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Bacterial Contamination in Cucumber (*Cucumis sativus*): Evidence from Saudi Arabia

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The use of waste water in agriculture and aquaculture was sensitive subject. Microbial contaminants in cucumber from the use of sewage water, the air during harvest and transportation stayed in cucumber. Samples were collected from Riyadh outside markets and from supermarkets, washed with tap water, 99% ethanol and sterilized distilled water. Cucumber endocarp sliced in circles and exocarp peels were placed on Mueller Hinton agar, incubated at 37°C overnight. The isolates from the peeled exocarp were Forty one sub cultured and colonies were gram stained. Bacterial colonies were studied morphologically, biochemically and antibiotic susceptibility. The colonies from outside cucumber markets and supermarkets in Riyadh were compared. Sections of the isolated colonies were investigated using scanning electron microscopy. Our study was aimed to identify some pathogenic bacteria in cucumber and provide advice to the consumers in limiting the transmission of infection and consequently help to reduce the risk of infection from eating contaminated cucumber.

Key words: Contamination, Bacteria, Cucumber peels, Riyadh market, Saudi Arabia, gram staining, SEM, Antibiotic susceptibility.

Cucumber is an economically important crop as well as a model system for sex determination studies and plant vascular biology.[1] Some diseases are caused by toxins produced by microorganism in the food- before being consumed and these toxins may damage human cells consequently the DNA. However, there is one of the most often used methods to monitor DNA repair in vivo called host cell reactivation assay.[2] Consumption of vegetables represents a route of direct human exposure to bacteria found in soil.[3] Drought is a worldwide phenomenon and is a major production constraint, reducing crop yields.[4] The emergence of multidrug-resistant (MDR) strains of *Mycobacterium tuberculosis* is one of the most critical issues facing tuberculosis researchers and clinicians today. Patients infected with drug resistant strains are less likely to be cured, and their treatment is more toxic and expensive than the treatment for patients infected with susceptible organisms.[5] Emergence of multidrug-resistant Mycobacterium tuberculosis strains and their global dissemination necessitate development, evaluation and comparison of the rapid molecular tests that target genetic determinants of bacterial drug resistance[6] The present study evaluated the complement of bacteria which are resistant to some antibiotics on vegetables often eaten raw.

Breidt F and coworkers studied and reported that Spoilage fermentations can result in significant economic loss for industrial producers and their data indicated that Gram-negative anaerobic bacteria supersede Gram-positive Fermincutes species after the pH rises from around 3.2 to pH 5, and propionic and butyric acids are

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produced.[7] A novel strain, designated J221(T), was isolated from the intestine of a sea cucumber, *Apostichopus japonicus*, collected from earthen ponds in Qingdao, China. The strain was Gram-negative, oxidase-positive, aerobic, rod-shaped and motile by means of one to several polar flagella. Growth of strain J221(T) was observed at temperatures between 10 and 40 °C with optimum growth between 25 and 28 °C. The pH range for growth was 5.0-9.0 with optimum growth at pH 7.5-8.0.[8] The National Food Surveillance System in Japan was formed in 1998 to monitor the contamination of retail foods with bacterial pathogens. Approximately 2000-3000 samples were tested annually, and the data from food categories that had more than 400 samples collected during 1998-2008 were analysed and the prevalence of Campylobacter jejuni/coli was 13.3% and 20.9% in chicken for raw consumption respectively. Salmonella was detected in cucumber, lettuce, sprout and tomato samples at a frequency of around 0.1-0.2%.[9] Sample preparation methods (pummeling, pulsifying, sonication, and shaking by hand) were compared for achieving maximum recovery of foodborne pathogens from iceberg lettuce, perilla leaves, cucumber, green pepper, and cherry tomato.[10]

There was no coherent corresponding increase in the abundance of antibiotic-resistant bacteria enumerated from any vegetable grown in manure-fertilized soil. Numerous antibiotic resistance determinants were detected in DNA extracted from vegetables grown in unmanured soil. A smaller number of determinants were additionally detected on vegetables grown only in manured and not in unmannered soil.[11] Some fresh minimally processed fruits and vegetables when eaten raw are sources of most food borne disease out breaks world wide. Cucumber (*Cucumis sativus*) is sold along major streets and markets in Saudi Arabia.[12] Strain JC164 (T) was isolated from a water sample from a rice field at Jamdih, Mau, and Uttar Pradesh, India. Colonies of strain JC164 (T) were brown-yellow and cells were Gram-stain-negative. Catalase, oxidase and amylase were present.[13.] Understanding the contamination of food crops by human pathogens and advising better management options to limit human infections is more important issue in developing and developed countries. Cucumber (*Apostichopus japonicus*) culture in China, and has been the subject of multiple studies by fishery scientists[14].

RESULTS

Samples were collected from Riyadh outside markets and from supermarkets, washed with tap water, 99% ethanol and sterilized distilled water. Cucumber endocarp sliced in circles and exocarp peels were placed on nutrient agar and incubated at 37^0C overnight. After overnight of incubation plated peels with exocarps had colonies while endocarps sliced in circles had no bacterial growth see Fig.1. Subculture colonies were smeared on Mueller Hinton agar and antibiotic discs of Polymyxin B (PB) 300 units, Neomycin (NE) 30

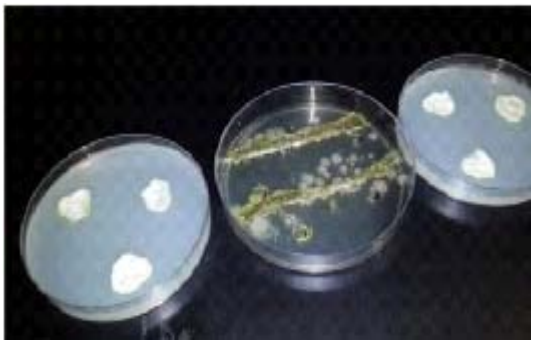

Fig. 1. From left is endocarp and in the middle is exocarp the last to the right is endocarp of cucumber after incubation overnight at 37°C. Exocarps are showing bacterial growth whille endocarps are not

Fig. 2. Colonies of the isolates were susceptible to Neomyein (NE) 30 micro grams, Gentamycin (Gm)10 micro gram, Penicilllin G (PG) 10 Units Chloramphenicol (C) 30 micro grams, Polymyxin B (PB) 300 units

micro grams, Gentamycin (GM) 10 micro grams, Penicillin G (PG) 10 Units, Tetracycline (T) 30 micro grams, Bacitracin (BA) 10 units, Chloramphenicol (C) 30 micro grams and Cotrimoxazole (TS) 25 micro gram were placed on and incubated at 37^0C overnight. The isolates were susceptible to Neomycin (NE) 30 micro grams, Gentamycin (GM) 10 micro grams, Penicillin G (PG) 10 Units Chloramphenicol (C) 30 micro grams, Polymyxin B (PB) 300 units respectively see Fig.2. The isolates were also susceptible to Novobicin disk. See fig.3. Colonies of the isolates after gram staining showed pink to red in color indicating that the isolates are gram negative see Fig.4. Results of scanning electron microscope of the isolates indicated the presence of *Escherichia coli* and *Streptococci* see fig. 5.

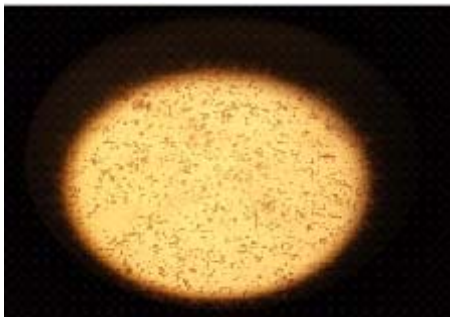

Fig. 3. Colonies of the isolates after gram staining showed rode shaped gram negative bacteria

Fig. 4. The isolates are showing susceptibility to Novobiocin disk 30 micro grams per unit

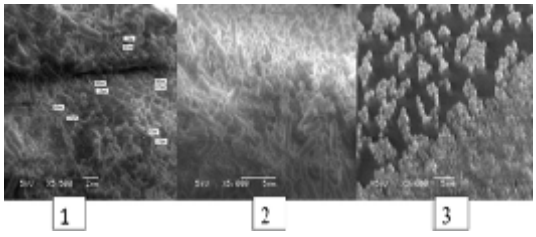

Fig. 5. Result of scanning microscopy of the isolates from left (1 & 2) indicates Escherichia coli and to the right (3) streptococci

Fungi and bacteria were isolated from surface disinfected leaf tissues of several citrus rootstocks. The principal bacterial species isolated were *Alcaligenes* sp., *Bacillus* spp. (including *B. cereus*, *B. lentus*, *B. megaterium*, *B. pumilus*, and *B. subtilis*), *Burkholderia cepacia*, *Curtobacterium flaccumfaciens*, *Enterobacter cloacae*, *Methylobacterium extorquens*, and *Pantoea agglomerans*, with *P. agglomerans* and *B. pumilus* being the most frequently isolated species.[15]

The present study evaluated the complement of bacteria resistant to some antibiotics on endocarp cucumber sliced in circles and peels that had been placed on nutrient agar, incubated at 37°C overnight. The isolates were sub-cultured, colonies were counted and gram stained. Bacterial colonies were morphologically, biochemically studied and the comparison between cucumber from outside markets and supermarkets in terms of colony number indicated that bacterial colonies of cucumber from outside markets were higher than that from supermarkets. Plasmids are main cause of antibiotic resistance. Marti R and coworker extracted DNA from soil and vegetables and evaluated by PCR for the presence of 46 gene targets associated with plasmid incompatibility groups, integrons, or antibiotic resistance genes.[11]

However, the detection of some determinants on vegetables grown only in freshly manured soil reinforces the advisability of pretreating manure through composting or other stabilization processes or mandating offset times between manuring and harvesting vegetables for human consumption.[11] On the other hand Subhash Y. and his colleagues investigated water samples from rice field in India isolated Strain JC164 (T). They reported that colonies of the isolated strains JC164 (T) were brown-yellow and cells were Gram-stain-negative. Catalase, oxidase and amylase were present[11] Characterization of the spoilage microbiota is an important first step in efforts to prevent cucumber fermentation spoilage.[13] In vegetables and fruit, Salmonella was detected in cucumber, lettuce, sprout and tomato samples

at a frequency of around 0.1-0.2%.[13] Sample preparation method for the recovery of foodborne pathogens from freshly produced food is paramount important. Kim SR et al compared sample preparation methods (pummeling, pulsifying, sonication, and shaking by hand) for achieving maximum recovery of foodborne pathogens from iceberg lettuce, perilla leaves, cucumber, green pepper, and cherry tomato.[10] Du ZJ and other researchers reported that they isolated a novel strain, designated J221 (T) from the intestine of a sea cucumbe[r. 8] In the present study we aimed to identify some pathogens in cucumber and their antibiotic susceptibility and provide advice to the consumers about the hazards so that transmission of infection can be limited and consequently help to reduce the risk of infection from contaminated cucumber.

CONCLUSION

Five cucumber samples from five outside cucumber markets and five from super markets were collected then were brought to the laboratory. All probable sources of laboratory recontamination, hand, knife, peeler and the working bench were disinfected with 70% ethanol. Samples were washed throw with tap water peeled the exocarp and the endocarp was sliced in circles, both endocarp and exocarp were washed with 99 % of ethanol for ten minutes and rewashed in sterile distilled water for 30 minutes, all of the working processes were curried near Bunsen burner. Cucumber peels and small sliced circles of exocarp were placed on nutrient agar incubated at 37^0C overnight. Colonies were sub cultured on nutrient agar and gram stained. Antibiotic suitability of the isolates was carried by using Mueller Hinton agar and disk antibiotics. Subculture colonies were smeared on Mueller Hinton agar and antibiotic discs of Polymyxin B (PB) 300 units, Neomycin (NE) 30 micro grams, Gentamycin (GM) 10 micro grams, Penicillin G

(PG) 10 Units, Tetracycline (T) 30 micro grams, Bacitracin (BA) 10 units, Chloramphenicol (C) 30 micro grams and Cotrimoxazole (TS) 25 micro gram were placed on and incubated at 37^0C overnight. Bacterial colonies were gram stained morphologically, biochemically studied and compared with colonies from outside cucumber markets and supermarkets in Riyadh. Section and whole colony of the isolates were investigated and compared by using scanning electron microscopy.

In the present study we aimed to identify some pathogens in cucumber and their antibiotic susceptibility and provide advice to the consumers about the hazards so that transmission of infection can be limited and consequently help to reduce the risk of infection from contaminated cucumber.

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