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C46 De novo design of antimicrobial and antibiofilm peptides starting from desert truffle *Tirmania pinoyi* peptides

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With the aim of discovering new routes in the research of antimicrobials, we focused on polypeptide-enriched extracts derived from edible desert truffle mushroom *Tirmania pinoyi*. The extracts showed an interesting activity with MIC=50 µg/mL against *Staphylococcus aureus* ATCC 29213 and *Pseudomonas aeruginosa* ATCC 15442. Through mass spectrometry analysis (RP-HPLC/nESI-MS/MS) the following eight novel peptides FDVVPKTAANFRAL, AVTVGASTLADERA, FLVGGASLKPEF, VARIFAVFNDTF, HLVDEPQNLLK, LGEYGFQNALLR, FAVNGGCAKET, SREDLHPKL were detected. To characterize them online websites were used: IAMPpred, DPBAAS, Cell-PPD, ToxinPred, HemoPI, PeptideCutter and HLP. The analysis indicated that some peptides showed negative or neutral charge, hydrophobic ratio between 42% and 67%, Boman Index < 2.25 kCal/mol. According to the "APD3: Antimicrobial Peptide Calculator and Predictor" tool of the Antimicrobial Peptide Database (APD) similarities (around 30-40%) with known antimicrobial peptides (AMPs) identified in amphibians were also detected. In contrast, the predicted antimicrobial, antifungal and antibiofilm activity was not significant.

In order to improve biological and physico-chemical properties, the sequences of natural peptides were modified using APD3, by replacing some hydrophilic and negative charged amino acids with hydrophobic and positive ones. The derivative sequences (GWDVVPKTWWKFRAL, KWTWGASTLAKKRA, FLRGGWSLKPKF, KWRIFWVFNKTF, HLVKRWQNLLK, KGKYRFWNALLR, FARWGGCAKRT, SRKWLHPWL) showed net positive charge between +2 and +4, hydrophobic ratio between 42% and 48%, Boman Index < 2.25 kCal/mol and high stability. Moreover, the predicted antimicrobial, antifungal and antibiofilm activity was high, without toxic or hemolytic effects.

In conclusion, bioinformatic analysis has demonstrated that novel peptides discovered in *T. pinoyi* may be considered new platforms for the design of novel antimicrobial and antibiofilm peptides to counteract multi-drug resistant pathogens.

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