HIV-1 Reverse Transcriptase Activity-Guided Isolation and Structural Characterization of Lichenicidin-Related Peptides from *Bacillus licheniformis* C55-8

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Abstract

The emergence of drug-resistant human immunodeficiency virus type 1 (HIV-1) strains highlights the need to explore new sources of reverse transcriptase (RT) inhibitors. Bacterial bioactive peptides represent promising natural products due to their structural diversity and potential biological activities. This study aimed to isolate and characterize HIV-1 RT-inhibitory peptides produced by *Bacillus licheniformis* C55-8 using an activity-guided purification strategy.

Ethyl acetate extracts from five *Bacillus* strains were initially screened for HIV-1 RT inhibitory activity using a colorimetric enzyme assay. *B. Licheniformis* C55-8 was selected for further investigation based on its pronounced inhibitory activity. Following optimization of fermentation conditions, the extract was subjected to chromatographic fractionation, and active fractions were monitored through HIV-1 RT inhibition assays. Peptide purification was performed using reversed-phase chromatography, followed by structural characterization through LC-MS/MS, high-resolution mass spectrometry, and NMR spectroscopy. Genome mining was conducted to investigate the biosynthetic origin of the isolated peptides.

The analyses revealed a group of structurally related peptides, designated Peptides A, B, and C, together with their oxidized derivatives. Peptide A exhibited a molecular mass of 666.3047 Da and was structurally associated with the N-terminal region of lichenicidin-α. Genome analysis identified a lichenicidin biosynthetic gene cluster supporting this structural relationship. The isolated peptides exhibited concentration-dependent HIV-1 RT inhibition, with IC_{50} values of 1.92 mM and 0.45 mM for Peptides A and C, respectively. Peptide C demonstrated the strongest inhibitory activity among the isolated compounds.

These findings demonstrate the potential of HIV-1 RT activity-guided screening for identifying structurally distinctive bacterial peptides. The isolated lichenicidin-related peptides provide a foundation for further investigations into their antiviral mechanisms and structure–activity relationships.

Keywords

Bacillus licheniformis, HIV-1 reverse transcriptase, bioactivity-guided isolation, bioactive peptides, lichenicidin, LC-MS/MS.

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