Characterizing Commas: Discovery and exploration of a novel family of pleomorphic Rhizobiales bacteria isolated from herbivorous ants

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Introduction

Ants are one of the most abundant, diverse, and ecologically important insects on the planet. Of particular interest to us are herbivorous ants, specifically within the genus Cephalotes (turtle ants). Interestingly, the evolution of herbivory in ants led to extensive diversification [1]. This is a somewhat surprising occurrence, as an herbivorous diet tends not to provide enough of certain necessary nutrients, like amino acids and nitrogen, for an organism to survive. Hence, we hypothesize that in the case of the Cephalotes ants, bacterial provisioning of nutrients allows an herbivorous lifestyle, and is a significant factor in ant diversification, allowing the ant to move into new niches and habitats where speciation can occur.

Five orders of bacteria are conserved in the guts of all herbivorous ants with bacteria in the order Rhizobiales being most closely linked to herbivory [2]. Interestingly, feeding studies have shown that when fed a diet of pollen, the number of Cephalotes increases significantly in the gut of Cephalotes varians, suggesting these bacteria have a role in ant nutrient provisioning [3]. To better understand this role, we isolated several novel Rhizobiales from Cephalotes varians ants throughout the Florida Keys and characterized key metabolic characteristics.

Interestingly, preliminary DNA sequencing of the Rhizobiales isolates suggested they may be members of a new family of bacteria. Hence we also use these metabolic characteristics to validate their placement within a new family of bacteria, as well as name and validly publish them as such.

Objectives

1. Physiologically characterize Rhizobiales cultivars and compare with members from the same family
2. Place Rhizobiales within a robust phylogenetic framework
3. Determine pectinolytic ability of Rhizobia cultivars

General Methods

Unless otherwise specified, cells were grown at room temp on TSA or TSA-pectin plates and in TSB liquid media. Gaseous environment alternated between 2% O2/5% CO2 (hypoxia) and 20% O2/1% CO2. After fixation in 2.5% glutaraldehyde/0.1M cacodylate solution, thin sectioning and transmission electron imaging were done at the MSU Center for Advanced Microscopy.

Growth Experiments

Figure 1. Effect of CO2 concentration on generation time of JR021-5. Since bacteria were isolated under an atmosphere of 5% CO2 (with 2% O2, 93% N2), we sought to determine if CO2 was a requirement for growth or was growth-stimulating. Under a starting headspace of 100% N2, Balch tubes were injected with pure CO2 and atmospheric air to obtain a final headspace composition of 0.5% CO2 in 0.5% and then 1% increments. O2 was maintained at 2% in each tube. Values are expressed as mean±SD of hours and were collected from 3 replicates.

Figure 2. To assess anaerobic, aerobic, or possible microaerophilic metabolism, we measured the effect of O2 and CO2 concentration on growth of JR021-5. Under a starting headspace of 100% N2, Balch tubes were injected with pure CO2 and atmospheric air to obtain a final headspace composition of 0% O2 with 0% CO2 (0/0), 5% O2 with 1% CO2 (5/1), 10% O2 with 1% CO2 (10/1), 20% O2 with 1% CO2 (20/1), and 20% O2 with 0% CO2 (20/0). Values are expressed as mean±SD of hours and were collected from 3 replicates. While growth occurred at 20/0 in liquid media, JR021-5 did not grow on solid media incubated on the bench top.

Microscopy and Phenotyping

Table 1. Substrate utilization profile of isolate JR021-5, compared with the well-characterized Rhizobium leguminosarum. The substrate utilization profile of JR021-5 clearly distinguishes it from other members of the Rhizobiaceae, gives a glimpse into its in situ environment and metabolic role as primarily a mono- and disaccharide degrader. However, it can utilize other non-sugar compounds such as L-lactic acid, citric acid, D-glucaronic acid, l-malic acid, α-hydroxybutyric acid, α-keto-glutaric acid, glucuronamide, puruvic acid methyl ester, and esculin ferric citrate, which suggests nutritional versatility, a possible benefit to the ant should it need to switch diets.

Conclusions

The morphology, substrate utilization, and DNA sequence of JR021-5 and its relatives place these bacteria well within a new family of the Rhizobiales order. A name has not yet been determined, but the genus will likely translate to the Latin form of “sent rod” and the species, likewise, to “esculin-swallowing.”

The CO2 growth data suggest that 3% CO2 yields significantly faster growth (p<0.05). Growth rates at 0% O2 and 20% O2 are not significantly different, suggesting a facultatively aerobic lifestyle that may allow JR021-5 to thrive in the varying conditions of the ant gut as the ant develops from larve to adult. Growth at 0% O2 suggests a need for further studies focused on the ability of JR021-5 to perform anaerobic respiration. Cell yield data is not clear on whether a greater O2 concentration facilitates more abundant growth. Further studies are needed.

The use of sugars by JR021-5 implies one function in the ant gut is to degrade sugar or sugar-polymers. Investigation with pectinase assays into the ability of Rhizobiales to degrade pectin is ongoing. JR021-5’s use of esculin may indicate an ability to degrade plant toxin, which would be beneficial for both the bacterium and the ant host.

This study lays the groundwork for the comparison of Rhizobiales isolated from Cephalotes varians (such as JR021-5) with Rhizobiales isolated from other Cephalotes species. These comparisons may shed light on metabolic differences that could have been important factors in ant speciation.

References and Acknowledgements


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