Physiological characterisation of the epiphytic bacterium *Marinobacter* sp. BPy-S1 isolated from sporophytes of the red alga *Pyropia yezoensis*.

Ryuya Matsuda[1], Shiori Furo[2], Katsuaki Takechi[3], Hiroyoshi Takano[3,4] and Susumu Takio[1]
[1] *Center for Water Cycle, Marine Environment and Disaster Management, Kumamoto University,*
[2]*Faculty of Science, Kumamoto University,*
[3]*Faculty of Advanced Science and Technology, Kumamoto University,*
[4]*Institute of Pulsed Power Science, Kumamoto University,*

E-mail: rmatsuda@kumamoto-u.ac.jp

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Abstract

The red alga *Pyropia yezoensis*, which has heteromorphic generations of sporophytes and gametophytes, is an important marine crop in Japan. Stable *P. yezoensis* cultures established for use in laboratory experiments coexist with epiphytic bacteria, some of which are essential for normal gametophyte morphogenesis. We isolated the epiphytic bacterium *Neptunomonas* sp. BPy-1, and found that it promoted gametophyte growth. The physiological properties of sporophyte epiphytes are unknown. Sporophytes are maintained as the seed source for gametophytes, which are cultivated in sea farms. Characterization of sporophyte epiphytes would facilitate sporophyte culture quality control. We investigated the physiological properties of the epiphytic bacterium BPy-S1 isolated from sporophytes in comparison with those of BPy-1.

The 16S rDNA sequence of BPy-S1 was 99% identical to that of *Marinobacter adhaerens* HP15, and 17 of 19 phenotypic characteristics of BPy-S1 were identical to those of *M. adhaerens* HP15. Therefore, BPy-S1 was classified as *Marinobacter* sp. BPy-S1. The 16S rDNA sequence analysis indicated the presence of at least six bacterial species in cultures of sporophytes and gametophyte, but BPy-S1 and BPy-1 were hardly detected. Moreover, a colony formation assay on nutrient-rich marine broth agar showed that most of the colonies derived from sporophytes and gametophytes were BPy-S1 and BPy-1, respectively. In summary, BPy-1 and BPy-S1 were minor components of the epiphytes of these hosts but exhibited the greatest activity on nutrient-rich medium. Because the nutrient-poor artificial seawater (ASW) used to cultivate host plants lacks a carbon sources, it did not support the growth of BPy-S1 or BPy-1. Furthermore, BPy-1, but not BPy-S1, grew well in ASW medium containing 0.1% glucose, indicating that BPy-S1 is less well adapted to nutrient-poor conditions than in BPy-1. Both BPy-S1 and BPy-1 grew in ASW medium containing 0.1% ethanol. Sporophyte culture in the ethanol-containing medium resulted in BPy-S1 overgrowth and marked inhibition of the photosynthetic activity of the sporophytes. Gametophyte culture in the ethanol-containing medium also led to BPy-1 overgrowth, but the photosynthetic activity of gametophytes was unaffected. These results indicate that BPy-S1 is capable of using ethanol as a carbon source under normal conditions but becomes saprophytic in an ethanol- enrich environment.