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Improvement of the Experimental and Informatic Pipeline for High-Throughput MS/MS Proteotyping of Pathogens

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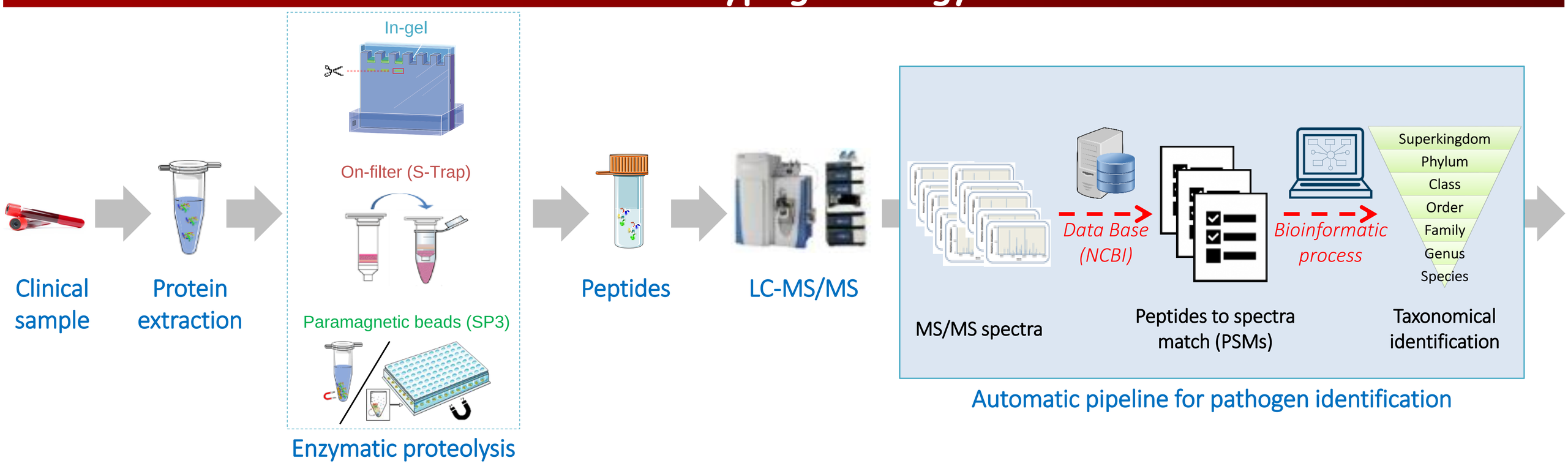
Abstract

Introduction: Quick identification of pathogens is crucial for efficiently fighting against infectious diseases. Mass spectrometry is a powerful and discriminative tool for this. Whole-cell MALDI-TOF is successful for most pathogens but requires a pure sample usually obtained after cultivation. Tandem mass spectrometry proteotyping has been shown to be an interesting alternative for complex samples and could avoid the cultivation step.

Objectives: Here, we present an optimization of the sample preparation for MS/MS proteotyping of any type of microorganisms, as well as an optimization of the data treatment for a fast result.

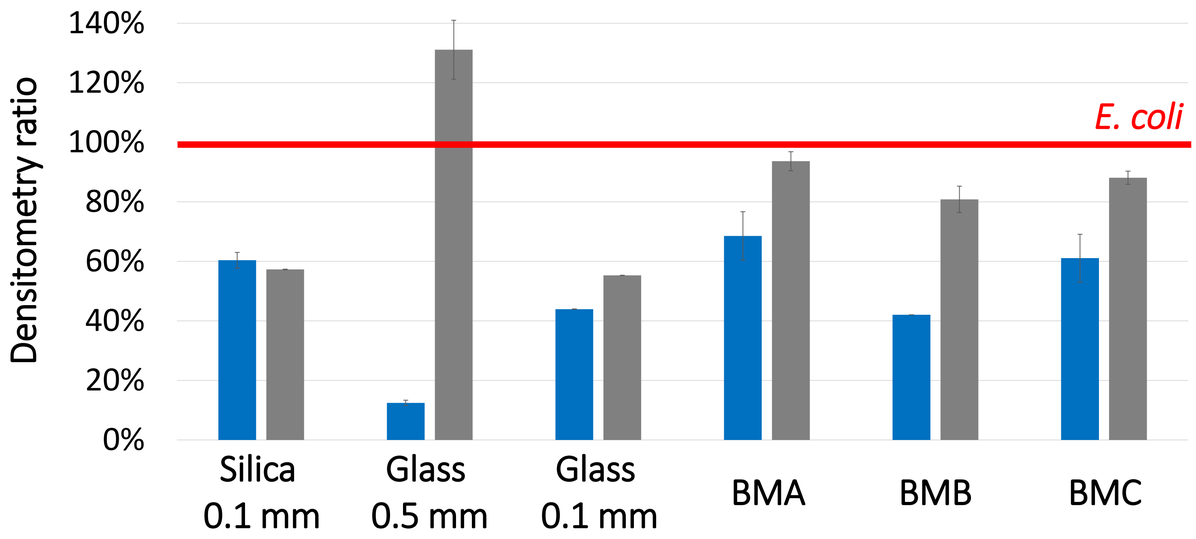
Methods: We proposed a bead-beating extraction of proteins and tested several protocols for fast trypsin proteolysis. A protocol based on magnetic beads was selected and further improved in order to achieve automatization of the sample preparation in 96-well plates. The bioinformatics pipeline for high-throughput MS/MS proteotyping was also optimized. The whole workflow is adapted for the identification of pathogens of clinical interest.

Proteotyping strategy



Optimization of sample preparation

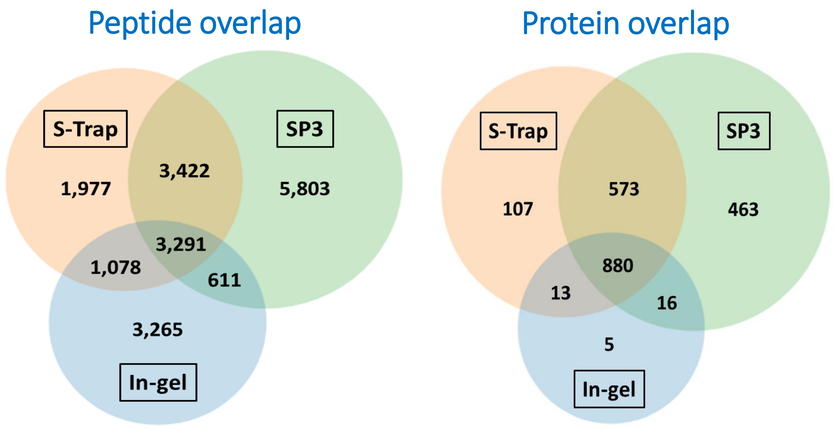
Protein extraction by bead-beating



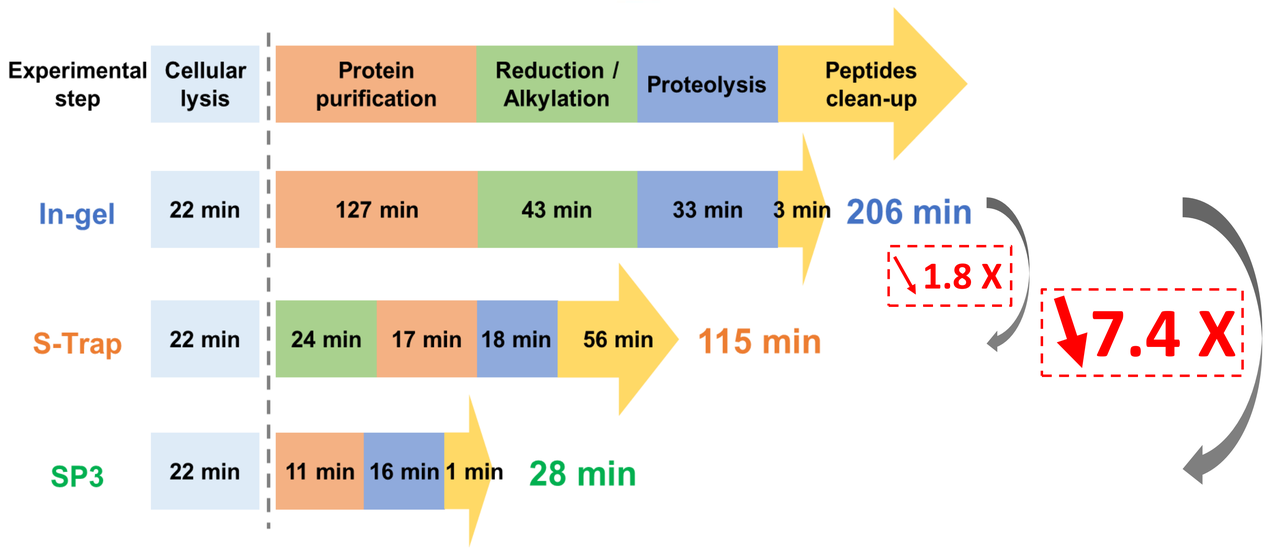
10,000 rpm ; 10 cycles of 30 seconds ; 30 seconds pause.
BMA: 2/3 silica beads 0.1mm + 1/3 glass beads 0.5mm ; **BMB:** 2/3 silica beads 0.1mm + 1/3 glass beads 0.1mm ;
BMC: 1/3 silica beads 0.1mm + 1/3 glass beads 0.5mm + 1/3 glass beads 0.1mm.

→ BMC : better bead mix for prokaryotic and eukaryotic cell lysis.

Comparison of proteolysis methods



SP3 → 68% of peptides and 94% of proteins.



Application for pathogens

Sample	Species-specific peptides	Unique peptides (species level)	PSMs (species level)	Identified species
1	15	3,918	5,607	*Bacillus cereus*
2	35	5,508	7,384	*Pseudomonas aeruginosa*
3	202	6,334	8,521	*Acinetobacter baumannii*
4	115	4,460	6,636	*Klebsiella aerogenes*

Conclusions & perspectives

Conclusions:

- Optimization of protein extraction from diverse microorganisms.
- Faster and better proteolysis using paramagnetic beads approach SP3.
- Bioinformatic software for automatic proteotyping.

→ Pathogen identification in 3h.

Perspectives:

- Optimized proteotyping approach for diagnostic tool in clinical microbiology.
- Application for high-throughput MS/MS proteotyping.

References

Armengaud J. (2016), Current Opinion in Biotechnology. **Grenga** et al. (2019), Clinical Mass Spectrometry, *In press*. **Hayoun** et al. (2019), Frontiers in Microbiology, *In press*.

Acknowledgements

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