

# Characterization of RNA modifications from *Cladosporium sphaerospermum*

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## Overview

The goal of this project is to explore the potential use of chitinase in facilitating the efficient lysis of *Cladosporium sphaerospermum* fungal cell wall so that transfer RNA (tRNA) can be purified for characterization of ribonucleoside modifications by liquid chromatography coupled with mass spectrometry (LC-MS/MS).

## Introduction

*Cladosporium sphaerospermum*, a melanin producing fungus, has been observed to grow in areas with high levels of ionizing radiation, such as the former Chernobyl Nuclear Power Plant. This radiation energy is used as increased reducing power to accelerate growth by stimulating metabolism.<sup>1</sup> However, characterization of ribonucleoside modifications is hampered by lack of suitable methods to facilitate extraction and purification of RNA from *C. sphaerospermum*. One principal reason is lack of efficient lysis of fungal cell wall without compromising the integrity of RNA as organisms are known to reprogram nucleoside modifications depending on growth habit.<sup>2</sup>

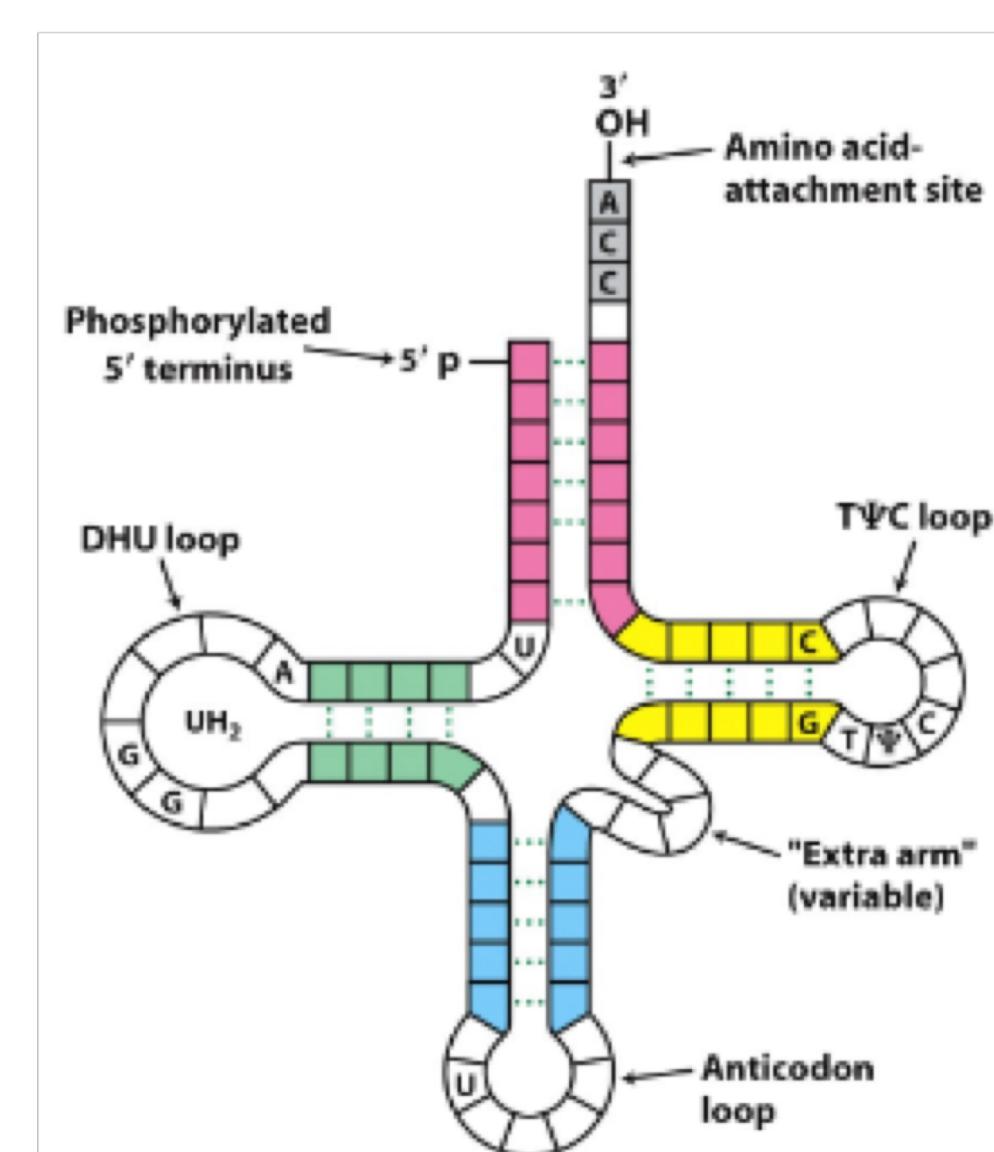


Figure 1. Clover leaf model of tRNA. The structure is identified by the presence of nucleoside modifications at key locations.

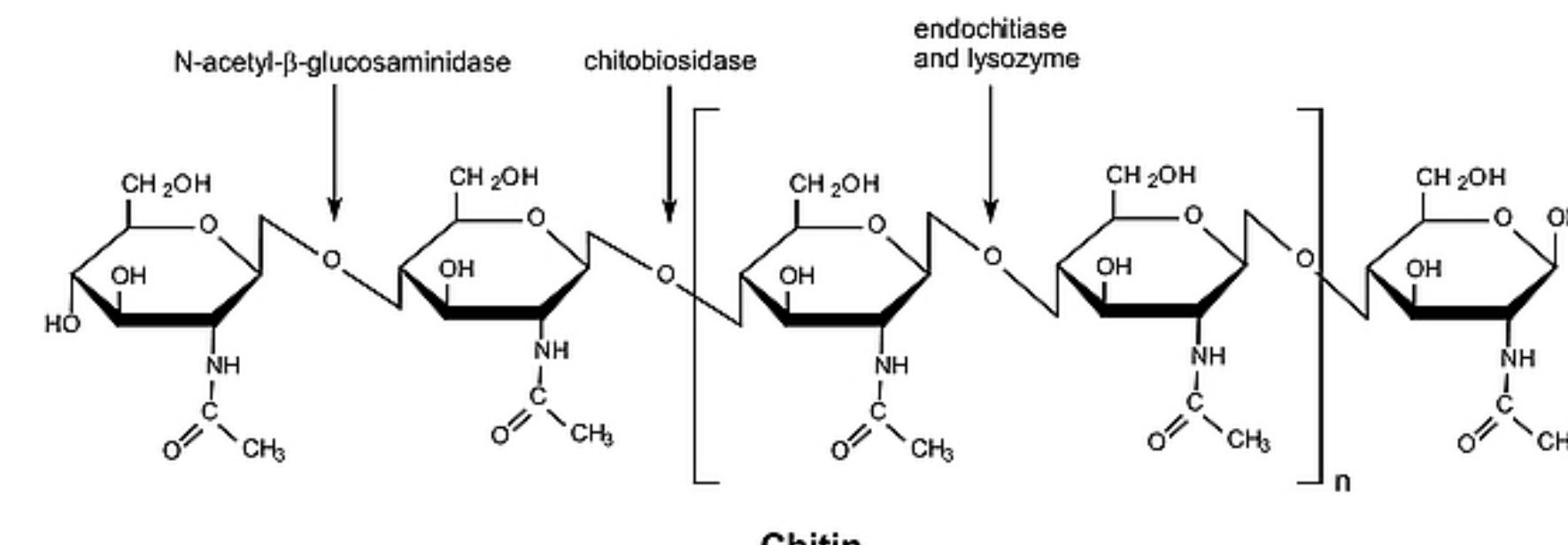
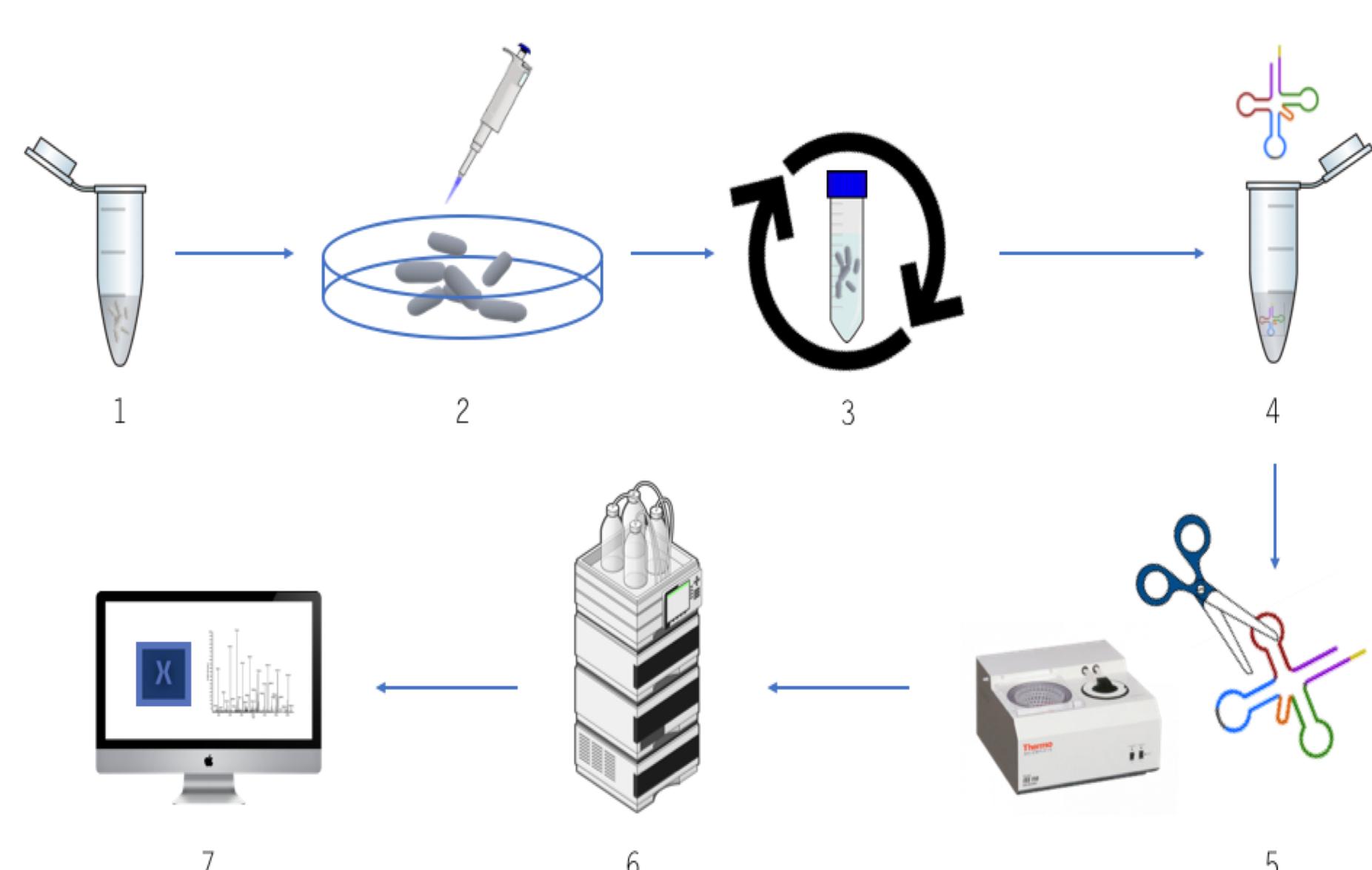
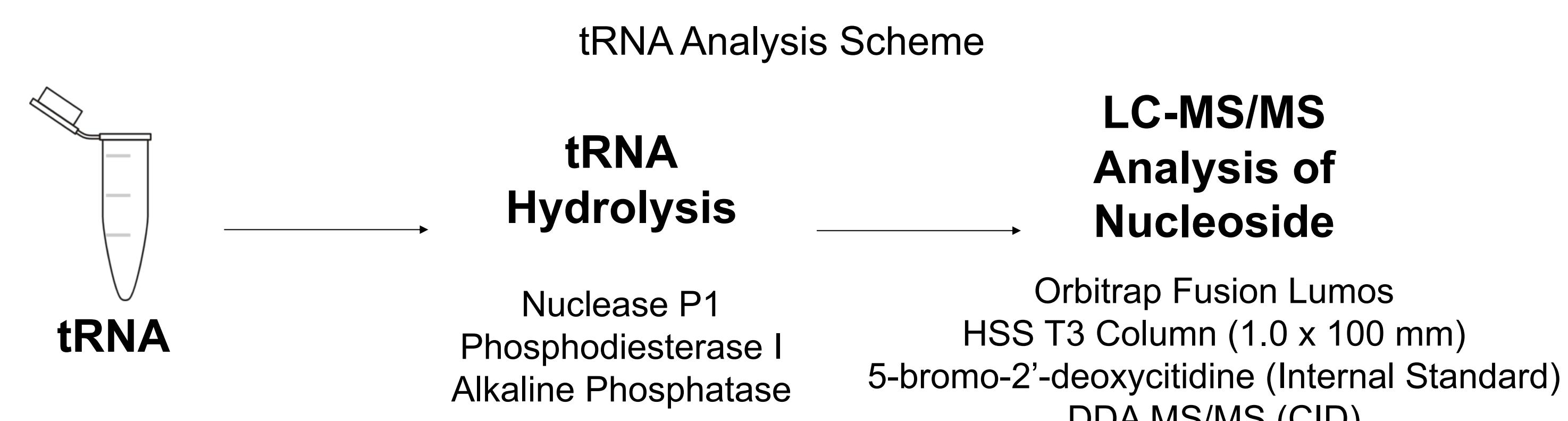


Figure 2. Structure of chitin and the cleavage of glycosidic bond by chitinase.<sup>3</sup>

## Experimental Scheme



1. The CBS1 strain of *C. sphaerospermum* was grown (2) on potato dextrose (pH 6.5) agar plate at 25°C to its log growth phase (~8 days) and harvested.
2. The fungus was incubated with 5 units of chitinase (Sigma Aldrich) for 72 hours at room temperature.
3. The cell suspension was homogenized and RNA isolated using a Qiagen RNeasy Plant Mini Kit.
4. The tRNA was purified using Nucleobond AX100 anion-exchange column. The purified tRNA was enzymatically hydrolyzed to ribonucleosides (scheme 1).<sup>4</sup>
5. The hydrolysate was analyzed by LC-MS<sup>4</sup> and (7) data processed by Xcalibur to identify the resident nucleoside modifications.



## Results

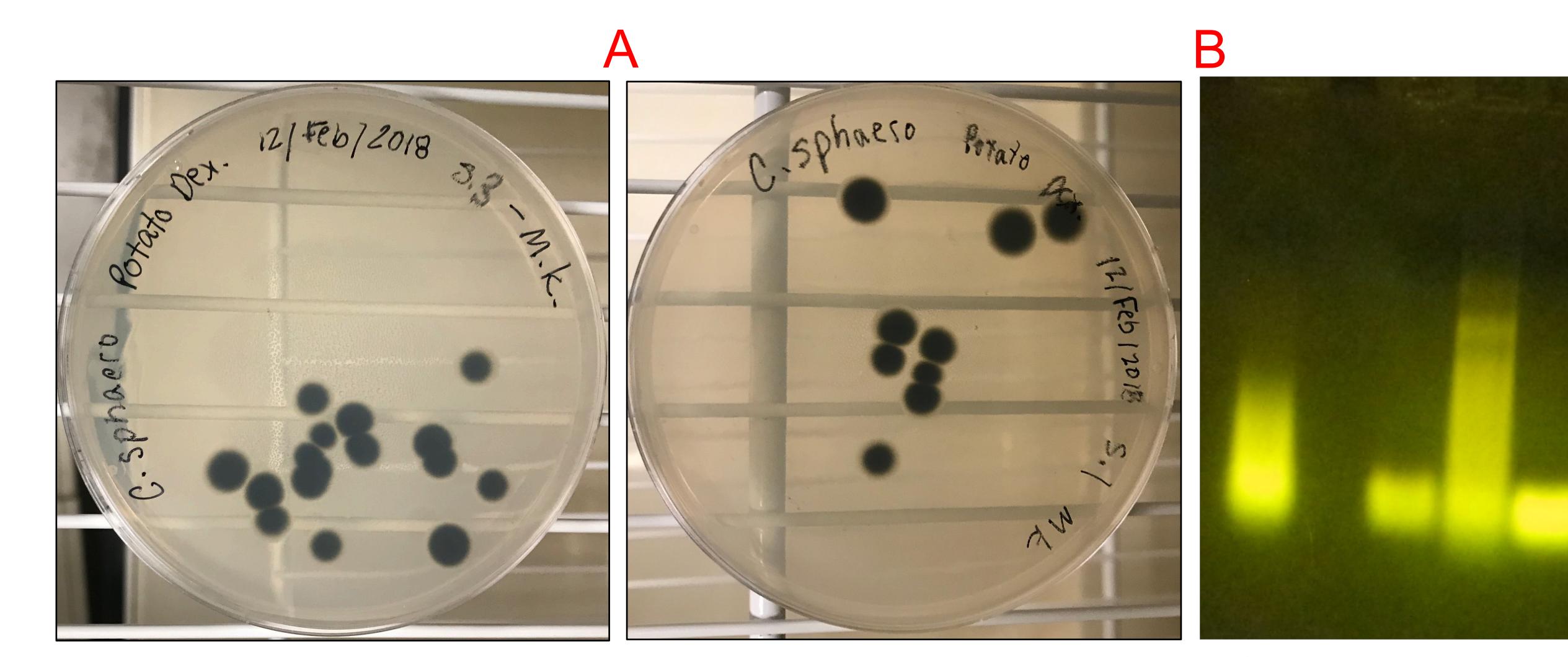


Figure 3. (A) Growth habit of *Cladosporium sphaerospermum*. (B) Agarose gel electrophoresis of purified RNA obtained by chitinase treatment and Qiagen kit based extraction. Lane 1: tRNA purified from *C. sphaerospermum*. Lane 2: standard tRNA, Lane 3: total RNA from *C. sphaerospermum*, Lane 4: tRNA from *B. subtilis*.

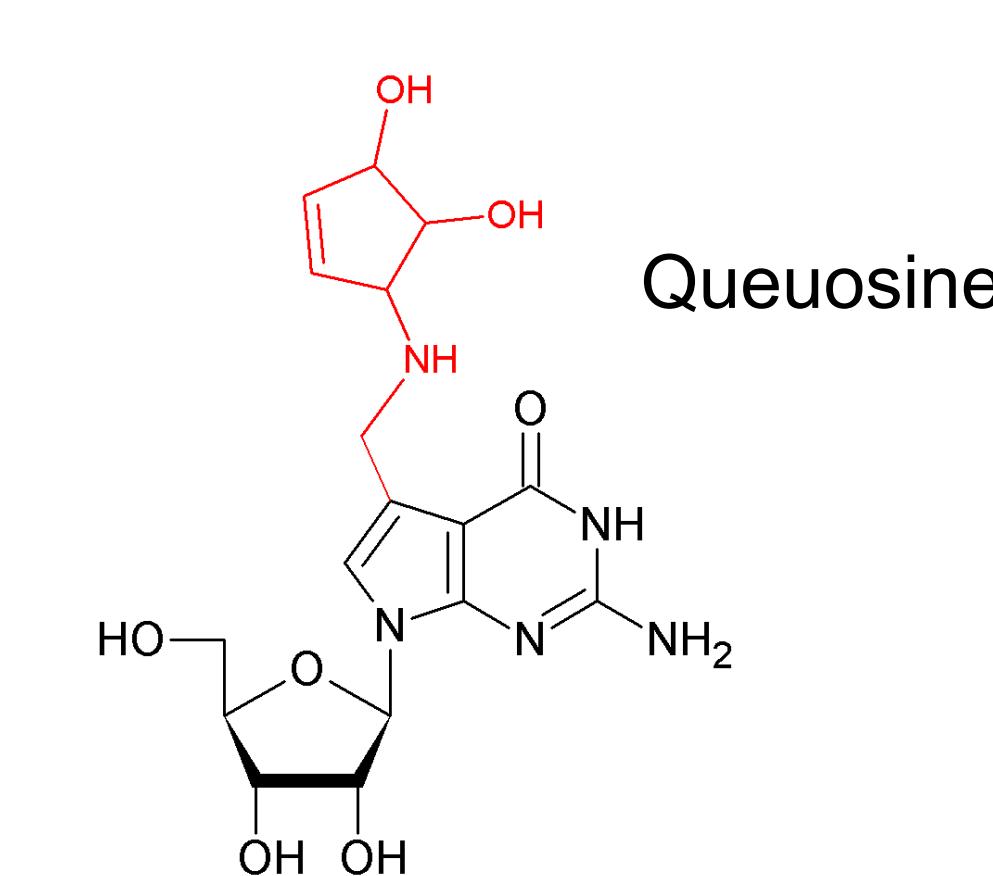
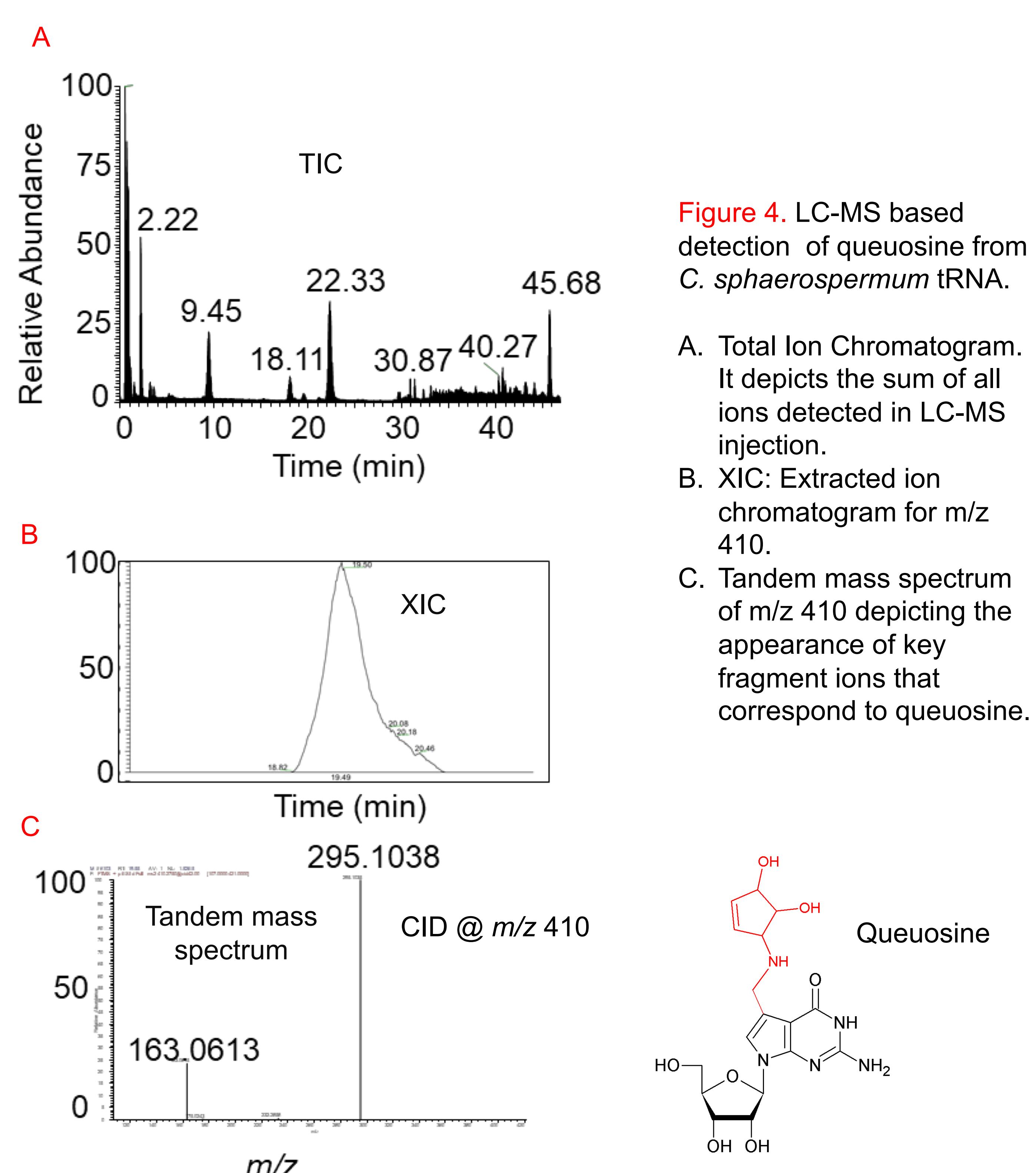


Table 1. Ribonucleoside modifications of *C. sphaerospermum* tRNA detected by LC-MS

Y	D	m4C	m5C
Cm	Um	m3U	m5U
I	Am	m2A	m1A
m1I	ac4C	hm6A	m2G
Gm	m7G	ncm5U	i6A
io6A	QtRNA	t6A	

Figure 3A depicts the growth habit of *C. sphaerospermum* when grown on potato dextrose agar. Figure 3B shows the agarose gel electrophoresis of total RNA and tRNA purified after chitinase treatment and Qiagen kit based extraction.

Figure 4. shows LC-MS based detection of modified ribonucleoside Queuosine as a representative example. Each ribonucleoside is scored based on the elution pattern from a reverse phase column and mass spectrometric behavior.

Table 1. lists the nucleoside modifications detected from *C. sphaerospermum* tRNA. They include modifications of all 4 canonical nucleosides (5).

## Conclusions

- This study demonstrates the successful utilization of chitinase in efficient lysis of fungal cell wall for subsequent purification of RNA
- Detection of queuosine, a predominantly bacterial modification in *C. sphaerospermum* is surprising. Its detection suggests similarities of decoding apparatus between bacteria and *C. sphaerospermum*.
- Future work includes mapping of these modifications to specific tRNAs and understand their abundance in the overall tRNA pool.

## References

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## Acknowledgments

The authors would like to thank the members of the Limbach Group for their assistance. Financial support of this work was provided by NSF (CHE1507357) and DTRA (HDTRA1-15-1-0033) to Patrick A. Limbach.