

Another look at green *Trichodesmium* colonies

Neveux et al. (2006) provide an important contribution on spectral diversity of phycoerythrins and diazotrophic abundance in tropical waters. As a minor component of the paper, Neveux et al. (2006) put forward two hypotheses about the spectral differences found between *Trichodesmium theibautii* and the “green” *Trichodesmium* colonies: (1) the green colonies are *T. theibautii* photoacclimated to low light, or (2) the green colonies may be a new species. In this commentary, we offer an alternative third hypothesis that was not considered by Neveux et al. (2006): the green *Trichodesmium* colonies may have been in senescence.

We participated in the 1999 cruise aboard the R/V *Ewing* (Neveux et al. 2006) and genetically characterized *Trichodesmium* colonies using three high-resolution molecular methods. The methods included whole genomic DNA fingerprinting using primers based on the highly iterated palindromic (HIP) repeat sequence (Orcutt et al. 2002). This polymerase chain reaction (PCR) technique allows for cyanobacterial species and strains to be distinguished and has been used to discriminate between species of *Trichodesmium* (Orcutt et al. 2002). Our technique uses a whole-cell approach where the entire colony is used in the PCR, and there is no DNA extraction step. The gel in Fig. 1 represents one entire PCR run using base-pair extended HIP primers (HIP-GC and HIP-CA) where all the samples were collected on the same day at Sta. 32 (one of the stations described in Neveux et al. 2006). All colonies were

prepared the same way and included the same green *Trichodesmium* as described in Neveux et al. (2006). Of the five green tuft and two green puff colonies examined, only the green puff colonies produced DNA amplicons. We found that the green *Trichodesmium* colonies that produced DNA amplicons were identical to other *T. theibautii* collected on the same day on the cruise (Fig. 1). Since all the other samples produced DNA amplicons, there was no indication that the PCR was inhibited by environmental factors or any of the reagents used in the reaction. The lack of DNA amplicons from the green tuft colonies suggests degenerated DNA, which is an indication that the colonies may have been in senescence.

The conclusion above was supported by the fact that the green colonies had no measurable nitrogenase activity (J. Burns and D. Capone, pers. comm.). The green colonies were collected between 50 and 120 m in the Coral Sea, and the presence of nitrate may have inhibited the rate of N₂ fixation. Holl and Montoya (2005) demonstrated that N₂ fixation in cultures of *T. erythraeum* were suppressed by up to 50–60% at high nitrate concentrations but never completely inhibited. Ohki et al. (1991) found that N₂ fixation was not suppressed after 7 h of incubation at 2 mmol L⁻¹ nitrate. Mulholland et al. (2001) however, reported decreases in N₂ fixation but only after additions in the order of 10 μmol L⁻¹ nitrate. The nitrate + nitrite profiles from Sta. 32 showed nitrate levels of 2 μmol L⁻¹ at

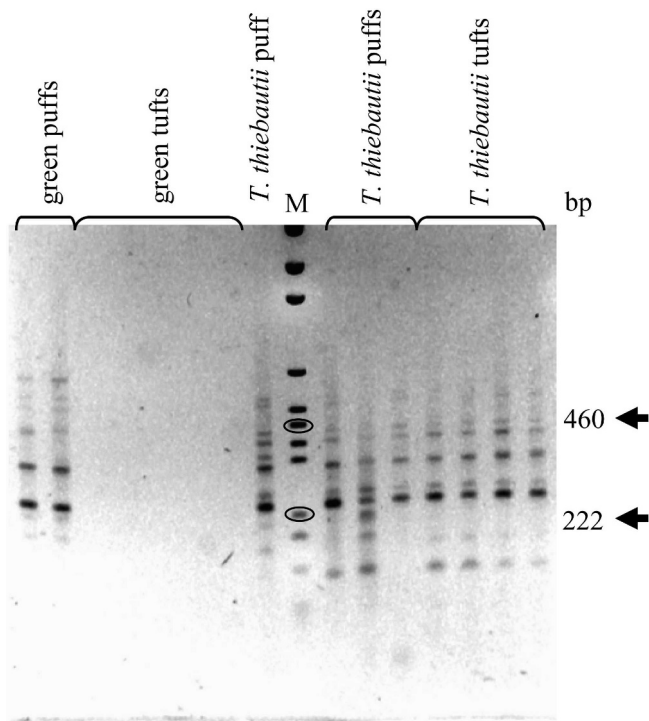


Fig. 1. DNA fingerprint patterns obtained with a combination of primers HIP-GC and HIP-CA for natural populations of green puff and tuft colonies and colonies of *T. thiebautii* puffs and tufts. The colonies were collected off North Australia, from Sta. 32 in the Coral Sea, during the R/V *Ewing* cruise in 1999. Lane M = molecular weight standards. Arrows shows the range of the primary bands (circled molecular markers) used for DNA fingerprint identification of *T. thiebautii*.

120-m depth (D. Capone, unpubl.). According to Holl and Montoya (2005), N_2 fixation in continuous cultures of *T. erythraeum* was inhibited 20–25% at this concentration level. Therefore, if the green colonies were viable and exposed to these levels of nitrate, a significant and detectable rate of nitrogenase activity would be expected.

If the colonies were in senescence, an alternate, third hypothesis to the green *Trichodesmium* colonies should be considered: a colony in some phase of senescence may absorb light inefficiently if compromised physiologically, and this would result in an inefficient electron transport system (fig. 7 in Neveux et al. 2006). The low light absorption in the UV region may also indicate stress in the green colonies (fig. 8 in Neveux et al. 2006), as *Trichodesmium* colonies have been shown to leak mycosporine-like amino acid molecules from the trichome sheath under physical stress (Subramaniam et al. 1999). The unusual fluorescence spectrum shown in fig. 1 in Neveux et al. (2006) may be the selective loss of phycoerythrin as seen in senescent *Trichodesmium* colonies. As demonstrated by Berman-Frank et al. (2004), varying degrees of senescence range from subtle (not always easily detected on visual inspection) to extreme changes within the cell in later phases. Typically, the programmed cell death pathway in *Trichodesmium* starts with the activation of endonucleases and proteases, while the cell membrane remains intact (Berman-Frank et al. 2004; Bidle and Falkowski 2004). One of the

early hallmarks of programmed cell death is degradation of the genome by endonucleases (Berman-Frank et al. 2004). By day 2 in their nutrient stress experiment, Berman-Frank et al. (2004, fig. 2) found that caspase activity (which appears concurrent with nuclease activity) was higher compared to the control, while the photosynthetic efficiency (Fv:Fm) remained near control level. The photosynthetic efficiency decreased after day 5 of the experiment, when caspase activity was extremely elevated. Another finding in Berman-Frank et al. (2004) was that natural populations of *Trichodesmium* exposed to environmental stress such as high light resulted in enhanced sinking. This type of environmental stress may be a reason why the green colonies were observed only between 50 and 120 m.

It was suggested in Neveux et al. (2006) that the high phycourobilin-to-phycoerythrobilin ratio (PUB:PEB) might be a result of low light adaptation in the green *Trichodesmium*. However, the high PUB:PEB ratio reported in Neveux et al. (2006) for the green *Trichodesmium* has also been observed in another study (Subramaniam et al. 1999) where diel measurements showed that this ratio was highest at midday under high light conditions. The Subramaniam et al. (1999) study suggests that the interconversion between PEB and PUB, with higher PUB under high light, is a mechanism to down-regulate the available energy supply to photosystem II. If the green colonies had a history of high light exposure, they may have entered senescence because of photoinduced stress. This photoinduced stress may have altered the pigment signature resulting in a down-regulated photosystem in the green colonies. As shown by Villareal (2004) for open ocean diatoms, photoinduced stress and a diminished relative electron transport rate may be a response to sustained high light. Villareal (2004) found high initial yields associated with depressed maximal relative electron transport rate in photoinhibited cells and suggested that this parameter could indicate light-induced stress. This scenario could explain the unusual pigment signature, location in the water column, and the low relative rate of electron transport found in the green colonies of *Trichodesmium*.

The speciation concept, particularly within the genus *Trichodesmium*, is poorly understood, and that is why we are compelled to write this commentary. Studies based on ultrastructure and morphology have shown an overlap with regard to *Trichodesmium* speciation by trichome cell width and length (Janson et al. 1995), necessitating the need for molecular tools (Janson et al. 1999; Orcutt et al. 2002; Lundgren et al. 2005). Using conserved sequences like structural genes (*NifH*, *HetR*) or 16S rRNA gene sequences, *Trichodesmium* species have been found to be genetically very similar (Ben-Porath et al. 1993; Zehr et al. 1990; Janson et al. 1999; Orcutt et al. 2002; Lundgren et al. 2005). Therefore, the diversity between closely related species within the genus *Trichodesmium* is better accomplished by using high-resolution genetic tools with highly variable regions of DNA, such as the intergenic transcribed spacer region between the 16S and 23S rRNA genes or repetitive DNA such as HIP repeat sequences (Smith et al. 1998; Orcutt et al. 2002).

We note that comparative analysis of conserved 16S rRNA sequences alone may result in an artificial classifi-

cation of cyanobacteria (Giovannoni et al. 1988; Nelissen et al. 1996; Palinska et al. 1996; Neilan et al. 1997). Therefore, in this commentary, we emphasize the importance of a polyphasic triangular approach that incorporates phenotypic, genotypic, and phylogenetic information to determine the speciation of microbes (Vandamme et al. 1996). As of today, *Trichodesmium* speciation is not well established, and it can be misleading if speculation of a new species relies only on physiological parameters such as pigmentation or photosynthetic response alone. Based on the presence of degenerated DNA, the identical genomic signature to *T. theibautii*, the low electron transport, and absence of nitrogenase activity, we suggest an additional hypothesis on green *Trichodesmium*. In addition to the possibility of photoacclimation to low light, we suggest that the green colonies in Neveux et al. (2006) are *T. theibautii* that could have been in a state of senescence.

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