Towards the elucidations of bacteriocins role in the shaping of ecology and evolution of Enterococcus faecium

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Background: Little is known about the diversity and distribution of bacteriocins in different Enterococcus faecium (Efm) populations, even though this species has been used in the food industry for decades. We aimed to create a Firmicutes bacteriocin database and to further evaluate its distribution in Efm and to unveil their potential role in the ecology and evolution of this species.

Materials/methods: Bacteriocin sequences (>22,000) were extracted from the Uniprot database, filtered by Efm. Confirmation was performed with InterPro/blastn/literature. Database sequences enrichment was performed using UNIREF_50/UNIREF_90-Firmicutes. Final bacteriocin sequences were established using clustal-omega (cut-off≥80.0%). 920 Efm genomes from GenBank/PATRIC and 14 from our laboratory (672-humans;102-animals;160-other) were included for bacteriocin analysis (custom-blastn/Geneious9.0). We performed a hierBAPS cluster analysis of the whole MLST database and the new STs found in this study (n=1457) as described for other bacterial species. This analysis was correlated with previous BAPS analysis performed for this species (correlation index=0.82). Bacteriocin clusters were defined by a heatmap generated by pheatmap-package (R-Studio).

Results: A homemade database of 76 Firmicutes’ bacteriocins (214 sequences;40 new bacteriocins), mostly belonging to classes I and II (Ila-IIc), was created. The most represented genera were Enterococcus (n=169), Bacillus (n=14), Lactobacillus (n=6) and Lactococcus (n=6) among other Firmicutes. Of these, 28 were present in Efm, being EntA (85.1%), Bac43 (38.6%), BacAS3 (37.9%) and BacAS32 (37.8%) the most prevalent. HierBAPS analysis revealed 9 clusters and 37 subclusters associated with different sources. Using a heatmap it was possible to define 6 main bacteriocin clusters (1 to 6) and 35 subclusters. Subclusters 1.1 (core:entA/bacAS3/bacAS3), 2.3 (core:entA/bac43) and 2.5 (core:entA) were the more prevalent (26.7%/20.3%/13.9%, respectively). These clusters were mostly present in hierBAPS 2.1a and 3.3 comprising most of the strains of hospital origin present in the MLST database. Less prevalent bacteriocins (≤20%), important for subclusters definition, might be associated with different hosts and environments.

Conclusions: This study enlarges the diversity of bacteriocins previously known in Firmicutes and its distribution in Efm lineages in particular. Further functional studies are required to unveil their spectrum activity and relevance in the evolution/lifestyle of particular Efm strains/hierBAPS clusters associated with specific hosts.