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Further Studies on the Microbial Content (Endomicrobiology, Endobacteriology) of Fruit and Vegetable Crops: the Study Continues!

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Abstract

Previous studies in our lab over the past several years on fruits and vegetables have identified various species of microorganisms, primarily bacteria, that appear to be endemic to the inner flesh/pulp of many crops. While consumption of these fruits and vegetables usually do not cause disease to the public in general, there may be a greater risk to immunocompromised individuals, especially upon consumption of crops that are eaten raw/uncooked or only slightly cooked. In this study, we examined various fruits, herbs, and vegetables that we had not previously examined for the presence of such microorganisms, and many/most of the crops we examined did indeed contain endemic microorganisms in their inner flesh/pulp. Our findings are herewith reported.

Keywords: bacteria, microorganisms, fruits, vegetables, crops, endomicrobiology

1. Introduction

Over the past several years have identified numerous species of microorganisms that inhabit the surface and/or flesh/pulp of various fruit and vegetable crops; terms we have coined topomicrobiology and endomicrobiology, respectively (Edelman and Lin 2011, 1013, 2016). These microorganisms were generally considered to be non-pathogenic commensal species, although in certain instances, such as immunocompromised individuals, some of these species can theoretically become opportunistic and pathogenic. We have continued this study to crops that we had not previously tested, and we hereby present the results in this report. Among the crops currently investigated are fruits and vegetables, edible mushrooms/fungi, aromatic/culinary herbs and spices, and some uncommon and tropical fruits and vegetables.

2. Materials & Methods

The microbial content of crops was isolated and identified using a combination of the methods previously described (Edelman and Lin (2011, 2013, 2016). These included inserting sterile cotton-tipped applicators into the flesh/pulp and/or homogenizing the crops or small sections of crops into blenders/food processors for one minute. Tryptic Soy agar plates (or in some cases, Saboraud agar plates, for fungal cultivation) were then swabbed with cotton tipped applicators and allowed to grow at room temperature for several days. Plates were then shipped to AvistaPharma Solutions, 104 Gold Street, Agawam, Massachusetts, 01001, U.S.A. for species identification using DNA sequence analysis.

3. Results and Discussion

While some crops were found to be sterile/microorganism-free in the inner flesh/pulp, many were indeed found to harbor bacteria and in some cases fungi. The bacterial/fungal content of such crops are shown in Table 1.

Table 1. Microbial species found in the inner flesh/pulp of crops tested in this study

Crop:	**Species present:**
Sage (Salvia officinalis)	Enterobacter cowanii
	Ignatzschineria indica
	Brachybacterium sp.
	Pseudomonas fulva
Oregano (Origanum vulgare)	Myroides odoratimimus
Dandelion (Taraxicum officinale)	Staphylococcus epidermidis
	Pseudomonas fulva
Green Ong Choy/Water Spinach	
(Ipomoea aquatica)	Enterobacter asburiae
Thyme (fresh,not dried)	
(Thymus vulgaris)	Pseudomonas straminea
	Pseudomonas fulva
	Microbacterium sp.
Rosemary (fresh, not dried)	
(Rosemarinus officinalis)	Bacillus subtilis
	Pantoea agglomerans
	Pantoea dispersa
Catnip (Neperta cataria)	Acinetobacter sp.
(used by humans as an herbal tea,	Exiguobacterium acetylicum
and culinary herb)	Enterobacter kobei
Arugula /Rocket, Garden Rocket,	Citrobacter freundii
Rugula, Rucola Colewort, Roquette	Alcaligenes faecalis
(Eruca sativa)	Corynebacterium variabile
Vanilla ("beans" and pods)	Bacillus subtilis
(Vanilla planifolia)	
	Bacillus amyloliquefaciens
Arrowhead Bulb (tuber)/Duck potato/	Chryseobacterium sp.
Katniss/Omodaka,swamp potato/tule	Stenotrophomonas maltophilia
potato/wapato	Pseudomonas fluorescens
(Sagittaria sagittifolia)	Stenotrophomonas sp.
Soursop/Guanabana/graviola	Pantoea agglomerans
(Annona muricata)	Enterobacter pyrinus
	Erwinia sp.
	Fungus: Aspergillus sp., (either A. oryzae (or)
	A. parasiticus (or) A. terricola)
Red Currant	Curtobacterium flaccumfaciens
(Ribes rubrum)	Pantoea agglomerans
Boysenberry	Glucanobacter cerinus
(Rubus ursinus X Rubus idaeus)	Candida railenensis (yeast)
	Bacillus altitudinis
	Geotrichum candidum
Nance Yellow Cherry	Acidovorax temporans
(Brysonima crassifolia)	Sphingomonas paucimobilis
	Sphingomonas abaci
Betel leaf/Paan/Lalot	Pseudomonas oryzihabitans
(Piper betle)	Rhizobium sp.
Watercress (Nastiurtium officinale)	Providencia rettgeri
Green Olive (fresh)	Lysinibacillus alkalisoli
Black Olive (semi-dried)	Providencia rettgeri
(Olea europaea))	Enterobacter kobei
	Achromobacter spanius
	Delftia acidovorans

Tarragon/Estragon	Exiguobacterium acetylicum
(Artmisia dracunculus)	Staphylococcus hominus
Lily Flowers (dried, packaged in	Bacillus cibi
cellophane, imported from China)	Bacillus flexus
(Hemerocallis fulva)	Bacillus amyloliquefaciens
	Bacillus megaterium
Swiss Chard (Beta vulgaris)	Acinetobacter johnsonii
	Pseudomonas chlororaphis
	Exiguobacterium actylicum
Feijoa (Acca sellowiana)	Methylobacterium hispanicum
	Stphylococcus hominis
	Many Fungal colonies: Penicillium camemberti (or)
	P. clavigerum (or) P.crustosum (or) P. commune (or)
	P. corylophilum
Stuffer/Stuffing Mushroom/Large White	Pseudomonas fluorescens
Button Mushroom (Agaricus bisporus)	Trichococcus collinsii
Shitake Mushroom (Lentinula edodes)	Ewingella americana
Seafood Mushroom/Enoki Mushroom/	Serratia grimesii
Enokitake/Gold Needle Mushroom	Achromobacter spaniu
(Flammulina velutipes)	
Bunapi Mushroom/White Beech Mushroom/	Serratia liquefaciens
Brown Beech Mushroom	Enterococcus avium
(Hypsizygus tessellatus)	Serrtatia quinivorans
	Pseudomonas extremorientalis
King Oyster Mushroom (Pleurotus eryngii)	Ewingella americana
	Staphylococcus pasteuri
Oyster Mushroom (Pleurotus ostreatus)	Pseudomonas fluorescens
Black Fungus/Tree Ear Fungus/Black Chinese	Bacillus thuringiensis
Fungus/Wood Fungus/Ear Fungus/Tree Ear Fungus	Serratia liquefaciens
(Auricularia polytricha/Hirneola polytricha)	Enterococcus casseliflavus
Morel (Morchella esculenta)	Pseudomonas tolaasii
Alfalfa Sprouts (Medicago sativa)	Enterobacter cancerogenus
	Stenoitrophomonas maltophilia
Peas (in pods/out of pods) (Pisum sativum)	Escherichia hermanii
	Enterobacter cancerogenus
Brown Russian Cucumber (Cucumis sativus)	Beutenbergia cavernae
	Microbacterium oleivorans
Red Water Lily (Nymphaea nouchali)	Klebsiella pneumoniae
(Southeast Asian food plant)	Enterobacter sp.
Dill (Anethum graveolens)	Enterobacter cowanii
	Lysinibacillus sp.
Mint (Mentha sp.)	Morganella sp.
	Pseudomonas pseudoalcaligenes
Basil/Sweet Basil (Ocimum basilicum)	Serratia fonticola
	Pseudomonas aeroginosa
Peanuts (Raw, Green) (Arachis hypogaea)	Serratia marcescens
	Pseudomonas mosselii
Rose Hips (freshly harvested) (Rosa rugosa)	Psychrobacillus sp.
	Pseudomonas oryzihabitans
	Curtobacterium flaccumfaciens
Garbanzo Bean/Chick Pea (Cicer arietinum)	Staphylococcus epidermidis
String Bean/Green Bean/Snap Bean	Arthrobacter sp.
(Phaseolus vulgaris)	Microbacterium esteraromaticum
Long Bean/Asparagus Bean/Chinese Long Bean/	Pantoea agglomerans

Yard Long Bean/Snake Bean	Pseudomonas fulva
(Vigna unguiculata)	Klebsiella pneumoniae
	Exiguobacterium acetylicum
Jamaican Naseberry/Sapodilla (Manikara zapota)	Phacidium sp. (Fungus)
Cilantro/Coriander/Chinese Parsley	Pseudomonas sp.
(Coriandrum sativum)	Stenotrophomonas sp.
	Bacillus thuringiensis
Red Amaranth (Amaranthus cruentus)	Myroides odoratus
	Arthrobacter nicotianae
	Arthrobacter sp.
Malabar Spinach (Basella rubra/B. alba)	Pantoea agglomerans type 3
	Arthrobacter pascens
	(Arthrobacter histidinolovorans (or)
	Arthrobacter nicotinovorans)
	Coccoloba uvifera acetylicum
	Exiguobacterium acetylicum
Mustard Greens (Brassica juncea)	Aeromicrobium fastidiosum
	Rhodococcus fascians
	Micrococcus sp.
Seaside Grape/Sea Grape/Bay Grape/	Pantoea dispersa
Beach Grape (Coccoloba uvifera)	Rhodococcus corynebacterioides
	Micrococcus sp.
	Staphyloccuc cohnii
	Fungus/mold: (unidentifiable)
Ba Cha/ Bac Ha/ Ba Ha/ Taro Stem/	Klebsiella pneumoniae
Elephant Ear Plant Stem	Stenotrophomonas sp.
(Colocasia gigantea)	Pseudomonas monteilii
Lemon Grass (Cymbopogon citratus)	Acinetobacter baumannii complex
Portobello Mushroom	Pseudomonas brenneri
(Agaricus bisporus)	(or) Pseudomonas fluorescens
Bamboo Shoots (raw, fresh)	Alcaligenes faecalis
(Bambusa vulgaris)	
Jackfruit (Artocarpus heterophyllus)	Exiguobacterium sp.
	Pseudomonas monteilii
Vietnam Garland Chrysanthemum/Edible	Enterobacter kobei
Chrysanthemum (Glebionis coronaria)	Pseudomonas sp.
	Exiguobacterium acetylicum
Asparagus Lettuce/Celtuce/Stem Lettuce/	Pantoea sp.
Celery Lettuce/Chinese Lettuce	Pseudomonas fulva
(Lactuca sativa var. augustana)	
Cremini Mushroom	Myroides odoratimimus
(Agaricus bisporus)	

Table 2. Fruit and Vegetable species in which the inner flesh/pulp was test:d and found to be sterile:

Watermelon (Golden Midget) (Citrullus lanatus)
Persimmon (Hachiya and Jiro cultivars) (Diospyros kaki)
Tejocote/Manzanita/Mexican Hawthorn (Crataegus mexicana)
Jocote Fruit/Mombin/Hog plum/Ciruela (Spondias purpurea)
Fava Bean(Vicia faba)
Drumstick Tree Pods (Moringa oleifera)
Loquat (Eryobotrya japonica)
Snake Gourd/Serpent Gourd(Trichosanthes cucumerina)
Chestnut(Castanea dentata)
Banana Blossom/BananaFlower (Musa acuminata Colla)
Sour Cherry/Montmorency Cherry(Prunus cerasus)
Ivory Gaya Melon (Cucumis melo inodorus 'Ivory Gaya')
Mango (Red & Yellow varieties) (Mangifera indica)
Indian Jujube/Ber Fruit/Chinese Date/Chinese Apple/Indian Plum/
Reji Pandu (Ziziphus mauritiana)
Carob/St. John's Bread/Locust Bean (Ceratonia siliqua)
Goldenberry/Cape Gooseberry/Pichuberry/
Peruvian Ground Cherry (Physalis peruviana)

Although most of the fruits and vegetables tested did contain one or several species of bacteria and fungi in their inner pulp/flesh, in general consumption of these crops with these organisms do not seem to cause human diseases. Many of these microorganisms are probably destroyed by hydrochloric acid and enzymes throughout our digestive system, while others are destroyed through the cooking process. However, immunocompromised individuals are most likely to be at a higher risk for infection, especially when these crops are eaten raw. In our previous study we noted numerous diseases that the microorganisms found in crops were capable of causing (Edelman and Lin, 2016), although their presence in fruits and vegetables do not necessarily cause infection. The purpose of this and our previous reports are merely to alert the consumers that these microorganisms are indeed present in such foods. Many consumers erroneously assume that if fruits and vegetables are washed before consumption they are automatically sterile, which we have shown not to be the case.

Another question which arises often is whether the organisms identified in the crops studied are pathogenic at all. While it is not the scope or objective of this report to investigate the possible pathogenicity of every organism identified, we have randomly selected a few species and canvassed the literature. We noted that almost all organisms identified in the fruits and vegetables can, under certain conditions, such as being immunocompromised, cause diseases and bacteremia. *Providencia rettgeri*, for example, (found in this study in watercress and green olives), have been found to cause bacteremia and bacteriuria (Wie, 2015). *Bacillus flexus*, (found in this study in dried lily flowers), has been found in an outbreak in a tertiary burn center (Ucar et al, 2016). *Acidovorax*, (found in this study in Nance yellow cherry), has been found in a case of sepsis (Shetty, et al., 2005). *Curtobacterium flaccumfaciens*, (found in this study in red currants), has been found in human infections (Funke, et al., 2005*). Enterobacter pyrinus,(* found in this study in soursop/guanabana), has also been found in human infections (Baylis et al., 2011). *Myroides odorotimimus (*found in this study fresh, undried oregano), has been found to cause bacteremia in a diabetic patient (Endicott et al., 2015) as well as soft tissue infections, septic shock, and pneumonia in other patients (Benedetti, et al., 2011). *Ignatzschineria indica, (*found in this study in the herb sage), has been implicated in Myiasis (Barker, et al., 2014). Infections involving *Acinetobacter baumanii*, (found in this study in lemon grass and catnip), have also been reported (Peleg et al., 2008*). Pseudomonas chlororaphis*, (found in this study in Swiss Chard), has been implicated in various human infections as reported by Faccone, et al., (2014). Further investigation of the literature on the other microbial species present in the fruits and vegetables used in this study will most likely also identify some cases of human infection. Again, this does not necessarily imply that such crops are dangerous to human health in general, but that they may, under certain circumstances, pose a risk to immunocompromised individuals.

4. Conclusions

The main idea of this report is to inform the public and scientific community that while some fruits and vegetables do seem to be sterile, many or most are not sterile, even after washing, as microorganisms are indeed present in their inner flesh/pulp. We plan to continue this investigation on other fruits and vegetables in the near

future.

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