**TITLE:** PURIFICATION AND ANTIMICROBIAL ACTIVITY OF ENTEROCIN TW21

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**ABSTRACT:**

Bacteriocins are antimicrobial peptides or proteins ribosomally synthesized by prokaryotes. Such compounds have been suggested as responses to the need for biopreservatives and new antimicrobials against emerging resistant bacteria. In 2008, Miguel and colleagues, searching for new natural antimicrobial substances, isolated from a meat pie sample a bacteriocin producing strain identified as *Enterococcus faecium* E86. In the bacteria genome, two bacteriocins genes were identified: enterocin P, a well-known peptide; and enterocin TW21 (EntTW21), which has been recently described and is still poorly characterized. Further studies developed by our research group in 2017 recognize the complete bacteriocinogenic cluster of enterocin TW21 in the *E. faecium* E86 genome ; and detected a mutation that leads to the truncation of the expression of the bacteriocin, making impossible to study EntTW21 in this strain. The present project aims to elucidate the three-dimensional structure (3D) of both TW21 and its hypothetical immunity protein (EntiTW21), as well as to characterize biotechnological properties of this enterocin, by heterologous expression of these compounds in *Escherichia coli* BL21 (DE3). EntTW21 and EntiTW21 were separately cloned in the plasmid pET-25(+) in constructions that include in the C-terminal region of each peptide a histidine tail (HisTag®) followed by GB1 protein and a thrombin cleavage site. The optimal expression of EntTW21 and EntiW21 were achieved in 4 hours at 16 ºC and at 37 ºC, with 1 mM IPTG. Both proteins were purified using nickel affinity chromatography and gel filtration, the protein EntTW21 only needed the first step to be purified. The expression yield for one liter was 2.05 mg for the bacteriocin and 22.9 mg for EntiTW21. Antimicrobial activity was observed for EntTW21 when attached to GB1. The bacteriocin presented a narrow spectrum, inhibiting *L. monocytogenes* and *Enterococcus* spp, but not *Micrococcus luteus*, *Staphylococcus aureus* and *E. coli.* The kinetic action of the EntTW21 construction was bacteriolytic for the strain *L. monocytogenes* 2968, with a minimal inhibitory concentration of 937,5 nM. Lastly, the stability of the peptide at 4 ºC was evaluated. The residual activity remained at 100% until the end of the first month and was reduced to 66.67% at the end of the second month. All characteristics presented by EntTW21 attached to the GB1 were compatible with those previously reported for other class IIa bacteriocins.

**Keywords:** Bacteriocin, heterologous expression, Histag®, enterocin TW21, antimicrobial activity

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