Mesophiles and Temperature Resistant Bacteria in Urban Technology and Their Impacts on Humans

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Introduction:

Bacteria have been found in the most extreme environments on our planet, both in the deepest depths of our oceans to the heights of our upper atmosphere. In my research, the focus was on the bacteria that have adapted to thrive in urban environments. Since the discovery of thermophilic bacteria by Thomas D. Brock at Yellowstone National Park in the 1970s, a high interest in bacteria that can thrive in extremely high or varying temperatures.Hot springs and geothermally heated waters are considered the ideal environments for thermophilic bacteria.

Technology today tends to have varying and adjustable temperatures. Things such as water boilers, stovetops, and hand dryers are able to drastically change temperatures within seconds. As bacteria can have many generations in short amounts of time, the bacteria present in these environments have evolved in order to adapt to natural environmental extremes as well as new modern extremes in the environment and bacteria's newer indoor environments.
Factors that influence the way in which bacteria function are temperature, pH, osmolarity and even radiation. Bacteria have evolved by changing these types of factors to be more suitable to their climates.

Not all bacteria are harmful, but it is good to be aware of what we are up against. With common methods of sterilization being centered around heat or radiation, it is important in the future to think of new methods. It is important to get a better understanding of what bacteria is found where, as well as what they can do and how they affect us.

Methods and Materials:

*Testing performed at Microbiology labs at University of Glasgow and Ramapo College of New Jersey
*Procedures were followed according to instruction by Dr. Sonya Taylor, Dr. Owen, and Dr. Olsen

Types of Testing Performed:

- Collecting samples with use of cotton swab and distilled water
- Steaking samples onto plates
- Isolating the samples (samples were isolated twice at Ramapo College to be sure colonies were isolated)
- Gram staining
- Endospore staining
- Differential media (Mannitol Salt, MacConkey, Sodium Azide Blood, Eosin Methylene Blue)
- Growth on media at the following temperatures (Glasgow): 37°C, 45°C, 55°C, 60°C 65°C, 70°C
- Growth on media at the following temperatures (Ramapo College): 25°C, 37°C, 45°C, 55°C
- Antibiotic Assay (different antibiotics depending on the sample (gram + or gram -))
- PCR
- DNA Sequencing (samples sent to outside sources for nucleotide sequencing of 16s rRNA)
- Use of BLAST for identification
- Dilution plating
- Database research on each identified sample and research through academic articles

Figure 2: Streaking Pattern for Isolating Bacteria

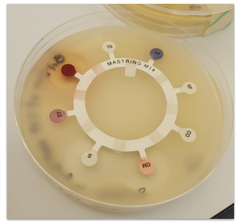
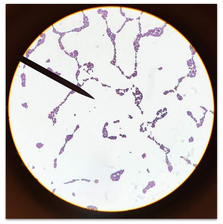


Figure 14: Antibiotic Assay showing susceptibility and resistance to bacterial sample



PHC: Gram positive (+)

Results Highlights:

Bacteria Type:	Temperatures for Growth:	Endospore Formation (only tested for samples in Glasgow)
Leptothrix	37-45	/
Stenotrophomonas maltophilia	37-60	At 60°C
Cupriavidus	37-60	At 55°C and 60°C
Staphylococcus haemolyticus	37-60	At 60°C
Dermacoccus nishinomiyaensis	37-65	At 65°C
Burkholderia mallei	37-65	At 60°C and 65°C
Sphingomonas paucimobilis	37-45	/
Micrococcus luteus	37-55	/
Kocuria	37-65	/
Brevibacillus Brevis	37-65	At 55°C, 60°C, and 65°C
Bacillus licheniformis	37-45	*At 37°C and 45°C*
Bacillus niabensis	37-55	/
Pantoea agglomerans	25-45	/
Staphylococcus hominis	25-45	/
Chryseobacterium takakiae	25-37	/
Chryseobacterium taihuense	25-37	/
Staphylococcus epidermidis	25-45	/
Klebsiella pneumoniae	25-55	/
Acinetobacter radioresistens	25-55	/

*For both Tables 4 and 5, the "R' signifies that the bacteria was resistant to the antibiotic, and the "S" signifies that the bacteria was susceptible to the antibiotic.

M13 (gram positive) Antibiotic Assay								
Samples:	C	E	FC	OX	NO	PG	S	T
DMW	S	S	S	S	S	R	S	S
DMO	R	R	S	S	S	R	S	S
DMR	S	S	S	S	S	S	S	S
BFW	S	R	R	R	S	R	S	S
BFY	R	S	R	R	S	R	S	S
K	R	S	S	S	R	S	S	S
KR	R	S	S	S	S	R	S	S
Scoop	R	R	S	R	S	R	S	S
BLB	R	R	S	S	S	R	S	S
Steam	R	S	S	S	S	R	S	S
M1W	S	R	S	S	S	S	S	S
M1Y	S	S	S	S	S	R	S	S
UVS	R	R	S	S	S	R	S	S
VUV	R	R	R	R	S	R	S	S
UVL	S	S	S	R	S	R	S	S
Total S	6	8	12	10	14	3	15	15
Total R	9	7	3	5	1	12	0	0

Table 4: M13 Antibiotic Assay for Gram Positive Bacterial Samples

M14 (gram negative) Antibiotic Assay								
Sample:	AP	KF	CO	GM	S	ST	T	TS
BRB1	S	S	S	R	R	R	S	S
BP	R	S	S	S	S	S	S	S
UVY	R	R	R	S	S	R	S	S
DMY	R	R	R	S	S	S	S	S
SM	R	R	R	S	S	R	S	R
BRB4	R	R	S	R	R	R	R	R
Vhd2	R	S	R	S	S	R	S	S
BRB6	R	R	R	S	S	R	S	S
Total R	7	5	5	2	2	6	1	2
Total S	1	3	3	6	6	2	7	6

Table 5: M14 Antibiotic Assay for Gram Negative Bacterial Samples

Results Highlights (cont.):

Sample Name:	Bacteria Identified:
BRB1	*Leptothrix*
BRB4	*Stenotrophomonas maltophilia*
BRB6	*Cupriavidus*
BP	*Staphylococcus haemolyticus*
BLB	*Dermacoccus nishinomiyaensis*
BFW	*Burkholderia mallei*
BFY	*Sphingomonas paucimobilis*
DMW	*Micrococcus luteus*
DMY	*Kocuria*
DMO	*Dermacoccus nishinomiyaensis*
UVL	*Brevibacillus brevis*
UVY	*Kocuria*
VUV	*Bacillus licheniformis*
M1W	*Staphylococcus haemolyticus*
M1Y	*Dermacoccus nishinomiyaensis*
K	/
SM	*Dermacoccus nishinomiyaensis*
SY	/
Vhd2	/
UVS	*Staphylococcus haemolyticus*

Table 8: List of names of the bacterial samples sequenced in UK

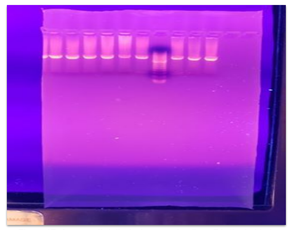


Figure 17: Agarose Gel displaying samples (lane 7 is the ladder)

Sample:	Bacteria Identified:
F2SY	*Acinetobacter radioresistans strain K2NRBAO003*
F1	*Chryseobacterium taihuense strain AMY_6.1.1*
F2W	*Klebsiella pneumoniae strain J41*
HDRG	*Staphylococcus epidermidis strain JST6*
LV1	*Chryseobacterium takakiae strain ab5*
LV2	*Staphylococcus hominis strain PRF50*
M410	*Bacillus niabensis strain F5.9*
PHC	*Staphylococcus haemolyticus*
NYPF	*Pantoea agglomerans strain Ns13*

Table 9: List of names of the bacteria samples sequenced in U.S.

Discussion

Through these tests, I have found not only heat resistant bacteria, but also bacteria that thrive in the cold, and bacteria that are radiation resistant. By looking at the resulting information acquired from bacteria collection, numerous methods of testing, and researching articles, there are important takeaways.

The bacteria found on the outside of a saucepan, a UV light on a restaurant's UV hand dryer, as well as on a phone battery pack, is known as *Staphylococcus haemolyticus*. It is an invasive and opportunistic pathogen, often found on skin and medical devices in a clinical setting. This bacteria, when infecting humans, causes sepsis, staph infection, bacteraemia, and peritonitis. This bacteria grows at a wide range of temperatures, and develops spores to protect itself from non-optimal conditions, displaying heat resistance. An interesting thing to note is that this bacteria was found in areas of both Glasgow and the United States. Though it could become a serious threat when humans are infected, this bacteria is common and widespread, and can survive in a wide range of environments.

Acinetobacter radioresistens is a particular bacteria of interest found through this research. The bacteria is radiation resistant, and shows resistance to ionization radiation. Ionizing radiation is the forms of radiation that have higher frequencies, such as X-rays, or microwaves. This sample was found in a 1100W microwave at Ramapo College. Since this radiation resistant bacteria is found in a microwave that uses ionization radiation, this further supports the idea that bacteria are evolving along with urban advancing technology. Not only are the heat resistant bacteria becoming more suitable for high temperature environments, or the cold resistant bacteria adapting to stay in cold areas such as water spouts, but bacteria are even able to survive in something with as much high frequency radiation as a microwave, to the point where it can live comfortably.

With current sterilization methods primarily making use of heat or radiation, it would be best to start exploring other options. Bacteria are constantly evolving in spite of new urban conditions arising, so it is important to create new sterilization methods to combat the rising strength of bacteria that can cause harm. Future testing will include a wider range of temperatures, to see the full temperature ranges at which each bacteria grow, as well as looking further into bacteria that have resistance to cold or radiation.

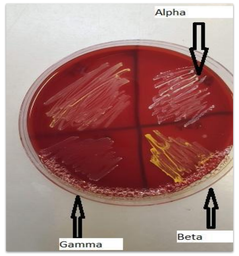


Figure 13: Sodium Azide Blood agar plates

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