Evaluation of FT-IR spectroscopy to discriminate Shiga-toxin-producing *Escherichia coli*

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Background and aim of the study

- ✓ Shiga-toxin-producing *Escherichia coli* (STEC) strains are zoonotic and waterborne pathogens, which represent a serious thread for public health. They can cause severe and life-threatening diseases like haemorrhagic colitis and haemolytic-uremic syndrome (HUS), especially in children and in elderly people.
 - ✓ Globally leading causes of diarrhea and even dysentery, extra-intestinal infections, and food-borne outbreaks.
 - ✓ Often associated to certain serotypes (i.e., O157:H7, O103:H21, O26:H11, etc.).

In this study, we evaluated the **discriminative power of Fourier-Transform Infrared (FT-IR) spectroscopy** to discriminate Shigatoxin producing *E. coli* strains at different intra-species level, and its potential to differentiate Shiga-toxin producing strains.

Materials and methods – FTIR spectroscopy

Fourier-Transform InfraRed Spectroscopy (FTIRS) → massively widespread technique in the field of analytical chemistry, for the identification of unknown substances through the detection of specific modifications in the vibrational modes of the covalent bonds induced by the absorption of IR light.



- ✓ Identification of covalent bonds \rightarrow functional groups \rightarrow molecules - \rightarrow biomolecules
- ✓ Each bacterial cell exhibits a unique FTIR spectrum, which is the sum of the "IR light absorption contributions" of all the molecules that form the cell → specific fingerprint signature
 - correlated with genetic information
 - showing also post-translational modification, and whatever is not genome-related (i.e., change in the expression level, acquisition of plasmids, etc.)
 - typing of microbial strains at different intra-species levels

Materials and methods – Dataset and workflow

Dataset: an international collection of well characterized *E. coli* strains (tot. N=179)

- ✓ N=30 from BfR
- ✓ N=149 from different centers in Germany
- \checkmark $\,$ From human and food sources
- ✓ 44 serogroups, 80 serotypes
- ✓ Different pathotypes
 - **n=50** STEC/EHEC (O26, O91, O103, O104, O111, O145, O157)
 - **n=32** EPEC
 - **n=2** EAEC
 - **n=95** non-enteropatogenic *E. coli*

FT-IR analysis: performed by the IR Biotyper[®] system (Bruker Daltonics GmbH & Co. KG, Germany)

- cultivation on Columbia sheep blood agar (24±2 h, 37 °C)
- **3 technical replicates** from **3 independent biological replicates**
- exploratory data analysis by HCA and PCA/LDA
- Three study phases:
 - A. Investigation of BfR strains (7 serogroups, 13 serotypes, different pathotypes, STs and toxin types)
 - B. Merging of BfR strains with strains from other centers
 - **C.** Addition of n=6 STEC with unknown serotype, to simulate the application of FTIR as classification method



Results – clustering BfR strains – all isolates (1)



✓ Complete correlation between IRBT clusters and O-serogroups (AR=1.000, AW=1.000).

✓ A further subclustering within the O-serogroups clusters is observed.

Results – clustering BfR strains – all isolates (2)



Results – clustering BfR strains – O26



Same serotype, pathotype, different ST



Same serotype, pathotype, ST



Same serotype, pathovar, ST



clones within the same ST?

 \rightarrow Differentiation related to ST or to \rightarrow Differentiation related to pathotype? \rightarrow Differentiation in relation to pathotype \rightarrow Possible differentiation of

Not for use in clinical diagnostic procedures.

toxin type?

Results – clustering BfR strains – O104



Same serotype, different ST



→ Differentiation between STEC (same toxin) and control strain, regardless the ST

Same serotype and toxin, different ST



→Differentiation between ST with the same toxin type

All strains



→ Differentiation between pathotypes possible (direct or via serotype?)

Results – clustering BfR strains – O157 and O111

Same serotype and ST, different pathotype



	Ec BfR-19987	O:157	O157:H7	ST11	stx2a + stx2c + eae
	Ec BfR-16094				stx1a + stx2a + eae
	Ec BfR-16926				undefined (Ec BfR-16926)
	Ec BfR-19664		O157:H19	ST4379	none

→Differentiation between STEC and control strain possible, despite the strains are very similar



 \rightarrow Clear discrimination of the EAEC ST111:H21 strain

Results – clustering BfR + other isolates (only EPEC & STEC)





- ✓ Merging the BfR strains with the EHEC previously measured, the same partition is observed → all O-groups are differentiated (AR=0.991, AW=1.000).
- ✓ A further subgrouping is again observed → will be investigated as soon as all metadata of the other strains will be available

Results – clustering BfR + other isolates (all)



✓ Merging the STEC strains with the non-pathogenic *E*. *coli* isolates, the STEC O-groups are clearly discriminated from all the others.

✓ The further subclustering within STEC O-groups previously observed is also kept.

Results – addition of unknown isolates



Conclusions

- ✓ FT-IR spectroscopy showed to be an innovative, promising and routine-friendly approach for typing of *E. coli*.
- ✓ The O-serogroups which to which most of STEC isolates belong are completely differentiated from all the others.
- ✓ In those O-serogroups comprising also pathotypes (STEC, EPEC, EAEC), a further discrimination, likely linked to serotype, sequence type and presence/absence of virulence factor is also observed.
- ✓ The addition of unknown STEC strains to a well characterized dataset allowed to clearly allocate them to STEC serogroups (confirmation ongoing) → background for the application of FTIR predictive models
- ✓ Further investigations, including more STEC strains, are necessary:
 - ✓ to confirm these promising preliminary results, and to **assess the discriminatory power of FT-IR**
 - ✓ to define the FT-IR clustering "drivers" (serotype, sequence type, toxin types/subtypes), also investigating different regions of the IR spectrum
 - ✓ to set up automated classifiers that, using machine learning, allows user-friendly typing method, at different intra-species level, suitable for laboratory routine workflows
- ✓ Collaboration partners are welcome ☺

Thanks to...

- All my coauthors, without whom this work would not have been possible
- Dr. A. Pranada, for his kind hospitality in his BSL3 lab
- All those who are attending my presentation today