Metabolic Conservation or Divergence in Turtle Ant Gut Bacteria?

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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.

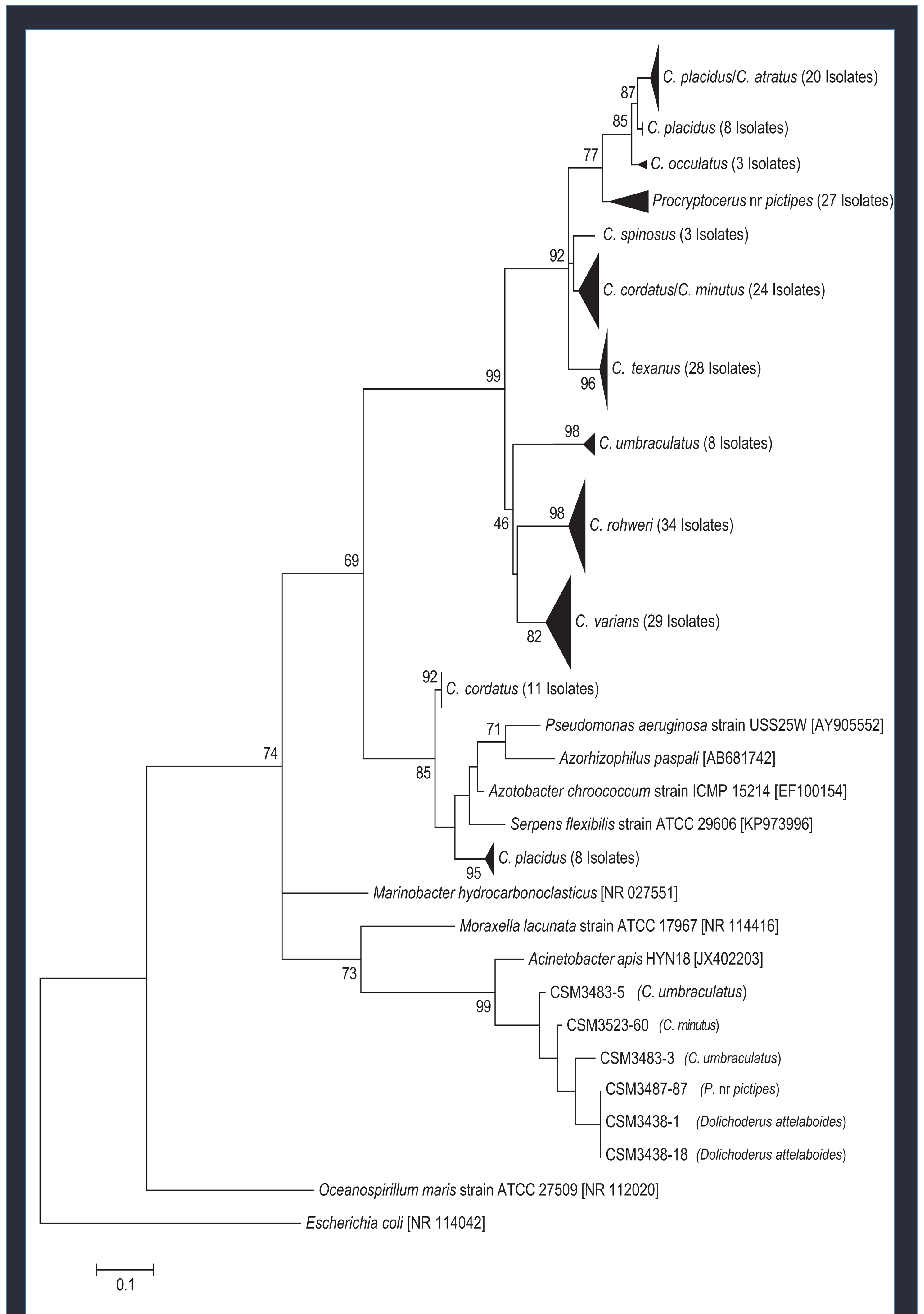

Figure 1. Maximum-likelihood 16S rRNA-based phylogenetic tree of the *Pseudomonadales* isolates from ants used in this study. The majority of isolates group within the novel family *Ventosamonadaceae*, and suggest a high degree of co-speciation with their ant host.

Ant Species	C. varians	C. rohweri	C. texanus	C. umbraculatus	C. minutus	C. cordatus	C. placidus	C. atratus	Procryptocerus
C. varians	99.0 ± 0.6								
C. rohweri	94.7 ± 0.3	99.9 ± 0.1							
C. texanus	94.0 ± 0.7	93.1 ± 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	93.1 ± 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	93.1 ± 0.2	93.3 ± 0.2	98.9 ± 0.3	99.7 ± 0.1	
Procryptocerus	92.1 ± 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	96.3 ± 0.1	99.6 ± 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	88.0 ± 0.1	88.0 ± 0.4

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:

1. Perform HPLC assays to study amino acid consumption and production by turtle ant ventosamonads
2. Assay urea production and degradation by ventosamonads
3. Assay for other potentially important metabolic functions such as plant toxin degradation
4. Assay Acinetobacter apis-like isolates for pectin degradation

Acknowledgements:
Jonathan Lin and Mark Schotanus.

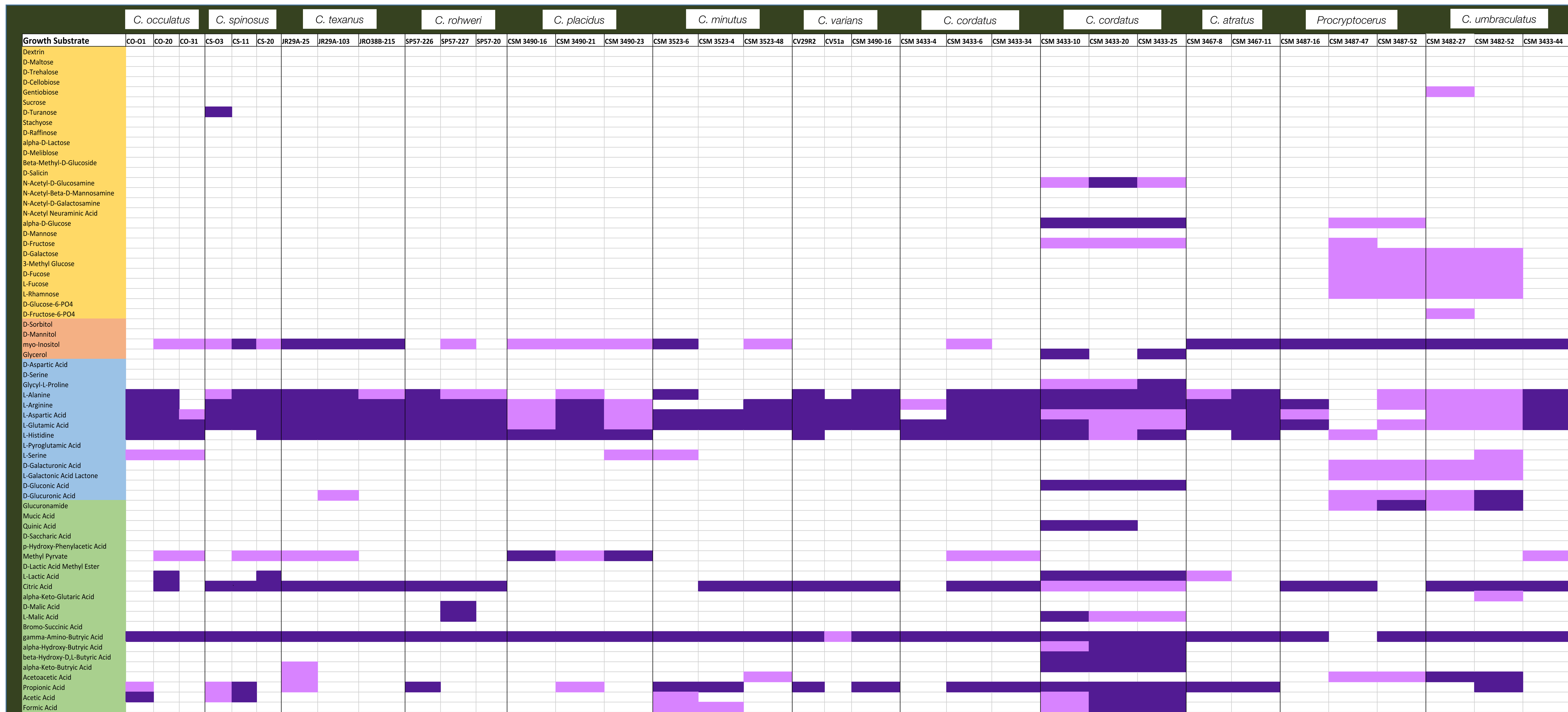

Figure 2. Inter- and intra-ant species analysis of 94 metabolic function of ventosamonad isolates. Metabolic data shows remarkable conservation of function across ventosamonads isolated from different species of ants. Consistently, ventosamonad function is focused on degradation of amino acids, citrate, and gamma-amino-butyric acid. This strongly suggests these molecules are available in the ant gut irrespective of species. There is inter- and intra-species metabolic variation of ventosamonads. Interestingly, the consumption of amino acids by the ventosamonads represents a nitrogen loss to the system and potential harm to the ant host. We expect the nitrogen loss is offset by production of essential amino acids or metabolites.

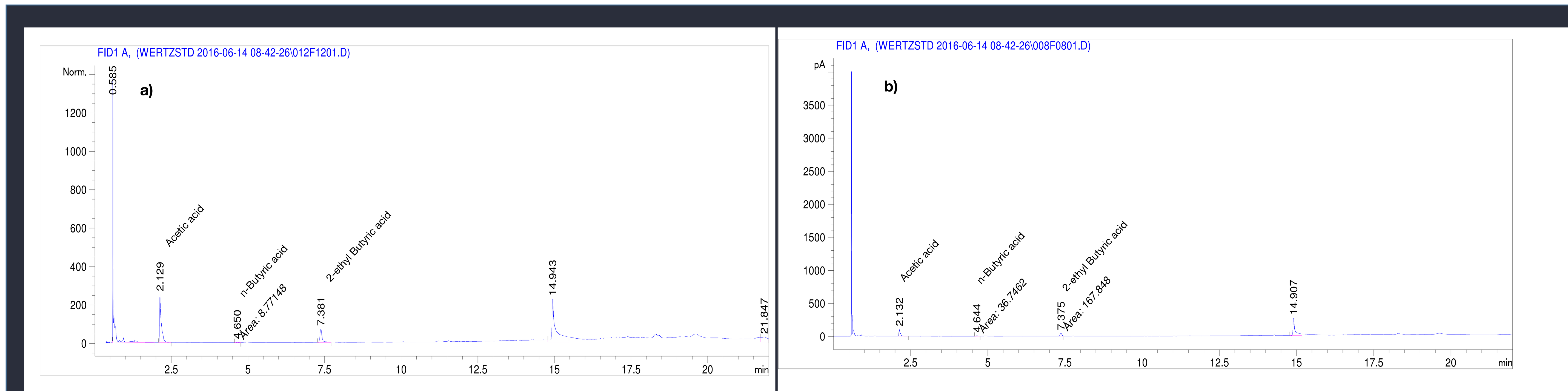

Figure 3. Short-chain fatty acid (SCFA) digestion and production by ventosamonad isolates. (A) uninoculated control. (B) representative ventosamonad spectra. Spectra consistently show acetate consumption (peak = retention time 2.45) and possibly butyrate production. Microbially-produced SCFA's are a major energy source for many insects.