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Transcriptomic Profiling of Intestinal Organoids Infected with *Mycobacterium avium* subsp. *paratuberculosis*

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Mycobacterium avium subsp. *paratuberculosis* (MAP) causes Johne's disease in ruminants and is a discussed zoonotic contributor to Crohn's disease, making it a key One Health target. However, epithelial responses to MAP remain poorly understood. We used human ileal organoids to characterize the epithelial transcriptomic response to MAP infection. Organoids were microinjected with MAP or PBS, treated with forskolin, or left untreated, and analyzed by RNA sequencing over 48 hours.

Culture-associated transcriptional drift was the dominant driver of gene expression, with patterns suggesting a progressive loss of metabolic and digestive programs and increased chromatin remodeling activity, likely masking pathogen-specific responses. Although forskolin facilitated microinjection, it transiently activated inflammatory and stress pathways, limiting its suitability as a neutral experimental aid. While MAP infection did not induce significant single-gene expression changes, pathway analyses suggested preservation of enterocyte-associated metabolic programs, indicating that MAP partially counteracts culture-induced drift. This may promote intracellular persistence and contribute to mucosal pathology in both Johne's disease and Crohn's disease.

These findings identify temporal drift and experimental perturbations as major confounders in organoid infection models and provide a framework for improving studies of enteric pathogens at the human-animal interface.

Keywords

Organoid technologies; Omics technologies; *Mycobacterium avium* subsp. *paratuberculosis*; Host-pathogen interactions

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Professional Status of the Speaker

PhD Student

Junior Scientist Status

Yes, I am a Junior Scientist.

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