The Effect of *Pediococcus pentosaceus* Bacteriocin on *Listeria monocytogenes* in Soft Cheese

Likaa Mahdi, Sana’a AL-Kakei, and Luma Zwain

**Abstract**—Among antimicrobial materials produced by *Pediococcus pentosaceus*, bacteriocin have gained the greatest interest in food preservation as a natural substance. A bacteriocin producing *P. pentosaceus* was isolated from vegetables and human stool in a previous study. Purification, activity measurement, and characteristics of the bacteriocin was also determined. The yield of the bacteriocin was 9.08% and the specific activity was 11266.67 Au/mg. The specific activity was increased by 15322 fold and the molecular weight was 39KDa.

The antimicrobial activity assay against *Listeria monocytogenes* was measured by agar well diffusion method. Different concentrations had prepared of crude and purified bacteriocin and the most effective one was the purified bacteriocin at a concentration 1000 ub/ml. The highest antilisterial activity of the crude bacteriocin in soft cheese was at concentrations 2000 and 1000 ub/ml at the tenth day which was 1.5 and 1.9 CFU/ml alternately. So, *P. pentosaceus* bacteriocin showed significant activity against *L. monocytogenes*

**Keyword**—bacteriocin, *Listeria monocytogenes*, *Pediococcus pentosaceus*, soft cheese

**I. INTRODUCTION**

The bacterium *Listeria monocytogenes* causes listeriosis which could be a non-invasive disease, but could happened primarily as invasive disease especially in persons with underlying disease such as immunosuppression and HIV/AIDS, pregnant woman, unborn or newly delivered infants, and elderly persons [1]. Because of its widespread occurrence in natural environments and its ability to tolerate environmental stresses such as low pH, low temperature, and high salt concentrations, the risk factor for post food processing contamination with *L. monocytogenes* high [2]. [3]. Bio control of *L. monocytogenes*in food could be achieved by adding bacteriocin —producing bacteria, bacteriocin – containing fermentate, bacteriocin crude extract, or purified bacteriocin. In fact, lactic fermentations are thought to be the oldest preservation way known to human [4]. Several LAB bacteriocins with broad spectra of inhibitory activity offer potential applications in food bio preservation replacing commonly used chemical preservatives [5], [6].

Currently, interesting in bacteriocins is huge because of their inhibitory activity against food spoilage and foodborne pathogenic bacteria such as *L. monocytogenes*[7].

In this research, we studied antimicrobial activity of a bacteriocin produced by *Pediococcus pentosaceus* against *L. monocytogenes* in vitro and in vivo.

**II. MATERIALS AND METHODS**

**A. Bacterial Isolates**

*P. pentosaceus* isolates were previously isolated from vegetables and human stool and identified using API 50 CHL micro-identification system in addition to microscopic and biochemical tests according to [8]. *L. monocytogenes* isolates were isolated from raw milk collected from local markets and diagnosed according to [8].

**B. Bacteriocin Production**

Eight isolates of *P. pentosaceus* were grown in MRS at 30 °C for 24h. The cultures supernatants collected and adjusted to pH 6.5, filtered through 0.45μm Millipore filter, concentrated to 0.1 volumes by polyethylene glycol dialysis (Sigma PEG 20000) and sterilized again by filtration. This material treated as crude bacteriocin and kept frozen at -20 °C until use [9].

**C. Bacteriocin Purification**

Crude bacteriocin was purified according to [9]. The purified bacteriocin was recovered from butanol extract by electroendosomatic preparative electrophoresis (EPE) according to [10] and kept freeze-dried.

**D. Bacteriocin Concentration Measurement**

Bacteriocin concentration was measured using dye-binding method [11].

**E. Purity and Molecular Mass Measurement**

Purity and molecular mass of purified bacteriocin was measured using 15% sodium dodecyl sulphate – poly acryl amide gel electrophoresis (SDS-PAGE) according to [12] and low molecular weight protein kit (Pharmacia chemical Co.) used as marker.

**F. Effect of Temperature, pH, and Enzymes**

Thermal stability of the bacteriocin was determined by exposing the bacteriocin to a range of temperature 25-90 O C for 30 min., 100 O C for 1-6min., 121 O C for 15 min., and frozen for up to 30 days. The activity of bacteriocin at different pH values was determined by adjusting the pH to pH 3-11. Sensitivity of bacteriocin to different enzymes was determined using purified bacteriocin treated with the
following enzymes: papein, trypsin, proteinase K, pronase E, α-amylase, β-glucuronidase, β-galactosidase, and N-acetylglucosaminidase then boiled for 2 min. to inactivate the enzymes. After each treatment, bacteriocin was tested for antimicrobial activity against L.monocytogenes [13].

G. Effect of Crude and Purified Bacteriocin on L.monocytogenes

Agar well diffusion method was used to detect antimicrobial activity of the crude and purified bacteriocin produced by P. pentosaceus against L. monocytogenes isolates at different concentrations (1000, 500, 250, and 125) ub/ml according to [14].

H. Antilisterial Activity of Crude Bacteriocin in Soft Cheese

Five gram of soft cheese sample was inoculated into 1ml distilled water and sterilized for 15min., after cooling it was inoculated with L. monocytogenes (10^5 cell/ml) and homogenized for 15 min. The antimicrobial activity of crude bacteriocin was quantified by treatment the homogenized suspension with (500, 1000, and 2000) ub/ml bacteriocin inoculated with L. monocytogenes

Treatment applied to cheese was:

1) L. monocytogenes alone (control)
2) L. monocytogenes + 2000bu/ml
3) L. monocytogenes + 1000bu/ml
4) L. monocytogenes + 500bu/ml

All the treatments were incubated at 4°C for (1, 2, 3, 7, and 10) days. After each incubation period serial tenfold dilutions were made. 100µl of each dilution was cultured on nutrient agar (Hi-media-India) and incubated at 37°C for 24-48h. The log^{10} CFU/ml was counted.

I. Statistical Analysis

Results were expressed as the mean ± standard deviation (SD). The intergroup variation was assessed by one way analysis of variance (ANOVA) P<0.05 using sigma stat statistical software.

III. RESULTS AND DISCUSSION

A. Production and Purification of P. pentosaceus Bacteriocin

Eight isolates of P. pentosaceus were isolated from vegetables and human stool samples. After identification, these isolates were found to produce antimicrobial material and the isolate P. pentosaceus P.5 showed the highest antimicrobial activity. Bacteriocin activity was purified up to 15322 folds, the overall yield and activity are summarized in TABLE I.

### TABLE I.

<table>
<thead>
<tr>
<th>Purification Stages</th>
<th>Culture Stage</th>
<th>Dialysis against PEG</th>
<th>Butanol Extraction</th>
<th>EPE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vol.(ml)</td>
<td>1000</td>
<td>100</td>
<td>18</td>
<td>0.8</td>
</tr>
<tr>
<td>Total Activity(AU)</td>
<td>38000</td>
<td>34000</td>
<td>7120</td>
<td>3380</td>
</tr>
<tr>
<td>Bacteriocin Activity(AU/ml)</td>
<td>39</td>
<td>450</td>
<td>395.55</td>
<td>4225</td>
</tr>
<tr>
<td>Total Protein(mg)</td>
<td>18900</td>
<td>147.50</td>
<td>0.83</td>
<td>0.3</td>
</tr>
<tr>
<td>Protein Conc.(mg/ml)</td>
<td>18.9</td>
<td>147.5</td>
<td>0.83</td>
<td>0.375</td>
</tr>
<tr>
<td>Specific Activity(AU/mg)</td>
<td>2.01</td>
<td>2.305</td>
<td>474.66</td>
<td>11266.66</td>
</tr>
<tr>
<td>Yield(%)</td>
<td>100</td>
<td>94.59</td>
<td>19.24</td>
<td>9.08</td>
</tr>
<tr>
<td>Purification(fold)</td>
<td>1</td>
<td>12.59</td>
<td>2434.1</td>
<td>15322</td>
</tr>
</tbody>
</table>

B. Effect of Temperature, pH, and Enzymes

In order to test the effect of enzymes, 4225 AU/ml bacteriocin was tested for sensitivity to several proteolytic enzymes. The bacteriocin was sensitive to proteinase K and pronase E, and was resistant to α-amylase, β-glucuronidase, β-galactosidase, and N-acetylglucosaminidase as shown in TABLE II.

### TABLE II.

<table>
<thead>
<tr>
<th>Enzymes</th>
<th>Untreated Bacteriocin</th>
<th>α-amylase</th>
<th>β-glucuronidase</th>
<th>β-galactosidase</th>
<th>N-acetylglucosaminidase</th>
<th>Proteinase K</th>
<th>Pronase E</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treatment</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
</tbody>
</table>

The bacteriocin was incubated for 30 min at different temperatures and residual activity was measured, it was stable at 80°C but the activity gradually decreased with the increase in temperature. The residual activity was 95% after incubation at 5°C for 30 min. and total loss of activity was observed after incubation at 85°C as shown in Fig.1 (a) and TABLE III. When the bacteriocin was incubated at 100°C, the residual activity was 80% after 2-3 min. but it was completely absent after 3 min. incubation Fig.1 (b). Bacteriocin activity was not lost by cooling and freezing storage.
The bacteriocin was active at pH (4.0-8.00), and it lost its activity outside this range as shown in Fig. 2.

**C. Bacteriocin Molecular Mass**

SDS-PAGE analysis revealed a band similar to the apparent M.wt. 39KDa. The molecular weight of the bacteriocin produced by *P. pentosaceus*CFRB19 was 4.8KDa [16]. *P. pentosaceus* ACCEL produced a bacteriocin with molecular weight of 17.5KDa, and another bacteriocin with molecular weight of 80KDa [17].

**D. Bacteriocin Antimicrobial Activity**

The results indicated that *P. pentosaceus* bacteriocin in all concentrations (125, 250, 500, 1000) ub/ml possesses significant antimicrobial activity against *L. monocytogenes* in contrast with control P<0.05 and the antimicrobial activity of crude and purified bacteriocin in concentration 1000 ub/ml was higher than other concentrations (125, 250, 500) ub/ml. The antimicrobial activity of purified bacteriocin was significantly higher than with crude bacteriocin P<0.05 as shown in TABLE IV. Reference [18] showed that the antimicrobial activity of cell free extract from *Lactococcus lactis* and *Lactobacillus lactis* on the growth of *L. monocytogenes* was higher than *P. pentosaceus*.

**TABLE IV**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Concentration (ub/ml)</th>
<th>Inhibition Zone Diameter (mm) Mean ± S.D</th>
</tr>
</thead>
<tbody>
<tr>
<td>Crude</td>
<td>1000</td>
<td>21±1.2 b</td>
</tr>
<tr>
<td></td>
<td>500</td>
<td>14±0.98 b</td>
</tr>
<tr>
<td>Bacteriocin</td>
<td>250</td>
<td>11±1.11 b</td>
</tr>
<tr>
<td></td>
<td>125</td>
<td>9±0.75 b</td>
</tr>
<tr>
<td></td>
<td>1000</td>
<td>25±0.22 a b</td>
</tr>
<tr>
<td>Purified</td>
<td>500</td>
<td>19±1.3 a b</td>
</tr>
<tr>
<td>Bacteriocin</td>
<td>250</td>
<td>17±0.73 a b</td>
</tr>
<tr>
<td></td>
<td>125</td>
<td>13±0.89 a b</td>
</tr>
<tr>
<td>Control D.W</td>
<td>0</td>
<td>0±0</td>
</tr>
</tbody>
</table>

a = Probability to crude P<0.05, b = Probability to control P<0.05
H. Antilisterial Activity of Crude Bacteriocin in Soft Cheese

The number of *L. monocytogenes* in untreated soft cheese was much higher than its number in treated soft cheese with *P. pentosaceus* crude bacteriocin. The antilisterial activity of crude bacteriocin at concentration 2000 ub/ml was higher than other concentrations; the CFU at zero time was 5.7 CFU/ml ended with 1.5 CFU/ml at the tenth day as shown in Fig. 3.

![Image](image_url)

**Fig. 3** Antilisterial activity of crude bacteriocin

REFERENCES


http://dx.doi.org/10.1111/j.1365-2672.2006.02966.x