

# A NOVEL HIGH MOLECULAR WEIGHT BACTERIOCIN PRODUCED BY A THERMOPHILIC BACTERIUM

Manta Vaičikauskaitė, Marija Ger, Mindaugas Valius, Eglė Lastauskienė, Lilija Kalėdienė, Arnoldas Kaunietis

Department of Life Sciences Center, Vilnius University, Lithuania  
[manta.vaicikauskaite@gmc.stud.vu.lt](mailto:manta.vaicikauskaite@gmc.stud.vu.lt)

We have revealed that *G. stearothermophilus* 15 secretes a novel high molecular weight antibacterial protein geobacillin 26, which belongs to the III class of bacteriocins. We successfully purified native bacteriocin and determined its amino acid sequence and structural gene based on MS/MS analysis and genome mining. Geobacillin 26 has no sequence similarities to any known function proteins. This is the first report of high molecular weight bacteriocin produced by thermophilic bacteria. Usually, producers of III class bacteriocins encode proteins responsible for immunity to the bacteriocin [1, 2, 3, 4]. The genomic context of *geo26* does not include any genes that might encode proteins related to the latter function. The cell wall lysis assay confirmed that geobacillin 26 is not a cell wall degrading enzyme, as some III class bacteriocins [5], most probably it is killing cells by non-lytic mode of action. Geobacillin 26 is the first bacteriocin of this class, which has activity against thermophilic bacteria. Moreover, it has narrow antibacterial spectrum against some thermophilic (*Para*)*Geobacillus* sp. strains. We determined the MIC value of geobacillin 26 inhibiting growth of thermophilic bacterium *P. genomospecies* 1 NUB36187 – 620 nM (16.3 µg/mL). It is thermo-labile bacteriocin, its antibacterial activity is reduced by 50% after incubation at 60°C and completely lost after incubation at 90°C temperature.

This study revealed the function of hypothetical protein encoded in *G. stearothermophilus* 15. Geobacillin 26 does not contain any conservative amino acid sequences and domain structures characteristic to other bacteriocins. Hypothetical proteins sharing sequence similarity with this novel bacteriocin are encoded in other thermophilic *Geobacillus* sp., *Anoxybacillus* sp. or mesophilic *Bacillus* sp. bacteria. Geobacillin 26 is very interesting subject for research on novel antibacterial proteins, which potentially may have a new mode of action. Further studies could focus on the characterization of antibacterial activity mechanisms of the bacteriocin, its targets in the cell or receptors. Moreover, geobacillin 26 could be applied as antibacterial agent against other thermophilic bacteria, which are undesirable in some food or biotechnological industry.

- 
- [1] Kiss, A. *et al.* Cloning and characterization of the DNA region responsible for megacin A-216 production in *Bacillus megaterium* 216. *J. Bacteriol.* **190**, 6448–6457 (2008).
- [2] Beukes, M., Bierbaum, G., Sahl, H. G. & Hastings, J. W. Purification and partial characterization of a murein hydrolase, millericin B, produced by *Streptococcus milleri* NMSCC 061. *Appl. Environ. Microbiol.* **66**, 23–28 (2000).
- [3] Swe, P. M., Heng, N. C. K., Cook, G. M., Tagg, J. R. & Jack, R. W. Identification of DysI, the immunity factor of the streptococcal bacteriocin dysgalactin. *Appl. Environ. Microbiol.* **76**, 7885–7889 (2010).
- [4] DeHart, H. P., Heath, H. E., Heath, L. S., LeBlanc, P. A. & Sloan, G. L. The lysostaphin endopeptidase resistance gene (*epr*) specifies modification of peptidoglycan cross bridges in *Staphylococcus simulans* and *Staphylococcus aureus*. *Appl. Environ. Microbiol.* **61**, 1475–1479 (1995).
- [5] Acedo, J. Z., Chiorean, S., Vederas, J. C. & Belkum, M. J. Van. The expanding structural variety among bacteriocins from Gram-positive bacteria. *FEMS Microbiol. Rev.* **42**, 805–828 (2018).