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**PURIFICATION OF BACTERIOCCIN FROM *ENTEROCOCCUS ITALICUS* ONU547 BY REVERSED PHASE-HIGH PERFORMANCE LIQUID CHROMATOGRAPHY**

**Abstract**

Pre-purified fractions of bacteriocin from *E. italicus* ONU547 were subjected to further purification by RP-HPLC with various methods. Two types of analytical columns (RP C<sub>8</sub> and C<sub>18</sub>), gradient and step elution methods were used for experiment. After using of both columns with gradient elution method on chromatograms two peaks were observed, which fraction exhibited antagonistic activity against *L. sakei* that could indicate the two component nature of the studied bacteriocin. The most effective was the RP-HPLC with C<sup>8</sup> column performed by step elution method resulted in bacteriocin activity given the zone of inhibition with diameter 1,75 sm. The purified fraction can be used for further study and characterization of bacteriocin.

Key words: bacteriocin, purification, *Enterococcus italicus*, reversed phase-high performance liquid chromatography

**Introduction**

Bacteriocins are ribosomally synthesized antimicrobial peptides produced by variety of bacterial species including lactic acid bacteria (LAB) [3]. The bacteriocins were suggested as biopreservatives in foods due to antagonistic activity against food-borne pathogens [4] and their anti-infective effect is a promising alternative to antibiotics [1]. Purification of bacteriocin is an important procedure needed for its study and characterization [7].

The aim of this study was to purify bacteriocin from *Enterococcus italicus* ONU547 by reversed phase-high performance liquid chromatography by various methods.

**Material and methods**

In the experiment were used the fractions of bacteriocin pre-purified before by ammonium sulfate precipitation, cation-exchange



and hydrophobic chromatographies according to Hwanhlem et al. (2013) [6] with some modifications. For performing of reversed phase-high performance liquid chromatography (RP-HPLC) the two types of analytical columns were used – C<sub>8</sub> (Symmetry C<sub>8</sub>, 3,5 μm, Ireland) and C<sub>18</sub> (Symmetry C<sub>18</sub>, 3,5 μm, Ireland). 100 μl of bacteriocin active fraction were injected into the analytical columns that were equilibrated with solvent A (98% MilliQ water / 2% acetonitrile / 0,05% TFA). The elution was performed by two methods: with the linear gradient of solvent B (20% MilliQ water / 80% acetonitrile / 0,04% TFA) and step elution by different concentrations of acetonitrile. The detected peaks were manually collected and the acetonitrile was evaporated by Speed-Vac concentrator, the pH was neutralized and activity of bacteriocin tested by agar well diffusion assay against indicator *Lactobacillus sakei* [5].

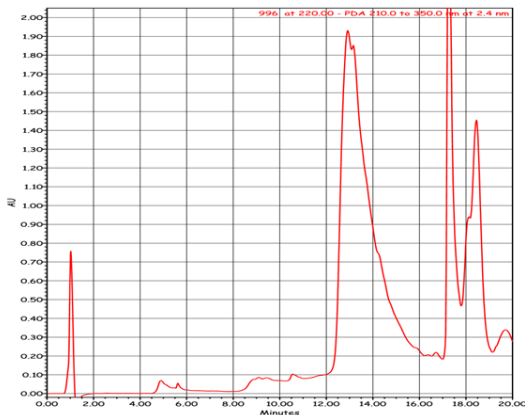
### Results and discussions

First method of elution was the gradient elution performed by solvent B. After using of C<sub>8</sub> as well as C<sub>18</sub> analytical columns two peaks were observed on chromatograms with retention time of 8-9 minutes. The fractions of two peaks were collected together and when tested by agar well diffusion assay against *L. sakei* showed inhibitory activity. It could indicate the two component nature of the studied bacteriocin. The diameter of inhibitory zone of pre-purified fractions before RP-HPLC was 1,7 sm and after using of C<sub>8</sub> column the diameter of inhibition zone was 0,9 sm and those of C<sub>18</sub> – 1,3 sm. It indicates the decrease of bacteriocin activity after purification with using of this method. The decreasing of total activity of bacteriocin after procedures of purification is in agreement with literature data [2].

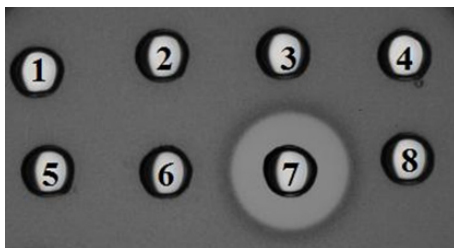
The next purification procedure was performed by RP-HPLC with step elution using different concentration of acetonitrile. In result, on obtained chromatogram several peaks were observed (Fig. 1) fractions of which were collected and activity was tested against *L. sakei*.

In case of C<sub>8</sub> column only first peak eluted by 100% acetonitrile showed antagonistic activity against target strain *L. sakei* (Fig. 2) with diameter of zone of growth inhibition of 1,75 sm. The fractions of all other peaks did not exhibit activity of bacteriocin that indicates the nature of contaminant which could be presented by non-bacteriocin proteins.

The activity of eluted fraction by this method was less in the case of C<sub>18</sub> analytical column and diameter of inhibition zone was only 1 sm that can be explained by higher hydrophobicity of column matrix and irreversible binding of bacteriocin to it resulted in decreasing of activity.



**Fig. 1. Chromatogram of purified bacteriocin by RP-HPLC with C<sub>8</sub> column performed by step elution with different concentration of acetonitrile: 1-20%, 2-30%, 3-40%, 4-5-100%**



**Fig. 2. Antagonistic activity of fractions after RP-HPLC with C<sub>8</sub> column tested by agar well diffusion assay against indicator *L. sakei* eluted by different concentration of acetonitrile: 1 – 20%, 2 – 3 – 30%, 4 – 6– 40%, 7 – 100% 1 peak, 8 – 100% 2 peak**

### **Conclusion**

After using of C<sub>8</sub> as well as C<sub>18</sub> analytical columns with gradient elution method two peaks were seen with retention time of 8-9 minutes and the fractions from these two peaks showed antagonistic activity against indicator *L. sakei* when tested by agar well diffusion assay. The most effective method of bacteriocin purification was RP-HPLC with C<sub>8</sub> column performed by step elution method resulted in bacteriocin activity given the zone of inhibition with diameter 1,75 sm. The purified fraction can be used for further study and characterization of bacteriocin.



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