Environmental Screening of A Cannabis Production And Processing Facility:

A Comparison of the PathogenDx Enviro^{X}™ Microarray and Traditional Microbiological Plating Methods

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ABSTRACT

As the cannabis industry continues to expand and become more heavily regulated, the need for screening tools which detect microbial contamination increases. While screening has primarily focused on screening of the raw product, there has been little emphasis on the actual facilities in which that product is processed, which has the potential to be a contaminating source for the cannabis product. The following case study was performed to demonstrate the utility and necessity of environmental screening in a cannabis production and processing facility. Samples were collected for assessment of microbial contamination across 11 locations throughout the facility. Each sample was assessed by traditional microbiological plating and the PathogenDx Enviro^{X} microarray to compare the effectiveness of both methods.

Comprised of 56 environmental samples, each tested positive for bacterial and fungal contamination when analyzed by both methods. While there were variations in the microbial species present within each of the rooms tested, Enviro^{X} detected contamination with higher sensitivity than traditional plating methods. Across the entire study, the most prevalent species detected were *Pseudomonas* spp., *Golovinomyces* spp., and *Cladosporium* spp., while several organisms were isolated to only a single room. Generated by the Enviro^{X} microarray, these species-level identifications and their distribution throughout the facility also revealed some temporal and spatial relationships between certain microbial contaminants.

INTRODUCTION

Environmental surveillance is increasingly appreciated for its utility in public health efforts, including those related to agriculture and quality control (Groseclose & Buckeridge, 2017). Presence of pathogenic organisms in production facilities is concerning, as it indicates that there may be a reservoir of the contaminating organism(s) within the facility (Bartz et al., 2017). Importantly, these pathogenic organisms may be capable of posing a significant risk to both human health and agricultural products. More specifically, the presence of agricultural pathogens in horticultural operations may increase the risk of agricultural disease, increasing the likelihood of agricultural losses and rejected batches of product. These concerns persist not only in agricultural operations, but also food and drug production.

Provided the potential ramifications of microbial contamination in such a facility, it is important that practices in risk reduction are diligently followed and maintained. An important component of risk reduction is the continuous screening and monitoring of a facility for microbial contamination. Importantly, while an initial investigation may be able to detect and identify contaminating organisms present within a facility, subsequent screening and surveillance is necessary to confirm the effectiveness of decontamination methods, verify sources of contamination, validate the cleanliness of a facility, as well as identifying any new instances of contamination that may occur.

Currently, the golden standard for the screening, detection, and assessment of microbial contamination has been through culturing on agar plates (Davey, 2011). While traditional plating methods offers a visual confirmation of microbial presence and viability, and does not require sophisticated equipment, this method does pose several disadvantages. For instance, plating is incredibly time-consuming, can be laborious, and requires the expertise of a skilled microbiologist. Additionally, a comprehensive microbiological analysis, which encompasses the detection of multiple types of organisms, often requires the use of multiple plates and media types, increasing material and labor costs; even so, species-level identifications require secondary methods of confirmatory testing.

Though their use has not yet become standard in the industry, the use of microarrays for broad-spectrum microbial detection and identification has been well-studied for its many applications in clinical and agricultural settings (Kostic & Sessitsch, 2012). Heavily implemented into the design of the PathogenDx Enviro^{X} array, microarray technology is able to identify microorganisms at the family, genus, and species-level of classification. Multiplexed in design, microarrays are capable of simultaneously detecting a multitude of microbial isolates within a single sample in a matter of a few hours, making the array more cost-effective and less labor-intensive than traditional plating methods. While microarrays do require specialized equipment and training in molecular techniques, the mechanism of recognition of specific DNA sequences imparts a high degree of both specificity and sensitivity in microbial detection.

This study was conducted in an effort to highlight the utility and necessity of environmental screening of an agricultural production and processing facility, and to compare the proficiency of traditional microbiological plating and the PathogenDx Enviro^{X} array in executing this screening.

METHODS

Sample Collection

56 swabs (each suspended in 4mL of buffered peptone water) were provided to the collaborating facility for this study. Sampling locations and intervals of sample collection were determined at the discretion of the facility contact. Immediately following sample collection (performed according to the procedure outlined in PathogenDx Product Insert: Enviro[X] Environmental Swab), swabs were stored at 4°C until return shipment to PathogenDx (samples shipped with ice packs to ensure samples remained cold over the course of delivery). All swabbing was conducted between March 7, 2019 and April 15, 2019, and samples were returned to PathogenDx over the course of three shipments shortly after collection time.

Sample collections spanned across 11 distinct rooms within the facility (listed below):

- **Veg 1**
- **Veg 2**
- **Propagation**
- **Post-Harvest**
- **Dry Room**
- **Mother Room**
- **Clone Room**
- **3rd Party Lab Sampling Room**
- **Packaging**
- **Packaging 2nd Room**
- **Inventory**

Documentation was provided for each sample collection, including date of collection, site of collection, and whether the swab site was swabbed prior to, or after, decontamination procedures were performed ("dirty" and "clean" designations, respectively). Schematics were also provided for seven of the above locations (Veg 1, Veg 2, Propagation, Post-Harvest, Dry Room, Mother Room, and the Clone Room).

Sample Analysis

Upon arrival, swab collections were homogenized by vortexing and aliquoted into 1mL samples for analysis via traditional microbiological plating and PathogenDx Enviro[X].

TRADITIONAL MICROBIOLOGICAL PLATING

Each sample was plated on a general medium for bacteria (Tryptic Soy Agar, TSA) and fungi (Sabouraud Dextrose Agar with chloramphenicol, SDA) to capture as many microbial contaminants as possible.

In addition to a neat sample, a 1:10 and 1:100 dilution was plated for each swab collection to ensure that individual isolates could be visualized in the event that heavy concentrations of microbial contaminants within the samples resulted in overgrowth of the plates. For each concentration, 100µL of sample was plated.

Both plate types were incubated at room temperature (25°C); TSA plates were incubated for two days and SDA plates were incubated for six days.

PATHOGENDX ENVIRO[X] MICROARRAY

Microarray analysis was performed on 1mL of the original sample according to the procedure outlined in the PathogenDx Product Insert: Enviro[X] Environmental Swab.

RESULTS

For each sample, the results of the traditional plating and Enviro^{X} methods were compared directly; a representative example of the comparative analysis is demonstrated in Figure 1, which displays the results for swab number one (located in Veg 2 within the facility). Microbial contamination is apparent, and robust, on both TSA and SDA plates, representing bacterial and fungal growth, respectively. While a dilution effect can be observed in the growth on the SDA plates, the growth observed on the TSA plates remained too concentrated to discern individual colonies. Interestingly, swab number one was collected after cleaning and disinfectant procedures had been conducted for this particular swab site ("clean" designation). While unable to definitively identify the contaminants from the plates alone, several categorical and species-level identifications were ascertained by the Enviro^{X} microarray, including Total Aerobic Bacteria (TAB), Total Enterobacteriaceae (TE), Bile-Tolerant Gram-Negative (BTGN), and Total Yeast and Mold (TYM); species-level identifications included *Aeromonas* spp., *Pseudomonas* spp., and *Cladosporium* spp. Similar trends were observed throughout the remaining study samples.

Of the 56 swab collections analyzed, all samples tested positive for microbial contamination through both traditional microbiological plating and Enviro^{X} analysis methods (**Table 1**). In comparing the number of swabs with confirmed growth on TSA (bacterial) to the number of confirmed detections on TAB (bacterial) on the Enviro^{X} array, Enviro^{X} displayed equal or greater sensitivity in detecting contaminating microorganisms as compared to the microbiological plating in all cases (**Table 2**). The same trend was observed, to a greater extent, in comparing the number of swabs with confirmed growth on SDA (fungal) as compared to the number of swabs with confirmed detection of TYM (fungal). Notably, TE and BTGN are more defined subcategories of TAB and consequently, were detected at a lower frequency than TAB.

The composition of microbial species detected was variable depending on the location analyzed (**Table 3**). Veg 1 presented with the highest degree of variability with the detection of seven species-level identifications (*Aeromonas* spp., *Pseudomonas* spp., *Pseudomonas aeruginosa*, *Fusarium oxysporum*, *Candida* spp., *Penicillium* spp., and *Mucor* spp.). In contrast, the Clone Room presented with the lowest degree of variability with the detection of only one species-level identification (*Pseudomonas* spp.).

Given the high degree of consistency in organisms detected across different locations within the facility, the frequency of locations which tested positive for each organism was calculated (**Table 4**). *Pseudomonas* spp. appeared to be the most prevalent microbial species within the facility, appearing in 82% of the locations tested (9 out of 11 locations). The next most prevalent species found was *Golovinomyces* spp., and was observed in 55% of the locations tested (6 out of 11 locations). Many microbial species observed were isolated to singular locations within the tested locations (*Pseudomonas aeruginosa*, *Mucor* spp., *Aspergillus terreus*, and *Aspergillus fumigatus*).

Provided the species-specific identifications from the Enviro^{X} analysis, temporal and spatial relationships in the distribution of microbial contaminants were evaluated. An observed temporal relationship is observed in Figure 2, which displays the contamination present at two sites within Veg 1, before ("dirty") and after ("clean") decontamination procedures were utilized. Looking at the two sets of agar plates, there is evident reduction of microbial burden after decontamination procedures were utilized, but they were not sufficient to remove all microbial contamination at these two sites. Interestingly, the microarray data indicated that four species of microbial contaminants were present before decontamination (*Aeromonas* spp., *Pseudomonas* spp., *Fusarium oxysporum*, and *Candida* spp.). After decontamination, only *Candida* had been removed from the sites, and the other three species still remained.

Among others, spatial relationships were observed in the distribution of *Golovinomyces* and *Cladosporium* spp. throughout the facility. As demonstrated in **Table 4**, *Pseudomonas* spp. were detected in 82% of the locations swabbed, and was the most widely distributed organism within the facility. *Golovinomyces* spp. were the second most prevalent, and present in 6 of the 11 locations swabbed. When broken down by location, it was observed that *Golovinomyces* spp. was detected in 86% of the swab sites within the Post-Harvest room (7 of 8 swab sites). In addition to the Post-Harvest room, *Golovinomyces* spp. were identified in the Dry Room, 3rd Party Lab Sampling room, Packaging, Packaging 2nd room, and the Inventory. Followed closely behind, *Cladosporium* spp. were present in 5 of the 11 locations swabbed, and was primarily concentrated in Veg 2, where all 9 of the swab sites within the room tested positive (**Figure 3**).

DISCUSSION

This study highlights the utility of environmental screening as a tool to evaluate potential microbial contamination within an agricultural production and processing facility. While largely harmless, many of the microorganisms detected in this surveillance carry potential risks to both human health and agricultural products and yields.

Highly ubiquitous in the environment and agricultural samples, it is unsurprising that *Pseudomonas* spp. were the most prevalent isolate in this study; while some species of *Pseudomonas* can be harmful, many serve as plant commensals and are not especially concerning (Sitaraman, 2015). Conversely, the detection of organisms such as *Golovinomyces* spp. and *Cladosporium spp*. are more concerning. The presence of *Golovinomyces* spp. are particularly problematic from an agricultural perspective, as these species are a major cause of powdery mildew in plants, which is capable of reducing or destroying agricultural yields (Lebeda & Mieslerova, 2011). By comparison, *Cladosporium spp*. are relatively ubiquitous in the air, but can be a significant allergen, and can pose health concerns in susceptible individuals (Bozek & Pyrkosz, 2017). Without environmental screening, microbial contamination such as this may go undetected, imposing risks to agricultural yields and human health. Provided such screenings, steps can be taken to reduce contamination, modify decontamination procedures as necessary, and monitor facilities to ensure rapid detection of any recurrence of contamination.

Further, this study emphasizes the advantages of utilizing the PathogenDx Enviro^{X} microarray technology in microbial detection, as compared to traditional microbiological plating. In addition to producing results in a more cost-effective and rapid manner as compared to traditional plating, the Enviro^{X} microarray displayed equal or greater sensitivity in detecting microbial contamination in all sample cases (**Table 2**). In fact, there were many cases in which the agar plates displayed no growth but Enviro^{X} detected contamination, for both bacterial and fungal isolates. Further, Enviro^{X} provided speciation of many of the contaminants present, a distinguishing characteristic that could not be determined by the agar plating alone. Notably, without these species-level identifications, the observed temporal and spatial relationships could not have been ascertained. While some patterns can be observed from the agar plates, the species-level identifications could not be made without further experimental analysis. Further, without species-level identifications, the degree of risk associated with the specific contaminants present cannot be fully appreciated. Taken together, these data support the usefulness and need for environmental screening in agricultural processing facilities, and highlights the critical advantages in utilizing microarray technology for microbial detection, as opposed to traditional microbiological methods.

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Figure 1: Representative comparison of traditional microbiological plating and PathogenDx Enviro^{X} for a single experimental sample

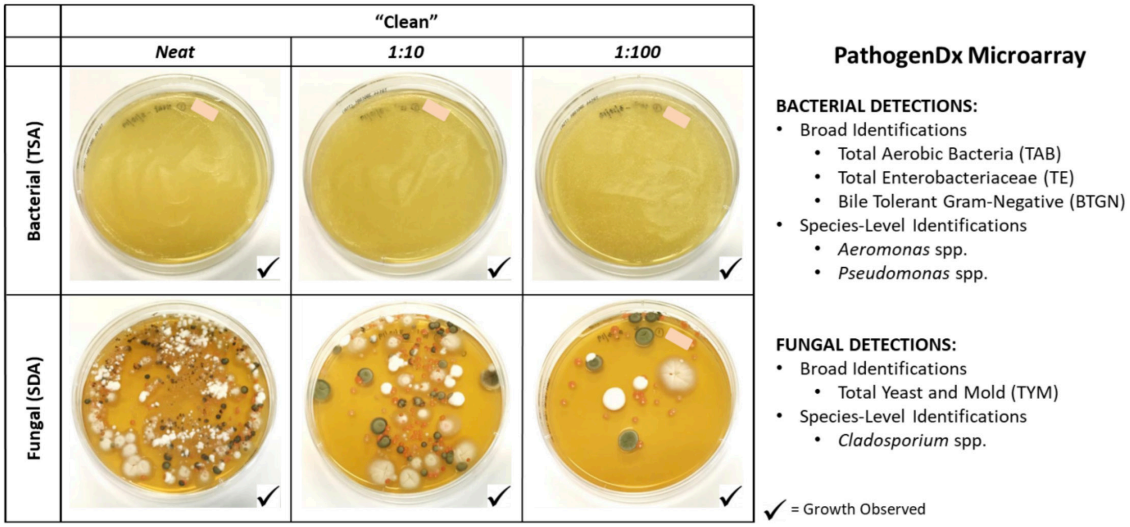

Figure 1: Bacterial-specific plates are displayed in the top row and the fungal-specific plates are displayed in the bottom row (concentration of sample decreases from left to right (neat, 1:10, and 1:100 of the original sample)). This sample has been designated as "clean", indicating that the sampling was performed after decontamination procedures had been conducted. The corresponding Enviro^{X} microarray data is displayed on the right. Above analysis represents swab sample number one, from the Veg 2 room.

Table 1: Microbial Characterization of Swabbing Collections by Traditional Microbiological Plating Methods and Enviro[X] Microarray Technology

		Micro Plating		Enviro[X]				
Swab Number	Location	TSA	SDA	TAB	TE	BTGN	TYM	Species-Level Identifications
1	Veg 2	√	√	√	√	√	√	*Aeromonas, Pseudomonas, Cladosporium*
2	Veg 2	√	√	√	√	√	√	*Aeromonas, Cladosporium*
3	Veg 2	√	√	√	√	√	√	*Aeromoans, Pseudomonas, Cladosporium*
4	Veg 1	√	√	√	√	√	√	*Aeromonas, Pseudomonas, Pseudomonas aeruginosa, Fusarium oxysporum, Candida*
5	Veg 1	√	√	√	√	√	√	*Aeromonas, Pseudomonas, Fusarium oxysporum, Candida*
6	Veg 1	√		√	√	√	√	*Pseudomonas, Penicillium*
7	Veg 1	√	√	√		√	√	*Pseudomonas*
8	Propagation	√	√	√	√	√	√	*Aeromonas, Pseudomonas, Fusarium oxysporum, Candida*
9	Propagation	√		√		√	√	*Pseudomonas*
10	Propagation	√	√	√	√	√	√	*Aeromonas, Pseudomonas, Candida*
11	Post-Harvest	√	√	√			√	*Golovinomyces, Penicillium*
12	Post-Harvest	√	√	√	√	√	√	*Pseudomonas, Golovinomyces*
13	Post-Harvest	√	√	√		√	√	*Pseudomonas, Golovinomyces*
14	Post-Harvest	√	√	√	√	√	√	*Aeromonas, Pseudomonas, Golovinomyces*
15	Veg 1	√	√	√	√	√	√	*Aeromonas, Pseudomonas, Fusarium oxysporum*
16	Veg 1	√	√	√	√	√	√	*Aeromonas, Pseudomonas, Fusarium oxysporum*
17	Veg 2	√	√	√	√	√	√	*Pseudomonas, Cladosporium*
18	Veg 2	√	√	√	√	√	√	*Pseudomonas, Cladosporium*
19	Dry Room	√		√			√	
20	Dry Room	√	√	√			√	*Golovinomyces*
21	Dry Room	√		√				
22	Dry Room	√		√				*Pseudomonas*
23	Post-Harvest	√		√			√	*Golovinomyces*
24	Dry Room	√		√			√	
25	Dry Room	√		√			√	*Golovinomyces*
26	Dry Room	√	√	√		√		
27	Dry Room	√		√				
28	Veg 1	√	√	√	√	√	√	*Pseudomonas, Pseudomonas aeruginosa*
29	Veg 1	√	√	√	√	√	√	*Pseudomonas, Mucor*
30	Veg 1	√	√	√		√	√	*Pseudomonas aeruginosa*
31	Veg 1	√		√		√	√	*Pseudomonas*
32	Mother Room	√		√		√	√	*Pseudomonas*
33	Mother Room	√	√	√		√	√	*Pseudomonas*

		Micro Plating		Enviro[x]				
Swab Number	Location	TSA	SDA	TAB	TE	BTGN	TYM	Species-Level Identifications
34	Mother Room	√	√	√	√	√	√	*Aeromonas, Pseudomonas, Cladosporium*
35	Mother Room	√	√	√	√	√	√	*Aeromonas, Cladosporium*
36	Mother Room	√	√	√	√	√	√	*Aeromoans, Pseudomonas, Cladosporium*
37	Clone Room	√	√	√	√	√	√	*Aeromonas, Pseudomonas, Pseudomonas aeruginosa, Fusarium oxysporum, Candida*
38	Clone Room	√	√	√	√	√	√	*Aeromonas, Pseudomonas, Fusarium oxysporum, Candida*
39	Clone Room	√		√	√	√	√	*Pseudomonas, Penicillium*
40	Veg 2	√	√	√		√	√	*Pseudomonas*
41	Veg 2	√	√	√	√	√	√	*Aeromonas, Pseudomonas, Fusarium oxysporum, Candida*
42	Veg 2	√		√		√	√	*Pseudomonas*
43	Veg 2	√	√	√	√	√	√	*Aeromonas, Pseudomonas, Candida*
44	Post-Harvest	√	√	√			√	*Golovinomyces, Penicillium*
45	Post-Harvest	√	√	√	√	√	√	*Pseudomonas, Golovinomyces*
46	Post-Harvest	√	√	√		√	√	*Pseudomonas, Golovinomyces*
47	3rd Party Lab Sampling Room	√	√	√	√	√	√	*Aeromonas, Pseudomonas, Golovinomyces*
48	3rd Party Lab Sampling Room	√	√	√	√	√	√	*Aeromonas, Pseudomonas, Fusarium oxysporum*
49	3rd Party Lab Sampling Room	√	√	√	√	√	√	*Aeromonas, Pseudomonas, Fusarium oxysporum*
50	Packaging	√	√	√	√	√	√	*Pseudomonas, Cladosporium*
51	Packaging	√	√	√	√	√	√	*Pseudomonas, Cladosporium*
52	Packaging	√		√			√	
53	Packaging 2nd Room	√	√	√			√	*Golovinomyces*
54	Packaging 2nd Room	√		√				
55	Inventory	√		√				*Pseudomonas*
56	Inventory	√		√			√	*Golovinomyces*

TSA = Tryptic Soy Agar; SDA = Sabouraud Dextrose Agar (with chloramphenicol); TAB = Total Aerobic Bacteria;
TE = Total Enterobacteriaceae; BTGN = Bile-Tolerant Gram Negative; TYM = Total Yeast and Mold

Table 2: Summary of Microbial Characterization of Swabbing Collections by Traditional Microbiological Plating and Enviro[X] Microarray Methods

		Traditional Plating (Number of Swabs with Confirmed Growth)		Enviro[X] (Number of Swabs with Confirmed Detection)			
Location	Number of Swab Sites at Location	TSA	SDA	TAB	TE	BTGN	TYM
Veg 1	10	10	8	10	7	10	10
Veg 2	9	9	9	9	5	9	9
Propagation	3	3	2	3	2	3	3
Post-Harvest	8	8	5	8	2	3	7
Dry Room	8	8	2	8	0	1	4
Mother Room	5	5	4	5	2	5	5
Clone Room	3	3	3	3	1	3	3
3rd Party Lab Sampling	3	2	1	3	0	0	3
Packaging	3	2	1	3	0	1	2
Packaging 2nd Room	2	0	1	2	0	0	2
Inventory	2	2	2	2	0	2	2

TSA = Tryptic Soy Agar; SDA = Sabouraud Dextrose Agar (with chloramphenicol); TAB = Total Aerobic Bacteria; TE = Total Enterobacteriaceae; BTGN = Bile-Tolerant Gram Negative; TYM = Total Yeast and Mold

Table 3: Microbial Species Identified at each Location

Location	Species-Level Identifications
Veg 1	*Aeromonas, Pseudomonas, Pseudomonas aeruginosa, Fusarium oxysporum, Candida, Penicillium, Mucor*
Veg 2	*Aeromonas, Pseudomonas, Cladosporium, Fusarium oxysporum*
Propagation	*Aeromonas, Pseudomonas, Fusarium oxysporum, Candida*
Post-Harvest	*Aeromonas, Pseudomonas, Golovinomyces, Penicillium*
Dry Room	*Pseudomonas, Golovinomyces*
Mother Room	*Aeromonas, Pseudomonas, Candida, Aspergillus terreus, Aspergillus fumigatus*
Clone Room	*Pseudomonas*
3rd Party Lab Sampling Room	*Cladosporium, Golovinomyces*
Packaging	*Pseudomonas, Golovinomyces, Botrytis, Cladosporium, Candida*
Packaging 2nd Room	*Cladosporium, Golovinomyces, Botrytis*
Inventory	*Pseudomonas, Cladosporium, Golovinomyces*

Table 4: Prevalence of each microbial species identified during study (11 locations total)

Microbial Species	Number of Locations that Tested Positive for Specified Microbial Species (%)
Aeromonas	5 (45)
Pseudomonas	9 (82)
Pseudomonas aeruginosa	1 (9)
Fusarium oxysporum	3 (27)
Candida	4 (36)
Penicillium	2 (18)
Mucor	1 (9)
Cladosporium	5 (45)
Golovinomyces	6 (55)
Aspergillus terreus	1 (9)
Aspergillus fumigatus	1 (9)
Botrytis	2 (18)

Figure 2: Effect of Decontamination on Burden and Composition of Microbial Contaminants

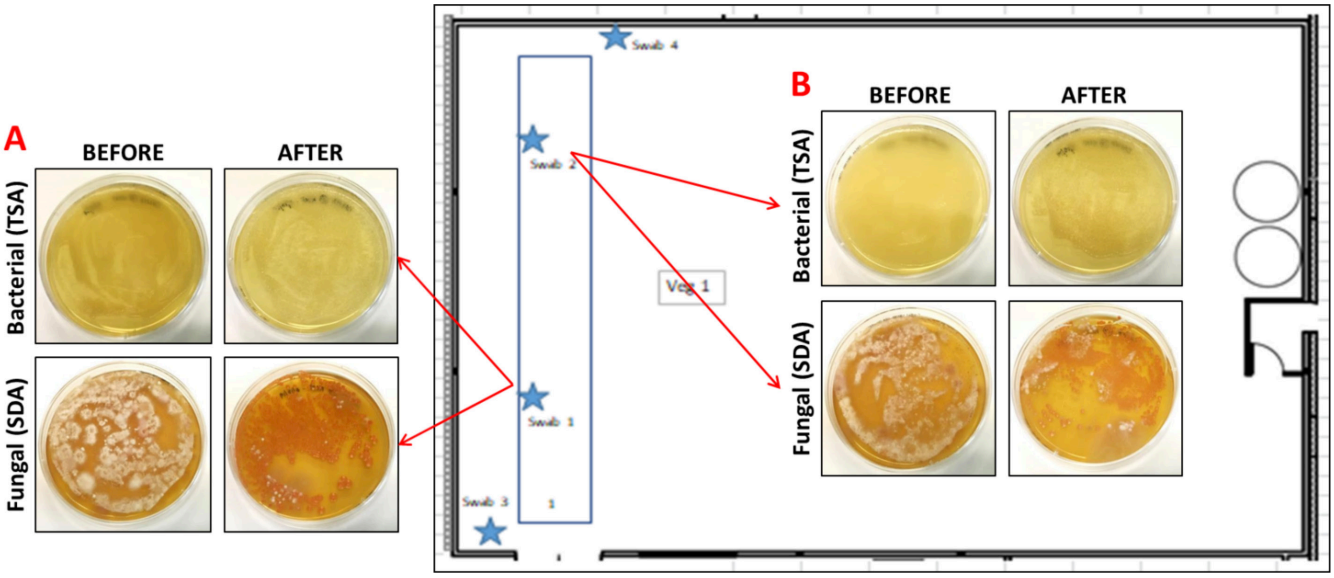

Figure 2: Panel A panel B represent swabbing from two different swab sites. Left columns of each panel are the bacterial and fungal plates from the sampling performed *before* decontamination. Right columns of each panel are the bacterial and fungal plates from the sampling performed *after* decontamination. Microbial species detected before decontamination: *Aeromonas* spp., *Pseudomonas* spp., *Fusarium oxysporum*, and *Candida* spp. (only *Candida* spp. was removed with decontamination procedure).

Figure 3: Spatial Distribution of *Cladosporium* spp. within facility

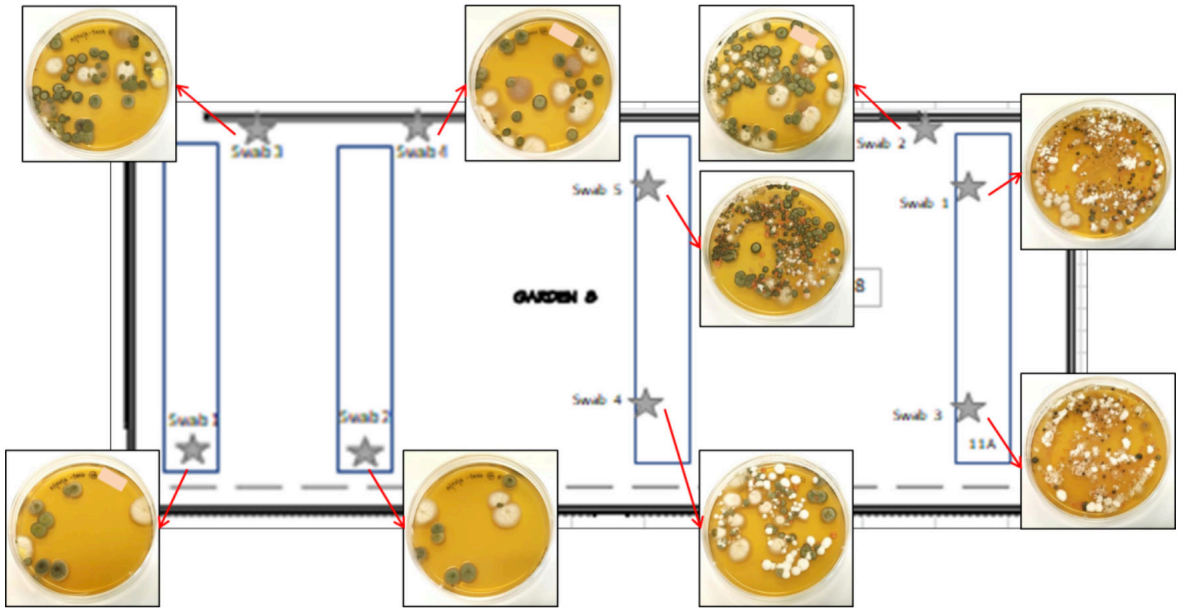

Figure 3: Distribution of *Cladosporium* spp. in the Veg 2 room of facility. Across all collections, *Cladosporium* spp. was identified in 100 percent of swab sites in Veg 2 room (9 swab sites total, represented above).