Bacterial Cell Surface Charge, Attachment and Decontamination on Melon Rind Surfaces

Dike O. Ukuku Ph.D.

FSIT-ERRC-ARS-USDA

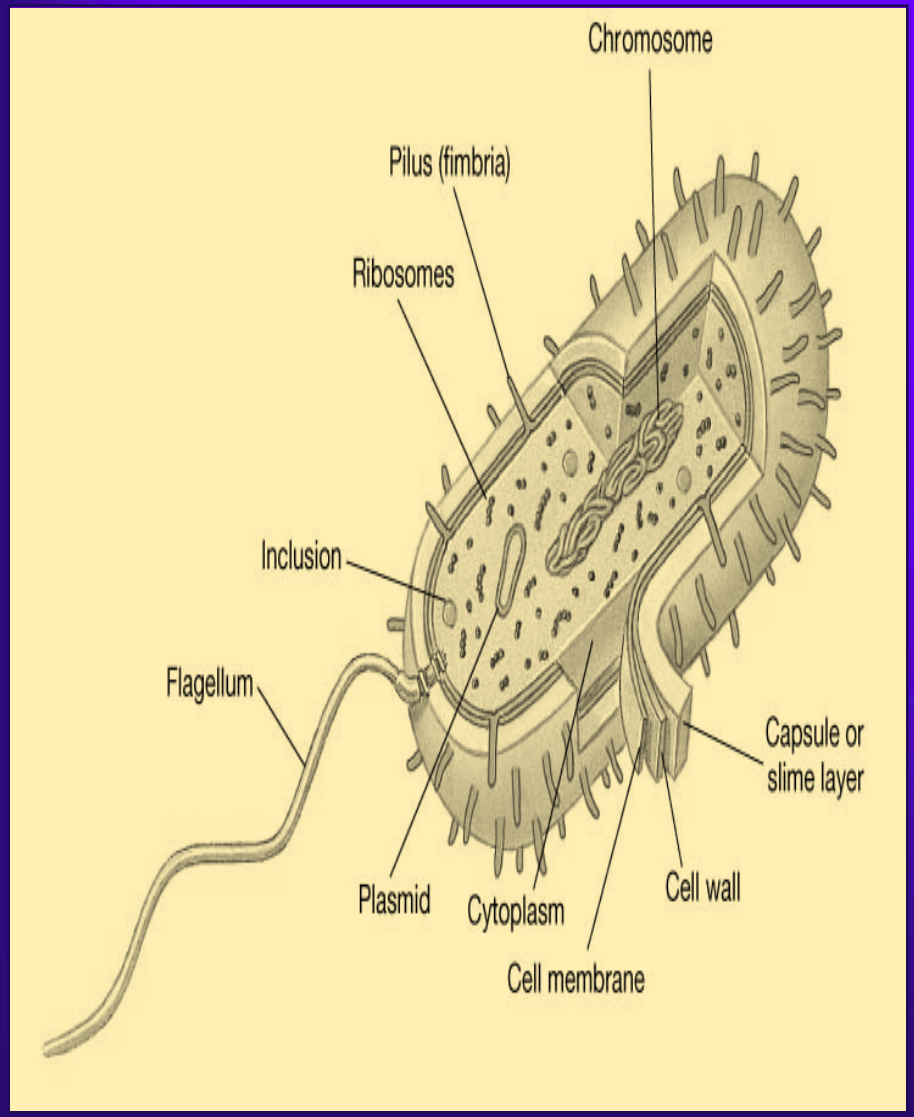
Wyndmoor, PA 19038

Eastern Regional Research Center

Background Information

- Ability of pathogenic bacteria to adhere to surfaces of fruits and vegetables continue to be a potential food safety problem for the produce industry and consumers alike
- Fruits and vegetables are frequently in contact with soil, insects, animals, and humans during growing, harvesting, and in the processing plant
- Presence of human bacterial pathogens in fresh produce and outbreaks of diseases has led to costly recalls

Bacteria Cell Surface



- Bacterial attachment to surfaces is influenced not only by cell surface charge and hydrophobicity but also by the presence of particular surface appendages such as flagella and fimbriae as well as extracellular polysaccharides

- Bacteria surfaces are heterogeneous with physicochemical properties determined primarily by teichoic acid (gram-positive strains) or other polysaccharides (gram-negative strains) along with proteinaceous appendages (fimbriae)

- Surface structure and biochemical characteristics of bacteria and of a substratum as, in this case, melon play a major role on how and where bacteria may attach

- Plant surfaces and microbes both have negative surface potential, which results in electrostatic repulsion between the two surfaces
- Most bacteria are readily suspended in aqueous media because of polar, hydrophilic moieties on bacterial cell surfaces (Mafu et al. 1991)
- Bacterial cell surface properties can only be measured indirectly, through phenomena that reflect more or less the nature of molecular interactions (Mozes and Rouxhet, 1987)

SEM observation of cantaloupe rind surfaces (Ukuku unpublished data)

fig. 1

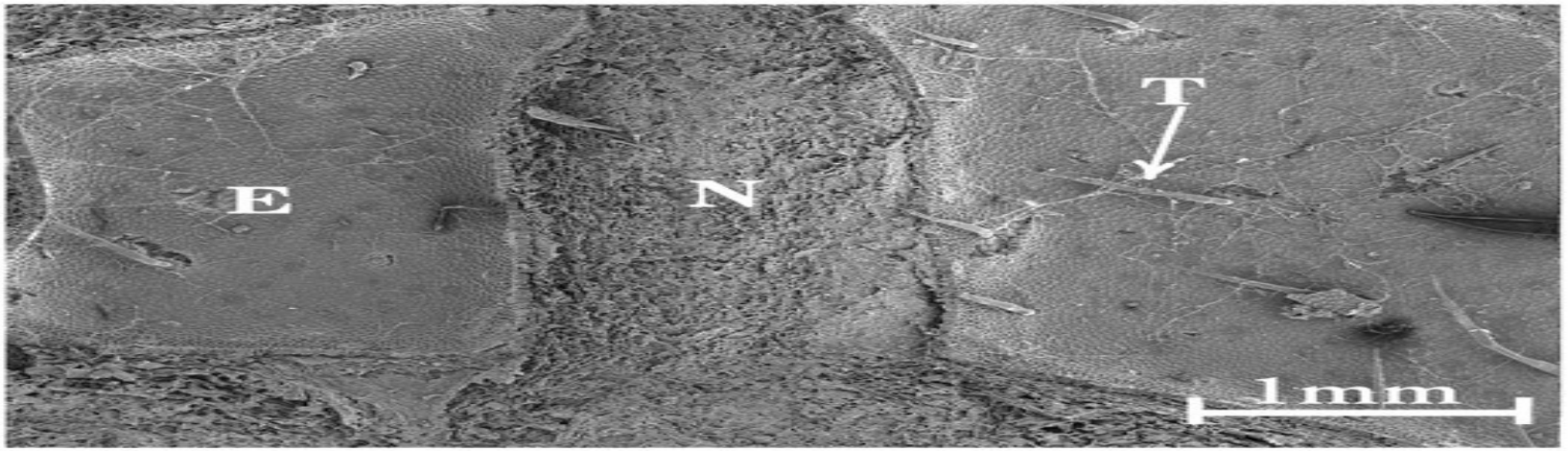
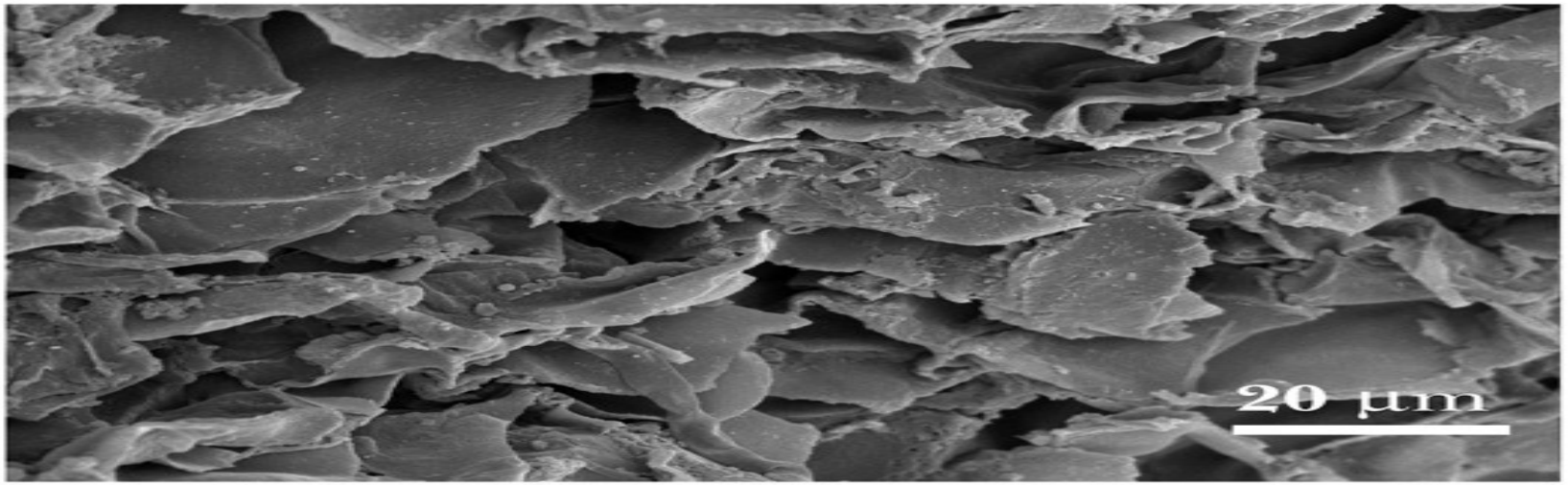


fig. 2

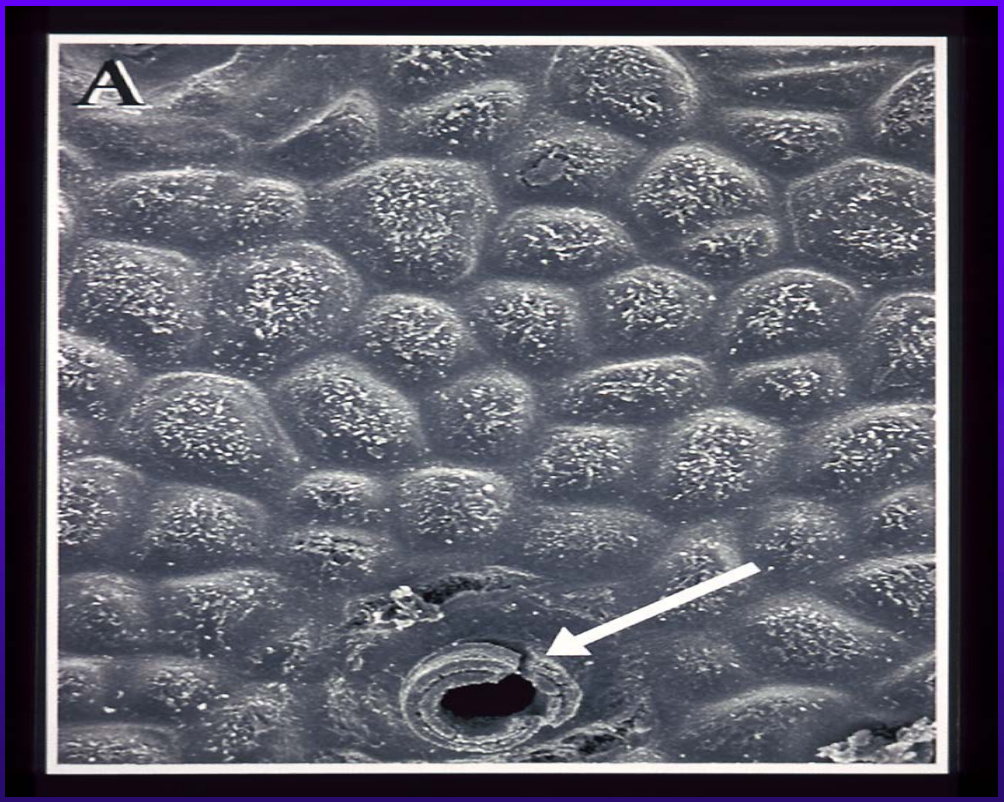


SEM Observation of Cantaloupe rind surface

Whole cantaloupe and fresh-cut pieces

Cantaloupe rind surface

Ukuku, unpublished data



- There are several techniques used for measuring bacterial cell surface charge

The most widely used techniques are:

- Hydrophobic interaction chromatography (HIC)
- Electrostatic interaction chromatography (ESIC)

Chromatography

- Hydrophobic interaction chromatography (HIC) were prepared according the procedure modified by Ukuku and Fett (2002) from Dahlback et al. (1981) and Pedersen (1980)

- Columns for HIC were packed with 8 ml of Octyl-Sepharose CL-4B gel (Sigma, St. Louis, MO) equilibrated overnight at 4^{o}C in 12 mL of 0.02 M $NaPO_4$, pH 6.8 buffer (bed volume = 0.6 ml)

- Electrostatic interaction chromatography (ESIC)

Prepacked columns:

Dowex chloride form (capacity, 1.2 meq/mL, 50 by 8, Bio-Rad Laboratories, Richmond, CA) was used for the anionic resin

Dowex hydrogen form (capacity, 1.7 meq/mL, 50 by 8, Bio-Rad Laboratories, Richmond, CA) was used for the cation resin

- The mesh size was 100 to 200 μm for both resins

Bacteria of interest in this study

- *L. monocytogenes*: Scott A (clinical isolate), CCR1-L-G (food isolate), ATCC 15313 (type strain) and H7888 (food isolate)
- *Salmonella* spp: *Salmonella* Stanley H0558 (alfalfa sprout-related outbreak), *Salmonella* Poona RM2350, *Salmonella* Saphra 97A3312 (cantaloupe-related outbreaks)
- *Escherichia coli:* ATCC 25922 (type strain), O157:H7 strains SEA13B88 and Oklahoma (apple juice cider-related outbreaks)

Bacteria strength of attachment

- The population remaining on the melon surface after washing treatment was described as strongly attached bacteria (S_R)
- The S_R value represents the percentage of total bacterial population strongly attached to the cantaloupe. S_R values were calculated as (strongly attached bacteria)/(loosely + strongly attached bacteria) as reported by Dickson and Koohmaraie (*9*).
- S_R-Value = Strength of attachment

RESULTS

- Table 1- Bacterial cell surface hydrophobicity (HIC) and charge (ESIC)

- Table 2- Bacterial attachment on melon surfaces in relation to S_R-Value at day 0

2

Bacteria	Hydrophobicity (g/e)	Surface charge (r/e) ESIC (-)	Surface charge (r/e) ESIC (+)
Salmonella			
Stanley (H0558)	0.338 ± 0.114[a]	21.48 ± 0.19	4.10 ± 0.10
Poona (RM2350)	0.486 ± 0.110	33.71 ± 0.30	1.82 ± 0.14
Saphra (97A3312)	0.629 ± 0.130	50.00 ± 0.15	6.08 ± 0.11
Escherichia coli			
ATCC 25922	0.233 ± 0.021	1.62 ± 0.12	0.12 ± 0.04
O157:H7 SEA13B88	0.207 ± 0.015	1.48 ± 0.10	0.18 ± 0.09
O157:H7 Oklahoma	0.220 ± 0.019	1.50 ± 0.13	0.16 ± 0.03
Listeria monocytogenes			
Scott A	0.284 ± 0.051	38.06 ± 0.12	0.40 ± 0.12
ATCC 15313	0.278 ± 0.029	38.11 ± 0.10	0.32 ± 0.08
CCR1-L-G	0.282 ± 0.059	37.68 ± 0.14	0.20 ± 0.04

Bacterium[a]	$\log_{10}$ CFU/cm^2	S_R-value[b]
Salmonella		
Stanley H0558	4.84 ± 0.10	0.920 ± 0.009
Poona RM2350	4.37 ± 0.11	0.939 ± 0.010
Saphra 97A3312	4.34 ± 0.18	0.942 ± 0.011
Escherichia coli		
ATCC 25922	5.53 ± 0.15	0.763 ± 0.052
O157:H7 SEA13B88	5.81 ± 0.21	0.750 ± 0.041
O157:H7 Oklahoma	5.20 ± 0.18	0.739 ± 0.059
Listeria monocytogenes		
Scott A	2.89 ± 0.09	0.826 ± 0.038
ATCC 15313	3.00 ± 0.10	0.798 ± 0.032
CCR1-L-G	3.12 ± 0.11	0.830 ± 0.021

Effect of treatments on bacterial cell surface charge and hydrophobicity of *Escherichia coli* [ND= not determined]

Treatment	Hydrophobicity (g/e)	Surface charge (r/e)	
		ESIC (-)	ESIC (+)
Thermal			
Room~ 21C	0.240 + 0.022 ^{D}	33.30 ± 0.14 ^{A}	0.12 ± 0.02 ^{A}
25^{o}C	0.245 + 0.023 ^{D}	33.27 ± 0.12 ^{A}	0.12 ± 0.02 ^{A}
60^{o}C	0.268+ 0.022 ^{C}	22.41 ± 0.14 ^{B}	0.09 ± 0.02 ^{A}
90^{o}C	0.348 + 0.020 ^{B}	16.12 ± 0.12 ^{C}	ND

Correlation coefficient between bacterial cell surface hydrophobicity or charge and strength of attachment to cantaloupe surfaces

	Correlation coefficient (r)		
	Surface charge (r/e)		Hydrophobicity (g/e)
Bacteria[a]	ESIC (-)	ESIC (+)	(HIC)
Salmonella			
cocktail	0.787	0.878	0.857
Escherichia coli			
cocktail	0.887	0.944	0.998
L. monocytogenes			
cocktail	0.995	0.984	0.956

Survival of *Salmonella* populations on cantaloupe rind surface stored at 5°C for 0, 3 or 7 days after sanitizer treatments [a]

Treatment	*Salmonella* on cantaloupe rind (log CFU/cm^2)[b] Day 0	Day 3	Day 7
Control	4.5 ± 0.3 D	4.2 ± 0.1D	4.0 ± 0.1D
Water	4.6 ± 0.2 D	4.4 ± 0.2D	4.2 ± 0.1D
250 ppm Cl_2	2.6 ± 0.1 B	2.4 ± 0.1B	2.4 ± 0.3B
3% H_2O_2	3.0 ± 0.1 C	3.1 ± 0.1C	3.3 ± 0.2C
H_2O (96 C)	0.9 ± 0.1 A	0.7 ± 0.2A	0.4 ± 0.4A

[a]Initial populations of *Salmonella* spp. in the inoculum was10^8 CFU/ml.
[b]Mean +/- SD data in each column not followed by the same letter are significantly different ($p<0.05$).

CONCLUSION

- The results of this study indicate that both surface charge and hydrophobicity influence attachment of human bacterial pathogens to cantaloupe rind surface
- It is difficult to predict the surface properties of human bacterial pathogens when the pathogens are first exposed to a plant surface as environmental conditions can significantly affect bacterial surface properties including charge and hydrophobicity
- Bacterial surface characteristics and attachment to other types of produce is currently under investigation

Take home message

- Proper modifications of treatment parameters that can disrupt the physicochemical properties and proteinaceous appendages of bacterial cell surface will help in decontamination process

- Such knowledge will allow for the development of much needed improved intervention strategies to help insure the microbial safety of produce

Acknowledgement

- Donyel M. Jones, Microbiologist
- Lee Chau, Biologist
- Dr. John Phillip, ERRC Statistician

For more information

Contact:
Dr. Dike O. Ukuku
Senior Scientist, FSIT- ERRC- ARS-USDA
600 E. Mermaid La, Wyndmoor, PA 19038
215-233-6427, Fax 215-233-6406
dike.ukuku@ars.usda.gov
http://www.ars.usda.gov/naa/errc

TEM observation of *E. coli* cells (A= control; B= Heat@60C; C= 90C