

A grayscale electron micrograph showing several rod-shaped Pseudomonas aeruginosa bacteria. The bacteria are arranged in a chain, with some showing flagella at their ends. The background is a textured, granular surface.

Pseudomonas aeruginosa

Myth or Menace?

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Why focus on *Pseudomonas aeruginosa*?

- ▶ Well known to environmental microbiologists
 - ▶ Indigenous microbiota of water and soil, plant tissues
 - ▶ Recently found to preferentially aerosolised from those sites into the atmosphere
- ▶ Well known to clinical microbiologists
 - ▶ Commonly isolated as a opportunistic pathogen in debilitated patients
 - ▶ Catheterised Urinary Tracts
 - ▶ Cystic fibrosis lung colonisation
 - ▶ Burn wounds, particularly large scale
 - ▶ Famously resistant to most antibiotics, very hard to treat
- ▶ Not well known to food quality microbiologists

Environmental Microbiology Research

Group study

- ▶ Pilot study regarding Salad Vegetables and *Pseudomonas aeruginosa* indicated potential of project
- ▶ Major study of sources of *Pseudomonas aeruginosa* colonization of CF lungs focussed on two potential sources:
 - ▶ Person to person: human source
 - ▶ Air/water/soil: environmental source
- ▶ Food-borne micro-organisms are often sourced from their environment, especially fruit and vegetable crops which can be contaminated by the air or water or soil or all three





And so to Salad Vegetables

- ▶ **On the premise that:**
 - ▶ Salad vegetables are generally eaten raw
 - ▶ Pathogens present in or on food may be aspirated or washed into the lungs
 - ▶ Vegetables are grown in soil or compost and irrigated by sprayed water, or hydroponically (in water)
- ▶ **We tested both the outer surface and inner pulp of the following salad fruit and vegetables:**
 - ▶ Lettuce – iceberg type (grown in soil or hydroponically)
 - ▶ Tomatoes
 - ▶ Mushrooms (organic and non-organic)
 - ▶ Alfafa sprouts





Treatment



- ▶ Vegetables were sourced from Supermarket, Greengrocer and Farmers' Market
- ▶ All samples were collected directly into a sterile stomacher bag using gloved hands, chilled for transport to the laboratory
- ▶ Outer surfaces were rinsed, rinse water filtered, plated out
- ▶ Roots of lettuce were removed for separate testing
- ▶ Flesh of all samples was stomached and plated out in same way as washings



Three levels of identification stringency





- ▶ **Level 1: Initial identification of *Pseudomonas aeruginosa* performed using Phenotypic tests – culture and biochemical characteristics**
 - ▶ This gives a presumptive identification
 - ▶ This level would usually be all that was done in food labs
- ▶ **Level 2: Genotyping such as Real-time PCR (RT-PCR)**
 - ▶ *Pseudomonas aeruginosa* duplex RT-PCR reaction assay (PA duplex)
 - ▶ Tests for conserved regions of genome, unique and exclusive to *Pseudomonas aeruginosa*
 - ▶ Confirms the identity of the organism, while decreasing the probability of misidentification.

Three levels of identification stringency

- ▶ **Level 3: Strain typing using Molecular (DNA) analysis**
 - ▶ Within the genome of confirmed *Pseudomonas aeruginosa* isolates, variation may still be found by this level of analysis
 - ▶ Variants are known as strains
 - ▶ Clones are strains that have been consistently found in a geographical area, in multiple patients
 - ▶ ERIC-PCR is a quick PCR method that is typically used to screen isolates for clonality
 - ▶ The resulting pattern provides a fingerprint of the organism which can be compared to each other to determine if any relationship exists between isolates
 - ▶ Discrimination between isolates was shown to be very high

Results – Level 1 Phenotypic ID

Table 1a. Presumptive and confirmatory identification data including number of genotypes of *Pseudomonas aeruginosa* recovered from sampled vegetables.



TYPE OF VEGETABLE		NUMBER OF SAMPLES	NUMBER OF PRESUMPTIVE ISOLATES
Lettuce	Leaves	30	74
	Roots	30	82
Mushrooms	Whole	48	45
Sprouts	Whole	36	10
Tomatoes	Whole	36	18
TOTAL		150*/180	227

* Indicates that lettuce roots were treated as individual samples during analysis, being the additional 30 samples shown in the current table.

Comments on Table 1a results

- ▶ All categories of retailers (greengrocer, farmers' markets, and supermarkets) contributed contaminated vegetables of some type.
 - ▶ Farmers' markets and supermarkets had contamination in/on all types of vegetables tested
 - ▶ Fruit and vegetable shop's mushrooms and tomatoes were uncontaminated



Level 2 and 3: Genotyping and strain typing summary

TYPE OF VEGETABLE		NUMBER OF SAMPLES	NUMBER OF PRESUMPTIVE ISOLATES	NUMBER OF CONFIRMED ISOLATES	NUMBER OF DIFFERENT GENOTYPES FOUND PER BATCH
Lettuce	Leaves	30	74	23	8
	Roots	30	82	29	10
Mushrooms	Whole	48	45	5	3
Sprouts	Whole	36	10	3	2
Tomatoes	Whole	36	18	14	5
TOTAL		150*/180	227	74	28



Level 1:

Level 2:

Level 3

Phenotyping:

Genotyping:

Strain typing

- ▶ Phenotypic methods are slow and subject to alterations in biochemistry profiles and phenotype expression
 - ▶ 227 isolates were identified by phenotyping as potential *Pseudomonas aeruginosa*
- ▶ Genotyping confirms the identity of the *Pseudomonas aeruginosa*, decreasing probability of misidentification
 - ▶ Of the 227 potential *P.aeruginosa* isolates, only 74 were confirmed using PA duplex (roughly 2/3 false positives)
- ▶ ERIC-PCR provides a fingerprint of the organism and compares strains to determine if any relationship exists between them
 - ▶ Of the confirmed 74 *Ps. a* isolates, 28 different genotypes were identified



Results of surface vs flesh tests

The great majority of the isolates were found on the outside of all of the vegetables (Table 2).

A very small number of confirmed isolates were found inside a tomato, one sprout package and the roots of the several lettuce.

Table 2: distribution of numbers of confirmed isolates recovered from vegetable tissue and on the external surfaces of various vegetables.

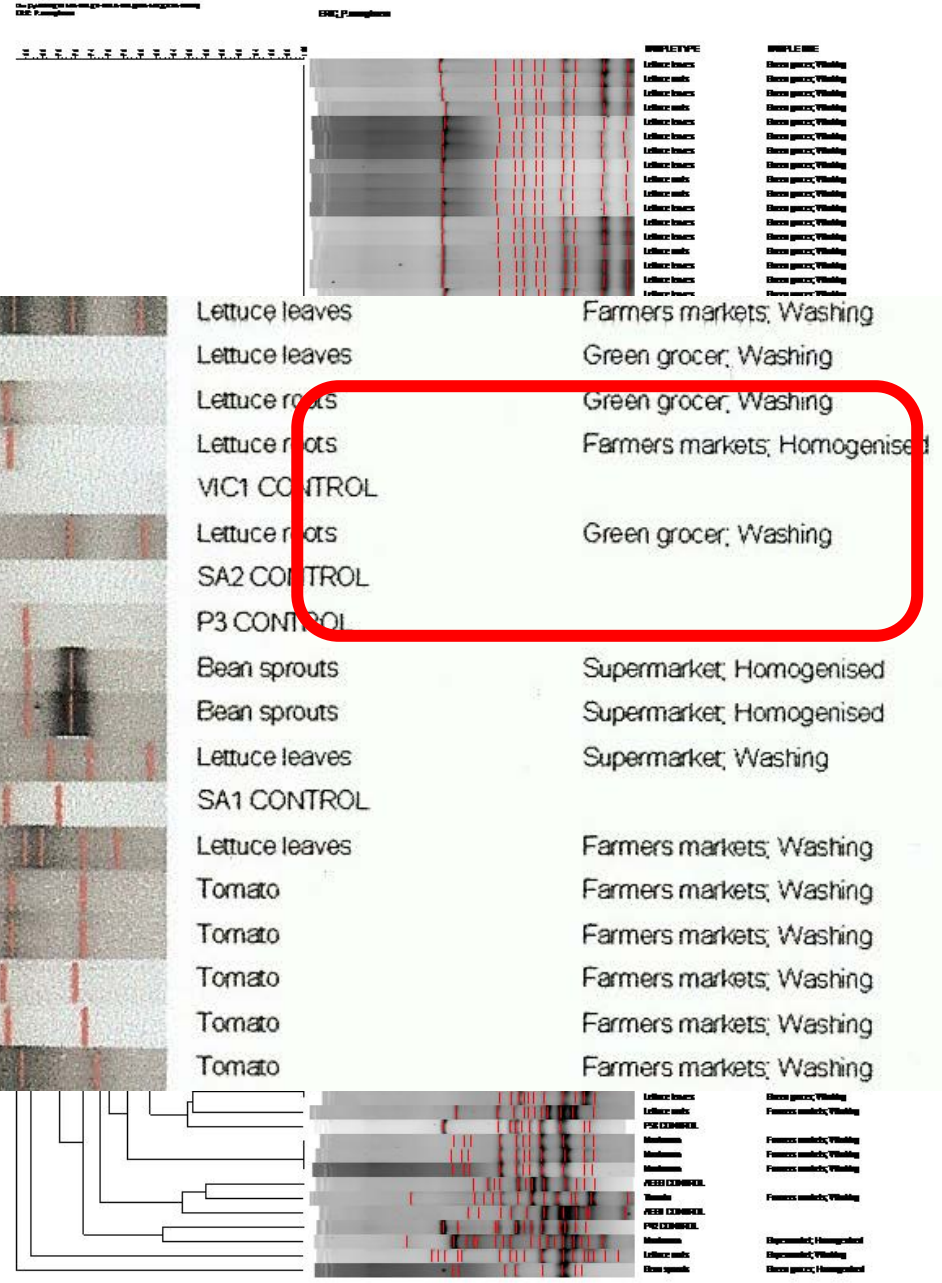
TYPE OF VEGETABLE		NUMBER OF CONFIRMED ISOLATES RECOVERED		
		SURFACE	TISSUE	TOTAL
Lettuce	Leaves	23	0	23
	Roots	23	6	29
Mushrooms	Whole	3	2	5
Sprouts	Whole	0	3	3
Tomatoes	Whole	13	1	14
TOTAL		62	12	74

Significance of surface vs flesh results



- ▶ The finding of a majority of isolates from surfaces of vegetable suggests that the contamination is most likely due to handling, washing or similar
- ▶ If the growth source of soil or water were found to be contaminated (results not shown here), we might have expected to find more plant tissue contamination
- ▶ Lack of cross contamination of genotypes between vegetables at any single retailer, indicates that the contamination is unlikely to have occurred there

Send in the Clones



Analysis of Cloning results

- ▶ No clonal genotypes of *P. aeruginosa* were found in or on any vegetable tested
- ▶ However, there has been a possibility suggested that isolated strains may mutate into clonal strains after infection of a human host, similar to the genetic adaptations in CF patients
- ▶ Recent evidence supports a theory that environmental strains of *P. aeruginosa* are able to move to other environments, such as the CF lung, and survive because the organism is forced to mutate due to natural selection (Rau *et al.*, 2010)
- ▶ Therefore, while the isolates recovered in this study may not have 100% correlation to commonly isolated clonal varieties, they may mutate into clonal strains, given favourable conditions.

Take home message

- ▶ Significance of proportion of isolates identified phenotypically as *Pseudomonas aeruginosa* that proved to be negative by PA-duplex (roughly 2/3).

This finding has major implications regarding the use of direct PCR methods for food quality testing!

- ▶ Genotyping results indicated that strains are found more consistently within a type of vegetable, than within a retail outlet.

This indicates that the contamination is more likely to have occurred on the farm, in storage or in transit to the retail outlet

And Finally – Myth or Menace

- ▶ Certainly not a myth – proved to be present on surface of many vegetables
- ▶ Degree of menace is yet to be confirmed
 - ▶ Clonal strains were not found in or on vegetables
 - ▶ Level of menace is likely to depend very much on host factors
 - ▶ If salad vegetables are to be eaten by a debilitated or immune-suppressed person, extra care should be taken with washing of the ingredients.



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