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. Incomplete denitrification pathways were found in all 25 isolates tested, with 5 strains displaying incomplete denitrification pathways terminating at nitrite (NO\textsubscript{2},-) or nitrous oxide (N\textsubscript{2}O), and possibly nitric oxide (NO).

**INTRODUCTION**

- Nitrogen cycles in geothermal environments are poorly understood.
- Many thermophilic microorganisms carry out denitrification.
- Temperature has strong impacts on ecosystem function and biogeochemical cycles, particularly within extreme environments such as geothermal springs above 60°C.
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**METHODS**

- **Microbial Cultivation**
- **Identification Measurements**
- **PCR Amplification**

**RESULTS & DISCUSSION**

- **Initial results from physiological experiments indicate 3 distinct denitrification phenotypes:**
  1. Group A produces nitrite (NO\textsubscript{2},-) as the only denitrification product. This physiological phenotype may be due to a missing or mutated nirK gene.
  2. Group B produces nitrous oxide (N\textsubscript{2}O) as a denitrification product, but has some unmeasured amount of nitrogen and nitrous oxide (N\textsubscript{2}O).

**REFERENCES**


**ACKNOWLEDGEMENTS**

This study was supported by the McNair Scholars Program and a grant from the National Science Foundation (EPS-1233131). We thank Dr. Brian P. Hedlund for his leadership, Dr. Robert A. Stahl for his guidance, and Dr. Lutz E. Woyke for his advice. The authors declare that they have no conflicts of interest.

**FUTURE WORK**

- Test and optimize PCR conditions.
- Analyze the diversity of denitrification genes in *Thermus* species from different geographic locations.
- Perform end-point experiments with 100% denitrification medium.
- Determine the role of nitric oxide in nitrous oxide production.

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

<table>
<thead>
<tr>
<th>Gene</th>
<th>Primers Used</th>
<th>Reference</th>
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<tbody>
<tr>
<td><em>nirK</em></td>
<td>nirKF2 TGC GCS CCS GGS GGS CA</td>
<td>This study</td>
</tr>
<tr>
<td><em>nirSF1</em></td>
<td>CAG ACS TGG CTS GTSGAC TA</td>
<td>This study</td>
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<tr>
<td><em>nirKR2</em></td>
<td>TTW GGG AAW WSG TTW CC</td>
<td>This study</td>
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<td><em>norB</em></td>
<td>cnorB6R GAA NCC CCA NAC NCC NGC</td>
<td>This study</td>
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<tr>
<td><em>nosZ</em></td>
<td>nosZ171 GGT GTG GGA GAT CAC</td>
<td>This study</td>
</tr>
<tr>
<td><em>narG</em></td>
<td>narG1960f TAY GTS GGS CAR GAR AA</td>
<td>Phillipot et al. 2002</td>
</tr>
<tr>
<td><em>narG2669r</em></td>
<td>TTY TCR TAC CAB GTB GC</td>
<td>Phillipot et al. 2002</td>
</tr>
</tbody>
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**Figure 1.** Detection of H\textsubscript{2}O\textsubscript{2} production by *Thermus* sp. JL-18 using the TUNEL test. *Thermus* sp. JL-18 incubated in liquid culture with 100 µg mL\textsuperscript{-1} cycloheximide showed H\textsubscript{2}O\textsubscript{2} production within 1 h. 

**Figure 2.** Detection of nitrosative stress in *Thermus* sp. JL-18 using the TUNEL test. *Thermus* sp. JL-18 incubated in liquid culture with 100 µg mL\textsuperscript{-1} cycloheximide showed nitrosative stress within 1 h.