Abstract

Bacteriocins are a widespread group of bacterial peptides and proteins that inhibit the growth of closely related (to the producer) species of bacteria. Currently, these compounds are mainly studied for their bactericidal activity and potential use in therapy, food industry or agriculture. However, their effect on host cells is still poor known.

Recently discovered peptide bacteriocin BacSp222, produced by the zoonotic *Staphylococcus pseudintermedius* 222 strain is the first known bacteriocin that undergoes succinylation at the ε-amino group of the lysine residues. BacSp222 exhibits antibacterial activity against Gram-positive bacteria, and, moreover, it also has the properties of a virulence factor and a peptide that stimulates the host's immune system.

The studies presented here have shown that BacSp222 succinylation by the producer strain depends on environmental conditions such as temperature, pH, access to nutrients, access to a selected carbon source, as well as the presence of a stressor such as nitric oxide. *In vitro* BacSp222 succinylation is a non-enzymatic reaction and the donor of the succinyl group is succinyl-coenzyme A (an important component of cell metabolism , e.g. in the Krebs cycle). On the other hand, desuccinylation reactions *in vitro* require the presence of a bacterial lysate and the oxidized form of nicotinamide adenine dinucleotide, what proves that this reaction is, most likely, enzymatic.

BacSp222 succinylation significantly reduces the lytic properties of bacteriocin, both against bacterial and eukaryotic cells. The modified forms of BacSp222 significantly reduce the killing activity against the producer cells, which suggests that the succinylation of the peptide is a form of self-protection against the bacteriocin toxic effects toward *Staphylococcus pseudintermedius* 222 cells. Given the known action of BacSp222 focused on bacterial membranes the observed drop in the lytic activity of the post-translationally modified forms of BacSp222 compared to the unmodified molecule is caused, most probably, by a change in the charge on the modified lysine residues from positive to negative. As a result, the molecule has lower affinity to positively charged bacterial cell membranes.

However, the pro-inflammatory activity of the succinylated forms of BacSp222 is identical to activity of the unmodified bacteriocin. The peptide is recognized by the TLR2/TLR6 heterodimer, causing MyD88-dependent activation of the NF-κB transcription factor. As a result of the stimulation of NF-κB, the synthesis of many pro-inflammatory cytokines is activated, including TNF (by mouse monocyte-macrophage cells) and IL-8 (by human neutrophils). Moreover, in the presence of IFN-γ, the peptide can stimulate mouse monocyte-macrophage cells to produce inducible nitric oxide synthase and, consequently, to secrete nitric oxide. Deprivation of the BacSp222 molecule of the N-terminal formylated methionine significantly reduces the inflammatory properties of the peptide, suggesting that the N-terminal fragment of the molecule is essential in the recognition of BacSp222 by the receptor.

BacSp222 is the first thoroughly tested bacteriocin that exhibits lytic properties against bacterial as well as eukaryotic cells. The presented experiments, in contrast to many other studies related to the

bacteriocins' subject described in the literature, were carried out using preparations thoroughly verified in terms of purity (including potential endotoxins' contamination). In addition, the pro-inflammatory properties of a bacteriocin molecule were demonstrated for the first time at carefully verified, non-toxic concentrations of BacSp222, while recognizing the receptor responsible for the interaction of the peptide with eukaryotic cells. The data obtained as part of the doctoral dissertation show potential for BacSp222 to be used in medicine. As a ligand of the TLR2 receptor, this molecule can be used in anti-cancer therapies or as an adjuvant in vaccines, but this requires further research.