Genomic Variations in *Hydrogenophilus thermoluteolus* Genomes Across a Small-Scale Hot Spring Soil Gradient and Global Geothermal Environments

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Abstract

The facultative, chemolithotrophic bacteria *Hydrogenophilus thermoluteolus* is an understudied thermophilic, hydrogen- and thiosulfate- oxidizing microorganism that has been found globally in hot spring environments. It was identified in a series of four soil samples collected around the Polloquere hot spring of Lauca National Park, Chile, in 10m intervals from the hot spring water line. Metagenome-assembled genomes (MAGs) of *H. thermoluteolus* were reconstructed from each sample, exhibiting high completion and a 98% average nucleotide identity with the reference genome of the cultured *H. thermoluteolus* isolate. In this study, we collected and analyzed publicly available genomes of *H. thermoluteolus* and other members of the *Hydrogenophilceae* family derived from cultures and metagenomes from a diverse set of geothermal environments for pangenomic comparison with the Polloquere MAGs. The Polloquere soils are characterized by distinct changes to the environmental chemistry and biology across the 30m distance from the hot spring. In particular, increased aridity and pH, as well as lower temperatures and biomass, coincided with a shift from a characteristic geothermal microbial population, to that of an arid desert community. Notably, however, the presence and relative abundance of *H. thermoluteolus* remained stable over the same distance (˜0.1% of the total community). Using pangenomics, we were able to deduce several genomic differences between soil samples closest (0m) and furthest (30m) from the hot spring, as well as between the Polloquere MAGs and the cultured reference. Functionally, the 30m MAG lacked carbon fixation capabilities, while all of the soil MAGs showed added genomic capacity for denitrification not present in the reference genome. These results contribute significantly to the pool of genomic data for *H. thermoluteolus*, adding to our understanding of the organism's high metabolic flexibility. The Polloquere MAGs also represent a rare example of this organism appearing in a dry, colder, soil environment, presumably transported from the local hot spring. This study investigates how the genomes and metabolisms of *H. thermoluteolus* vary between environments from a biogeographical perspective, both globally and across a small spatial distance defined by a steep environmental gradient.

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INTRODUCTION

- ***Hydrogenophilus thermoluteolus* (*H. thermo*):** thermophilic, facultative chemolithoautotroph; found globally in **hot spring environments**
- **Site:** Polloquere (PQ) hot spring, Lauca National Park, Chile; downwind side
- **Only species** with **consistent** presence and **relative abundance** (~0.1% of total community) in a set of four site soil samples

Table 1 Comparing *H. thermo* optimal growth and PQ3 conditions

Conditions	pH	Temp. (°C)	Relative *H. thermo* abundance (%)
Optimal *H. Thermo* growth	7	50–52	–
PQ, 0m conditions	2.57	(mean annual) 2.9 ± 15	0.14%
PQ, 30m conditions	8.43	(mean annual) 2.9 ± 15	0.11%

CENTRAL QUESTIONS:

- Any genomic differences between PQ samples to explain *H. thermo* persistence?
- Major genomic differences between PQ samples and cultured reference or other published genomes for *H. thermo*?

METHODS

- **Pangenome** (Anvi'o v7): *H. thermo* metagenome-assembled genomes (MAGs) (1 per sample) and all publicly available, environmentally relevant genomes
- **Completeness of metabolic pathways**
- Gene clusters that appeared:
  - Only in **closest or furthest** samples
  - Only in **cultured reference** or **PQ samples**
- Gene clusters with high geometric but low functional homogeneity (cluster **structurally consistent** but individual **amino acids may change** in a way that **impacts functionality**)

Hydrogenophilus thermoluteolus Pangenome

Items order: Presence absence (D: Euclidean; L: Ward)

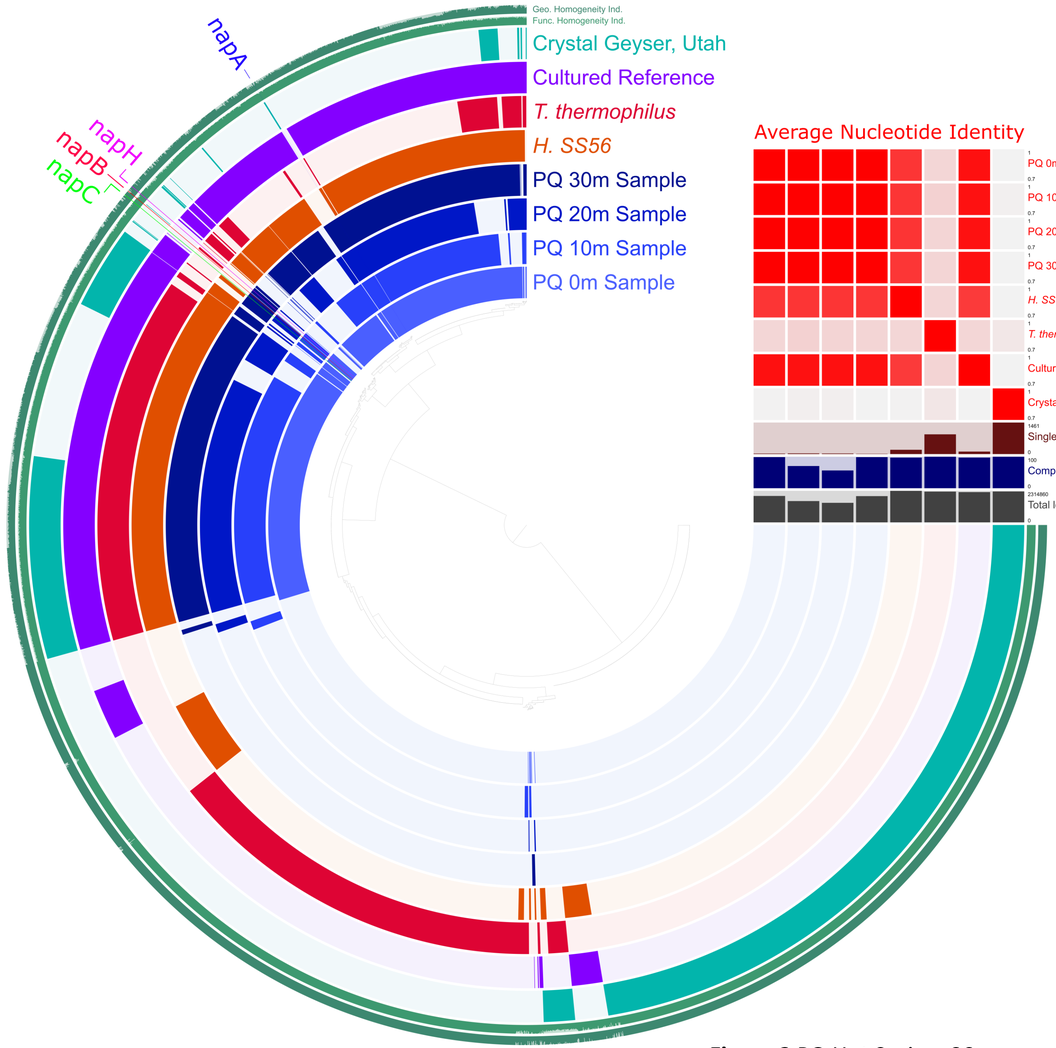
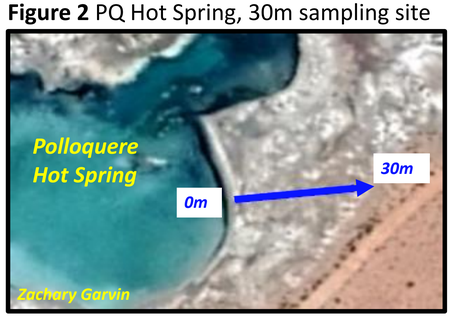


Figure 1 Pangenomic analysis from Anvi'o software, v7. Four inner-most circles represent the genomes derived from the PQ samples from 0m to 30m in 10m intervals. Three outer circles represent externally gathered genomes (outer-most: cultured reference, related organism *T. thermophilus*, related organism *H. SS56*). Genes are organized by similarity (inner tree). Average Nucleotide Identity computed using PyANI, Pritchard et al. (DOI: 10.1039/C5AY02550H)

Figure 2 PQ Hot Spring, 30m sampling site



RESULTS + DISCUSSION

- **Denitrification** (NO_3^- → N_2), **dissimilatory nitrate reduction (**NO_3^- → NH_4^+) pathways more complete in 0m and 30m samples compared to the cultured reference
  - napA, B genes (code for enzymes critical for NO_3^- → NO_2^-) present in PQ samples, not in cultured ref.
  - napH, C genes (code for membrane quinone oxidases) also present, **potential for nitrate respiration** (Sparacino-Watkins et. al, 2013)
- Total Nitrogen increases: 0.032% to 0.079% from 0m to 30m
- NO_3^- (mg/kg) increase: 12.8 mg/kg to 25.6 mg/kg from 0m to 30m
- Genomes included more similar than different — metabolic flexibility ingrained in genome?

FUTURE

- Transcriptomics; nap operon transcription
- Further investigation of nitrate respiration
- Comparison to MAGs for individuals from colder locations

SOURCES

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