CLEANING LABORATORY EVALUATION SUMMARY

SCL #:	2021
DateRun:	08/31/2021
Experimenters:	Alicia McCarthy, Aditi Patel
ClientType:	Cleaner Manufacturer
ProjectNumber:	Project #1
Substrates:	Stainless Steel
PartType:	Coupon
Contaminants:	Food
Cleaning Methods:	Manual Wipe
Analytical Methods:	ATP Measurement, Organism count
Purpose:	To evaluate the growth curve by ATP and colony counts for a probiotic product at multiple time points on contaminated and uncontaminated stainless steel surfaces.

Experimental Procedure:

Six autoclaved stainless-steel coupons per time point were labeled as one positive (P+), two negatives (N-), test one (T1), and test two (T2). Table 1 is an overview of the coupon descriptions.

Table 1: Overview of stainless steel coupon designation

Coupon	Description
P+	Culleoka Product Only
N-	Grape Juice (GJ)
N-	Tryptic Soy Broth (TSB)
T1	GJ
T2	TSB

Table 1: Overview of Coupon Designation

100 microliters of grape juice or TSB were applied and spread on the surface of the negatives, T1, and T2 coupons, and air-dried overnight. The P+, T1, and T2 coupons were treated with 1000µl of Culleoka product to the surface for two minutes and wiped dry with a microfiber towel. Coupons were tested at the following time points (hours): 0, 4, 8, 24, 48, 72, and 96.

ATP Surface Testing

ATP Testing was performed using the Hygiena UltraSnap Surface ATP Test Device Swabs and Hygiena SystemSure Plus Luminometer ATP Bio-Contamination Testing Meter. Swabs were removed from the fridge fifteen minutes before testing to allow them to reach closer to the room temperature. At the designated time points, a new swab was used to measure the P+, N- GJ, N- TSB, T1 GJ, and T2 TSB surfaces, and the results from the meter were recorded.

Colony Counts

Treated coupons at the designated time points were transferred with forceps into 15 ml of D/E neutralizing broth into four separate 50ml conical tubes labeled P+, N- GJ, N-TSB, T1 GJ, and T2 TSB. Conical tubes were placed on the shaker for 10 minutes. Dilution tubes were filled with 900µl of 1X PBS, and serial dilutions were made for P+, T1 GJ, and T2 TSB from 10^{-3} to 10^{-8} using 100µl of the shaken solution. The stock and serial dilution solutions were plated and spread evenly over the surface of the solid tryptic soy agar (TSA) using a metal spreader. Finished plates were placed into a clean labeled zip lock bag and incubated at 37°C overnight. Isolated colonies were counted, and colonies over 300 were determined as too numerous to count (TNTC).

Results:

ATP Results:

ATP results showed that the Culleoka product did not have an ATP reading but did not completely interfere with readings when mixed with nutrients. This does not mean that there wasn't an inhibitor in the cleaner that prevented ATP readings. Based on the results below in Figure 1, ATP is not an appropriate method to determine the growth curve of the Culleoka organism.

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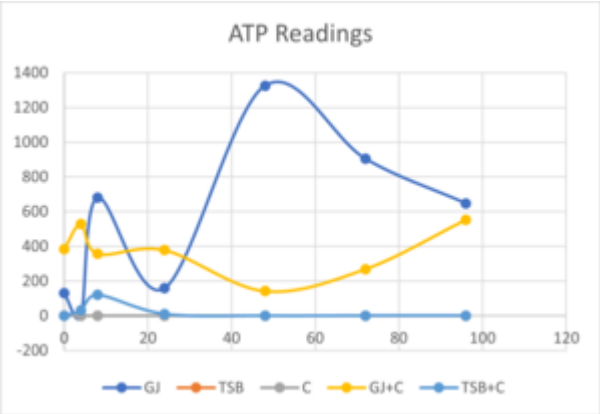


Figure 1: ATP readings for Culleoka only (C), grape juice (GJ), tryptic soy broth (TSB), grape juice with Culleoka (GJ+C), and tryptic soy broth with Culleoka (TSB+C) treated stainless steel surfaces over 0, 4, 8, 24, 48, 72, and 96 hour time points.

Colony Counts:

The growth curve in Figure 2 shows that both GJ and TSB are appropriate nutritional mediums that have similar growth curves at the designated time points. There was some variance in the colony counts of GJ and TSB at the eight-hour time point. Figure 3 and Table 2 are of the recovery of probiotic bacteria when the product was used alone on a clean stainless-steel surface at the designated time points. When the product was placed on a clean surface, it kept a 6-7 Log throughout each of the time points tested.

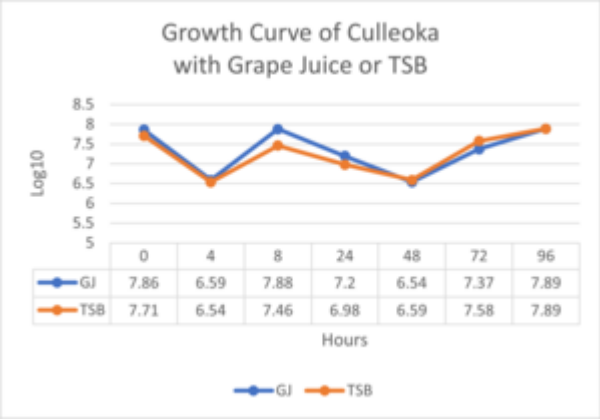
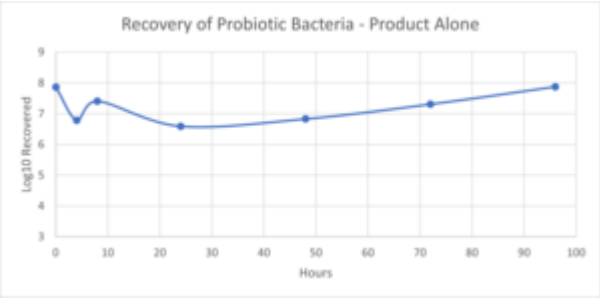


Figure 2: Colony Count Growth Curve for Culleoka on grape juice (GJ) and tryptic soy broth (TSB) treated stainless steel surfaces over 0, 4, 8, 24, 48, 72, and 96-hour time points.



Probiotic Bacteria Alone

Figure 3: Colony Count Growth Curve for Culleoka product alone on stainless steel at 0, 4, 8, 24, 48, 72, and 96-hour time points.

Hours	Log10
0	7.87
4	6.79
8	7.41
24	6.59
48	6.83
72	7.31
96	7.88

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Table 2: Log results after each time point of the recovered probiotic bacteria, using the product alone and without a food source, on a clean stainless-steel surface.

Summary:

Substrates:	Stainless Steel				
Contaminants:	Food				
Company Name:	**Product Name:**	**Conc.:**	**Efficiency:**	**Effective:**	**Observations:**
Culleoka Co	Culleoka Natrual Based Cleaner	100%	0.00	☑	

Conclusion:

Culleoka did show growth over time when nutrients were introduced to the surface. The best method to verify based on this testing is using colony counts as the analytical method. ATP was not consistent with the growth seen on the plates, and there may be an inhibitor in the product slightly interfering with ATP readings.