IN VITRO EVALUATION OF THE ANTAGONISTIC ACTIVITY OF *PEDIOCOCCUS ACIDILACTICI* ATCC 8042 AGAINST *PSEUDOMONAS AERUGINOSA* AND *LISTERIA MONOCYTOGENES*

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Introduction. Some Lactic Acid Bacteria produce bacteriocins; *Pediococcus spp.* produces one named pediocin (1,2), nevertheless Llorente *et al.* (3) demonstrated that the strain *P. acidilactici* ATCC 8042 had not the gen *pedB,* so it is incapable to synthesize pediocin, but it can produce an extracellular proteolytic and peptidoglycan hydrolase (110 kDa) (3). García-Cano *et al.* (4), detected a 99 kDa protein, their work showed that such protein is an N-acetylmuramidase. Through zymograms (3), agar diffusion assay and broth dilution assay (4) proteins lytic activity against many Gram (+) and few Gram (–) bacteria was detected.

The aim of this work was to evaluate *in vitro* the antagonistic activity of proteins produced by *P. acidilactici* against pathogen (*L.* monocytogenes, Gram +) and spoilage food microorganism (*P. aeruginosa,* Gram -).

Methods. *Pediococcus acidilactici* ATCC 8042 and its supernatant was growth and obtain according to Llorente *et al.* (3). Target strains were *Listeria monocytogenes* ATCC 15313 and *Pseudomonas aeruginosa* ATCC 9027. Supernatant of *P. acidilactici*, was obtained also according to LLorente *et al.* (3)' and its protein quantification was performed according Bradford (5) For agar diffusion assay (6) were added 160 µL of supernatant (246 µg/well of protein). For critical dilution assay (7) double dilutions in each well were placed, 1:2 (123 µg), 1:4 (61.5 µg), 1:8 (30.75 µg) and 1:16 (15.37 µg). Broth dilution assay (8) carried out as indicated the Table 1.

Table 1. Broth dilution assay for *L. monocytogenes* and *P. aeruginosa.*

Str.	Tube	1	2	3	4	5	6	Nega-tive	Positive
Psudomonas aeruginosa	*Nutrient Broth (50 µL)*	4.5 mL	4.0 mL	3.5 mL	3.0 mL	2.5 mL	2.0 mL	5.0 mL	5.0 mL
	Supernatant resuspended	0.5 mL	1.0 mL	1.5 mL	2.0 mL	2.5 mL	3.0 mL	------	------
	Lactic Acid 2%	----	----	----	----	----	----	------	0.114 mL
Listeria monocyto-genes	BHI Broth (50 µL)	3.5 mL	3.0 mL	2.5 mL	2.0 mL	1.5 mL	1.0 mL	4 mL	4 mL
	Supernatant resuspended	0.5 mL	1.0 mL	1.5 mL	2.0 mL	2.5 mL	3.0 mL	------	------
	Lactic Acid 2%	----	----	----	----	----	---- --	------	0.091 mL

Results. In agar diffusion assay, the supernatant of *P. acidilactici* produced inhibition halos of 28.33 ± 0.57 mm for *L. monocytogenes* culture (Fig. 1 a), and 16.33 ± 2.51 mm halos for *P. aeruginosa* culture (Fig. 1 b), obtained a protein specific activity of 115.27 ± 16.01 mm/mg for *L. monocytogenes* and 65.68 ± 5.46 mm/mg for *P. aeruginosa*. In critical dilution agar assay for *L. monocytogenes* were formed inhibition halos of 14.66 ± 2.08 mm at 1:4 dilution (Fig. 2 d) with a Minimal Inhibitory Concentration (MIC) of 25 Arbitrary Units/mL (UA/mL)*;* for *P. aeruginosa* assay were formed 9.66 ± 2.64 mm halos at 1:2 dilution with a MIC of 12.5 UA/mL(Fig. 1 e).

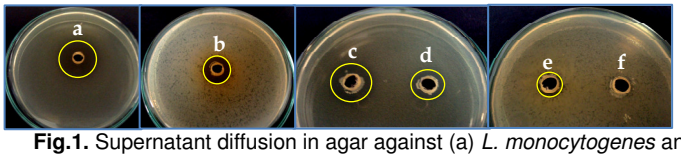

Fig.1. Supernatant diffusion in agar against (a) *L. monocytogenes* and (b) *P. aeruginosa*. Critical dilution test for *L. monocytogenes* (c) 1:2 and (d) 1:4. *P. aeruginosa,* (e) 1:2 and (f) 1:4

Broth dilution assay of *P. aeruginosa* (Fig. 2 a) showed a logarithmic phase growth rate decrease (1.0 to 2.0 mL treatment); a log phase increase (2.5 and 3.0 mL treatment) and CFU reduction from 10^9 to 10^8 log (at 23 h of 3.0 mL treatment). Also was observed a lag phase time increase and a CFU reduction, from 10^9 to 10^6 log at 23 h (3 mL treatment) for *L. monocytogenes* (Fig. 2 b).

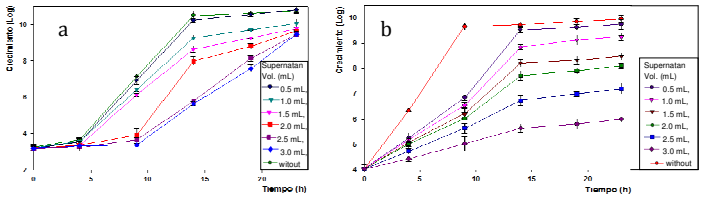

Fig.2. (a) *P. aeruginosa* and (b) *L. monocytogenes* growth with different supernatant volumes.

Conclusions. The proteins produced by *Pediococcus acidilactici* ATCC 8042 were able to inhibit target strains. Specific protein activity and CIM were obtained. The inhibitory effect was stronger for *L. monocytogenes* than for *P. aeruginosa,* which reduce visible agar pigmentation at 3.0 mL treatment (data not shown).

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