ECMC-P 2025 Conference

The 1st International Electronic Conference on Medicinal Chemistry and Pharmaceutics

01–30 November 2025 | Online

MDPI

Engineering a Bacterial Platform for Efficient Expression of Histatin 5: A Promising Candidate for Antimicrobial Therapy

V.S. Novikova[1,2], O.O. Klychkova[1], A.A. Lee[1], D.A. Kashirskikh[1], V.A. Khotina[1], V.A. Gasanov[1]

[1] Koltzov Institute of Developmental Biology of Russian Academy of Sciences

[2] Institute of Agrobiotechnology, Russian State Agrarian University - Moscow Timiryazev Agricultural Academy

INTRODUCTION & AIM

Antibiotic resistance remains a key medical challenge, highlighting the need for alternative antimicrobial agents. Antimicrobial peptides are promising candidates due to their broad activity and high selectivity. Histatins produced by salivary gland cells are notable for their fungicidal, bacteriostatic, wound-healing, and immunomodulatory properties. Recombinant production of histatins may offer a more feasible approach than chemical synthesis. This study aimed to develop a bacterial expression system for histatin 5 (Hst5), including cassette constructs designed to enhance expression.

METHOD

Plasmids pH5 and p10xH5 were constructed using the pET30(a) vector. Cloning was performed in *E. coli* XL1-Blue, followed by plasmid purification and restriction analysis. Peptide expression was performed in *E. coli* BL21(DE3) with IPTG induction. Peptides were detected by PAGE and purified using SP-Sepharose ion-exchange chromatography. Cassette peptide was hydrolyzed with cyanogen bromide to release Hst5 monomers. Peptide concentration was determined by the Bradford assay. Antimicrobial activity was assessed against *E. coli* using agar diffusion assay.

The 10xHst5 peptide was successfully purified using a two-step ion-exchange chromatography procedure. Hst5 was eluted at 170–290 mM NaCl. PAGE analysis confirmed the high purity of the target peptide (Figs. 4, 5).

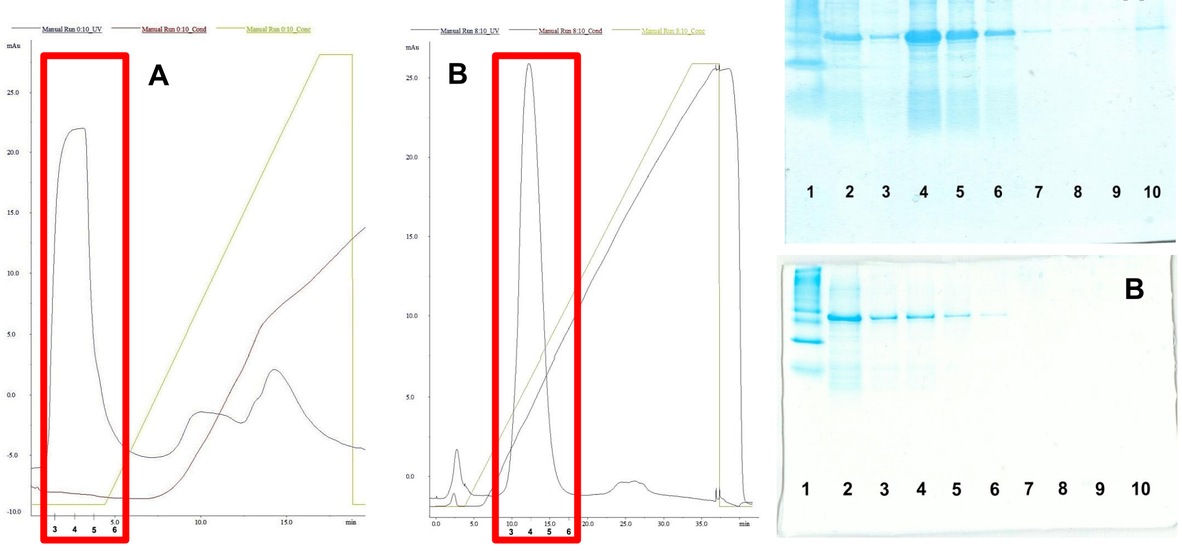

Figure 4. Chromatograms of the 10×H5 peptide. Q-Sepharose chromatography: the peptide peak elutes at 0–125 mM NaCl (**A**). SP-Sepharose chromatography: the peak elutes at 170–290 mM NaCl (**B**).

Figure 5. PAGE analysis of fractions after Q-Sepharose (**A**) and SP-Sepharose (**B**) chromatography.1 — molecular weight marker, 2 — initial sample before purification, 3–6 — target peptide fraction, 7-10 — late fractions.

RESULTS & DISCUSSION

The pH5 plasmid, containing the Hst5 gene sequence, and the p10xH5 plasmid with a cassette insertion containing ten repeats of this gene, were constructed (Figs. 1, 2).

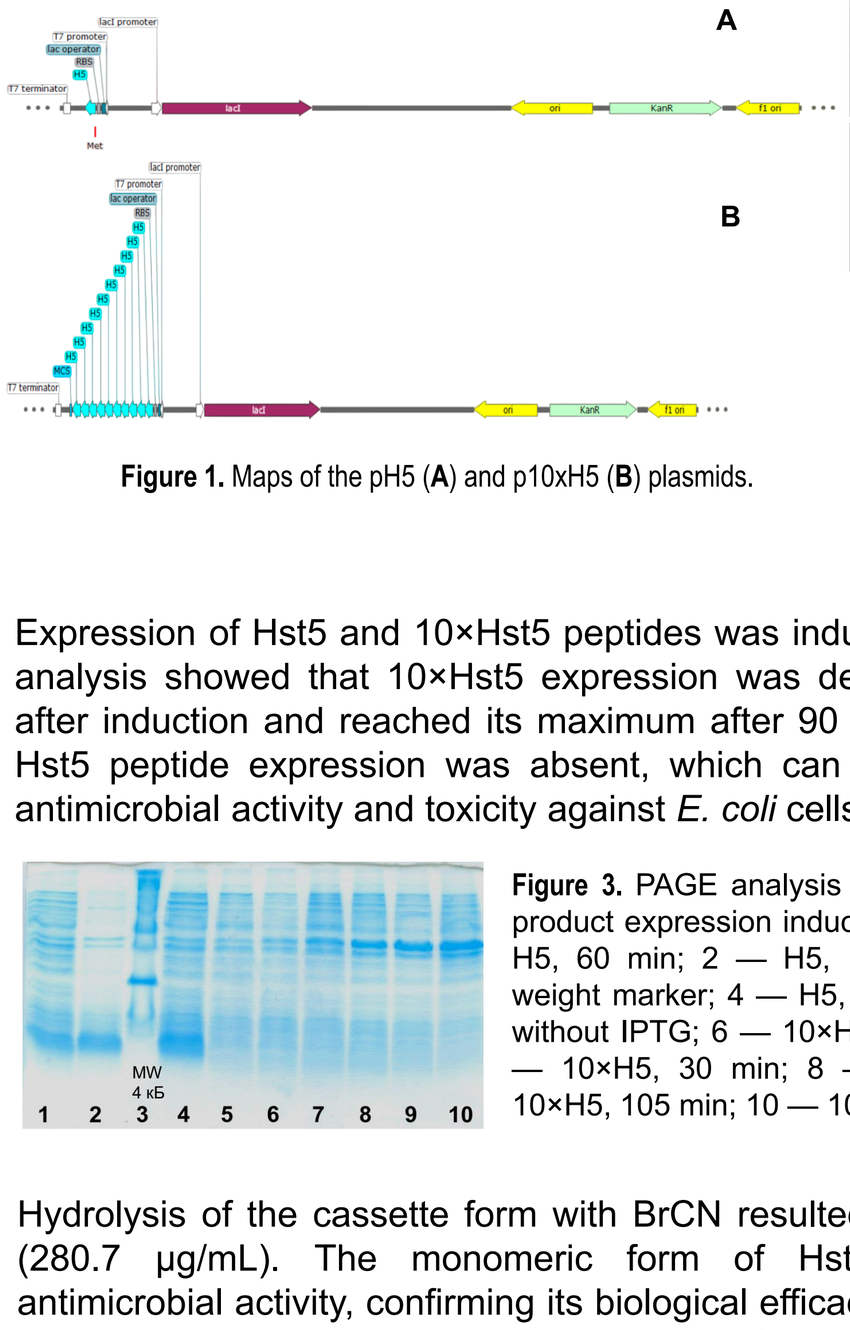

Figure 1. Maps of the pH5 (**A**) and p10xH5 (**B**) plasmids.

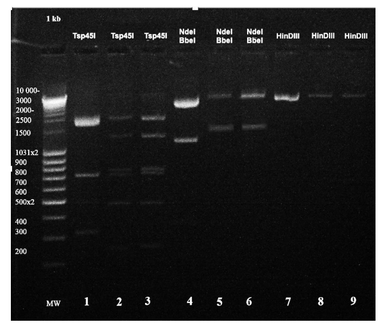

Figure 2. Restriction analysis of the pET30a, pH5, and p10xH5 plasmid fragments after ligation. Lanes: M – Molecular weight marker (MassRuler DNA Ladder Mix, 1 kb); 1, 4, 7 – pET30a; 2, 5, 8 – pH5; 3, 6, 9 – p10xH5.

Expression of Hst5 and 10×Hst5 peptides was induced by IPTG. PAGE analysis showed that 10×Hst5 expression was detectable 60 minutes after induction and reached its maximum after 90 minutes. In contrast, Hst5 peptide expression was absent, which can be explained by its antimicrobial activity and toxicity against *E. coli* cells (Fig. 3).

Figure 3. PAGE analysis of the Hst5 and 10×Hst5 product expression induced by 200 mM IPTG.1 — H5, 60 min; 2 — H5, 105 min; 3 — Molecular weight marker; 4 — H5, 135 min; 5 — H5 control without IPTG; 6 — 10×H5 control without IPTG; 7 — 10×H5, 30 min; 8 — 10×H5, 60 min; 9 — 10×H5, 105 min; 10 — 10×H5, 135 min.

Hydrolysis of the cassette form with BrCN resulted in Hst5 monomers (280.7 µg/mL). The monomeric form of Hst5 exhibited potent antimicrobial activity, confirming its biological efficacy, whereas the Hst5 cassette construct remained inactive. The BrCN control sample showed weak antimicrobial activity. Comparison of the inhibition zones against *E. coli* growth indicated that a 25 mg/mL ampicillin solution corresponded to the activity of a 55 mg/mL Hst5 peptide solution. Thus, the activity of 1 mg of Hst5 was equivalent to that of approximately 2.1 mg of ampicillin (Fig. 6).

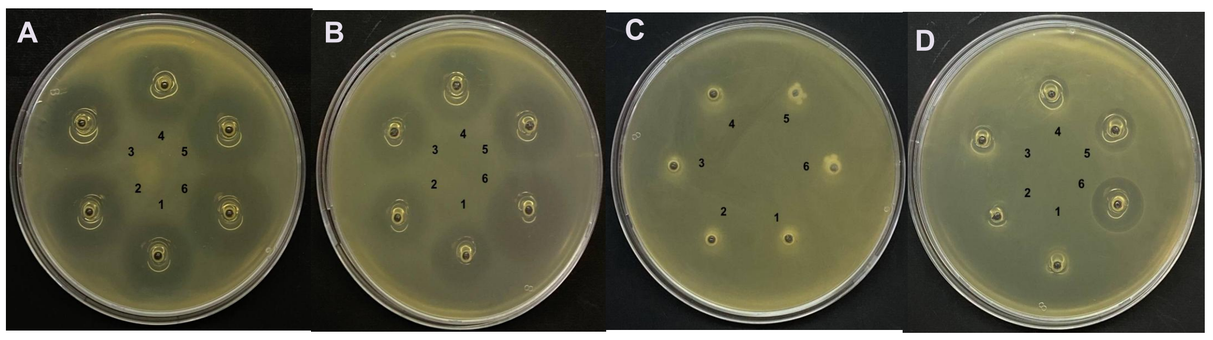

Figure 6. Analysis of antimicrobial activity against *E. coli* of 10×H5 peptide treated with 20 mM BrCN for 20 hours (**A**), with the antibiotic ampicillin (50 µg/ml) applied (**B**), 10×H5 peptide without BrCN treatment (**C**), 20 mM BrCN solution (**D**).
Wells contain serial dilutions of the sample: 1 — 1:32, 2 — 1:16, 3 — 1:8, 4 — 1:4, 5 — 1:2, 6 — undiluted.

CONCLUSION

The results demonstrate the feasibility of producing biologically active Hst5 in a bacterial expression system. This approach offers a cost-effective strategy for generating histatins, supporting the development of novel therapeutics against resistant pathogens. This work was supported by the Ministry of Science and Higher Education of the Russian Federation in 2025 (Agreement No. 075-15-2025-464).