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Expression, Purification, and Activity of Peptidyl-tRNA Hydrolase 1 of *Rickettsia rickettsii*

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Introduction

Peptidyl-tRNA hydrolase 1 (Pth1) cleaves the ester bond of peptidyl-tRNA thus recycling peptidyl-tRNAs produced by the expression of short ORFs and minigenes or the pre-mature termination of translation. Pth1 is a vital and highly conserved enzyme in bacteria possessing no essential eukaryotic homolog making it a favorable antibacterial target.This study investigates Pth1 from the bacterial species *Rickettsia rickettsii* most commonly known as the cause of Rocky Mountain spotted fever. Expressing, purifying, and characterizing the activity of recombinant *R. rickettsii* Pth1 (RrPth1) with a 6xHis-tag would be the first step in developing new and effective treatments towards this disease.

Methods

Transformed RrPth1 into BL21 *E. coli* cells for expression

Grew cultures at 37˚C in M9 minimal media to OD_{600} 0.6

Induced overnight at 30˚C with 0.5 mM IPTG

Lysed cells by the addition of lysozyme and sonication

Purified protein using Ni^{2+} affinity chromatography

Dialyzed and concentrated purified protein for activity assay

Results

Overexpression and size of RrPth1 was confirmed through SDS-PAGE. RrPth1 was exactly confirmed by a Western blot.

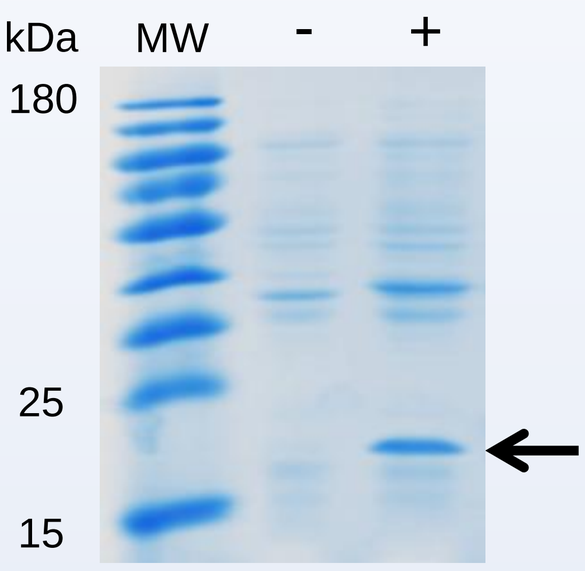

Fig. 1. SDS-PAGE expression gel showing an uninduced (-) sample and an induced (+) sample. The arrow denotes RrPth1.

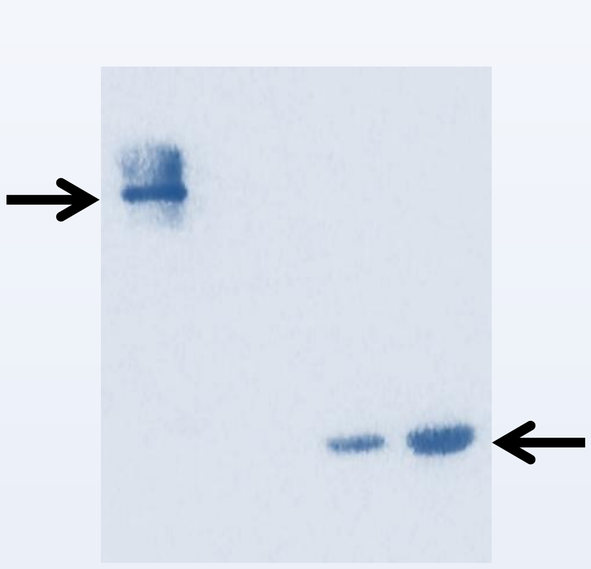

Fig. 2. Western blot with antibody control and diluted RrPth1 samples. The left arrow denotes primary HisTag antibody. The right arrow denotes RrPth1.

A yield of >7 mg of purified RrPth1 per liter of minimal media was attained.

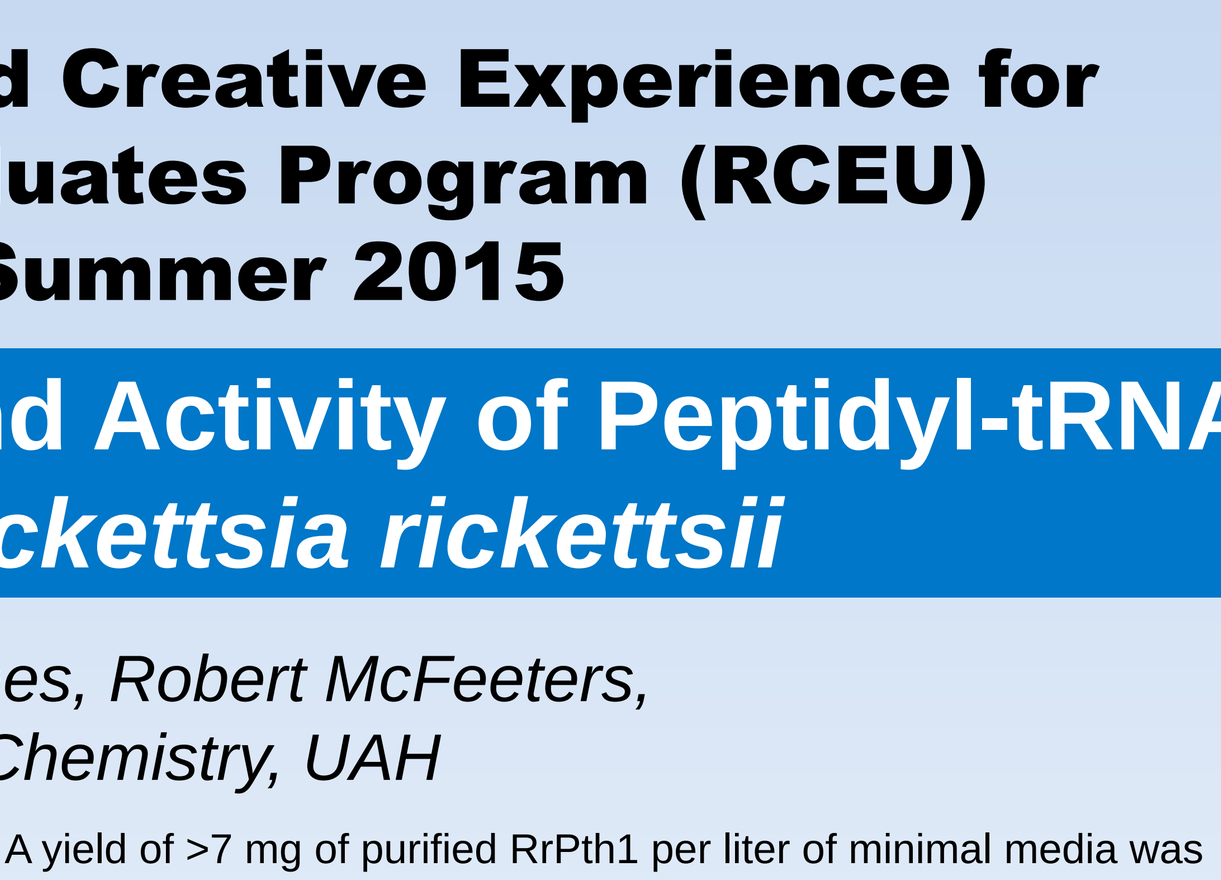

Fig. 3. RrPth1 SDS-PAGE purification gel. The arrow denotes RrPth1.

A reliable and rapid activity assay using urea gel electrophoresis was performed to qualitatively assess if RrPth1 was active. *E. coli* Pth1 was the positive control displaying 100% cleavage, and peptidyl-tRNA with a 5S reference band was the negative control displaying 0% cleavage.

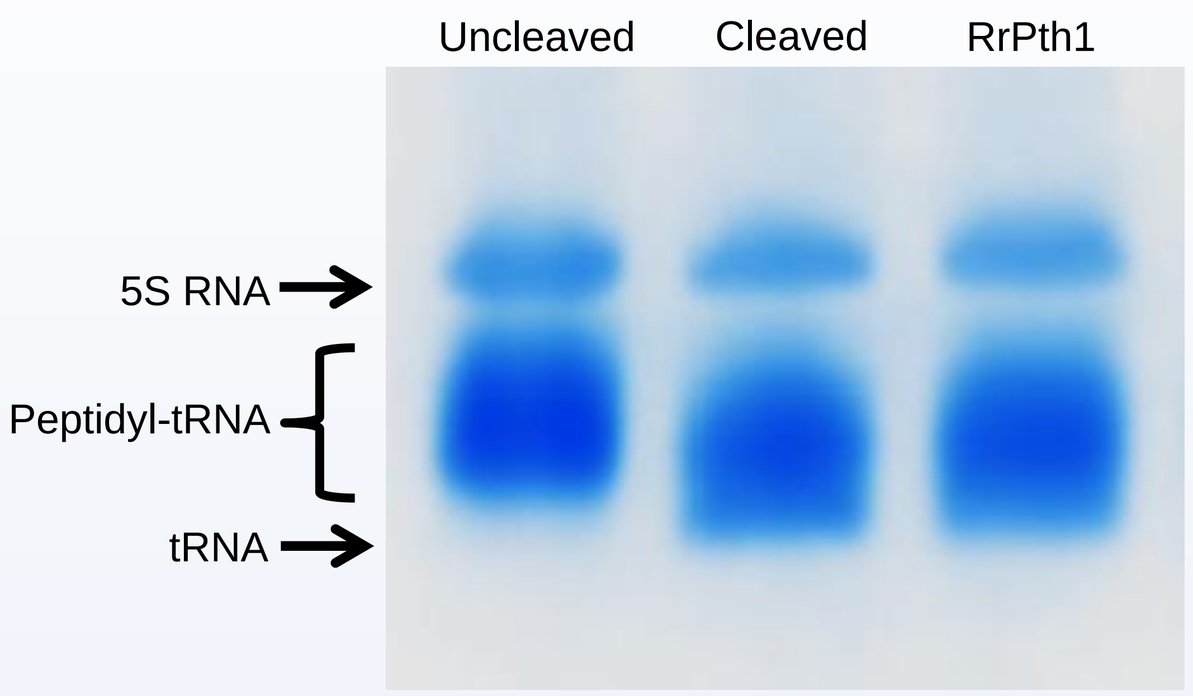

Fig. 4. Acid urea gel stained with methylene blue dye showing peptidyl-tRNA cleavage by RrPth1.

Conclusion & Future Work

Recombinant *R. rickettsii* Pth1 was successfully expressed and purified. Activity of the enzyme was also confirmed; however further assays will be performed to establish enzyme kinetic parameters. Furthermore, buffer conditions will be tested using the hanging drop method in order to obtain an optimal storage buffer. Ultimately, natural products will be screened for inhibitory activity and small molecule inhibitors identified.

Acknowledgements

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