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Research Note

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Symbiosis between *Rhizobium* and the non-legume, *Polypleurum wallichii*

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ABSTRACT

Polypleurum wallichii is an aquatic plant found in East- Khasi Hill District of Meghalaya, in the Northeast India. The endophyte was successfully cultured using Tryptone-yeast medium. Holdfast as well as thallus showed Gram-negative bacterium resembling the bacterioids of *Rhizobium,* (designated as Polypleurum type-I) similar to nodules from *Glycine max* and other tropical legumes. The plant body of *Polypleurum* has poor vasculature and lack intercellular space, hence, no formation of distinct nodules. The observations indicate that the plant is a nitrogen fixing non-legume, and the endophyte bacterium is member of *Rhizobium*

Key words: *Polypleurum wallichii,* Podostemaceae, *Rhizobium*, Meghalaya.

Polypleurum wallichii is aquatic plant growing on the rocks in the streams of Janiaw, East-Khasi Hill District of the state of Meghalaya, in the Northeast India. *Polypleurum* belongs to the family Podostemaceae. The plant body is thallus like liverworts, attached by holdfast, which is about 5-15 mm long by 3-5 mm diameter. Plants were collected during October–November from the year 2001 to 2003. Culture of the endophyte was attempted on Tryptone-yeast medium[1,2] without adequate combined nitrogen after complete sterilization. The abundant growth indicated that these organisms were present in large number. Holdfast as well as thallus examined by phase contrast microscopy showed rods resembling the bacterioids of *Rhizobium,* (designated as Polypleurum type-I) similar to nodules from *Glycine max* and other tropical legumes. The bacterium is motile, gram negative and measures 1.6 µm to 3 µm X 0.5µm to 0.9 µm. The samples were also observed with TEM (Fig. 1); the bacteria are mainly straight but some times curved, occurring singly or in groups inside the vesicles, with two to three refractile areas near the ends and sometimes in the middle of cells.

Unlike the legumes, the *Polypleurum* do not form nodules because of the lack of proper root system. The endophytes are intracellular and grow inside the hapteron and sub-dermal layers of the thalli. *Rhizobium* strain was tested for its

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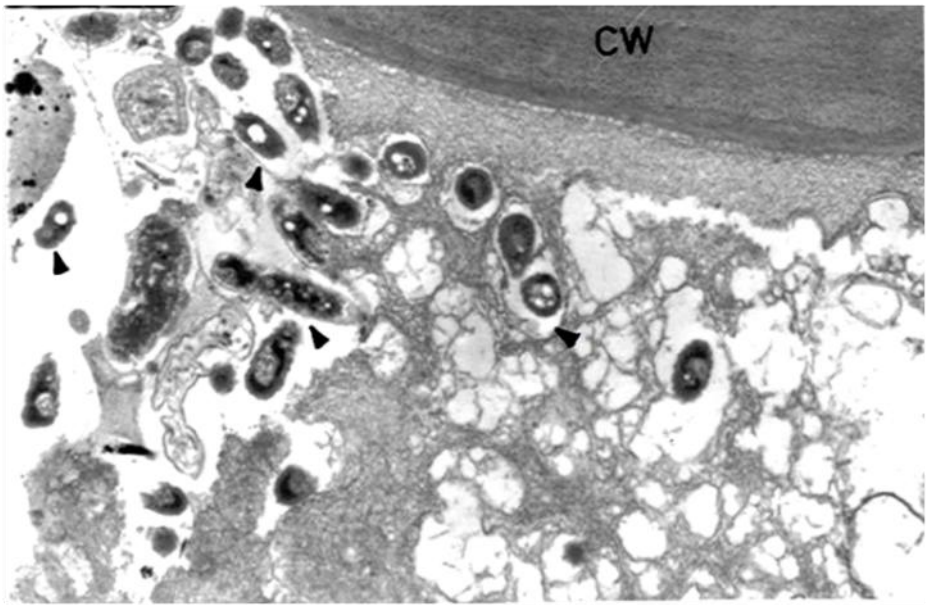

Figure 1. Electron micrograph of the peripheral cytoplasm of holdfast protoplast, cell wall (CW); bacteriods are present in vesicles in the cytoplasm (arrow heads). X 11,000.

ability to grow in seedlings of *Polypleurum*. All treatments were in triplicate as per Mohanram and Sehgal[3] with bacteria and the germinated seedlings also contained the same strains of *Rhizobium.*

Acetylene reduction assays for nitrogenase activity (4) was carried out after surface sterilization with 1.5% sodium hypochlorite/0.1% Triton X100 for five minutes and thoroughly washed with sterile water. Nitrogenase activity showed more in the holdfast (58.56 n mol C_2H_4/g^{-1} dry wt/ h^{-1}) than in the thallus (19.95 n mol C_2H_4/g^{-1} dry wt/ h^{-1}). The plant body of *Polypleurum* has poorly developed vasculature and lack of intercellular space. Therefore, the formation of distinct nodules, like other legumes, is absent.

So far, the ability of a plant to establish a nitrogen-fixing symbiosis is restricted to legumes with two exceptions; the genera *Parasponia* (5) and *Trema* (6) of the Ulmaceae family. Our results show that *Polypleurum wallichii* may now be added to the list of nitrogen fixing non-legume, aquatic plant, and that the endophyte is bacterium. The bacteria satisfied the criteria necessary to classify them as *Rhizobium* species.

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