Yakult International Symposia Beneficial Microbes
March 27th and 28th, 2025
27 e 28 de Março, 2025
WYNDHAM SÃO PAULO IBIRAPUERA CONVENTION PLAZA
SÃO PAULO - BRAZIL

Characterization of a novel circular bacteriocin, streptocyclin BTW, and its diversity in the Streptococcus genus

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Streptococcus is a genus of gram-positive, ubiquitous bacteria that can also be found in the mouth, upper respiratory tract, and lower genital tract of humans. This group of microorganisms has been associated with severe infections and the development of resistance to antibiotics[1,2]. However, another characteristic that has been studied regarding this group is the production of bacteriocins. These compounds can be defined as ribosomally synthesized multifunctional peptides produced by prokaryotes, which can present a narrow or broad inhibitory spectrum. This class of peptides is usually hydrophobic and cationic, with the bacteriocinogenic strain presenting immunity to its own antimicrobial peptide. Bacteriocins can be divided into two main classes: (i) those with post-translational modifications and (ii) non-modified peptides[3]. Many class I bacteriocins have been described for the *Streptococcus* genus, especially lanthipeptides; however, only one circular bacteriocin was described for the group (uberolysin)[4]. The lack of characterized peptides of this group represents a promising field of knowledge to be explored. Due to this, this study has as its objective the characterization of streptocyclin, a 64-amino-acid circular bacteriocin produced by *Streptococcus devriesei* DSM 19639, as well as its diversity in the *Streptococcus* genus. The deferred antagonism assay was applied in order to understand the inhibitory spectrum of strains, which included *Enterococcus*, *Streptococcus*, *Micrococcus*, *Lactococcus*, *Listeria*, and *Clostridium* strains. Important to highlight that strain presented a pronounced activity against the *Streptococcus* genus. After, the colony mass spectrum of the strain was collected, where the mass of 6118.75 Da was observed, which matched with the predicted mass of the streptocyclin BTW. The genomic analysis revealed that the streptocyclicin BTW gene cluster is composed of five genes: core peptide (*stpA*), membrane protein (*stpB*), DUF95 family protein (*stpC*), ATP-binding protein (*stpD),* and putative immunity protein (*stpE*). When compared to other characterized circular bacteriocins, its closest relatives are caledonicin (50%), amylocyclicin (43.75%), amylocyclicin CMW1 (42.19%), and enterocin NKR-5-3B (40.63%). To further understand the distribution of this bacteriocin in the *Streptococcus* genus, a sequence similarity network was used with the propeptide of streptocyclicin BTW as the driver sequence. The results reveal the presence of streptocyclin-BTW-like circular bacteriocins was found only in *Streptococcus orisasini*, *Streptococcus equi*, *Streptococcus pneumoniae*, *Streptococcus pseudopneumoniae*, and *Streptococcus mitis* species. Purification of streptocyclin was done using the Jupiter Proteo semi-preparative column, running a 30-55% gradient over 5 minutes followed by a 55-100% gradient over 45 minutes, confirming its molecular mass of 6118 ± 1 Da. As far as we are concerned, this is the first report of a bacteriocin produced by the *Streptococcus devriesei* species and the second circular bacteriocin from the *Streptococcus* genus.

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Acknowledgements: The present work as funded by the European Union (ERC, BACtheWINNER, 101054719). Views and opinions expressed are however those of the author(s) only and do not necessarily reflect those of the European Union or the European Research Council. Neither the European Union nor the granting authority can be held responsible for them. This project has received funding from the European Union's Horizon 2020 Research and Innovation Programme under the INSPIRE COFUND Marie Sk?odowska-Curie grant agreement No. 101034270.