PRINCIPAL INVESTIGATOR:

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Objectives:

1. **Development of qPCR-based detection methods for pathogenic Burkholderia bacteria**
2. **Investigation of species from vegetables and catfish: isolation and identification**

Milestones for FY2016-17:

1. **Development of Rapid Detection Systems for Pathogenic *Burkholderia* spp. from Fresh Vegetable and Catfish**
2. **Milestones of the project was met participially.**

The research activities of qPCR primer design and specificity tests were delayed due to the difficulty to hire qualified research associate. One research associate who agreed to work on the project left to Cornell University as a postdoctoral research fellow with three years contract. The biggest challenge is to hire a qualified researcher just for one year. One part time research associate (committed 1/4 time to the project) was employed. Therefore, more than $20,000 funds were returned to MAFES.

Progress report:

Totally 88 sequenced genomes or genome drafts of *Burkholderia* in GenBank were compared to identify the unique genetic regions of *Burkholderia* bacteria (Figs. 1-4). From the unique regions of *B. multivorans* and *B. cenocepacia*, four pairs of PCR primers (BcF/R1, BcF/R2, BmF/R 1 and BmF/R2) were designed (Table 1). The results showed that BcF/R2 is specific to *B. cenocepacia* (Fig. 5). Additional two pairs (BmF/R3 and BmF/R4) were designed for *B. multivorans*. The new primers are being tested.

Extensive efforts were made to select selective culture media and to optimize isolation procedure. We ordered *Burkholderia cenocepacia* BAA245 and *Burkholderia multivorans* ATCC BAA247 as controls (table 2). Totally 45 samples were collected from the local grocery stores. The CB medium was used for screening for *Burkholderia* strains. The suspected isolates were further identified by DNA sequence analysis. *Burkholderia contaminans* was recovered from the samples sweet onion and celery stalk (Table 3).

Accomplishments:

As of May 20, 2017, totally 88 sequenced genomes or genome drafts of the *Burkholderia* strains in NCBI GenBank were genomewide compared to identify unique regions for primer design. We have identified one pair of PCR primers that produces a PCR product from the species *B. cenocepacia*, not the rest of bacterial tested. Additional two pairs are under investigation for the species *B. multivorans.* From 45 samples collected from the local grocery stores, which included lettuce, cucumber, cabbage, celery, onion, and catfish, The bacterial isolates (16FS-27-1, 2 and 16FS28-1, 2) of *B. contaminans* was recovered from the samples sweet onion and celery stalk (Table 2). In addition, some animal and human pathogens, such as *Brucella* sp. (the isolate 16FS11-2), was recovered from a celery stalk (Table 2).

Significant Activities that Support Special Target Populations:

Many studies have showed that some *Burkholderia* species could cause respiratory complications for cystic fibrosis patients. Quality food should not be contaminated by the bacteria. However, no approach is available for detection of pathogenic *Burkholderia* from fresh vegetables and catfish. This study will develop qPCR-based detection methods (qPCR primers and probes) and optimize bacterial enrichment procedures that will be used for rapid and accurate detection of *Burkholderia* from food materials. This study has identified genetic regions of *Burkholderia* spp. for species-specific qPCR primers. In addition, we have found some isolates that are *Burkholderia* spp. and other opportunistic pathogens. The research will address the post-harvest food safety issue, which is aligned up with the Action Plans of National Program 108 of USDA-ARS.

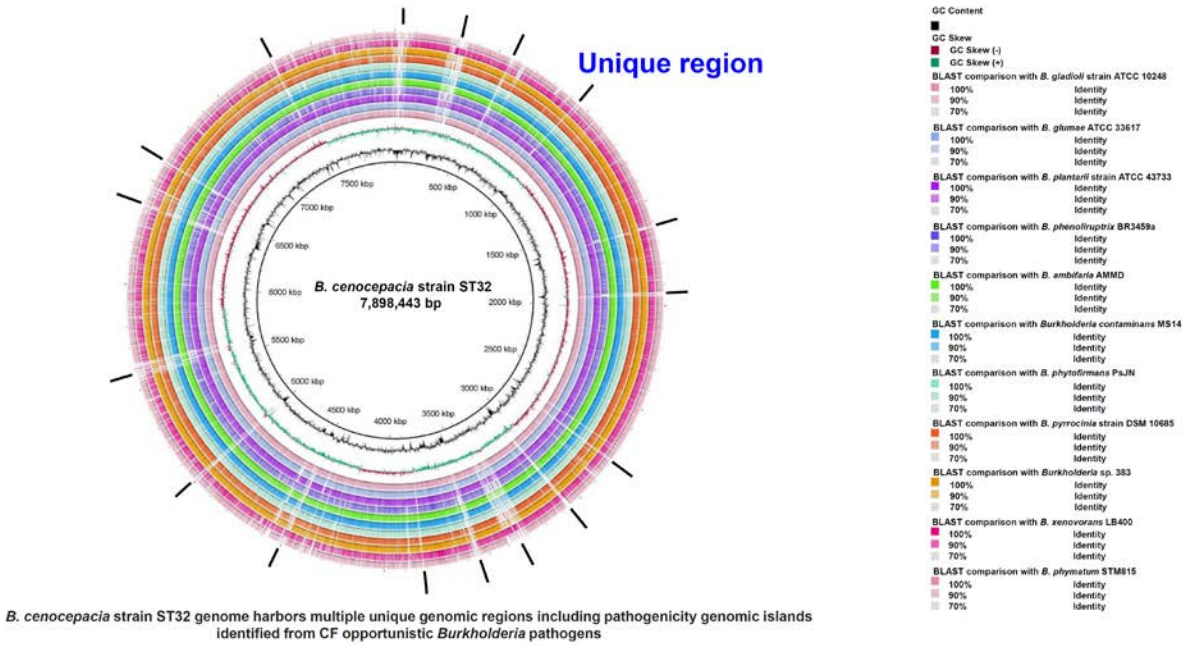
Technology Transfer: This is the first year to conduct the research. I had a hard time to identify a qualified research associate just for one year. So, no technology has been developed yet at this moment.

International Cooperation / Collaboration: None as of this moment.

Publications: A manuscript is being prepared to report the PCR –based detection of *Burkholderia canocepacia.*

Presentations:

Lu, S.-E. (2016, May).Development of Rapid Detection Systems for Pathogenic *Burkholderia* spp. from Fresh Vegetable and Catfish, invited by the MSU Institute Safety, Mississippi State, Mississippi.



B. cenocepacia strain ST32 genome harbors multiple unique genomic regions including pathogenicity genomic islands identified from CF opportunistic *Burkholderia* pathogens

Fig. 1. *Burkholderia cenocepacia* strain ST32 Genome Comparison with Other *Burkholderia* Species

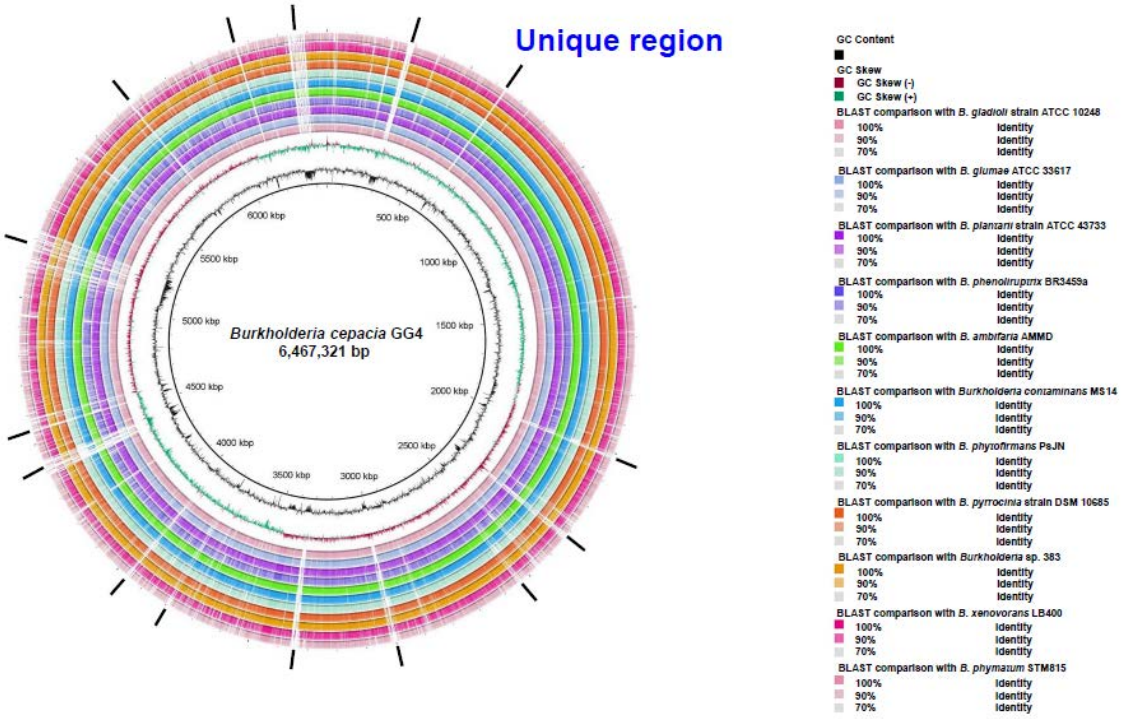


Fig. 2. *Burkholderia cepacia* GG4 Genome Comparison with Other *Burkholderia* Species

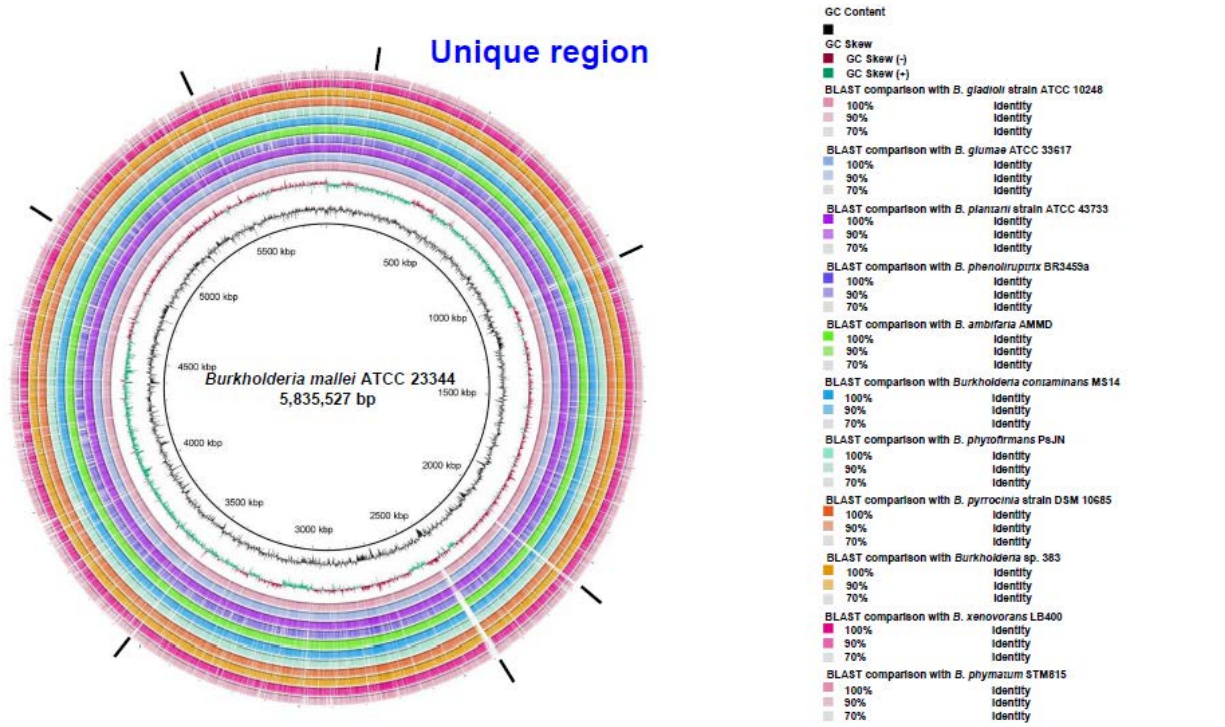


Fig. 3. *Burkholderia mallei* ATCC 23344 Genome Comparison with Other *Burkholderia* Species

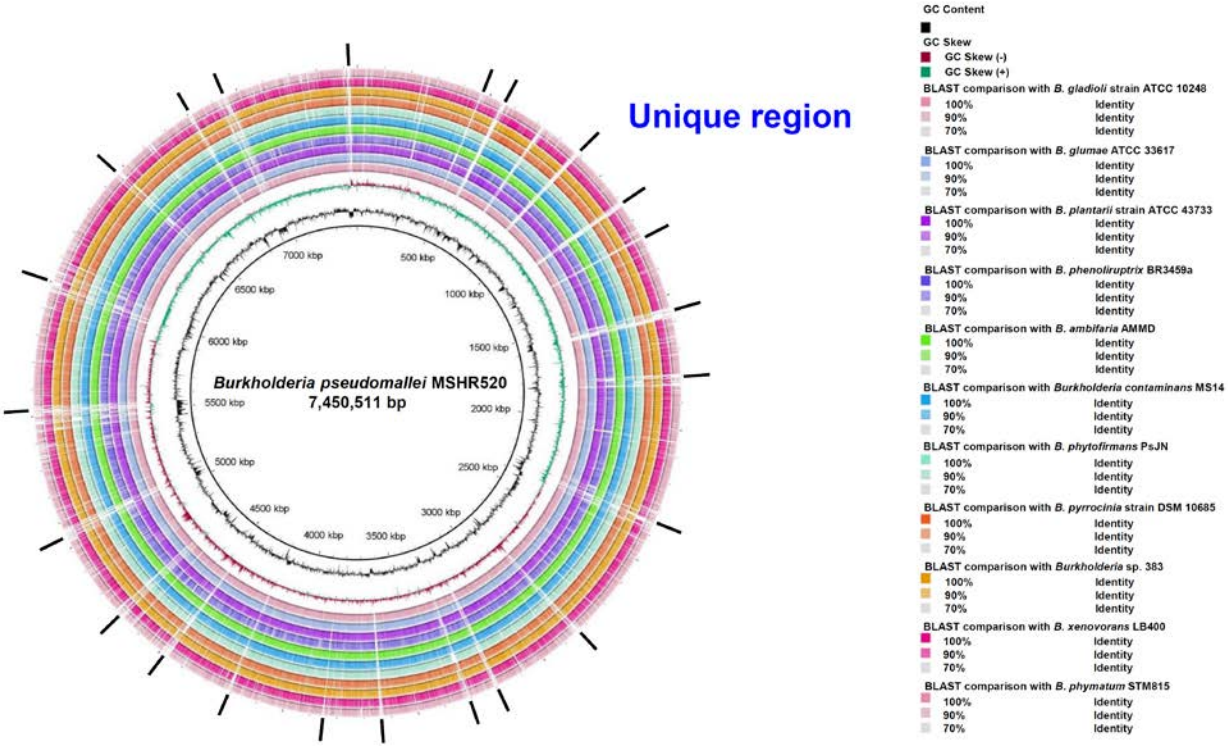


Fig. 4. *Burkholderia pseudomallei* MSHR520 Genome Comparison with Other Burkholderia Species

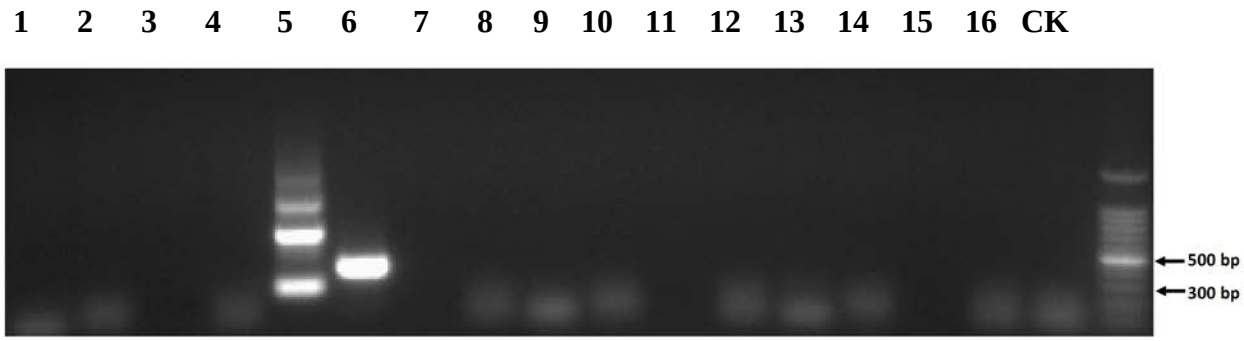


Fig. 5. PCR analysis of specificity of the primer candidates for detection of *Burkholderia* spp. Genomic DNA samples of *B. multivorans* (Lanes 1-4), *B. cenocepacia* (Lanes 5-8), *B. ambifaria* (lanes 9-12) and *B. contaminans* (Lanes 13-16) were used for testing primer specificity. Four sets of PCR primers (BcF/R1 and BcF/R2 for *B. cenocepacia*; BmF/R1 and BmF/R2 for *Burkholderia maltivorans*) were tested for their specificity. Primers BcR/F1: Lanes 1, 5, 9, and 13; Primers BcF/R2: Lanes 2, 6, 10, and 14; Primers BmF/R1: 3, 7, 11 and 15; Primers BmF/R2: Lanes 4, 8, 12 and 16. CK: No DNA templated with the mixuture of the four pairs of primers. Primers BcR/F2 shows a single band from *B. cenocepacia* but not from others. The experiments were replicated three time and the results were consistent.

Table 1. Primers designed for detection of *Burkholderia* spp.

Primer	Sequence	product size	Target bacteria
BcF1	5’-CGTTGCAGCGAGCGTAGTG	260 bp	*Burkholderia cenocepacia*
BcR1	5’-GATCTGTCGAGCGGAACCAT		
BcF2	5’-TCTCGGTCGTGTGCTGGGTGAT	350 bp	
BcR2	5’-ACCATCGGCCTCGTCCAGCAGT		
BmF1	5’-GCGATCCAGGTCAGTTACGAG	294 bp	*Burkholderia multivorans*
BmR1	5’-TGCGAAGGAGAAGCCGAAGGT		
BmF2	5’-TGAATGCCGGGTTTGTCCAGTTT	266 bp	
BmR2	5’-CGTTGACTGCTCGGAAAGGATGT		
BmF3	5’-AGCTTGCCGGGCGTGTCTG	250 bp	*Burkholderia multivorans*
BmR3	5’-GGCTACTTTGCGGCGTTGAT		
BmF4	5’-AGCGGTCCTTTCCCTGATTG	274 bp	
BmR4	5’-CGACCTCCGCCGATTCCTT		

Table 2. Identity confirmation of the ATTC strains of *Burkholderia*

Order Date	Isolate	Gene (Primers)	Identity
2016-6-20	BAA245-1	RecA (BCRF/BCRR)	*Burkholderia cenocepacia* strain 842 at 99% identity, 1744 Total Score, 97% Query Cover; BAA-245 not in the list of hits but LMG 16656 is at 99% identity, 1722 Total Score, 96% Query Cover
	BAA245-2		*Burkholderia cenocepacia* strain 842 at 99% identity, 1740 Total Score, 99% Query Cover; BAA-245 not in the list of hits but LMG 16656 is at 99% identity, 1700 Total Score, 96% Query Cover
	BAA245-3		*Burkholderia cenocepacia* strain 842 at 99% identity, 1768 Total Score, 100% Query Cover; BAA-245 not in the list of hits but LMG 16656 is at 99% identity, 1742 Total Score, 99% Query Cover
	BAA245-4		*Burkholderia cenocepacia* strain 842 at 99% identity, 1731 Total Score, 100% Query Cover; BAA-245 not in the list of hits but LMG 16656 is at 99% identity, 1711 Total Score, 97% Query Cover
	BAA245-5		*Burkholderia cenocepacia* strain 842 at 99% identity, 1742 Total Score, 99% Query Cover; BAA-245 not in the list of hits but LMG 16656 is at 99% identity, 1716 Total Score, 98% Query Cover
	BAA247-1		*Burkholderia multivorans* ATCC BAA-247 at 99% identity
	BAA247-3		*Burkholderia multivorans* ATCC BAA-247 at 99% identity
	BAA247-4		*Burkholderia multivorans* ATCC BAA-247 at 99% identity

Notes: BAA245 (*Burkholderia cenocepacia*) and BAA247 (*Burkholderia multivorans*) were purchased from ATCC. The bacteria were recovered from the lyophilized material received. The gDNA was extracted and the *recA* PCR for sequencing analysis. Note that BAA245 was not in the list of "hits" on the BLAST but this is because the *recA* sequence for that strain is not in the NCBI database. BAA245 = LMG 16656 and its *recA* is included in the database and the "hits".

Table 3. Bacterial isolates obtained from fresh foods in 2016

2016-7-21	16FS11-2	16S (27F/1492R)	*Chryseobacterium indoltheticum* (one sp. & strain at 99% identity; several other spp. in the same genus were at 97% identity and all others were ≤96%)
	16FS16-2		*Sphingobacterium faecium* (two strains within this species were at 99% identity; one strain in each of two other spp. in the genus were at 98% identity and all others were ≤94%))
	16FS17-1		*Delftia tsuruhatensis* (two strains of this species were at 99% identity; five strains in four spp. in the same genus were at 98% identity and all others were ≤96%)
	16FS18-1		*Sphingobacterium faecium* (two strains within this species were at 99% identity, as was one strain of *S. kitohiroshimense*; one strain of *S. anhuiense* as at 98% identity and all others were ≤94%)
	16FS20-2		*Chryseobacterium indoltheticum* (one sp. & strain at 99% identity; five strains in four other spp. in the same genus were at 97% identity and all others were ≤96%)
	16FS20-3		*Delftia tsuruhatensis* (one strain of this species and five strains in three other spp. were at 99% identity; three strains in two spp. in the same genus were at 98% identity and all others were ≤96%)
	16FS21-3		*Sphingobacterium faecium* (two strains within this species were at 99% identity, as was one strain of *S. kitohiroshimense*; one strain of *S. anhuiense* as at 98% identity and all others were ≤94%)
2016-8-8	16FS13-1	16S (27F/1492R)	*Janthinobacterium svalbardensis* (four strains from three spp in this genus, all at 99% identity; strains from several genera and spp had 97-98% identity)
	16FS13-5		*Janthinobacterium lividum* (three strains other from two spp in this genus, all at 99% identity; two strains from two other genera were at 97% identity)
2016-8-23	16FS11-2s*	16S (27F/1492R)	*Ochrobactrum thiphenivorans* (several spp & strains in this genus, all at 99% identity; 2 species and 3 strains within the same genus had 98% identity)
	16FS11-2L*		*Pseudomonas marginalis* (several spp & strains in this genus, all at 99% identity)
	16FS21-3WP**		*Janthinobacterium lividum* (four strains from three spp in this genus, all at 99% identity; strains from several genera and spp had 97-98% identity)

	16FS21-3Y**		*Sphingobacterium faecium* (2 strains of *S. faecium* and one of *S. anhuiense* had 99% identity; 1 strains of *S. kitahiroshimense* was 98%; all others were ≤95%)
2016-10-24	16FS27-1	RecA (BCRF/BCRR)	*Burkholderia contaminans*, 99% (all BLAST returns were >97% and all were strains of spp. within the *B. contaminans* group)
	16FS27-2		*Burkholderia contaminans*, 99% (all BLAST returns were >97% and all were strains of spp. within the *B. contaminans* group)
	16FS28-1		*Burkholderia contaminans*, 99% (all BLAST returns were >97% and all were strains of spp. within the *B. contaminans* group)
	16FS28-2		*Burkholderia contaminans*, 99% (all BLAST returns were >97% and all were strains of spp. within the *B. contaminans* group)
2016-11-21	16FS32-4	16S (27F/1492R)	bad sequencing read
	16FS34-1		no identities >94%
	16FS36-3		bad sequencing read

*16FS11-2 was identified previously as *Chryseobacterium indolthecium* based on 16S sequencing. When it was streaked again from freezer stock, two distinct sizes (L= large, s= Small) of colonies grew out. The bacteria were picked and streaked individually onto fresh NBY. They still maintained the colony size difference, so we did 16S sequencing on both colony sizes.

**16FS21-3 was identified previously as *Sphingobacterium faecium* based on 16S sequencing. When it was streaked again from freezer stock, two distinctly different colony colors were present. The bacteria were picked up and streaked individually onto fresh NBY. They maintained the color difference. WP has white colonies that turn a very intense purple with age. Y has yellow/orange colonies.