

1 **Isolating mycobacteriophage for the biocontrol of Johne's disease.**

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8 **Abstract**

9 Johne's disease is a chronic gastroenteritis condition that affects ruminants that results in
10 animal death and major economic losses in the agriculture sector. The etiological agent of
11 Johne's disease, *Mycobacterium avium* sbsp. *paratuberculosis* (MAP), has also been
12 associated with human diseases, such as Alzheimer's, Crohn's and Parkinson's. Several
13 mycobacteriophage-based diagnostic assays have been developed to detect MAP in clinical
14 samples. In an effort to contribute to the available bank of MTU mycobacteriophages, with the
15 additional goal of isolating viruses capable of infecting MAP, a simple screening study of
16 environmental samples was conducted. Mycobacteriophage buffer was mixed with (mainly
17 soil) samples and dense organic material was allowed to settle before the buffer was decanted,
18 filtered and enriched. Traditional plaque- and spot-assays were used to confirm the presence or
19 absence of mycobacteriophage in the enriched samples. The study was successful in isolating
20 a novel Fishburnevirus, Nix22, from leaf litter collected in Phoenix Park, Co. Dublin, that
21 demonstrates mycobactericidal activity against *Mycobacterium smegmatis* mc²155. Several
22 MAP strains are currently being cultivated to explore the host range of this phage. Should
23 Nix22 have activity against MAP, it could be a useful candidate for targeted phage-based
24 diagnostic strategies for Johne's disease. As a consequence of improving diagnostics, early
25 interventions can be implemented, which will greatly improve current control strategies, animal
26 welfare and agricultural profit.