Acidovorax avenae: Causal Agent for Soybean Yield Decline in South Carolina.

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Background and Justification:

In South Carolina, 35% of the soybean acreage is non-rotated and over 50% of the acreage is double-cropped with wheat (Norsworthy, 2002). In research conducted at the Pee Dee REC from 1997 to 1999, soybean growth was stunted per visual observations and yields were decreased 32% in non-rotated, double-cropped soybean compared to double-cropped soybean rotated with corn (Frederick et al., 2001). Part of this yield loss could be due to the presence of deleterious bacteria on the roots, which inhibit root functions. Twenty-five genera and 34 species of rhizosphere bacteria were isolated from soil around soybean roots when crop rotation was used; whereas, 19 genera and 28 species of rhizosphere bacteria were isolated from soybean roots in the non-rotated plots.

Acidovorax avenae, a bacterial pathogen (Schaad et al., 2001), was found in a high proportion when rotation was not used (38% of root bacteria isolated), but in a much lower proportion when soybean was rotated with corn (4% of rhizobacteria isolated). *Acidovorax avenae* apparently displaced other major species on the soybean roots. Pathogenic strains of *Acidovorax avenae* produce foliar blight symptoms on other crops (Schaad et al., 2001); however, crop stunting was the main response noted in these plants with little or no foliar blight symptoms. These preliminary data suggest that at least a portion of the soybean seed yield increase due to crop rotation was the result of significantly fewer (10-fold less) *Acidovorax* bacteria in the rhizosphere. These data are also supported by decreased soybean yields from the same plots in 2001 and decreased yields in double-cropped wheat. The objective of this research was to determine if there is a cause and effect relationship between the surge in *Acidovorax avenae* and lower soybean yields when growers do not use crop rotations.

Identification of bacteria associated with yield decline of soybean:

In an effort to identify a soilborne bacterium associated with soybean yield decline in South Carolina, 78 bacterial isolates were selected for evaluation. Seventeen of these isolates failed to grow when re-cultured on solid growth agar (King's medium B (KMB)).

All of the isolates were previously identified as *Acidovorax avenae* subsp. *avenae* or *A. avenae* by fatty acid methyl ester profile analysis. *A .avenae ssp avenae* is a Gram-negative bacterium that grows slowly and appears cream to off white on King's B medium. It is more often associated with foliar diseases of grasses including corn and rice. To confirm the identity of the strains, polymerase chain reaction (PCR) using two sets of genus specific oligonucleotide primers, and substrate-utilization profiles were analyzed using the BIOLOG (Hayward CA) system.

Early on, it was clear that not all of the strains would be *A. avenae* since many displayed varying colony morphologies on KMB. Several produced a fluorescent green pigment, indicative of certain Pseudomonas species. Nevertheless, 44% (27/61 viable isolates) of the strains yielded an appropriately sized DNA product when tested with the PCR primer pair, WFB1/2 (Walcott and Gitaitis, 2000). WFB1/2 is specific for the genus Acidovorax. So while it can direct the amplification of DNA from species in the *Acidovorax-genus* it cannot distinguish between the species and subspecies. Hence data generated with its primer set has limited utility.

To narrow the focus of identification, attempts were made to amplify DNA from all of the strains using RST49/51, a primer set designed at the University of Florida by Dr. Bob Stall's research group. Like WFB1/2, RST49/51 reacts with several *Acidovorax* species, however, when the PCR product is digested with *Hae* III restriction enzyme, a unique pattern is produced for *A. avenae* subsp. *avenae* that distinguishes it from other *Acidovorax* spp. Approximately 10% (6/61) of the strains yielded an amplicon with RST49/51. However, the restriction digestion patterns for these strains were not identical to that of a reference strain of *A. avenae* subsp. *avenae*.

When the substrate utilization profiles were analyzed for the strains, a wide range of bacterial names was generated (Table 1). Of the 61 strains, only Weed 4 M15, a root bacterium from crabgrass, was identified as *A. avenae* subsp. *avenae*. Hypersensitive response assays conducted on a sub-sample of the strains on tobacco indicated that they were all capable of causing plant disease. Unfortunately, this data does not indicate if the strains would be pathogenic on soybean.

Table 1. Reaction of SC bacterial isolates to PCR primers and substrate utilization.

Bacterial Isolate	WFB1/2	RST49/51	Biolog ID	Probability	Similarity
Plot 2 M15	negative	negative	All positive		
Plot 2 M19	negative	negative	Ralstonia picketti	100	0.67
Plot 2 M26	negative	negative	All positive		
Plot2 M36	positive	**positive**	Achromobacter cholinophagum	100	0.59
Plot 2 M38	positive	negative	Achromobacter cholinophagum	0	13.33
Plot 2 M39	positive	negative	Janthinobacterium lividum	0	0.16
Plot 21 M19 9/17/98	positive	negative	Achromobacter cholinophagum	0	0.32
Plot 21 M19 8/30/99	negative	negative	All positive		
Plot 2 M22	positive	negative	Pseudomonas aurantiaca	0	0.18
Plot 21 M34	positive	negative	Achromobacter cholinophagum	0	0.4
SB Plot 2 M34	negative	negative	Ralstonia picketti	100	0.66
SB Plot 2 M38	positive	negative	Brevunidomas vesicularis	0	0.38
SB Plot 2 M16	positive	negative	Achromobacter cholinophagum	0	0.38
SB Plot 19 M17	negative	negative	Pseudomonas spinosa (Burkholderia)	0	0.47
SB Plot 21 M11	negative	negative	All positive		
SB Plot 21 M39	positive	negative	Sphingomonas sanguinis	0	0.14
SB Plot 2 M39	positive	negative	Pseudomonas fluorescens biotype A	0	0.16
SB Plot 2 M24	negative	negative	Pseudomonas putida biotype B	99	0.75
SB R1 P7 M7	positive	**positive**	Pseudomonas putida biotype B	0	0.24
SB Plot 8 M1 7/14/99	negative	negative	All positive		
SB Plot 8 M39 7/14/99	negative	negative	All positive		
SB Plot 19 M30 7/14/99	negative	negative	All positive		
SB Plot 21 M31 7/14/99	negative	negative	Chryseobacterium gleum/indologenes	97	0.65
8 CCbp CU-M29 9/1/98	positive	**positive**	Pseudomonas fluorescens biotype G	0	0.23
15CP-1 M31 11/1/00	positive	**positive**	Pseudomonas fluorescens biotype G	0	0.42
16CT R2 M10	positive	negative			

16RP-R2 M10 11/1/00	positive	negative			
Weed 4 M15 7/27/98	**positive**	**positive**	**Acidovorax avenae subsp. avenae**	**90**	**0.63**
Weed 4 M27 7/27/98	positive	negative			
Tobacco R3 M25 7/27/98	positive	**positive**			
SB Plot 8 M21 8/30/99	negative	negative	CDC group II-H	0	0.28
SB Plot 8 M28	negative	negative	Xanthomonas campestris pv begonia A	0	0.22
SB Plot 8 M22 8/30/99	negative	negative	Pseudomonas fboreopolis	0	0.35
SB Plot 8 M3 8/30/99	negative	negative	Xanthomonas campestris pv dieffenbachiae	0	0.23
SB Plot 8 M31 8/30/99	negative	negative			
SB Plot 21 M32 7/13/98	negative	negative	Burkholderia pyrrocinia	93	0.73
SB Plot 21 M20 7/13/98	negative	negative	Sphingomonas parapaucimobilis	0	0.11
SB Plot 21 M26 7/13/98	negative	negative	Sphingomonas sanguinis	0	0.23
SB Plot 2 M31 8/30/99	negative	negative			
SB Plot 2 M30 8/30/99	negative	negative	Vibrio alginolyticus	0	0.17
SB Plot 8 M32 8/30/99	negative	negative	Sphingomonas sanguinis	0	0.29
SB Plot 2 M37 8/30/99	negative	negative			
SB Plot R1P7 M11 7/25/97	negative	negative	Xanthomonas campestris pv hyacinthi	0	0.45
SB Plot 8 M13 8/30/99	negative	negative	Sphingomonas sanguinis	0	0.21
SB R1 P7 M16 7/25/97	negative	negative	Vibrio alginolyticus	0	0.06
SB Plot 8 M7 9/17/98	negative	negative	Brevunidomas vesicularis	0	0.32
SB Plot 8 M24 9/17/98	negative	negative	CDC group II-E subgroup A	0	0.28
SB Plot 2 M29 9/17/98	negative	negative	Brevunidomas vesicularis	0	0.47
SB Plot 8 M28 9/17/98	negative	negative	Sphingomonas sanguinis	0	0.17
SB Plot 2 M20 9/17/98	negative	negative	Vibrio furnissii	0	0.23
SB Plot 2 M2 9/17/98	negative	negative			
SB R1 P7 M38 7/25/97	negative	negative			
SB Plot 8 M31 8/30/99	positive	negative	Vibrio furnissii	0	0.28
SB Plot 2 M31 8/30/99	positive	negative	Vibrio fluvialis	90	0.58
SB Plot 2 M30 8/30/99	positive	negative	Rhizobium radiobacter	100	0.86

SB Plot 8 M21 8/30/99	positive	negative			
SB Plot 8 M3 8/30/99	positive	negative			
SB Plot 8 M32 8/30/99	positive	negative	Vibrio mediterranei	0	0.28
SB R1 P7 M38 7/25/97	positive	negative	no id		
SB Plot 2 M37 8/30/99	positive	negative	no id		
SB R1 P7 M16 7/25/97	positive	negative			
SB R1 P7 M11 7/25/97	negative	negative	Ralstonia paucula	0	0.11
SB Plot 8 M38 9/17/98			did not grow		
SB Plot 21M34 9/17/98			did not grow		
SB Plot 2 M36 9/17/98			did not grow		
SB Plot 2 M22 713/98			did not grow		
SB Plot 2 M15 8/30/99			did not grow		
SB Plot 2 M26 8/30/99			did not grow		
SB Plot 2 M19 8/30/99			did not grow		
SB Plot 19 M7 8/30/99			did not grow		
SB Plot 2 M24 8/30/99			did not grow		
SB Plot 2 M39 7/14/99			did not grow		
SB Plot 2 M34 8/30/99			did not grow		
SB R1 P7 M7 7/25/97			did not grow		
SB Plot 21 M39 7/13/98			did not grow		
SB Plot 2 M38 9/17/98			did not grow		
SB Plot 2 M16 8/30/98			did not grow		
SB Plot 21 M11 8/30/98			did not grow		
SB Plot 2 M11 9/17/98			did not grow		

Conclusions:

Despite the fact that all of the strains were identified as *Acidovorax avenae* or *A. avenae* ssp. *avenae* by fatty acid methyl ester profile analysis, only one of these strains was confirmed as *A. avenae* subsp. *avenae* by PCR and substrate utilization profile analysis. It is clear that the strains evaluated are not *A. avenae* subsp. *avenae*. One possible explanation for the discrepancies is that the cultures may have been contaminated with faster growing saprophytic organisms that made it difficult to recover *A. avenae* subsp. *avenae* or *A. avenae*. In this case, the samples may still be identified as *Acidovorax* but the BIOLOG results would have indicated another organism.

The failure to confirm a majority of the strains as *A. avenae* subsp. *avenae* by independent assays suggests that this bacterium is not associated with soybean yield decline. This is not an unexpected conclusion since *A. avenae* subsp. *avenae* is a foliar pathogen of many grasses including corn, rice and pearl millet. There have been no report of *A. avenae* subsp. *avenae* causing disease on soybean and in general, it is likely that *A. avenae* subsp. *avenae,* as a phyllosphere bacterium, would not survive well in the soil environment due to competition with native soil microflora. Indeed, closely related soilborne *Acidovorax* species may be associated with soybean yield decline.

Another possible explanation is that the strains associated with soybean yield decline are not *A. avenae* subsp. *avenae*, but they may be closely related organisms. A closely related but previously unidentified organism would be named as *A. avenae* subsp. *avenae* by fatty acid analysis because it is a close relative. However, the organism may yield different results in PCR and restriction digestion assays as well as BIOLOG.

Since no strong foliar blight symptoms on soybean had been observed in the field, it was not unexpected that the GC FAME identification as *A. avenae* subsp. *avenae* or *A. avenae* was in error or at least questionable. Additional research is required to identify the causal agent for the soybean yield decline.

References:

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