



Relationship between redox conditions and the abundance of photosynthetic pigments and synthesizing genes in microbial communities including thermophilic filamentous photosynthetic bacteria

【 Approaching the origin the first photosynthetic organism 】

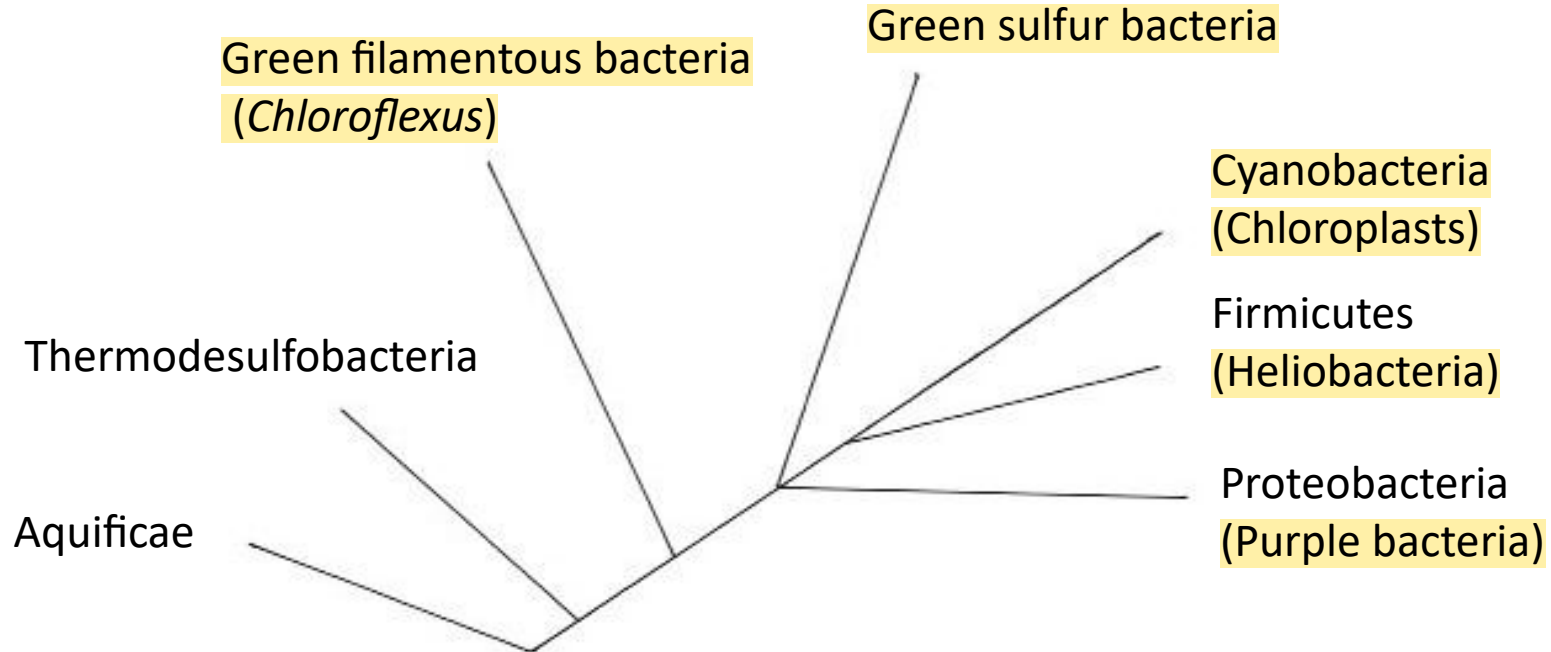
Katsumi Matsuura^{1), 2)} , Shawn E. McGlynn²⁾ , Shigeru Kawai³⁾

¹⁾ Inst. Early Metabolic Evol. , ²⁾ ELSI , Tokyo Tech., ³⁾ JAMSTEC

Chloroflexus is a phylogenetically old photosynthetic organism

The green non-sulfur bacteria: A deep branching in the eubacterial line of descent

H.Oyaizu, B.Debrunner-Vossbrinck, L.Mandelco, J.A.Studier, C.R.Woese
(1987) Systematic and Applied Microbiology 9, 47-53



Chloroflexus as a candidate for the first photosynthetic organism

1. The deepest divergence in photosynthetic organisms in 16S rRNA phylogenetic analysis.
2. Growing up to the highest temperature (around 70°C) as a photosynthetic organism.
3. Growing well by heterotrophic photosynthesis and aerobic respiration.
4. In 2019, *Chloroflexus aggregans* was shown to grow well in hydrogen-sulfide-dependent autotrophic photosynthesis and slightly in chemosynthesis.



Kawai, Nishihara, Matsuura, Haruta, 2019, FEMS Microbiol. Lett. 366, fnz122

Kawai, Kamiya, Matsuura, Haruta, 2019, Front. Microbiol. 24, 1550

Redox conditions, hydrogen sulfide, oxygen and growth of *Chloroflexus* (expected)

Aerobic

Microaerobic

Anaerobic

Oxygen concentration

Sulfide concentration

Redox potential (E_h)

+ 100 mV

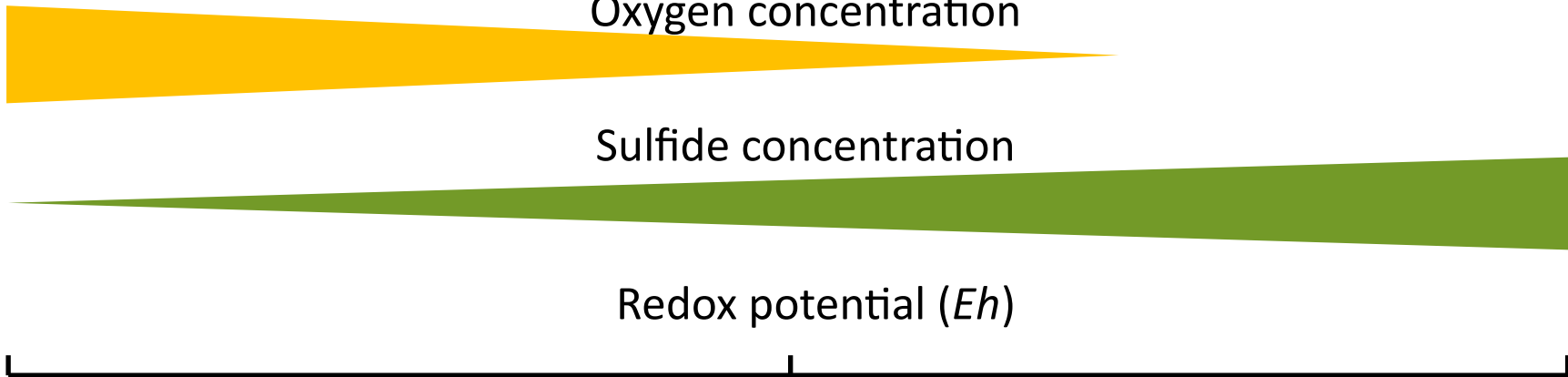
0 mV

- 100 mV

Aerobic respiration

従属栄養光合成

硫化水素依存の独立栄養光合成



Background and purpose

Background :

1. No studies have reported on the growth of *Chloroflexus* in the field relation to environmental conditions.
2. Understanding the relationship between photosynthesis and other energy metabolism in *Chloroflexus* may provide hints for the emergence of photosynthesis.

Purpose :

3. Study the relationship between growth and redox conditions in a microbial community where *Chloroflexus* is growing as a sole photosynthetic organism.
4. Examine the growth by the absorption spectrum of photosynthetic pigments, the photosynthetic reaction center gene, and bacteriochlorophyll synthesis genes.

Steps for investigation

July-September 2020

- Adjust the flow and temperature of hot spring water to stabilize communities and environmental conditions.

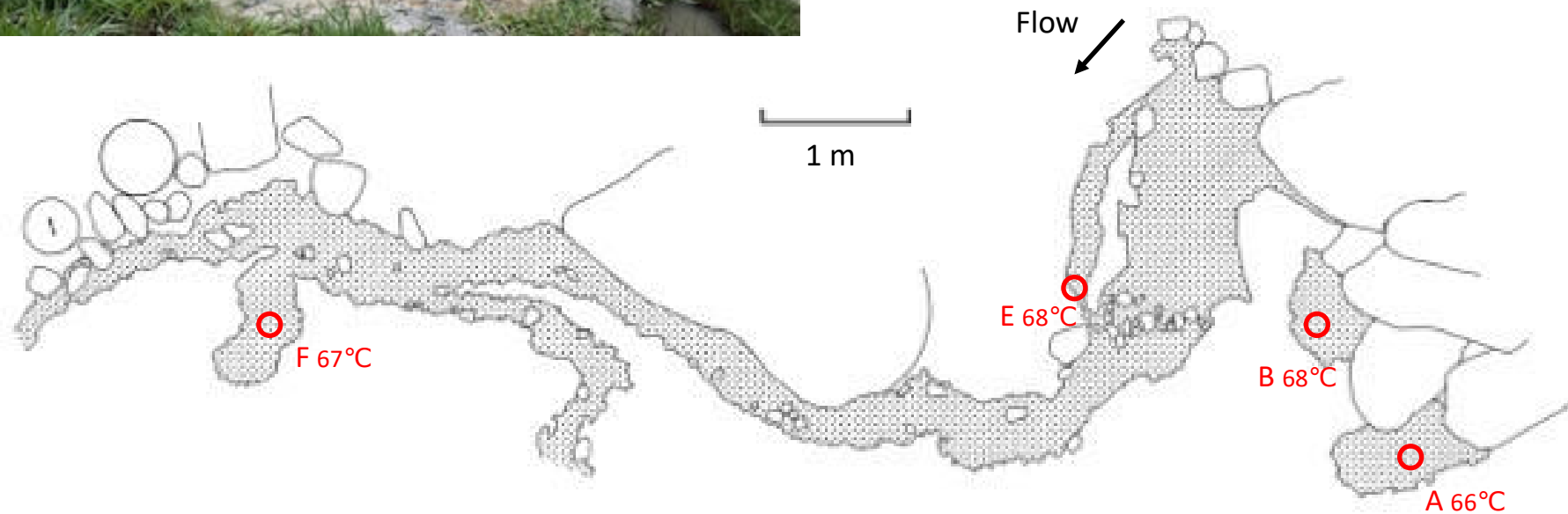
September 26, 2020 – September 28, 2020

- Collect 7 types of microbial communities containing Chloroflexus with different colors and distances from the source
- Measure hydrogen sulfide concentration, oxygen concentration, and redox potential in the water around the collected sites

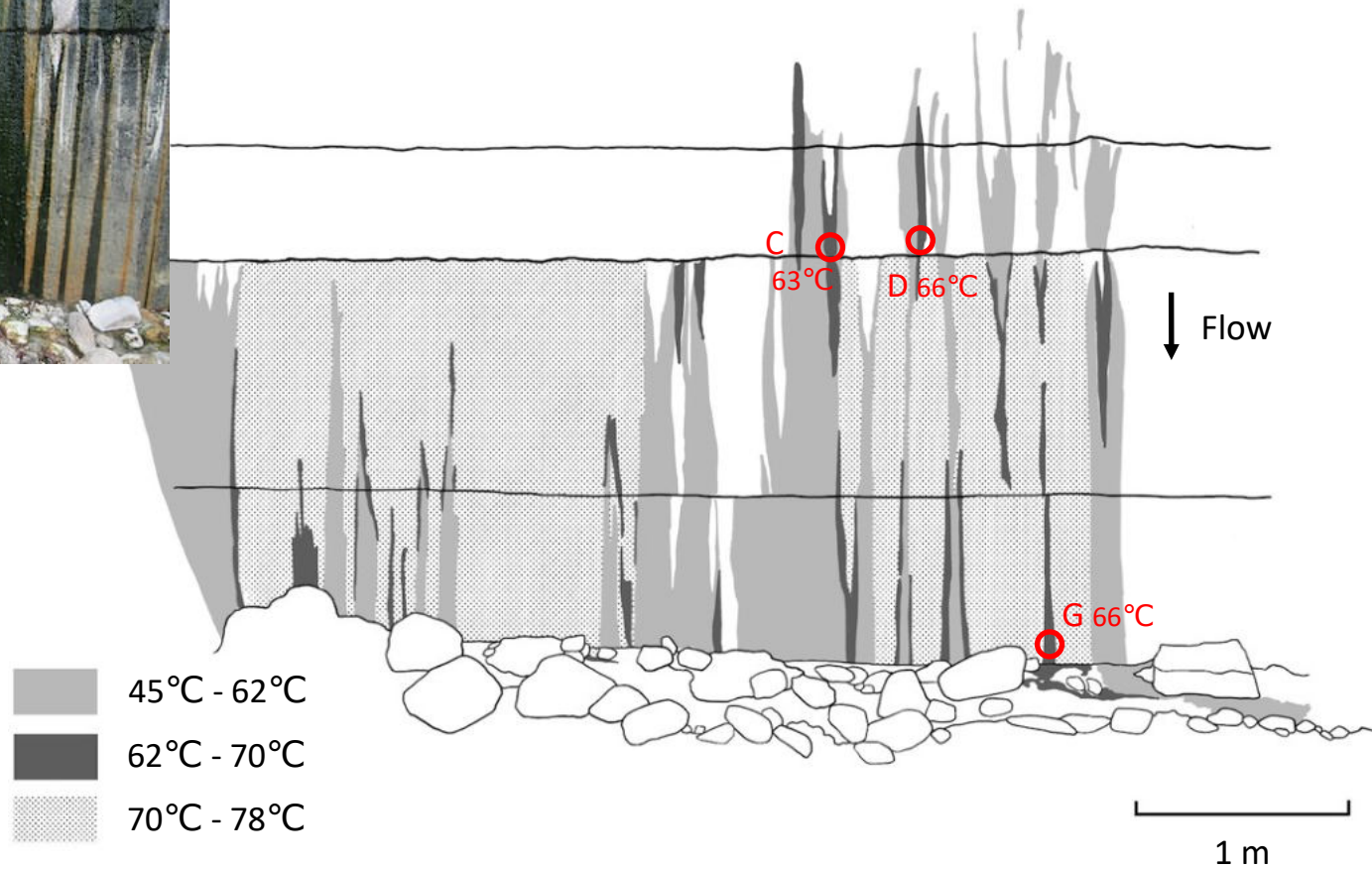
After October 2020

- Quantify relative values of absorption spectrum of photosynthetic dye by second derivative
- Quantify photosynthetic reaction center gene puf by real-time quantitative PCR
- Quantify bacteriochlorophyll synthesis genes, bchX and bchL, by amplicon sequence analysis

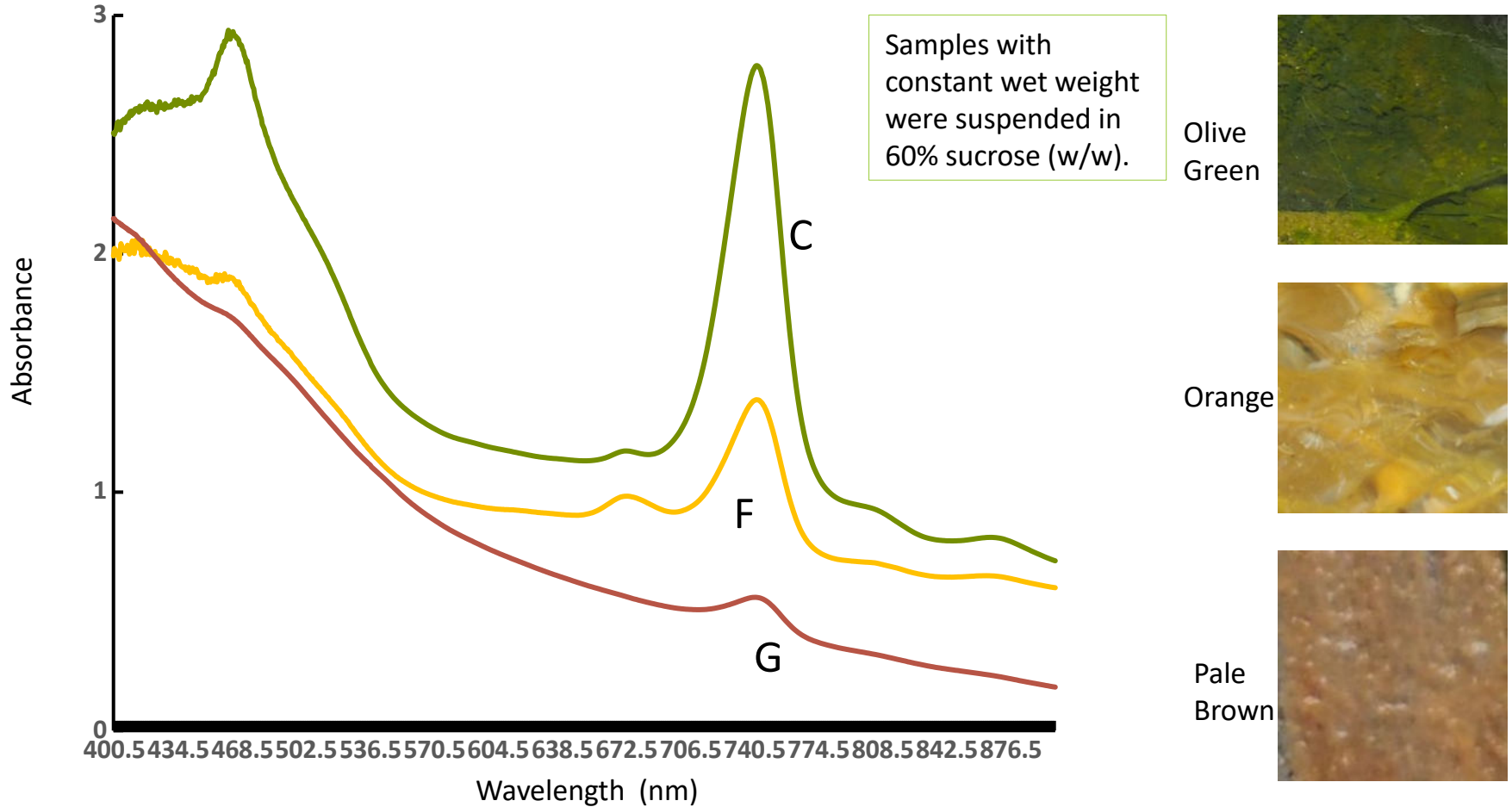
Research site 1: Nakabusa / Kassen spring: Flow on the sandy ground



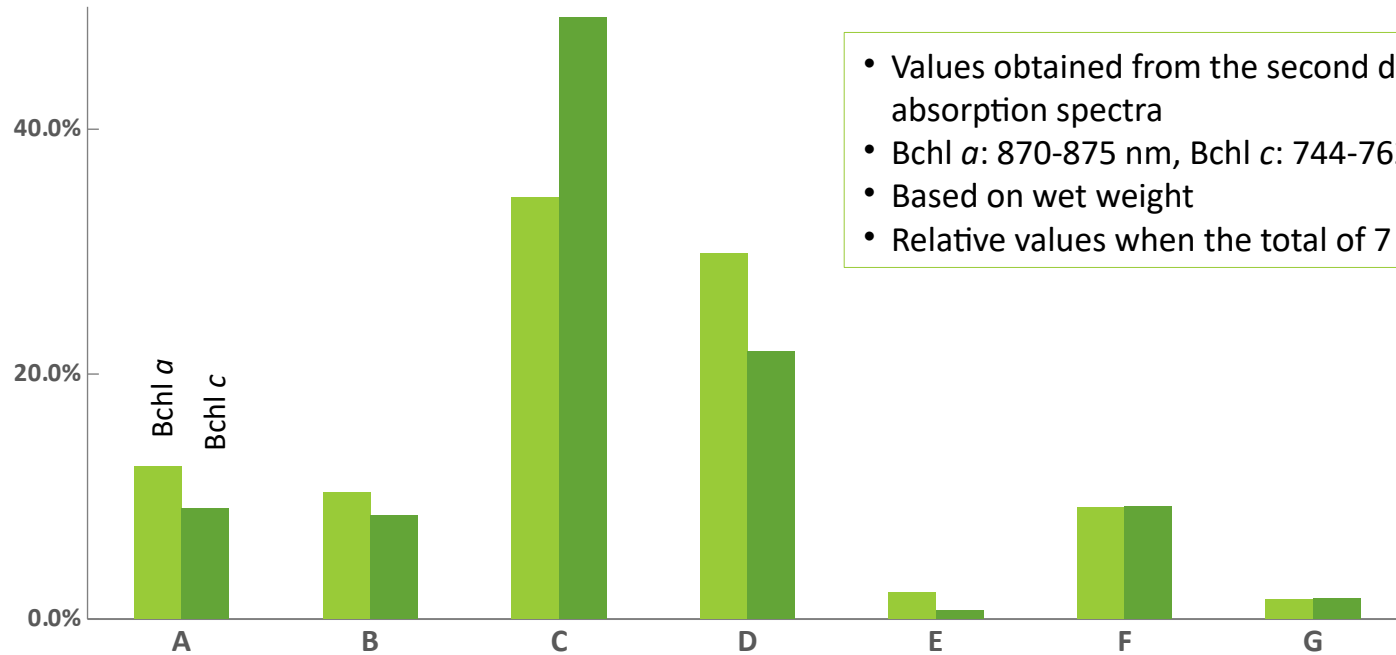
Research site 2: Nakabusa / Kojiki spring: Flow on the concrete wall



Absorption spectra of photosynthetic pigments in community suspensions



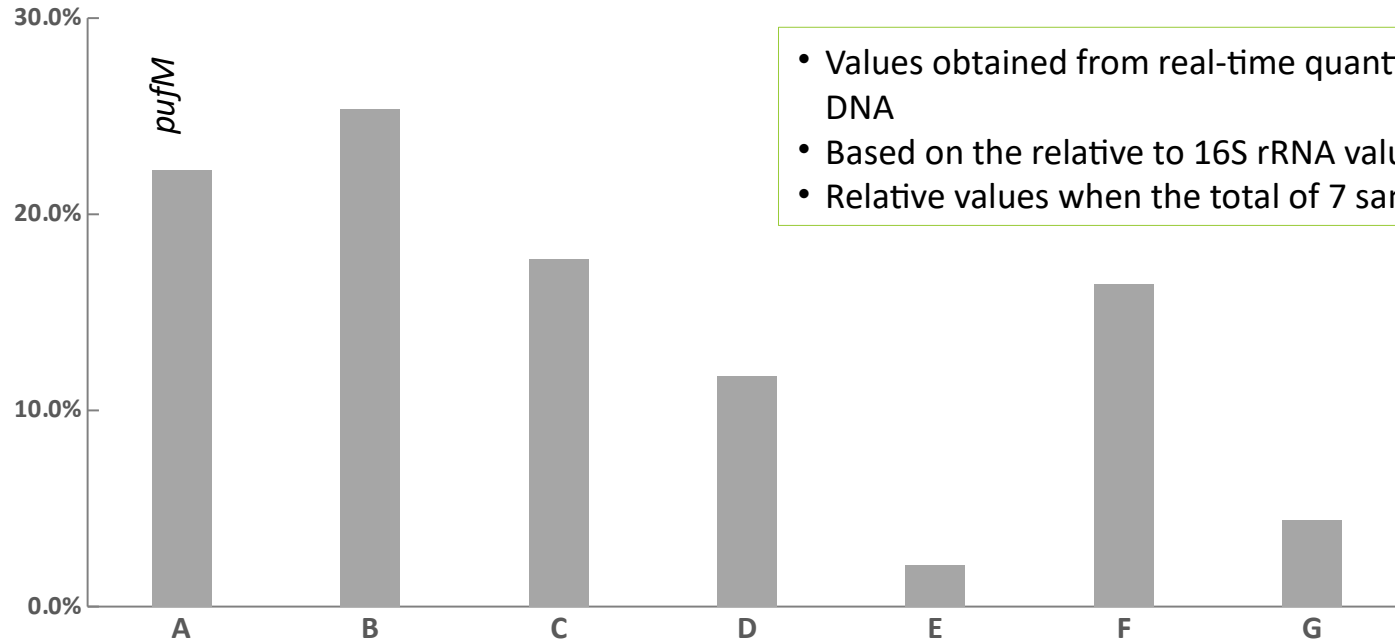
Relative amounts of photosynthetic pigments and redox conditions



- Values obtained from the second derivatives of the absorption spectra
- Bchl a: 870-875 nm, Bchl c: 744-762 nm
- Based on wet weight
- Relative values when the total of 7 samples is 100%

| | | | | | | | |
|------------------------|--------------|--------------|-------------|-------------|------------|--------|------------|
| redox, Eh mV | -68 | -50 | -45 | -36 | -6 | 46 | 64 |
| SH ⁻ μmol/L | 109 | 202 | 146 | 58 | 22 | 14 | 3 |
| O ₂ μmol/L | 33 | 42 | 84 | 116 | 130 | 74 | 134 |
| Color | Olive Orange | Olive Orange | Olive Green | Olive Green | Pale Brown | Orange | Pale Brown |
| Flow mm/s | 2 | 6 | 300 | 400 | 80 | 8 | 450 |

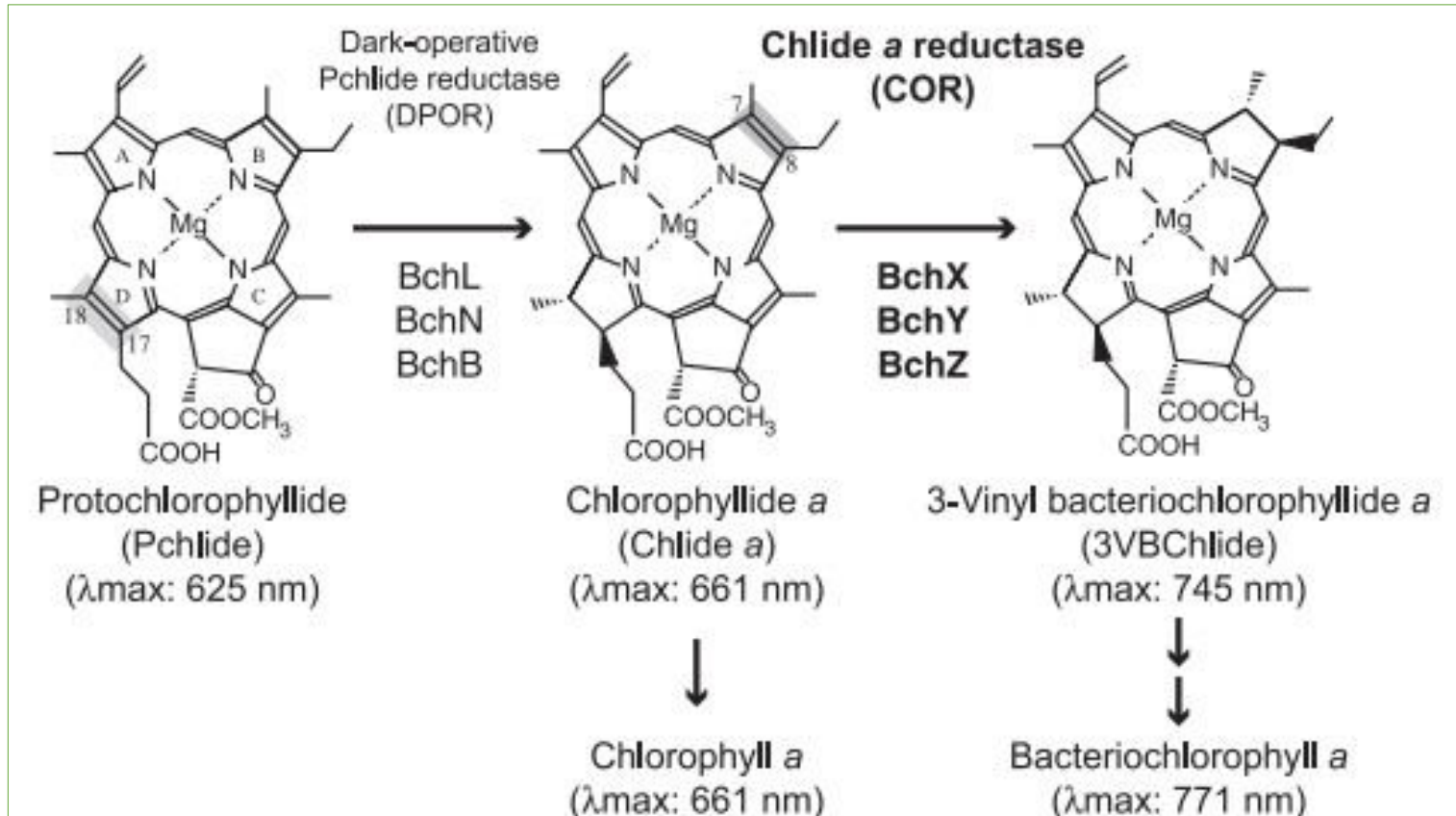
Relative amounts of photosynthetic RC gene *pufM* and redox conditions



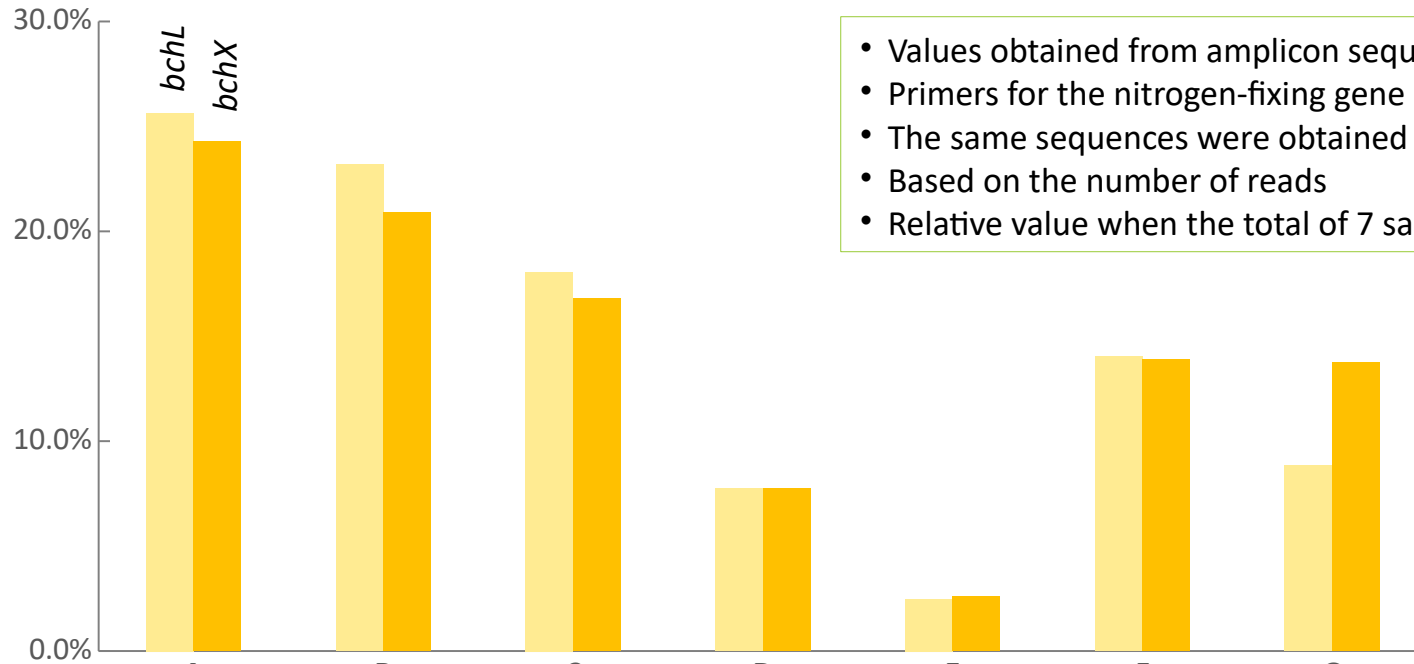
- Values obtained from real-time quantitative PCR of DNA
- Based on the relative to 16S rRNA value
- Relative values when the total of 7 samples is 100%

| | | | | | | | |
|------------------------|--------------|--------------|-------------|-------------|------------|--------|------------|
| redox, Eh mV | -68 | -50 | -45 | -36 | -6 | 46 | 64 |
| SH ⁻ μmol/L | 109 | 202 | 146 | 58 | 22 | 14 | 3 |
| O ₂ μmol/L | 33 | 42 | 84 | 116 | 130 | 74 | 134 |
| Color | Olive Orange | Olive Orange | Olive Green | Olive Green | Pale Brown | Orange | Pale Brown |
| Flow mm/s | 2 | 6 | 300 | 400 | 80 | 8 | 450 |

Reaction pathway of BchL and BchX in bacteriochlorophyll synthesis



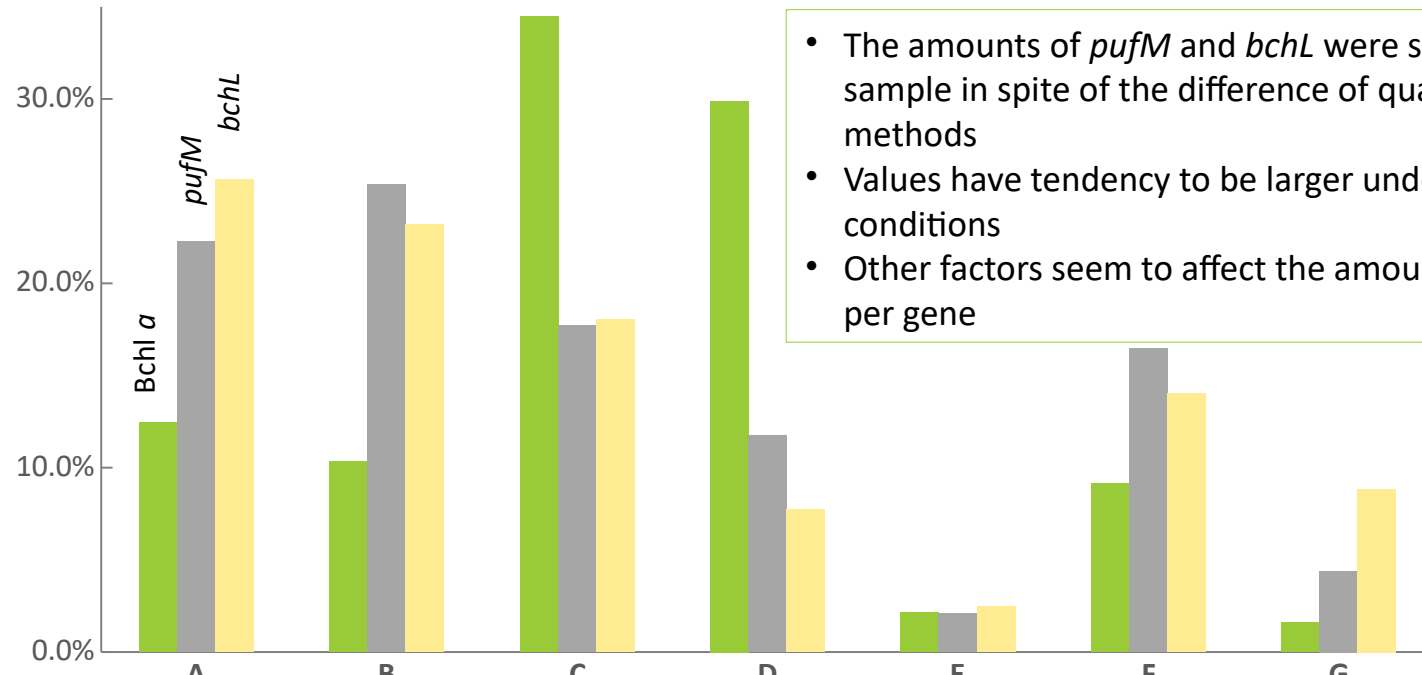
Relative amounts of *bchL* and *bchX* and redox conditions



- Values obtained from amplicon sequence analysis
- Primers for the nitrogen-fixing gene *nifH* were used
- The same sequences were obtained in all samples
- Based on the number of reads
- Relative value when the total of 7 samples is 100%

| | A | B | C | D | E | F | G |
|------------------------|--------------|--------------|-------------|-------------|------------|--------|------------|
| redox, Eh mV | -68 | -50 | -45 | -36 | -6 | 46 | 64 |
| SH ⁻ μmol/L | 109 | 202 | 146 | 58 | 22 | 14 | 3 |
| O ₂ μmol/L | 33 | 42 | 84 | 116 | 130 | 74 | 134 |
| Color | Olive Orange | Olive Orange | Olive Green | Olive Green | Pale Brown | Orange | Pale Brown |
| Flow mm/s | 2 | 6 | 300 | 400 | 80 | 8 | 450 |

Data summary: Amounts of Bchl α , *pufM*, and *bchL* and redox conditions



- The amounts of *pufM* and *bchL* were similar in each sample in spite of the difference of quantifying methods
- Values have tendency to be larger under reducing conditions
- Other factors seem to affect the amount of pigment per gene

| | | | | | | | |
|-----------------------------------|--------------|--------------|-------------|-------------|------------|--------|------------|
| redox, Eh mV | -68 | -50 | -45 | -36 | -6 | 46 | 64 |
| SH ⁻ $\mu\text{mol/L}$ | 109 | 202 | 146 | 58 | 22 | 14 | 3 |
| O ₂ $\mu\text{mol/L}$ | 33 | 42 | 84 | 116 | 130 | 74 | 134 |
| Color | Olive Orange | Olive Orange | Olive Green | Olive Green | Pale Brown | Orange | Pale Brown |
| Flow mm/s | 2 | 6 | 300 | 400 | 80 | 8 | 450 |

Conclusions

1. The same *Chloroflexus* grew under various redox conditions, hydrogen sulfide concentration, and oxygen concentration in Nakabusa hot springs.
2. The relative abundance of *Chloroflexus* was higher under low redox potential, high hydrogen sulfide concentration, and low oxygen concentration.
3. *Chloroflexus* at Nakabusa hot spring was mainly growing through hydrogen sulfide-dependent autotrophic photosynthesis.
4. Further research may bring us closer to understanding of the emergence of the first photosynthesis.

