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Characterization of Bacteriophages as Indicators of Bacterial Contamination in Marketed Leafy Vegetables from Riyadh, Saudi Arabia

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This paper presents the results of the evaluation of bacteriophage detection as a Rapid Indicator of contamination of leafy vegetables in Riyadh, Saudi Arabia. In this study analyzed a variety of leafy vegetables and collected randomly from local markets. The types of equipment generally available for microbiological analysis, to detect the presence of phage in supernatant, using the plaque assay methods. These results are observed highest rate of bacterial contamination from the packaged salad and were successfully isolated from vegetables juice. The highest rate of bacteriophage was detected during the spring and lowest rate during the winter. Plaques formed diameters were less than (1mm).This study indicates the likely bacterial contamination of plants irrigated with sewage water using bacteriophage as indicator.

Key word: Leafy vegetable, Bacterophage, Contamination, Riyadh, Saudi Arabia.

Outbreaks of illness due to human enteric pathogenic bacteria via fresh vegetables warrant intensive research on changing strategies of these bacteria in alterning their hosts for survival. The systemic infection of human pathogenic bacteria in plants and the plant growth stage at which they establish endophytic relationship is poorly understood. Green leafy vegetables are important part of daily diet invarious parts of the world. The present study was carried out to evaluate some of the leafy vegetable plants sold in local markets for human consumption to check whether they harbor foodborne pathogens. Since leafy vegetables are major vegetables consumed in the form of unprocessed salads in Saudi Arabia, our study aimed at determination of detection of bacterial contamination in leafy vegetables from local markets Riyadh city.

During our present age there is an increasing demand for water, and throughout the world, and in the Kingdom of Saudi Arabia in particular, sewage water starts playing a crucial role in the management of water resource as an alternative for drinking water in agriculture. Thus the treated sewage water can be implemented in a diversity of activities and the drinking water can be directed towards household needs. Fresh vegetables are regarded as important part of a healthy diet. In many countries such leafy plants are eaten raw or lightly cooked to preserve taste and this practice may also favour the likelihood of foodborne pathogens[9,12].

Current agricultural production in developed countries takes a special introduction

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care about food safety and quality. In the last few years in most countries, as in Saudi Arabia, the consumer's awareness of food safety issue is also increased. With an increasing demand for "safe food" in perception of majority of consumers this means that vegetables or other crops have to be produced in "safe (clean) environment"[4]. Therefore, the interest of growers is to have good agricultural practices and to avoid any contamination of their products. Their interest is also that their products are certified according to standards. This helped them to improve their export in more and more competitive food markets. Microbiological criteria of food quality are considered of great importance due to the significance of public health and safety. Contamination of fruits and vegetables can arise as a result of presence of microorganisms as bacteria, viruses and parasites[7].

Leafy vegetables were accorded the highest priority based on the ranking criteria. The available data varied by completeness, but the there was sufficient information to indicate that, from a global perspective. leafy vegetables currently presented the greatest concern in terms of microbiological hazards. Leafy vegetables are grown and exported in large volume, have been associated with multiple outbreaks with high numbers of illnesses in at least three regions of the world, and are grown and processed in diverse and complex ways, ranging from in-field packing to pre-cut and bagged product. Such post-harvest activities contribute to the possibility of amplification of foodborne pathogens[9].

Consumption of fresh vegetable produce in the form of salads has increased in recent years and a large number of minimally processed fresh-cut vegetables are available in supermarkets, food service facilities and are also prepared household. Unfortunately, the increase in the consumption of raw vegetables has resulted in the increase in frequency of outbreaks of illness[4]. Experimental studies examining *Salmonella spp* have been isolated from several raw vegetables from many countries[11]. Microbial contamination depends on different factors including soil conditions that could be the reservoir of foodborne pathogens as Bacillus cereus or water for irrigation. Water may be source of different pathogenic as *Salmonella spp*., or *E. coli* O157:H7[17].

They could be transferred from water to the fruits or vegetables[5]. Organic fertilizers (sewage sludge, animal manure, compost) may also be potential risk for contamination of fruits or vegetable. Fruits and vegetables could also be contaminated during harvest (due to the lack of sanitary facilities to workers or dirty storage capacity) or post harvest treatment (handling, storage and transportation)[10] . Therefore, to minimize the risk of infection or intoxication associated with raw fruits and vegetables, potential sources of contamination from the environment should be identified and specific measures and interventions to prevent and/or minimize the risk of contamination should be considered and correctly implemented[16]. Irrigation water was implicated as a source of *E. coli* detected on cabbage seedlings. irrigated with sewage-contaminated water because none were found on seedlings in an adjacent field irrigated with municipal water[11]. Experimental studies examining contamination of lettuce with *E. coli* O157:H7 demonstrated that irrigation water could effectively transmit *E. coli* to lettuce plants. Contact with soil was not required for the lettuce plants to become contaminated, suggesting that the bacteria were taken up through the root system. In addition, the *E. coli* was visualized throughout the lettuce tissues, including areas that would be inaccessible to postharvest washing[15]. 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[12,4]. 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[2,9].

The increasing consumption of leafy vegetable plants by many people in Saudi Arabia has urged undertaking this study to investigate the microbial contamination in leafy vegetable from local markets Riyadh city.

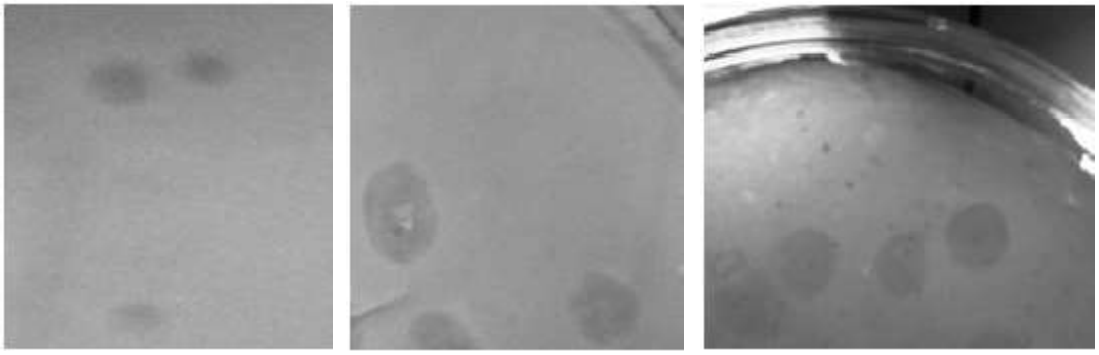
MATERIALS AND METHODS

Samples of four groups which are produced locally were collected from local grocery stores. Radish, Green Onions, Lettuce, and Packaged Salad Samples were freshly purchased and processed. The part of plant leaves were washed separately with tap water followed by sterile distilled water. Juices were prepared separately by juicer machine, and were clarified by filtration. Then collected it in conical flask. The final volume of 500ml of the juice samples were clarified by filtration through Millipore filters 0.45 µm them 0.22 µm. the filtrate was saved at 4°C for further work. Culture media YT broth, Standard strain obtained from Central Laboratory, Department of Botany and Microbiology, King Saud University. For colony development, cultures were incubated at 73°C then stored at -80 ^{O}C and when necessary, maintained on nutrient agar slants at 4 ^{O}C[8].

Isolation of Bacteriophage: Leafy vegetables samples were collected; centrifuged at (10,000 rpm for 10 min at 4 °C) and supernatants were filter sterilized (0.45 µm pore size Millipore filter). 50 ml filtered samples and 50 ml sterile nutrient broth were mixed with 5.0 ml overnight culture of and incubated at 37 °C overnight. The bacteria were removed by centrifugation; supernatant was filter sterilized and checked for the presence of phages. To detect the presence of phages in supernatant, spot test was carried out . The phage titer was determined by plaque assay by employing double agar overlay technique. Briefly, each of the phage suspension was serially diluted. 100 µl diluted phage and 100 µl host bacterium (108 CFU/ml) were mixed with 5.0 ml molten soft agar (0.75 % agar, w/v) and poured quickly on top of the solidified nutrient agar plate. The numbers of plaques were counted after incubating the plates overnight at 37 °C[1,15,8].

RESULTS

Lytic bacteriophages were successfully 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 (Fig. 1. A, B and C) below. Plaques formed on some of the bacteria were very distinct in that they were tiny in size and turbid (Fig. 1. A and Fig. 1. C). Diameters were less than (1mm). The results are shown of samples collected from leafy vegetables examined, were positive for bacteriophage. The highest rate of bacterial contamination was reported from the packaged salad and only samples collected from cabbage did not reveal any bacteriophages, and were successfully isolated from leafy vegetables juice. The lytic activity of the phages in the whole sample is shown in (Fig. 1. A; Fig. 1. B), (Fig. 1. C) indicates the degree of bacterial lysis by the specific phage isolates. Phage typing refers to the use of phages to differentiate between strains of



A B C

Fig. 1(A,B,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

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. It is not surprising that packaged salad was among the group with the highest detection rate. One possible reason for this is packaged salad is handled more than other groups of produce. The highest rate of bacteriophage was detected during the spring while the lowest rate was detected during the winter.

DISCUSSION

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[3]. In another study[8], carrot samples were tested along various steps in harvesting for the presence of bacteriophage. The field, the truck on the way to processing, and the processing shed were all tested. The processing shed, where workers repeatedly handled the carrots, had the highest number of positive samples compared to the other two locations. Other bacteria were shown to be present, but no *E. coli* was found. As confirmed by *Biomeriuex API 20 E* strips, bacteria such as *Klebsiella, Citrobacter*, and *Enterobacter* were found on the samples[13].

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 for replication. 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 contamination[14].

CONCLUSION

This paper presents the evaluate some of the leafy vegetable plants sold in local markets for human contamination using evaluation of bacteriophage detection as a Rapid Indicator of contamination of leafy vegetables with sewage water. The results of the study indicated that the procedure is a rapid, inexpensive method for enumerating bacteriophages. The coliphage levels can then be related to coliforms to give a quick indication of the number of coliform bacteria in potentially contaminated samples. These results are observed differences in the recovery of bacteriophages from foods as a function of sample suspension technique may be related to morphological or size differences between these phages. Additionally, indicates the likely bacterial contamination of plants irrigated with sewage water using bacteriophage as indicator of the presence of bacteria in a variety of leafy vegetables sold in the local markets in Riyadh city, Saudi Arabia.

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