

雪氷環境における好冷性微細藻類の共生細菌 *Hymenobacter nivis*のゲノム解析及び代謝機構の解明

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氷河や高山雪原では雪解けが進む春、雪の表面をピンクに染める赤雪現象が起こる。この現象は藻類増殖によるものであり、低温、強光、高紫外線、低栄養、凍結による乾燥などのストレス下においても藻類が生育している。赤雪には微細藻類のほか、細菌も検出されており、藻類が生産した有機物を消費していると思われる。南極の赤雪からは真核緑藻と一緒に従属栄養性細菌の *Hymenobacter nivis* が特異的に検出されている。本研究では、赤雪に優占している *H. nivis* が南極赤雪の厳しい環境へ適応するメカニズムを解明するため、ゲノム解析、プロテオーム解析及び培養実験を行った。その結果、光に反応するタンパク質（プロテオロドプシン、クリプトクロム等）の遺伝子がゲノム上に含まれていることが明らかになった。さらに、光と暗環境下とで本菌の増殖を比較すると、光環境下の方が速かった。また、*H. nivis* のプロテオームを解析したところ、光反応のタンパク質が発現されていることがわかった。このような光反応タンパク質により、*H. nivis* は光を認識し、利用することが可能であり、南極赤雪の低温・強光環境に適応し、優占していると考えられる。

Hymenobacter nivis was isolated from algae-rich red snow in Antarctica (Kojima et al., 2016). *H. nivis* was found to be by far the most dominating bacterium in red snow, suggesting that it is well-adapted to flourish in the snow surface environment (Fujii et al., 2010). This study aims to understand the adaptive strategy of *H. nivis* in low temperature and light-exposed environments through genome and proteome analyses as well as growth tests.

I. Analyzing the genome of *Hymenobacter nivis* P3^T

- The genome of *H. nivis* P3^T was sequenced using PacBio (Takara Bio).
- Genome sequencing reveals the presence of pathways adapted for cold and high light environments.
- H. nivis* P3^T genome contains the carotenoid biosynthesis pathway and light-reactive proteins such as proteorhodopsin, cryptochromes, a phytochrome, deoxyribodipyrimidine, and photolyase.

Table 1. Genomic features of *Hymenobacter nivis* P3^T

Features	
Genome size (bp)	5,027,597
Contig	1
DNA GC content (%)	62.5
Total protein-coding genes	4604
Protein-coding genes with function prediction	2563
Protein-coding genes of hypothetical function	2041
rRNA	9
tRNA	43
Number of subsystems	364
Protein-coding genes in subsystems	1324

II. Growth of *Hymenobacter nivis* P3^T is enhanced under light

- Growth of under light and dark conditions were compared at 5 °C and 15 °C
- Cell densities were determined using flow cytometry
- Both the speed and capacity of growth were enhanced under the light
- These results indicate that *H. nivis* can utilize light positively for growth

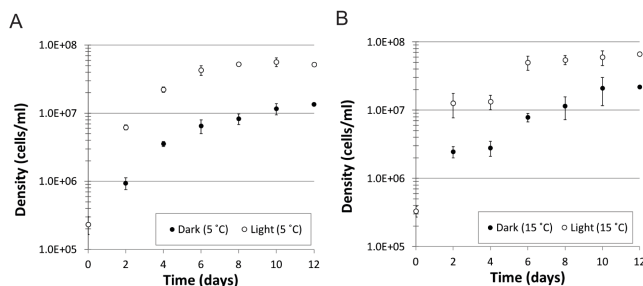


Fig. 1. Growth of *H. nivis* P3^T monitored under dark and light (200 $\mu\text{mol}/\text{m}^2\text{s}$) conditions at 5 °C (panel A) and 15 °C (panel B).

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References:

Fujii et al., 2010, *Microb Ecol*, 59(3):466-75; Kojima et al., 2016, *Int J Syst Evol Microbiol*, 66(11):4821-4825; Terashima et al., 2019, *Environ Microbiol Rep*, 11(2):227-235.

III. *H. nivis* cells grown in the dark produce more EPS

- Total carbohydrates and protein levels in the extracellular polymeric substances (EPS) were determined with cells at stationary growth phase.
- EPS was higher under the dark conditions on a per cell basis.

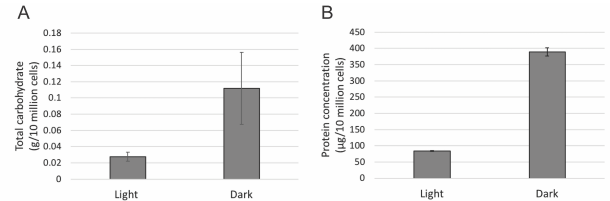


Fig. 2. Total carbohydrate (panel A) and protein (panel B) concentrations from extracellular polymeric substances (EPS) extracted from *H. nivis* P3^T cells at stationary phase after growth at 5 °C. $P=1.4 \times 10^{-4}$ (panel A) and $P=1.8 \times 10^{-6}$ (panel B) by non-paired two sample T-test.

IV. Light-reactive proteins are expressed in the proteome

- Total protein was extracted from *H. nivis* P3^T cultured in the light and dark conditions at 5 °C.
- The proteome was analyzed through mass spectrometry using a Q-Exactive plus Orbitrap mass spectrometer (Thermo Fisher Scientific).
- The proteome confirmed the expression of light reactive proteins such as proteorhodopsin, phytochrome, photolyase and cryptochromes.

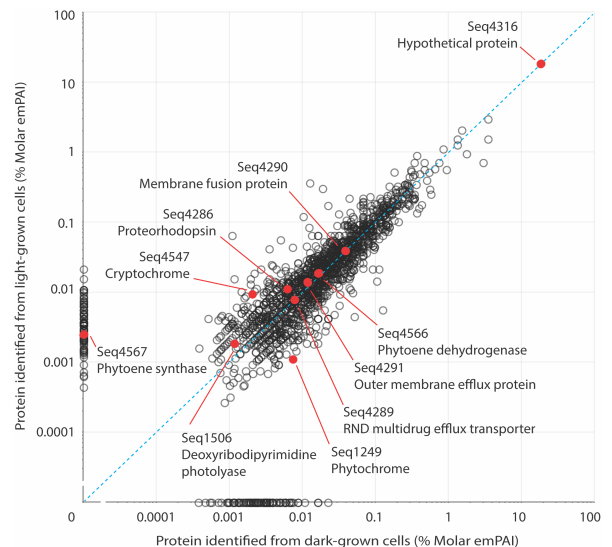


Fig. 3. Proteome of *H. nivis* P3^T under dark and light growth conditions. 1724 proteins are plotted on the graph and the values plotted represent the % molar emPAI for each protein identified with two or more unique peptides.

V. Conclusions

The genome of a Antarctic heterotrophic bacterium *Hymenobacter nivis* P3^T was sequenced, revealing several genes encoding for putative light-reactive proteins. Growth rates were enhanced under the light, while EPS accumulation was higher in the dark. Further analysis of the proteome of dark- and light-grown cells at 5 °C identified that the proteome remains similar between the two conditions. Furthermore, the expression of putative light-reactive proteins were confirmed. These identified proteins were: proteorhodopsin, cryptochrome, phytochrome, and deoxyribodipyrimidine photolyase. The light-enhanced growth of *H. nivis* P3^T and the expression of proteorhodopsin and other putative light-reactive proteins suggest that this bacterium is likely to sense and utilize light, resulting in a growth advantage under the light-exposed conditions of Antarctic red snow. The results from this study has been published (Terashima et al. 2019).