring speed on the growth and cell viability of P. pentosaceus using a 2^2 factorial design. We found that in the range of 34 to 40°C, the temperature affected the specific growth rate, biomass production and cell viability of probiotic-bacterium P. pentosaceus, being 37°C the optimum temperature condition for the culture of the bacterium. In bioreactor cultures, we observed that the stirring speed and pH not affected the biomass production and cell viability. The specific growth rate only was affected by the pH and we obtained the equation: $\mu = 0.95 + 0.064$ pH with an R-squared value of 0.964. These findings allow establishing the conditions of suitable cultures to advantage the growth of probiotic-bacterium P. pentosaceus increasing biomass production and their cell viability.

Keywords

Probiotic, Optimization, *Pediococcus pentosaceus*, Cell viability.

Introduction

Probiotics are defined as live microorganism which, when administered in adequate amounts, confer a health benefit on the host [1]. *Pediococcus spp.* are lactic acid bacteria (LAB) commonly found in the mammalian gut microbiota, and some strains are classified as probiotic [2,3]. In particular, *Pediococcus pentosaceus* is a probiotic-bacterium able to synthetized compounds as 3-phenyllactic acid and bacteriocins [4]. By this reason, the application of this bacterial group in the pharmaceutical and food industries has been increasing given that bacteriocin synthesis, resulting in the protection of fermentation products against spoilage and/or pathogenic bacteria. Another characteristic of *P. pentosaceus* is that this bacterium can prevent the pathogen adhesion to intestinal cells as well as could tolerate bile salts, gastric and intestinal conditions [5].

Emerging evidence suggests that the gut microbiome can influence the core symptoms of neuropsychiatric disorders, such as stress, depression and anxiety, giving rise to the concept of 'psychobiotics', which are defined as probiotics that, when ingested in appropriate quantities, confer mental health benefits [6-8]. In particular, there are evidence that *P. pentosaceus* attenuates the severity of colitis and improve the intestinal permeability [9]. When LAB bacteria are grown in liquid cultures, diverse factors as composition of culture medium, temperature, pH, aeration are important to obtained maximum biomass concentration in a minor culture time and high viability cell [10-12].

When attempting to discover the important factors and then optimize a response by tuning these factors, experimental design gives a powerful suite of statistical methodology. Experimental design and response surface methodology are useful tools for studying the effects of variables on parameters of interest with the advantage of minimal experimental runs [13]. By this reason is important stablishing the adequate conditions for the growth of probiotic-bacterium *P. pentosaceus*. The aim of the present study was evaluated the effect of temperature, pH and stirring speed on the growth of the probiotic-bacterium *P. pentosaceus* with the objective to obtained a maximum biomass production with and

cell viability using a central composite design 2^2.

Materials and Methods

Microorganism and culture medium

Pediococcus pentosaceus was isolated from breastmilk and identified by the 16s RNA method. It was cryopreserved at -70ºC in a 30 % glycerol solution. The probiotic bacterium was cultured in MRS medium (Difco) with the following composition in g L-1: Proteose Peptone No. 3 (10.0); Beef Extract (10.0); Yeast Extract (5.0); Dextrose (20.0), Polysorbate 80 (1.0); Ammonium Citrate (2.0); Sodium Acetate (5.0); Magnesium Sulfate (0.1); Manganese Sulfate (0.05); Dipotassium Phosphate (2.0). 55 g of MRS Broth was weighted and dissolved in 1.0 liter (L) of distilled water and after the solution was sterilized at 121ºC during 15 minutes.

Culture conditions

Flasks cultures

P. pentosaceus was cultivated under anaerobic conditions in 250 mL Erlenmeyer flasks, with a filling volume of 100 mL MRS medium at 100 rpm in a shaking incubator (LabTech). In these cultures, the effect of temperature was evaluated in the range from 34 to 40ºC. The bacterial growth was measurement by optical density at a wavelength of 620 nm and was correlated with a patron curve for determinate biomass concentration in g L^{-1}. The samples were taken each hour in the cultures and the glucose and lactate concentration were measured in YSI equipment (YSI 2900 Series Biochemistry Analyzers).

Bioreactor cultures

P. pentosaceus cultures were grown in an Applikon bioreactor containing 2 L of MRS medium at 37 ± 0.5°C, with variable stirring speed from 100 to 300 rpm. The bioreactor was equipped with two impellers (Di/T = 1/3), where Di was the impeller diameter, and T the bioreactor diameter. The cultures were done under fully anaerobic conditions and to guarantee these conditions in the bioreactor, a constant flow of nitrogen of 10 vvm was injected by the head of the bioreactor during all culture time. The pH was measured with an Ingold probe (Applikon, ADI 1010) and controlled at 5.5, 6.25, and 7.0 ± 0.1 by an on/off system using a peristaltic pump and adding 4 N NaOH or 20 % (v/v) H_3PO_4 solutions. According to the central composite design 2^2 used in this study, each experimental condition was evaluated by duplicate and the results presented are the average of the independent runs.

Evaluation of the effect of pH and stirring speed through a central composite design 2^2

The effect of the two factors (pH and stirring speed) on specific growth rate (μ), biomass production and cell viability was analyzed using a central composite design 2^2 (Table 1). The pH was studied at the level -1 (5.5), level +1 (7.0) and one central point (6.25), meanwhile that the stirring speed was evaluated at the level -1 (100), level +1 (300) and one central point (200) rpm. Each experimental condition was performed twice and differences between treatments were assessed through ANOVA analysis (p = 0.05), using the Design-Expert software version 8.0 (Stat-Ease, Inc., Minneapolis, MN).

+	100 7.0		300 7.0
pH		200 6.25	
-	100 5.5		300 5.5
	-	**Stirring speed (rpm)**	+

Table 1: Central composite design 2^2 that was used in this study in the which pH and stirring speed were the evaluated factors.

Analytical determinations: biomass concentration, optical density measurement and cell viability

The biomass concentration (X) was estimated using a correlation between the biomass concentration and optical density. The biomass concentration was measurement as cell dry weight (in grams per liter) and the optical density was measurement at a wavelength of 620 nm (Victor X3 multimode plate reader, Perkin Elmer). For this reason, a patron curve was made in the interval of optical density from 0.1 to 0.9 absorbance units and biomass concentration from 0.2 to 3.5 g/L. The equation obtained experimentally used to estimate the biomass concentration was X (g L^{-1}) = (Optical density – 0.1841)/ 0.1908.

The cell viability was measurement by plate counting using MRS-Agar Petri dishes. A serial dilution in the order since 10^6 to 10^9 was done and only the dishes where the colonies forming units were between 25 and 250 were considerate in this study. Later of plate the dishes were incubated at 37°C during 48 h at least and later, the colonies form units (CFU) were count.

Results

Effect of temperature on specific growth rate and biomass production in shaken flaks cultures

With the aim of evaluate the effect of temperature on specific growth rate cultures were carried out in 250 mL Erlenmeyer flasks with 100 mL of MRS medium at 34, 37 and 40ºC. In the temperature conditions of 34 and 40ºC, the maximum biomass concentration was 0.8 ± 0.015 g L^{-1}, meanwhile the specific growth rate was 0.73 ± 0.01 and 1.02 ± 0.03 h^{-1} at 34 and 40ºC, respectively (Figure 1). In the condition of 37ºC, a maximum biomass concentration of 0.86 ± 0.016 g L^{-1} was obtained and the specific growth rate was 0.95 ± 0.07 h^{-1}. These findings indicate that in the range of 34 to 40ºC the temperature had an effect on the growth of probiotic-bacterium *P. pentosaceus* in shake flask cultures, since the maximum concentration of biomass and the specific growth rate were different in all culture conditions evaluated. In summary, at 37ºC was possible to obtain a higher maximum biomass concentration compared with the obtained at 34 and 40ºC. On the contrary, the specific growth rate obtained at 37 and 40ºC was similar in both conditions.

Effect of temperature on the cell viability in shaken flaks cultures

The cell viability was measurement during the early stationary phase (8 h of cultivation time) in the experiments under different temperature conditions. When the bacterium was cultivated at 34°C, the cell viability was of 1.65 ± 0.12 x 10^9 CFU mL^{-1},

meanwhile for the temperature conditions of 37 and 40°C, there are not differences in the cell viability. The cell viability was 2.1 ± 0.42 x 10^9 CFU mL^{-1} and 2.7 ± 0.29 x 10^9 CFU mL^{-1} for the culture condition of 37 and 40°C, respectively. Interestingly, the cell viability was higher in the temperature conditions of 37 and 40°C compare with the cell viability obtained in the temperature condition of 34°C. Due that in the cultures development at 37°C, the biomass production obtained was maximum (0.86 ± 0.016 g L^{-1}) and because the cell viability was similar at 37 and 40°C, the temperature condition select for work in bioreactor was 37°C.

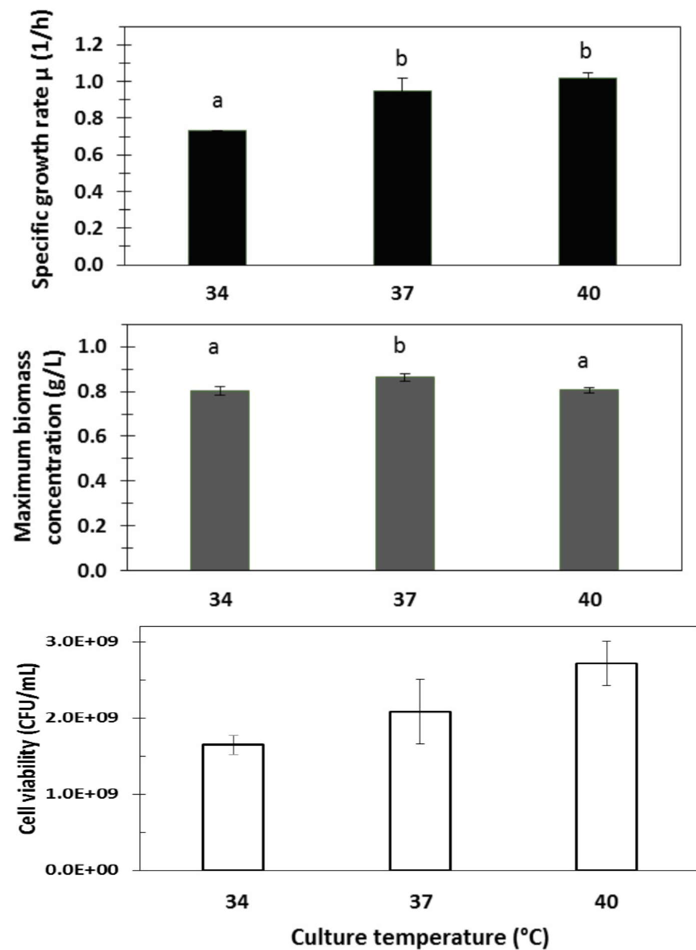

Figure 1: Specific growth rate (μ), maximum biomass concentration and cell viability obtained in cultures in 250 mL Erlenmeyer flask with 100 mL of MRS medium under anaerobic conditions at 34, 37 and 40ºC.

Glucose consumption and lactate production in shaken flasks cultures

In the cultures of shaken flasks under conditions of 37ºC the glucose was uptake fully later of 7 h of cultivation. This behavior corresponded with the exponential growth phase, and in this phase, the volumetric uptake glucose rate was 2.3 g glucose per liter per hour. The production of lactate was fully associated with bacterial growth. The maximum lactate concentration was 7.6 g L^{-1} obtained at 5 h of culture time and probably the bacterial growth was stop due at low pH in the medium. During the stationary phase the lactate concentration was remained constant and this behavior indicated that lactate was not produced later of 5 h of cultivation.

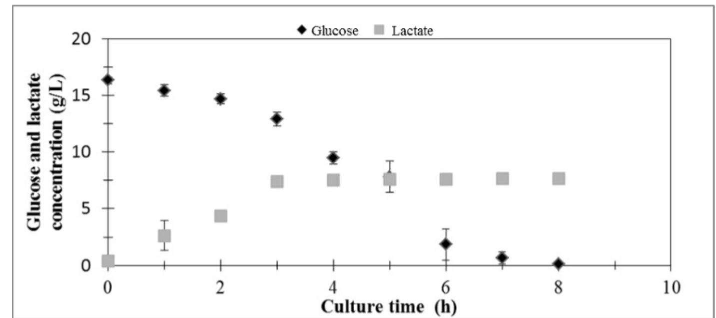

Figure 2: Glucose consumption and lactate production in cultures in 250 mL Erlenmeyer flask with 100 mL of MRS medium under anaerobic conditions at 37 ºC and 100 rpm.

Effect of pH and the stirring speed on biomass production and specific growth rate in bioreactor cultures

In bioreactor cultures, we evaluated the effect of pH and the stirring speed on biomass production and specific growth rate of *P. pentosaceus* using a central composite design 2^2. Two levels and one central point were evaluated in this study and for each condition evaluated two experiments were done. For all the conditions evaluated both pH as stirring speed, we found that the biomass concentration was between 2.8 and 3.0 g L^{-1}. Only for the condition of pH of 5.5 (level -1) and stirring speed of 300 rpm (level +1), the maximum biomass concentration of 3.4 ± 0.3 g L^{-1} was obtained. With respect to the specific growth rate (μ), this parameter was estimated of 1.0 ± 0.01 h^{-1} in the conditions of pH of 6.25 and 200 rpm (central point) and pH of 7.0 (level +1) and stirring speed of 100 rpm (level -1).

On the contrary, the μ was of 0.85 ± 0.03 h^{-1} when the bacterium was cultivated in conditions of low pH (5.5, level -1) independently of the stirring speed evaluated (100 and 300 rpm). This evidence indicated that the pH is the main factor that influence on the growth of *P. pentosaceus* when was evaluated in bioreactor cultures. With the experimental data obtained the following equation was proposed to correlate the pH and the specific growth rate with an Rsquared value of 0.964.

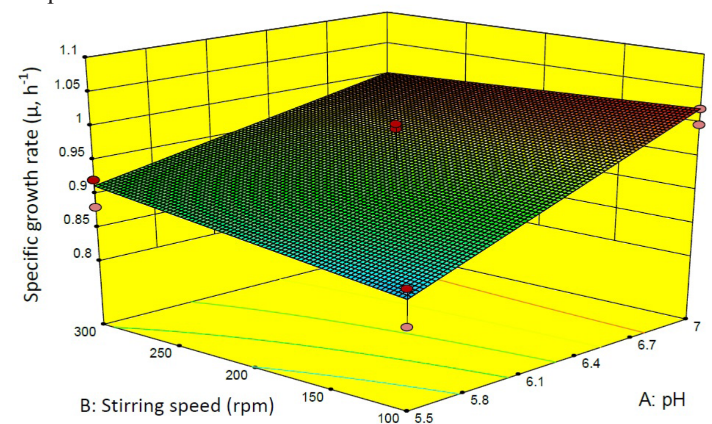

Figure 3: Surface response graphic generate with the experimental data obtained using a central composite design 2^2, where the factors evaluated were pH (factor 1) and stirring speed (factor 2).

Effect of pH and the stirring speed on cell viability in bioreactor cultures

In the bioreactor cultures, the cell viability was measurement at 6 h of culture time by plate counting. For all the conditions evaluates in this study, the cell viability measurement was similar, obtained values between 6.63 ± 0.97 x 10^8 CFU mL^{-1} and only for the condition of low pH (5.5, level -1) and high stirring speed (300 rpm, level +1) the cell viability increased to 1.13 ± 0.45 x 10^9 CFU mL^{-1}, however, in this experimental conditions the standard error was of 4.5 x 10^8 CFU mL^{-1}. For this reason, is necessary to perform more experimental work for confirmed this differences.

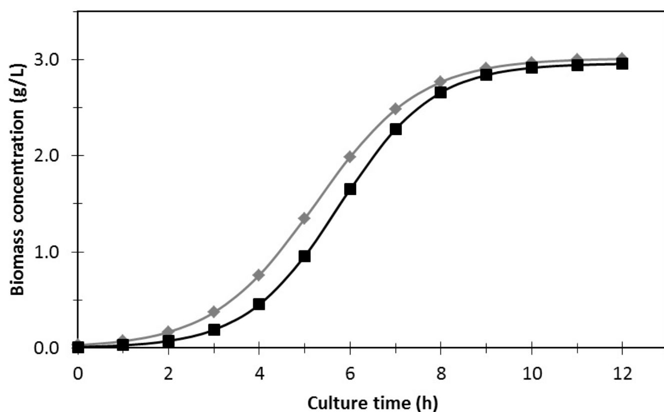

Figure 4: Kinetics of biomass production in bioreactor wit 2 L of MRS medium under conditions of controlled pH at 7.00, 100 rpm and 37ºC.

Discussion

P. pentosaceus is a probiotic bacteria with many to which effects such as inhibiting *Salmonella, Listeria monocytogenes* and *Clostridium difficile* growth, as well as anti-colitis activity have been attributed [14-16]; in addition, benefits have been attributed to different farm animals and possible uses for food preservation. Therefore, we consider that it has wide use as a probiotic in preserving the health of consumers.

In this study we evaluated the effect of temperature on specific growth rate, biomass production and cell viability in shaken flaks culture; as well as the effect of pH, stirring speed on biomass production, specific growth rate and cell viability in bioreactor cultures. The optimal condition of temperature for the growth of the probiotic-bacterium *P. pentosaceus* was in the interval of 37 to 40°C and this behavior is probably due that this strain was isolated from human breastmilk. Also, this result was similar to the previously reported for the strain *Lactobacillus plantarum* BC-25 in bioreactor cultures with 4.5 L of MRS medium [17]. At 37°C *P. pentosaceus* grown adequately because the biomass concentration and the specific growth rate were maximum and both lower and higher temperature the growth of the probiotic-bacterium decreased. This behavior is similar to observed in *L. plantarum* cultures where the optimal temperature condition for the growth of lactobacilli was 35ºC because the specific growth rate was maximum [18].

Other critic parameter for the growth of probiotic bacteria is the pH of the culture. Generally, lactic acid bacteria such as *P. pentosaceus* generate high amount of organic acids during growth. These acids are protonates and easily reenter the cells where it dissociates, which could alter the intracellular pH and in these conditions of low pH, *P. pentosaceus* would be in an environment of stress, causing a negative effect on the bacterial growth and for this reason a decrease of the specific growth rate was observed when the culture pH in the bioreactor was controlled at low values. In the case of aerobic bacteria cultures, the oxygen concentration is an important parameter that influences the growth bacterial. A way to manipulate the oxygen concentration and the mass and energy transfer in the culture system is through of the stirring speed. In the case of *P. pentosaceus* due that is a facultative anaerobic bacterium in this study were not observed effects of the stirring speed on the growth. However, under anaerobic conditions the biomass production is favored in cultures of *P. pentosaceus* [4]. High stirring speed conditions helped to keep homogeneous condition of energy and nutrients in the bioreactor cultures. Even though there are evidences that dissolved O_2 directly affects on pediocin production, the oxygen concentration does not interfere with cell growth of *Pediococcus acidilactici* NRRL B5627cultures [18]. Also, has been reported that in *P. acidilactici* cultures, in conditions of low pH and when the specific growth rate is low, the pediocin synthesis is favored [15] and in this conditions the carbon source is directed towards the synthesis of metabolites and not towards the biomass production.

Conclusion

In the shaken flasks cultures, we found that at 37ºC, the biomass concentration and the specific growth rate was maximum. By other hand, in bioreactor cultures at 37ºC the pH was the main factor that has a positive effect on the specific growth rate (μ) of the probiotic bacterium *P. pentosaceus*. On the contrary, the stirring speed not has a significance effect on the μ. In this study, any effects were observed of both factors (pH and stirring speed) on the parameters of culture such as biomass production and cell viability. With those evidences is possible establishing the adequate conditions cultures for the growth of *P. pentosaceus* which have probiotic properties and can be exploited for formulation of functional foods for human consumption.

References

1. Hill C, Guarner F, Reid G, et al. Expert consensus document. The International Scientific Association for Probiotics and Prebiotics consensus statement on the scope and appropriate use of the term probiotic. Nat Rev Gastroenterol Hepatol. 2014; 11: 506-514.
2. Cremon C, Barbaro MR, Ventura M, et al. Pre- and probiotic overview. Curr Opin Pharmacol. 2018; 43: 87-92.
3. Rout GK, Jayanta KP, Sushanto G, et al. Benefaction of probiotics for human health: A review. J Food Drug Anal. 2018; 26: 927-939.
4. Anastasiadou S, Papagianni M, Filiousis G, et al. Growth and metabolism of a meat isolated strain of Pediococcus pentosaceus in submerged fermentation Purification, characterization and properties of the produced pediocin SM-

1. Enzyme Microb Technol. 2008; 43: 448-454.
5. Amara AA, Shibl A. Role of probiotics in health improvement, infection control and disease treatment and management. Saudi Pharm J. 2015; 23: 107-114.
6. Rodríguez-Fragoso L, Sandoval-Ocampo A, Corbalá-Nava M, et al. Evaluation regarding the efficacy and safety of a probiotic mixture in healthy volunteers with evacuation disorders. Food Nutr Sci. 2012; 3: 117-122.
7. Dinan TG, Stanton C, Cryan JF. Psychobiotics: a novel class of psychotropic. Biol Psychiatry. 2013; 74: 720-726.
8. Pärtty A, Kalliomäki M, Wacklin P, et al. A possible link between early probiotic intervention and the risk of neuropsychiatric disorders later in childhood: a randomized trial. Pediatr Res. 2015; 77: 823-828.
9. Torres-Aguilar L, Rodríguez-Fragoso L, Reyes-Esparza J. P. pentosaceus administration attenuates the severity of dextran sulfate sodium-induced colitis and improve the intestinal permeability. Microbiol Res J Int. 2017; 19: 1-12.
10. Kiviharju K, Leisola M, Eerikainen T. Optimization of a Bifidobacterium longum production process. J Biotechnol. 2005; 117: 299-308.
11. Lew LC, Liong MT, Gan CY. Growth optimization of *Lactobacillus rhamnosus* FTDC 8313 and the production of putative dermal bioactives in the presence of manganese and magnesium ions. J Appl Microbiol. 2013; 114: 526-535.
12. Romero-Gil V, Bautista-Gallejo J, Rodríguez-Gómez F, et al. Evaluating the individual effects of temperature and salt on table olive related microorganisms. Food Microbiol. 2013; 33: 178-184.
13. Wallenius J, Uuksulainen T, Salonen K, et al. The effect of temperature and pH gradients on *Lactobacillus rhamnosus* gene expression of stress-related genes. Bioprocess Biosyst Eng. 2011; 34: 1169-1176.
14. Bamba S, Takahashi K, Imaeda H, et al. Effect of fermented vegetable beverage containing *Pediococcus pentosaceus* in patients with mild to moderate ulcerative colitis. Biomed Rep. 2018; 9: 74-80.
15. Xu Q, Gu S, Chen Y, et al. Protective effect of Pediococcus pentosaceus LI05 against *Clostridium difficile* infection in a mouse model. Front Microbiol. 2018; 9: 1-12.
16. Rossi F, Lathrop A. Effects of *Lactobacillus plantarum*, *Pediococcus acidilactici*, and *Pediococcus pentosaceus* on the Growth of *Listeria monocytogenes* and *Salmonella* on Alfalfa Sprouts. J Food Prot. 2019; 82: 522-527.
17. Zhou K, Zeng Y, Han X, et al. Modelling growth and bacteriocin poduction by *Lactobacillus plantarum* BC-25 in response to temperature and pH in batch fermentation. Appl Biochem Biotechnol. 2015; 176: 1627-1637.
18. Anastasiadou S, Papagianni M, Filiousis G, et al. Pediocin SA-1, an antimicrobial peptide from *Pediococcus acidilactici* NRRL B5627: production conditions, purification and characterization. Bioresour Technol. 2008; 299: 5384-5390.

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