Are Incomplete Denitrification Pathways a Common Trait in Thermus species from Geothermal Springs in China?
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ABSTRACT
Temperature has strong impacts on ecosystem function and biogeochemical cycles, particularly within extreme environments such as geothermal springs above 60°C. The primary focus of this study was to investigate the denitrification pathways of Thermus (Bacteria) isolates from geothermal springs from Tengchong, China. This study tested the hypothesis that incomplete denitrification is a common characteristic of the genus Thermus, regardless of geographic origin or species affiliation. We found evidence to support this hypothesis. We isolated Thermus strains from Geothermal Springs in China and characterized their denitrification pathways. The Thermus strains were isolated from geothermal springs using enrichment cultures. Denitrification measurements were made using colorimetric and gas chromatography assays. We tested the hypothesis that Thermus species are capable of incomplete denitrification pathways using denitrification gene profiling and other preliminary tests. The results show that Thermus species from geothermal springs are capable of incomplete denitrification pathways. The Thermus species from geothermal springs are capable of incomplete denitrification pathways. The Thermus species from geothermal springs are capable of incomplete denitrification pathways.

INTRODUCTION
- Nitrogen cycles in geothermal environments are poorly understood.
- Many thermophilic microorganisms carry out denitrification.
- Denitrification is the process of reducing nitric oxide to molecular nitrogen.
- Truncated denitrification pathways are known to terminate at nitrous oxide (N2O).
- Thermus thermophilus and T. scotoductus from U.S. Great Basin have been shown to have incomplete denitrification pathways terminating at nitrous oxide (N2O).
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- A positive relationship has been observed between temperature and nitrous oxide flux from hydrothermal vents.
- Hot spring environments serve as sources of nitrous oxide, a strong greenhouse gas.
- Temperature has strong impacts on ecosystem function and biogeochemical cycles, particularly within extreme environments such as geothermal springs above 60°C.
- The primary focus of this study was to investigate the denitrification pathways of Thermus (Bacteria) isolates from geothermal springs from Tengchong, China.
- This study tested the hypothesis that incomplete denitrification is a common characteristic of the genus Thermus, regardless of geographic origin or species affiliation.
- We found evidence to support this hypothesis. We isolated Thermus strains from Geothermal Springs in China and characterized their denitrification pathways.
- The Thermus strains were isolated from geothermal springs using enrichment cultures.
- Denitrification measurements were made using colorimetric and gas chromatography assays.

METHODS
- Microbial Cultivation
- Denitrification Measurements
  - Nitrate, nitrite and ammonium ions were measured in each sample using colorimetric assays.
  - Detection by Electron Capture
    - Sample
    - Oven
    - Gas Chromatography
  - Isolates were grown anaerobically on tryptic soy agar (Becton Dickinson). Strains were inoculated into 10 mL of medium (H2O-NH4Cl, Na2HPO4, KNO3, MgSO4, and FeSO4) at various concentrations of nitrogen. The nitrogen source was nitrate, nitrite, or ammonium.
  - Gas Chromatography
    - Isolates were grown anaerobically on tryptic soy agar (Becton Dickinson). Strains were inoculated into 10 mL of medium (H2O-NH4Cl, Na2HPO4, KNO3, MgSO4, and FeSO4) at various concentrations of nitrogen. The nitrogen source was nitrate, nitrite, or ammonium.
  - Detection by Electron Capture
    - Sample
    - Oven
    - Gas Chromatography

RESULTS & DISCUSSION
- One previous study indicates that some T. thermophilus isolates can partially denitrify completely to dinitrogen (Cava, 2008).
- In contrast, the study from Hedlund (2011) investigated several isolates of T. thermophilus and T. scotoductus from the U.S. Great Basin, which displayed incomplete denitrification pathways due to the absence of the nitrous oxide reductase (nosZ) gene (Murugapiran, 2013).
- This study showed highly variable denitrification phenotypes among members of the genus Thermus, which suggests that denitrification genes are part of the dispensable genome in Thermus. Denitrification gene islands are found on conjugative plasmids in some Thermus strains (Ramirez-Arcos, 1998), which have been shown to evolve significantly faster than the chromosome (Bruggemann, 2006).
- This study demonstrates that incomplete denitrification pathways are common phenotypes in Thermus and that Thermus may have a role in greenhouse gas production.

FUTURE WORK
- Initial results from physiological experiments indicate that distinct denitrification pathways are common in Thermus species.
- The physiological phenotype may be due to a missing or mutated nosZ gene.
- This interpretation refers to nosZ, nosX and nosY are present.
- Group A produces nitrous oxide (N2O) as a denitrification product, but has some unmeasured amount of nitrogen and may be due to nitric oxide (NO). This physiological phenotype may be due to a missing or mutated nosZ gene. This interpretation refers to nosZ, nosX and nosY are present.
- Group B produces nitrous oxide (N2O) as a denitrification product, but has some unmeasured amount of nitrogen and may be due to nitric oxide (NO). This physiological phenotype may be due to a missing or mutated nosZ gene. This interpretation refers to nosZ, nosX and nosY are present.
- Group C produces nitrous oxide (N2O), but has missing N. This physiological phenotype may be due to a missing or mutated nosZ gene. This interpretation refers to nosZ, nosX and nosY are present.

ACKNOWLEDGEMENTS
- Data of isolates obtained through thermal enrichment and/or isolation from Geothermal Springs in China, including DNA extraction, PCR generation, and characterization through several methods including GC and GC-ECD.
- Data of isolates from Thermus scotoductus and Thermus thermophilus were obtained from Huang et al. (2006).
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REFERENCES