

# Metabolic Conservation or Divergence in Turtle Ant Gut Bacteria?

Our project is centered around the question of "how has the microbial component to the Cephalotes ant holobiont system contributed to ant evolution?" 117 different species of Cephalotes (turtle) ants are found in neotropical areas, such as the Caribbean, American Southwest, and South America. Cephalotes have a carbohydrate-rich and nitrogen-poor diet, which is nutritionally problematic for the ant. In addition, Cephalotes are unique among ants because different species share a remarkably similar community of gut bacteria. We recently isolated members of a novel family of Pseudomonadales bacteria from 10 different species of turtle ant and two sister genera. Our particular objective for this project is to determine if the Pseudomonadales isolates have conserved metabolic function or if the metabolism of the bacteria have diverged as the ant hosts have speciated. This should give insights into the role and importance of these bacteria to the ant, and whether functional differences reflect diet and habitat difference between ant species.



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Figure 1. Maximum-likelihood 16S rRNA-based phylogenetic tree of the *Pseudomonadales* isolates from ants used in this study. The majority of isolates group within the novel family Ventosamonadaceae, and suggest a high degree of cospeciation with their ant host.

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Ant Species	C. varians	C. rohweri	C. texanus	C. umbraculatus	C. minutus	C. cordatus	C. placidus
C. varians	99.0 ± 0.6						
C. rohweri	94.7 ± 0.3	99.9 ± 0.1					
C. texanus	94.0 ± 0.7	93.I ± 0.3	99.4 ± 0.3				
C. umbraculatus	94.7 ± 0.6	92.8 ± 0.2	92.8 ± 0.6	99.1 ± 0.7			
C. minutus	94.2 ± 0.6	93.I ± 0.2	96.9 ± 0.2	92.8 ± 0.2	98.8 ± 0.1		
C. cordatus	94.6 ± 0.7	92.3 ± 0.4	96.4 ± 0.6	93.1 ± 0.6	98.3 ± 0.5	98.7 ± 0.5	
C. placidus	91.8 ± 0.5	90.6 ± 0.3	93.9 ± 0.5	88.9 ± 0.3	93.3 ± 0.2	93.5 ± 0.3	99.1 ± 0.4
C. atratus	91.8 ± 0.1	90.5 ± 0.3	94.1 ± 0.5	89.2 ± 0.2	93.I ± 0.2	93.3 ± 0.2	98.9 ± 0.3
Procryptocerus	92.I ± 0.3	91.9 ± 0.3	94.0 ±0.4	89.5 ± 0.4	94.3 ± 0.2	94.2 ± 0.2	96.2 ± 0.2
P. aeruginosa	87.1 ± 0.4	86.0 ± 0.3	85.2 ± 0.8	84.1 ± 0.3	85.5 ± 0.3	85.9 ± 0.6	88.7 ± 0.6

	С. с	occulatus	C. spinosus	C. texa	nus	(	C. rohwe	ri	C. placidu	IS		C. minutus	C	c. varians	;	(	C. cordatus		C. cordatus	С. ғ	itratus	Procry	otocerus	С. (	umbraculatus	
Growth Substrate	CO-01	CO-20 CO-31	CS-O3 CS-11 CS-20	JR29A-25 JR29A-103	3 JRO38B-215	SP57-226	SP57-227	SP57-20 CSM 3490-	16 CSM 3490-21	CSM 3490-23	CSM 3523-6	CSM 3523-4 CSM 3523-	48 CV29R2	CV51a CSN	VI 3490-16	CSM 3433-4	CSM 3433-6 CSM 3433-34	CSM 3433-10	CSM 3433-20 CSM 3433-2	5 CSM 3467-8	CSM 3467-11	CSM 3487-16 CSM 3	487-47 CSM 3487-52	CSM 3482-27	CSM 3482-52 CSM 34	33-44
Dextrin D-Maltose																										
D-Trehalose D-Cellobiose																										S
Gentiobiose Sucrose																										n
D-Turanose Stachyose																										
D-Raffinose																										
D-Meliblose																										N
Beta-Methyl-D-Glucoside D-Salicin																										re
N-Acetyl-D-Glucosamine N-Acetyl-Beta-D-Mannosamine																										fr
N-Acetyl-D-Galactosamine N-Acetyl Neuraminic Acid																										
alpha-D-Glucose D-Mannose																										V
D-Fructose																										C
3-Methyl Glucose																										Q
D-Fucose L-Fucose																										fv
L-Rhamnose D-Glucose-6-PO4																										
D-Fructose-6-PO4 D-Sorbitol																										
D-Mannitol																										C
Glycerol																										b
D-Aspartic Acid D-Serine																										S
Glycyl-L-Proline L-Alanine																										2
L-Arginine L-Aspartic Acid																										
L-Glutamic Acid L-Histidine																										
L-Pyroglutamic Acid																										iş
D-Galacturonic Acid																										n
L-Galactonic Acid Lactone D-Gluconic Acid																										
D-Glucuronic Acid Glucuronamide																										¥
Mucic Acid Quinic Acid	-																									U
D-Saccharic Acid p-Hydroxy-Phenylacetic Acid																										a
Methyl Pyrvate																										r r
L-Lactic Acid																										tľ
Citric Acid alpha-Keto-Glutaric Acid			·																							¥
D-Malic Acid L-Malic Acid							-																			
Bromo-Succinic Acid gamma-Amino-Butryic Acid																										n
alpha-Hydroxy-DL-Butyric Acid																										p
alpha-Keto-Butryic Acid																										
Acetoacetic Acid Propionic Acid																										
Acetic Acid Formic Acid																										







Table 1. Average (± standard deviation) percent similarities in 16S RNA sequence from Ventosamonadaceae isolates from each ant species studied. A <98% similarity denotes separate bacterial species. This shows that each species of ant has its own coevolved genus within the Ventosamonadaceae, evidence that this is an ancient symbiosis.

## Future Directions:

Perform HPLC as
study amino acid
consumption and
production by turt
ventosamonads
Assay urea produ
degradation by
ventosamonads
Assay for other po
important metabo
functions such as
degradation
Assay Acinetobac
like isolates for pe
degradation

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ire 2. Inter- and intra-ant cies analysis of 94 abolic function of osamonad isolates. abolic data shows arkable conservation of tion across osamonads isolated from rent species of ants. sistently, ventosamonad tion is focused on radation of amino acids, te, and gamma-aminoric acid. This strongly gests these molecules are lable in the ant gut pective of species. There ter- and intra-species abolic variation of osamonads. Interestingly, consumption of amino s by the ventosamonads esents a nitrogen loss to system and potential harm he ant host. We expect the gen loss is offset by uction of essential amino s or metabolites. Figure 3. Short-chain fatty acid (SCFA) digestion and production by ventosamonad isolates. (A) uninoculated control. (B) representative ventosamonad spectra. Spectra consistently show acetate consumption (peak = retention time 2.45) and possibly butyrate



production. Microbiallyproduced SCFA's are a major energy source for many insects.