

Why competitive exclusion?

- There is some evidence that eliminating or greatly reducing background micro-organisms with the use of strong disinfectants may increase the risk of cross-contamination.
- This could be particularly true if sprouts are grown by people without hygienic training, or in an unsanitary environment, or using potentially contaminated water.

- A research project done in 2002 by B. Ingham et al. showed that *Listeria monocytogenes* grew to much higher levels on disinfected seed soon after the treatment, when background levels were very low, than on day 5, when background levels were much higher.

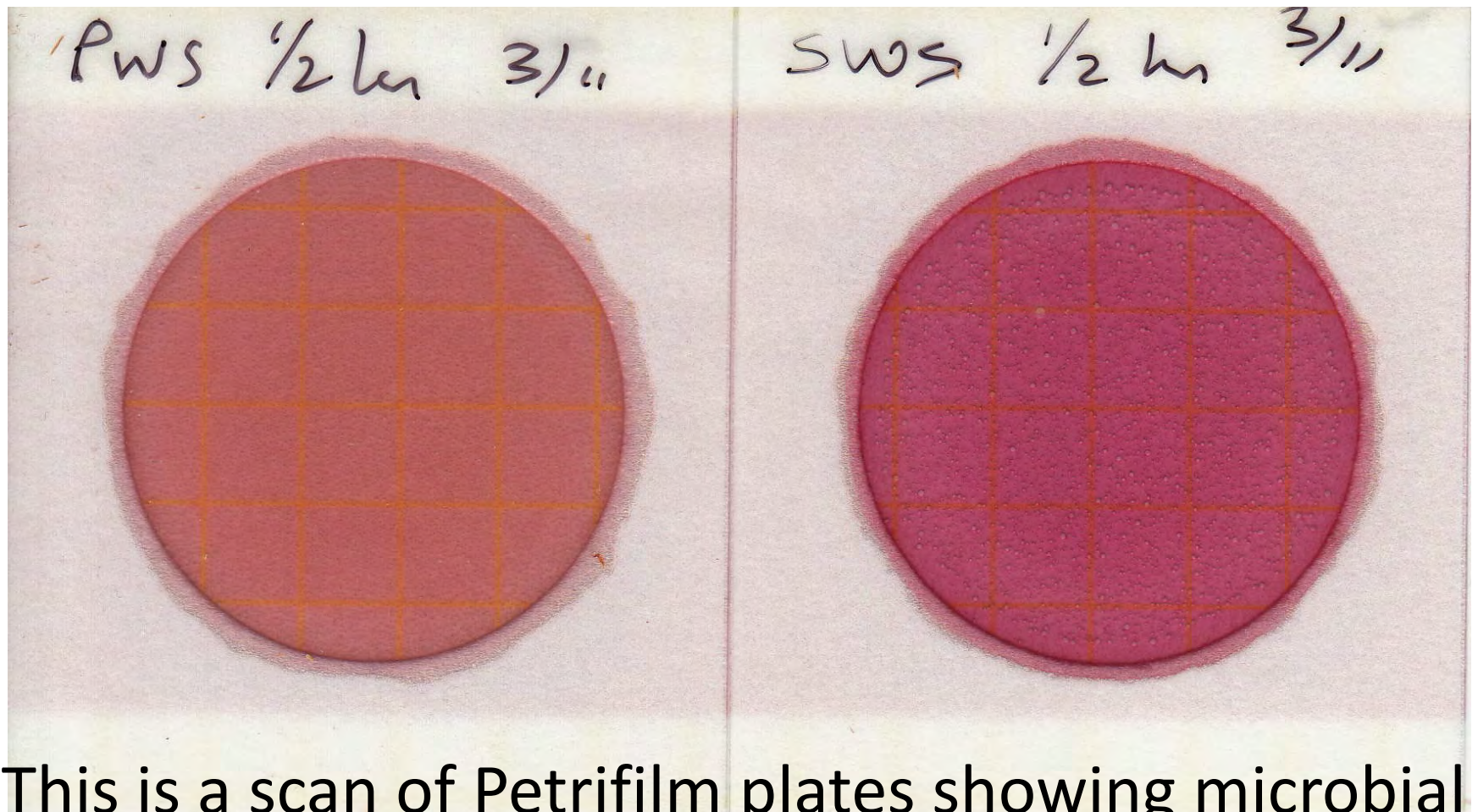
- When the seeds were inoculated with a cocktail of *L. monocytogenes* (log 5 CFU/10 ml) on day 0 or 1, the population of the pathogen increased dramatically, to within 1 to 2 logs of the total, and remained high during refrigerated storage. When sprouted seeds were inoculated with *L. monocytogenes* later in the process (day 5), the inoculum survived but did not grow more than ca. 1 log CFU/g, regardless of whether the inoculation level in each jar was low (10³) or high (10⁵).
- Ingham et al. JFP Vol 65, NO.8, 2002 “**Assessment of the Potential for *Listeria monocytogenes* Survival and Growth during Alfalfa Sprout Production and Use of Ionizing Radiation as a Potential Intervention Treatment**”

- From 2009 email to Dr. Barbara Ingham:
- Dear Dr. Ingham,
- During the past year there have been two recalls of sprouts following detection of *L. mono* in routine product sampling at retail or in distribution warehouses.
- Although appropriate interventions should be carried out to make sure *L. mono* is not present in a growing environment, I wonder if, in environments where *L. mono* might be present in low numbers, the use of 20,000 ppm chlorine seed soaks actually increases the possibility of product contamination?

Reply from Dr. Barbara Ingham:

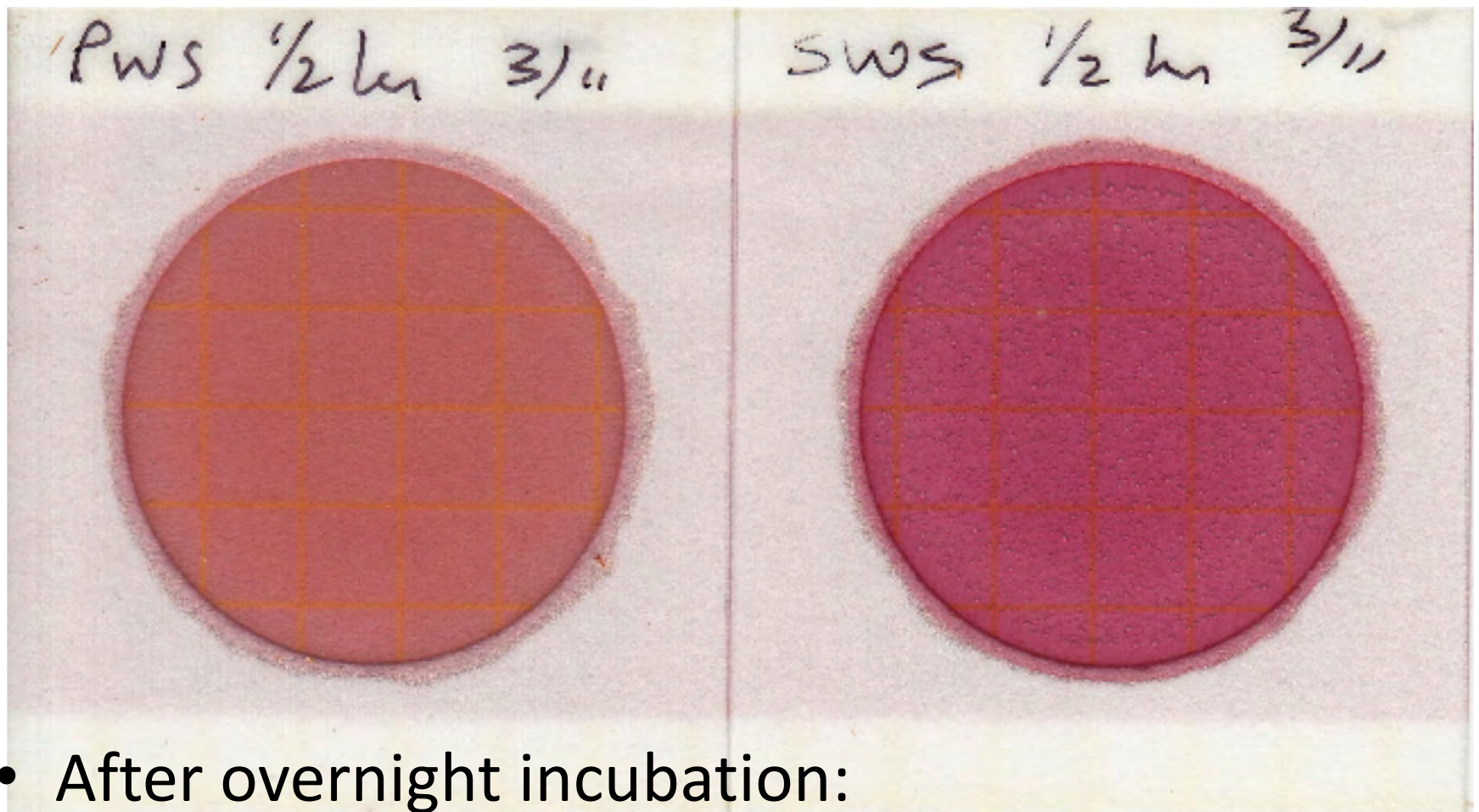
Certainly as competitive flora is reduced due to high chlorine levels, LM (being a natural processing plant contaminant) could very likely colonize plant material and become a problem. Do you know if this work is being done? I find this intriguing and wonder if I might find a student looking for an independent study project to do this work in the fall term

- There are two ways in which competitive exclusion can be effective:
- One way is to introduce organisms that actually kill pathogens (“antagonistic”)
- The other way is to introduce organisms early in the process that simply out-number and out-compete pathogens, if present.



This is a scan of Petrifilm plates showing microbial growth in 1 ml of rinse water taken from each of 2 samples of alfalfa seed $\frac{1}{2}$ hour after treatment with 20,000 ppm Calcium hypochlorite.

- After rinsing off the chlorine to an undetectable level from both seed samples, one was immersed in plain water, and the other was immersed in spent irrigation water from a production crop of alfalfa sprouts.
- Both samples were agitated, and 1 ml of water from each was plated on a Petrifilm E. coli/coliform plate
- The samples were put in a 40C incubator overnight

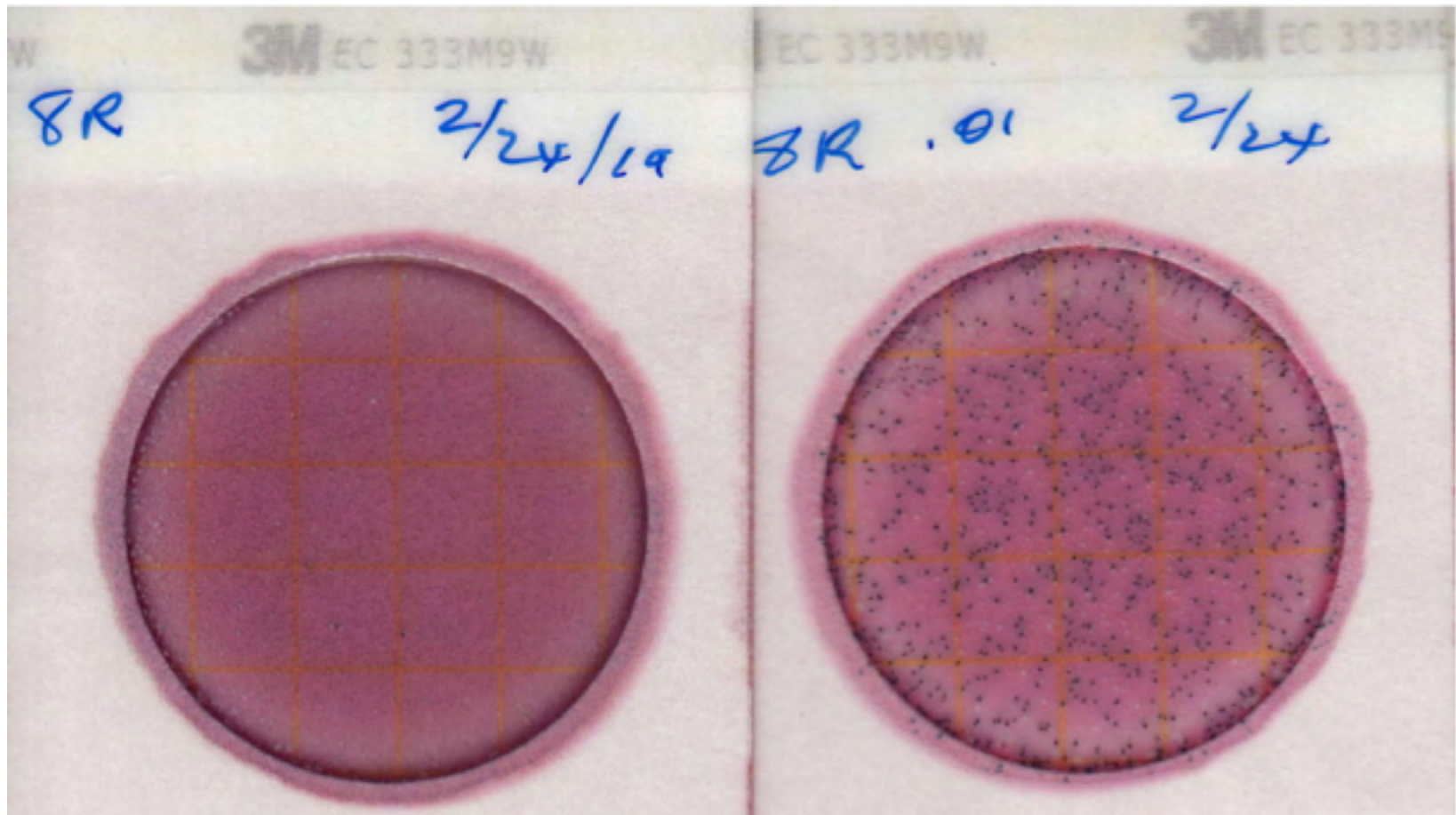


- After overnight incubation:
 - The plate on the left was from the sample soaked in plain water; the plate on the right was from the sample soaked in the spent irrigation water from the production crop

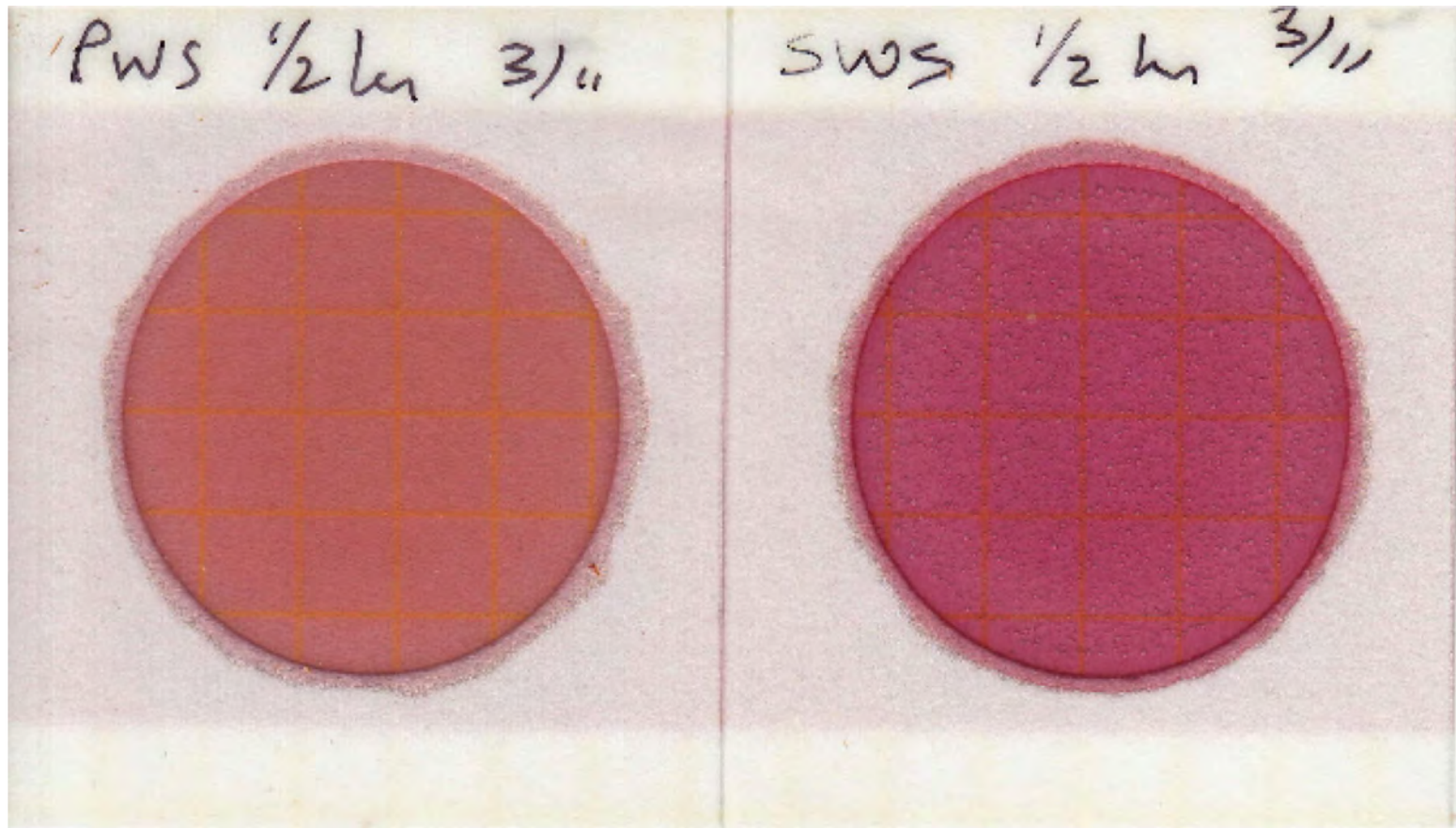
- The light pink color on the left slide indicates that very few organisms grew on the seed sample treated with the chlorine, rinsed thoroughly, and immersed in plain water, sampled and incubated overnight.
- The darker color on the right slide indicates coliform bacteria “TNTC”- “Too numerous to count” on the sample immersed in spent irrigation water after chlorine treatment, sampled, and incubated overnight.

- The darker color on the right slide indicates numbers of organisms in the range of 100,000 or more “cfu/ml” (colony forming units per milliliter.)
- This high number of “cfu/ml” is typical of spent irrigation water from healthy sprouts.
- These sprouts would in all likelihood be good quality, with a good shelf-life.
- This characteristic of sprouts was first observed and published by a researcher at Cornell in 1983.

- Here's how a "too numerous to count" slide would look if you diluted it. Left is full strength, right is 100 x dilution.



Again, the previous slide is from an earlier project, and is included in order to show how the darker color in the picture below shows TNTC microorganisms in the right-hand platem, made with spent irrigation water from healthy sprouts.



Normal Good Quality Sprouts

- “Repeated surveys of a factory producing vegetable sprouts showed that these foods commonly yielded aerobic plate counts of 10^8 /g and coliform counts of 10^7 /g. Most of the microbial growth occurred during the first two days of the germination process. Mung beans germinated in the laboratory in sterilized containers yielded comparable counts indicating that growth of the bean microflora rather than insanitary conditions was responsible. Populations were reduced to a limited extent with germicidal rinses”
- [D. F. SPLITTSTOESSER](#) et al. THE MICROBIOLOGY OF VEGETABLE SPROUTS DURING COMMERCIAL PRODUCTION JFP, Volume 5, Issue 2 June, 1983

- Unfortunately, the prevailing Food Safety treatments do not distinguish between harmless or possibly beneficial microorganisms, and harmful ones, and this perspective results in the idea that since we can't tell the difference, we'd better kill as many microorganisms of all sorts as possible.

- However, if reducing background organisms to very low levels may increase the likelihood of cross-contamination following treatment...
 - whether or not pathogen levels are high enough to cause illness -
 - positive test results obtained in routine regulatory sampling may continue to plague the sprout industry with required recalls, big expenses, and negative publicity.

- Since something is going to grow on sprouts, isn't it better to know what it is, and know that it's beneficial or at least safe, than to just hope for the best?
- Research has already identified some candidate organisms that could be used to lower the risk of damaging positive test results obtained from very low level contamination, and could also lower the likelihood of sprout-associated illnesses.

- A continuing expression of interest by the ISGA in developing competitive exclusion as a risk-reduction strategy could help provide an incentive for this work to be implemented.
- 18 years ago, some top food safety scientists expressed optimism that competitive exclusion could provide a significant way to improve the safety of sprouts:

“Highly effective antagonists will be taxonomically identified and studies on the mechanism(s) of action of effective antagonists to be used in a competitive exclusion product will be initiated. A patent on the technology applied for and discussion with an industrial partner concerning development of a CRADA that will lead to commercialization of a competitive exclusion product will be initiated and finalized.”

- *New Technologies for Decontaminating Sprouting Seed and Produce with Easily Damageable Surfaces, USDA ARS Food Safety Progress Report 2001, Section 10: CRIS: 1935-41420-006, Fett WF, Liao C-H., Ukuku D, Matos A*

Following up on the optimism of these scientists, expressed nearly 20 years ago, could lead to a transformation for the sprout industry.

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