MICROBE
DETECTIVES

Microbial Analysis Report

"Bourbon Gumbo"
10/13/2016

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Disclaimer

The information provided in this report is based on the latest DNA sequencing methodologies and is for research purposes only. Microbe Detectives takes no responsibility for decisions made based upon this information.

Executive Summary

Bourbon Street in New Orleans frequently has stagnant water sitting along the curbs at all hours of the day rain or shine. This phenomenon is somewhat unusual and is likely due to inadequate drainage as the street is more than 100 years old and doesn't slope adequately in many places. Due to the nature and atmosphere of Bourbon Street it begs the question of what exactly is in that water. Some locals refer to this water as Bourbon Gumbo. Microbe Detectives decided to investigate. We were told that the street is cleaned nightly, therefore we assumed the water may not be exotic as one might think. Maybe just a mishmash of bacteria consuming soap residue. Turns out, it was a bit more interesting than that.

A sample of grey water was collected from a small puddle on Bourbon Street, New Orleans on Wednesday September 28, 2016 at 1pm following the WEFTEC conference. The water was analyzed using DNA sequencing to identify the bacteria present. The high abundance of the Firmicutes Phylum is unusual for clean surface water and indicates potential fecal contamination. Though no obvious pathogenic genera were detected, 32% of identified bacteria was dominated by fecal-associated bacteria, especially *Prevotella*, *Bacteroides* and many others. Most of these bacteria live in mammal intestines including the human gut. However, *Prevotella* is generally not that abundant in the human gut in the Western Hemisphere (though it is very common in some developing countries with diets lower in fat and protein). As a result, it seems that the presence of these fecal bacteria are likely predominantly due to manure from a large mammal. Several of the bacteria are associated with ruminate mammals. Since Bourbon Street hosts many celebrations and parades, it's likely we're seeing the aftermath of some horse manure festering in the puddles on the street.

Background

The following samples were received: Bourbon Gumbo, Date Sampled: 09/28/16

Results

DNA data are displayed in the figures and tables below. The data in these tables is a subset of the total data, focusing on bacteria that are typically important in wastewater systems. The full data is also provided in a spreadsheet. Data presented are bacterial relative abundance (% of total bacteria in the sample). For example 0.12% *Actinobacteria* means that 0.12% of bacteria in the sample are *Actinobacteria*.

Please note, the raw data spreadsheet identifies each bacteria with a taxonomic string compromised of Domain>Kingdom>Phylum>Class>Order>Family>Genus>Species. Typically, most bacteria are identified to the genus level. If there is a strong match with our database they may be identified to the species level. On the other hand, if there are weaker matches to our database, they may be identified only to the family, order or class level. Many bacteria remain unstudied and unknown to science, but our database continues to grow and improve with the rapid advancement of DNA-based microbiology.
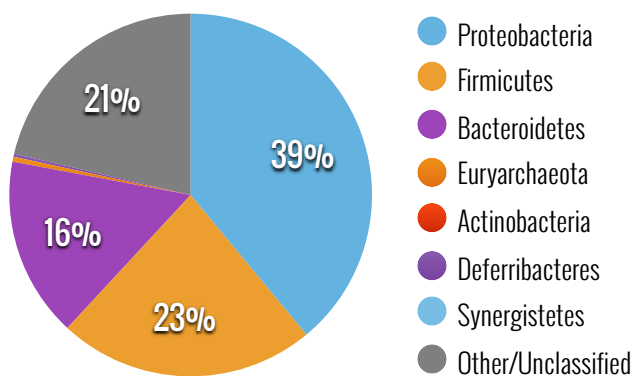
Figure 1. Phlya Analysis

- Proteobacteria
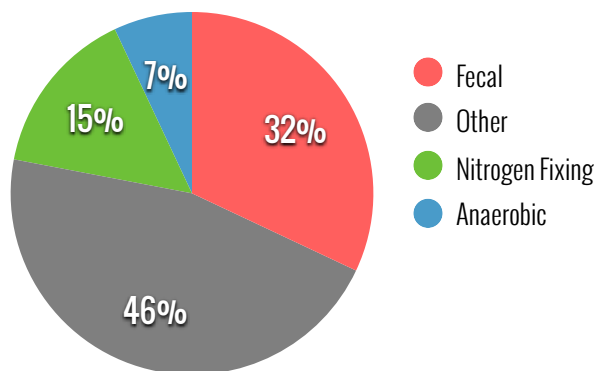- Firmicutes
- Bacteroidetes
- Euryarchaeota
- Actinobacteria
- Deferribacteres
- Synergistetes
- Other/Unclassified

Figure 2. Indicator Analysis

- Fecal
- Other
- Nitrogen Fixing
- Anaerobic

Table 1. Most Abundant Bacteria

Kingdom	Phylum	Class	Order	Family	Genus	WEFTEC-09_28_16-BourbonGumbo	Indicator
Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Prevotellaceae	Prevotella	11.75%	Fecal
Bacteria	Firmicutes	Clostridia	Clostridiales	Veillonellaceae	Veillonella	5.54%	Fecal
Bacteria	Firmicutes	Clostridia	Clostridiales	Veillonellaceae	Anaerovibrio	3.41%	Fecal
Bacteria	Firmicutes	Clostridia	Clostridiales	Veillonellaceae	Propionispira	3.10%	Fecal
Bacteria	Firmicutes	Clostridia	Clostridiales	Veillonellaceae	Mitsuokella	2.52%	Fecal
Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Bacteroidaceae	Bacteroides	2.28%	Fecal
Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Porphyromonadaceae	Parabacteroides	1.44%	Fecal
Bacteria	Firmicutes	Clostridia	Clostridiales	Veillonellaceae	Schwartzia	1.12%	Fecal
Bacteria	Firmicutes	Clostridia	Clostridiales	Veillonellaceae	Megasphaera	1.11%	Fecal
Bacteria	Proteobacteria	Gammaproteobacteria	Pseudomonadales	Pseudomonadaceae	Azotobacter	7.33%	N Fixing
Bacteria	Proteobacteria	Gammaproteobacteria	Pseudomonadales	Pseudomonadaceae	Azorhizophilus	6.13%	N Fixing
Bacteria	Proteobacteria	Gammaproteobacteria	Pseudomonadales	Pseudomonadaceae	Azomonas	1.33%	N Fixing
Bacteria	Proteobacteria	Epsilonproteobacteria	Campylobacterales	Campylobacteraceae	Sulfurospirillum	3.35%	S Reducer
Bacteria	Proteobacteria	Deltaproteobacteria	Desulfovibrionales	Desulfovibrionaceae	Desulfovibrio	2.15%	S Reducer
Bacteria	Firmicutes	Clostridia	Clostridiales	Veillonellaceae	Succinispira	1.18%	Anaerobic
Bacteria						46.26%	Other

Coliforms

Sample ID: 09/28/16 – *Citrobacter*, 0.16% was detected in this sample.

Coliform bacteria have been used as a crude indicator of fecal contamination for several decades. Coliform culturing is the most widely used drinking water test to test for the presence of fecal contamination and the microbial safety of drinking water. The test is relatively inexpensive and easy to perform, typically costing just a few dollars for supplies and a few minutes of time to inoculate the culture.

Coliform bacteria are defined as rod-shaped gram negative non-spore forming bacteria that can ferment lactose with the production of acid and gas at 35-37C. Most coliform bacteria are harmless and their presence simply indicates the possibility that fecal contamination may be present in the sample which could include pathogenic organisms. One challenge of coliform testing is that some coliforms are able to live in the environment in biofilms that can be found in drinking water distribution system pipes and well casings. As a result, a positive coliform result from a water sample does not necessarily indicate fecal contamination. It could occur due to naturally occurring bacteria. DNA testing is now able to reveal the organisms in a sample and provide either confirmation of the presence or absence of fecal contamination.

Common genera of coliform bacteria include *Citrobacter*, *Eschericia* (i.e. E. coli), *Enterobacter*, *Hafnia*, and *Klebsiella*.

Non-Coliforms that can trigger Coliform test

Sample ID: 09/28/16 – *Acinetobacter* 0.21%, *Pseudomonas* 9.76%, *Shewanella* 0.21% were detected in this sample.

Some bacteria, not considered coliforms, can trigger a positive coliform result (Zhang et al, 2015). These include common environmental genera such as Staphylococcus, Streptococcus, Acinetobacter and others.

Fecal Indicator Bacteria

Sample ID: 09/28/16 – *Prevotella* 11.75%, *Bacteroides* 2.28% were detected in this sample.

Non-coliform bacteria can also be indicators of fecal contamination. These bacteria include genera that are abundant in the intestinal tract of animals and/or humans such as *Bacteroides*, *Prevotella* and others. The presence of these bacteria may indicate fecal contamination of the sample as these bacteria are generally strictly anaerobic and cannot survive well in aerobic environmental conditions.

Potential Pathogens

Sample ID: 09/28/16 – *Clostridium* 0.21% was detected in this sample.

Potentially pathogenic genera include some fecal-associated bacteria such as *Eschericia* (E. coli) and Salmonella and other non-fecal genera such as *Helicobacter* (ulcers) and Legionella (respiratory pathogen). It should be noted that only certain species and strains within these genera are actually pathogenic and the presence of the genera does not necessarily indicate the presence of the pathogenic variety. Additionally, the presence of bacterial DNA does not indicate the presence of a viable pathogen bacteria. The DNA can be present within a dead or dying bacterial cell.

Freshwater or Marine Bacteria (potential sign of surface water intrusion)

Sample ID: 09/28/16 - None were detected in this sample.

Certain bacteria such as *Polynucleobacter*, *Pelagibacter* and acI *Actinobacteria* live strictly in surface waters (fresh or marine). The presence of these bacteria in a sample typically indicate the presence of surface water. If the sample was collected from a groundwater well, this likely indicates surface water intrusion or fractured bedrock that allows a direct connection between a nearby surface water source and the well. The presence of surface water in a well could allow for a mechanism to carry fecal contamination from the surface into the well. If surface water is detected, the water should be treated with disinfection prior to consumption.

Nitrogen Fixing Bacteria

Sample ID: 09/28/16 – *Azotobacter* 7.33%, *Azorhizophilus* 6.13%, *Azomonas* 1.33% *Clostridium* 2.15%, and *Desulfovibrio* 0.2% were detected in this sample.

Some bacteria are capable of converting inorganic atmospheric nitrogen into organic nitrogen to build their cells. As a result, they do not require nitrogen in solution (as most bacteria do) in order to grow. They can also provide a source of organic nitrogen for bacterial growth as they decay and release their nitrogen. These bacteria can provide a critical supply of nitrogen for biofilm growth in environments that are otherwise nitrogen limited. The presence of nitrogen fixing bacteria likely indicates that nitrogen is a limiting nutrient in the system as they would likely not have a competitive niche if nitrogen is abundant.

Carbon Fixing Bacteria

Sample ID: 09/28/16 - None were detected in this sample.

Some bacteria are capable of converting inorganic carbon into organic carbon to build their cells. As a result, they do not require organic carbon in solution (as many bacteria do) in order to grow. These bacteria can also provide a source of organic carbon for bacterial growth as they decay and release their carbon. These bacteria can provide a critical supply of organic carbon for biofilm growth in environments that are otherwise carbon limited. The presence of carbon fixing bacteria likely indicates that organic carbon is a limiting nutrient in the system as they would likely not have a competitive niche if organic carbon is abundant. Some of the most common carbon fixing bacteria found in potable water systems are iron oxidizing bacteria and ammonia oxidizing bacteria as well as photosynthetic bacteria such as cyanobacteria.

Ammonia Oxidizing Bacteria

Sample ID: 09/28/16 - None were detected in this sample.

A small number of bacteria are able to obtain energy converting ammonia to nitrite. These bacteria can cause problems in distribution systems that utilize chloramines as a residual disinfectant. The bacteria will consume the residual as a source of food which could result dangerously low residual levels. This activity can also drive up operational expenses as more and more chemical is needed to maintain a disinfection residual.

Nitrite Oxidizing Bacteria

Sample ID: 09/28/16 - None were detected in this sample.

A small number of bacteria are able to obtain energy converting nitrite to nitrate. These bacteria typically live alongside ammonia oxidizing bacteria. These bacteria can cause problems in distribution systems that utilize chloramines as a residual disinfectant. The bacteria will consume the residual as a source of food which could result dangerously low residual levels. This activity can also drive up operational expenses as more and more chemical is needed to maintain a disinfection residual.

Iron Oxidizing Bacteria

Sample ID: 09/28/16 - None were detected in this sample.

Iron oxidizing bacteria consume reduced iron for energy in aerobic conditions, typically producing a rust color in the water. These bacteria can also play a role in corrosion. *Gallionella* is one of the most well-known iron oxidizing which is frequently found in drinking water wells. The presence of iron oxidizing bacteria indicates the presence of dissolved iron.

Sulfate Reducing Bacteria

Sample ID: 09/28/16 – *Desulfovibrio* 0.2% was detected in this sample.

The presence of sulfate reducing bacteria indicates the presence of sulfur. Sulfate reduction can also produce hydrogen sulfide gas which may cause aesthetic issues due to the rotten egg odor. The presence of sulfur-associated bacteria could increase corrosion in certain environments where it produces corrosive sulfuric acid.

Sulfur Oxidizing Bacteria

Sample ID: 09/28/16 – *Thiodictyon* 0.22%, *Sulfurospirillum* 3.35% were detected in this sample.

The presence of sulfur oxidizing bacteria indicates the presence of sulfur. The presence of sulfur could cause taste and odor issues and possibly corrosion problems if sulfuric acid is formed.

Biofilm Slime Formers

Sample ID: 09/28/16 - *Pseudomonas* 9.76% was detected in this sample.

Sphingomonas is well known as a first colonizer that is vital to establish a biofilm community. Pseudomonas is also well known as a slime forming bacteria, comprising substantial portions of some biofilms. The presence of biofilms in wells or distribution systems can harbor anaerobic environments and may shield pathogens such as Legionella and enteric pathogens from disinfectants. Biofilms can also exhibit a demand on the residual disinfectant and could result in dangerously low levels of disinfection residual.

Table 2. Bacterial Relative Abundance (% of Total Bacteria)

Bolded or highlighted text indicates bacteria that were found greater than 0.2% of total bacteria.

Phylum	BourbonGumbo-09_28_16
Proteobacteria	38.98%
Firmicutes	22.93%
Bacteroidetes	16.05%
Euryarchaeota	0.44%
Actinobacteria	0.00%
Deferribacteres	0.27%
Synergistetes	0.00%
Other/Unclassified	21.34%
Coliforms	
Pantoea	0.00%
Escherichia	0.00%
Enterobacter	0.00%
Serratia	0.00%
Citrobacter	0.16%
Klebsiella	0.00%
Hafnia	0.00%
Proteus	0.00%
Kluyvera	0.00%
Buttiauxella	0.00%
Erwinia	0.00%
Hafvia	0.00%
Obesumbacterium	0.00%
Morganella	0.00%
Providencia	0.00%
Rahnella	0.00%
Raoultella	0.00%
Trabusiella	0.00%
Yersinia	0.00%

Non-Coliforms	BourbonGumbo-09_28_16
Streptococcus	0.00%
Acinetobacter	0.21%
Aeromonas	0.00%
Bacillus	0.00%
Chryseobacterium	0.00%
Herbaspirillum	0.00%
Microbacterium	0.00%
Plesiomonas	0.00%
Pseudomonas	9.76%
Shewanella	0.21%
Stenotrophomonas	0.00%
Staphylococcus	0.00%
Vibrio	0.00%
Wautersia	0.00%

Fecal Indicators	
Prevotella	11.75%
Veillonella	5.54%
Anaerovibrio	3.41%
Propionispira	3.10%
Mitsuokella	2.52%
Bacteroides	2.28%
Parabacteroides	1.44%
Schwartzia	1.12%
Megasphaera	1.11%
Escherichia	0.00%
Ruminococcaceae	0.00%
Enterococcus	0.00%
Lachnospiraceae	0.00%
Leptospira	0.00%

Contamination Indicators	BourbonGumbo-09_28_16
Enterobacteriales	0.00%
Clostridia	0.00%
Spirochaetae	0.00%
Fusobacterium	0.00%
Streptococcus	0.00%
Staphylococcus	0.00%
Leptotrichia	0.00%
Rothia	0.00%
Bifidobacterium	0.00%
Aggregatibacter	0.00%
Trichococcus	0.00%
Gemella	0.00%
Porphyromonas	0.00%
Capnocytophaga	0.00%
Arcobacter	0.47%
Potential Pathogens	
Mycobacterium	0.00%
Helicobacter	0.00%
Haemophilus	0.00%
Legionella	0.00%
Escherichia Shigella	0.00%
Salmonella	0.00%
Campylobacter	0.00%
Aeromonas	0.00%
Alternaria	0.00%
Clostridium	0.20%
Freshwater or Marine Bacteria	**BourbonGumbo-09_28_16**
Polynucleobacter	0.00%
Prochlorococcus	0.00%
Limnohabitans	0.00%
Pelagibacter	0.00%
Freshwater Actinobacteria	0.00%

Nitrogen Fixation	
Azospira	0.00%
Azospirillum	0.00%
Acidithiobacillus	0.00%
Agrobacterium	0.00%
Bosea	0.00%
Bradyrhizobium	0.00%
Desulfovibrio	2.15%
Methylosoma	0.00%
Clostridium	0.20%
Azotobacter	7.33%
Azorhizophilus	6.13%
Azomonas	1.33%

Carbon Fixation	BourbonGumbo-09_28_16
Sediminibacterium	0.00%
Sideroxydans	0.00%
Nitrospira	0.00%
Acidithiobacillus	0.00%
Sulfuricurvum	0.00%
Jettenia	0.00%
Nitrosomonas	0.00%
Sulfuricella	0.00%
Geobacter	0.00%
Gallionella	0.00%
Brocadia	0.00%
Scalindua	0.00%
Leptospirillum	0.00%
Crenothrix	0.00%
Nitrospinaceae	0.00%
Ferritrophicum	0.00%
Candidatus_Nitrosoarchaeum	0.00%
Nitrosococcus	0.00%
Nitrosopumilus	0.00%
Sulfurivirga	0.00%
Ammonia Oxidation	**BourbonGumbo-09_28_16**
Nitrosospira	0.00%
Jettenia	0.00%
Nitrosomonas	0.00%
Brocadia	0.00%
Scalindua	0.00%
Pirellula	0.00%
Gemmata	0.00%
Planctomyces	0.00%
Candidatus_Nitrosoarchaeum	0.00%
Planctomycetaceae	0.00%
Isosphera	0.00%
Candidatus_Kuenenia	0.00%
Anammoxoglobus	0.00%
Nitrosococcus	0.00%
Nitrosopumilus	0.00%
Nitrosocaldus	0.00%
Nitrososphaera	0.00%
Nitrite Oxidation	**BourbonGumbo-09_28_16**
Nitrospira	0.00%
Nitrobacter	0.00%
Nitrospina	0.00%
Nitrococcus	0.00%

Iron Oxidation	
Ferribacterium	0.00%
Sediminibacterium	0.00%
Sideroxydans	0.00%
Acidithiobacillus	0.00%
Geobacter	0.00%
Gallionella	0.00%
Leptospirillum	0.00%
Crenothrix	0.00%
Ferritrophicum	0.00%
Ferrovibrio	0.00%
Acidiferrobacter	0.00%
Sulfate Reduction	**BourbonGumbo-09_28_16**
Thermodesulfobium	0.00%
Desulfuromonas	0.00%
Desulfurivibrio	0.00%
Desulfovibrio	2.15%
Desulfocapsa	0.00%
Desulfomonile	0.00%
Desulfobacteraceae	0.00%
Desulfobulbaceae	0.00%
Desulfobacterium	0.00%
Sulfur Oxidation	
Thiodictyon	0.22%
Thiobacillus	0.00%
Thiothrix	0.00%
Sulfuritalea	0.00%
Sulfuricurvum	0.00%
Sulfuricella	0.00%
Sulfurospirillum	3.35%
Magnetovibrio	0.00%
Biofilm Slime Formers	
Pseudomonas	9.76%
Sphingomonas	0.00%