2020 Center for Produce Safety Research Symposium
Session 4
July 14, 2020

As a result of the ongoing Coronavirus pandemic, the 11th Annual CPS Research Symposium is being conducted virtually over the course of five consecutive weeks. In Session 1 held on June 23, 2020, we explored the use of computer-based modeling to help address two burning issues for the produce industry: understanding potential *Listeria* growth and persistence in whole produce commodities and the development of sampling strategies to support the validity of assumptions surrounding microbial testing needs and design of acceptable protocols (Key Learnings Session I). In Session 2 conducted on June 30, 2020, we expanded our knowledge base on *Listeria monocytogenes* and its persistence and growth on specific commodities and fresh-cut products and examined novel methods to control *Listeria* growth on food contact surfaces (Key Learnings Session II). In Session 3 we explored projects that took a wholistic, systems approach to solving challenges with pest intrusion into leafy greens fields, pathogen transference on co-managed farms and the impact of traits associated with concepts of soil health on pathogen persistence. We also examined *Cyclospora* presence in the irrigation canal systems in the Yuma, AZ production region (Key Learnings Session III)insert link to key learnings from session 3). Session 4 held on July 14, 2020, featured the use of genomics and metagenomics in three final project reports and one interim results presentation. These projects focus on the challenges of identifying new or revisited indicators and index testing-targets of human viral pathogens that may ultimately be used in the produce industry, the distribution and relatedness of *Listeria* species in the U.S. and the use of that information to better understand source-risk related to facilities and product, identification of competitors of *Listeria monocytogenes* that might control that organism in composts and build our knowledge base of bacterial pathogen persistence and rates of genetic diversification in the Yuma and Salinas vegetable production regions. An executive summary and the key learnings from these outstanding presentations and the discussions that followed are described below.

Executive Summary:

- **Genomics and metagenomics are important tools to help fill produce safety knowledge gaps**. Genomics and metagenomics are powerful technologies being leveraged to address critical and biologically complex produce safety challenges. It is important to understand how these technologies can be used and what they will mean for the produce industry moving forward.
- **Research builds better research**. The four research projects highlighted in Session 4 all have their roots in previously funded CPS projects. Research is a process where the learnings from one project are leveraged to ask better questions and fuel development of new tools to address those questions.
- **Potential indicators for human enteric viruses have been identified**. The presence of crAssphage and six other indicator organisms have been correlated with the presence of

various human enteric viruses in agricultural water influenced by or blended with reclaimed wastewater using genomic approaches to dissect the complex microbial community associated with these water sources.

- ***Listeria spp*. distribution has been mapped throughout the U.S.** 31-percent of soil samples, collected from non-production public sites, were positive for *Listeria* and 12-percent were positive for *Listeria monocytogenes* (Lm). 14 known and 5 new *Listeria* species were identified. The greatest occurrence of *Listeria* spp. was found in the Mississippi River Basin.
- ***Listeria* whole genome sequencing data can be leveraged in source tracking**. Soil isolates of Lm collected from these natural environments were sequenced, compared to a public database, and some clinical isolates were found to be closely related. These sequence data can be used to provide context for interpreting whole genome sequencing results during outbreak source tracking and indicate that low single nucleotide polymorphism (SNP) differences (evidence of high similarity) between human, food, facility, or regionally obtained environmental isolates do *not* necessarily imply a causal link to a firm's product.
- **Composts contain competitor bacteria that demonstrate lab-based anti-Lm properties**. Genomic and metagenomic approaches were used to dissect the diverse microbial communities in dairy and poultry composts to identify competitive exclusion (CE) organisms with anti-Lm activity. When composts were inoculated with Lm, *Bacillus* strains, among others, demonstrated antimicrobial molecule production shown to be inhibitory to Lm. While long-term and speculative, CE may offer new opportunities for Lm control in farm inputs used by organic and conventional systems.

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Key Learnings:

The four research presentations of Session 4 of the 2020 CPS Symposium were linked together by the use of genomics and metagenomics to address four industry knowledge gaps. As we review the session it is important to understand what is meant by the terminology:

- ***Genomics*** is an interdisciplinary field of biology focusing on the structure, function, evolution, mapping, and editing of genomes. A ***genome*** is an organism's complete set of DNA including all of its genes. In contrast to genetics, which refers to the study of individual genes and their roles in inheritance, genomics aims at the collective characterization and quantification of all of an organism's genes, their interrelations and influence on the organism.

- ***Metagenomics*** is the use of DNA sequencing techniques to study DNA extracted directly from environmental samples. It is a culture-independent tool for studying environmental microorganisms. In addition to the information about individual identity and comparative diversity, metagenomics gives insight into the collective functional traits of the organisms, as it includes measures of the physiological and biochemical activities at the sample site.

We are familiar with the use of genomics in breeding and selecting new plant varieties and as a tool employed by CDC and FDA to help identify specific pathogens during illness outbreaks and we have witnessed how genomics and metagenomics have been leveraged in research funded by CPS: to track *Listeria monocytogenes* (Lm) movements in citrus packing environments (Suslow 2018), to measure tolerance and physiological responses of pathogens to sanitizers (Wiedmann 2017) and to detect *Cyclospora* in irrigation waters (Lopez 2019). But in Session 4, we learned how genomics and metagenomics are coming to the forefront in produce safety research.

If you have been regularly involved with CPS over the years, you know that a number of projects have been funded in search of indicators for *E. coli* O157:H7, *Salmonella* and Lm. Indicator organisms are non-pathogenic and are used like "canaries in a coal mine" to indicate environments suitable for survival or growth and for potential presence of pathogens in foods or the production environment. Indicators like generic *E. coli* have been used as an indicator for STECs in closed irrigation water systems and a variety of indicators have been used to assess and verify sanitation practices in facilities and on equipment.

Thus, our first presentation of Session 4 was by ***Gloria Sánchez Moragas*** from IATA-CSIC in Spain, titled "*Metagenomics to identify viral indicators in the produce chain*" (Sanchez Moragas 2019). We sometimes forget that Norovirus is the most frequent cause of foodborne illness, overall, and because human viruses represent significant challenges to culture or predict an infectious from a non-infectious state, they are harder to work with than pathogenic bacteria. It has long been recognized that fecal bacterial indicators are not reliable indicators of human

enteric viruses. Therefore, this project focused on identifying biological and chemical indicators or markers that could quickly and reliably indicate the presence or absence of human pathogenic viruses in agricultural inputs, within the on-farm agricultural environment, on produce commodities and in produce handling facilities. The research leveraged genomics and metagenomics to characterize the diverse community of viruses and bacteriophages (the "virome") from archived and newly collected irrigation water samples from wastewater reuse sources, produce, and feces in order to identify indicators of the presence or absence of human pathogenic viruses. Key learnings include:

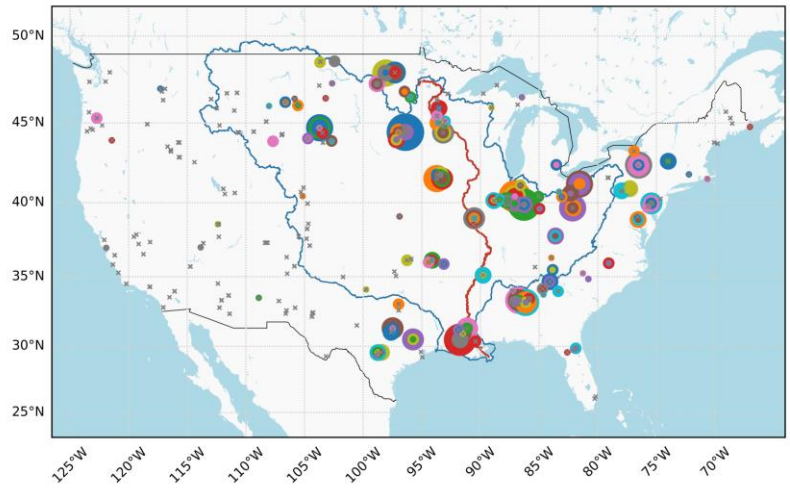
- ***Methods for concentration and detection developed***. A detailed procedure for extraction and purification of the virome permitted generation of sequencing libraries for each sample. Irrigation, wastewater, and produce samples were collected from two different fresh produce growing regions in Spain over a two-year period.
- ***Human enteric viruses detected in water samples***. Human enteric viruses were highly detected in "reuse" irrigation water and wastewater samples. The viral community composition from 235 archived and newly collected samples was characterized. There was a general correlation between occurrence of human enteric viruses and the presence of statistically predictive viral indicators.
- ***crAssphage presence was significantly correlated to human enteric virus presence***. The recently proposed viral indicator crAssphage (Bibby 2018) was significantly correlated with the presence of human enteric viruses. Metagenomic profiling and comparison of the viral community in positive and negative samples for enteric viruses suggest cross-assembly phage or crAssphage was significantly correlated with the presence of human enteric viruses in 'reuse' irrigation water, stool, and produce samples. Applicability to other ag-water sources is reasonable. Ideally, an indicator for human enteric viruses would be found 100-percent of the time in positive samples and not found in negative samples. But biology is almost never that absolute, so crAssphage was found in 89-percent of samples positive for Norovirus, 33-percent in samples negative for Norovirus, 82-percent of samples positive for human enteric viruses, and 23-percent of samples negative for human enteric viruses.
- ***Six additional phages night be indicators of human enteric viruses***. The metagenomics data from this study also identified six new phages as potential indicators to predict enteric virus contamination. This collection of six phages was associated with positive samples for Norovirus in 74 to 93-percent of the samples, 18 to 40-percent of the negative Norovirus samples, 65 to 89-percent of the positive enteric virus samples and 9 to 29-percent of the negative enteric virus samples. Further studies and application of a business risk management and public health perspective are needed to confirm their suitability in predicting enteric virus presence for decision-making. These perspectives are necessary prior to investment in the essential steps to develop rapid and commercially practical methods for their application.

The Sánchez Moragas project demonstrated the power of genomics and metagenomics to examine the complex microbial community of waste impacted irrigation water and identify potential indicators for human enteric viruses, which could not be accomplished as readily by traditional microbiological approaches.

Moving ahead, the produce industry has been laser-focused on the risk of Lm for over a decade. CPS has prioritized and funded 46 projects at 21 institutions to help build the industry knowledge base on *Listeria,* its presence in facilities, residency in biofilms, persistence and movement in various production environments, and sanitation strategies to control the risk it represents. Staying with the theme of genomics and metagenomics to address industry challenges, ***Martin Wiedmann*** from Cornell University presented his latest project, "*Listeria whole genome sequence data reference sets are needed to allow for improved persistence assessment and source tracking"* (Wiedmann 2019). Whole genome sequencing of Lm has been used by public health and regulatory agencies to identify and define outbreaks, integrate with epidemiological data, and conduct targeted foodborne disease surveillance assignments. Characterization of Lm isolates obtained from foods, food processing facilities, and food-associated environments for the last several years has expanded the public database. Despite these efforts, due to a lower priority, there is only limited information on the distribution and diversity of Lm and *Listeria* whole genome sequence-based subtypes in non-food associated environments. Owing to the fact that whole genome sequence data is limited to foods and food production environments, our interpretation of genome sequencing data is hindered because we cannot adequately assess the likelihood of closely related Lm and *Listeria* spp. being isolated from different sources and differentiate *Listeria* persistence in produce fields, packinghouses, or processing facilities from re-introduction. This project involved the collection of more than 1,000 soil samples from across the US, focusing on non-agricultural and natural environments, followed by testing of samples for Lm and *Listeria* spp. and performing genomic sequencing on representative isolates. The result was the collection of 659 Lm and 1,195 *Listeria spp*. isolates, with whole genome sequence data generated for 177 Lm isolates and 417 *Listeria spp*. isolates. Key learnings include:

- ***New Listeria species and future opportunities***. The project developed a national map of *Listeria* and enriched our collection of *Listeria* by 1,854 isolates, which represents opportunities for future research and listeriosis tracking. The project expanded the taxonomy of *Listeria* with the identification of five new species.
- ***Prevalence of Listeria in soil from the natural environment (mostly state parks and public areas) was about 31%, with 12% of soil samples positive for Lm***. Results showed that the diversity of *Listeria* in soil was very high, with 14 known species and 5 potential new species identified. Lm was the most prevalent species, followed by *L. welshimeri* and *L. seeligeri*, though these results may be, in part, an unintended bias of the cultural enrichment process. *L. seeligeri* was only found in the northern US. *L. booriae* was isolated for the first time from soil and ranked fourth in prevalence and was noteworthy in that it had a surprisingly large genome size (3.5-4.0 Mbp).
- ***The highest concentration of Listeria was in the center of the US***. Distribution of *Listeria* in soil across the US was delineated by longitude and elevation and mainly driven by soil moisture and sodium content. *Listeria* was particularly prevalent in and around the Mississippi River Basin. A total of 6 Lm isolates from soil were found to be closely related to clinical isolates submitted to the NCBI public database mentioned above (<20 cgMLST mismatches). *Listeria* spp. from non-food associated environments and from food-associated environments were not very closely related except for *L.*

seeligeri. Different Lm lineages and *Listeria spp.* exhibited heterogenicity in prevalence, geographic clustering pattern, and distance-decay relationships.



Sampling map and isolation of Listeria spp. across the U.S. showing concentration of isolate recovery in the Mississippi Basin and the middle of the country. Diagram courtesy of Martin Wiedmann, Cornell University.

- ***Lm was detected in soil***. Three different lineages of Lm were detected in soil, a well-known environmental source. While prevalence of Lm lineage I and II was not high in soil, they do represent a high risk for virulence to humans**.** Illness from direct soil transfer to an individual is not held to be a significant risk. Lm lineage III was the most prevalent.
- ***Lm isolates from the environment and humans are genetically similar***. Whole genome sequence data shows that Lm isolates from natural environments may differ from clinical or human isolates by as few as 10 cgMLST alleles. cgMLST alleles are commonly used to measure the number of shared genes between organisms. So, this data demonstrates that the Lm isolates taken from around the country in natural settings, are remarkably genetically similar, which is further evidence that low single nucleotide polymorphism (SNP) differences between human and food or environmental isolates does <u>*not*</u> necessarily imply a causal link in a recall, outbreak, or archival clinical isolate.

Genomics tools have been applied to give us a greater understanding of *Listeria* spp. and their distribution in the environment, and have enabled sequence data to be added to a growing database that can be used for research and to permit improved source tracking during listeriosis outbreaks. In particular, the comparison sequencing data from environmental Lm isolates and clinical Lm isolates may be fairly small and underscores the cautions the industry and regulatory agencies should observe. While SNP differences are valuable in creating links between outbreak strains and product or facility-level samples, they may not be sufficient. Epidemiological data, traceback, and investigative observations remain critical to identifying the source of a listeriosis outbreak.

Moving through Session 4 we examined the complexities of composts, with a specific focus on control of Lm survival and transmission through application of soil amendments. ***Xiuping Jiang***

from Clemson University presented her final project report on *"Identifying competitive exclusion microorganisms against Listeria monocytogenes from biological soil amendments by metagenomic, metatranscriptomic and culturing approaches"* (Jiang 2019). We know that in-process or non-composted animal waste–based biological soil amendments are nutrient sources that promote plant growth, improve soil texture, and may also be sources of microorganisms that benefit soil health. However, if composts are not prepared properly, they can potentially be a source of contamination by human pathogens, including Lm. This project was focused on identifying competitive exclusion (CE) microorganisms against Lm, in other words microorganisms that have the ability to inhibit the growth of Lm, if present in composting organic matter, by various mechanisms. The research team hypothesized that "composts are excellent sources of CE microorganisms" and that next-generation sequencing technology could be employed to speed up the discovery of CE mitigations for Lm. Key learnings include:

- ***Studies confirmed that composts are microbially diverse***. Six different dairy and six different poultry-based composts were analyzed at both the active and finished stages. Genomic and metagenomics approaches were employed to identify 800 bacteria and 180 fungal species recovered from different composts.
- ***CE bacteria were identified by genomic and metagenomic techniques***. Lm presence in composts enabled genomic and metagenomic tools to identify genera and species which appeared to react to Lm by producing inhibitory compounds, including bacteriocins, that prevent Lm growth. Composts were inoculated with Lm to elicit genetic responses from the microbial community of in the composts and these were measured by recovery and sequencing of 16S (bacteria) and 18S (fungal) rRNAs. The introduction of Lm to composts changed the abundance of some microbial members and gene expression levels associated with bacteriocin production. Among the top genera found were *Ureibacillus*, *Sphaerobacter* and *Bacillus* in all dairy composts, and *Bacillus* and *Paucisalibacillus* in poultry composts. Metagenomic sequencing analysis confirmed that the five most abundant phyla accounted for at least 91-percent of all reads in the active dairy compost samples.
- ***Lm growth was inhibited by co-culturing with putative CE isolates***. Through different culturing methods**,** a total of 40 bacterial isolates from 22 species were confirmed with anti-Lm activities. Using traditional culturing methods, 50-percent of the isolated CE strains were *Bacillus*. Though Lm survived and remained viable, growth of Lm was prevented by co-culturing with CE strains in both dairy and poultry compost extracts under all incubation conditions. Growth-inhibition effect from CE strains increased in more concentrated compost extracts at 35°C. Though very preliminary, these results indicate that CE organisms can potentially be used as biological tools to control Lm populations if contamination occurs in compost, and possibly in other applications.
- ***Metatranscriptomics data demonstrates bacteriocin synthesis in CE organisms challenged with Lm***. From the metatranscriptomic sequencing data, comparisons of gene expression changes associated with specific microbial functional pathways in compost with and without Lm demonstrated an increased level of bacteriocins production in the compost samples containing Lm, which indicates that the interactions between Lm and compost microflora may include competition for compost nutrients and the presence of antimicrobials produced by CE bacteria.

The last application of genomics and metagenomics covered in Session 4 of the CPS Symposium was presented by ***Kerry Cooper*** of the University of Arizona in his interim report, *"Illuminating the role of whole genome sequencing in produce safety"*. This research project aims to study human pathogen persistence in vegetable production environments and to determine their mutational rates, leading to diversification. This research can help the industry begin to understand if the pathogens are actively replicating in these environments or simply surviving. Ultimately, the research team hopes to examine the influence that the microbiome has on survivability of pathogens in different agricultural environments representing different geospatial conditions. This information could lead to a better understanding of pathogen die-off rates in different environments and help elucidate mutational rates to enable a better understanding of single nucleotide polymorphism differences between isolates and their degree of relatedness. Some preliminary learnings are:

- ***Microcosms used to simulate field conditions may provide predictive models***. Since human pathogens cannot be released into open vegetable production fields, microcosms set up in incubators have been established to represent Yuma, AZ, growing conditions from September to February and Salinas, CA, production regions from January to June. Fidelity to pathogen behavior in field conditions is a long-term and critical goal.
- ***Arizona growing conditions appear to diminish pathogen survival in soil***. In the initial microcosm studies looking at Arizona conditions, in one soil type, where temperatures are warm and humidity low, all three pathogens—*Salmonella*, *E. coli* O157:H7 and *Listeria*—died every 2 weeks. There was some low-level cycling in January and February conditions.
- ***Pathogen survival in California soils appears to be more robust***. The microcosm studies simulating California Central Coast conditions, in one soil type, resulted in *Salmonella s*urvival for 12 weeks in soil and 16 weeks in irrigation water and survival, with cycling every 2 weeks. *E. coli* O157:H7 survived at 26 weeks in the soil and irrigation water while *Listeria* survived 10 weeks in soil, 12 weeks in irrigation water and survive cycling every 2 weeks.
- ***More to come***. Since this was an interim report, there will be much more to come in terms of the measures of the impact of these production environments on genome mutation and persistence of pathogens under changing environmental conditions.

Why are these projects important to the industry? Owing to the challenges of delivering perishable products across great distances, variable production environments and the pressures to make orders every day of the year, we sometimes fail to take the time to look out beyond a day or a week, let alone look ahead to next year or the year after. Session 4 was an opportunity to look ahead two or three years and reflect on how much produce safety science has evolved and the significance of how maturing genomic and metagenomic technologies are presenting opportunities to advance the science still further. The four research programs that comprised Session 4 employed genomic and metagenomic approaches to address key produce safety knowledge gaps. The subject areas are not new to us and we have certainly seen significant progress in the search for indicator organisms, *Listeria* biology and control,

composting process management, and pathogen die-off rates in the environment in recent years. But to evolve the science to meet these industry challenges more fully, we are at a point where there is an opportunity to employ new technology to ask questions in novel ways, develop knowledge and insights previously unattainable, and drive us toward science-based actionable steps. So, while Session 4 does not provide instant solutions to key produce safety challenges, it does alert us to proactively get ready to be able to beneficially leverage knowledge generated by genomics and metagenomics.

For example, the Sánchez Moragas project on the search for indicators of human enteric viruses creates awareness about this public health risk and notifies growers that the advent of viral indicators may mean another measurement for microbial water quality that will need to be added to their produce safety plans in the next few years. It serves to reinforce grower attention on knowing their water sources and taking every precaution to prevent any possible contamination from wastewater or other sources that may carry human waste harboring enteric viruses. It is important to note that in the discussion following the presentation, Dr. Sánchez Moragas reported her limited testing of agricultural irrigation water samples where the group found human enteric viruses as well as crAssphage. This project reminds growers that it is important to stay current with this area of research so they can understand what it means and what it does not mean. crAssphage and the six putative phage indicator candidates for enteric viral pathogens are not presently culturable. Without the ability to culture the indicator or enteric viruses, we cannot know if the organisms are dead or alive, as the detection is based only on the presence of DNA or RNA. However, being able to find their DNA or RNA certainly indicates that they were present at some point and that your water source is being impacted by human waste in some fashion, a caution for any grower or packer of produce. By following this area of research and knowing its strengths and weaknesses, growers can be positioned to leverage future developments to enhance their produce safety efforts.

Similarly, the Wiedmann research should create awareness for the produce industry, and at a high level the results re-affirm the oft spoken adage that "*Listeria* is everywhere". But it also tells growers that while *Listeria* may be everywhere, its distribution is certainly variable and likely dependent on weather conditions, soil types, and other factors. The silver lining is that this variability and the data derived from this project and other *Listeria* genomic study activities are contributing to a database that may have value in source tracking outbreaks more effectively. Surprisingly, *Listeria* was detected less frequently on the West Coast. Before West Coast growers and packers celebrate, it must be remembered that this project was carried out over one year and that a low frequency of detection does not mean there is no risk of *Listeria* contamination in the West. In fact, we know that Lm can be found in Western fruit and vegetable production environments from previous research projects and incidents of product contaminations and recalls due to Lm contamination emanating from Western production locations.

This project is also a call to action for growers, packers, processors, and distributors that there is power to adding additional samples to the existing genomic database. Extension of our knowledge of the geographic distribution of *Listeria* spp. and Lm will ultimately enable more

effective source tracking that could help resolve outbreaks more quickly which in turn might permit more timely root-cause evaluations so that the occurrence of future outbreaks can be reduced. But building *Listeria* genomic databases inclusive of meta data (the information associated with the specific sample location and source) to provide context requires input and help from the industry. *Listeria*-positive samples or isolates need to be provided to researchers so they can be speciated and sequenced to help build the fidelity of the *Listeria* database. There has been reluctance by some to provide isolates to public entities due to the fear that these isolates could eventually be used to implicate the donor in a future outbreak. While that is certainly possible, one also must consider that the detection of a *Listeria* positive in a facility should spark a "search and destroy" response to eliminate the source of the contamination and follow-up testing to make sure the contamination is not resident and recurrent. It is also important to note that a genomic database could also exonerate those that are not involved in an outbreak, freeing specific production regions or even specific growers, packers or processors thereby narrowing the scope of an incident and minimizing damage to the industry and consumers.

The Jiang project on the identification of CE organisms from composts to control Lm in fields and facilities is an example of the value of genomic and metagenomic tools. Gaining a deeper understanding of how microorganisms interact with each other physiologically in composts may present opportunities in the future to use CE organisms to leverage their defense mechanisms to control Lm.

The Cooper work is still in early days but reinforces the notion that die-off rates are dependent on many environmental factors. The use of controlled environments to simulate growing regions and the deployment of genomics to track pathogen persistence may help lay a foundation for the key drivers of pathogen persistence and help the industry build region by crop models.

Why are these projects important to the research community? While the biology and the procedures of genomics and metagenomics are well known to the research community, the Session 4 projects are good examples of how these tools can be applied to real-world industry challenges. A recurrent theme of the 2020 CPS Symposium webinar series has been the importance of collaboration between industry and the research community. The four research teams that participated in Session 4 have a long history of industry collaboration and outreach that has enhanced their research efforts. Collaboration can be access to fields, facilities, or equipment and it can also mean exchange of industry knowledge and scientific expertise. During the question and answer period at the end of the session there were several inquiries to Dr. Cooper about the soil types and the variability of conditions in the Salinas Valley in California and how they might impact persistence and mutation rates. Indeed, there are probably more variables (e.g. tillage, nutrients, UV exposure, humidity, temperature, CE organisms) than can be addressed in a single study and "mission creep" can be a problem, but it was reassuring to listen to this exchange and witness industry willingness to provide input and a researcher responding with enthusiasm.

It was also striking that the knowledge we gain from using advanced genetic technologies to examine critical industry knowledge gaps drives us to ask better questions for the research community to address. For example, the identification of putative viral indicator organisms places the focus on verifying their utility in agricultural waters and developing *in vitro* culture systems. The *Listeria* distribution work and the pathogen persistence projects both speak to the mutation rates in pathogens and the interpretation of SNPs to determine whether isolates are from the same source during source tracking in outbreaks. The CE project raises yet again the potential use of bacteriocins to mitigate human pathogens and with it the complexities of working within biological systems to find conditions that permit commercial scale outcomes.

As we have witnessed in previous sessions, the use of new tools like computer-based modeling, antimicrobial plastics, bio-fencing, and genomics means that a multifunctional, broader array of scientific expertise is being and will need to be brought to bear to meet industry produce safety research needs.

Why are these projects important to regulators? Session 4 provides the regulatory community with an update on how genomics and metagenomics are being leveraged to address key industry produce safety challenges. Regulatory agencies have been actively engaged in genomics for source tracking during illness outbreaks, and undoubtedly the *Listeria* data on similarities between environmental and clinical Lm samples will guide future directions on determining strain commonality. The data can clearly help to improve interpretation of whole genome sequencing results going forward, but it also supports the ongoing importance of strong epidemiological data as a foundation to provide context for genomic interpretations of isolate identity and source tracking. The call to action to contribute to genomic databases has been discussed in reference to growers, but FDA and others need to continue to find ways to make isolate donations easy for donators and to educate transparently on how the databases are to be used and how decisions will be made based upon the data.

Lastly, the research projects of Session 4 provide the regulatory community with the same opportunity it does the grower, shipper, processor community to look out ahead to see how emerging technologies are driving the next generation of produce safety knowledge. This awareness is critical to prepare regulators and other government agencies to: innovate novel preventive controls that might arise from these projects, support next steps towards adoption of better tools to detect viral contamination in agricultural waters, form a better understanding of pathogen persistence in key produce production environments, and consider how CE organisms or anti-*Listeria* compounds from those CE organisms might be used to control Lm.

Join us for Session 5 of the 2020 CPS Research Symposium on July 21, 2020, at 1:00 PM Eastern and 10:00 AM Pacific time. The program will feature two final research reports and three interim reports focusing on a variety of important topics from agricultural water pathogen die-off rates, produce safety in shade houses, the impacts of sanitizers on viable but not culturable (VNBC) bacteria, agricultural water treatment methods, and the development of models to

predict the impact of sediments on microbial quality of irrigation water. For information on how to register for Session 5, see the CPS website: www.centerforproducesafety.org.

***Acknowledgements**: The Center for Produce Safety would like to thank the researchers who made presentations during session 4 of the 2020 Research Symposium. Their presentation of research results and their discussion of what that research might mean to the produce industry certainly informs the content of this paper. More detail on these research projects can be found at www.centerforproducesafety.org. This discussion of key learnings contained here is meant to inform and provoke thought with an eye towards inspiring readers to examine their own produce safety programs and to use the research to make improvements. It is not meant as a directive on what must be done to produce safe food. Produce safety needs to be determined on an operation by operation basis; there are no one size fits all solutions. If you have additional questions, please feel free to contact Bonnie Fernandez-Fenaroli (Bonnie@centerforproducesafety.org). Thank you.*