

INTRODUCTION

Salted beef is commonly known as the “food of sailors” because it was the staple for centuries on European sailing vessels. Nearly every shipboard account from the 16th to 18th centuries mentions the consumption of salted beef. Though many historical accounts of sailors’ health and diet exist, little is known about the microbes that were present and consumed in their diet. Salted beef was recreated using historical, archaeological, and ethnographic sources. The beef was stored in barrels and subjected to a 6-week simulated voyage on *Elissa* in Galveston, Texas. Samples of salted beef were collected regularly and taken to the USDA Agricultural Research Service laboratory in College Station, Texas for microbiological analysis. The purpose of this research is to determine identities of sampled microbes and their possible effects on sailors.

HYPOTHESIS

Microbes hypothesized to grow in the salted beef include: *Halamonas salina* and *Pediococcus halophilus* (halophiles), *Lactobacillus* and *Pediococcus homari* (halophiles and heat-tolerant), *Halobacterium halobium* ($a_w=0.75$), *Listeria monocytogenes* ($a_w=0.92$), *Micrococcus halodenitrificans* ($a_w=0.85$), *Staphylococcus aureus* ($a_w=0.86$), and *Virbrio costicola* ($a_w=0.85$). In addition, due to the lack of proper sanitation and sterilization, most 17th-century sailors likely experienced gastrointestinal issues resulting from *Salmonella* or *Staphylococcus aureus* infections via consumption of beef.

MATERIALS & METHODS

- An antibiotic and hormone-free grass-fed steer was butchered on August 5, 2017
- The beef was salted and brined according to John Collins’ 1682 recipe
- On August 18, 2017, the barrel of salted beef was transported to Galveston and loaded into the dock of *Elissa*
- Samples of the salted beef (both inside and outside washes) were collected regularly over a 6-week period
- These samples were plated on differential and selective media
- All cultures were purified and subjected to 16s rRNA sequencing and BLAST analysis

Directions for Salting of Fleish and Fish for long keeping, viz. Beef and Pork, according to the common way.

1. AN Ox being driven in cool one day, is slaughtered the next, quartered, and after it hath hung a convenient time to cool, is cut into four pound pieces.
2. The fume are very well rubb'd with Bay Salt, then put into Lins, Cribs, or Receptacles like Mangers, and almost buried in Salt, for the space of a Fortnight, three weeks, or longer, that the bloody Pickle may run away wait.
3. Then the Meat is again well rubb'd and pack'd or trodden into Cask, on a Cloth or Skin, with Salt betwixt every Line or Lay, and being headed up is thrown by in Store-houses, for six weeks or two Months time, but sometimes will not keep a Fortnight, which is known by the scent at the Bung.
4. Then when the fume is to be Repack'd, they turn the Bung of the cask downward, that all the bloody pickle may drain away into a waft Current.
5. Then all the Meat is taken out to be packt into Cask, smelling each piece, which is again well rubb'd with Salt, each lay being clove packt with Salt between; and when full is roll'd to the pickling place to be pickling.

The Pickle is thus made.

1. Dissolve Bay Salt in fresh-water, to long and to such a height, till upon Boiling with a brisk Fire, and summing off great quantities of Dirt and Filth, it is in a readiness to Kern or turn to Salt again, which is known by a Cream or Ice at the top. Then empty it into Coolers.
2. When the fume is thoroughly Cold, often fill up the cask at the Bung-hole and the Meas is cured.



Figure 1 (Left): A folio from John Collins’ 1682 discourse on salt, showing the salted beef recipe used in this experiment.
Figure 2 (Right): *Elissa*, a 19th-century tallship in Galveston, Texas.

RESULTS & DISCUSSION

- Microbial activity decreased over time in all media. (Table 1.1-1.3)
- A total of 7 pure culture isolates from the bacteria have been identified thus far (Table 2), most of which are commonly found in soil and water environments and are opportunistic pathogens
- This suggests that while eating the beef does not warrant any danger, wounded or sick sailors were likely at a high risk of infection
- The majority of identified microbes produce histamines possibly leading to decreased sensitivity to histamine over time, and therefore a stronger immune system



Figure 1: Salted beef submerged inside barrel filled with brine.
Figure 2: Color comparison between the inside and outside of a salted beef sample.
Figure 3: Salted beef samples plated onto several differential media.

SAMPLE ID	BLOOD AGAR				
	DAY 01	DAY 03	DAY 18	DAY 32	DAY 48
BEEF NO H2O	TNTC	*	TNTC	1382666.67	577333.33
BEEF IN H2O	TNTC	*	TNTC	TNTC	TNTC
INSIDE WASH	TNTC	*	106415	223014.67	27945
COOKED BEEF	553333.33	*	40	44200	8

Table 1.1: Averages of microbial counts on Blood Agar in logarithmic format. Data for inside washes was condensed from three replicates. Some samples were unable to be plated due to technical difficulties.

SAMPLE ID	BLOOD AGAR
	DAY 64
BEEF NO H2O	452666.67
INSIDE WASH	452
BEEF IN H2O 10	TNTC
BEEF IN H2O 20	TNTC
BEEF IN H2O 30	TNTC
BEEF IN H2O 40	TNTC
COOKED BEEF 10	3008.33
COOKED BEEF 20	0
COOKED BEEF 30	0
COOKED BEEF 40	0

Table 1.2: Averages of microbial counts on Blood Agar in logarithmic format. Data for inside washes was condensed from three replicates. Samples cooked between 10-40 minutes to determine minimal microbe activity.

SAMPLE ID	ROGOSA
	DAY 64
BEEF NO H2O	321.75
INSIDE WASH	57.2
BEEF IN H2O 10	TNTC
BEEF IN H2O 20	TNTC
BEEF IN H2O 30	TNTC
BEEF IN H2O 40	TNTC
COOKED BEEF 10	124
COOKED BEEF 20	0
COOKED BEEF 30	0
COOKED BEEF 40	0.2

Table 1.3: Averages of microbial counts on Rogosa in logarithmic format. Data for inside washes was condensed from three replicates. Samples cooked between 10-40 minutes to determine minimal microbe activity.

Microbe	Klebsiella oxytoca	Citrobacter freundii	Bacillus mirabilis	Proteus mirabilis	Kosakonia *	Acinetobacter *	Micrococcus
Morphology	Gram-negative rod	Gram-negative rod	Gram-positive rod	Gram-negative rod	Gram-negative rod	Gram-negative rod	Gram-positive cocci
Pathologies: Mode of Transmission	Opportunistic Wound/airway	Opportunistic Direct contact	None	Secondary Infection: UT, Wounds, Airway	Unknown	Pathogenic: Direct contact	Opportunistic Contact
Commonly Found	GI system	Soil/water	Soil/water and GI tract	Soil/water	Soil/water	Soil/water	Skin
Histamine producer?	Yes	Yes	Yes	Yes	Possibly	Probably	Probably
Nitrogen	Fixer	Fixer	Fixer	Fixer	Fixer	Fixer	Fixer
Temperature sensitivity?	No	No (heat resist)	No	No	Unknown	Unknown	Unknown
Salty Environments?	Yes	Yes	Endospore	Yes	Unknown	Yes (slightly)	Yes

Table 2: A chart of characteristics noted for the sequenced microbes based on morphology, pathology, mode of transmission, origin, production of histamine, nitrogen fixation, temperature sensitivity, and salt tolerance.

CONCLUSIONS & NEXT STEPS

Most of the microbes are considered opportunistic pathogens that could cause issues in wounded and immunocompromised sailors but primarily through direct contact and not ingestion. In the upcoming months, the project will strive to interpret the microbiological and nutritional findings in order to better understand the Hygiene Hypothesis. The Hygiene Hypothesis attributes the proliferation of allergies and other diseases in modern society to the lack of exposure to infectious agents and the waning diversity of gut flora. By understanding what 17th-century sailors were regularly exposed to in their diet, it may be possible to determine the way their diet impacted their immune system. Furthermore, the project continues to sequence and characterize microbes from the shipboard food in hopes of discovering novel strains of probiotics that could offer health benefits for many today.

REFERENCES

- Ananthalakshmy, V.K., Ramesh A., Venugopalan V.K. Bacterial production of histamine in some tropical fish. *Microbios*. 63 (1990): 71-7. Web.
- Hostacká, A & Klokočnicková, L. Characteristics of clinical Acinetobacter spp. strains. *Folia microbiologica*. 47 (2002):579-82. 10.1007/BF02818801.
- Joneja, J. M. Histamine intolerance, Diamine Oxidase Activity, and Probiotics. Retrieved from <http://www.allergynutrition.com/wp-content/uploads/2014/05/Histamine-DAO-and-Probiotics-Revised.pdf> (2004):.1-13.
- Settanni, L., and A. Corsetti. The use of Multiplex PCR to Detect and Differentiate Food- and Beverage-Associated Microorganisms: A Review. *Journal of Microbiological Methods* 69.1 (2007): 1-22. *TOXLINE*. Web.
- Taylor S.L. Histamine food poisoning: toxicology and clinical aspects. *Crit Rev Toxicol*. 17 (1986): 91-128. Web.
- The influence of water activity (aw) in meat products. (2008). [ebook] EQUINLAB, p.2. Available
- Ventosa A, Nieto JJ, Oren A. Biology of moderately halophilic aerobic bacteria. *Microbiol Mol Biol Rev*. 1998;62(2):504-544.

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