Determining the regulation and control of saxitoxin production in *Pyrodinium bahamense* in the Indian River Lagoon

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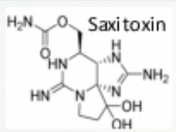
Abstract

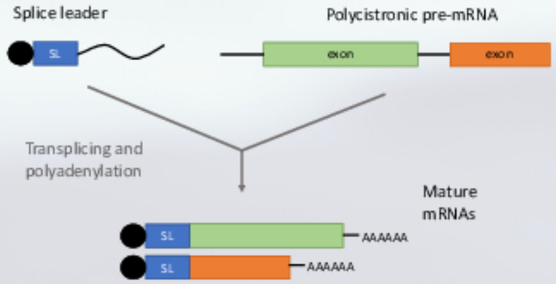


- As one of the most severe HAB toxins, saxitoxin is a global issue.
- While production of saxitoxins has been widely studied in cyanobacteria and *Alexandrium* dinoflagellates, little is known about its production in *Pyrodinium bahamense,* the major saxitoxin producer in the Indian River Lagoon (IRL).
- **Objectives:** Characterize the biosynthesis of saxitoxin in *P. bahamense* through analysis of the transcription and related saxitoxin production using lab cultures of *P. bahamense* strains collected from the IRL.
- Determine potential genetic regulators that control the production of saxitoxins in *P. bahamense* in the IRL.

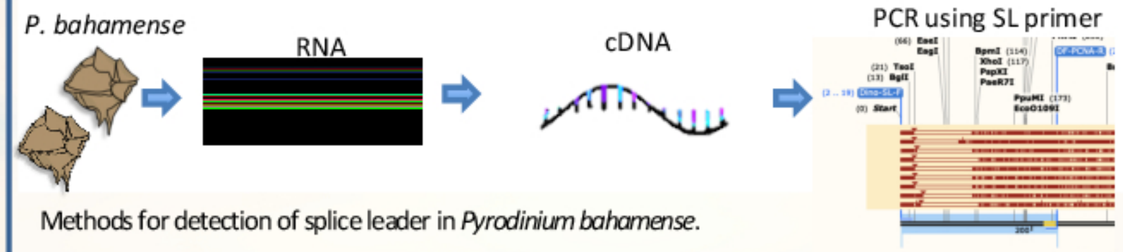
Introduction

- Dinoflagellates have massive genomes which can be several times greater than that of humans; thus, research has been focused on using transcriptomics to study gene expression and regulation in dinoflagellates.
- Most transcripts undergo splice leader trans-splicing: an mRNA maturation process in which a small RNA is added to the 5' end of a pre-mRNA.
- A 22 nt conserved splice leader has been documented in species from all major orders of dinoflagellates; however, the function of the splice leader is still unknown.
- Here, we use techniques in biotechnology to determine the presence of the conserved splice leader in *Pyrodinium*.

  Additionally, we sequence the transcriptome of *P. bahamense* grown in nutrient replete and deplete conditions to reveal genes involved in the regulation of toxin production.



Results



Methods for detection of splice leader in *Pyrodinium bahamense*.

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S.goreaui-PCNA          TCCGTAGCCATTTTGGCTCAAGGATCTTGGCTCGAACTTGTTTTAGGTGTAGCAT-----
K.micrum-PCNA           TCCGTAGCCATTTTGGCTCAAGGGTACG-------------------------------
K.brevis-PCNA           TCCGTAGCCATTTTGGCTCAAGTGCTGCTTCAGTGCTTAGTAAG---------------
P.bahamense21A-PCNA     TCCGTAGCCATTTTGGCTCAAGCATTTCGATCCGTTC-----------------------
P.bahamense23B-PCNA     TCCGTAGCCATTTTGGCTCAAGCATTTCAATCCGTTT-----------------------
A.affine-PCNA           TCCGTAGCCATTTTGGCTCAAGGCG----------TTATTGGTTCAGGTCAGTCTTAAA
A.fundyense-PCNA        TCCGTAGCCATTTTGGCTCAAGCTTTTGGCTCAAGCCATTTTGCTCAAGTCAGTCTCGAC
P.minimum-PCNA          TCCGTAGCCATTTTGGCTCAAGCCTTTGGCTCAAGGACTCCAGCGCTGCCC---------
P.foliaceum-PCNA        TCCGTAGCCATTTTGGCTCAAGCCCCTCACGCAGTCGCATCAGC---------------
K.rotundatum-PCNA       TCCGTAGCCATTTTGGCTCAAGCCCTAGGCACCCAAGGGAGCGCCCTCCTCGCATTG---
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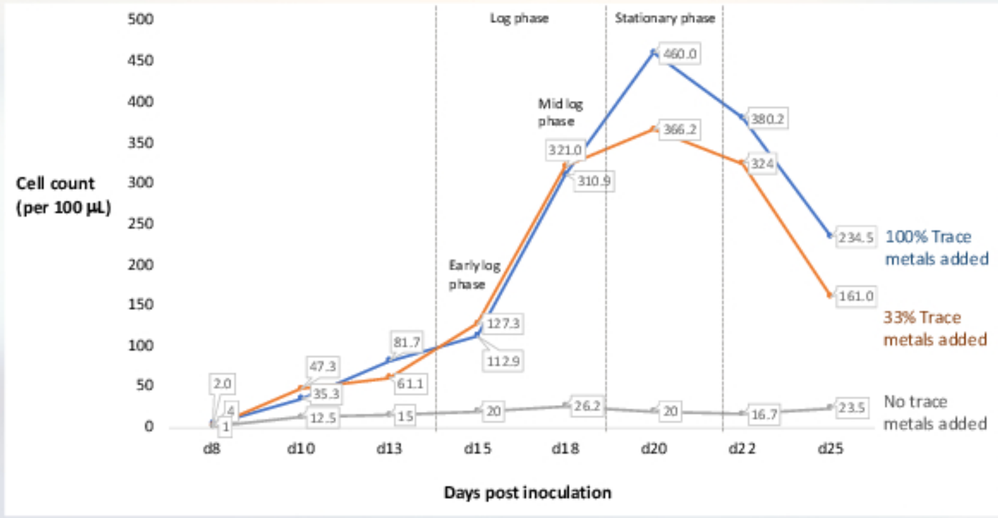
Pairwise alignment of the 5' end of cDNAs from multiple dinoflagellate orders including *Pyrodinium bahamense strains* 21A and 23B. Stars reveal the highly-conserved 22nt splice leader.



Pyrodinium bahamense grown in three levels of trace metals. Samples were taken for RNA-sequencing and toxin quantification on d15, d18 and d20.

Conclusion

- Like other dinoflagellates, *Pyrodinium bahamense* transcripts contain the conserved 22nt splice leader attached at the 5' end.
- Trace metals are essential for the growth of *Pyrodinium bahamense*.
- Optimal methods for RNA isolation from *Pyrodinium bahamense* require heat shock and bead beating.

Future direction

- Determine the function of the conserved 22 nt splice leader through next generation RNA silencing technology.
- Evaluate RNA-seq data to identify the percentage and types of transcripts containing the conserved splice leader.
- Analyze transcripts for differential gene expression and its relation to toxin production for cells grown in 100 versus 33 percent trace metals.

References

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- Zhang, H., Y. Hou, L. Miranda, D. A. Campbell, N. R. Sturm, T. Gaasterland and S. Lin (2007). "Spliced leader RNA trans-splicing in dinoflagellates." Proceedings of the National Academy of Sciences 104(11): 4618-4623.

Acknowledgements

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