Next Generation Sequencing Impact on the Food Industry

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NGS and Food Industry: Irrational Exuberance, Illogical Fears

- NGS (SM 2) sequel to PFGE (SM 1)
- NGS has awakened the same fears that the industry experienced two decades ago with PFGE

NGS IE/IF: Epidemiology

- Epidemiology without Epidemiology
- Linking a food company to an outbreak by mistake (White Water Outbreak)
- Enhanced ability to link a food processor to small clusters, and a cluster of one
- Linkage to historical clusters: you are linked to an outbreak in 2006, statute of Limitation?????

Epidemiology: Ability to Detect Foodborne Outbreaks

- Is based on an antiquated system, which is chronically underfunded, and has relied on clinical laboratories to culture pathogens
- Most clusters of foodborne illnesses can not be linked to a given food or a food producer
- NGS is a tool to shortcut the system

Epidemiology without Epidemiology

- Can my products be linked to an outbreak purely based on NGS data
  - No
  - While NGS can be used to link an isolate from your food or production facility to human cases, in the absence of consumption/exposure history linkage can not be made

Ability to link a food processor to small clusters, cluster of one..

- NGS has increased the resolution of the Public Health Radar
- While PFGE can be used and was used to link sporadic cases, the lack of precision often resulted in false clustering
- NGS brings in a high level of precision compared to PFGE

Linkage to decade old outbreaks

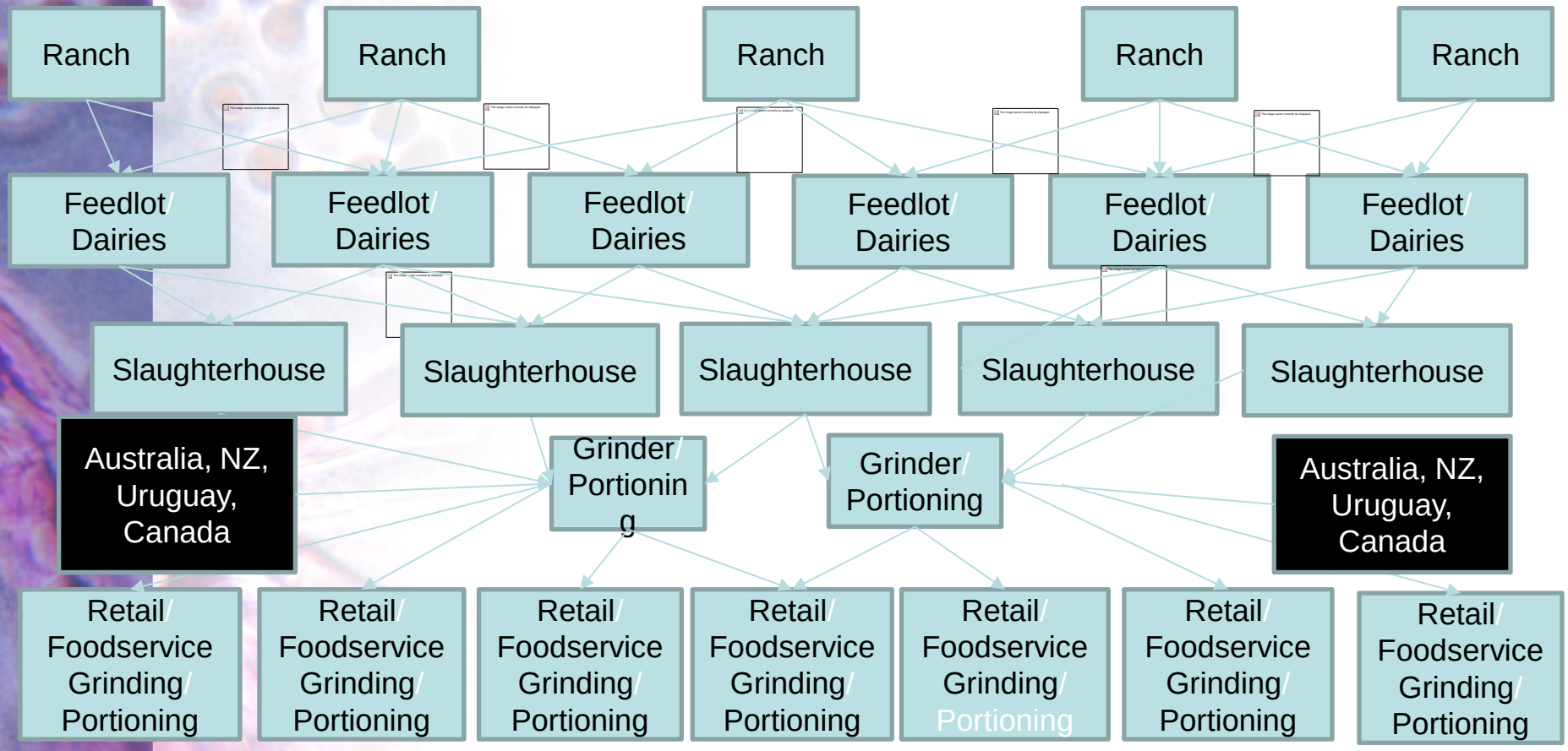
- Theoretically possible
- The ability to connect the dots goes back in time as far back as there are preserved pathogen cultures of food/environment and patients
- Microbes last a long time

IE/IF: Molecular Epidemiology

- Trademarking a clone: This is Company A's clone
  - While a given clone can be isolated from products/environment of company A, due to the complex distribution paths, company A doesn't hold a patent or a trademark on the Clone, it can present from other sources simultaneously

IE/IF: Molecular Epidemiology

- Assertion that a production facility is colonized by a pathogen:
  - Yes, No, Maybe
  - Repeated isolation of members of the same clone from a given food or production plant can be due to:
    - ✓ Harborage
    - ✓ Or Frequent Transient Passage through constant seeding by a raw ingredient
    - ✓ Or a finding positives overtime from a single contamination event


Ranch
Ranch
Ranch
Ranch
Ranch
Feedlot/ Dairies
Feedlot/ Dairies
Feedlot/ Dairies
Feedlot/ Dairies
Feedlot/ Dairies
Feedlot/ Dairies
Slaughterhouse
Slaughterhouse
Slaughterhouse
Slaughterhouse
Slaughterhouse
Australia, NZ, Uruguay, Canada
Grinder/ Portioning
Grinder/ Portioning
Australia, NZ, Uruguay, Canada
Retail/ Foodservice Grinding/ Portioning
Retail/ Foodservice Grinding/ Portioning
Retail/ Foodservice Grinding/ Portioning
Retail/ Foodservice Grinding/ Portioning
Retail/ Foodservice Grinding/ Portioning
Retail/ Foodservice Grinding/ Portioning
Retail/ Foodservice Grinding/ Portioning

Case study of WGS: *Salmonella* cross-contamination in a food laboratory

Isolate information	ATCC lab strain	Test strain	IEH control strain	Predicted serotype	O antigen prediction	H1 antigen prediction	H2 antigen prediction
ATCC lab strain	0	1	203	Typhimurium	O-4	i	1,2
Test strain	1	0	204	Typhimurium	O-4	i	1,2
IEH control strain	203	204	0	Typhimurium	O-4	i	1,2

The discriminatory power provided by SNP analysis of WGS data helped us further verify that the client sample was contaminated with laboratory strain (ATCC lab strain)

Note: SNP differences closer to Zero indicate that the isolates are indistinguishable and possibly clones of the same strain

Case study of WGS: SNP analysis of *Salmonella* genomic DNA isolated from agarose plug samples

Agenda was to see how closely the strains are related to each other by extracing gDNA from agarose plug samples.

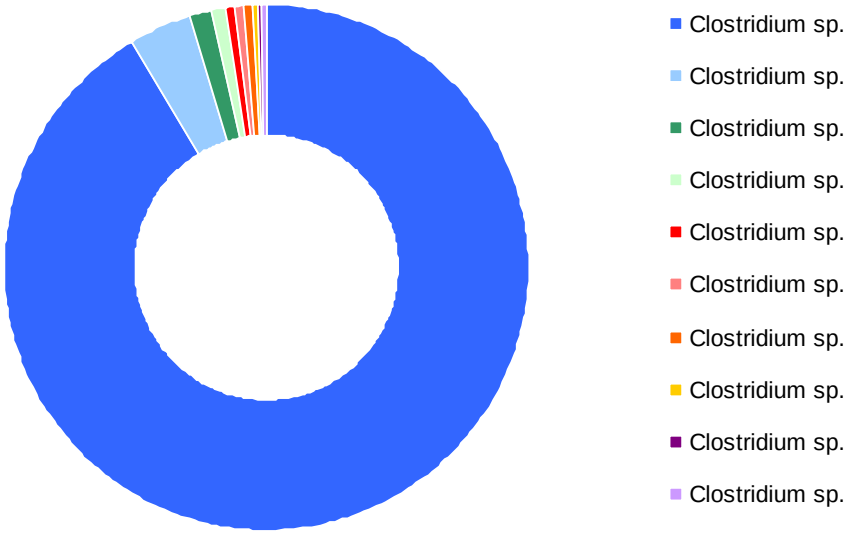
Table 2. Number of of SNP's calculated based on Senftenberg genome as reference using CFSAN SNP pipeline

	85273-13a	85273-37a	85273-41a	85299-1A	85310-2A	85358-16A	85358-17A	85470-11b	85525-37b	85525-50b	85614-44c	Predicted serotype	O antigen prediction	H1 antigen prediction (fliC)	H2 antigen prediction (fljB)
85273-13a	0	1	1	3	5	47347	46941	0	1	0	0	Senftenberg	O-1, 3, 19	g, s, t	-
85273-37a	1	0	3	4	7	47343	46941	2	2	2	1	Senftenberg	O-1, 3, 19	g, s, t	-
85273-41a	1	3	0	4	6	47347	46942	2	2	1	1	Senftenberg	O-1, 3, 19	g, s, t	-
85299-1A	3	4	4	0	5	47347	46933	3	4	3	3	Senftenberg	O-1, 3, 19	g, s, t	-
85310-2A	5	7	6	5	0	47313	46907	6	6	5	5	Senftenberg	O-1, 3, 19	g, s, t	-
85358-16A	47347	47343	47347	47347	47313	0	37489	47338	47339	47343	47342	Braenderup	O-7	e,h	e,n,z15
85358-17A	46941	46941	46942	46933	46907	37489	0	46928	46935	46940	46938	Enteritidis	O-9	g,m	-
85470-11b	0	2	2	3	6	47338	46928	0	1	2	0	Senftenberg	O-1, 3, 19	g, s, t	-
85525-37b	1	2	2	4	6	47339	46935	1	0	1	1	Senftenberg	O-1, 3, 19	g, s, t	-
85525-50b	0	2	1	3	5	47343	46940	2	1	0	0	Senftenberg	O-1, 3, 19	g, s, t	-
85614-44c	0	1	1	3	5	47342	46938	0	1	0	0	Senftenberg	O-1, 3, 19	g, s, t	-
Senftenberg_ LN868943_ref erence	135	134	135	133	107	46812	46677	132	131	133	133	Senftenberg	O-1, 3, 19	g, s, t	-

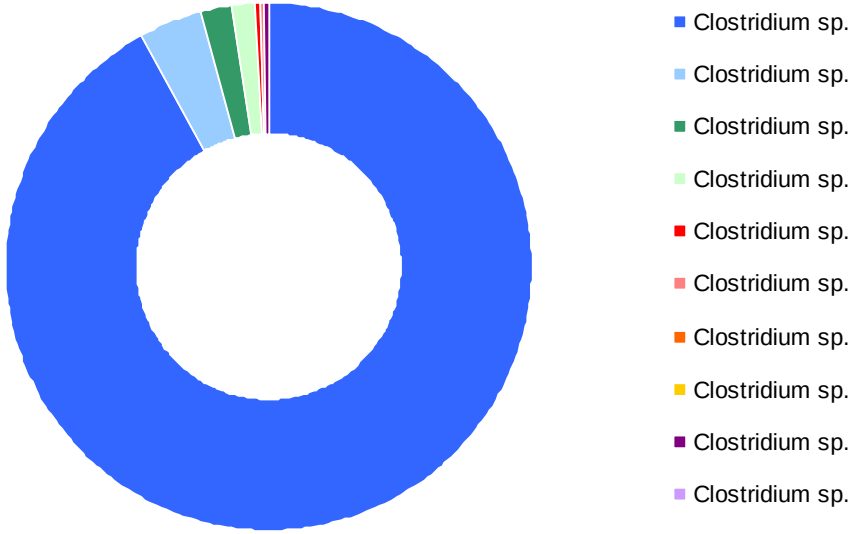
SNP differences closer to Zero indicate that the isolates are indistinguishable and possibly clones of the same strain

Metagenomics of Spoiled Roasted Beef

- Direct



- After 10 day enrichment

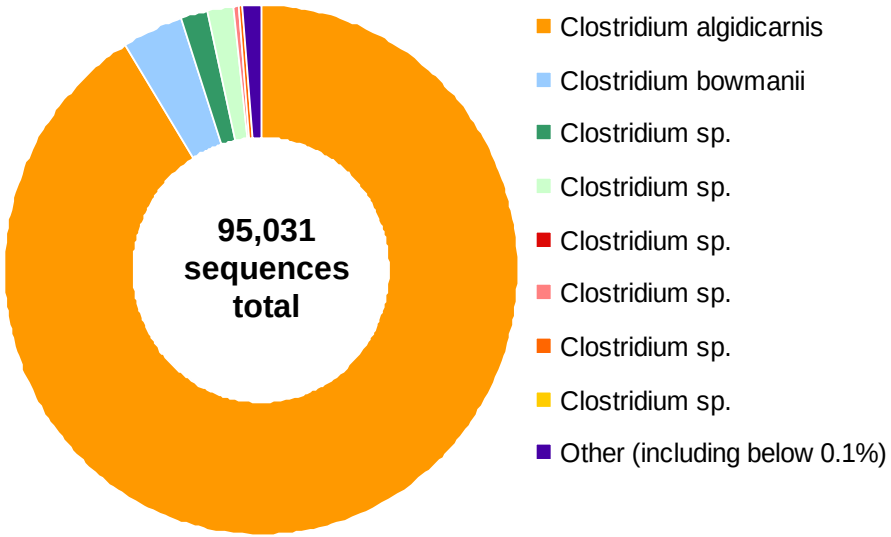


More than 12 different Clostridium sp. lineages identified in spoiled roasted beef samples.

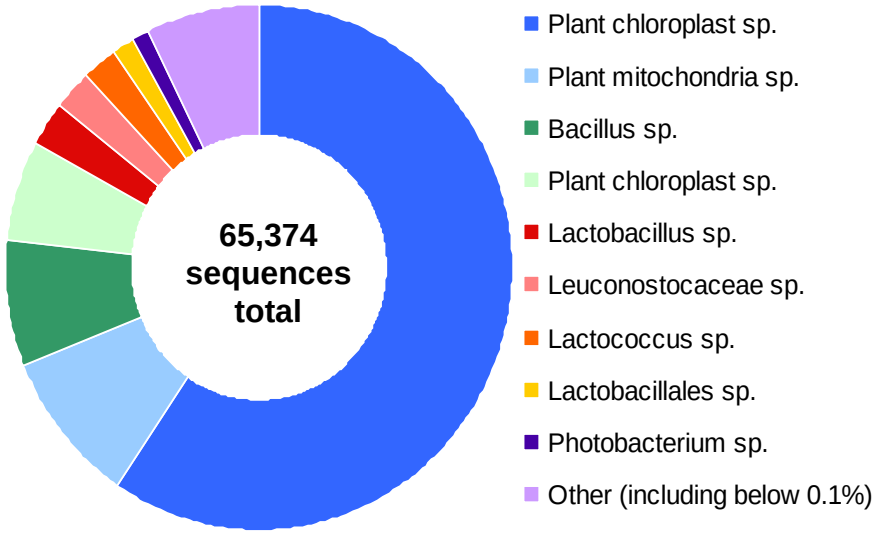
Identification of food spoilage organisms by shotgun metagenomics

- Example: Spoiled Roasted Beef “Blown Pack”

Spoiled Sample



Unspoiled Control Sample

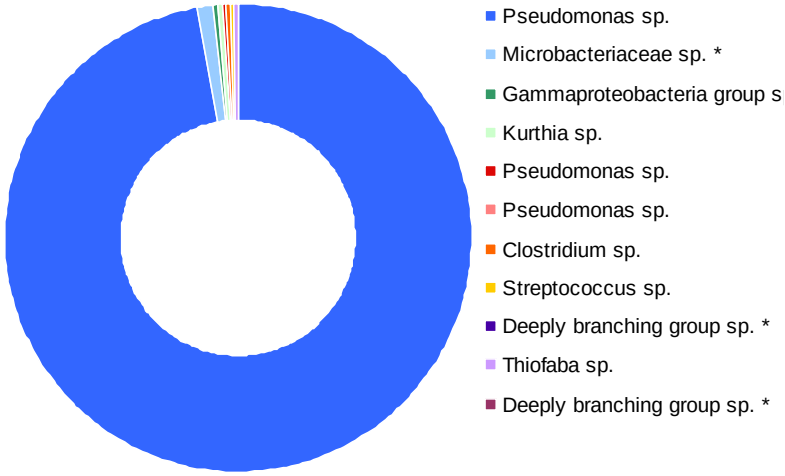


- Direct Identification of *Clostridium algidicarnis* as causative spoilage organism w/o cultivation.

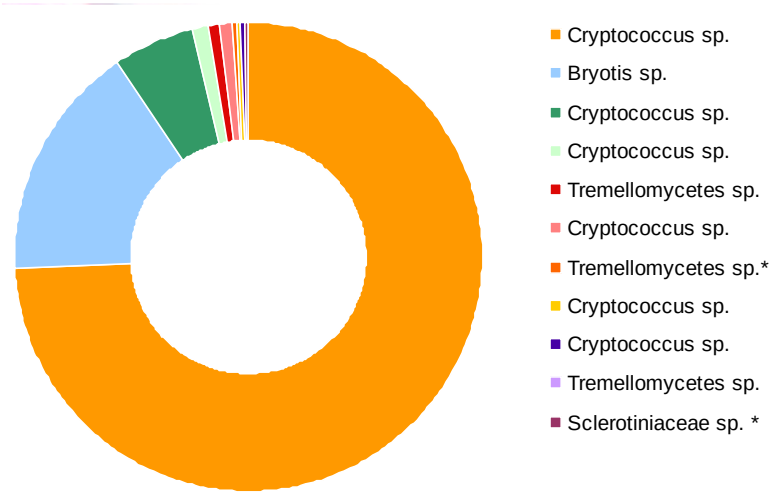
Identification of *Cryptococcus* sp. as the main spoilage organism in veggie puree by metagenomics

Spoiled sample

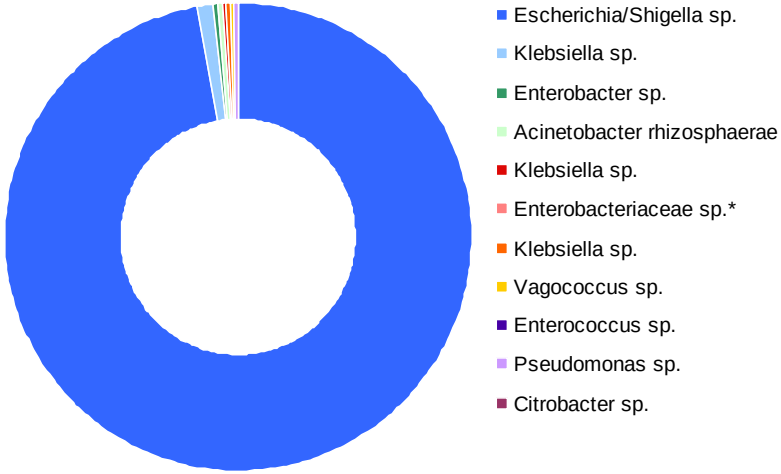
Bacteria



Fungi



Unspoiled Control Sample



Not detected

Identification of New Delhi Metallo-beta-lactamase-1(NDM-1) producing *E. coli* from a case patient's environment by whole genome sequencing (WGS)

- Carbapanem-resistant *Escherichia coli* bacteria was isolated from a case patient's environment.
- The sequence type of the isolated strain (IEH71520) was identified as ST131 by WGS.
- New Delhi Metallo-β-lactamase (NDM-1) and fluoroquinolone resistance genes were found in the isolated strain.
- The NDM-1 gene was located on a plasmid, indicating horizontal gene transfer.
- The isolate (IEH71520) is the first reported NDM-1 positive strain belonging to the *E.coli* O25b-ST131 group.

Application of WGS: Identification of NDM-1 producing *E.coli* from case patient's environment

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Draft Genome Sequence of *bla*$_{NDM-1}$-Positive *Escherichia coli* O25b-ST131 Clone Isolated from an Environmental Sample

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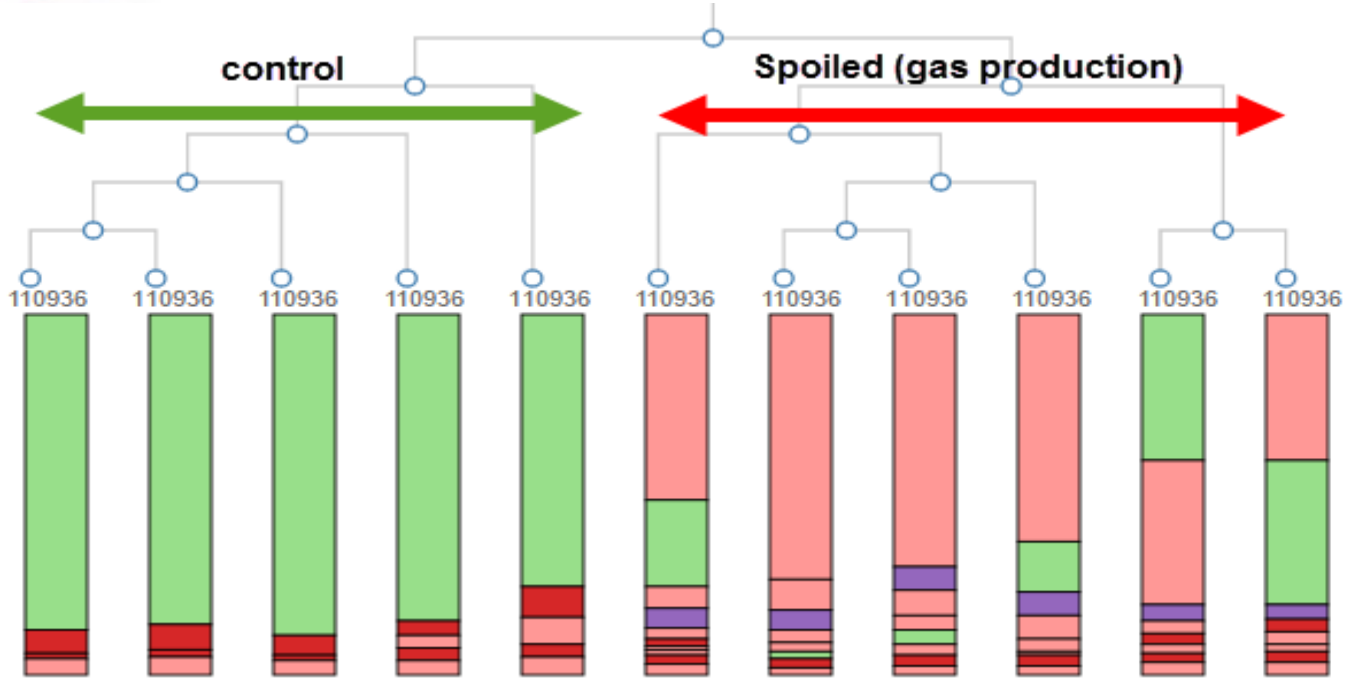
Classifications

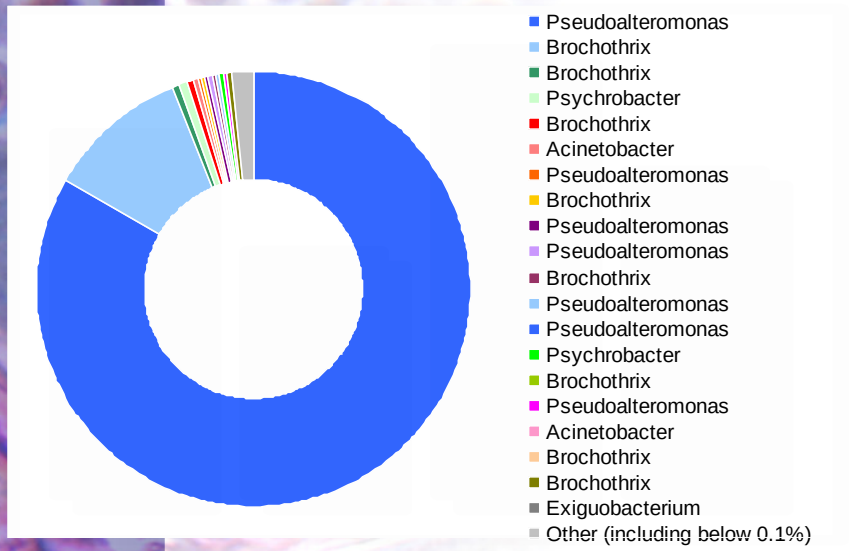
Prokaryotes

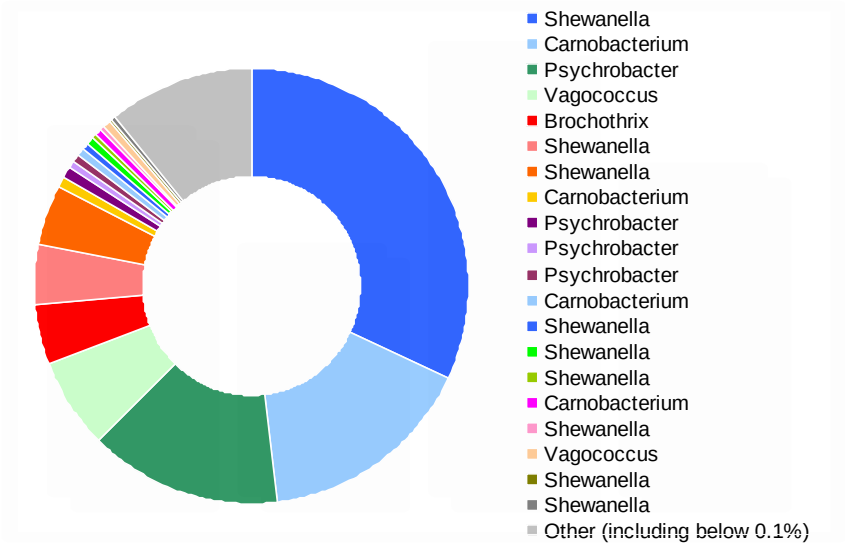
Identification of *Leuconostoc citreum* as the primary spoilage organism in a juice sample by metagenomics

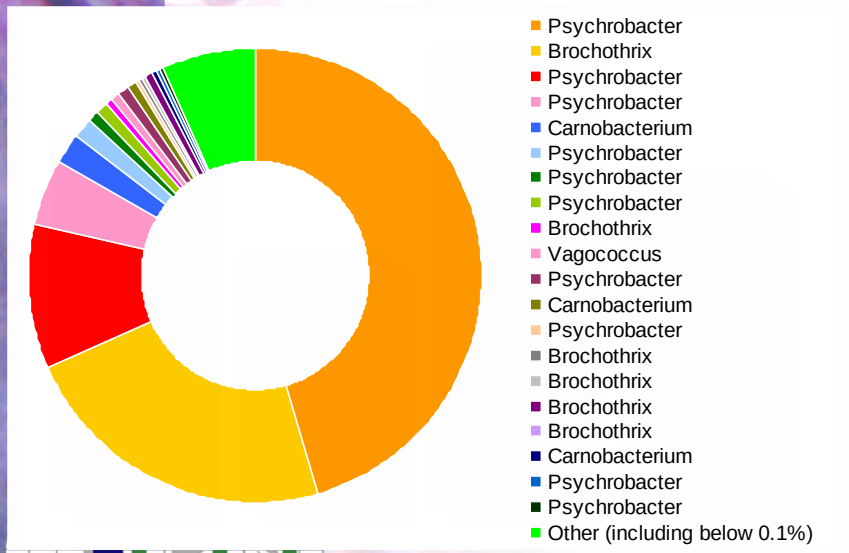
	Spoiled					Control				
IEH work order #	1109360-0(	1109360-0(	1109360-0(	1109360-01	1109360-012	1109360-01	1109360-01	1109360-01	1109360-02	1109360-024
Species	IEH-MG-00	IEH-MG-00	IEH-MG-00	IEH-MG-00	IEH-MG-0000149	IEH-MG-00	IEH-MG-00	IEH-MG-00	IEH-MG-00	IEH-MG-00001
Calothrix parietina	45%	46%	15%	27%	4%	89%	94%	99%	99%	99%
Leuconostoc citreum	46%	45%	70%	58%	77%	9%	4%	0%	0%	0%
Fructobacillus pseudoficulneus	5%	5%	7%	6%	8%	1%	0%	0%	0%	0%
Sulfurimonas denitrificans	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%
Leuconostoc palmae	2%	2%	4%	3%	5%	0%	0%	0%	0%	0%
Leuconostoc gasicomitatum	1%	1%	1%	1%	3%	0%	0%	0%	0%	0%



Analysis of Market Shrimp by 16S Metagenomics



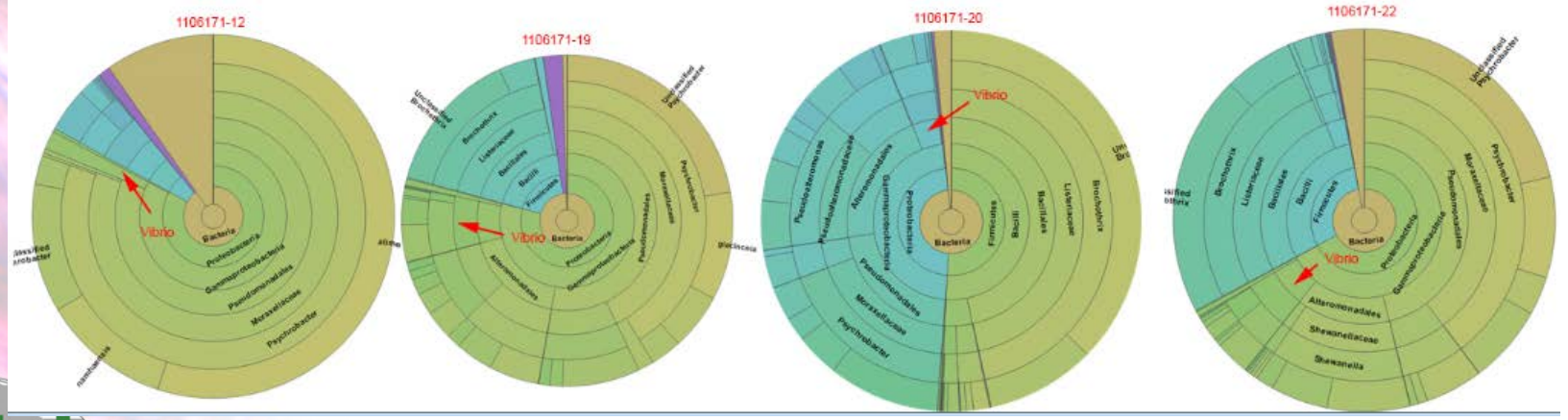




- Three typical bacterial diversity patterns observed in shrimp samples.
- *Psychrobacter*, *Brochothrix*, *Shewanella*, and Pseudomonas are the dominant genera identified.
- *Vibrio sp*. were detected in 12 samples in low numbers.

Identification of *Vibrio cholerae spp.* from shrimp samples by metagenomics

A	5	6	9	10	11	12	13	14	15	16	17	18	19	20	22	23	24	25	26	27	28	29
Thermus																						
Thioalkalimicrobium																						
Thioalkalivibrio																						
Thiobacillus																						
Thiobacter																						
Thiocapsa																						
Thiohalorhabdus																						
Thiomonas																						
Thiorhodospira																						
Thiothrix																						
Tindallia																						
Tolumonas		0.07%				0.03%			0.02%	0.01%			0.01%		0.04%					0.02%	0.04%	0.26%
Treponema																						
Trichococcus		0.28%	0.02%				0.11%		0.03%								0.02%					0.02%
Turicibacter																						
Uliginosibacterium																						
Ureibacillus																						
Vagococcus		7.09%	0.11%	2.06%	0.28%	0.02%	0.05%	0.04%	0.75%	0.34%	0.84%	0.08%	0.35%	1.49%	2.38%	0.24%	0.28%		0.30%	0.01%	0.16%	0.24%
Variovorax																						
Vibrio						0.87%							0.09%	4.24%	2.30%							0.14%
Virgibacillus																						
Viridibacillus																						
Vitreoscilla													0.05%									
Vogesella																						
Waddlia																						
Wautersiella						0.03%																
Weissella						0.15%										0.02%			0.03%		0.03%	0.43%
Xanthobacter																						
Xanthomonas																						
Xenophilus																						
Yersinia											0.02%				0.02%						0.04%	
Zhihengliuella																						
Zoogloea																						



Analysis of Market Shrimp by 16S Metagenomics

Sample #	Description	Source	Vibrio sp.	Strains isolated
1106171-5	Cooked shrimp, peeled, w tail, 26/30, frozen	Brand A		
1106171-6	Raw shrimp, w shell, w tail, 51/60, frozen	Brand B	+	
1106171-9	Raw white shrimp, w shell, w/o tail, 61/70, frozen	Brand C		
1106171-10	Raw white shrimp, 61/70, frozen	Brand D		
1106171-11	White shrimp, w/o head, w shell, w tail, 51/60, froz	Brand E		
1106171-12	Cooked shrimp, w head, w tail, frozen	Brand F	+	
1106171-13	Raw white shrimp, w head, w/o tail, 61/70, frozen	Brand G	+	
1106171-14	Raw shrimp, w head, w tail, 60/70, frozen	Brand H		
1106171-15	Raw shrimp, w head, w tail, frozen	Brand I	+	
1106171-16	Raw shrimp, w/o head, w tail, 51/60, frozen	Brand J		
1106171-17	Freshwater shrimp	Brand K	+	
1106171-18	Raw white shrimp, w head, w tail, 30/40, frozen	Brand E	+	Vibrio cholerae
1106171-19	Raw shrimp, unknown	Unknown	+	Vibrio parahaemolyticus
1106171-20	Raw shrimp, w head, w tail, 30/40	Local market	+	
1106171-22	Raw shrimp, w head, w tail, 51/60	Local market	+	
1106171-23	Raw shrimp, w head, w tail, frozen	Unknown	+	
1106171-24	Raw shrimp, w head, 4/6, frozen	Brand L		
1106171-25	Raw shrimp, w head, w tail, 30/40, frozen	Brand M		
1106171-26	Raw shrimp, w head, w/o tail, 31/40, frozen	Brand D		
1106171-27	Raw shrimp, 41/50, frozen	Brand D		Vibrio cholerae
1106171-28	Raw shrimp, peeled, 71/90, frozen	Brand N	+	Vibrio parahaemolyticus
1106171-29	Cooked shrimp, peeled, 100/200, frozen	Brand O	+	

- Out of 22 samples tested Vibrio sp. were found by Metagenomics in 12 samples.
- *V. cholerae* strains were isolated from 2 samples included in metagenomics and one additional sample.

Analysis of *V. cholerae* and *V. parahaemolyticus* Isolates by Whole Genome Sequencing

Gene detected based on genome analysis		V.cholerae IEH isolates								V.cholerae References				V.parahaemolyticus IEH isolates						Other	
		1106171-0007A	1106171-0007C	1106171-0018A	1106171-0018B	1106171-0018C	1106171-0018D	1106171-0027A	1106171-0027D	MEI 67133-1	Vibrio cholerae O1 biovar El Tor str. N16961	VC_PS15	VC_VCC19	1106171-0007B	1106171-0019A	1106171-0019B	1106171-0028A	1106171-0028B	1106171-0028D	Vibrio_parahaemolyticus_BB22OP	Vibrio mimicus MB-451
Transcriptional activator ToxR	*toxR*	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+
Thermostable hemolysin delta-VPH	*dth*	+	+	+	+	+	+	+	+	+	+	+	-	+	-	+	+	+	+	+	+
Outer membrane protein OmpU	*ompU*	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+
Exotoxin A precursor (NAD-dependent ADP-ribosyltransferase)	*chxA*	-	-	+	+	+	+	+	+	-	-	+	-	-	-	-	-	-	-	-	-
MSHA pilin protein MshA	*mshA*	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+
Cytolysin and hemolysin, HlyA, Pore-forming toxin	*hlyA*	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-	-	+	+	+	+
Toxin co-regulated pilin A	*tcpA*	-	-	-	-	-	-	-	-	+	+	+	-	-	-	-	-	-	-	-	-
Type III secretion inner membrane channel protein (LcrD,HrcV,EscV,SsaV)	*vcsv2*	+	-	-	-	-	-	+	-	-	-	-	+	+	+	+	+	+	+	+	-
Flagellum-specific ATP synthase FliI	*vcsn2*	+	+	+	+	+	+	+	+	-	-	+	+	+	+	+	+	+	+	+	-
putative type III secretion system EscC protein	*vcsC2*	+	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	+	-
Type III secretion host injection protein (YopB)	*vspD*	+	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	+	-
Vibrio seventh pandemic island I	VSP-I	+	+	+	+	+	+	+	+	+	+	-	+	+	-	+	+	+	+	partial	+
Vibrio seventh pandemic island II	VSP-II	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-	-	-	-	partial	-
accessory cholera enterotoxin	*ace*	-	-	-	-	-	-	-	-	+	+	-	-	-	-	+	-	-	-	-	+
cholera enterotoxin A subunit	*ctxA*	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-
cholera entrotoxin B subunit	*ctxB*	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-
rtx toxin	*rtxA*	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-	-	-	-	+	+
rtx toxin	*rtxC*	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-	-	-	-	-	+
zona occludens toxin	*zot*	-	-	-	-	-	-	-	-	+	+	-	-	+	-	+	+	-	-	-	+
outer membrane protein	*ompW*	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	rstR	+	+	+	+	+	+	+	+	+	+	-	+	-	-	-	-	-	-	-	-
Thermolabile hemolysin	*tlh*	+	+	+	+	+	+	+	+	+	+	-	+	+	-	+	+	+	+	+	+
Thermostable direct hemolysin	*tdh*	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-
Thermostable related hemolysin	*trh*	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
***V.cholerae* MLST analysis**	*adk*	2	2	3	3	3	3	1	24	7	7	3	17	28	105	60	116	116	116	11	NA
	gyrB	5	5	56	56	56	56	1	5	11	11	5	33	138	147	29	76	76	76	48	NA
	mdh	11	11	14	14	14	14	12	7	4	4	14	51	144	170	192	149	149	149	48	NA
	metE	88	88	40	40	40	40	65	10	37	37	41	31	26	230	61	45	45	45	26	NA
	pntA	6	6	3	3	3	3	2	13	12	12	66	58	177	251	147	62	62	62	48	NA
	purM	1	1	9	9	9	9	1	1	1	1	1	10	116	312	147	72	72	72	43	NA
	pyrC	1	1	1	1	1	1	65	65	20	20	66	29	61	171	110	26	26	26	26	NA
	ST	unknown	unknown	unknown	unknown	unknown	unknown	unknown	unknown	69	69	unknown	unknown	810	unknown	unknown	247	247	247	88	NA

Identification of a new species of Clostridium in spoiled pear by metagenomics and whole genome sequencing

Preliminary identification

- Identified the pear spoilage organism as *Clostridium sp* from 16S community metagenomics.

Isolation & Characterization

- Whole genome sequencing (WGS) of the bacterial isolate revealed a new species of *Clostridium* named *C. pearianum* Sp. Nov, a close relative of *C.pasteurianum.*

Development of a detection kit

- Developed a PCR based detection system using two distinct genetic markers obtained from WGS

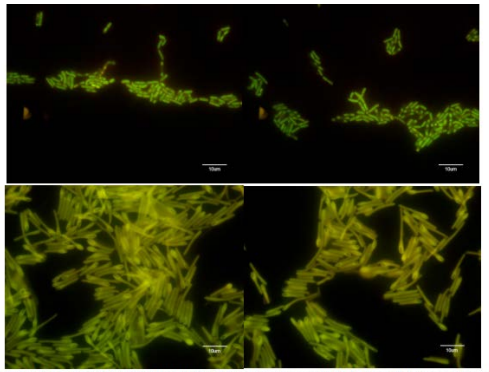


Figure 1. Spore maturation stage by Epi-fluorescence microscopy (1000x Zeiss, SyBr Green stained).

Figure 2. The strain was isolated on PYG-CaCO3 agar plate. This plate has been incubated at 37°C for 5 days in anaerobic condition. The clear zone was appeared around umbonate colonies that indicated this strain is producing acids in anaerobic condition.

Identification of Clostridium tepidum sp. nov. by metagenomics and whole genome sequencing

- Novel species isolated from non-dairy protein shakes in bloated bottles.
- Related to *C.sporogenes* by 35.7-36%.
- More thermophilic (temperature range for growth: 30–55˚ C) and less halotolerant [growth range: 0–2.5% (w/v) NaCl] than *C. sporogenes* and *C.botulinum* .

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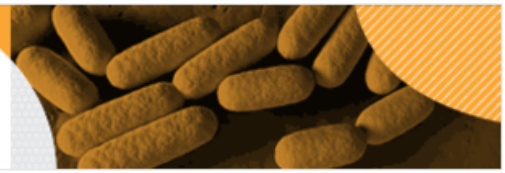

S *Clostridium tepidum* sp. nov., a close relative of *Clostridium sporogenes* and *Clostridium botulinum* Group I

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NGS Impact on the Industry

- Epidemiology:
  - Increases the chance of connecting a food processor to an outbreak
  - A Single case can be connected to foods
- Regulatory:
  - It allows for micromanaging the micro data
  - Increased regulatory actions based on WGS
- Plant and Animal Genetics
- Shelflife extension
- Food Microbiology