PRODUCTION OF BACTERIOCIN-LIKE INHIBITORY SUBSTANCE FROM LACTIC ACID BACTERIA UTILIZING CULTURE MEDIUM FORMULATED WITH CORN STEEP LIQUOR AND SUGAR CANE MOLASSES

Taciana Freire de Oliveira[1]*, Guilherme Medrado dos Santos Silva[1], Taís Mayumi Kuniyoshi[1], Eliônio Galvão Frota[1] Fernando Moises Mamani Sanca[1] & Ricardo Pinheiro de Souza Oliveira[1]

[1] *Department of Pharmacy, University of São Paulo, São Paulo, Brazil.*
** Corresponding author's email address: taciana.oliveira@usp.br*

ABSTRACT

Considering the current scenario of increased demand for more natural foods and sustainable products, this study aimed to evaluate growth and Bacteriocin-like inhibitory substance (BLIS) production of seven lactic acid bacteria (LAB) using an alternative culture medium formulated with molasses and corn steep liquor (CSL). The results showed the potentiality of these by-products as substrates to produce antimicrobial compounds with activity against *Listeria monocytogenes*, an important foodborne pathogen.

Keywords: Keyword 1. Bacteriocin 2. Agro-industrial by-products 3. Antimicrobial activity 4. Foodborne pathogens

1 INTRODUCTION

The growing search for clean lable products has enhanced the demand for natural additives and food ingredients manufactured using sustainable sources. In this context, bacteriocins, an antimicrobial peptides produced by lactic acid bacteria, have gain attention due to its antimicrobial activity against foodborne pathogens and potential as safe bio-preservers in foods (NEGASH et al., 2020). However, the conventional culture medium used for LAB cultivation is often costly and impractical for commercial production. Some authors have already described that the use of agro-industrial wastes to produce bacteriocins as substitutes for culture media would result in a low-cost and environmentally friendly process (MUSSATI, et al. 2020). In this way, this study aimed to assess cell growth of bacteriocinogenic LAB, using an alternative media, that containing molasses and steep corn liquor, and to evaluate the antimicrobial activity of the produced BLIS against important pathogenic bacteria in food industry.

2 MATERIAL & METHODS

Sugarcane molasses and corn steep liquor were characterized according to the sugar and organic acid profile by high-performance liquid chromatography (HPLC) and the total nitrogen and protein content by the Kjeldahl method. No additional treatments were carried out before using these substrates in the culture medium.

LAB strains (Table 1) were obtained from Laboratory of Microbial Biomolecules (University of São Paulo, Brazil). The cell growth was evaluated in MRS medium as a standard condition and compared with an alternative medium (M1), with the following composition (g/L): molasses, 20; CSL, 6; peptone, 4; yeast extract, 2, tween 80, 1 and supplemented with the same salts solution of MRS medium. The growth profile was obtained by optical density (OD) readings at 600 nm in 96-well plates incubated in a microplate reader at 37 °C. To verify the BLIS production, the samples of each studied conditions were prepared according to Sabo et al. (2018) and the antimicrobial activity was performed through agar diffusion and broth dilution methods (SHARIR et al., 2024). For that, it was used as bioindicators *Listeria monocytogenes* CECT 934 and *Salmonella Heidelberg* IOC 969/17, cultivated in BHI medium, at 37 ºC. Each condition was tested in triplicate.

3 RESULTS & DISCUSSION

Firstly, it was evaluated the sugar content (sucrose, glucose and fructose) and protein concentration of the agro-industrial by-products used in this study. The results showed that molasses contain great amount of fermentable sugars (69 %, w/w) and CSL is richer in organic nitrogen source (21.06 %, w/w). After characterization of the substrates, it was formulated an alternative culture medium, considering molasses and CSL in the equivalent amounts of carbon and nitrogen supplied in the MRS broth. The kinetic of microbial growth was evaluated and, according to Figure 1, it was observed that all strains were able to growth in the M1 medium. The growth profile was compared and showed that the maximum OD (600 nm) in M1 medium was lower than in commercial medium, however, it was not observed longer lag phase for BALs cultivated in M1 medium.

It is known that the conditions that maximized LAB growth do not guarantee an increase in bacteriocins production (ÜNLÜ et al., 2015). The results of antimicrobial production in both medium (Table 1) showed that, excepted for *m3a, A1A* and *C 195,* the BLIS produced in M1 medium was able to inhibit more than 90 % of the *L. monocytogenes* growth. Besides, the BLIS produced by *BT2*, *BE2, M1A* and *C173* resulted in an increase in inhibition percentage compared to MRS medium. These results support the fact that increasing cell growth does not always result in higher bacteriocin production and the alternative medium seem to increase BLIS production for some strains, under studied conditions. Molasses and CSL have a complex composition, which

contain other nutrients that could be used for microbial nutrition and bioproducts production, such as vitamins and aminoacids, Garmasheeva et al., (2023) studied the screening of *Enterococcus* strains using a low cost medium prepared with molasses and CSL. According to the authors, the alternative medium can significantly reduce the cost of industrial bacteriocin production.

Regarding antimicrobial activity against *S. Heidelberg*, it was not observed inhibition effect for any BLIS. This results are in line with other studies (OLIVEIRA, et al., 2024) and could be probably due to most bacteriocins isolated from Gram-positive bacteria inhibit closely related species to the producer (SABO et al., 2018).

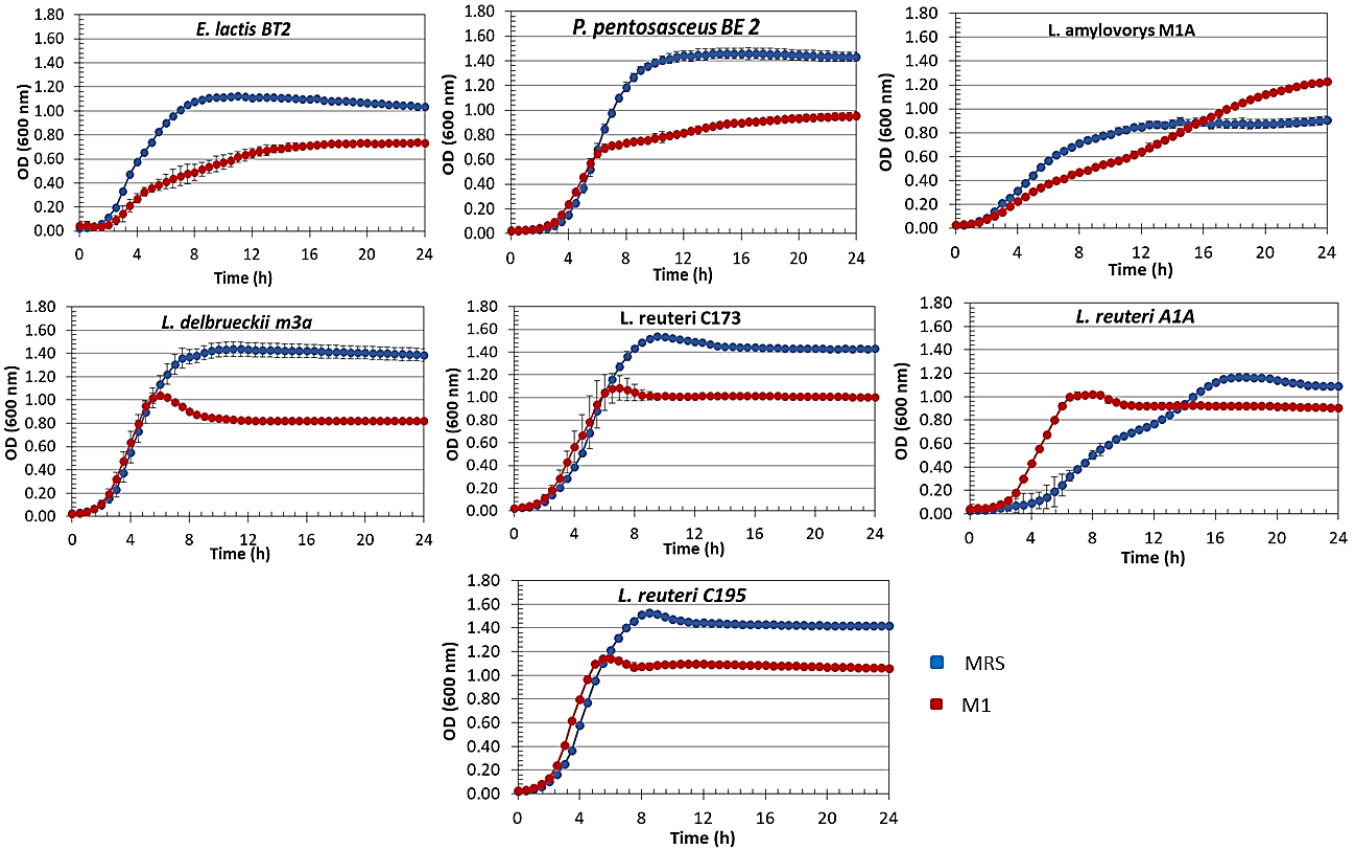

Figure 1 Microbial growth of BALs in MRS and M1 medium.

Table 1 Inhibition percentage of BLIS produced by LAB against *L. monocytogens* CETC 934.

LAB strains	MRS	M1
	Inhibition percentage, %	Inhibition percentage, %
Enterococcus lactis BT2	94.41% ± 0.2	99.03% ± 0.9
Pediococcus pentosaceus BE2	93.31% ± 0.8	98.21% ± 1.1
Lactobacillus amylovorys M1A	90.75% ± 4.2	97.72% ± 1.9
Lactobacillus delbrueckii m3a	38.51% ± 5.0	18.84% ± 2.1
Lactobacillus reuteri A1A	92.24% ± 3.1	16.91% ± 6.4
Lactococcus lactis C173	94.31% ± 0.1	95.65% ± 0.3
Lactococcus lactis C195	4.14% ± 7.2	22.77% ± 2.9

In this way, this study showed preliminary results, which indicated a potential for molasses and CSL to be used as carbon and nitrogen sources, respectively, for LAB cultivation and BLIS production with antilisterial activity. However, to expand the applications of bacteriocin in the food industry, more studies are needed in order to investigate the optimal concentrations of the alternative medium and to assess the efficacy of bacteriocins against additional foodborne pathogens (e.g. *Staphylococcus aureus, Campylobacter sp., Bacillus cereus*, etc).

4 CONCLUSION

Sugar-cane molasses is a by-product from sugar industry and corn steep liquor is considered a residue from corn processing. Although they are already being used mainly for animal nutrition, the utilization of these products to generate biomolecules with high added value is a great and current strategy to reduce the manufacture costs of bioproducts production, diversify their biorrefinery and promote the circular economy. In this context, the results of this work were in line with these concepts and contribute to the study of bacteriocin production and its application as a bio-preserver in food industry.

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