（博士課程）
Doctoral Program

論 文 要 旨

THESIS SUMMARY

系・コース： Department of, Graduate major in	応用化学 系 応用化学 コース	申請学位（専攻分野）： Academic Degree Requested	博士 Doctor of Science
学生氏名： Student's Name	PRONDZINSKY Alannah Paulina	指導教員（主）： Academic Supervisor(main)	豊田 栄
		指導教員（副）： Academic Supervisor(sub)	

要旨（英文 800 語程度）
Thesis Summary（approx.800 English Words ）

Methane producing archaea, methanogens, are anaerobic microorganisms whose physiology is found in wide ranges of pH, salinity, pressure, and temperature. Detailed studies on the organisms' adaptive features to their diverse habitats can help us understand their contributions to global methane cycling today, as well as their role throughout Earth's biogeochemical history. Here, the adaptive features observable at the genome level or through different growth phenotypes were investigated.

Chapter 1 gives a brief introduction to methanogenesis, and its impact on the global carbon cycle on Earth today as well as its biogeochemical role through Earth's history.

In **Chapter 2,** differences in genome structure, composition, and function between psychrotolerant and thermotolerant methanogens were observed across 86 cultured species using comparative genomics approaches: the conserved genomic core of methanogens makes up around one third of a genome on average, and the shared genome content of two species decreases with increasing phylogenetic distance. Throughout the core- and pangenomes, charged amino acids, leucine, and isoleucine content increase with temperature, while polar uncharged amino acids are more abundant at lower temperatures. Thermotolerant methanogens are enriched in metal and other transporters, and psychrotolerant methanogens are enriched in proteins related to structure and motility. Overall, physiology seems to shape genome content more than phylogenetic relatedness in the

methanogens.

Chapter 3 focuses on physiological responses to temperature at the single cell level. Using stable isotope probing in combination with high resolution spatial mass spectrometry, physiological responses at the single cell level were assessed for two psychrotolerant, two mesophilic and two thermotolerant, methylotrophic species grown in pure cultures. The species were chosen based on their common carbon substrate, methanol, and their growth temperature ranges: methylotrophic methanogenesis spans from - 2.5° C to 70 ° C and the organisms analyzed here were grown either at 4 ° C, 37 ° C, or 55 ° C for interspecies comparisons. Additionally, the psychrotolerant species *Methanococcoides burtonii* was grown at its optimal growth temperature (23 ° C) and the mesophile *Methanosarcina acetivorans* was grown at its temperature limits of 15 ° C and 45 ° C, for intraspecies comparisons.

Growth is slow at lower temperatures, and slow growth is often associated with stressful or limiting growth conditions. However, when considering single cell phenotypic heterogeneity, carbon uptake heterogeneity is high in cold growth and nitrogen uptake heterogeneity increases with temperature. Increases in heterogeneity have been attributed to stressful growth conditions, but the results show that while low temperatures slow down growth, they do not necessarily induce cellular stress responses.

Spatial heterogeneity was also assessed for *M. burtonii* at 4 ° C and 23 ° C, which occur in microcolonies. As in free-living cells, carbon heterogeneity is higher at the lower temperature, and nitrogen heterogeneity is higher at the higher growth temperature. Spatial heterogeneity was observed in the cold, with cells on the outside of the aggregates incorporating more substrate than the inner cells.

To my knowledge this is the first investigation of phenotypic heterogeneity in archaea, and the first of any organism which documented the effects of temperature on single cell variations. The causes of single-cell variability in isogenic populations are not yet clear, though authors have attributed this heterogeneity to stochastic gene expression, asymmetric cell division, or cellular interactions.

Chapter 4 introduces the use of metabolic modeling to study the different metabolic modes responsible for the observed phenotypes of *M. burtonii*, giving an insight into metabolic flux variations and differences, in an attempt to explain the causes of phenotypic heterogeneity.

At first, a core metabolic model for methylotrophic methanogens was created in a top-down approach from an existing genome scale model, by maximizing random gene deletions while keeping sufficient biomass and methane output, reducing the number of genes from 807 to 339.

Flux boundaries in the core model were then modified based on media composition and observed cell growth. These modifications did not affect the simulated growth of the model, showing that growth was not limited by media composition.

Finally, flux balance analyses were conducted to sample for the observed single cell uptake rates of methanol and ammonia at 4° C and 23° C. The flux simulation for the phenotype with the mean carbon and nitrogen assimilation rates at 23° C was found to be overall similar to carbon limited phenotypes of both temperatures, with methanogenesis fluxes remaining high.

In this thesis adaptive mechanisms of methanogens to varying growth temperatures were studied from three standpoints: first, genomes of all available methanogens were compared showing compositional, functional, and structural differences. Second, single cell phenotypes and population heterogeneity were assessed for selected methylotrophic species. An increase in carbon heterogeneity at low temperatures and nitrogen heterogeneity at high temperatures, as well as an increase in spatial heterogeneity at low temperatures were observed.

Finally, the use of metabolic models to explain the underlying metabolic modes of single cells was proposed, using a core methylotroph model.

The analyses showed that microorganisms' general physiologies rather than phylogenies play an important role in temperature adaptation.