

IN VIVO STUDY OF A NOVEL THOERIS EFFECTOR

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Bacteria are constantly exposed to viral (bacteriophage) infections and have therefore evolved a diverse repertoire of innate immune defense systems [1]. Among these, the Thoeris antiviral defense system relies on Toll/interleukin-1 receptor (TIR) domain-mediated immune signaling [2]. Thoeris systems consist of two components: a TIR-domain-containing protein that senses phage invasion and generates a specific ADP-ribose-based immune signaling molecule, and an associated effector protein activated by this signal to induce cellular growth arrest or cell death, thereby aborting phage replication. Although several Thoeris system types have been characterized, large-scale genomic analyses have uncovered numerous Thoeris loci encoding previously uncharacterized effector proteins with diverse domain architectures [3]. The frequent genomic association of TIR domains with genes encoding proteins of unknown function suggests that many Thoeris effectors act through yet undescribed molecular mechanisms.

In this study, we investigated the *in vivo* function of a previously uncharacterized Thoeris effector protein to define its role in TIR-mediated antiviral immunity. For functional analysis, the effector gene was cloned into *E. coli* expression vectors and introduced either alone or together with a TIR-domain-encoding gene via dual-plasmid electroporation, thereby establishing single-expression and co-expression conditions. Bacteriophage plaque assays demonstrated that expression of the effector alone did not confer antiviral protection, whereas co-expression with the TIR-domain protein resulted in phage resistance. These results indicate that the effector functions as a TIR-activated antiviral protein. Next, the effector protein was heterologously expressed in several *E. coli* strains, and expression was systematically evaluated across a range of induction conditions and growth temperatures to determine optimal protein production. Following optimization, the protein was purified by affinity chromatography.

These findings establish a functional framework for this previously uncharacterized Thoeris effector and lay the groundwork for future *in vitro* studies aimed at determining its molecular mechanism of action. Defining the function of this effector will expand our understanding of the mechanistic diversity of Thoeris defense systems and provide broader insight into conserved principles of TIR-mediated immune signaling across diverse biological domains.

[1] F. Tesson et al., "Exploring the diversity of anti-defense systems across prokaryotes, phages and mobile genetic elements," *Nucleic Acids Research*, vol. 53, no. 1, Dec. 2024, doi: 10.1093/nar/gkae1171.

[2] S. Doron et al., "Systematic discovery of antiphage defense systems in the microbial pangenome," *Science*, vol. 359, no. 6379, Jan. 2018, doi: 10.1126/science.aar4120.

[3] E. Yirmiya et al., "Systematic discovery of TIR-based immune signaling systems in bacteria," *bioRxiv*, Dec. 2025, doi: 10.64898/2025.12.03.692087.