

Characterization and phylogenetic analysis of a cDNA encoding an EF-hand domain-containing calumenin-like protein in *Strongyloides ratti*

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Abstract

Strongyloides ratti serves as an experimental model for diagnosing strongyloidiasis in humans. The secretory EF-hand-containing calumenin-like protein functions as calcium signaling molecule from rat was characterized. An 849-nucleotide cDNA fragment was amplified using RT-PCR, and a GenBank search revealed that the amplified product shared 98.54% identity with an incomplete mRNA sequence for *S. ratti*. Using this fragment as a probe, a search was conducted in the Expressed Sequence Tags (EST) database, identifying a 998 bp cDNA fragment through overlapping regions designated as SrCAL. The encoded protein consists of 314 amino acids and has a molecular weight of 36.74 kDa, and an isoelectric point (pI) of 4.61. The sequence includes a signal peptide, which has a cleavage site located between amino acids 21 and 22. A protein domain database search identified five highly conserved EF-hand calcium-binding motifs (cd16226), classifying SrCAL within the Efh_CREC_Calumenin-like protein superfamily, spanning amino acids 29 to 296. Structural prediction revealed that SrCAL is composed of 39.18% alpha-helices, 18.21% beta-strands, and 42.61% loops. Three dimensional (3D) structural modeling focused on 232 amino acids, covering 74% of the total coding sequence, with 99.8% confidence, showing strong structural resemblance to the crystal structure of an EF-hand protein from *Danio rerio*. Genetic distance and phylogenetic analysis revealed that SrCAL shares the lowest genetic distance (0.19%) with the only known protein sequence isolated from *S. ratti* (XP_024499492.1). SrCLA plays a crucial role in calcium signaling and may serve as a housekeeping gene with strong potential as a nematode-specific drug target.

Key words: Nematodes, EF-hand domain, *Strongyloides ratti*, RT-PCR, Calumenin-like proteins

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