LFFM WGS THROUGHPUT TRACK AND PROJECT UPDATES - SOUTH DAKOTA STATE UNIVERSITY ADRDL

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SOUTH DAKOTA STATE UNIVERSITY

SDSU-ADRDL LFFM projects year 6 and 7

1. Foodborne pathogens in the dairy processing environment
2. Foodborne pathogens in the grain-associated environment
   - Grain-associated environment
   - Grain-associated wildlife samples

Target organisms for both: *L. monocytogenes*, *Salmonella*, and *Cronobacter*



Foodborne pathogens in the dairy processing environment

Foodborne pathogens in the dairy processing environment

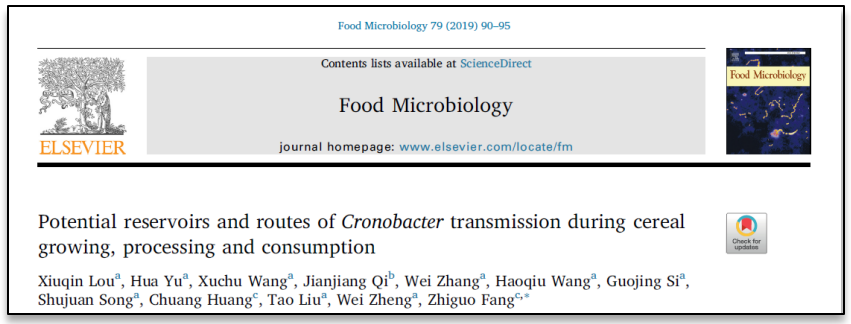
- The Davis Dairy Plant on the SDSU campus processes 15,000 pounds of milk per week and produces cheese, butter, beverage milk, and over 20 flavors of ice cream for retail sale.
- Sampling efforts (monthly):
  - 75 samples (year 6)
  - 36 samples (year 7 to date)
  - Non-food contact surfaces: Production floors, floor drains, handwashing, sink drains, table surface swabs
  - All samples were negative for *Cronobacter*, *Listeria*, *Salmonella*.



Foodborne pathogens in the grain-associated environment

Foodborne pathogens in the grain-associated environment

- SDSU uses more than 17,000 acres of land across the state for scientific research via the SD Agricultural Experiment Station. The SDSU Farm Department maintains the equipment used for harvest along with some storage for seed stocks.
- Fairly little data available particularly on *Cronobacter* in domestic agricultural settings/environments.





Foodborne pathogens in the grain-associated environment

- SDSU Farm Department/Foundation Seed Stocks Division
- Sampling effort: We took 61 samples (year 7)
- Dust/grain dust at production and grain handling sites, Dust/grain dust from large agricultural equipment. Sampling started pre-harvest and will continue post-harvest. Crops: soy, corn, and wheat.
- 40 samples from grain-associated wildlife (*will be tested next quarter*)

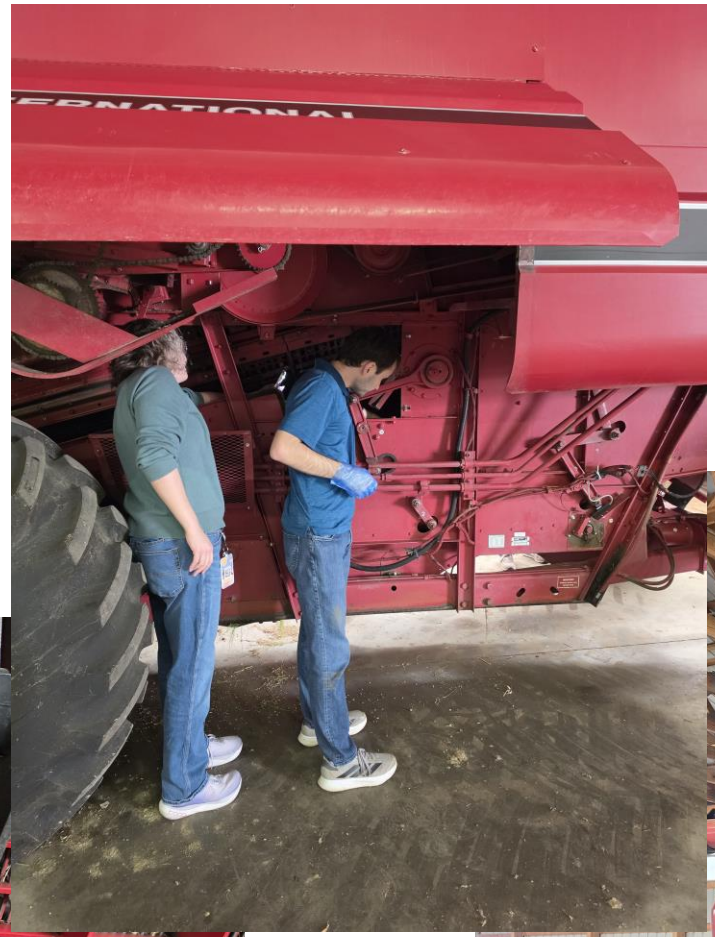


Foodborne pathogens in the grain-associated environment












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Foodborne pathogens in the grain-associated environment

- Sampling effort: We took 61 samples, 3 sampling time points
- Dust/grain dust at production and grain handling sites, dust/grain dust from large agricultural equipment/machinery.
  - 24 *Cronobacter* isolates – 39% of samples were positive
  - 6 *Siccibacter (S. colletis* and *S. turicensis)*
  - 5 *Listeria welshimeri*
  - 3 *Kosakonia cowanii*
  - 3 *Leclercia adecarboxylata*
  - 1 *Listeria fleishmanii*

Foodborne pathogens in the grain-associated environment

- First results from 20 sequenced *Cronobacter* isolates: 9 *C. sakazakii*, 9 *C. dublinensis*, 2. *C. malonaticus*

Strain	Species	MLST ST	CC	Assembly size [Mbp]	no. of contigs	Plasmids
Cronobacter LFFM-26-FS-005-C	*C. dublinensis*	4 MM*		4.538	39	
Cronobacter LFFM-26-FS-006-C	***C. sakazakii***	**ST31**		**4.507**	**30**	**2.8 kbp; 103 kbp**
Cronobacter LFFM-26-FS-008-C	***C. sakazakii***	**ST4**	**CC4**	**4.606**	**24**	**118 kbp; 116 kbp**
Cronobacter LFFM-26-FS-013-C	***C. sakazakii***	**ST31**		**4.507**	**30**	**2.8 kbp; 103 kbp**
Cronobacter LFFM-26-FS-014-C	***C. sakazakii***	**ST31**		**4.507**	**30**	**2.8 kbp; 103 kbp**
Cronobacter LFFM-26-FS-020-C	*C. dublinensis*	ST179		4.496	39	
Cronobacter LFFM-26-FS-025-C	*C. dublinensis*	ST455		4.5	32	
Cronobacter LFFM-26-FS-031-C	***C. sakazakii***	**ST4**	**CC4**	**4.552**	**29**	**116 kbp; 118kbp**
Cronobacter LFFM-26-FS-032-C	*C. dublinensis*	6 MM*		4.586	41	
Cronobacter LFFM-26-FS-033-C	*C. dublinensis*	ST1118		4.532	33	
Cronobacter LFFM-26-FS-035-C	***C. sakazakii***	**ST4**	**CC4**	**4.615**	**38**	**116 kbp; 118kbp**
Cronobacter LFFM-26-FS-037-C	*C. dublinensis*	4 MM*		4.485	29	129 kbp; 2.8 kbp
Cronobacter LFFM-26-FS-038-C	***C. sakazakii***	**ST226**	**CC8**	**4.351**	**24**	**51 kbp**
Cronobacter LFFM-26-FS-040-C	***C. sakazakii***	**ST264**	**CC264**	**4.404**	**40**	**51 kbp; 3.2 kbp**
Cronobacter LFFM-26-FS-041-C	*C. dublinensis*	5 MM*		4.746	61	194 kbp; 2.9 kbp
Cronobacter LFFM-26-FS-042-C	*C. dublinensis*	4 MM*		4.485	28	129 kbp; 2.8 kbp
Cronobacter LFFM-26-FS-043-C	*C. malonaticus*	ST129		4.552	42	129 kbp
Cronobacter LFFM-26-FS-046-C	***C. sakazakii***	**ST4**	**CC4**	**4.605**	**21**	**116 kbp; 118kbp**
Cronobacter LFFM-26-FS-049-C	*C. dublinensis*	ST908		4.514	28	157 kbp; 66 kbp
Cronobacter LFFM-26-FS-051-C	*C. malonaticus*	ST129		4.552	41	129 kbp

* Number of mismatches to closest hit in PubMLST

Foodborne pathogens in the grain-associated environment

- Some *C. sakazakii* isolates are clonal (based on average nucleotide identity (ANI) and on SNPs (NCBI Pathogen Detection))

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Genome Cart | ANIb Result | ANIm Result | Tetra Result | TCS Result

ANIm Result Matrix | ANIm Result by Genome

Show ANIm and [aligned nucleotides] [%] | Download as .csv

Legend: Above cutoff (> 95%) | Below cutoff (< 95%) | Suspicious alignment!

ST31

	Cronobacter LFFM-26-FS-006-C.fasta	Cronobacter LFFM-26-FS-013-C.fasta	Cronobacter LFFM-26-FS-014-C.fasta	Cronobacter LFFM-26-FS-008-C.fasta	Cronobacter LFFM-26-FS-031-C.fasta	Cronobacter LFFM-26-FS-035-C.fasta	Cronobacter LFFM-26-FS-046-C.fasta
Cronobacter LFFM-26-FS-006-C.fasta	*	99.99 (100.00)	99.99 (100.00)	98.00 (94.32)	98.04 (93.84)	98.00 (94.32)	98.00 (94.32)
Cronobacter LFFM-26-FS-013-C.fasta	99.99 (100.00)	*	100.00 (100.00)	98.00 (94.31)	98.04 (93.84)	98.00 (94.31)	98.00 (94.31)
Cronobacter LFFM-26-FS-014-C.fasta	99.99 (100.00)	100.00 (100.00)	*	98.00 (94.31)	98.04 (93.84)	98.00 (94.32)	98.00 (94.31)
Cronobacter LFFM-26-FS-008-C.fasta	98.01 (92.56)	98.01 (92.56)	98.01 (92.56)	*	99.50 (95.37)	99.99 (99.99)	100.00 (100.00)
Cronobacter LFFM-26-FS-031-C.fasta	98.05 (93.14)	98.05 (93.15)	98.05 (93.14)	99.51 (96.38)	*	99.51 (96.37)	99.51 (96.38)
Cronobacter LFFM-26-FS-035-C.fasta	98.01 (92.36)	98.01 (92.36)	98.01 (92.36)	99.99 (99.92)	99.50 (95.17)	*	99.99 (99.92)
Cronobacter LFFM-26-FS-046-C.fasta	98.00 (92.55)	98.01 (92.56)	98.00 (92.56)	100.00 (100.00)	99.50 (95.37)	99.99 (99.99)	*

ST4

Foodborne pathogens in the grain-associated environment

- There seems to be a diverse set of *Cronobacter* species in the grain-associated environment
- 39% of the samples were positive for *Cronobacter*
- In addition, we also isolated related isolates (which were previously classified as *Enterobacter*)
  - *Siccibacter colletis, S. turicensis*, *Kosakonia cowanii*
  - 36/61 (59%) samples were positive for *Cronobacter*, *Siccibacter*, and *Kosakonia*
- The presence of ST4/CC4 *C. sakazakii* may be of concern
- All *C. sakazakii* isolates had plasmids
  - Some plasmids are highly similar to characterized *C. sakazakii* plasmids (e.g. pESA3), that are important for virulence and stress (desiccation) survival

Foodborne pathogens in the grain-associated environment

Next steps:

- Two additional sampling time points
- Sequence additional *Cronobacter* isolates
- More detailed genomic analysis of the *Cronobacter* strains
  - Obtain closed genomes for some of the *Cronobacter* isolates
  - Potentially include some of the *Siccibacter* isolates in genome sequencing and analysis.

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