Exploring environmental reservoirs and microbial interactions of mycobacteria in bovine tuberculosis-affected farms

Nerini Marta, *University of Florence – Biology Department*

Mycobacterium bovis is the causative agent of bovine tuberculosis (bTB), a chronic infectious disease in cattle that leads to substantial economic losses and is subject to rigorous European Union eradication programs. Transmission is believed to occur primarily through aerosol inhalation and the ingestion of contaminated environmental materials. This study aims to investigate the environmental occurrence of mycobacteria, identifying preferred ecological niches and survival microenvironments within farm settings.

Six dairy farms were selected for the study: three with a documented history of bTB outbreaks and three officially tuberculosis-free. For each farm, soil samples were collected from comparable functional areas. Key physico-chemical parameters—including temperature, redox potential (ORP), electrical conductivity, salinity, and pH—were measured *in situ*. Cultivable bacteria were isolated and subjected to competition assays with *Mycobacterium smegmatis* (a non-pathogenic surrogate) to identify antagonistic or synergistic effects. In parallel, total DNA was extracted and analyzed via Full-length 16S rRNA gene sequencing (NGS) to characterize community composition and identify taxa associated with bTB-positive environments.

Multivariate statistical analysis (PERMANOVA) identified pH and ORP as the primary environmental drivers shaping the soil microbial communities. While no antagonistic strains were found in the competition assays, several isolates were identified as strong promoters of *M. smegmatis* growth. Furthermore, a decision tree model identified low *Ornithinimicrobium* abundance combined with high ORP values as a strong predictor of TB positivity in the sampled environments.

Understanding these ecological relationships provides valuable insights into the environmental reservoirs of mycobacteria, supporting the development of improved biosecurity and prevention strategies for bovine tuberculosis.