Bulk Nitrogen Fixation Rates and Optimal Temperature Range of the Globally Important Diazotroph *Candidatus* Thalassolituus haligoni

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Introduction

- The **global nitrogen cycle** (Fig 1) plays a fundamental role in supplying essential nutrients needed to sustain life, controlling marine productivity and the earth's climate.
- The most abundant form of nitrogen found within the ocean is dissolved atmospheric nitrogen, N2, however, it is not **bioavailable** (Gruber 2008).
- **Diazotrophs** are a select group of microorganisms that can convert atmospheric N2 gas into ammonia (NH3) using the oxygen sensitive enzyme **nitrogenase** (Sohm et al 2011; EQN 1).
  - Further divided into cyanobacterial (CDs) vs. non-cyanobacterial (NCDs)
    - characterized by the *nifH* gene
- ***Candidatus* Thalassolituus haligoni** (nov. BB40) is a globally distributed, model diazotroph that belongs to a large group of unknown/uncultured NCDs within Oceanospirillales (Rose et al 2024).

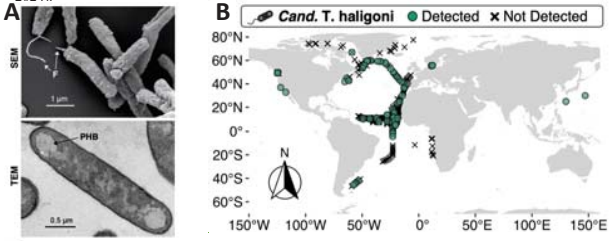


Figure 2 (A) Scanning electron microscopy, SEM (top) and transmission electron microscopy, TEM (bottom) of *Candidatus* Thalassolituus haligoni. (B) Presence and absence of *Candidatus* Thalassolituus haligoni based on *nifH* qPCR. Figures obtained from Rose et al 2024.

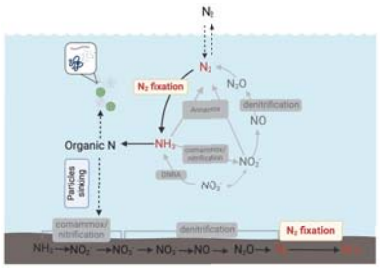


Figure 1 Schematic representation of the marine nitrogen (N) cycle. Image created by Sonja Rose using BioRender.

$$N_2 + 8H^+ + 16ATP + 8e^- \rightarrow 2NH_3 + H_2 + 16ADP + 16P_i$$

EQN 1; Biological Dinitrogen Fixation

Objectives & Hypotheses

Part 1: Temperature range of BB40

Objective: investigate the temperature range for *Cand*. T. haligoni when under the presence of fixed nitrogen (NO3 and NH3) and N2 conditions.

Hypothesis:

a) I hypothesize that the growth rate of *Cand*. T. haligoni will increase to a maximum and then decrease because the species temperature threshold is reached.

b) I hypothesize that the *nifH* transcript will show a linear relationship early on as temperature increases, however higher temperatures could result in 1 of 3 options (linear, monod, parabolic).

Part 2: Nitrogen Fixation rates of BB40

Objective: investigate how the bulk N2 fixation rates change when *Cand*. T. haligoni is grown under various nitrogen (NH3, NO3 and N2) and oxygen (anoxic and oxic) conditions.

Hypothesis : I hypothesize that N2 fixation rates will be highest in N2 conditions and lowest in NH3 conditions due to NH3 uptake being energetically cheaper than N2 fixation.

Methods

Part 1: Temperature range of BB40

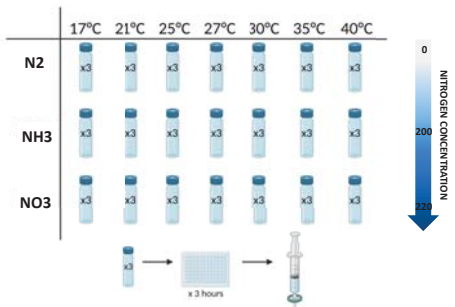


Figure 3 Schematic of temperature block used for growing *Cand*. T. haligoni cultures. Temperatures ranged from 17 deg C to 40 deg C, with biological replicates for each nitrogen condition (N2, NO3, NH3). 4 deg C cultures were kept in a separate incubator and grown in triplicate for each nitrogen condition. Image created using BioRender.

Part 2: Nitrogen Fixation rates of BB40

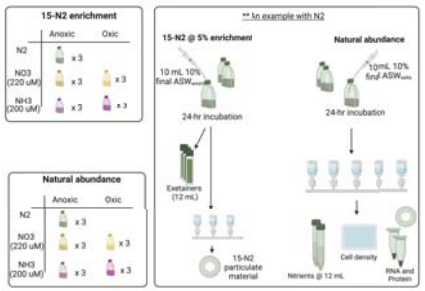


Figure 4 Schematic of set up for nitrogen fixation experiments (N2 as an example). Methods adapted from the study completed by Rose et al (2024). Cultures include five conditions with three replicates per each condition: N2 (anoxic), NO3 (anoxic and oxic), and NH3 (anoxic and oxic). Natural abundance samples involve collection of RNA, Protein, particulate material, nutrients, and cell density. Enriched samples involve collection for exetainers, and particulate material (15-N2). Image created using BioRender.

Results

Part 1: *Cand.* T. haligoni grows on a temperature range comparable to what is seen within the environment

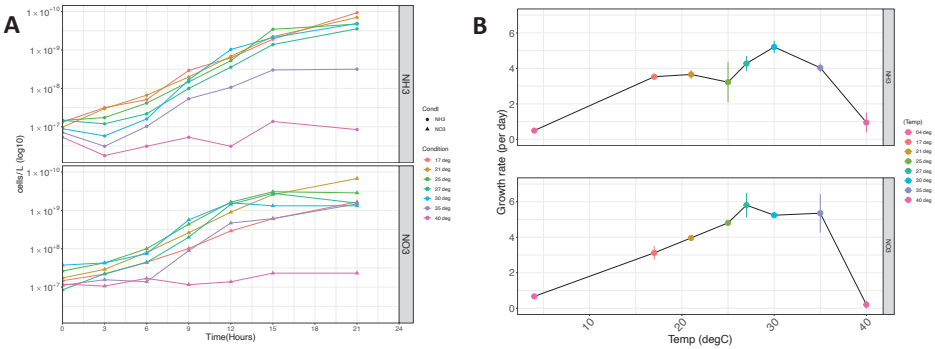
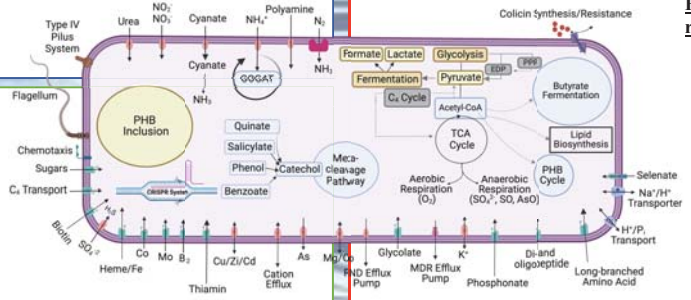


Figure 5 (A) Average measured cell densities (cells/L) ± 1 standard error of *Candidatus* Thalassolituus haligoni for nitrogen (NO3 and NH3) and temperature conditions (17 deg C to 40 deg C). Error bars include biological replicates. Cell densities were measured every three hours on a Novocyte 3000 paired with the NovoSampler Pro (Agilent Technologies, CA, USA). (B) Average growth rate (per day) of *Candidatus* Thalassolituus haligoni for nitrogen (NO3 and NH3) and temperature conditions (17 deg C to 40 deg C) ± 1 standard error. Error bars include biological replicates. Note 4 deg growth rate was obtained from Rose et al 2024 but growth curve is not displayed.



$$N_2\ fixation\ rate = \frac{\left(A_{PN}^{final} - A_{PN}^{t=0}\right)}{\left(A_{N_2} - A_{PN}^{t=0}\right)} \times \frac{[PN]}{\Delta t}$$

EQN 2; Nitrogen Fixation Rate Equation

Part 2: *Cand.* T. haligoni fixes N across various conditions and ranges at rates comparable to other globally significant diazotrophs

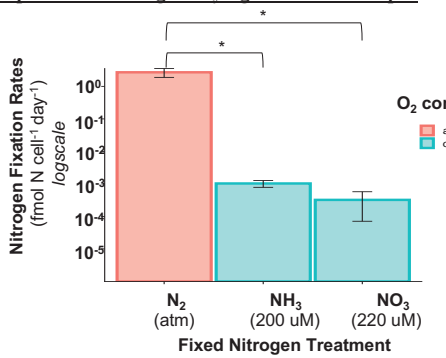


Figure 6 Average bulk nitrogen fixation rates (fmol N cell^{-1} day-1) of *Candidatus* Thalassolituus haligoni for various nitrogen conditions (N2, NH3, NO3) and oxygen conditions (anoxic and oxic) plotted on log scale. Nitrogen fixation rates were calculated using EQN 2, where N_2 fixation rate is the relationship of atom percent (%) of the particulate 15N at the final point of incubation (A_{PN}^{final}) and at the beginning of the incubation ($A_{PN}^{t=0}$), A_{N2} is the atom % of the dissolved 15N in the nitrogen pool, PN is the concentration of the particulate nitrogen (PN) of the natural abundance and Δt is the length of incubation time. Stars indicate significant difference between treatments and was calculated using students T-test, $\alpha = 0.05$.

Discussion/ Next steps…

- Growth curve of Cand. T. haligoni for fixed N sources (NO3 and NH3).
- **Optimal growth temperature** (T opt) between 27 °C – 30 °C for NO3 and 30 °C – 35 °C for NH3.
- **Maximum growth temperature** (T max) for fixed N sources was 35 °C.
- Significant difference found between bulk NFR measurements, with N2 conditions being **3 orders of magnitude higher** than oxic fixed N conditions.
- *Cand.* T. haligoni fixes N at rates **comparable to other globally significant diazotrophs** (Figure 7).
- ***Next Steps:***
  - Optimal temperature range for anoxic N2 cultures
  - Bulk fixation rates for anoxic fixed N conditions (NO3 and NH3)
  - RNA extraction and RT- qPCR to evaluate *nifH* transcript abundance across conditions

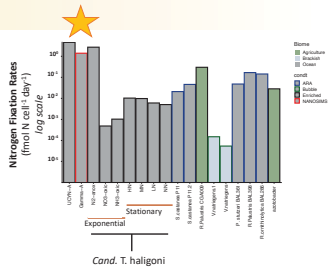


Figure 7 Literature comparison of nitrogen fixation rates (fmol N cell^{-1} day^{-1}) to *Candidatus* Thalassolituus haligoni under various conditions and various methods. Note y-axis is log scale. Figure modified from Rose et al 2024.

References: (1) Gruber N. 2008. The marine nitrogen cycle: overview and challenges. Nitrogen in the Marine Environment. 2:1-50.
(2) Rose SA, Robicheau BM, Tolman J, Fonseca-Batista D, Rowland E, Desai D, Rattan JM, Kantor EJ, Comeau AM, Langille MGI, et al. 2024. Nitrogen fixation in the widely distributed marine γ-proteobacterial diazotroph *Candidatus* Thalassolituus haligoni. Sci Adv. 10(31):eadn1476.
(3) Sohm JA, Webb EA, Capone DG. 2011. Emerging patterns of marine nitrogen fixation. Nat Rev Microbiol. 9(7):499-508.

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