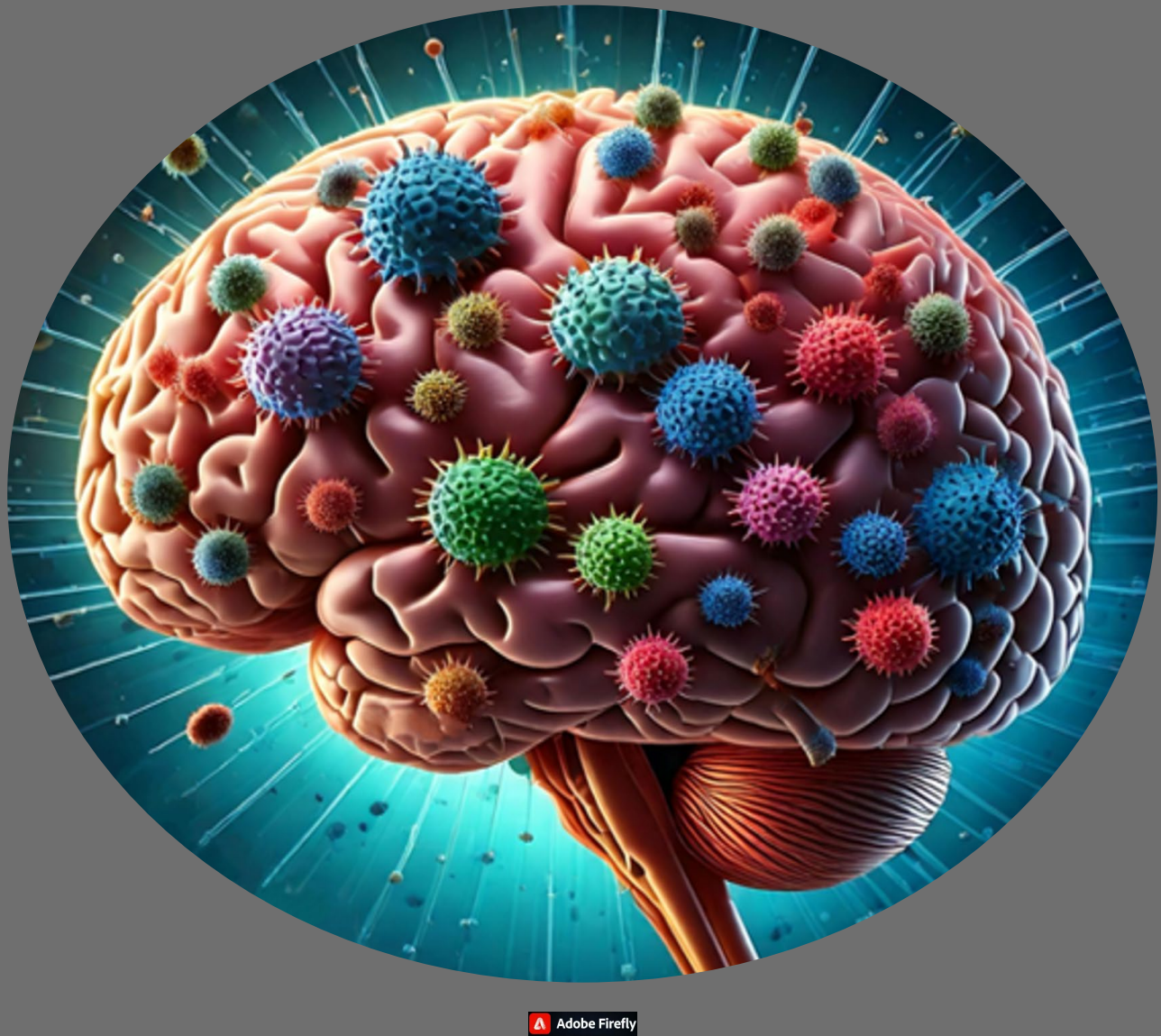


Pathogen-driven Alzheimer's Disease?

High-resolution Microbial Profiling Of Postmortem AD Brain Tissue



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Background

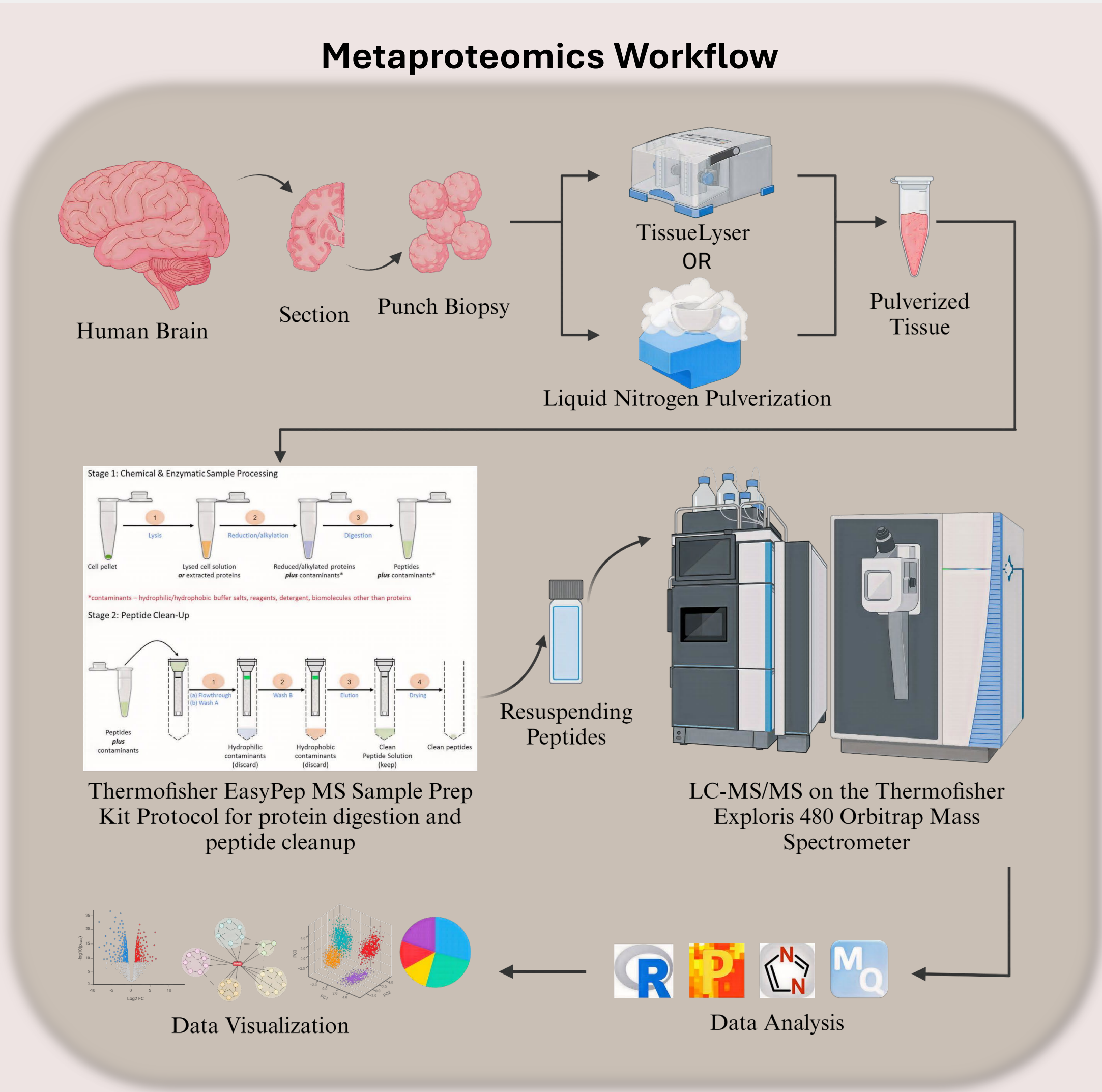
- The infectious hypothesis of Alzheimer’s disease (AD) proposes that microbes may contribute to AD pathology, challenging the assumption of a sterile brain.
- Investigations of postmortem brain have reported bacteria in both AD & controls (e.g. Hu *et. al.*, 2023; Emery *et. al.*, 2022).
- But findings remain inconclusive, possibly due to methodological variability and microbial contamination (Bhattacharya *et. al.*, 2025).
- Metaproteomic profiling offers high sensitivity and specificity yet remains an underexplored alternative; this study addresses this gap.

Aims

- To optimize the processing & analysis techniques for metaproteomic profiling of human post-mortem brain tissue samples.
- To assess if the brain microbiome exists in neuropathologically normal & AD cases using metaproteomic methods.

Methods

- 6 Control & 6 Cases – Medial Temporal Cortex (MTC) – 30mg each
- Zymo Microbial Mock Community used as +ve control & spike-in sample for the limit of detection experiment.
- Multiple other operational negative controls acquired at each step to account for potential contamination.



Results

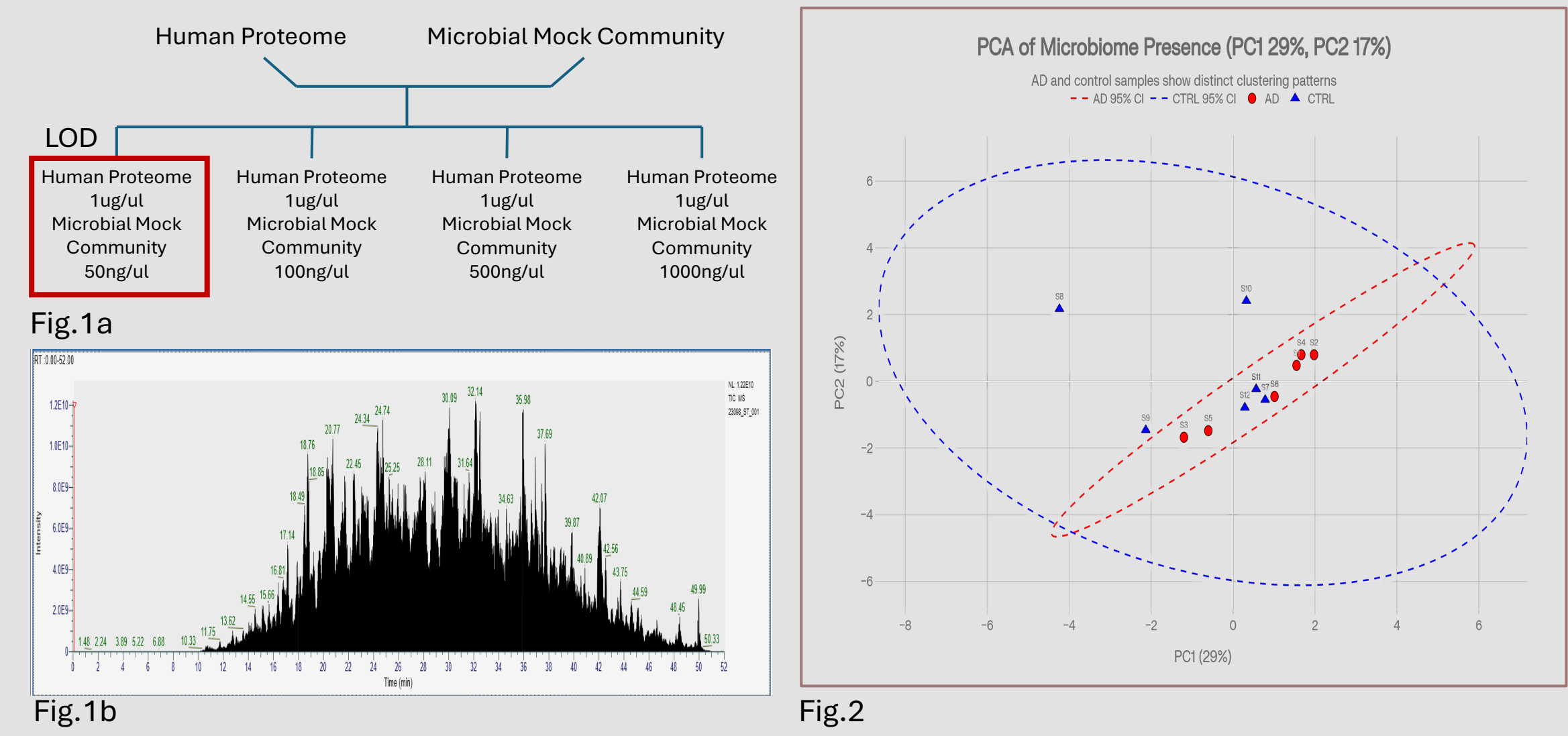


Table 1: List of identified bacterial species from pilot samples.

Control Only (n=13)	AD Only (n=20)	Shared (n=42)
Arthrobacter sp.	Acidothermus cellulolyticus	42 bacterial species including <i>Bartonella quintana</i> , <i>Brucella anthropi</i> , <i>Campylobacter fetus</i> , <i>Rickettsia rickettsii</i> , <i>Salmonella typhimurium</i> , <i>Salmonella paratyphi C</i> , <i>Streptococcus pyogenes</i> , <i>Proteus mirabilis</i> , and <i>Clostridium perfringens</i> , among others.
Borrelia duttonii	Acinetobacter baylyi	
Bradyrhizobium diazoefficiens	Agrobacterium fabrum	
Bradyrhizobium elkanii	Azoarcus sp.	
Clostridium novyi	Bifidobacterium adolescentis	
Corynebacterium diphtheriae	Brucella ovis	
Flavobacterium psychrophilum	Caldanaerobacter subterraneus	
Haemophilus ducreyi	Caldicellulosiruptor bescii	
Mycobacterium sp.	Escherichia coli	
Neisseria meningitidis	Geobacillus kaustophilus	
Petrogala mobilis	Neisseria meningitidis	
Sinorhizobium medicae	Parabacteroides distasonis	
Treponema pallidum	Rhizobium etli	
	Rhodospirillum rubrum	
	Rickettsia massiliae	
	Rickettsia typhi	
	Sodalis glossinidius	
	Solibacter usitatus	
	Thermosipho melanesiensis	
	Xanthomonas campestris	

Fig. 1a: Limit Of Detection (LOD) experiment to find the minimum quantity of microbial peptides identifiable under a constant background of human peptides using mass spectrometry. Fig. 1b: Mass Spectrometry spectra of 1ug human+bacterial peptides. Fig. 2: Principal Component Analysis (PCA) of the two groups. AD samples (red) cluster together on the 'rich & diverse' side, while healthy (blue) scatter everywhere, suggesting AD brains share a unique bacterial fingerprint.

- Limit of detection experiment (Fig. 1a & 1b) – the lowest quantity of bacterial peptides identified was 50ng.
- Bacterial & fungal peptides were detected in all samples (controls & AD) (Fig. 2, Table 1). Peptides from environmental & lab-introduced organisms were detected in nearly all samples and were removed bioinformatically downstream.
- In the AD only list organisms like *Neisseria meningitidis*, *Escherichia coli* (especially K1), & *Rickettsia typhi* have known BBB translocation mechanisms & are involved in infections such as meningitis.
- Many of the identified pathogens have previously reported in the AD hippocampus and cingulate cortex.

Conclusion

- These preliminary results suggest that microbiome proteins can be detected in the human brain beyond that accounted for by contamination alone, implying that the brain is not strictly sterile & may harbour a low-biomass microbiome.
- Integrating high-sensitivity metaproteomics, including contamination checks, & organism-level risk categorisation, provides the methodological framework for future studies to evaluate the infection-AD hypothesis in postmortem brain tissue.
- Future studies could consider using mass-spectrometry-based metaproteomics for its high sensitivity while ensuring that contamination-derived signals are identified and accounted for at least in the analysis stage.

References

- Emery *et. al.* 2022, *Frontiers in Aging Neuroscience*, 14:1026260
- Hu *et. al.*, 2023, *BioRxiv*, 527297
- Bhattacharya *et. al.* 2025, *Current Opinion in Psychiatry*, 38(3):p 252-257

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