Optimization of Bacteriocin Production by Batch Fermentation of *Lactobacillus plantarum* LPCO10

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Optimization of bacteriocin production by *Lactobacillus plantarum* LPCO10 was explored by an integral statistical approach. In a prospective series of experiments, glucose and NaCl concentrations in the culture medium, inoculum size, aeration of the culture, and growth temperature were statistically combined using an experimental 2^{3-1} fractional factorial two-level design and tested for their influence on maximal bacteriocin production by *L. plantarum* LPCO10. After the values for the less-influential variables were fixed, NaCl concentration, inoculum size, and temperature were selected to study their optimal relationship for maximal bacteriocin production. This was achieved by a new experimental 3^{2-1} fractional factorial three-level design which was subsequently used to build response surfaces and analyzed for both linear and quadratic effects. Results obtained indicated that the best conditions for bacteriocin production were shown with temperatures ranging from 22 to 27°C, salt concentration from 2.3 to 2.5%, and *L. plantarum* LPCO10 inoculum size ranging from $10^{7.3}$ to $10^{7.4}$ CFU/ml, fixing the initial glucose concentration at 2%, with no aeration of the culture. Under these optimal conditions, about 3.2×10^4 times more bacteriocin per liter of culture medium was obtained than that used to initially purify plantaricin S from *L. plantarum* LPCO10 to homogeneity. These results indicated the importance of this study in obtaining maximal production of bacteriocins from *L. plantarum* LPCO10 so that bacteriocins can be used as preservatives in canned foods.

Lactic acid bacteria (LAB) are widely used as starter cultures in dairy, meat, and vegetable fermentations (1, 7, 36). One major reason for their wide use is the wide range of antimicrobial substances that they are able to produce which efficiently contribute to the preservation of the fermented products (13, 15, 31). Of these antimicrobial substances, bacteriocins are one of the most promising natural food preservatives produced by LAB (10, 12, 26, 33, 34). This preservation potential could be achieved either by using a bacteriocin-producing starter culture or by applying the bacteriocin itself as a food additive. The latter will necessarily require optimization of their production, for this is dependent on multiple factors which are usually strain specific (9, 39).

In the past, several studies have pursued this goal for a number of different bacteriocins. They have generally focused on the effects of pH, temperature, composition of the culture medium, and general microbial growth conditions (in vitro as well as in natural fermentations) on maximal bacteriocin production (2, 4, 6, 8, 11, 14, 24, 25, 28–30, 38–40). However, although some of these studies claim validation by a statistical test, usually variance analysis, the combination of variables and their values and limits are arbitrarily chosen, based mainly on subjective personal experience. Thus, usually no previous prospective experimental design is performed to optimize the information that can be gained from subsequent experiments.

In this work, we explore the valuable power of statistical experimental design to optimize the production of bacteriocins by *Lactobacillus plantarum* LPCO10 (21, 22, 24, 35) to allow the use of bacteriocins as natural food additives in canned vegetables and other food systems.

MATERIALS AND METHODS

Bacterial strains. *L. plantarum* LPCO10 (produces plantaricin S [PLS] and T [PLT]) and *L. plantarum* 128/2 (PLS and PLT sensitive) belong to our culture collection and have been previously described (21). *Lactobacillus fermentum* ATCC 14933 (PLS and PLT sensitive) was obtained from the CNRZ Culture Collection, Jouy-en-Josas, France.

Culture media. For maintenance purposes and bacteriocin sensitivity assays, the strains were grown at 30°C in MRS medium (Oxoid, Unipath Ltd., Basingstoke, Hampshire, England). For the optimization experiments, MRS broth prepared in our laboratory was used. MRS broth contained the following (in grams per liter of deionized distilled water): peptone (E. Merck, Darmstadt, Germany), 10.0; Lab-Lemco powder (Oxoid), 8.0; yeast extract (Oxoid), 4.0; K_2HPO_4 (Fluka Chemie AG, Buchs, Switzerland), 2.0; $NaCH_2COOH \cdot 3H_2O$ (Fluka), 5.0; diammonium hydrogen citrate (Fluka), 1.84; $MgSO_4 \cdot 7H_2O$ (Merck), 0.2; $MnSO_4 \cdot H_2O$ (Merck), 0.04; and Tween 80 (Fluka), 1 ml. When required, 3 to 5% (wt/vol) NaCl (Merck) was added, and the pH was adjusted to 6.2 with 12 N HCl (Fluka). The volume was adjusted to 950 ml with deionized distilled water and then sterilized by autoclaving at 121°C. Fifty percent (wt/vol) filter-sterilized glucose (Fluka) was added to bring the final glucose concentration to 1 or 2%, and the final volume of the medium was brought to 990 ml by the addition of sterile distilled water. The medium was brought to the final volume before each experiment by addition of 10 ml of the *L. plantarum* LPCO10 inoculum in sterile saline.

Inoculum preparation. A single colony of *L. plantarum* LPCO10 growing in MRS agar (Oxoid) was inoculated into 20 ml of MRS broth, prepared as described above, containing 2% glucose, and brought to the final volume with sterile water. This solution was incubated at 30°C overnight and then used to inoculate 2 liters of fresh MRS medium. After the medium was incubated at 30°C, the cells were collected by centrifugation ($5,000 \times g$, 10 min) when the absorbance at 600 nm reached 0.7 (ca. 10^8 CFU/ml), washed with sterile saline,

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TABLE 1. Fractional factorial ($2^{3-2}_{(5-2)}$) diagnostic experimental design and coded values used to test the effects of several fermentation parameters over the production of bacteriocin by *L. plantarum* LPCO10

Run no.	Factor level ^a					Response ^b	
	Glucose	Initial inoculum size	Aeration	Temp	NaCl	A	B
1	-1	-1	-1	+1	+1	0.0 ^c	2.44
2	+1	-1	-1	-1	+1	2.90	5.05
3	-1	+1	-1	+1	-1	2.44	4.10
4	+1	+1	-1	-1	-1	3.35	7.03
5	-1	-1	+1	-1	-1	3.35	5.28
6	+1	-1	+1	+1	-1	2.14	3.95
7	-1	+1	+1	-1	+1	2.60	4.82
8	+1	+1	+1	+1	+1	1.30	2.74

^a The actual factor levels, coded as values of -1 and +1 in the table, were as follows: for glucose, 1% (-1) and 2% (+1) glucose (wt/vol); for initial inoculum size, $\log_{10}$ CFU/ml of 5 (-1) and 7 (+1); aeration (1 liter/min) (-1) and no aeration (+1); temperature of 25°C (-1) and 35°C (+1); and 3% (-1) and 5% (wt/vol) (+1) NaCl.

^b Responses obtained with every run are shown. *L. plantarum* 128/2 (A) and *L. fermentum* ATCC 14933 (B) were used as the indicator strain. Each response is the logarithm of maximal bacteriocin activity in that run ($\log_{10}$ maximal AU per milliliter).

^c No bacteriocin activity was detected with this strain in this run.

and resuspended in 200 ml of 15% (vol/vol) glycerol. Aliquots were made and stored at -80°C until use.

Fermentation experiments. A 2-liter Biostat MD fermentor (B. Braun-Biotec, Melsungen, Germany) containing 1 liter of the MRS medium described above was used to study the kinetics of bacteriocin production by *L. plantarum* LPCO10. For every experiment, one of the stock aliquots generated as described above was thawed and the necessary amount of cells was collected by centrifugation ($5,000 \times g$, 10 min), washed, and finally resuspended in 10 ml of sterile saline, which was then used to inoculate the fermentor. When appropriate, aeration was set by bubbling filter-sterilized air at a rate of 1 liter/min. Cultures were stirred at 40 rpm throughout the fermentation.

Analysis of the samples. At convenient time intervals, samples were aseptically withdrawn from the fermentation vessel to determine glucose concentration, viable cell number, bacteriocin activity, and lactic acid production. Furthermore, pH value was monitored online by the fermentor probe. Glucose concentration was determined by the D-glucose/D-fructose Enzymatic BioAnalysis kit (Boehringer Mannheim GmbH, Mannheim, Germany), according to the manufacturer's instructions. Viable cell counts were determined by plating serial dilutions of the samples onto MRS agar (Oxoid) by a Spiral System (Interscience, Saint-Nom-La Breteche, France) and incubating at 30°C for 48 h. Bacteriocin activity was quantified with a microtiter plate assay system as described previously (19, 22), using *L. plantarum* 128/2 and *L. fermentum* ATCC 14933 as the indicator strains, averaging two- and threefold dilution series for every sample (17, 22), and expressed as arbitrary activity units per milliliter (AU/ml). Lactic acid concentration (expressed as millimoles per liter) was determined acidimetrically by titration with 0.1 N NaOH to an endpoint pH of 6.8.

Experimental design. Initially, the responses, i.e., inhibitory activity due to bacteriocin production by *L. plantarum* LPCO10, were detected by using *L. plantarum* 128/2 and *L. fermentum* ATCC 14933 as indicator cultures. Bacteriocin production was measured as the $\log_{10}$ AU per milliliter, and it was assumed to be under the influence of the glucose concentration, inoculum size, aeration, temperature, and NaCl concentration. Their effects were tested in the diagnostic experiment by the $2^{3-2}_{(5-2)}$ fractional factorial two-level design shown in Table 1 (5, 23). In this design, the temperature column was obtained by combining the glucose concentration and aeration columns, and the NaCl concentration column was obtained by combining the inoculum size and aeration columns, respectively. Physical and coded levels are given in Table 1, footnote a. In general, coded values for continuous variables were obtained as follows: coded value = [physical value - 1/2(highest value + lowest value)]/1/2(highest value - lowest value).

For qualitative variables, coded values were assigned at random. All experiments were randomly run. For responses, the logarithm of maximal bacteriocin activity detected in the supernatants for every fermentation run was used. It was a measure of the total inhibitory activity that can be collected at that sampling point and represents the maximal bacteriocin production for that fermentation

run. Moreover, two responses were obtained for each experiment, one for each indicator strain used to titrate the bacteriocin activity. The logarithmic transformation was used to reduce variance due to the wide range of activity values obtained.

After the analysis of the previous experiment, a new three-level $3^{2-1}_{(3-1)}$ fractional factorial design (Statistica for Windows computer program manual; StatSoft Inc., Tulsa, Okla.) with replication at the central point was performed (Table 2). Values of the variables under study were selected to include the region of highest bacteriocin activity, as concluded from the diagnostic experiments (see Results). The responses considered were the same as in the previous diagnostic design. Results were analyzed for linear and quadratic effects and used to build the response surfaces.

Statistical analysis. Results were analyzed by the Experimental Design Module of the Statistica software package (Statistica for Windows computer program manual; StatSoft Inc.).

RESULTS

Diagnostic experimental design of bacteriocin production by *L. plantarum* LPCO10. When the effects of the individual factors (glucose concentration, inoculum size, aeration of the culture, temperature of incubation, and NaCl concentration) were assessed, none of the factors showed a statistically significant effect on bacteriocin production by *L. plantarum* LPCO10 when *L. plantarum* 128/2 was used to analyze the response. Thus, due to the limitations of the design, no information on the response in the design region could be obtained. However, when *L. fermentum* ATCC 14933 was used, temperature and NaCl concentration were found to have significant ($P < 0.05$) effects on the responses (Fig. 1). These estimated effects were negative in both cases, thus indicating that the response decreased when the temperature or NaCl concentration increased, and conversely, into the limits of the coded values used in this study (Table 1, footnote a). On the other hand, glucose concentration, inoculum size, and aeration did not show significant effects on the responses ($P < 0.05$) (Fig. 1). However, the effects of the first two factors were positive, indicating that an increase in glucose concentration or in the

TABLE 2. Experimental design and coded values used to obtain a response surface of the maximal bacteriocin production by *L. plantarum* LPCO10 as a function of NaCl concentration, temperature, and inoculum size

Run no.	Factor level ^a			Response ^b	
	Temp	NaCl	Initial inoculum size	A	B
1	-1	-1	-1	2.90	3.95
2	-1	0	+1	3.35	4.55
3	-1	+1	0	3.05	4.15
4	0	-1	+1	3.05	4.45
5	0	0	0	3.35	4.55
6	0	+1	-1	1.85	3.65
7	+1	-1	0	3.35	4.55
8	+1	0	-1	2.75	3.95
9	+1	+1	+1	2.90	4.41
10	0	0	0	3.51	5.05

^a The actual factor levels, coded as values of -1, 0, and +1 in the table, were as follows: temperature of 22°C (-1), 25°C (0), and 27°C (+1); 1% (-1), 3% (0), and 5% (wt/vol) (+1) NaCl; for initial inoculum size, $\log_{10}$ CFU/ml of 6 (-1), 7 (0), and 8 (+1).

^b Responses obtained with every run are shown. *L. plantarum* 128/2 (A) and *L. fermentum* ATCC 14933 (B) were used as the indicator strain. Each response is the logarithm of maximal bacteriocin activity in that run ($\log_{10}$ maximal AU per milliliter).

FIG. 1. Bar graph of standardized estimated effects of the different variables tested in the prospective experiment on bacteriocin production by *L. plantarum* LPCO10. *L. fermentum* ATCC 14933 was used as the indicator strain. The variables tested were temperature (T), NaCl concentration (S), initial glucose concentration (G), initial inoculum size (IS), and aeration (A). The point at which the effect estimates were statistically significant (at $P = 0.05$) is indicated by the vertical solid line.

inoculum size would follow only a slight favorable effect on the response within the limits studied.

A predictive estimation of the expected bacteriocin production by *L. plantarum* LPCO10 at the limits of the coded values of temperature and NaCl concentration showed that the highest titers of bacteriocins would be obtained when the values of these parameters were around 25°C and 3% NaCl (data not shown), whatever the values of the other parameters studied, within the limits described in Table 1, footnote *a*. Actually, run 4, which was the one obtaining the highest responses (Table 1), was repeated to confirm the results. The responses were again, as expected, the highest (data not shown).

The purpose of this diagnostic two-level design was to obtain experimental data which served as an initial approach to final optimization of bacteriocin production, establishing which factors had significant effects on responses (temperature and NaCl concentration, in this case) and whether these effects were positive or negative. With respect to the other variables, their levels did not statistically affect the responses. However, there were slightly smaller responses to aeration, and on the other hand, slightly higher responses were obtained when 2% glucose or 10^7 CFU/ml was used. Consequently, in further experiments, the culture was not aerated, the glucose level was fixed at 2%, and the inoculum size was tested again to investigate a possible correlation between bacterial growth and bacteriocin production.

Study of quadratic effects and response surfaces for maximal bacteriocin production by *L. plantarum* LPCO10. To estimate not only linear effects but also quadratic effects and to build response surfaces for maximal bacteriocin production in the region defined by the previous diagnostic experiments, a new experimental design at three levels was planned, and the corresponding experiments were performed. The cultures were not aerated, and the initial glucose concentration in the medium was fixed at 2%. Temperature, NaCl concentration, and inoculum size were selected as the variables (Table 2). The coded values for these variables were different from those used in the exploratory experiment, and the physical levels were

selected taking into account the results from the diagnostic design. Thus, the limits for some variables were decreased or increased, according to the previous results: temperature and NaCl concentration were decreased, while the limit for inoculum size was increased (Table 2, footnote *a*). The central values (coded value of 0) were those found to promote the highest bacteriocin production in the previous diagnostic round of experiments for that variable.

Responses obtained now were different depending on the bacteriocin-sensitive strain used (Table 2) and on the effects of every variable within the new limits. In general, responses obtained with *L. plantarum* 128/2 were higher than those obtained in the exploratory experiments which allowed the detection of effects and the application of the statistical analyses. Thus, when *L. plantarum* 128/2 was used as the indicator strain, NaCl concentration (linear) and inoculum size (linear and quadratic) were found to have significant ($p < 0.05$) effect on the response (bacteriocin production). The corresponding response surface is shown in Fig. 2, where a point for maximal bacteriocin production can be found. The conditions corresponding to this point can be easily deduced from the contour lines at the bottom of Fig. 2 (plane NaCl concentration – $\log_{10}$ inoculum size) and are defined by a NaCl concentration of 2.5% and $10^{7.3}$ CFU of *L. plantarum* LPCO10 per ml. On the other hand, temperature did not show a significant effect in the interval considered for this variable in the second experimental design. Therefore, the lowest limit might be used, since their energetic requirements are lower.

When *L. fermentum* ATCC 14933 was used as the indicator strain, the responses were again higher than those obtained with strain 128/2, although response trends maintain similar proportionalities in all runs (Table 2). No new significant effects of the variables were found in this second round of experiments, indicating that the region of optimal conditions, as detected by strain ATCC 14933, had already been included in the diagnostic design. However, the same variables still showed the highest influence, as shown by the response surfaces that can be obtained for every parameter combination regarding

FIG. 2. Response surface of bacteriocin production by *L. plantarum* LPCO10, estimated as the $\log_{10}$ of maximal AU per milliliter, and contour lines showing the optimal region as a function of NaCl concentration and the *L. plantarum* LPCO10 inoculum size. *L. plantarum* 128/2 was used as the indicator strain.

strain ATCC 14933 responses. The relationship between temperature and NaCl concentration is shown in Fig. 3. As was found with *L. plantarum* 128/2, temperature had a negligible effect in the interval selected (from 22 to 27°C), as shown by the virtually horizontal line corresponding to the temperature axis. On the other hand, NaCl concentration showed a stimulating influence on the response up to 2.5% (as with strain 128/2), but concentrations above this level tended to decrease bacteriocin production.

The relationship between NaCl concentration and inoculum size is shown in Fig. 4. From the contour lines shown in Fig. 4, it is easily deduced that maximal bacteriocin production could be obtained with a combination of 2.5% NaCl and $10^{7.4}$ CFU of *L. plantarum* LPCO10 per ml, again virtually the same result as with *L. plantarum* 128/2.

Finally, the relationship between temperature and inoculum size (Fig. 5) points out again the negligible influence of temperature within the proposed limits, as well as the fact that when the inoculum size of *L. plantarum* LPCO10 is more than $10^{7.4}$ CFU/ml, there is no further increase in bacteriocin production and even a slight decrease.

Relationship between bacteriocin production and growth of *L. plantarum* LPCO10. It could be argued that conditions for maximal bacteriocin production should correspond to those conditions that also allow maximal cell density of the producer strain, as bacteriocin is usually a primary metabolite (11, 14, 25). Thus, the three variables found to have a significant effect on bacteriocin production (NaCl concentration, temperature, and inoculum size) and whose relationship has been optimized also showed a significant linear effect on the maximal *L. plantarum* LPCO10 cell density. This is shown by the response surfaces obtained by using the $\log_{10}$ CFU per milliliter at the points of maximal bacteriocin production in every run as the response. These response surfaces are very simple, virtually planes, indicating that there is no correlation between both

responses ($\log_{10}$ of maximal bacteriocin activity produced and $\log_{10}$ of CFU per milliliter at the same points) in the interval of values assayed for the variables under study. As an example, Fig. 6 shows the relationship between NaCl concentration and temperature when the response is now the CFU per milliliter of the LPCO10 strain at the points of maximal bacteriocin production. Figure 6 shows that the cell density decreases linearly as the temperature increases and that the cell density decreases as the NaCl concentration increases (although with a more pronounced slope).

DISCUSSION

Optimization of bacteriocin production by *L. plantarum* LPCO10 in a laboratory fermentor as a preliminary step to producing bacteriocin for use as a preservative in canned foods (mainly vegetables), was accomplished. As large amounts of bacteriocin are necessary to test their preservative efficiency in natural environments (2, 10, 13, 33, 36), establishment of the factors and levels influencing maximal production would lead to a more effective recovery of these antimicrobial compounds from a defined laboratory culture medium.

The study was accomplished in two consecutive steps: first, a diagnostic two-level design was performed to determine the variables that could affect bacteriocin production by *L. plantarum* LPCO10; later, a 3^{2-1}_{III} fractional factorial three-level design with replication at the central point was performed to estimate linear and quadratic effects and the corresponding response surface. In general, responses obtained using *L. plantarum* 128/2 as the indicator strain were lower than those obtained with *L. fermentum* ATCC 14933; this difference was an average of 2 log units, and the behavior was similar in all runs. This result was expected, since *L. fermentum* ATCC 14933 has been shown to be more sensitive than *L. plantarum* 128/2 to PLS and PLT (21). Sensitivity to the bacteriocins is apparently

FIG. 3. Response surface of bacteriocin production by *L. plantarum* LPCO10, estimated as the $\log_{10}$ of maximal AU per milliliter, as a function of temperature and NaCl concentration. *L. fermentum* ATCC 14933 was used as the indicator strain.

FIG. 4. Contour plot showing the optimal region of bacteriocin production by *L. plantarum* LPCO10, estimated as the $\log_{10}$ of maximal AU per milliliter, as a function of the inoculum size and NaCl concentration. *L. fermentum* ATCC 14933 was used as the indicator strain.

an intrinsic characteristic of each strain and does not depend on the conditions of the culture medium from which bacteriocin was obtained. Thus, an interesting conclusion of the first part of this study was the need for selecting an appropriate bacteriocin indicator strain. If related studies on optimization of bacteriocin production are to be developed using statistical approaches, it seems advisable, if not absolutely necessary, to select those strains which are most sensitive to the bacteriocin under study. In other terms, strains which can offer the highest responses must be selected. In our case, for the parameters and

the limit values used, results obtained from *L. fermentum* ATCC 14933 showed the effects better than *L. plantarum* 128/2 did, although both are quite sensitive to the bacteriocins studied. An adequate quantitative titration method for inhibitory activity is also necessary. We suggest using the microtiter plate method described previously (19), averaging the values for two- and threefold dilution series for every sample (17, 22).

The second part of this study indicates that the best conditions for bacteriocin production by *L. plantarum* LPCO10 (in the fermentor and with the culture medium described in this study) are a NaCl concentration of about 2.5%, temperature ranging from 22 to 27°C, and inoculum size of about $10^{7.4}$ CFU/ml, with no aeration and an initial glucose concentration

FIG. 5. Response surface of bacteriocin production by *L. plantarum* LPCO10, estimated as the $\log_{10}$ of maximal AU per milliliter, and contour lines showing the optimal region as a function of temperature and initial inoculum size. *L. fermentum* ATCC 14933 was used as the indicator strain.

FIG. 6. Response surface of *L. plantarum* LPCO10 cell density ($\log_{10}$) as a function of NaCl concentration and temperature.

between 1 and 2%. Interestingly, these results clearly correlate with the empirical method by which the olives are processed. In traditional fermentation, the fruits are covered with a brine of around 5 to 6% NaCl (final concentration). The containers of about 10 tons of fruits and 5,000 liters of brine are placed outdoors; therefore, many times the fermentation mixture is at temperatures ranging from 20 to 25°C (16, 18). Also, aeration is not a usual practice in the green olive fermentation, and the concentration of sugars in the fruits is estimated to be about 2 to 6% of the pulp weight, so there is no carbon nutrient limitation for lactobacilli. On the other hand, the *L. plantarum* LPCO10 strain used in this work, originally isolated from a green olive fermentation (21), has been successfully used as a starter culture for olive fermentations previously (24, 34). In these cases, it has been clearly demonstrated that the strain dominated the epiphytic microflora and that this domination was due to the ability of *L. plantarum* LPCO10 to produce bacteriocin (34). Thus, the statistical study applied here, even if a synthetic culture medium has been used, generates results which resemble those obtained at the natural environment of *L. plantarum* in green olive fermentations. This parallelism could indicate that the best conditions established here for maximal bacteriocin production by *L. plantarum* LPCO10 in a laboratory medium perhaps are quite similar to the best ones for bacteriocin production in natural olive fermentations. Moreover, recently obtained results (M.V. Leal-Sánchez, J. L. Ruiz-Barba, A. H. Sánchez, L. Rejano, R. Jiménez-Díaz, and A. Garrido, submitted for publication) indicate that the traditional fermentation of Spanish-style green table olives could be dramatically improved by using *L. plantarum* LPCO10 as a starter culture at $\geq 10^7$ CFU/ml (final concentration in brine) and a starting brine of 4% NaCl. These results obtained at an experimental, semi-industrial level again correlate with those conditions obtained in this study for maximal bacteriocin production by *L. plantarum* LPCO10 in the laboratory.

On the other hand, results obtained indicate that there is not a clear-cut correlation between maximal bacteriocin production and the number of *L. plantarum* LPCO10 cells at the same points. However, it has been shown here that at the interval points of maximal bacteriocin production, the cell density decreases as the temperature increases and the cell density decreases as the NaCl concentration increases (Fig. 6). From this, it can be concluded that maximal production of bacteriocin by *L. plantarum* LPCO10 is related not only to the cell number but also to additional factors. For instance, the salt concentration has been found to play an important role in the release of bacteriocins from different bacteriocin-producing strains of LAB during growth, most probably by influencing the adsorption of the bacteriocins to the cell envelope of the bacteriocin producer itself (3, 20, 21, 25, 27, 32, 37).

Finally, when *L. plantarum* 128/2 was used as the indicator strain, maximal bacteriocin production by *L. plantarum* LPCO10 is shown by conditions described in run 10 (3,200 AU/ml; Table 2). This amount of bacteriocin is about 3.2×10^4 times the amount from which purification to homogeneity of PLS was achieved, using the same 128/2 strain as the sensitive strain to monitor the purification steps (22). Thus, results obtained here clearly indicated the importance of this study in the production of bacteriocins from *L. plantarum* LPCO10 for application in canned foods.

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