4-25-2019

Bacillus cereus & Bacillus pumilus Harvested From Copper Roof Inhibit Growth of Other Organisms

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Bacillus cereus & Bacillus pumilus Harvested From Copper Roof Inhibit Growth of *Other Organisms*

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Abstract

The goal of this project was to isolate bacteria from unusual places, as these bacteria may have distinct adaptations to allow them to grow in challenging environments. Bacteria samples were obtained from the copper roof of Simons Hall in Collegeville, MN during November, 2018. These samples are of interest because bacterial growth is typically inhibited by copper. Once isolated and grown in culture, some of the collected bacterial samples displayed the ability to out-compete other bacterial samples. A polymerase chain reaction was used to identify bacteria samples 1 and 2 as *Bacillus cereus* and bacteria 4 as *Bacillus pumilus*. Growth curve experiments show that these isolates are capable of inhibiting other bacterial species. The results from our growth curve experiments depict similar inhibitory effects on unknown bacteria samples during all stages of the growth curve. Our results support previous studies which suggest *Bacillus* have the capability of inhibiting or killing other organisms within their environment.

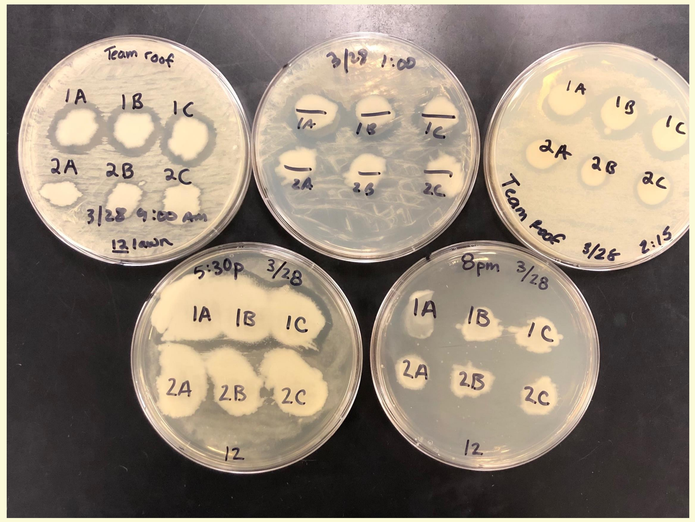
Introduction

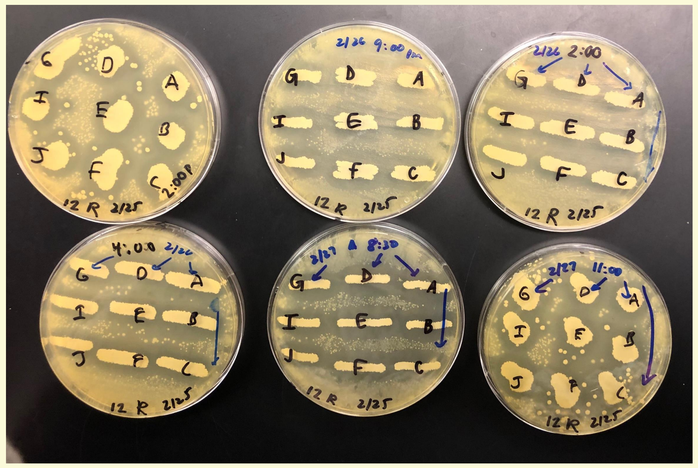
Previously, it has been thought that bacteria are unable to survive for more than a few hours on copper surfaces. Being that bacteria may have the ability to survive on stainless steel surfaces for weeks, many hospitals and labs have implemented the use of copper surfaces in an effort to eliminate possibilities for contamination or spread of unwanted bacteria.

However, some bacteria may have unique adaptations allowing them to survive on copper surfaces. A sample was isolated from a copper roof in Collegeville, Minnesota and identified as *Bacillus cereus* and *Bacillus pumilus* through a polymerase chain reaction. These two strains of bacteria were then tested against other bacteria and have given us reason to believe that *Bacillus cereus* and *Bacillus pumilus* may possess the ability to inhibit or even kill other bacteria (figures 6 & 7). We expected to observe that *Bacillus cereus* and *Bacillus pumilus* were emitting an inhibitory molecule during a specific time on their growth curves.

Methods

Bacteria collected from Simons Hall copper roof at Saint John's University. Placed bacteria on agar plates. Isolated three target bacteria. Performed PCR tests to isolate portion of 16S gene. Utilized PCR test results for gel electrophoresis. Sequenced purified DNA through GeneWiz using Sanger method. Analyzed results to identify microbial isolate. Inserted PCR results into BLAST to determine genus/species of bacteria. Created bacterial growth curves for each of the three bacteria's utilizing the spectrophotometer. Tested each of the three bacteria against another unknown bacteria that was spread on a lawn (TSA plates) and simultaneously created additional growth curves to understand in which phase of the growth curve, the bacteria are affecting the unknown on the lawn.





Figures 1 & 2. TSA plates of *Bacillus cereus* (top) and *Bacillus pumilus* (bottom) on unknown bacteria lawn at various times of the growth curves. In fig. 1 lawns were made minutes before *Bacillus cereus* was added. In fig. 2 lawns were made one day prior.

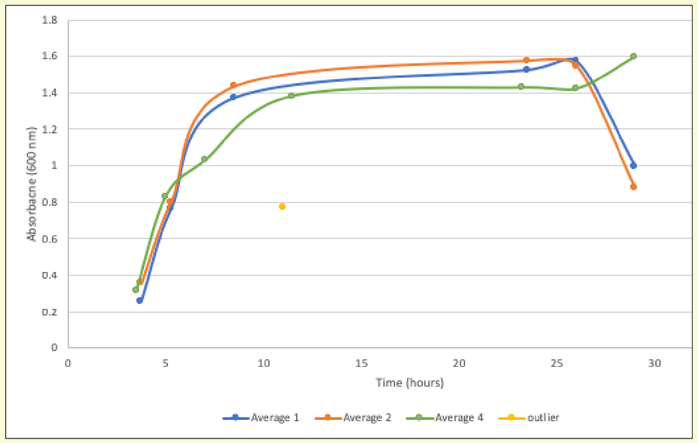


Figure 3. Average growth curves for *Bacillus cereus* (1,2) and *Bacillus pumilus* (4).

Results

PCR results processed in BLAST identified bacteria 1 & 2 as *Bacillus cereus* and bacteria 4 as *Bacillus pumilus.*

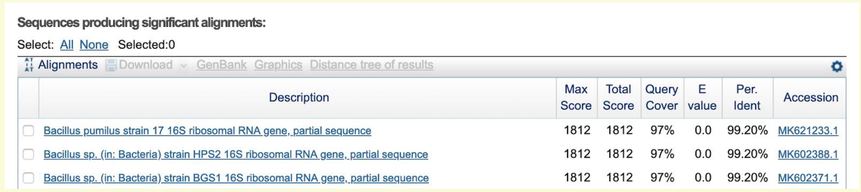
Measurable zones of inhibition were apparent for *Bacillus cereus* and *Bacillus pumilus* on the unknown bacteria lawn throughout all stages of their growth curves. Zones of inhibition are similar sizes, regardless of whether or not lawns were made one day prior or right before *Bacillus* were introduced.

Sequences producing significant alignments:

Select: All None Selected:0

Alignments Download GenBank Graphics Distance tree of results

Description	Max Score	Total Score	Query Cover	E value	Per. Ident	Accession
Bacillus cereus strain SKH 16S ribosomal RNA gene, partial sequence	1840	1840	96%	0.0	99.60%	KJ685393.1
Bacillus sp. G96 strain g96 16S ribosomal RNA gene, partial sequence	1838	1838	96%	0.0	99.60%	JQ661039.1
Bacillus cereus strain WJH 16S ribosomal RNA gene, partial sequence	1834	1834	96%	0.0	99.50%	MK675099.1



Sequences producing significant alignments:

Select: All None Selected:0

Alignments Download GenBank Graphics Distance tree of results

Description	Max Score	Total Score	Query Cover	E value	Per. Ident	Accession
Bacillus pumilus strain 17 16S ribosomal RNA gene, partial sequence	1812	1812	97%	0.0	99.20%	MK621233.1
Bacillus sp. (in: Bacteria) strain HPS2 16S ribosomal RNA gene, partial sequence	1812	1812	97%	0.0	99.20%	MK602388.1
Bacillus sp. (in: Bacteria) strain BGS1 16S ribosomal RNA gene, partial sequence	1812	1812	97%	0.0	99.20%	MK602371.1

Figures 4 & 5. BLAST results for bacteria 1 and 2 (top) and bacteria 4 (bottom).

Discussion

Bacillus are known to inhibit gram negative bacteria, yeasts, fungi, and some gram positive bacteria through peptides known as bacteriocins. This inhibitory behavior suggests it may be beneficial to harvest and identify the compound(s) *Bacillus* are producing. Results suggest *Bacillus cereus* and *Bacillus pumilus* inhibit growth of unknown bacteria samples throughout all stages of the growth curve. This potentially provides a competitive advantage for *Bacillus* in resource-limited environments. Additionally, it would be useful to identify what inhibitory compound(s) are being produced and what specific organisms they inhibit. To do so, future research should include the identification of the unknown bacteria lawn through PCR and BLAST techniques.

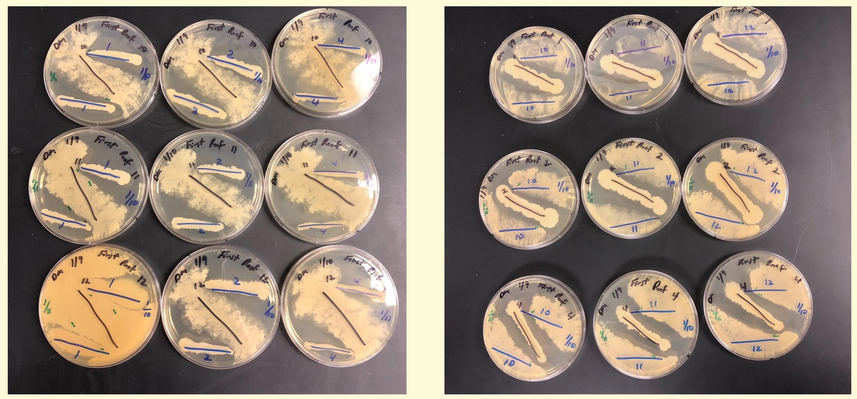


Figure 6 & 7 . The initial inhibitory behavior of unknown bacteria that led to the hypothesis for this study.