

Short communication

Isolation of Bacteriophage from the Environment and Characterization of Isolates for Potential Control of Food borne Pathogens

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Introduction

Bacteriophages, (phages), have been used for the control of pathogens or in phage therapy for almost 100 years. First discovered by Felix d'Herelle in the early 1900s, phages were originally tested as potential agents for the control of diseases in the field of veterinary medicine (17). Phage therapy in these early studies was limited by the inadequate information available on phages at the time. It was not until several decades later when research was done on temperate/virulent life cycles, the narrowness of phage host range, and appropriate purification methods for phage preparations that more effective clinical testing could be conducted. Despite these early limitations, phage therapy has continued with greater interest in Eastern Europe compared with the rather limited work in the United States (4). Phage control has also been evaluated for on-the-farm application to live animals (2) and compost (10) with some demonstrated success in controlling pathogens at the pre-harvest stage of food production. Phage mediated post-harvest biocontrol of food borne pathogens and spoilage microorganisms has been explored in produce (6, 13, 16), meat (3, 7, 8), and dairy products (5, 9). Given the recent outbreaks of food borne illness associated with fresh produce, and the limited available options for pathogen control in fresh products, phage treatment may represent a viable option for the food industry to reduce the risk of outbreaks in fresh foods. Efforts to utilize phage for pathogen control and therapy are aided by the ubiquity of a diverse population of phages in the environment. Although not generally recognized as a living organism, bacteriophages may be the most abundant element in the micro biome, present at even greater levels than their bacterial hosts (5). Phage can be found at high levels in environments where the host bacteria can be expected to be found replicating, which include water, soil, feces, and food (and at particularly high levels in fermented food). Phages have been detected at levels of up to 107 particles/ml in aquatic environments (18) and 108 viable phage/g of meat product (11). Phage in the environment not only exists in high density, it is also present in great diversity.

Additionally, the composition of the phage community present in a given environment varies with the composition of the bacterial community. A single environmental sample may be expected to yield phages of different species, specific for different bacteria present in the environment (18). Additionally, a single environmental sample may contain multiple phage isolates specific for some of the same bacteria, but with a slight variation in cross-reactivity among different strains of the same bacterial species (10).

Initial studies on the application of bacteriophage for control of pathogens require the identification and isolation of an appropriate phage from the multitude of phages that exist in the environment. Phages specific for pathogenic *E. coli* and *Salmonella* have often been isolated from sewage and manure samples (10, 12) and following isolation, characterization of the candidate isolates is necessary to determine the most promising candidate for pathogen control. Spectrum testing is used to evaluate the host range of phage isolates which is generally expected to be fairly narrow, and often limited to specific strains of the target microorganism (4, 5). Since effective control of the target would depend on the sensitivity of multiple strains to phage infection to prevent outbreaks from a wide range of pathogens, cocktails of phages have been used to address the limitation of narrow host range for any individual phage isolate (10, 13, 15).

Assuring the lytic nature of phage candidates is also an important parameter to characterize as growth of the pathogen would likely not be inhibited by lysogenic phages (15). The objective of this study was to characterize phage isolates from manure and sewage samples to determine the most promising candidate for use in food challenge studies.

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