

## Isolation of Bacteriophage from Mentha species in Riyadh, Saudi Arabia

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Investigation of the microbial pollution in different *Mentha* species available commercially in local markets of Riyadh, Saudi Arabia. *Mentha* samples were collected from the local markets of Riyadh, Saudi Arabia. *Mentha* juices were prepared from both stems and leaves. Detection of bacteriophages was done as indicated in the materials and methods. A wide spectrum of bacteriophages was found in the *Mentha* samples by using standard strains of *E. coli*. In addition, results indicate the presence of different bacteriophages since different plaques were seen in the plates. Therefore economical plants like *Mentha* were polluted by a variety sources of pollutants. This criterion, which is linked to the high or low number of microbes, can be affected according to increasing distance from polluting source. Thus, bacteriophages could be a good indicator to detect the bacterial pollution when using sewage waters to irrigate plants like *Mentha* species. This report shows that sewage waters were used to irrigate some economical plants including *Mentha* species. The significance of this study comes from the importance of selecting an easy and quick method of detecting bacterial pollution in plants. The method detects bacteriophages in *Mentha* juice by their reaction to the host bacteria (*E. coli*). This study draw attention to the three sources of microbial pollution i.e. sewage waters, human and animals wastes.

**Key word:** *Mentha* species, Bacteriophage, *E. coli*, Sewage, Economical plants.

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Bacteriophages (phages) are viruses that infect prokaryotes. Like all viruses, phages are obligate intracellular parasites, which have no intrinsic metabolism and require the metabolic machinery of the host cell to support their reproduction. Contact with the host cell occurs by passive diffusion. Phage adsorption and entry are mediated by specific receptors such as carbohydrates, proteins and lipopolysaccharides on the surface of the host cell<sup>16</sup>. The specificity of interaction between phage attachment structures and host-cell surface receptors influences the bacterial host range. Host range is generally assumed to be narrow for aquatic phages<sup>3</sup>. Virtually

any type of cell is susceptible to virus infection; viruses cause disease in plants and animals, and can also infect prokaryotes and unicellular eukaryotes<sup>7</sup>. Viruses that infect prokaryotes are known as bacteriophages, or phages, because when they were first discovered, they appeared to eat bacterial cells, generating a clearing, or plaque, on a lawn of susceptible bacteria<sup>13</sup>. Therefore, bacteriophages use as indicators of contamination in food .

Microbial contamination throughout the world is increasing, and therefore more attention is being focused on the evaluation of the associated health hazards and their effective control. In this connexion, bacteriological indicators of food quality have proved to be unsatisfactory in respect of bacterial contamination<sup>2</sup>.

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*Mentha spicata* L. (spearmint) , is a creeping rhizomatous, glabrous and perennial herb with a strong aromatic odor. The oil of *M. spicata* is rich in carvone and presents a characteristic spearmint odor<sup>12</sup>. The fresh and dried plants and their essential oils are widely used in food, cosmetic, confectionary, chewing gum, toothpaste and pharmaceutical industries<sup>15</sup>. It is locally known as “ mint”, that is the focus of this study.

Spearmint herb and its volatile oil are used as flavouring agents for many kinds of food products and beverages, carminative, mouth preparations, gargles, tooth pastes and pharmaceuticals. Japanese mint is cultivated in India for its menthol rich essential oil used in medicine, cosmetics food as flavor industry. Peppermint has been used extensively for a variety of complaints, including sore throat, diarrhea, tooth aches, cramp and indigestion. The oil is used as flavouring agent in foods and pharmaceuticals especially in massage lotions for aching muscles and rheumatic joints. Aroma therapists recommend a footbath of diluted peppermint oil for tired feet. It is mildly anesthetic, giving the cooling, numbing sensation experienced when smelling or tasting<sup>18,14</sup>. also regardless of the production system used, are grown in environments that have a wide range of accidental or intentional inputs that are potential sources of microbial food borne hazards and may lead to contaminated produce<sup>4,6</sup>. The major potential inputs identified were wildlife, livestock, human activity and wastes, water, soil and soil amendments, seeds and plant stocks. Other inputs identified that may affect the risk of contamination were climate and flooding, topographical features of growing fields, and prior use of the growing field land. contamination risk of leafy vegetables and herbs risk . Faecal waste, urine and hair from live animals and carcasses of dead animals in the field may directly contaminate produce while growing in the field. In addition, human waste may be a source of direct contamination if deposited in the growing field. Alternatively, environmental contamination with pathogens from these sources may be transferred indirectly to produce via contaminated water, insects, workers, or fomites such as dust, tools and equipment.

These studies emphasize the importance of using good quality irrigation water for ready-to-eat crops. Evidence that pathogens present in

irrigation water can contaminate not only fruit and vegetables but can also cause disease in humans is found in greater incidences of disease observed in populations practicing wastewater irrigation in which the wastewater receives little or no treatment before use. foodborne pathogenic are among the most common infections worldwide<sup>5,17</sup>. Various epidemiological studies indicated that the prevalence of microbial contamination was high especially in developing countries, although in many of these, the environmental risk factors have not been clearly elucidated.

In an effort to find suitable virus indicators, bacteriophages have often been studied , but further research is required . The purpose of the present field study was to compare levels of bacterial contamination through the recovery of bacteriophages in different types of *Mentha* sp. sold in the local markets in Riyadh city, Saudi Arabia.

## MATERIAL AND METHODS

### Sample collection and processing

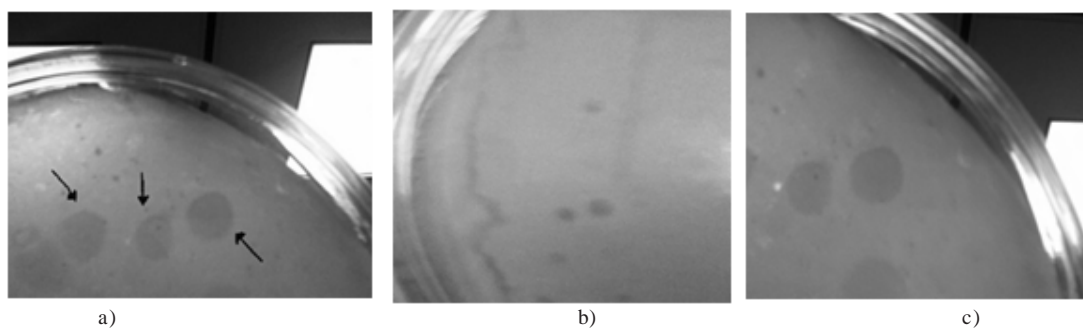
This study includes isolation of Bacteriophages from Plant samples . The Plant is *Mentha* species that was collected from local markets of Riyadh, Saudi Arabia. The part of plant stems and leaves of *Mentha* samples. Were washed separately with tap water followed by sterile distilled water. Approximately 300g, of fresh stems, leaves of *Mentha* samples. Juices were prepared separately by juicer machine, and were clarified by filtration. Then collected it in conical flask. The final volume of 500ml. The juice samples were clarified by filtration through Millipore filters 0.45  $\mu\text{m}$  then 0.22  $\mu\text{m}$ . The filtrate was saved at 4°C further work<sup>8</sup>. *Mentha* samples juice was the source for screening phages capable of lysing *E. coli* by the method described previously for isolating *E. coli* phages<sup>9</sup>. Phage strains were purified by repeatedly plating and picking individual plaques and were concentrated by plate lysate and polyethylene glycol 6000-NaCl centrifugation.

## RESULTS

Bacteriophages isolated by plaque assay using the double agar overlay. This was evident from the clear zones (plaques) on bacterial lawns

formed after hours of incubation of agar plates. Differences in plaque morphology, clear or turbid and sizes were observed. Variation in plaque morphology indicated the presence of several strains of phages because this feature is specific for different phages. Plaques of different sizes were obtained from plates on which different bacterial hosts were grown. This is indicated in (Figure 1. A, B, C) below. Plaques formed on some of the bacteria were very distinct in that they were tiny in size, turbid and diameters were less than 1mm (Fig. 1 B). Bacteriophages were successfully isolated from *Mentha* species. The lytic activity of the phages

in the whole sample is shown in (figure 1. A, C and B) indicates the degree of bacterial lysis by the specific phage isolates. Phage typing refers to the use of phages to differentiate between strains of bacteria. The absence or presence of receptors determines whether the virus is able to bind to the potential bacterial host. This technique has been successfully employed in bacterial species such as *E.coli*. The phage typing would also be particularly interesting to investigate with the isolates from the sewage samples whose host range was broad compared to some samples, this has also been seen in similar studies<sup>11</sup>.



**Fig. 1.** A, B and C. Plaques formed on soft-agar overlay. An enrichment sample containing plaque-forming activity was diluted, and 1 ml of each dilution was plated with *E. coli* cells by the soft-agar overlay technique



**Fig. 2.** D and E. Isolation of Bacteria from *Mentha* samples

The age of the host bacterial culture affects the ability of the filtration method to detect the phage (Fig. 2. D and E), broth solution of *E. coli* was prepared and incubated at 37 C for up to 12 hr prior to use in the filtration method before any resultant decrease in titer was observed, 1 to 2-hr bacterial growth period was used in all experiments to keep the overall time requirements to a minimum. The optimal quantity of bacteria in a filtered sample was about  $10^9$  to  $10^{10}$  bacteria.

## DISCUSSION

The results obtained here also demonstrate the ease at which bacteriophage can be detected from fresh produce samples. Even if the bacteriophage is present in low levels on the produce, the enrichment step of the detection protocol allows of cross-contamination. The methods used are quick, require minimum lab equipment, and are easy to perform, this study

draws attention to the threat of bacteria as a risk to public health when they are present in food. Pathogenic bacteria require special attention because they behave differently, and because currently used control measures typically either have not been validated and there is not a good understanding of their efficacy towards bacterial contamination in plants, or are not effective in controlling bacterial<sup>10</sup>. When bacterial and phage growth are not inhibited and when there are sufficient but not excessive amounts of bacteria on the filter, the filtration method can be used for the detection and enumeration of the phage. The results shown in Fig. 1 are similar to those observed by<sup>1</sup>, who found that the physiological state of the host bacteria will significantly affect resultant numbers of plaques formed. The bacteriophages are used to detection of Microbial contamination depends on different factors including soil conditions that could be the reservoir of foodborne pathogens as *Bacillus cereus* or water for irrigation. The final verdict on whether the filtration method and the coliphage can be used to measure the virological quality of water will only come after investigation of the relationship of coliphage to coliform organisms and specific enteric viruses in polluted and potable water.

The chopping/slicing/preparing of the product allows for more opportunities for handling and possibly more opportunities for cross-contamination. This has also been seen in similar studies<sup>2</sup>. Selection of phages for detailed investigation to select a group of phages that were representative of the phages isolated, phage-host cross-reaction tests were performed with the phages and bacterial isolates obtained from Mint.

This study draws attention to the threat of bacteria as a risk to public health when they are present in food. Pathogenic bacteria require special attention because they behave differently, and because currently used control measures typically either have not been validated and there is not a good understanding of their efficacy towards bacterial contamination in plants, or are not effective in controlling bacterial contamination. Therefore, this study draws attention to the three major routes for bacterial contamination in *Mentha*. Human sewage and faces, infected food handlers, and animals. This report indicate the use of sewage water to irrigate those plants, like *Mentha*.

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