



Complete niche separation in closely related bacteria: obligate endosymbionts and obligate free-living *Polynucleobacter* strains

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The *Polynucleobacter necessarius*-cluster is a monophyletic group of bacteria belonging to the class *Beta-Proteobacteria*. The name of the cluster derives from the only validly described species of the group, namely *P. necessarius*. This bacterium is an obligate endosymbiont of the ciliate *Euplotes aediculatus*. Several bacteria belonging to the genus *Polynucleobacter* were found to be symbionts of different *Euplotes* species. For all symbiotic *Polynucleobacter* it was demonstrated that they are necessary for the division process of their hosts, thus demonstrating the high degree of specialization of this symbiosis. In recent years, several studies by culture-independent 16S rRNA gene targeted techniques indicated abundant occurrence of *Polynucleobacter* cluster bacteria in the pelagic of freshwater habitats. Recently, cultivation and characterization of isolates belonging to this cluster was successfully performed. A comparative investigation between symbionts and freshwater isolates was performed in order to understand their phylogenetic, physiological and ecological relationships. The endosymbionts and the freshwater isolates are closely related (> 98.5% 16S rRNA similarity). Due to a high degree of conservation of sequences, neither 16S rRNA gene, nor 16-23S ITS sequence analyses resolve satisfactorily the phylogenetic relationships amongst the closely related endosymbionts and the strains isolated from freshwater habitats. On the other hand, cultivation attempts of endosymbionts, performed with the techniques successfully used for the isolated strains, failed. This showed that growth requirements of *Polynucleobacter* symbionts differ from those of their isolated relatives. Furthermore, environmental detection of symbionts and of isolated strains by FISH

(Fluorescence *In Situ* Hybridization) showed that the two groups of organisms do not share the same habitat and that isolated strains really represent free-living organisms. Our results show that while symbiotic and free-living *Polynucleobacter* bacteria are close phylogenetic relatives, they show completely different physiological and ecological traits. We found no evidence for switching between endosymbiotic and free-living growth. Therefore, we conclude that these closely related bacteria occupy completely separated ecological niches.