THE APPLICATION OF (META)GENOMICS TO BACTERIOCIN RESEARCH

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Introduction. As a consequence of the advent of next-generation sequencing technologies, genomic and metagenomic data can now be easily generated and a vast quantity of information can be easily accessed from public databases. These developments have had a major impact on the strategies employed to identify novel antimicrobial producers and antimicrobial-encoding gene clusters.
Here the bacteriocins, i.e. ribosomally synthesised antimicrobial peptides (1,2), will be used as an example to highlight how sequence-based advances have considerably enhanced our ability to identify novel representatives of the lantibiotic (bacteriocins distinguished by the presence of lanthionine bridges), sactibiotic (bacteriocins distinguished by the presence of sulphur to α-carbon linkages) and other classes within the bacteriocin family.

Methods. Fully sequenced microbial genomes were screened for the presence of type 1 and type 2 lantibiotic gene clusters and for sactibiotic gene clusters using the most highly conserved component of these clusters as the driver sequences.
In addition, in several instances the genes encoding putatively novel bacteriocin operons were identified through genome sequencing and bioinformatic analysis of the data generated.

Results. Screening of sequenced microbial genomes resulted in the identification of over 100 novel type 1 and type 2 lantibiotic clusters which unexpectedly were associated with species, genera and even phyla of bacteria which have not previously been associated with lantibiotic production (3, 4). This resulted in the ultimate identification of the novel lantibiotics, haloduracin (5) and lichenicidin (3). A similar strategy has also revealed that sactibiotic gene clusters are much more common than was originally appreciated (6).
With respect to genome sequencing, we have most recent employed this approach to identify a gene cluster in a *Lactobacillus salivarius* strain that was ultimately found to encode, Bactofencin. This novel bacteriocin is notable by virtue of more closely resembling eukaryotic defencin-like antimicrobial peptides than typical bacteriocins (submitted).

Conclusions. Screening of genomic and metagenomic databases provides a valuable means of identifying novel antimicrobial-encoding gene clusters. In this instance, multiple lantibiotic and sactibiotic gene clusters were identified at a frequency that suggests that these antimicrobials are much more widespread than previously thought. These clusters represent a rich repository which can yield a large number of valuable novel antimicrobials and biosynthetic enzymes.
Similarly, genome sequencing has revolutionized the approaches that we take upon the identification of putatively novel bacteriocins. Rather than relying of peptide purification and reverse genetics, mutagenesis or the creation of expression libraries, we can now rapidly identify the corresponding gene cluster through genome sequencing of the strain of interest. This information can be hugely valuable when deciding how best to purify the antimicrobial, predicting its antimicrobial spectrum and determining how best to commercially utilize the associated strain or bacteriocin.

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