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Isolation & Characterization of Bacteria in the Built Environment: Measuring The Effect of Pharmaceuticals on Growth

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Abstract
This work reports the isolation and characterization of bacteria from the built environment at Gettysburg College in Gettysburg, PA. Surfaces of a water fountain on campus were swabbed and serially streaked to isolate multiple bacteria on R2A agar. Following multiple rounds of growth, the unknown microbial candidates were narrowed to two visiblydistinct organisms. Morphological characterization and phylogenetic identification based on 16S rDNA sequencing revealed that the isolates were Chryseobactierum hispalense and Microbacterium maritypicum. We report synergistic biofilm formation between Chryseobactierum hispalense and Microbacterium maritypicum. The contamination of drinking water with varying levels of personal care products and pharmaceuticals (PCPPs) is well documented. Additionally, these environmental pollutants and their derivatives affect aquatic life, as illustrated with effect of the antidepressant fluoxetine on mudsnails. To determine if previously reported contaminants affect freshwater bacteria, we assessed both planktonic growth and biofilm formation following exposure to nalidixic acid (nonfluorinated quinolone antibiotic), diphenhydramine (overthecounter drug Benadryl), and fluoxetine (Prozac).

Keywords
Chryseobactierum hispalense, Microbacterium maritypicum, personal care products and pharmaceuticals, fluoxetine, nalidixic acid, synergistic biofilm formation, biofilm formation, Microbiology

Disciplines
Bacteriology | Biology | Microbiology

Comments
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Isolation & Characterization of Bacteria in the Built Environment: Measuring the Effect of Personal Care Products & Pharmaceuticals on Growth

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BACKGROUND

The contamination of drinking water with varying levels of personal care products and pharmaceuticals (PCPPs) is well documented. Additionally, these environmental pollutants and their derivatives affect aquatic life, as illustrated with effect of the antidepressant fluoxetine on molluscs and crustaceans (Fong and Ford 2014). To determine if previously reported contaminants affect freshwater bacteria, we isolated and characterized unknown bacteria from the built environment at Gettysburg College in Gettysburg, PA. Additionally, we measured both planktonic growth and biofilm formation.

ISOLATION OF MICROBES

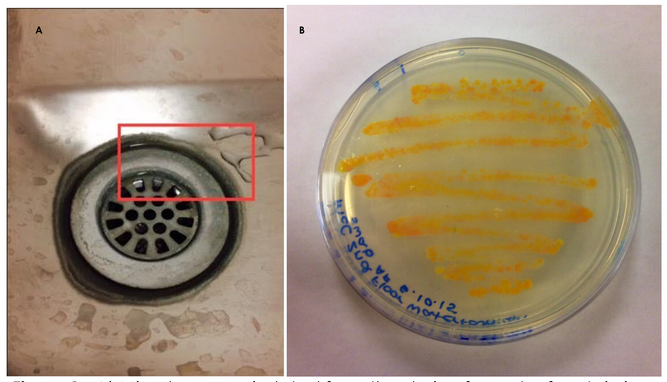


Figure 1. A) Microbes were isolated from the drain of a water fountain in an academic building (McCreary Hall) at Gettysburg College. B) Swabs from sample were streaked and grown on R2A agar at room temperature for 3 days.

MOLECULAR IDENTIFICATION

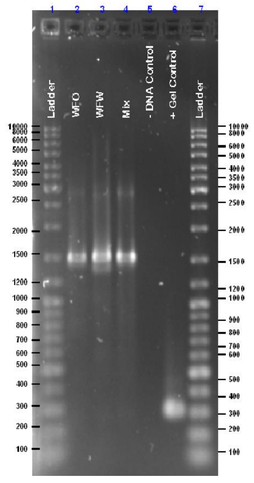


Figure 2. Unknown microbes were identified via PCR amplification of 16S rDNA and subsequent sequencing. Colony PCR with primers U1 (5'-ACGCGTCGACAGAGTTTGATCCTGGCT-3') and U2 (5'-CGCGGATCCGCTACCTTGTTACGACTT-3') yielded ~1500 bp PCR products. PCR products were visualized on a 1% agarose gel, shown left. See amplification of desired 16SrDNA isolated species (Wells 2-4) and absence of product in the Negative Control (Well 5).

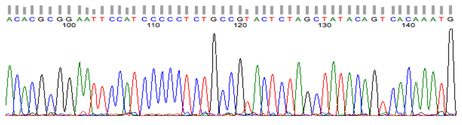


Figure 3: PCR products shown in Figure 2 were sequenced by Eton Biolabs using primers U1 and U2. Representative results are shown above.

Genus Species **WFW**	e value	Percent Identity	Length (bp)
Microbacterium maritypicum strain DSM 12512 ref|NR_114986.1|	0.0	910/910(100%)	1345
Microbacterium maritypicum strain DSM 12512 ref|NR_042351.1|	0.0	910/910(100%)	1437
Microbacterium oxydans strain DSM 20578 ref|NR_044931.1|	0.0	909/910(99%)	1466
Genus Species **WFO**	e value	Percent Identity	Length (bp)
Chryseobacterium hispalense strain AG13 ref|NR_116277.1	0.0	769/769(100%)	1480
Chryseobacterium taeanense strain NBRC 100863 ref|NR_113951.1|	0.0	745/771(97%)	1442
Chryseobacterium taeanense strain PHA3-4 ref|NR_043254.1	0.0	745/771(97%)	1424

Table 1. Phylogenetic Analysis of 16S rDNA. Using NCBI Blast, sequences were compared to a database of 16S rDNA to determine the closest matches.

RESEARCH QUESTION

We ask what bacteria are present in the built environment? Additionally, how are these organisms affected by pharmaceutical and personal care products known to contaminate drinking water?

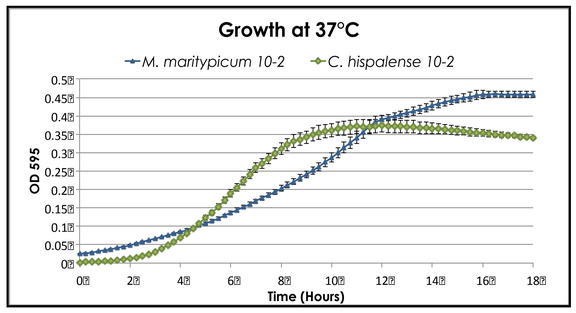


Figure 5. *M. maritypicum* and *C. hispalense* grow @ 37°C. Overnight cultures were diluted 100 fold in R2A broth and incubated with shaking every 780 seconds. Growth was measured in a 96-well microtiter plate, with OD595 recorded every 15 minutes for 18 hours.

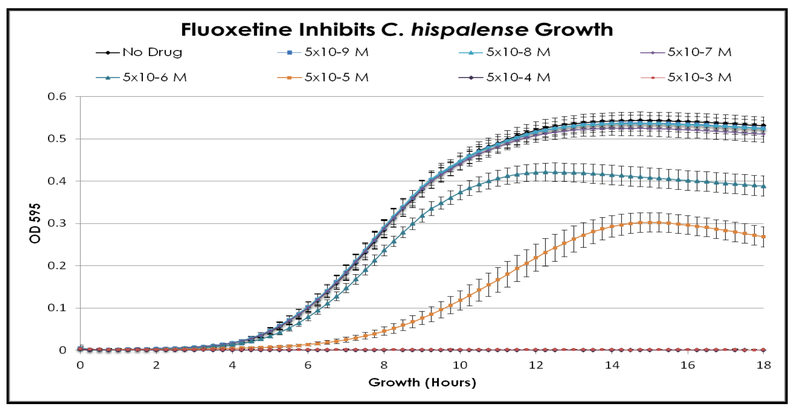


Figure 6. At high concentrations ($5x10^{-3}$M – $5x10^{-4}$M), fluoxetine completely inhibits growth. Growth defects are also seen at more dilute concentrations ($5x10^{-5}$ – $5x10^{-6}$M). Overnight cultures were diluted and growth was monitored as explained above. Results are from 3 separate trials, n=14.

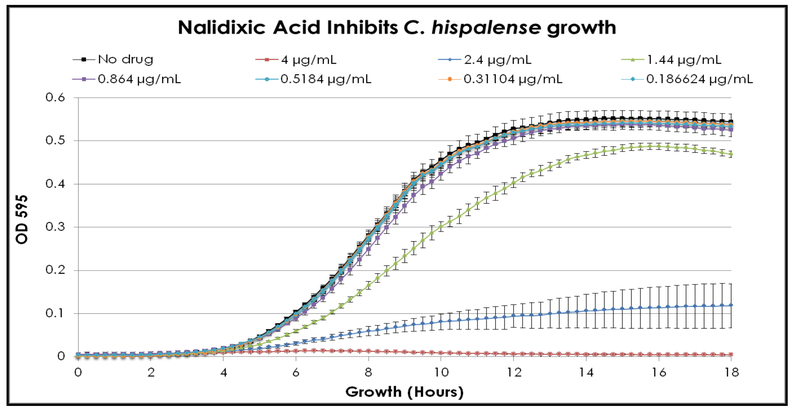
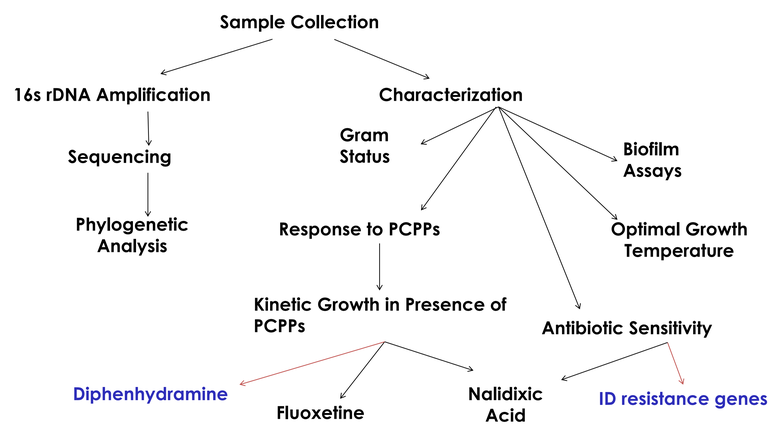


Figure 7. Nalidixic acid impairs growth at higher concentrations (4 µg/mL -1.44 µ g/mL).

Antibiotic	*Chryseobacterium hispalense*	*Microbacterium maritypicum*
Rifampin	Susceptible 28 mm	**Resistant** 14 mm
Erythromycin	Susceptible 21 mm	Susceptible 19 mm
Vancomycin	Susceptible 20 mm	Susceptible 25 mm
Tetracycline	Susceptible 24 mm	**Resistant** 8 mm
Penicillin	Susceptible 15 mm	Susceptible 27 mm

Table 2. Antibiotic resistance or susceptibility was determined using a standard filter disk assay on Mueller-Hinton agar. Following overnight incubation, zones of inhibition were measured and categorized based on Clinical Laboratory and Standards Institutes regulations.

EXPERIMENTAL DESIGN



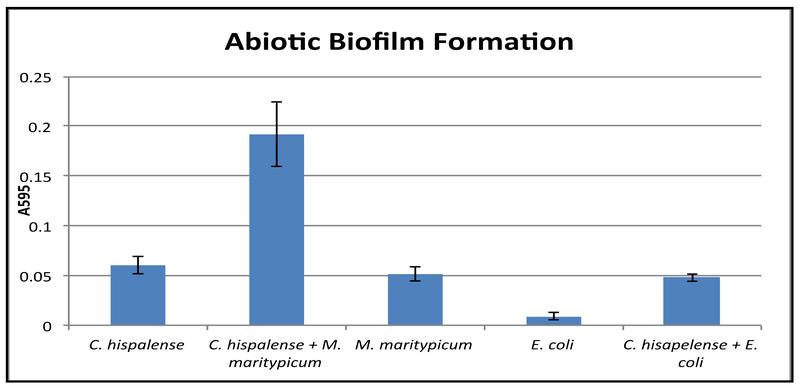


Figure 8. *C. hispalense and M. maritypicum* exhibit synergistic biofilm formation. This synergism appears specific to these species, as *C. hispalense* & *E. coli* co-incubation does not yield biofilm at similar levels. Static cultures were incubated 3 days @ room temperature. Planktonic bacteria was removed and biofilm was stained with crystal violet. Crystal violet was solubilized in 30% acetic acid and measured at 595nm. Results represent 1 trial, n=5.

CONCLUSIONS

We identified two unknown microbes as *Microbacterium maritypicum* and *Chryseobacterium hispalense*. Identification was confirmed by phenotypic & phylogenetic analysis of 16S rDNA (Fig 2-3 & Table 1). We found *M. maritypicum* was resistant to rifampin and tetracycline, while *C. hispalense* was susceptible to all antibiotics tested (Table 2). We report a previously uncharacterized growth impairment in response to fluoxetine, a serotonin reuptake inhibitor (Figure 6). This finding was interesting and unexpected. Additionally, abiotic biofilm assays indicate specific synergism between *C. hispalense* and *M. maritypicum* (Figure 8).

Future experiments include additional tests to understand the effect of fluoxetine and other known PCPP contaminants on freshwater bacteria. We are particularly interested in the effects of these compounds on biofilm formation. Does drug exposure shift the composition of biofilms? How does drug exposure affect gene transfer within these communities? Do trace levels of combinations of drugs create a measurable response? These questions are particularly important when considering how contamination affects both drinking water and freshwater ecosystems.

REFERENCES

Fong P, Ford A. The biological effects of antidepressants on the molluscs and crustaceans: a review. Aquat Toxicol. 2014;151:4–13.