

ISOLATION AND CHARACTERIZATION OF POTENT LYTIC PHAGE SPECIFIC TO STERNE STRAIN OF *BACILLUS ANTHRACIS*

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ABSTRACT

Bioterrorism is a biological attack which involves intentional release of bacteria, viruses in naturally occurring form or a human modified form that sicken or kill people, livestock or crops. *Bacillus anthracis*, is the causative agent of anthrax and is most commonly used agent in a biological attack. Anthrax spores are easily found in nature, can be produce in lab, and can last for a long time in the environment. Infection occurs when the spores, the dormant form of the bacteria, are ingest, inhaled, or come into contact with a lesion on the skin. Some *Bacillus anthracis* strains are antibiotic resistant or not targeted by vaccines. Therefore, to overcome this problem phages specific to the *Bacillus anthracis* species can be isolated as an alternative method to antibiotic treatment. The objective behind this study is isolation, characterization, screening and purification of potent lytic phages specific to the host. The *Bacillus anthracis* (sterne strain) was previously isolated and identified by cultural and biochemical characteristics using Bergey's manual of Determinative Bacteriology. Its morphological and biochemical characters were studied for the identification of host. Isolation and enrichment of phages was carried out which involves mixing environmental samples (source of phage) and specific host strain in enrichment media (Phage broth). Double agar layer plaque assay method was carried out to calculate the phage titer. Potent lytic phages were successfully isolated from the soil sample against *Bacillus anthracis* which will be useful in anthrax therapy or in reducing spore inoculums in combination with vaccines.

Keywords: Bacteriophages, *Bacillus anthracis*, Double layer agar method, MOI, Spot assay method

INTRODUCTION

Bacillus anthracis, the organism which is causative agent of anthrax caused in humans and animals, derives its name from the Greek word for coal, because of its ability to cause black, coal-like cutaneous eschars. *Bacillus anthracis* belongs to the group *Bacillus cereus sensu lato*, is a Gram positive, spore forming, aerobic or facultatively anaerobic and rod shaped organism, which appears in chains.^[1,2] This bacterium exists in two forms, vegetative cells present inside the host and spore form which persist in the soil or environment. This organism has the ability to infect humans by gastrointestinal, cutaneous or respiratory routes. The most common is cutaneous anthrax, which accounts about 90% of all human cases and which is acquired through a lesion on the skin. The other two forms include gastrointestinal anthrax and pulmonary anthrax,

also known as inhalation, anthrax. Cutaneous anthrax infections are the least symptomatic and have mortality rate of around twenty percent. Gastrointestinal anthrax has mortality rate of around sixty percent. Whereas, Inhalation anthrax is the most severe form of anthrax with a near eighty percent mortality rate. Serious clinical development is Meningitis, which may follow any of the three form of anthrax^[3].

The anthrax spores are easy to store, transport, and disseminate and may survive in soil for many decades. This durable spores can be used as a idea biological weapon for mass destruction. Due to this feature, *Bacillus anthracis* was used as bioterrorist weapon against animals in world war I and both animals and man in world war II. Bioterrorism, in the form of letters containing *B. anthracis* spores was used due to which 2 cases of inhalation anthrax and 11 cases of cutaneous anthrax was being identified^[1,4].

