

Mining the Mouse Microbiome

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Hirschsprung's Disease



Normal



Hirschsprung's

Complication of Hirschsprung's

- Hirschsprung's-associated enterocolitis (HAEC)
 - Can occur before or after surgery

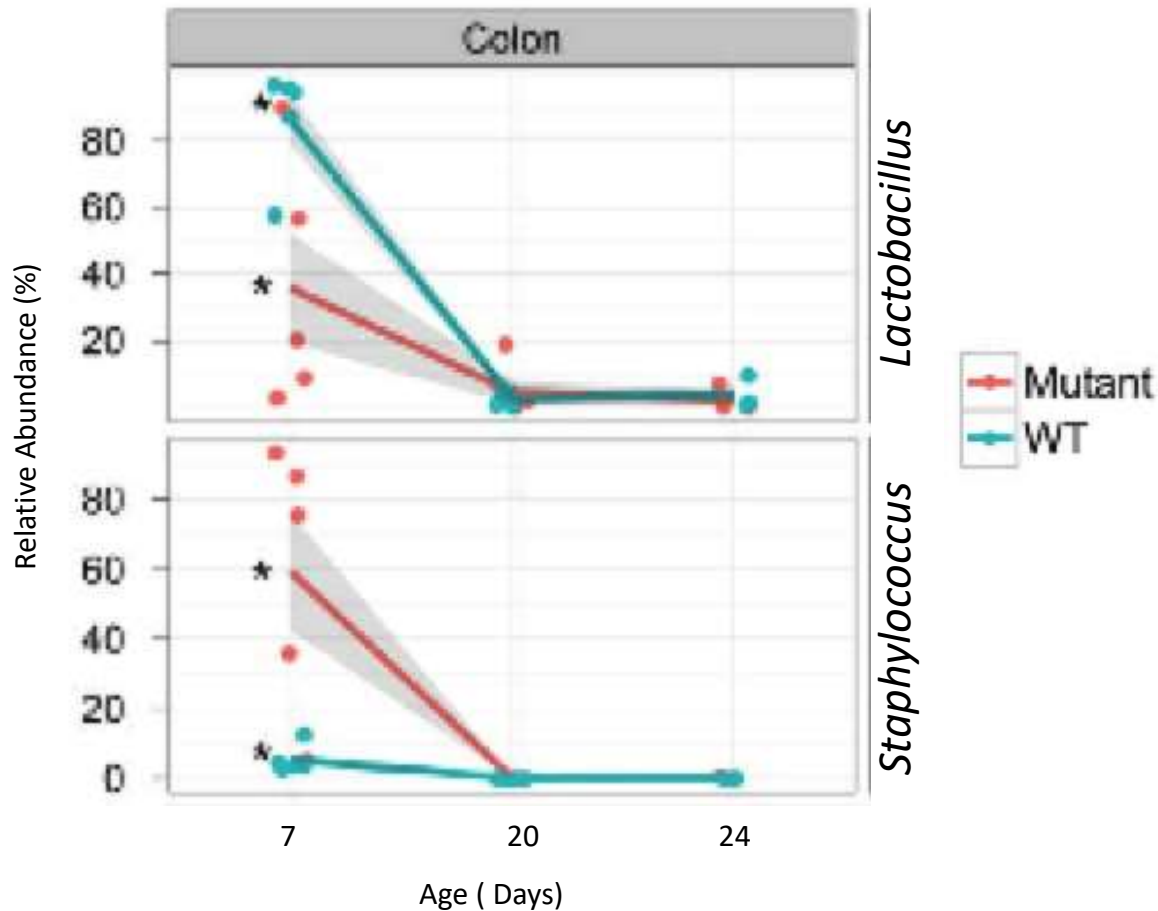
- Symptoms:
 - All patients: diarrhea, distension, fever
 - Some patients: bacterial translocation, sepsis, and death



Previous Work in the Ward Lab

- 2012 Study on the diversity of mouse microbiomes
 - Ednrb^{-/-} mouse
 - Mice most closely resemble the phenotype of human HD and HAEC
 - “Colorectal aganglionosis is associated with early and sustained disruption of the normal colonic and fecal microbiome”
- 2015 Study on the impact of antibiotics on the microbiome of the GI tract (unpublished data)

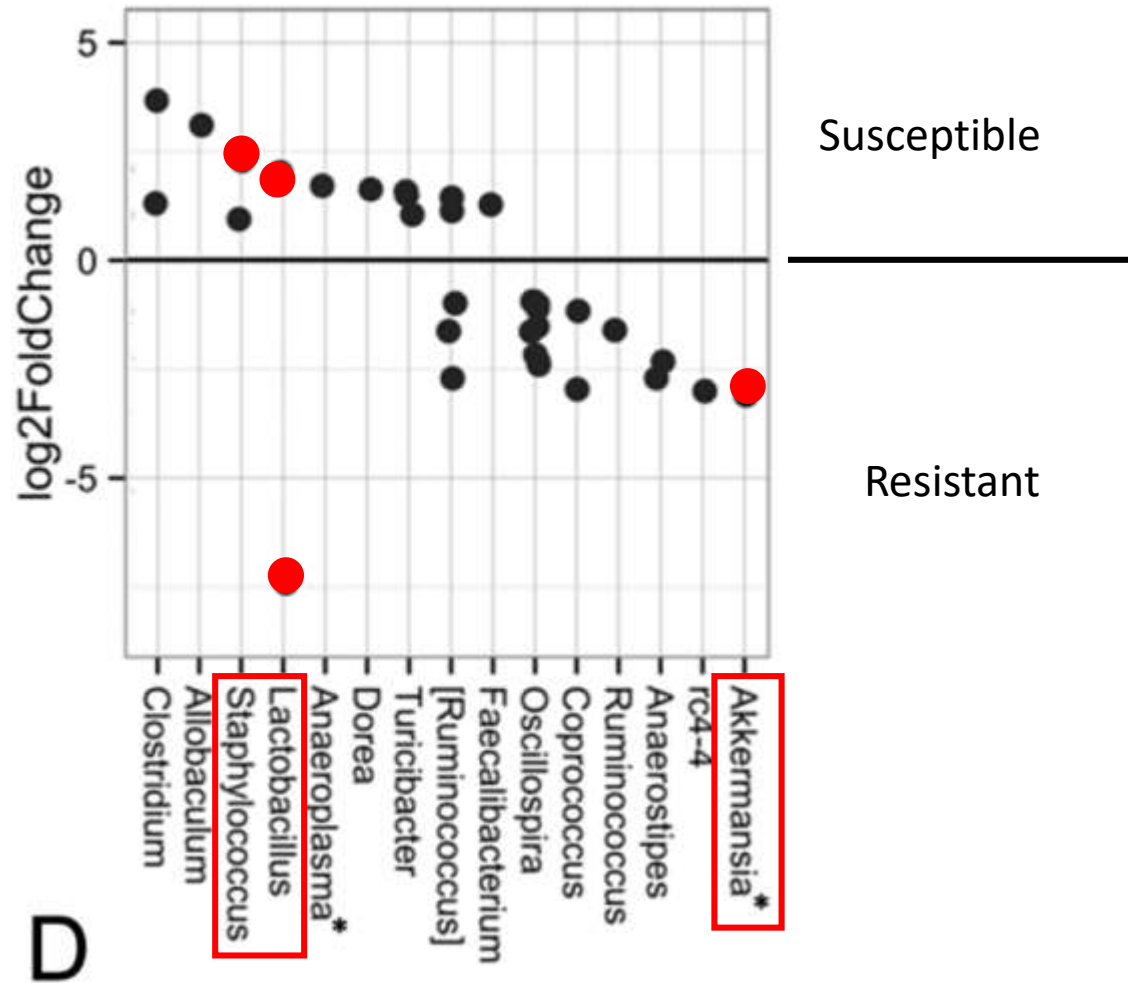
Variance in Diversity at the Genus Level in Wild Type And KO Mice



* = $P < 0.005$

shading = standard error

Occurrence of Genera in relation to severity of colitis symptoms

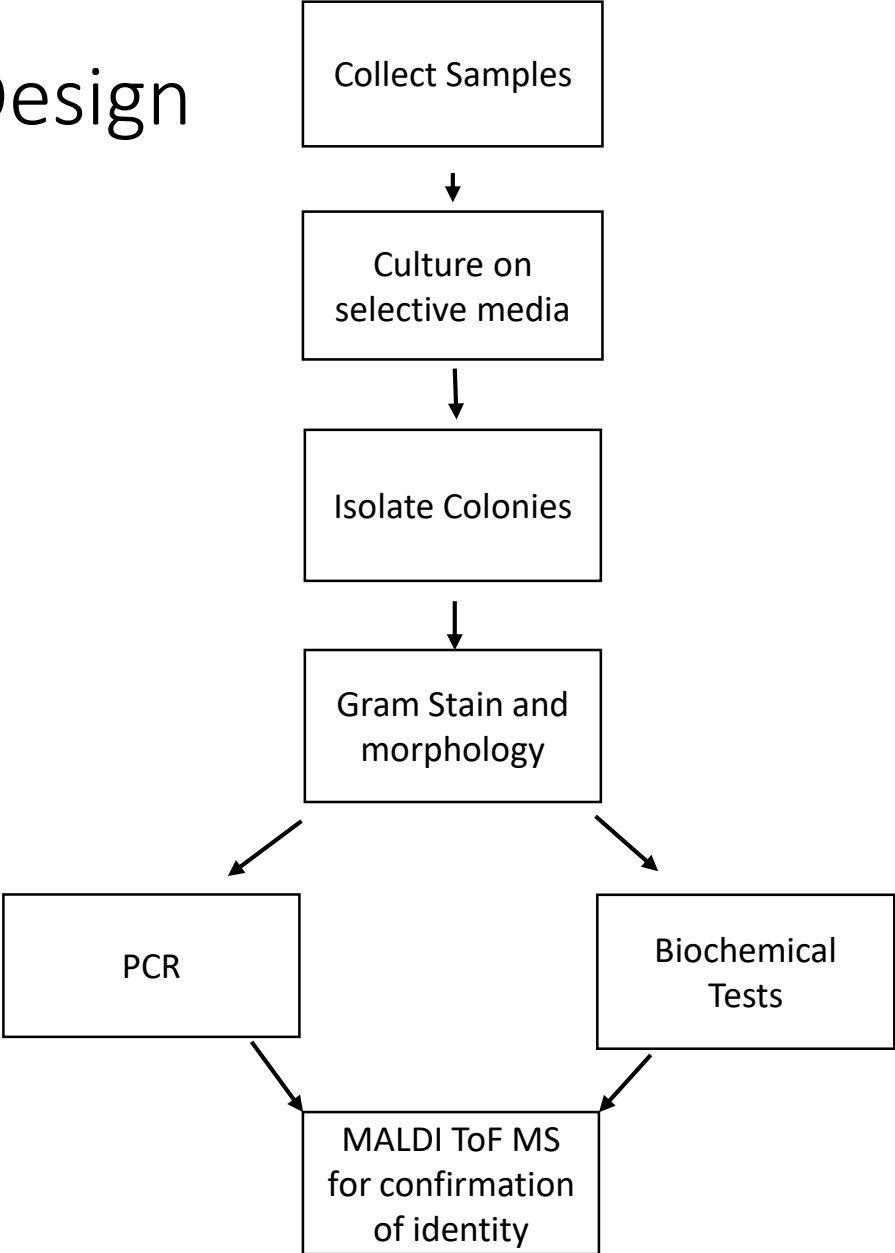


Project Purpose

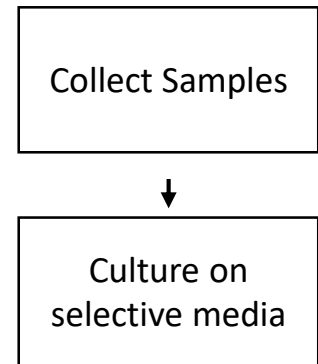
- To collect, isolate and positively identify species from the genera of *Lactobacillus*, *Staphylococcus*, and *Akkermansia*
 - Provide a reference bank for future research projects in the Ward lab
 - Create a building block for further understanding of the role of the microbiome in both HAEC and other types of colitis



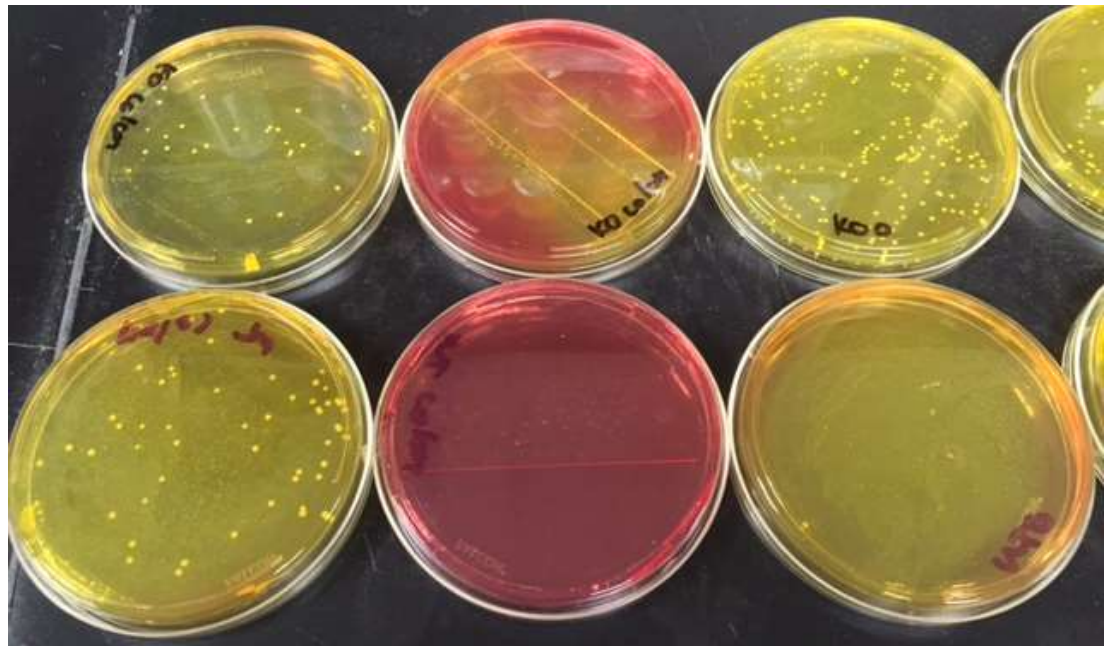
Methods and Design



Initial Cultures

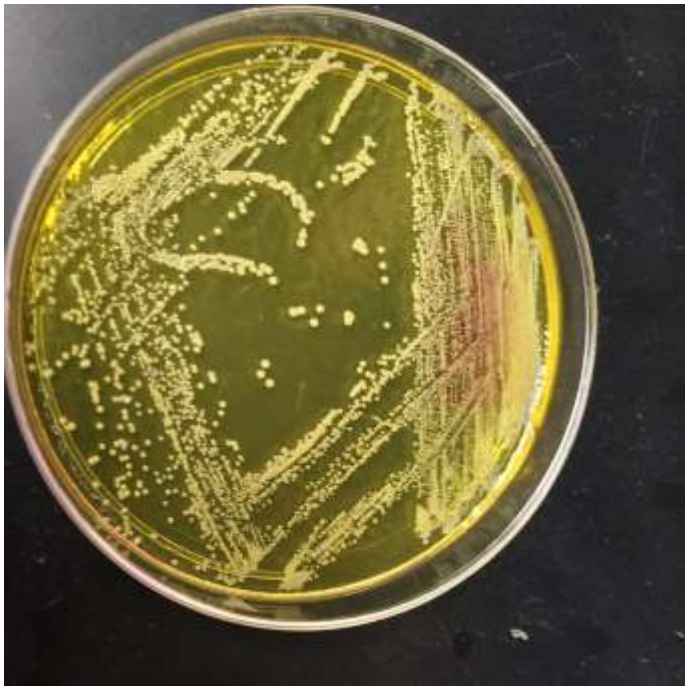
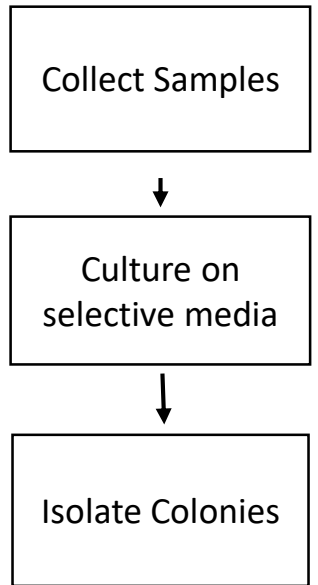


- Culture on Mannitol Salt Agar (MSA) plates for *Staphylococcus*
- Samples collected from both wild type and mutant mice
 - Homogenized feces
 - Scraping from colon



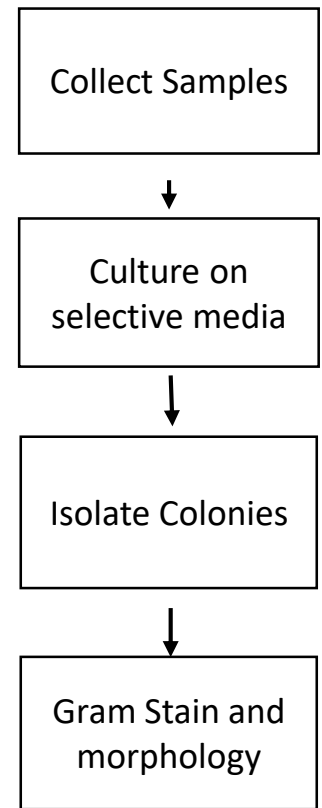
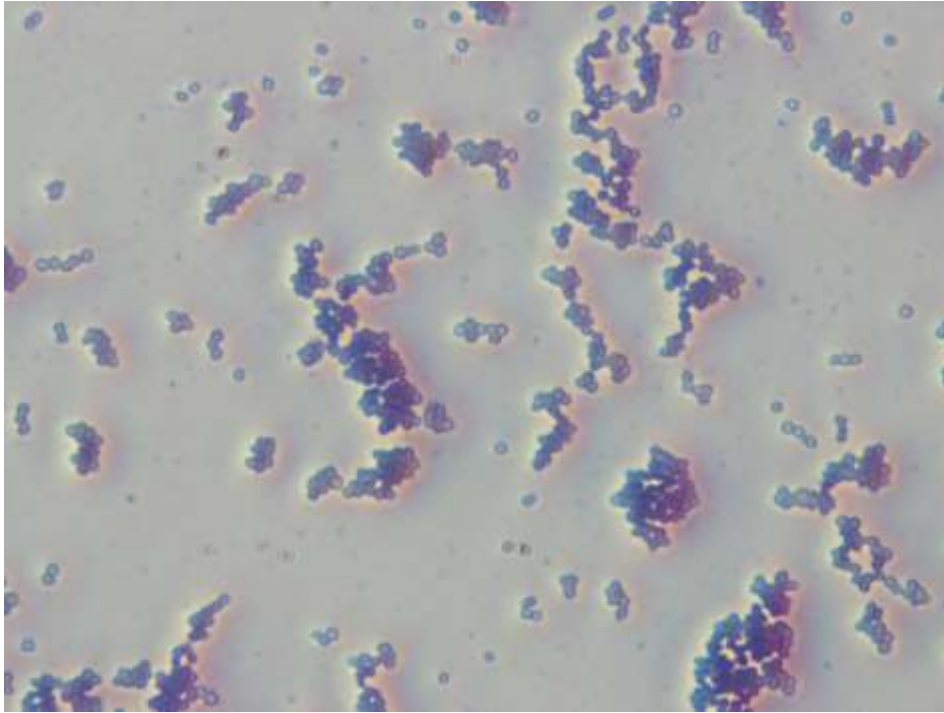
Isolations

- Species isolated using standard isolation techniques
- Isolated on MSA plates
 - MSA is partially selective for *Staphylococcus*
- All species also grown in both aerobic and anaerobic environments



Strain	Identity/Source	MSA Fermentation
BHK1	Staph. from KO feces	(+) yellow
BHW1	Staph. from WT colon	(+) yellow
MF2	Staph. from KO feces	(+) yellow

Gram Stains and Colony Morphology

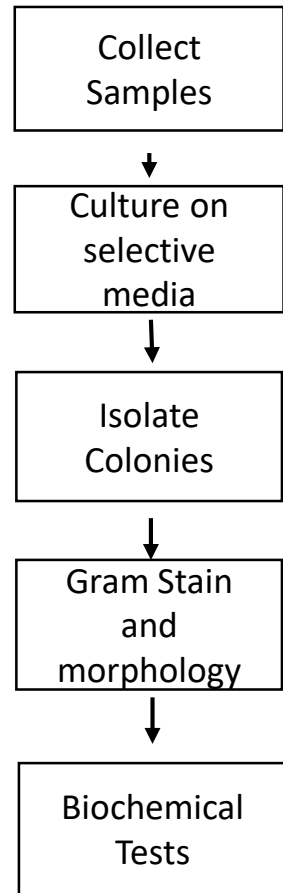


Strain	Identity/Source	Colony morph.	Gram-Stain
BHK1	Staph. from KO feces	round white	purple coccus
BHW1	Staph. from WT colon	round white	purple coccus
MF2	Staph. from KO feces	round white	purple coccus

Catalase Test

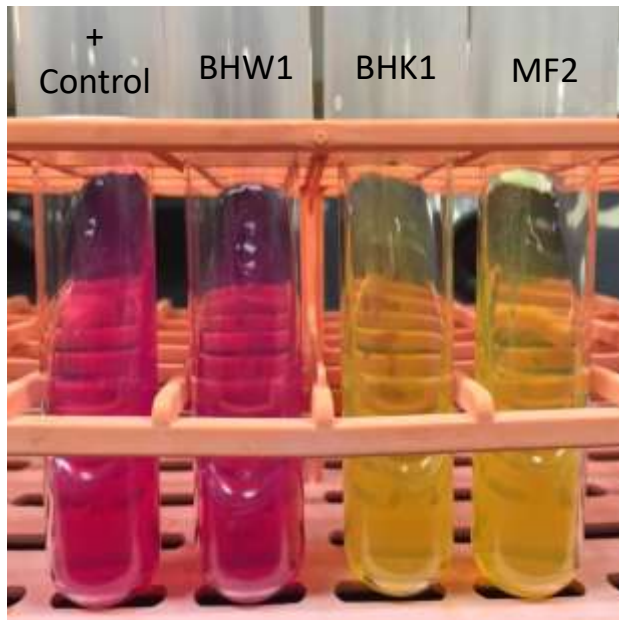
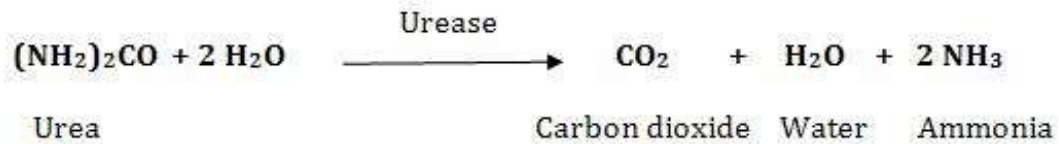


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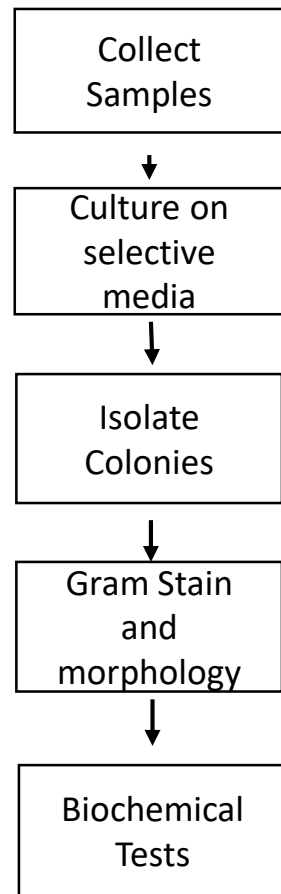


Strain	Identity/Source	Catalase
BHK1	Staph. from KO feces	(+)
BHW1	Staph. from WT colon	(+)
MF2	Staph. from KO feces	(+)

Urease Test

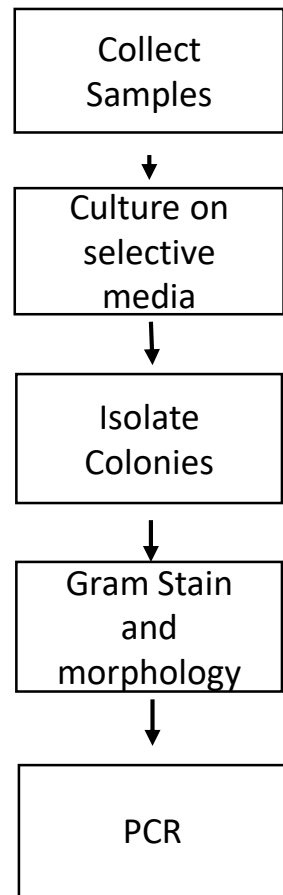
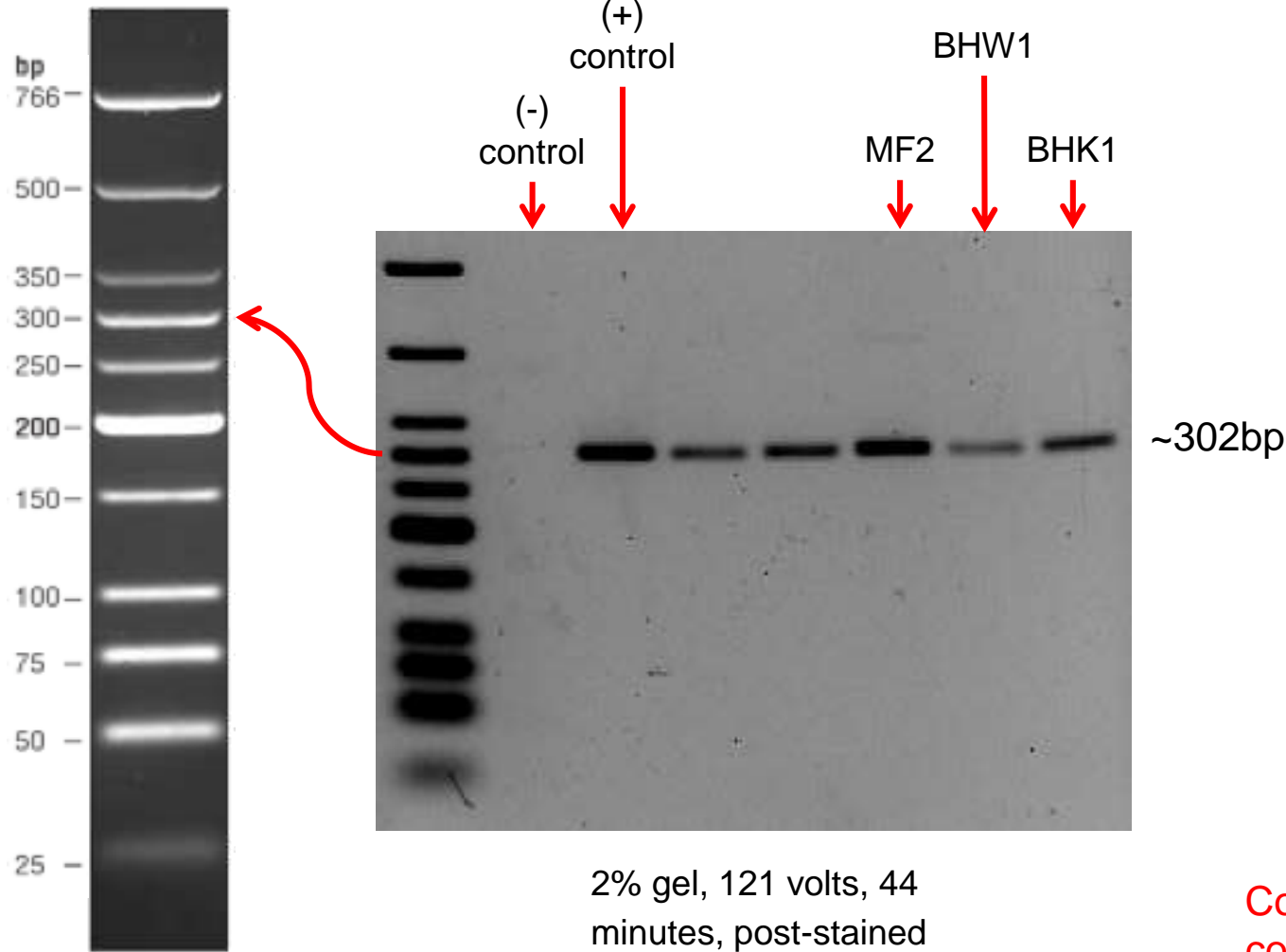


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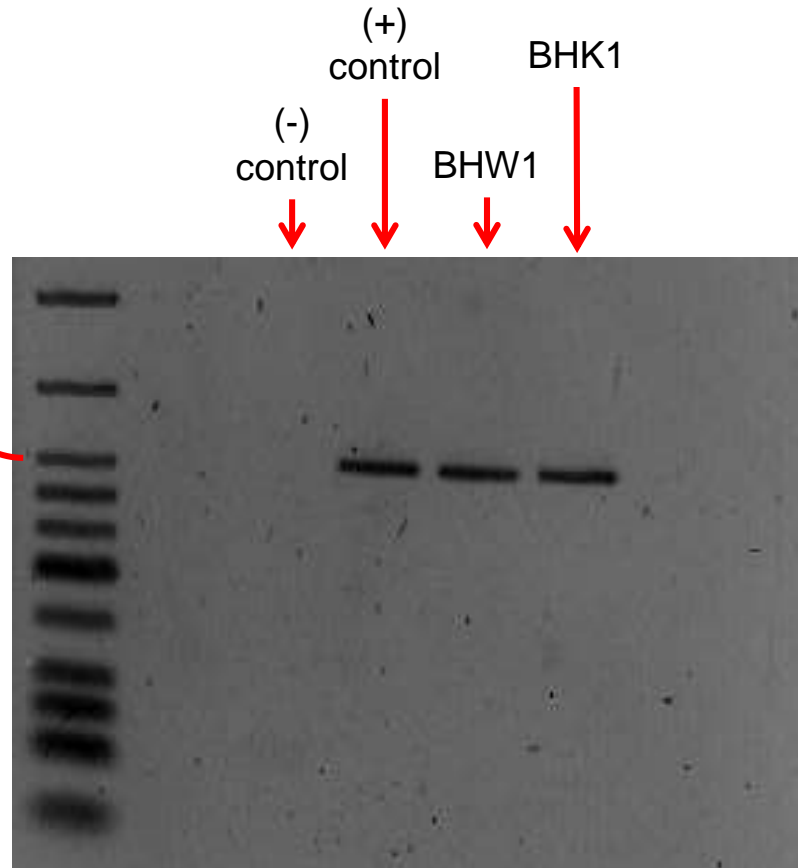
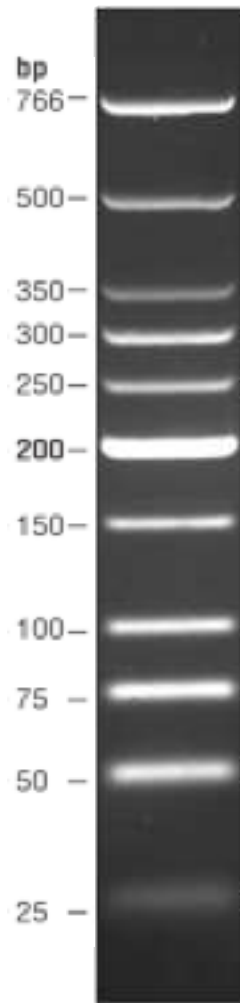
Strain	Identity/Source	Urease
BHK1	Staph. from KO feces	(-)
BHW1	Staph. from WT colon	(+)
MF2	Staph. from KO feces	(-)

Staphylococcus specific 302bp amplicon



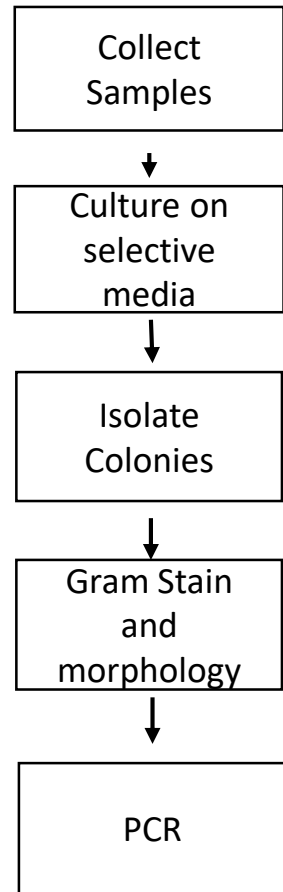
Corresponds to highly conserved region within *Staphylococcus eno* gene, which encodes the enzyme enolase

Staphylococcus specific 370bp amplicon



2% gel, 121 volts, 44 minutes, post-stained

Corresponds to highly conserved region within *Staphylococcus tuf* gene, which encodes the elongation factor Tu



Conclusions

Strain	Identity/Source	Colony morph.	Gram-Stain	MSA Fermentation	Staph. Eno.	Catalase	Urease
BHK1	Staph. from KO feces	round white	purple coccus	(+) yellow	(+)	(+)	(-)
BHW1	Staph. from WT colon	round white	purple coccus	(+) yellow	(+)	(+)	(+)
MF2	Staph. from KO feces	round white	purple coccus	(+) yellow	(+)	(+)	(-)

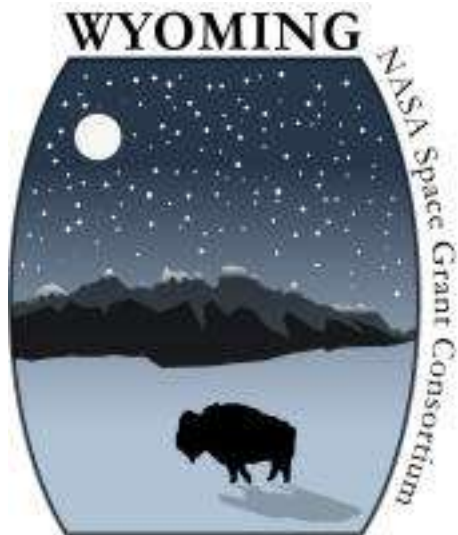
- Two verified species of *Staphylococcus* isolated from initial samples
 - BHK1 and BHW1 most likely different species based on production of urease
- One confirmed species of *Staphylococcus* from previous isolation

Future Directions

- Awaiting results of MALDI TOF MS on BHK1, BHW1, and MF2
- Isolated 4 potential *Lactobacillus* strains
 - Biochemical analyses, PCR analyses, and then MALDI TOF MS
- *Akkermansia* Isolations

Acknowledgments and Funding

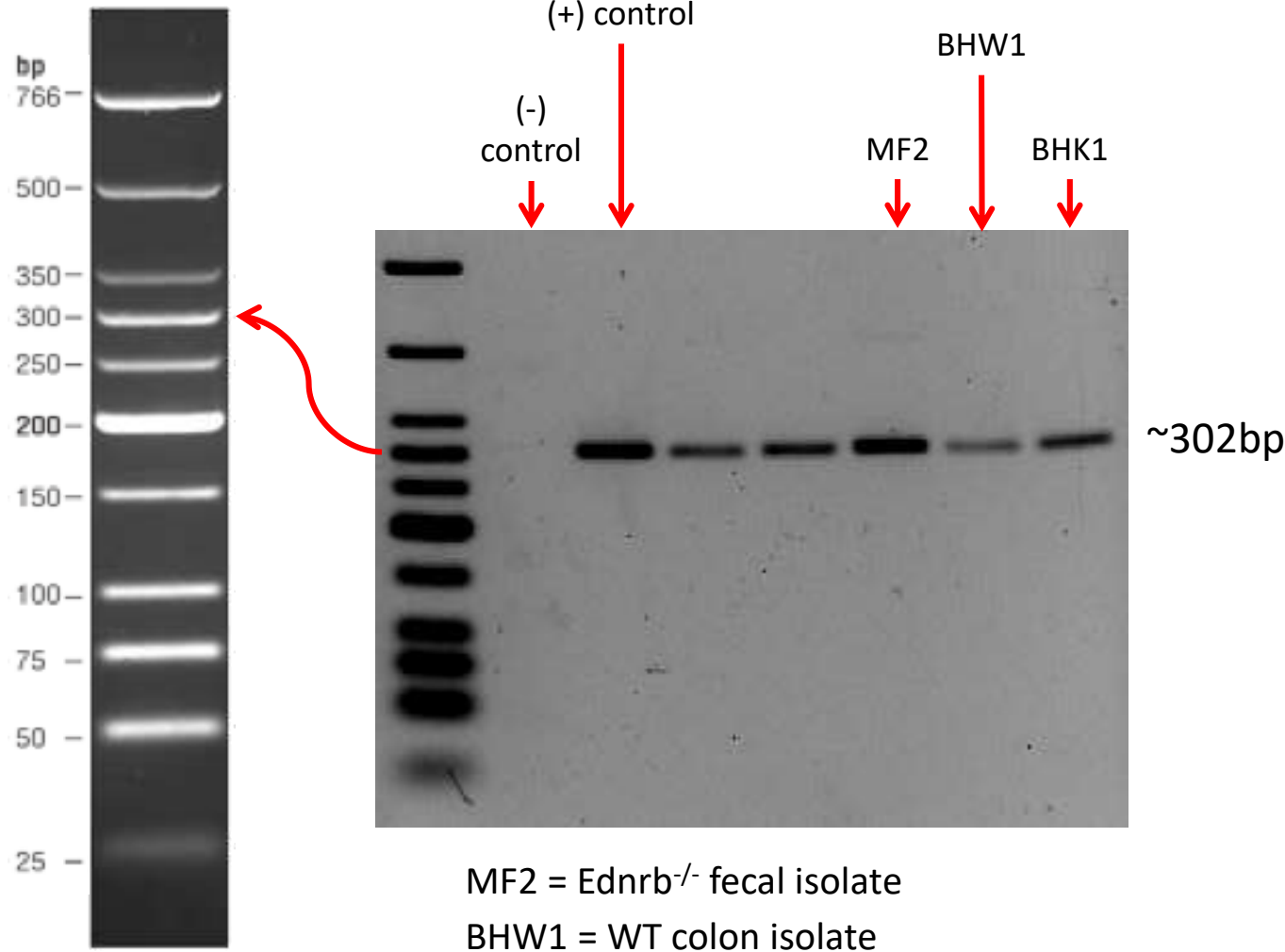
- Dr. Naomi L. Ward
- Kristopher Parker
- Sean Stettner



Citations

- Ward, N.L., Pieretti, A., Dowd, S.E., Cox, S.B., & Goldstein, A. M. (2012). Intestinal aganglionosis is associated with early and sustained disruption of the colonic microbiome. *Journal of Neurogastroenterology & Motility*. 1365-2982.
- Couto, I., Pereira, S., Miragaia, M., Sanches, I.S., & De Lancastre, H. (2001). Identification of Clinical Staphylococcal Isolates from Humans by Internal Transcribed Spacer PCR. *Journal of Clinical Microbiology*. 39. 3099-3103.
- Patel, R. (2013). Matrix-Assisted Laser Desorption Ionization-Time of Flight Mass Spectrometry in Clinical Microbiology. *Medical Microbiology*. 564-572.
- Bäckhed, F., Ley, R.E., Sonnenburg, J. L., Peterson. D.A., & Gordon. J. I. (2005). Host-Bacterial Mutualism in the Human Intestine. *Science*. 307. 1915-1920.

Staphylococcus specific 302bp amplicon



MF2 = *Ednrb*^{-/-} fecal isolate
BHW1 = WT colon isolate
BHK1 = *Ednrb*^{-/-} fecal isolate

