

## Phylogenomic and physiological exploration of novel clades of the phylum *Chloroflexi* from Great Boiling Spring, Nevada, USA

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The physiologically diverse, deeply branching bacterial phylum *Chloroflexi* harbors clades that are important players in biogeochemical cycling on a global scale. Here we report on the diversity of two novel classes (*Thermoflexia* and yet to be named) and one order (*Kallotenuales*) of *Chloroflexi* found within Great Boiling Spring (GBS) in Nevada, USA. Genomic predictions and physiological experiments suggest diverse heterotrophic lifestyles. Additional insight into the physiology of *Thermoflexus hugenholtzii*, the only representative of the novel class *Thermoflexia*, are gained through metabolic probing and exometabolomics. Genomic predictions indicate complete central carbon pathways yet metabolic probing suggests disconnects between pathways and an inactive pathway. Exometabolomics provided insight into the consumption of organic compounds, the ability to evaluate genomic and metabolic predictions, and detailed molecular characterization of an organic extract obtained from GBS water, with evidence for extract-contained indole derivatives playing a major role in growth stimulating properties. In combination, the phylogenomic and metabolic diversity of *Chloroflexi* have been expanded and significant strides have been made toward the discovery of a defined medium to aid in future studies of *T. hugenholtzii*.