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Paper Poster Session IV

Drug discovery - Gram-positives

Discovery of an unusual antimicrobial peptide, NI04, active against antibiotic-resistant Gram-positive bacterial pathogens

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Objective: Spread of antibiotic resistance (AMR) among bacteria, combined with diminished new antibiotic discovery, is an increasing threat to human health. Bacterially derived antimicrobial peptides (AMP) hold excellent potential as potent novel therapeutics. This study embraces natural antimicrobial product screening, combined with modern techniques that led to the discovery of peptide NI04 a promising AMP, produced by *Bacillus pumilus*.

Methods: Peptide NI04 was purified using solid phase extraction followed by high pressure liquid chromatography (HPLC) and its physicochemical properties were confirmed using enzyme and heat stability tests. Spectrum of activity and minimum inhibitory concentrations (MIC) was determined using a wide range of Gram positive pathogens, including antimicrobial resistant species. Toxicity of NI04 was determined towards human keratinocyte and monkey Vero cell lines using trypan blue exclusion and neutral red uptake assays and using mouse blood erythrocytes. The mass of the peptide was determined using MALDI TOF, mass spectrophotometry (MS). The draft genome sequence of the producer strain was obtained and the peptide sequence and underlying gene responsible for production was determined through a de novo peptide sequencing approach, using tags from tandem MS fragmentation of the tripsinised peptide using an Orbitrap MS system.

Results: NI04 inhibits a wide spectrum of Gram positive pathogens including Meticilin resistant *Staphylococcus aureus* and vancomycin resistant *Enterococcus* species at MICs of 20 ug/ml. In addition, no toxicity or haemolytic activity is observed up to 18x MIC concentrations. The MALDI-TOF MS analysis of the HPLC purified peptide revealed NI04 has a mass of 10722.993 Da. Sequence tags obtained were matched to the draft genome sequence. The gene responsible for the production of peptide NI04 has strong similarities to the ESAT-6/WGX100 peptide superfamily and more specifically with ESX-A peptide, which has been associated with virulence in a number of organisms including *S. aureus* and *Mycobacterium tuberculosis*; in some bacteria, the function has not been identified.

Conclusions: Our findings reveal an interesting observation as, to the best of our knowledge, there have been no antimicrobial peptides identified from this family and peptide NI04 does not fit with any existing bacteriocin classification scheme, as it is larger than 10 kDa while still being heat stable. We believe that further study of this peptide may reveal interesting new facts about bacterially derived AMPs.