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Influence of several gums on the growth and the production of a bacteriocin like substance from *Lactobacillus curvatus/sakei* ACU-1

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Abstract

The study was meant to evaluate the influence of xanthan gum, λ-carrageenan, arabic gum and tragacanth gum on the growth and *in situ* production of bacteriocin like inhibitory substances from *Lactobacillus curvatus/sakei* ACU-1. *Listeria innocua* ATCC 33090 and *Staphylococcus aureus* FBUNT were used as indicator microorganisms. The growth of, and bacteriocin production by, *Lactobacillus sakei/curvatus* ACU-1 strain was assessed using MRS broth supplemented with 0.5 g /100ml of each gum. Results showed that xanthan and arabic gum did not significantly influence bacterial growth rate while tragacanth and λ-carrageenan promoted it. The effect of the presence of gums on bacteriocin production was dependent upon the type of gum, i.e. compared to the control system, arabic gum diminished it, and the rest of the gums showed an enhancing effect. Arabic gum interfered with bacteriocin activity and thus its use in food products would be conditioned.

Keywords: bacteriocin; gum; interaction; lactobacilli; food biopreservation

1. Introduction

Gums are traditionally the type of molecules considered as food hydrocolloids. These long-chain complex polysaccharide molecules have played a significant role in foodstuffs since ancient times on account of their texturizing and water-structuring properties. Their sources are as varied as their functionality ranging from microorganisms (xanthan gum), algae (carrageenans) and vegetal exudates (tragacanth gum and arabic gum), among others. Xanthan gum is an anionic heteropolysaccharide which forms aggregates. Xanthan solutions are highly pseudoplastic and exhibit high viscosity (Katzbauer, 1998; Viebke & Williams, 2000). This gum is commonly added to oil-in-water emulsions to enhance the viscosity of the continuous phase and to retard creaming

(Dickinson, 2009). Carrageenan is a water soluble polysaccharide consisting of potassium, sodium, magnesium and calcium sulphate esters of galactose and 3,6-anhydrogalactose copolymers. There are different kinds of carrageenan: kappa (I and II), iota and lambda, with varying numbers and positions of the sulphate groups on the galactose dimmer (van de Velde, 2008). Tragacanth is one of the most important gums from commercial viewpoint (Stephen and Churms 1995). It is an exudate composed basically of high molecular weight polysaccaharides (galactoarabans and acid polysaccharydes), which contains galacturonic acid. Gum arabic is a branched, neutral or slightly acidic, complex polysaccharide obtained as a mixed calcium, magnesium, and potassium salts. It has excellent emulsifying properties and its solutions are characterized by a low viscosity, allowing the use of high gum concentrations in various applications (Dziezak, 1991; Verbeken et al., 2003). Mentioned gums are all generally recognized as safe (GRAS) additives.

Food biopreservation is based upon the use of antagonistic substances produced by microorganisms to set an additional hurdle against spoilage and pathogenic microflora. The production of these substances can be done *in situ* or *ex situ*, being the latter a hard accomplishment since it needs the approval of official regulation agencies which takes time and money. Conversely, the *in situ* production of bacteriocin-like substances is undertaken by the bacteriocinogenic bacteria within the food, fact that is already approved, i.e. the producing bacteria is considered GRAS. In the light of this advantageous scenario, the use of lactic acid bacteria as a source of antimicrobial compounds has been growing in the last decades as a natural alternative for food preservation.

Bacteriocin production and effectiveness are both dramatically influenced by the food matrix. Several reports showed bacteriocin interactions with proteins, fat (Aasen et al., 2003) and macromolecules (Dicks & Todorov, 2004) that jeopardized the antimicrobial activity of these

substances. Furthermore, the production of bacteriocin-like substances (BLIS) has been slowed or inhibited by environmental factors such as pH, temperature and salts (Verluyten et al., 2003; Sarantinopoulos et al., 2002). To our knowledge, there are no data available up to now about the effects of gums on the production and effectiveness of BLIS. Therefore, the primary objective of this study was to determine whether the presence of xanthan gum, carrageenan, tragacanth and arabic gum influence the growth and *in situ* production of BLIS by *Lactobacillus sakei/curvatus* ACU-1.

2. Materials and methods

2.1 Bacterial strains and culture conditions

The strain *Lactobacillus sakei/curvatus* ACU-1, previously isolated from artisanal dry sausages, was the bacteriocin-producing strain used in this study. *Listeria innocua* ATCC 33090 (in lieu of *Listeria monocytogenes*) and *Staphylococcus aureus* FBUNT (obtained from clinical isolates and identified by the Microbiology Department of Facultad de Bioquímica, Química y Farmacia, Universidad Nacional de Tucumán (FBUNT), Argentina) were used as indicator microorganisms. The strains were maintained as frozen stocks at -30°C. Prior to being used the indicator strains were recovered in Brain Heart Infussion (BHI, Biokar Diagnostics, Beauvais, France) at the convenient temperature for each one (*L. innocua* ATCC 33090 at 30°C and *S. aureus* FBUNT at 37°C) while the bacteriocin-producing strain was recovered in MRS broth at 30°C.

2.2 Effect of several thickening agents on growth and bacteriocin activity

The growth of, and bacteriocin production by, *L. sakei/curvatus* ACU-1 strain was assessed using MRS broth supplemented with 0.5 g/100 ml of the following gums: xanthan, arabic, tragacanth and λ-carrageenan, all of them from Sigma-Aldrich (USA). These additives were added to MRS

broth by stirring (1400 rpm) at room temperature during 1 hour to ensure complete hydration of the polymer. The pH was adjusted to 6.4 $\pm$ 0.2 with drops of 4N HCl if necessary. Each suspension was placed in tubes and sterilized in autoclave for 15 min. Tubes containing 15 ml of the different formulations were inoculated at a concentration of 1 ml/100ml with an overnight culture of the bacteriocin-producing strain (~ 10^8 cfu/ml) and incubated at 30°C. A positive control consisting of MRS broth without gums was assessed. Aliquots were retrieved after 0, 3, 6, 9, 12, 15, 24 and 36 h to determine bacterial count, pH and the production of bacteriocin along the bacterial growth. Cell numbers were determined by spreadplating on MRS agar, incubating the plates at 30°C for 72 h. By means of a glass electrode attached to a pHmeter (Oakton®, Eutech Instruments, Singapore), pH of each BLIS was measured. Bacteriocin production was determined from 6 hours of storage by the agar well diffusion assay (AWDA) described by Schillinger and Lücke (1989) using *L. innocua* and *S. aureus* as indicator microorganisms. Arbitrary units (AU) per ml were calculated as *AU= (1000/v)/d*; being *v*: volume seeded in the well and *d*: dilution (Kouakou et al., 2009).

2.3 Statistical analyses

All experiments were carried out in duplicate and replicated twice. The maximum specific growth rate (μ_{max}) was estimated from the experimental data which were fitted to the modified-Gompertz equation (Zwietering et al., 1990) with the Marquardt algorithm by using STATGRAPHICS® Plus version 4.0 software (Statistical Graphics Corp., USA). A variance analysis (ANOVA) was applied to establish whether significant differences ($p < 0.05$) existed between the values obtained for the means of every trial conducted.

3. Results

The fitting of the experimental data to the modified Gompertz equation helped to find *L. sakei/curvatus* ACU-1 growth parameters for each of the different environmental conditions provided by the four gums tested in this study (Table 1). Specific growth rate (μ) of the strain was not significantly affected by the presence of xanthan gum compared to the control system without added gum. Nevertheless, tragacanth, λ-carrageenan and arabic gum increased the specific growth rate of the strain, even duplicating the value (tragacanth gum's) when compared to xanthan gum. Regarding maximal decimal logarithm counts (N_{max}), all of them were around 8 log [CFU/ml].

Maximum titres of the BLIS produced by *L. sakei/curvatus* ACU-1 were obtained for all systems after 36 h of incubation and are depicted in table 2. It can be observed that, all the values were the same for each indicator microorganism. This fact is of relevance since this BLIS showed to have dissimilar sensibility towards *L. innocua* and *S. aureus* (Castro et al., 2011). Xanthan gum, tragacanth and λ-carrageenan promoted the release of BLIS into the medium duplicating the titre of the control system against both indicator microorganisms. Arabic gum was the only one that diminished bacteriocin production giving half of the titre of the control system. The pH of BLIS obtained from 36 h culture was within the range of 4.21-4.31 systems.

4. Discussion

The growth parameters considered herein were selected as a practical tool to compare the influence of the gums in the growth of the bacteriocin-producing bacteria *L. sakei/curvatus* ACU-1, and consequently, in the production of its BLIS. It has been assumed that the gums tested had no significant effect on bacterial growth since only a slight effect on bacterial growth rate was

131 detected and similar maximum population at the stationary phase were found. From a rheological
132 point of view, the studied gums have dissimilar characteristics. To illustrate, arabic gum solutions
133 have low viscosity and a Newtonian behavior while xanthan gum solutions have high viscosity and
134 a pseudoplastic behavior (Verbeken et al., 2003). Despite this fact, the rheology of gum solutions
135 seemed to have no influence in the availability of nutrients since no effect on *L. curvatus/sakei*
136 ACU-1 growth was observed.

137 Regarding bacteriocin production, the three gums promoting it share a chemical pattern, i.e.
138 lateral chains with a high degree of substitution and repulsion effects. Bacteriocin molecules are
139 bound to the producer cell by means of hydrogen bonds (Abee et al., 1995). These charged
140 hydrocolloids could interact with the cellular membrane releasing the bacteriocin into the medium
141 which would promote the increase of bacteriocin production. In contrast, the low bacteriocin
142 production obtained with the arabic gum solution system suggests another hypothesis. This gum is
143 a neutral or slightly acid salt of a complex polysaccharide. It has excellent emulsifying properties,
144 thanks to its hydrophobic polypeptide backbone that strongly adsorbs at the oil–water interface
145 (Verbeken et al., 2003). Adsorbed gum to the cellular membrane could lower the release of
146 bacteriocin into the medium.

147 Although the specific growth rates (μ) showed statistical significant differences between them and
148 the control system in some cases, it could not be possible to correlate them with the BLIS titres of
149 the systems. The discussion among the nature of bacteriocins is still controversial, oscillating
150 between primary and/or secondary metabolites (Delgado et al., 2005; 2007). Our results suggest a
151 secondary metabolite pathway since bacteriocin production reached maximum values during late
152 exponential phase of growth and/or early stationary phase of growth (data not shown). Several
153 authors highlighted the intimate connection between textural characteristics of the environment

where bacterial cells are grown and their behavior against inhibitory substances (Brocklehurst et al., 1995; Castro et al., 2009). So, the regulation of bacteriocin production in a gum solution as well as its relationship to growth is far from being completely understood. New experiments are currently underway in order to investigate it in more detail.

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Table 1. *L. sakei/curvatus* ACU-1 growth parameters derived from Gompertz equation.

Estimated parameters	μ_{max}±SD (h^{-1})	N_{max}±SD (log CFU/ml)
Control	0.25^{a}±0.01	8.72^{b}±0.01
Xanthan Gum	0.23^{a}±0.01	8.60^{a}±0.04
Tragacanth Gum	0.46^{b}±0.02	8.76^{b}±0.01
Arabic Gum	0.29^{c}±0.01	8.74^{b}±0.03
λ-Carrageenan	0.33^{d}±0.01	8.76^{b}±0.05
pV	$< 1x10^{-4}$	0.0006

μ_{max} :maximum specific growth rate; N_{max}: maximal decimal logarithm counts. SD: standard deviation. Within columns, different letters indicate significant differences.

Table 2. *L. sakei/curvatus* ACU-1 BLIS titres against indicator microorganisms after 36 h of incubation.

System	***L. innocua*** (AU/ml)	***S. aureus*** (AU/ml)
Control	266	266
Xanthan Gum	533	533
Tragacanth Gum	533	533
Arabic Gum	133	133
λ-Carrageenan	533	533

AU: Arbitrary units.

Average of three trials is informed