Evaluation of Contaminated Bulk Soap Dispensers for Biofilm Bacteria: Comparison of Two Methods of Analysis and Effectiveness of Dispenser Washing Procedures

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Lindsey Lorenz[1], Brad Ramsay[1], Matthew Fields[1], Darla Goeres[1], Carrie Zapka[2]
[1]Center for Biofilm Engineering, Montana State University, Bozeman, MT
[2]GOJO Industries, Inc., Akron, OH

MONTANA STATE UNIVERSITY
Center for Biofilm Engineering

a National Science Foundation Engineering Research Center in the MSU College of Engineering

INTRODUCTION

Bulk refillable soap dispensers are manually refilled with bulk soap through an opening in the top, Figure 1.

Previous research demonstrated that up to 25% of bulk hand soap dispensers are contaminated with approximately 6 LOG_{10}(CFU/mL) heterotrophic bacteria based upon samples collected from the bulk soap[1]. The contamination results from extrinsic sources and occurs when the preservative system in the soap is overcome.

This poster presents the results of a two-phase project. The goal of Phase 1 was to determine if biofilm growth within the dispensers contributed to bulk soap contamination, and Phase 2 investigated if washing the dispensers effectively reduced bacterial contamination .

Figure 1. Bulk refillable soap dispenser

PHASE 1 – BIOFILM TESTING

Viable plate counts paired with biochemical identification assays and molecular methods were used to determine the amount of biofilm present and the ecology of the biofilm communities found in three types of dispensers. The dispenser types tested were: plastic counter-mount (from a shopping center), plastic wall-mount (from an elementary school), and stainless steel wall-mount (from middle/high schools). All dispensers tested were previously determined to be contaminated in the field.

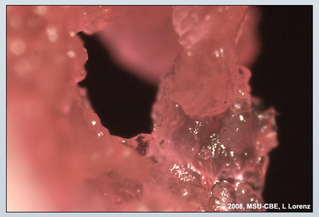


Figure 2. Stereoscope image of dried soap on the spigot opening of a plastic wall-mount dispenser. 5X

Viable Plate Count & Biochemical Identification Methods

Dispensers were received and visually inspected for any damage during shipment. Samples were collected and analyzed at three distinct steps:

- Sample A: the bulk soap (suspended bacteria)
- Sample B: the rinsed solution (loosely attached cells rinsed from the dispenser surfaces)
- Sample C: the scraped solution (surface associated cells scraped from the dispenser surfaces)

The three samples were then:

- Disaggregated and neutralized in D/E neutralizer (Disaggregation methods included sonicating and vortexing the sample with sterile 3mm glass beads, for 1 minute each, alternating with three repeats.)
- Diluted and plated for heterotrophic and coliform plate counts
- Filtered for total cell counts (Figure 3)

When possible, stereoscope images of the dispenser were taken between the rinse and scraping steps, with careful attention paid not to disrupt the biofilm within the dispenser (Figures 2 ,4 ,5 and 7).

Isolated colonies were picked from the heterotrophic and coliform plate counts and were sent in for biochemical organism ID.

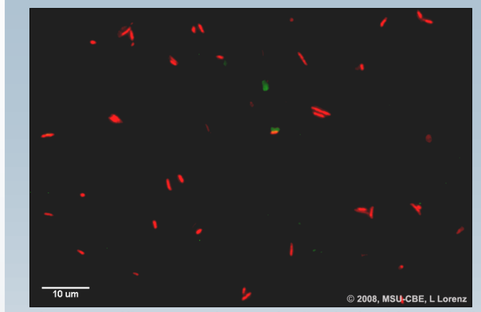


Figure 3. Epifluorescent image of cells obtained from a bulk refillable soap dispenser, filtered onto a polycarbonate membrane, and stained with Live/Dead for total cell counts. Total cell counts were an important way of determining the efficacy of the disaggregation methods. Disaggregation was determined to be efficient when single cells were seen, as shown above. 100X

Community Analysis Molecular Methods

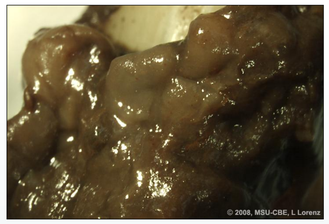
The community analysis approach was broken down into four steps:

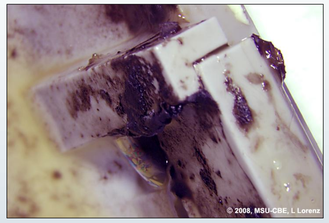
Biomass collection
- Collect pooled bulk and surface associated pellets

DNA preparation
- Cell lysis
- Removal of cell debris via centrifugation
- Precipitate proteins

Clone library construction
- Clone gene of interest (SSU rRNA gene via PCR)
- Ligation into plasmid & transformation into *E. coli*
- Screen/pick colonies

Organism identification
- Sequencing
- Bioinformatic analysis





Figures 4 and 5. Stereoscope images of unknown brown material found in all types of dispensers studied. Shown here: internal tubing from a counter-mounted dispenser (top) and lid of a plastic wall-mounted dispenser (bottom). 7.5X

BIOFILM TESTING RESULTS

Results indicated that (Figure 6) :

- The bulk soap, Sample A, was contaminated with 4-7 LOG_{10}(CFU/mL) bacteria.
- Samples B (loosely surface associated) and C (surface associated) contained 4-7 LOG_{10}(CFU/cm^2), (n=6).
- Total cell counts ranged from 4-8 LOG_{10}(CFU/cm^2) for all dispensers and sample types.

These results were Independent of dispenser type or construction material.

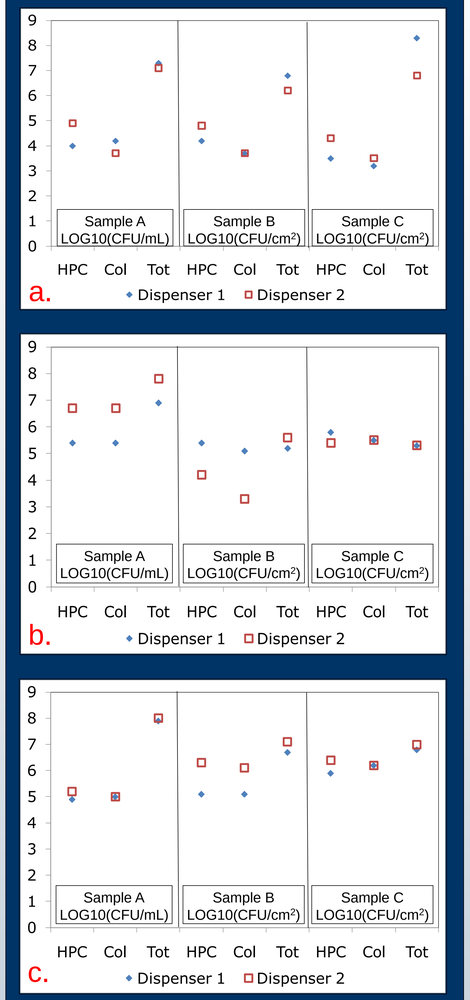


Figure 6. Panel a. is the counter-mounted dispenser results, b. the plastic wall-mounted dispenser results, and c. the stainless steel wall-mount dispenser results. For the viable plate count results, HPC refers to heterotrophic plate counts, Col refers to coliform counts and Tot refers to total cell counts. Samples A, B and C depict the bulk soap, loosely surface associate, and the surface associated biofilm counts, respectively.

METHODS COMPARISON

Overall, the results of the bacterial identification based upon biochemical assays versus molecular methods were comparable at the genus level, but some differences were observed (Table 1).

The biochemical profiling from all dispensers tested identified :
- 14 unique bacterial species
- 11 different genera

Whereas the molecular methods identified :
- 13 unique genera
- Possibly dozens of different species

All microorganisms observed are considered opportunistic pathogens and are mostly gram negative. The organisms identified were surprisingly consistent, and were independent of type and location of dispenser.

Table 1. Panel a. is an indirect comparison of field versus biochemically isolated microbes identified from the plastic wall-mounted dispensers. Panel b. is an indirect comparison of field identified microbes versus microbes identified using molecular methods from the plastic wall-mounted dispensers. Panel c. is a direct comparison of microbes identified using biochemical assays versus molecular based methods from stainless steel wall-mounted dispensers. Molecular ID isolates were based on DNA found in the dispensers, thus, viability of identified organisms could not be assessed.

a.

Dispenser #	Field ID	Biochemical ID	Molecular ID
1	*Providencia rettgeri*	*Providencia rettgeri*	
	Pseudomonas aeruginosa	*Pseudomonas aeruginosa*	
	Citrobacter koseri	*Citrobacter freundii*	
	Serratia oderifera	*Serratia liquefaciens*	
		Klebsiella pneumoniae	
2	*Pseudomonas aeruginosa*	*Pseudomonas aeruginosa*	
	Stenotrophomonas maltophilia	*Burkholderia cepacia*	
	Aeromonas hydrophila	Yeast, not *C. albicans*	
		Gram positive bacillus (no further ID available)	

= same genus and species
= same genus

b.

Dispenser #	Field ID	Biochemical ID	Molecular ID
3	*Pseudomonas aeruginosa*		*Pseudomonas aeruginosa*
	Providencia rettgeri		*Providencia rettgeri*
	Serratia rubidae		*Serratia rubidaea*
			Achromobacter xylosoxidans
			Alcaligenes xylosoxidans
4	*Stenotrophomonas maltophilia*		*Stenotrophomonas sp.*
	Pseudomonas fluorescens		*Pseudomonas aeruginosa*
	Pseudomonas luteola		*Citrobacter sp.*
	Pseudomonas stuzeri		*Curvibacter sp.*
	Enterobacter cloacae		*Enterobacter sp.*
			Leptothrix sp.
			Pelomonas sp.
			Delftia acidovorans
			Rubrobacter xylanophilus

= same genus and species
= same genus

c.

Dispenser #	Molecular ID	Biochemical ID
22	*Pseudomonas sp.* – probably *P. aeruginosa*	*Pseudomonas aeruginosa*
	Providencia sp. – probably *P. rettgeri*	*Providencia/Proteus rettgeri*
	Serratia sp. – probably *S. liquefaciens*	*Serratia marcescens*
40	*Providencia sp.*	*Providencia/Proteus rettgeri*
	Stenotrophomonas sp.	*Stenotrophomonas maltophilia*
	Pseudomonas sp.	*Pseudomonas fluorescens/putida*
	Serratia sp.	*Serratia liquefaciens*
	Alcaligenes/Achromobacter sp.	*Acinetobacter lwoffii*

= same genus and species
= same genus

PHASE 2 – DISPENSER WASHING STUDY

Washing studies were completed to determine if dispensers could be washed or sanitized to eliminate future contamination. The methods used were selected to mimic options that could be available during routine restroom maintenance by janitorial staff. Three washing procedures were analyzed for plastic wall mounted bulk refillable soap dispensers:

1) a simple hot water rinsing technique
2) a hot water rinsing and scrubbing technique
3) a hot water rinse, scrub, 5,000mg/L bleach treatment, hot water rinse combination

Positive and negative control dispensers were drained and refilled with sterile soap.

Samples were collected from the rinse steps and evaluated for heterotrophic and coliform plate counts. Bulk soap sampling was performed for up to two weeks to determine washing procedure efficacy.

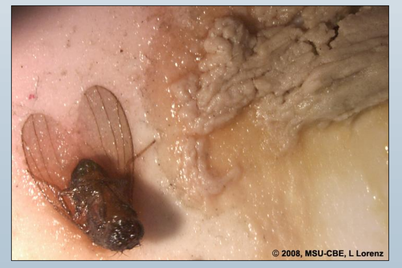


Figure 7. Stereoscope image of a fly found in the bottom dispenser assembly of a plastic wall-mounted dispenser. 7.5X

WASHING STUDY RESULTS

The washing study results (Figures 8-9) showed that bacterial counts in the bulk soap returned to pre-wash levels within two weeks of cleaning a dispenser and subsequently rinsing it with 5,000 mg/L bleach. The purple and blue X symbols represent the positive and negative control results, respectively.

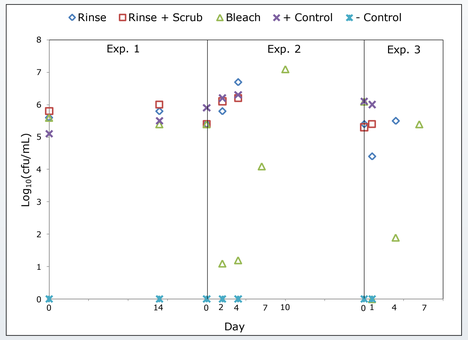


Figure 8. Dispenser washing study results: coliform counts

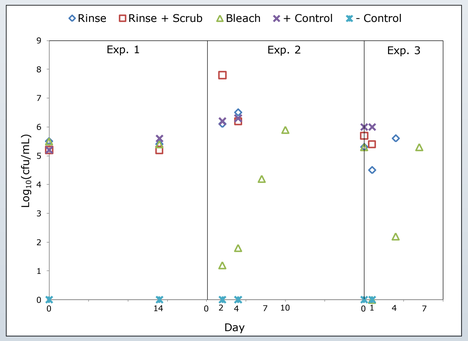


Figure 9. Dispenser washing study results: heterotrophic plate counts

CONCLUSIONS

- Dispensers contaminated with bacteria in the bulk soap also had high levels of biofilm bacteria.
- While the bacterial diversity was relatively low compared to other environments, detection of SSU rRNA gene sequences suggested the presence of organisms not detected via cultivation-based techniques (for some samples).
- The washing study results showed that bacterial counts in the bulk soap returned to pre-wash levels within two weeks regardless of the washing procedure used, although the bacterial counts in the dispensers rinsed with bleach did recover more slowly.

ACKNOWLEDGEMENTS

Funding was provided by GOJO Industries, Inc., Akron, OH.

[1]Gerba CP, and Maxwell SL, "Bacterial contamination of liquid hand soaps used in public restrooms," Poster Presentation at NEHA 71st Annual Educational Conference & Exhibition, Atlantic City, NJ, 2007.