DOI: 10.25205/978-5-4437-1843-9-232

IDENTIFICATION AND FUNCTIONAL CHARACTERISATION OF NEW ANTIMICROBIAL PEPTIDES FROM MEDICINAL LEECH BY EXPRESSING THEIR CODING GENES IN *ESCHERICHIA COLI* *

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Abstract

The medicinal leech is an organism with a complex system for regulating its interactions with microorganisms. Its saliva contains a variety of molecules, including antimicrobial peptides. This study used a novel *recombinant genes expression*-based high-throughput screening method to identify new antimicrobial peptides finded in the *Hirudo medicinalis* genome.

The search for new methods to combat infectious diseases remains a relevant task for modern science. In this context, antimicrobial peptides (AMPs) represent great potential for the development of innovative approaches. The medicinal leech (*Hirudo medicinalis*) is a rich source of bioactive molecules, yet its potential as a source of new AMPs remains largely untapped. This study aimed to investigate the antimicrobial properties of peptides found in the medicinal leech genome and determine their potential, using a novel primary screening method based on the expression of recombinant AMP genes in *Escherichia coli* [1]. This method allows for the direct detection of AMP activity within bacterial cells, bypassing the limitations and complexities of traditional peptide synthesis and purification methods.

BLAST analysis of the *H. medicinalis* genome revealed 10 sequences homologous to known AMPs: five to lumbricin (*Lumbricus rubellus*, UniProt ID: O96447), four to neuromycin (*H. medicinalis*, UniProt ID: A8V0B3) and one to theromycin (*H. medicinalis*, UniProt ID: A8I0L8). Upon confirming the correspondence between the sequences of the potential antimicrobial peptides found in the genome and real data, it was revealed that most peptides matched completely, with the exception of LBrHM11, for which two variants were identified. One of these variants (LBrHM11*) contained a single nucleotide polymorphism that led to the substitution of leucine (L) for proline (P).

The analysis itself does not provide information on real antimicrobial activity and requires experimental verification. We evaluated the antimicrobial properties of the expressed AMPs using two complementary detection methods: measuring the kinetic optical density (OD_{600}) in a liquid medium and drop-plating serial dilutions onto agar. OD600 measurement allows for the quantitative assessment of bacterial cell growth in the presence or absence of an AMP gene transcription inducer. Drop plating of serial dilutions, on the other hand, allows for a visual assessment of bacterial colony density, making it possible to detect even the weakest antimicrobial activity. Combining these approaches increases the reliability of the results and provides a comprehensive assessment of the antimicrobial potential. Among lumbricin homologues, only LBrHM1 exhibited significantly higher antimicrobial activity than lumbricin I (UniProt ID O96447) [2], comparable to the activity of the well-known AMP melittin. For peptides from the macin group, however, the observed effect was less pronounced, remaining at the level of positive control peptides.

The sequence of lumbricin LBrHM1 was examined in detail based on the antimicrobial activity analysis, with a particular focus on the unique N-terminal fragment LBrHM1(1-23) and the fragment LBrHM1(27-55). LBrHM1 was selected for further study due to its unique activity profile. Furthermore, the presence of a region with no analogues in known antimicrobial peptide databases in the LBrHM1 sequence presents additional interest for research. The N-terminal fragment LBrHM1(1-23) exhibited high activity against *E. coli* and *B. subtilis*, whereas LBrHM1(27-55) predominantly acted against *B. subtilis*. This indicates that the different peptide regions contribute differently to the antimicrobial properties of the peptide.

These results highlight the significant potential of *H. medicinalis* as a source of new AMPs with diverse activity profiles. The *E. coli* expression system provides a reliable and efficient platform for the high-throughput screening and characterisation of these peptides. The discovery of LBrHM1(1-23) warrants further investigation as a potential therapeutic agent due to its unique sequence, broad-spectrum activity and lack of homology to existing AMPs.

References

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* The study is supported by the Russian Science Foundation (project #23-15-00084).