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 evaluated this hypothesis by testing three strains from Geothermal Springs in China and one Thermus strain from U.S. Great Basin. We cultured 25 isolates, including known Thermus species, and measured the stoichiometry of nitrogenous products of nitrate reduction using gas chromatography and colorimetric assays. We also designed custom primers for polymerase chain reaction (PCR) amplification of denitrification genes including narG, nirS, nirK, and nosZ to screen for the genetic capacity for each step in denitrification. Experimental results show that all Thermus strains tested display incomplete denitrification pathways terminating at nitrite (NO2−) or nitrous oxide (N2O), and possibly nitric oxide (NO).

METHODS

- The microbial cultivation of strains was performed on nutrient media containing nitrate or nitrite as electron acceptors. O2 was excluded from anaerobic media. Isolates were grown anaerobically on 9 mM nitrate-amended Castenholz medium D (CMD).

RESULTS & DISCUSSION

- One previous study indicates that some Thermus isolates can denitriﬁc completely to dinitrogen (Cava, 2008).

- In contrast, the study from Hedlund (2011) investigated several isolates of T. thermophilus and T. oshimai from the U.S. Great Basin, which avoid complete nitrous oxide production. Incomplete denitrification pathways were also observed in Thermus strains from geothermal springs in China.

- Initial results from physiological experiments indicate 3 distinct denitrification product patterns: 
  1) Group A produces Nitrite (NO2−) as the only denitrification product. 
  2) Group B produces Nitrous oxide (N2O) as a denitrification product, but has some unmeasured amount of nitrogen and nitric oxide (NO). This interpretation infers that only nosZ gene is present. 
  3) Group C produces Nitrite (NO2−) but has missing N. This phenotype may be due to nitric oxide (NO) as a denitrification product.

- In the study, we found that all strains produced nitrite (NO2−) and nitrous oxide (N2O) as the main denitrification products, with some strains also producing nitric oxide (NO).

- The data show that the Thermus strains from geothermal springs in China can display incomplete denitrification pathways terminating at nitrite or nitrous oxide, and possibly nitric oxide. The presence of nosZ gene suggests the possible formation of nitric oxide.

FUTURE WORK

- Testing and optimizing PCR conditions
- Testing diversity of denitrification genes
- Isolating and culturing different Thermus strains from different geographic locations
- Performing controlled experiments with nitrous oxide intermediates in the absence of nitrous oxide

ACKNOWLEDGEMENTS

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REFERENCES


Braker & Tiedje, 2003. Denitrification gene nosZ is present.

**Table 1. Selected primers used for amplification for nitrate-reducing genes**

<table>
<thead>
<tr>
<th>Group</th>
<th>Primers Used</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>TTR GGT TAW WRG WGR TTR CC</td>
<td>This study</td>
</tr>
<tr>
<td>B</td>
<td>TTR GGT TAW WRG WGR TTR CC</td>
<td>This study</td>
</tr>
<tr>
<td>C</td>
<td>TTR GGT TAW WRG WGR TTR CC</td>
<td>This study</td>
</tr>
</tbody>
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**Figure 1. Gas Chromatography**

- Time course experiment. Nitrate and nitrite assays along with nitrous oxide GC measurements showed that Thermus isolates to reduce nitrate to nitrite and, in some cases, nitrous oxide. Endpoint and time course data show the reduction of nitrate to nitrite and possible formation of nitric oxide.