Comparison of *Listeria* Isolates from Environmental Sampling using Whole Genome Sequencing, Pulsed Field Gel Electrophoresis, and the RiboPrinter® System

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INTRODUCTION

- Whole Genome Sequencing (WGS) is transforming food safety as it allows for rapid and reliable differentiation between strains of foodborne illness culprits
- WGS is used by academia / regulatory authorities to:
  - Characterize and link foodborne outbreaks
  - Trace pathogen source
  - Compare against epidemiological data
- WGS industry use is still fairly new and somewhat limited, but can aid in:
  - Implementing a seek and destroy (S&D) process (ex: *Listeria* in the manufacturing facility environment)
  - Taking corrective actions for quick eradication
- There are several methodologies to subtype *Listeria* isolates, though most often, subtyping of swab isolates is limited to speciation
- Despite *Listeria* speciation and even with a S&D culture, it can still be difficult to determine a root cause for its presence, confirm whether the strains are persistent or transient, assess whether the strains are resistant to certain chemicals, and get rid of it
- WGS has the potential to provide insight into these gaps and more meaningful data at the manufacturing facility level to improve food safety

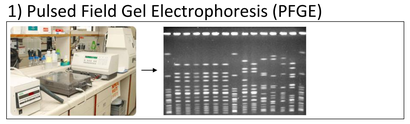
PURPOSE

- Compare three subtyping methodologies by analyzing isolates collected from environmental swabs in a manufacturing facility
- Determine any differences in results that may impact investigation and influence future method selection

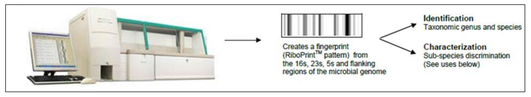
METHODS

- Thirty-five *Listeria* isolates were assessed following:

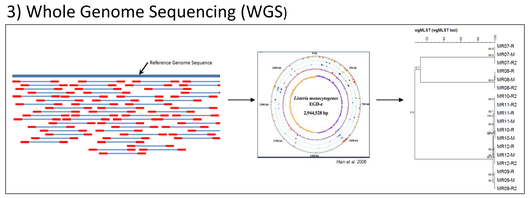
1) Pulsed Field Gel Electrophoresis (PFGE)



2) RiboPrinter



3) Whole Genome Sequencing (WGS)

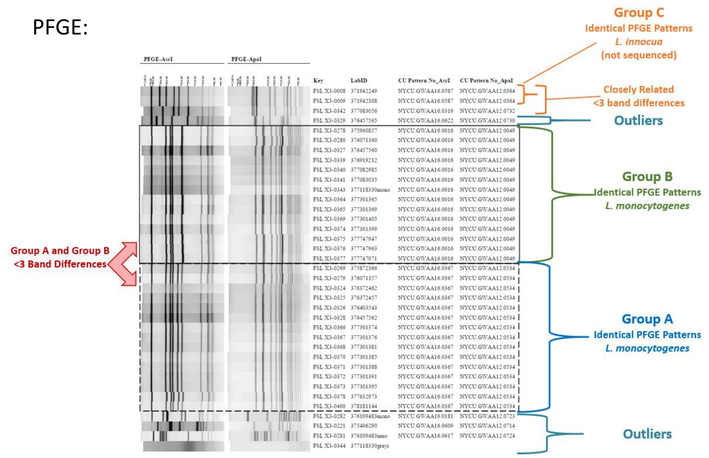


RESULTS

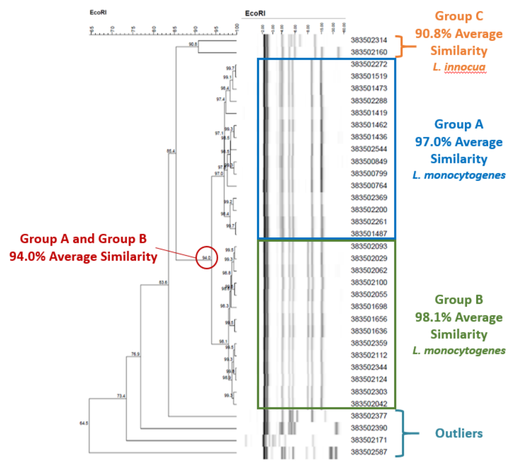
- Isolates clustered into two groups using PFGE, WGS, and RiboPrinting
  - Group A included 15 isolates that clustered by PFGE (identical band patterns – deemed indistinguishable), wgMLST (92.4% allele similarity), and RiboPrinting (97.0% average similarity)
  - Group B included 14 isolates that clustered by PFGE (identical band patterns – deemed indistinguishable), wgMLST (84.9% allele similarity), and RiboPrinting (98.1% average similarity)
- The remaining 6 isolates did not cluster well by PFGE, wgMLST, or RiboPrinting, with the exception of 2 isolates, which clustered by RiboPrinting (90.8% average similarity)

Figure 1. Comparison of PFGE, WGS, and RiboPrinting results for groups A and B.

PFGE:



RiboPrinting:



WGS:

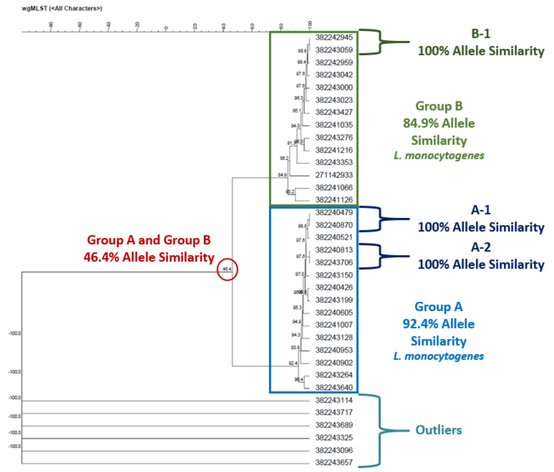


Table 1. Similarity criteria for PFGE, WGS, and RiboPrinting results.

Method	Similarity Description	Criteria
PFGE	Indistinguishable by PFGE and high probability the isolates are related	Identical PFGE patterns
	Closely related and have a potential of sharing a common ancestor	PFGE patterns differ by three bands or less
WGS	Indistinguishable by wgMLST	Average allele similarity is 100%
	Highly similar by wgMLST	Average allele similarity is between 99.1% and 99.9%
RiboPrinter	Indistinguishable by RiboPrinter	Average similarity ≥ 99.2%

DISCUSSION

- WGS offers a higher level of discrimination than PFGE and RiboPrinting
  - Groups A and B were closely related when analyzed by PFGE and RiboPrinting
  - Based on WGS analysis, their relatedness appears more remote which suggests that they were from a different source or introduced at different time points
- WGS results also provided insight into traffic patterns
  - Groups A-1, A-2, and B-1 had 100% allele similarity and were from locations that were in close proximity to each other
  - Group A-2 was from locations in close proximity, with swabs taken two months apart, suggesting a more robust strain able to persist in the manufacturing facility environment

CONCLUSION

- It would not be possible to gain these insights based on the PFGE or RiboPrinting results for these isolates
- WGS has a place for usage within the food industry and using PFGE or RiboPrinting results alone may potentially link isolates that are not as similar as they appear, which could lead to incorrect root cause analysis

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