氏　　　　名	カワイ　シゲル 河合　繁
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学位論文題名	Novel modes of autotrophy of the thermophilic anoxygenic photosynthetic bacteria in the genus *Chloroflexus* *Chloroflexus* 属の好熱性酸素非発生型光合成細菌における新しい独立栄養生育様式（英文）
論文審査委員	主査　教授　春田　伸 委員　教授　花田　智 委員　准教授　得平　茂樹 委員　生命工学領域領域長補佐　鎌形　洋一（産業技術総合研究所）

【論文の内容の要旨】

The genus *Chloroflexus* are thermophilic and ancient anoxygenic photosynthetic bacteria belonging to the deeply branched phylum *Chloroflexi*. These bacteria are distributed in terrestrial hot springs and their modes of heterotrophic growth via photosynthesis and aerobic respiration have been well studied. However, their ability to grow autotrophically has not been elucidated yet. Genome studies indicate all known *Chloroflexus* spp. possessed a gene set for the carbon fixation pathway. In natural hot spring water, which is rich in sulfide and poor in organic compounds, *Chloroflexus* are frequently found in microbial communities as a dominant member to form cell aggregates known as “microbial mats”. These observations strongly suggest that *Chloroflexus* can grow autotrophically and show sulfide-dependent autotrophy in natural environments. In this study, I evaluated the autotrophic growth of *Chloroflexus* isolates under various conditions and examined their growth modes in microbial mats in natural environments by transcriptomic analysis.

Chloroflexus strains were isolated from sulfidic hot springs at Nakabusa, Nagano, Japan, to examine their autotrophic growth. All isolates (nine isolates belonging to *Chloroflexus aggregans* and three isolates of *Chloroflexus aurantiacus*) grew photoautotrophically under H_2 atmosphere. Additionally, autotrophic growth of all

the isolates was observed under 80% H_2 and 5% O_2 conditions in the dark. This is the first report showing that *Chloroflexus* grew under both photoautotrophic and chemolithotrophic conditions.

On the other hand, no isolates showed remarkable photoautotrophic growth on sulfide as the sole electron source. Based on the idea that sulfide is oxidized to elemental sulfur by *Chloroflexus* and the accumulation of elemental sulfur may have an inhibitory effect on their growth, I examined the co-culture effects with an elemental sulfur-disproportionating bacterium. A sulfur-disproportionating bacterium, *Caldimicrobium thiodismutans* strain TF1, which is known to convert elemental sulfur to sulfide and sulfate and isolated from Nakabusa Hot Spring, was co-cultivated with *Chloroflexus aggregans* in this study. After successive subcultivations, both species successfully grew in inorganic medium containing sulfide as the sole electron source in the light. During growth periods, the simultaneous consumption of sulfide and the accumulation of sulfate. *C. thiodismutans* likely works as an elemental sulfur scavenger enabling photoautotrophic growth of *C. aggregans* on sulfide. *C. aggregans* oxidizes sulfide derived from *C. thiodismutans* and works as a sulfide scavenger allowing *C. thiodismutans* to grow on elemental sulfur.

Diel changes in transcript profiles of *Chloroflexus* within cyanobacteria-inhabiting microbial communities in a natural environment were further examined. Total RNAs were extracted from microbial mats collected in Nakabusa Hot Spring and transcription levels of energy metabolism-related genes were analyzed. Genes encoding key enzymes for carbon fixation were highly transcribed during low light in the morning as well as in the late afternoon when the mats became anoxic due to suppression of oxygenic photosynthesis by co-existing cyanobacteria. Highest peaks of relative transcription levels of genes encoding uptake hydrogenase and sulfide:quinone oxidoreductase were also observed in the late afternoon. In combination with these results, H_2 and HS^- are supposed to be utilized by *Chloroflexus* spp. for their photoautotrophic growth in the mats. Low transcription levels of cytochrome *c* oxidase genes were detected during mid-day when the microbial mats were hyperoxic. However, relative transcription levels of gene encoding uptake hydrogenase, key enzymes for carbon fixation, as well as respiratory complexes peaked in the early morning before sunrise. These results suggest that *Chloroflexus* spp. employ chemoautotrophy on H_2 before sunrise, then, change to photoautotrophic growth at low light period and photoheterotrophic growth during midday.

In this study, autotrophic growth capabilities of *Chloroflexus* were characterized both in the laboratory using pure culture and co-culture conditions with other bacteria and in the natural environment. The results obtained in this study clearly indicate that *Chloroflexus* can utilize H_2 solely and HS^- symbiotically as electron sources, in addition to organic compounds. Furthermore, metatranscriptomic studies revealed that changes in growth modes depend on sunlight and oxygen concentration and suggested to adjust their growth modes by changing transcriptomic activity.