DECLARATION

I declare that this dissertation is my own, unaided work. It is being submitted for the Degree of Master of Science in the University of Witwatersrand, Johannesburg. It has not been submitted before any degree or examination in any other University.

(Signature of Candidate)……

……day of……..2009

ABSTRACT

The *Mycobacteria* are a genus of bacteria which are acid-fast, non-motile, grampositive rods. The genus comprises several species classified into three main groups. Firstly, the major group of these organisms, which poses the biggest threat, is the *M. tuberculosis* complex which can cause tuberculosis-like disease. These include *M. bovis*, *M. africanum* and *M. microti*. Members of the *M. tuberculosis* complex are not found in the environment. The second group is *M. leprae* which is the causative agent of leprosy. The last group constitutes the nontuberculous mycobacteria (NTM), which are all the environmental mycobacteria that can cause various diseases resembling tuberculosis. Due to the importance of environmental mycobacteria, 15 mycobacteria isolates were isolated from environmental samples such as soil, water and drinking water biofilms. After PCR amplification of the *hsp65* gene using genus specific primers *hsp65*, the isolates revealed sequences similarities when compared with the well characterized mycobacteria in the GenBank. Alignment of the nucleotide sequences and homology analysis were done with Clustall. It has been suggested that mycobacteria-associated phages (mycobacteriophages) may make an important contribution to the evolution of pathogenic mycobacteria. Spontaneous induction of phage associated with mycobacteria isolates using overlay and indicator plate methods was not successful to detect the presence of any inducible phage. A phage was isolated from soil samples that was designated the name A22. After purification and characterization. A22 phage was compared morphologically to well characterized L5 phage using electron microscopy. Morphological studies revealed that A22 mycobacteriophage had a non-contractile tail approximately 150 nm long with an isometric head approximately 60 nm, the phage could be assigned to the family *Siphoviridae*, According to these criteria, both of the phages (A22 and L5) belong to the order *Caudovirales* (tailed bacteriophages). Based on PCR amplification of A22 phage DNA using L5 gp71 specific primers and the infection of *M. smegmatis* L5 lysogen, we believe that this novel A22 phage differs from L5 phage.

KEYWORDS: Environmental mycobacteria; Mycobacteriophages; Spontaneous induction

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LIST OF ABBREVIATIONS

A22	Mycobacteriophage A22 isolates
D29	Mycobacteriophage D29
EFM	Epifluorescene microscopy
L5	Mycobacteriophage L5
MAC	*Mycobacterium avium* complex
NTM	Non-tuberculous mycobacteria
OD	Optical density
ORFs	Open reading frames
PCR	Polymerase chain reaction
PEG	Polyethylene Glycol
PFU	Plaque forming Unit
Phage	Bacteriophage
SDS	Sodium dodecyl sulfate
TEM	Transmission electron microscopy
TM4	Mycobacteriophage TM4
ZN	Ziehl-Neelsen