INTRODUCTION

Worldwide, food-borne illnesses caused by pathogenic microorganisms are among the largest cause of mortality, killing several millions of persons each year (WHO, 2006). In the most developing countries, frequent ones (diarrhea, cholera and typhoid fever) are transmitted by contaminated water and foodstuffs (Kuete, 2010). Antibiotics are still widely used for treatments, and the result is an alarming increase and spread of pathogenic bacteria that are resistant to multiple antibiotics, a growing threat in medicine nowadays. Therefore, there is a pressing need to find or develop novel natural antimicrobial agents. Lactic acid bacteria (LAB) have long been used as supplements to restore intestinal balance disorders by altering the gut flora as well as by their antimicrobial properties (using API 50 CHL kits, BioMérieux, Marcy l’Etoile France) and biochemical characteristics (using API 50 CHL kits, BioMérieux, Marcy l’Etoile France) and biochemical characteristics (using API 50 CHL kits, BioMérieux, Marcy l’Etoile France) and biochemical characteristics (using API 50 CHL kits, BioMérieux, Marcy l’Etoile France) and biochemical characteristics (using API 50 CHL kits, BioMérieux, Marcy l’Etoile France) and biochemical characteristics (using API 50 CHL kits, BioMérieux, Marcy l’Etoile France) and biochemical characteristics (using API 50 CHL kits, BioMérieux, Marcy l’Etoile France) and biochemical characteristics (using API 50 CHL kits, BioMérieux, Marcy l’Etoile France). The acid and bile tolerant strain of Lactobacillus plantarum 2S isolated from sha’a, a maize-based traditionally fermented beverage from Cameroon, was screened for bacteriocin properties and its viability in local pasteurized honey. Bacteriocin activity were checked after their treatment with different enzymes, organic solvents, sodium chloride (NaCl) and detergents as well as their heat stability and effect of pH was studied. This strain produced an antimicrobial substance sensitive to proteolytic enzymes (trypsin and pepsin), thus confirming its proteinaceous nature. pH changes and heat treatment up to 121°C had no effect on the activity of the bacteriocin produced. This bacteriocin inhibited the growth of various indicator organisms with Lactobacillus plantarum 5S having the widest inhibition spectrum. Besides, it showed broad antibacterial activity spectrum against Gram-positive and Gram-negative pathogens including several that are classified as Especially Dangerous Infections by World Health Organization. The mode of action against Lactobacillus plantarum 5S and Salmonella enteritidis subsp. enterica serovare Typhi ATCC 6539 is bacteriostatic. After 28 days of storage at 4 °C or 25°C, the viable cell numbers of all bacterial strain in both samples were not changed. Lactobacillus plantarum 2S exhibited best viability in honey stored at 4 °C for 28 days and did not altered the physico-chemical characteristics of honey.

Keywords: Lactobacillus plantarum, probiotic, bacteriocin, honey, viability.

MATERIAL AND METHODS

Bacterial strains, media and growth conditions

The strain Lactobacillus plantarum 2S used in this study was obtained from our laboratory collection. In our previous studies, this strain was isolated from “Sha’a”, a Cameroonian maize-based traditionally fermented beverage, and identified as Lactobacillus plantarum based on physiological and biochemical characteristics (using API 50 CHL kits, BioMérieux, Marcy l’Etoile France) and rep-PCR genomic fingerprinting; It showed bacteriocinogenic activity using triple agar layer test and proved to be free from virulence factors like hemolytic and gelatinase activities as well as antibiotics resistance (Kakhtam et al., 2012a). Lactobacillus plantarum 2S strain was maintained as frozen stocks at –20 °C in MRS broth (Biolife®, Milano, Italy) supplemented with glycerol (30% v/v) and propagated twice (1% inoculum) in MRS broth for 14 – 16 h at 37 °C before experimental use. Agar and soft agar media were prepared by the addition respectively of 1.5% and 0.75% (w/v) granulated agar (Merck, Darmstadt, Germany) to broth media. For bacteriocin production, a modified medium (mMRS), was used. The mMRS broth was prepared by dissolving all the ingredients (from Oxoid, Basingstoke, UK; Merck, Darmstadt, Germany; and Sigma, St Louis, USA) of the MRS broth, except ammonium citrate which was replaced by ammonium sulfate. After adding all medium components, the mixture was autoclaved. The other bacteria strains used as indicator organisms were revived from frozen stocks (at -20 °C on slants) and grown using the appropriate media and temperature as indicated in Table 3. Lactobacillus plantarum 5S is a bacteriocin’s highly sensitive LAB strain resulting from our previous studies.

Assessment of the ability of the strain to resist conditions of the gastrointestinal tract (GIT)

Acid Tolerance

Strain L. plantarum 2S was preliminary selected amongst many other lactobacilli using a rapid test according to the method described by Pelinescu et al., (2009).
For real assessment of the acid tolerance, strains were cultured on MRS agar medium for 24 h at 37 °C. Colonies were collected and suspended in 0.2 mol.l\(^{-1}\) citrate buffer pH 3 (used as representative gastric pH value), and the turbidity of cells suspensions adjusted to 4 Mc Farland standard (12 × 10\(^{10}\) CFU/ml). This prepared cells suspension was incubated with 0 h and 6 h at 37 °C, followed by centrifugation (3500g, 15 min), washing twice using 0.5 mol.l\(^{-1}\) phosphate buffer pH 7 and re-suspension in physiological saline. The viable cells were enumerated by pour plate technique. Appropriate serial dilutions in 0.1% peptone water were done and plates were incubated anaerobically in GasPak anaerobic jar (Genbox anae; BioMérieux, France) at 37 °C for 48 h (Verdenelli et al., 2009). Results were expressed as the percent (log CFU) of resistant cell.

Bile salt tolerance

Bile salts tolerance was also evaluated as described by Verdenelli et al., (2009). L. plantarum 2S was cultured on MRS agar slant for 24 h at 37 °C. Colonies were collected and suspended in 0.5 M phosphate buffer, pH 7 supplemented with 0.4% (w/v) bile salts (50 % sodium taurocholate and 50 % sodium glycocholate; Sigma, St Louis, USA) and the turbidity of cells suspensions adjusted to 8 Mc Farland standard (24 × 10\(^{8}\) CFU/ml). The resulting cells suspension was treated as previously described.

Detection and partial characterization of the bacteriocin produced by L. plantarum 2S

Bacterial growth and bacteriocin activity assay

A 14-hour-old culture of L. plantarum strain 2S was inoculated (2% v/v) into mMRS broth and incubated at 30 °C without agitation. Changes in cell density (O.D\(_{600}\)) were recorded every 2 h up to 14 h; the pH of the culture and bacteriocin activity were measured at the same time interval. Bacteriocin activity in liquid medium was detected using Agar Well Diffusion Assay (AWDA) according to Schillinger and Lücke (1989); the culture was centrifuged at 4000 rpm for 30 min at 4 °C and the supernatant was collected, adjusted to pH 6.5 with 6 mol.l\(^{-1}\) NaOH, heated at 80 °C for 10 min, and filter-sterilized through a 0.22 µm pore-size membrane filter (Millipore Corporation, Bedford, MA, USA) using an appropriate syringe to prepare Neutralized Cell-Free Supernatant (NCFS). Soft MRS agar seeded with the indicator strain L. plantarum 5S (approximately 10\(^{5}\) CFU/ml) was overlaid onto pre-poured MRS agar plates; six millimeter diameter wells were punched in the plates and filled with 50 µl of supernatant. After incubation of the plates anaerobically at 30 °C for 24 h, diameters of zones of inhibition were measured using a caliper. Experiments were performed in triplicate.

Sensitivity of the bacteriocin to enzymes, temperature and pH

In this set of experiment, neutralized cell-free supernatant was treated with the following enzymes in 1mg/ml final concentrations: trypsin (Fluka Biochemika, Buchs, Switzerland) in 0.05 M Tris-HCl buffer, pH 8.0; pepsin (Sigma-Aldrich, Steinheim, Germany) in 0.05 mol.l\(^{-1}\) citrate buffer, pH 2.0; α-Amylase (Sigma-Aldrich, Steinheim, Germany) in 0.05 M phosphate buffer, pH 7.0; lipase (Sigma-Aldrich, Steinheim, Germany) in 0.05 mol.l\(^{-1}\) phosphate buffer, pH 7.0; and l-lysylzme (Fluka Biochemika, Buchs, Switzerland) in 0.05 M phosphate buffer, pH 7.0. Incubation was held at 37 °C for 2 h and enzymes reactions were terminated by boiling for 5 min. To determine the effect of pH, the neutralized cell-free supernatant was adjusted to pH 2.0 - 12.0 (at increments of two pH unit) using 6 mol.l\(^{-1}\) HCl or NaOH. After incubation at 37 °C for 2 h, the treated samples were neutralized before tested for residual activity. The effect of temperature on the bacteriocin was assayed by heating the neutralized cell-free supernatant at 37 °C and 100 °C for 30, 60, 90, 120 min, and at 121 °C for 15 min. Cell-free supernatants treated with buffers only and sterile MRS broth treated with enzymes served as controls; after each treatment, the residual activity was determined by AWDA using L. plantarum 5S as indicator strain. All experiments were performed in triplicate.

Spectrum of inhibitory activity

The antibacterial activity of the neutralized cell-free supernatant was tested against Gram-positive and Gram-negative bacteria. The indicator strains (0.5 Mac Farland suspensions) were inoculated in the appropriate soft agar media (~10\(^{9}\) CFU/ml) and the antibacterial activities were determined by AWDA as previously described. All experiments were conducted in triplicate.

Mode of bacteriocin action

Twenty milliliter of neutralized cell-free supernatant was added to 100 ml of a 3 h-growing cultures of Salmonella enterica serovar Enteritidis L74 stock. The cultures were harvested at 55% respectively in Mueller-Hinton broth and MRS broth at 37 °C. Changes in cell density were recorded at 600 nm hourly for 8 h, and the number of viable cells (CFU) was determined by plating the samples on Salmonella-Shigella agar and MRS agar (1.5% w/v) followed by incubation at 37 °C for 48 h. Growing cultures in which 20 ml of sterilized distilled water was added served as controls.

Assessment of the stability of the bacteriocin-producing L. plantarum 2S strain in honey

Preparation of honey samples and inoculation

The honey sample used in this study was a wild polyfugal type, procured from a local beekeeper of the sudano-guinean zone of West-Cameroun. It was obtained by draining the honey after manually uncapping the comb frames, and pasteurized at 63 °C for 30 min.

In another experiment, L. plantarum culture were propagated twice in MRS broth and incubated at 37 °C for 16 h. Seventy milliliter of broth media were inoculated with 10% overnight, activated culture of strain L. plantarum 2S. The growth of the culture in broth media was monitored using spectrophotometer (Pharmacia LKB NOVASPEC II) for 6 h. At the early stationary growth phase, bacterial cells were harvested from the media by centrifugation of the culture (4000 g, 10 min, 4 °C). The pellet was then washed twice with 0.01 mol.l\(^{-1}\) potassium phosphate buffer pH 7.0. The resultant pellet was suspended in 100ml sterile potassium phosphate buffer 0.01mol.l\(^{-1}\) and stored at –20°C until used. The viable cell counts of this suspension was determined by enumeration on MRS plate agar and incubation at 37 °C for 48 h in anaerobic. 120 µl of this concentrate (about 2.8 × 10\(^{10}\) CFU/ml) was introduced into 100 ml of pasteurized honey and thoroughly homogenized. Non-inoculated samples were used as control. Inoculated and non inoculated pasteurized honey samples were stored at 25 °C (room temperature) and 4 °C respectively for 28 days.

Assessment of L. plantarum 2S viability in pasteurized honey

From the inoculated pasteurized honey samples, an aliquot of 10 ml was taken at 1-week interval for viable count. Ten-fold serial dilutions were done in 0.1% peptone water while homogenizing by vortexing, followed by viable cell count by pour plate technique using MRS agar. Plates were incubated anaerobically at 37°C for 48h.

Evaluation of physico-chemical characteristics of honey samples during storage

Physico-chemical characteristics frequently used as best indicators for quality and stability of honey such as pH, moisture, free acidity were determined (Codex Alimentarius, 1981). Moisture was determined using IUPAC method (IUPAC, 1979). The pH was measured by a pH-meter (Searchtech) on 10% (v/v) honey sample in CO\(_2\) free distilled water (Moogning et al., 2011). The density of samples was determined by the method described by AFNOR (1981). For the determination of free acidity, the harmonized method of the International Honey Commission was used (Bogdanov et al., 1997). In this respect, 10 g of each sample was dissolved in 75 ml of CO\(_2\)-free water in a 250 ml beaker, stirred to homogenize and the initial pH of the solution recorded. Afterwards, the solution was titrated with 0.1 mol.l\(^{-1}\) NaOH to pH 8.30. The free acidity, express as milliequivalents or millimoles acid.kg\(^{-1}\) honey represented the volume (in ml) of 0.1 mol.l\(^{-1}\) NaOH used to bring the initial pH of the solution to 8.30, multiplied by the factor 10. All these experiments were performed at 1-week interval.

Statistical analysis

The data obtained were subjected to Analysis of Variance (ANOVA) and multiple comparisons were done by Student-Newman-Keuls test, using the software GraphPad InStat (GraphPad Software Inc, V3).

RESULTS AND DISCUSSION

Tolerance to acidic pH and bile salts

Acid and bile salts tolerance are prerequisite for probiotics to resist the stressful conditions of the stomach and the upper intestine during their journey to the gastro intestinal tract. L. plantarum 2S demonstrated a high tolerance to acidic pH after 6 h of exposure in citric acid at 37 °C, by showing a survival percentage of 80.29. It also showed high tolerance to high bile salts concentration (3.7%) after 6 h of incubation at 37 °C, since a survival percentage of 86.32 was recorded.

Probiotics are health-promoting microorganisms. The criteria used to select potential probiotics are related to acid and bile tolerance, production of antimicrobial substances, cholesterol metabolism, production of useful enzymes and safety for food and clinical use (Ouwehand et al., 1999). In vitro survival of bacterial strains in low pH is a more accurate indication of the ability of strains to survive passage through the stomach. The organisms taken orally have to face stresses from the host which begin in the stomach, with pH between 1.5 and 3.0 (Corzo et al., 1999). Resistance to bile salts is generally considered as an
essential property for probiotic strains to survive the conditions in the small intestine. Results suggest that strain *L. plantarum* 2S could successfully transit the human stomach and may be capable of reaching the intestinal environment and functioning effectively there. In fact, many other studies demonstrated the capacity of *L. plantarum* strains to tolerate gastric acid and intestinal bile salts conditions (Khalili et al., 2009; Sirilun et al., 2010; Sieladie et al., 2011). Bile resistance of lactobacilli may be due to their ability to de-conjugate bile salts in the duodenum (De Smet et al., 1995).

**Kinetics of bacterial growth and bacteriocin production**

Bacteriocin activity of *L. plantarum* 2S was detected at early logarithmic growth phase (4 h) in mMRS broth at 30 °C (Figure 1). Maximum bacteriocin production (14 mm) was reached after 8 h of incubation (end of logarithmic phase) and the antibacterial activity slowly decreased afterwards. During cultivation (16 h), the pH of the medium decreased from 6.85 to 4.14 and the cell density increased from 0.13 to 2.11 (O.D 600nm). In this study, the detection of bacteriocin activity at early logarithmic growth phase may suggest that the peptide is secreted as a primary metabolite.

![Bacteriocin Activity vs pH and O.D 600 nm](image)

**Figure 1** Growth of *L. plantarum* 2S, change in pH and bacteriocin production in mMRS broth at 30 °C for 16 h without agitation. Legend: (●) = growth; (▲) = change in pH; the bars indicate diameters of zone of inhibition.

**Sensitivity of the bacteriocin to enzymes, temperature and pH**

The effects of proteolytic and non-proteolytic enzymes, temperature and pH on the bacteriocins activity were determined (Tab. 1). Complete inactivation of the antimicrobial activity was observed when the neutralized cell-free supernatant was treated by trypsin and pepsin, showing that the antibacterial substance was of a proteinaceous nature. Treatments with α-Amylase, lipase and lysozyme did not affect the antimicrobial activity, suggesting that the bacteriocin activity does not depend on a carbohydrate or lipid moiety; this bacteriocin might belong to Class I or Class II of the classification of bacteriocins. The stability of the bacteriocins at 37 °C suggest that it could fully retain its activity once produced in the GIT; its heat stability (up to 121 °C for 15 min) which is advantageous for transport and use as drug, could arise from the complex patterns of disulphide intramolecular bonds that stabilize secondary structures by reducing the number of possible unfolded structures (Deraz et al., 2009); but more investigation is required to confirm.

**Table 1** Effect of enzymes, pH and heat treatment on the antibacterial activity of the bacteriocin produced by *L. plantarum* 2S

<table>
<thead>
<tr>
<th>Factors</th>
<th>Diameter (mm) of zone of inhibition</th>
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</thead>
<tbody>
<tr>
<td>Enzymes</td>
<td></td>
</tr>
<tr>
<td>Without enzymes</td>
<td>13.00 ± 0.00</td>
</tr>
<tr>
<td>Trypsin and pepsin (1 mg/ml)</td>
<td>–</td>
</tr>
<tr>
<td>α- Amylase (1 mg/ml)</td>
<td>12.66 ± 0.57</td>
</tr>
<tr>
<td>Lipase (1 mg/ml)</td>
<td>12.66 ± 0.57</td>
</tr>
<tr>
<td>Lysozyme (1 mg/ml)</td>
<td>13.00 ± 0.00</td>
</tr>
<tr>
<td>pH</td>
<td></td>
</tr>
<tr>
<td>2 to 6</td>
<td>13.33 ± 0.57*</td>
</tr>
<tr>
<td>8-10</td>
<td>12.33 ± 0.57*</td>
</tr>
<tr>
<td>12</td>
<td>12.00 ± 0.00*</td>
</tr>
<tr>
<td>Heat treatment</td>
<td></td>
</tr>
<tr>
<td>37 °C for 30, 60, 90 and 120 min</td>
<td>14.00 ± 0.00*</td>
</tr>
<tr>
<td>100 °C for 30 and 60 min</td>
<td>13.66 ± 0.57*</td>
</tr>
<tr>
<td>100 °C for 90 and 120 min</td>
<td>12.33 ± 0.57*</td>
</tr>
<tr>
<td>121 °C for 15 min</td>
<td>11.66 ± 0.57*</td>
</tr>
</tbody>
</table>

* ± : With respect to the effect of pH, values of the activity with the same symbol are not significantly different (P>0.05).
* †: With respect to the effect of temperature, values of the activity with the same symbol are not significantly different (P>0.05).

Diameters of zone of inhibition were mean of triplicate, including the diameter of the wells (6 mm). ‘‘*’’- no inhibition zone was observed.

**Spectrum of inhibitory activity**

The antibacterial activity of the bacteriocin produced by *L. plantarum* 2S was evident not only on Gram-positive bacteria, but also on Gram-negative bacteria. This bacteriocin inhibited several food spoilage bacteria and foodborne pathogens, including *Staphylococcus aureus*, *Bacillus cereus*, *Streptococcus mutans*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Salmonella enterica* subsp. *enterica* serovar Typhi. However, no activity was detected against many other lactobacilli as well as an *Enterococcus faecium* strain tested (Tab. 2). The bacteriocin had a broad spectrum activity against a range of Gram-positive and Gram-negative pathogens, including several classified as Especially Dangerous Infections (EDI) by the World Health Organization (WHO). Interestingly, the bacteriocin was not active against several related bacteria, including *L. plantarum*, *L. rhamnosus* and *E. faecium*, suggesting that *L. plantarum* 2S could share the same niche with other useful LAB. Several other studies reported similar features observed with other bacteriocins produced by *L. plantarum* strains (Todorov et al., 2007, 2008; Gong et al., 2010; Kaktcham et al., 2012b), but they were for application as food biopreservatives and not to treat bacterial infections. Nevertheless, the studies of Charernjiratrakul et al., (2010) and Mkitchyan et al., (2010) reported cases of *L. plantarum* strain that could be used against infectious pathogens such as *vibrio parahaemolyticus* and *Salmonella enterica* serovar *Typhimurium ATCC 14028* NA respectively.
dependent experiments and the error bars
Figure 4
it is worth noting that the addition of sterile distilled
6
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Hernàndez
ced by

6539. Although the majority of the bacteriocins produced by LAB showed a

6
bacteriocin produced by

2S on the
growth of

2S in pasteurized local

2011; Xie et al., 2011). It is however worth noting that the
determination of the bactericidal or bacteriostatic effect in vitro is strongly
influenced by several aspects of the assay such as the number of arbitrary units,
the buffer and the culture medium, the purity of the antimicrobial agent,
the indicator strain and the cell density used (Hernàndez et al., 2005).

Figure 3 The effect of the bacteriocin produced by

2S on the
growth of

2S over a period of 8 h. Optical density recorded in the

2S in pasteurized local honey
Figure 4 showed the viable cell counts of

2S did not show significant changes (P>0.05) after storage at 4 °C for 28 days, as
a slight but not significant decrease (from 2.78 × 108 CFU/ml to 5.62 × 107 CFU/ml) was observed (Figure 4). The estimated survival percentage at this storage temperature was 90.66%. When the inoculated honey was stored at 25 °C, a significant decrease (P<0.05) of the number of viable cell was recorded, going from 2.78 × 108 CFU/ml to 6.30 × 107 CFU/ml after 28 days of storage. At this storage temperature, the estimated survival percentage was 64.47%.

Mode of action of the bacteriocin produced by

2S
The addition of the neutralized cell-free supernatant containing bacteriocin to
cells of

serovare Typhi ATCC 6539 in their early logarithmic growth phase (3 h-old) resulted in a significant decrease (P<0.05) in the
number of viable cells and cell density 1 h after the treatment; Afterwards, the
viable cell number remained constant whereas the cell density slightly but not
significantly increase over the following 4 h (Figure 2).

Figure 2 The effect of the bacteriocin produced by

2S on the
growth of

2S (Figure 3). It is worth noting that the addition of sterile distilled
water (control) instead of bacteriocin resulted in continuous increase of the
number of viable cells and cell density of the two indicators strains. The
bacteriocin produced by

2S has a bacteriostatic mode of action against

5S and

serovare Typhi ATCC 6539. Although the majority of the bacteriocins produced by LAB showed a
lethal effect (with or without cell lysis), a bacteriostatic effect has also been
described for plantarics (Athih et al., 2001; Hernàndez et al., 2005;

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Table 2 Antibacterial activity spectrum of the bacteriocin produced by

2S

<table>
<thead>
<tr>
<th>Indicator strains</th>
<th>Sources</th>
<th>Growth conditions</th>
<th>Diameters (mm)* of zone of inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lactic acid bacteria</td>
<td>Our collection</td>
<td>MRS®, 30 °C</td>
<td>14.00 ± 0.00</td>
</tr>
<tr>
<td>L. plantarum 5S</td>
<td>Our collection</td>
<td>MRS®, 30 °C</td>
<td>–</td>
</tr>
<tr>
<td>L. plantarum 9S</td>
<td>Our collection</td>
<td>MRS®, 30 °C</td>
<td>–</td>
</tr>
<tr>
<td>L. plantarum 11S</td>
<td>Our collection</td>
<td>MRS®, 30 °C</td>
<td>–</td>
</tr>
<tr>
<td>L. plantarum 29V</td>
<td>Our collection</td>
<td>MRS®, 30 °C</td>
<td>–</td>
</tr>
<tr>
<td>L. rhamnosus 18S</td>
<td>Our collection</td>
<td>MRS®, 30 °C</td>
<td>–</td>
</tr>
<tr>
<td>L. rhamnosus 1K</td>
<td>Our collection</td>
<td>MRS®, 30 °C</td>
<td>–</td>
</tr>
<tr>
<td>E. faecium DSM 13596</td>
<td>DSM</td>
<td>MRS®, 30 °C</td>
<td>–</td>
</tr>
<tr>
<td>Other Gram-positive bacteria</td>
<td>ATCC®</td>
<td>TB, 37 °C</td>
<td>13.66 ± 0.57</td>
</tr>
<tr>
<td>S. aureus ATCC 25923</td>
<td>ATCC</td>
<td>TB, 37 °C</td>
<td>12.66 ± 0.57</td>
</tr>
<tr>
<td>B. cereus ATCC 11778</td>
<td>ATCC</td>
<td>TB, 37 °C</td>
<td>13.00 ± 0.00</td>
</tr>
<tr>
<td>S. mutans DSM 20523</td>
<td>DSM</td>
<td>MH, 37 °C</td>
<td>12.66 ± 1.15</td>
</tr>
<tr>
<td>Gram-negative bacteria</td>
<td>ATCC</td>
<td>MH, 37 °C</td>
<td>13.66 ± 0.57</td>
</tr>
<tr>
<td>P. aeruginosa ATCC 27853</td>
<td>ATCC</td>
<td>TB,PCA,37 °C</td>
<td>14.33 ± 0.57</td>
</tr>
<tr>
<td>K. pneumoniae (Clinical isolate)</td>
<td>CPY®</td>
<td>TB,PCA,37 °C</td>
<td>14.00 ± 1.00</td>
</tr>
<tr>
<td>S. flexneri (Clinical isolate)</td>
<td>CPY®</td>
<td>N°, 37 °C</td>
<td>–</td>
</tr>
<tr>
<td>S. enterica subsp. enterica serovar Typhi ATCC 6539</td>
<td>ATCC</td>
<td>MH, 37 °C</td>
<td>13.66 ± 0.57</td>
</tr>
<tr>
<td>P. mirabilis (Clinical isolate)</td>
<td>CPY®</td>
<td>TB,PCA,37 °C</td>
<td>–</td>
</tr>
</tbody>
</table>

*Inhibition zone Diameters are means of three independent experiments. Wells (6 mm in diameter) were filled with 50 µl of Neutralized Cell-free supernatant (NCS). --*: no inhibition.

**DSM**: Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH, Brau. **ATCC**: American Type Culture Collection. **CPY**: Centre Pasteur Yaroude. **MRS**: de Man, Rogosa and Sharpe broth and agar. **TB**: Tryptone broth. **PCA**: Plate Count Agar. **MH**: Mueller Hinton Broth and Agar. **N**: Nutrient broth and agar.

A similar situation was observed when the neutralized cell-free supernatant containing bacteriocin was added to an early logarithmic growth phase culture of

5S (Figure 3). It is worth noting that the addition of sterile distilled
water (control) instead of bacteriocin resulted in continuous increase of the
number of viable cells and cell density of the two indicators strains. The
bacteriocin produced by

2S has a bacteriostatic mode of action against

5S and

serovare Typhi ATCC 6539. Although the majority of the bacteriocins produced by LAB showed a
lethal effect (with or without cell lysis), a bacteriostatic effect has also been
described for plantarics (Athih et al., 2001; Hernàndez et al., 2005;
The moisture, pH, free acidity, and density of honey were evaluated using the Codex Alimentarius [12]. The moisture content was less than the maximum 20% recommended by Codex Alimentarius [12], while the values of the density were in agreement with the standard value defined by the Harmonised Methods of the European Honey Commission, Apidologie [109].

Changes in physico-chemical characteristics of inoculated honey during storage

Free acidity, pH, moisture content and density are amongst the physico-chemical characteristic commonly used as main indicators of quality and stability of honey. After 28 days of storage at 4 °C and 25 °C, no significant change was observed in the physico-chemical parameters of the honey samples (Tab. 4). The moisture, pH, free acidity and density ranged from 17.25 to 17.90 %, 2.42 to 4.28, 101.0 to 102.5 mEq/kg, and 1.35 to 1.39 g/ml, respectively, for the honey samples stored at 25 °C. For the samples stored at 4 °C, the values of these parameters ranged from 17.35 to 18.10, 4.22 to 4.25, 101.25 to 102.50, and 1.34 to 1.39 g/ml respectively (Tab. 3).

**CONCLUSION**

In this study we demonstrated the ability of *L. plantarum* 2S to resist stressful conditions of the stomach and small intestine. The properties of its bacteriocin (broad antibacterial activity spectrum, heat stability, resistance to acidic and alkaline pH) indicate that it can be an alternative organism to use as probiotic in the treatment of infectious diseases. Pasteurized locally produced honey has been found to be a good matrix to convey this probiotic strain into the GIT of Humans or animals, provided that it is being stored at 4 °C. These results are promising and more studies are necessary to investigate the survival of other probiotic strains in local honey and the *in vivo* antimicrobial activity of the inoculated honey samples.

**REFERENCES**


