MICROBE DETECTIVES

Microbial Analysis Report

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Submitted to
Sample Drinking Water Client

METAGENOMICS • DATA SCIENCE • BIOBANKING

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Microbial DNA Data Background

What is the value of this information?

Microbial issues such as corrosion, nitrification and fecal contamination cost water utilities millions of dollars every year. These problems are difficult to solve with conventional tools. Traditional microbial investigation tools such as microscopy and culturing are able to identify only about 1% of bacteria in a sample. DNA methods are able to identify and quantify nearly 100% of bacteria in a sample, providing much more information to solve these costly microbial issues.

Example: A small water utility was experiencing taste and odor complaints from residents in the city. The utility had performed extensive coliform testing to attempt to understand the issue but all tests were negative. The utility performed microbial DNA testing on 8 samples from various locations around the distribution system and found suspicious microbes (i.e. Salmonella and other fecal associated bacteria) at one location in particular. The utility followed up with further inspections of nearby facilities and found failed backflow preventers in a poultry processing facility, a courthouse and a school. DNA data revealed a hidden problem and may have saved the utility from a major public health issue.

What can be learned from this information?

Microbial DNA data is able to identify and quantify nearly all bacteria in a sample. This data can be used to detect fecal contamination, nitrification and surface water intrusion.

How should this information be used?

DNA data is valuable for tracking microbial communities in one location over time. Samples can be shipped in one batch to provide weekly data from a single location for trending purposes. The ability to visualize these trends can allow operators and engineers to better understand and manage the microbial communities by understanding impacts caused by various control methods (i.e. increase disinfection dosage, pipe cleaning, etc.) Every system is different. Data is most useful when compared over time at one location.

Who should use this information?

Drinking water engineers, operators and laboratory professionals can use microbial DNA information to improve their understanding of water quality in their systems.

Where should this method be applied?

Microbial DNA methods can be applied to better understand water quality from groundwater wells and also in potable water distribution systems.

What problems can be solved?

Distribution System Nitrification

DNA data can shed light on issues with nitrification in distribution systems that consumes disinfection residual (especially chloramine).

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Distribution System Backflow

DNA data can indicate potential issues with untreated water entering distribution systems via backflow or other routes. Coliform testing will typically not detect any issues until the problem is substantial enough to consume the chlorine residual and cause potential health issues. DNA data, on the other hand, can detect bacterial contaminants such as E. coli and other fecal-associated bacteria even when a chlorine residual renders these bacteria non-viable. This information is helpful for identifying problems in a distribution system before they become major public health issues.

Well Surface Water Intrusion

DNA data can indicate the presence of surface water in groundwater wells. Surface water entering wells could be a cause for concern as it could harbor fecal contamination. Without DNA data, it is very difficult to detect surface water entering a well.

Aesthetics (taste/odor) issues

DNA data can shed light on taste and odor issues such as sulfur and iron. This data reveals the identity of sulfur oxidizing bacteria, sulfate reducing bacteria and iron oxidizing bacteria.

Positive Coliform Tests

DNA data can be used to provide more information in cases where positive coliform tests have occurred. The DNA data can indicate whether or not the coliforms are naturally occurring environmental bacteria or are associated with other fecal bacteria in the sample.

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Data

DNA results and analysis are presented below.

Bacteria Name	Lateral.1	Lateral.2	Lateral.3	Lateral.4
Phylum Analysis				
Proteobacteria	53.56%	66.48%	65.84%	75.61%
bacteroidetes	1.95%	9.32%	16.75%	11.51%
firmicutes	11.98%	7.23%	2.92%	3.09%
verrucomicrobia	12.36%	4.13%	4.32%	1.89%
cyanobacteria	7.12%	3.02%	1.78%	1.55%
actinobacteria	4.97%	3.49%	2.32%	1.28%
acidobacteria	4.34%	2.48%	2.39%	1.24%
chloroflexi	1.99%	1.03%	1.32%	0.94%
planctomycetes	0.47%	0.57%	0.21%	2.40%
nitrospirae	0.86%	0.73%	0.56%	0.03%
chlamydiae	0.01%	0.45%	0.60%	0.23%
elusimicrobia	0.00%	0.50%	0.26%	0.07%
spirochaetes	0.00%	0.19%	0.16%	0.02%
Other	0.40%	0.38%	0.58%	0.14%

Coliforms	Lateral.1	Lateral.2	Lateral.3	Lateral.4
Pantoea	0.00%	0.00%	0.00%	0.00%
Escherichia	0.00%	0.00%	0.00%	0.00%
Shigella	5.08%	0.39%	0.02%	0.15%
Enterobacter	0.00%	0.00%	0.00%	0.00%
Serratia	0.00%	0.05%	0.00%	0.00%
Citrobacter	0.00%	0.00%	0.00%	0.00%
Klebsiella	0.00%	0.00%	0.00%	0.00%
Hafnia	0.00%	0.00%	0.00%	0.00%
Proteus	0.00%	0.00%	0.00%	0.01%
Kluyvera	0.00%	0.00%	0.00%	0.00%
Buttiauxella	0.00%	0.00%	0.00%	0.00%
Erwinia	0.00%	0.00%	0.00%	0.00%
Hafvia	0.00%	0.00%	0.00%	0.00%
Obesumbacterium	0.00%	0.00%	0.00%	0.00%
Morganella	0.00%	0.00%	0.00%	0.00%
Proteus	0.00%	0.00%	0.00%	0.01%
Providencia	0.00%	0.00%	0.00%	0.00%
Rahnella	0.00%	0.00%	0.00%	0.00%
Raoultella	0.00%	0.00%	0.00%	0.00%
Trabusiella	0.00%	0.00%	0.00%	0.00%
Yersinia	0.00%	0.00%	0.00%	0.00%

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Non-Coliforms that can trigger Coliform test	Lateral.1	Lateral.2	Lateral.3	Lateral.4
Streptococcus	0.00%	0.00%	0.00%	0.27%
Acinetobacter	0.52%	0.04%	0.01%	0.14%
Aeromonas	0.00%	0.00%	0.00%	0.00%
Bacillus	0.88%	0.40%	0.08%	0.04%
Chryseobacterium	0.00%	0.00%	0.00%	0.00%
Herbaspirillum	0.00%	0.01%	0.11%	0.01%
Microbacterium	0.00%	0.00%	0.00%	0.00%
Plesiomonas	0.00%	0.00%	0.00%	0.00%
Pseudomonas	0.33%	0.22%	0.06%	0.21%
Shewanella	0.00%	0.00%	0.00%	0.00%
Stenotrophomonas	0.14%	0.01%	0.01%	0.00%
Stephylococcus	0.00%	0.00%	0.00%	0.00%
Vibrio	0.00%	0.00%	0.00%	0.00%
Wautersia	0.00%	0.00%	0.00%	0.00%
Fecal Indicators	**Lateral.1**	**Lateral.2**	**Lateral.3**	**Lateral.4**
Bacteroides	0.00%	0.00%	0.02%	0.10%
Prevotella	0.00%	0.01%	0.00%	0.00%
Escherichia	0.00%	0.00%	0.00%	0.00%
Shigella	5.08%	0.39%	0.02%	0.15%
Ruminococcaceae	0.00%	0.00%	0.00%	0.00%
Enterococcus	0.00%	0.00%	0.00%	0.00%
Lachnospiraceae	0.00%	0.00%	0.00%	0.00%
Leptospira	0.00%	0.00%	0.00%	0.00%

Contamination Indicators	Lateral.1	Lateral.2	Lateral.3	Lateral.4
Enterobacteriales (O	5.65%	0.54%	0.04%	0.19%
Clostridia (Class, str	8.13%	5.57%	2.39%	1.84%
Spirochaetae (Phylu	0.00%	0.19%	0.16%	0.02%
Fusobacterium	0.00%	0.00%	0.02%	0.00%
Streptococcus	0.00%	0.00%	0.00%	0.27%
Staphylococcus	0.00%	0.31%	0.01%	0.10%
Leptotrichia	0.00%	0.00%	0.00%	0.02%
Rothia	0.00%	0.00%	0.00%	0.00%
Bifidobacterium	0.00%	0.00%	0.01%	0.23%
Aggregatibacter	0.00%	0.00%	0.00%	0.00%
Trichococcus	0.00%	0.00%	0.00%	0.00%
Gemella	0.00%	0.00%	0.00%	0.00%
Porphyromonas	0.00%	0.05%	0.02%	0.00%
Capnocytophaga	0.00%	0.00%	0.00%	0.00%
Arcobacter	0.00%	0.01%	0.01%	0.00%
Potential Pathogens	**Lateral.1**	**Lateral.2**	**Lateral.3**	**Lateral.4**
Mycobacterium	0.00%	0.00%	0.00%	0.00%
Helicobacter	1.35%	0.29%	0.12%	0.35%
Haemophilus	0.00%	0.00%	0.00%	0.00%
Legionella	0.01%	0.20%	0.14%	0.08%
Escherichia	0.00%	0.00%	0.00%	0.00%
Salmonella	0.57%	0.06%	0.00%	0.03%
Campylobacter	0.00%	0.00%	0.00%	0.00%
Aeromonas	0.00%	0.00%	0.00%	0.00%
Alternaria	0.00%	0.00%	0.00%	0.00%
Clostridium	0.42%	0.54%	0.34%	0.30%

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Freshwater or Marine Bacteria (potential sign of surface water intrusion)	Lateral.1	Lateral.2	Lateral.3	Lateral.4
Polynucleobacter	6.73%	1.87%	0.34%	3.23%
Prochlorococcus	5.46%	2.09%	1.11%	0.82%
Limnohabitans	0.00%	0.00%	0.00%	0.00%
Pelagibacter	0.00%	0.00%	0.00%	0.00%
acl Freshwater Actin	0.00%	0.00%	0.00%	0.00%
Nitrogen Fixation	**Lateral.1**	**Lateral.2**	**Lateral.3**	**Lateral.4**
Azospirillum	0.00%	0.02%	0.05%	0.03%
Acidithiobacillus	0.00%	0.00%	0.00%	0.00%
Rhizobiales (Order)	0.00%	0.00%	0.00%	0.00%
Desulfovibrio	1.17%	0.11%	0.14%	0.01%
Azospira	0.00%	0.00%	0.00%	0.00%
Bradyrhizobium	0.00%	0.07%	0.04%	0.11%
Methylosoma	0.00%	0.00%	0.00%	0.00%
Clostridium	0.42%	0.54%	0.34%	0.30%

Carbon Fixation	Lateral.1	Lateral.2	Lateral.3	Lateral.4
Sediminibacterium	0.54%	6.04%	9.30%	9.37%
Sideroxydans	0.49%	1.08%	1.06%	0.74%
Nitrospira	0.40%	0.60%	0.46%	0.02%
Acidithiobacillus	0.00%	0.00%	0.00%	0.00%
Sulfuricurvum	0.00%	0.00%	0.00%	0.00%
Jettenia	0.00%	0.00%	0.00%	0.00%
Nitrosomonas	0.00%	0.00%	0.00%	0.00%
Sulfuricella	0.00%	0.00%	0.00%	0.00%
Geobacter	0.62%	0.41%	1.06%	0.53%
Gallionella	5.82%	6.88%	13.55%	5.97%
Brocadia	0.00%	0.00%	0.00%	0.00%
Scalindua	0.00%	0.00%	0.00%	0.00%
Leptospirillum	0.07%	0.00%	0.00%	0.01%
Crenothrix	0.00%	0.00%	0.00%	0.00%
Nitrospinaceae	0.00%	0.00%	0.00%	0.00%
Ferritrophicum	0.00%	0.00%	0.00%	0.00%
Candidatus_Nitroso	0.00%	0.00%	0.00%	0.00%
Nitrosococcus	0.00%	0.08%	0.02%	0.00%
Nitrosopumilus	0.00%	0.00%	0.00%	0.00%
Sulfurivirga	0.00%	0.00%	0.00%	0.00%

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Ammonia Oxidation	Lateral.1	Lateral.2	Lateral.3	Lateral.4
Jettenia	0.00%	0.00%	0.00%	0.00%
Nitrosomonas	0.00%	0.00%	0.00%	0.00%
Brocadia	0.00%	0.00%	0.00%	0.00%
Scalindua	0.00%	0.00%	0.00%	0.00%
Pirellula	0.26%	0.02%	0.04%	0.50%
Gemmata	0.00%	0.06%	0.02%	1.55%
Planctomyces	0.09%	0.29%	0.10%	0.12%
Candidatus_Nitroso	0.00%	0.00%	0.00%	0.00%
Planctomycetaceae	0.00%	0.00%	0.00%	0.00%
Isosphera	0.00%	0.00%	0.00%	0.00%
Candidatus_Kuenen	0.00%	0.00%	0.00%	0.00%
Anammoxoglobus	0.00%	0.00%	0.00%	0.00%
Nitrosococcus	0.00%	0.08%	0.02%	0.00%
Nitrosopumilus	0.00%	0.00%	0.00%	0.00%
Nitrosocaldus	0.00%	0.00%	0.00%	0.00%
Nitrososphaera	0.00%	0.00%	0.00%	0.00%
Crenarchaeum	0.00%	0.00%	0.00%	0.00%
Nitrite Oxidation	**Lateral.1**	**Lateral.2**	**Lateral.3**	**Lateral.4**
Nitrospira	0.40%	0.60%	0.46%	0.02%
Nitrobacter	0.00%	0.00%	0.00%	0.00%
Nitrospina	0.00%	0.18%	0.07%	0.00%
Nitrococcus	0.00%	0.00%	0.00%	0.00%

Iron Oxidation	Lateral.1	Lateral.2	Lateral.3	Lateral.4
Ferribacterium	0.00%	0.00%	0.00%	0.00%
Sediminibacterium	0.54%	6.04%	9.30%	9.37%
Sideroxydans	0.49%	1.08%	1.06%	0.74%
Acidithiobacillus	0.00%	0.00%	0.00%	0.00%
Geobacter	0.62%	0.41%	1.06%	0.53%
Gallionella	5.82%	6.88%	13.55%	5.97%
Leptospirillum	0.07%	0.00%	0.00%	0.01%
Crenothrix	0.00%	0.00%	0.00%	0.00%
Ferritrophicum	0.00%	0.00%	0.00%	0.00%
Ferrovibrio	0.00%	0.00%	0.00%	0.00%
Acidiferrobacter	0.00%	0.00%	0.00%	0.00%
Sulfate Reduction	**Lateral.1**	**Lateral.2**	**Lateral.3**	**Lateral.4**
Desulfuromonas	0.00%	0.00%	0.01%	0.00%
Desulfurivibrio	0.00%	0.00%	0.00%	0.00%
Desulfovibrio	1.17%	0.11%	0.14%	0.01%
Desulfocapsa	0.00%	0.00%	0.00%	0.00%
Desulfomonile	0.00%	0.09%	0.02%	0.00%
Desulfobacteraceae	0.00%	0.00%	0.00%	0.00%
Desulfobulbaceae	0.00%	0.00%	0.00%	0.00%
Desulfobacterium	0.00%	0.00%	0.01%	0.00%
Desulfosporosinus	0.00%	0.00%	0.01%	0.00%

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Sulfur Oxidation	Lateral.1	Lateral.2	Lateral.3	Lateral.4
Thiobacillus	0.00%	0.08%	0.01%	0.00%
Thiothrix	0.35%	0.02%	0.02%	0.00%
Thiobacillus	0.00%	0.08%	0.01%	0.00%
Sulfuritalea	0.00%	0.00%	0.00%	0.00%
Sulfuricurvum	0.00%	0.00%	0.00%	0.00%
Sulfuricella	0.00%	0.00%	0.00%	0.00%
Sulfurospirillum	0.00%	0.00%	0.00%	0.00%
Sulfuritalea	0.00%	0.00%	0.00%	0.00%
Magnetovibrio	0.00%	0.00%	0.00%	0.00%

Methane Oxidation	Lateral.1	Lateral.2	Lateral.3	Lateral.4
Methanosaeta	0.00%	0.00%	0.00%	0.00%
Methanoculleus	0.00%	0.00%	0.00%	0.00%
Methyloversatilis	0.01%	0.24%	0.39%	0.25%
Methylophilaceae	0.00%	0.00%	0.00%	0.00%
Methylosoma	0.00%	0.00%	0.00%	0.00%
Methylococcus	1.11%	0.56%	0.44%	0.47%

Biofilm Slime Formers	Lateral.1	Lateral.2	Lateral.3	Lateral.4
Pseudomonas	0.33%	0.22%	0.06%	0.21%
Sphingomonas	0.09%	0.01%	0.01%	0.40%

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Results

DNA data are displayed in Figure 1 and the Microbe Detectives tables. The data in these tables is a subset of the total data, focusing on bacteria that are typically important in drinking water 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 comprised 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.

Phylum

Gram-Negative

Proteobateria are gram negative bacteria that include many environmental bacteria, especially freshwater and marine bacteria. This phyla includes anaerobic, aerobic and facultative bacteria (facultative means they can survive in aerobic or anaerobic environments). Many bacteria important in drinking water treatment fall into the Proteobacteria phyla including ammonia oxidizing bacteria, sulfate-reducing bacteria, and phosphorus accumulating bacteria.

Bacteroidetes is a very common phyla of gram negative environmental bacteria that includes both aerobic and anaerobic bacteria. One of the most well-studied genera in this phyla is *Bacteroides* a very common inhabitant of the human large intestine. The presence of *Bacteroides* in particular likely indicates the presence of fecal matter from warm-blooded animals and possibly humans.

Cyanobacteria are gram-negative bacteria that obtain most or all of their energy from sunlight and typically utilize inorganic carbon to build cell materials. These bacteria are commonly called blue-green algae, though they are actually bacteria, not eukaryotic algae. Most "algal blooms" are caused by Cyanobacteria. Some of these bacteria such as *Microcystis* contain neurotoxins. As a result, algal blooms can be dangerous to mammals. Algal blooms are typically triggered by warm, stagnant water and high nutrients (nitrogen and phosphorus). These blooms produce oxygen during daylight but consume oxygen during night-time and as cells die and decay. This decay can lead to oxygen-starved waters like those found in the Gulf of Mexico dead zone which is inhospitable to fish and most life forms.

Nitrospirae are a phyla of gram-negative bacteria that are important in the nitrogen cycle. These bacteria are very important in drinking water treatment and other freshwater and marine environments where they oxidize nitrite to nitrate and play a crucial role in removing ammonia from drinking water. These bacteria are able to compete well at low concentrations of nitrite and oxygen. In the past, it was thought that *Nitrobacter* was the primary nitrite-oxidizing bacteria found in drinking water treatment plants. This mistake was based upon culture studies that were able to grow *Nitrobacter* from drinking water samples. However, scientists have more recently learned (based on DNA methods) that *Nitrobacter* is not typically common in drinking water treatment plants and doesn't compete well at low concentrations of nitrite even though it grows well in culture (which usually contains high concentrations of nitrite).

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Synergistetes are a phyla of bacteria involved in methane production. These bacteria are frequently symbionts with methanogenic archaea.

Chloroflexi are a somewhat new and unstudied phyla. Some of these bacteria may be important filaments in drinking water treatment.

Verrucomicrobia are a somewhat new and unstudied phyla. These bacteria are frequently correlated with cyanobacterial blooms in freshwaters and likely consume simple carbohydrates released from cyanobacteria or other sources. They tend to be fast growing bacteria.

Planctomycetes are a unique phyla of bacteria that are ovoid and reproduce by budding. This phyla includes most or all of the bacteria capable of performing anammox (anaerobic ammonia oxidation) in drinking water treatment which is a novel method to remove ammonia with substantially lower energy consumption than conventational processes that rely on nitrifiers and denitrifiers.

Gram-Positive

Firmicutes are gram-positive and are generally rod-like (bacilli) or round (cocci) cells. Many Firmicutes produce endospores that are resistant to drying and can survive extreme conditions. One example is *Clostridia tetani*, the bacteria that survives in soil and can cause tetanus. Firmicutes are important in beer, wine and cider spoilage. The primary subgroups of Firmicutes include the Clostridia class which are strictly anaerobic and the Bacilli which are obligate or facultative aerobes. In drinking water treatment, the presence of Firmicutes may indicate anaerobic conditions and could also indicate the presence of fermenting bacteria which are important for the production of volatile fatty acids necessary for biological phosphorus removal.

Actinobacteria are gram-positive and are frequently soil-dwelling bacteria, though a major portion of freshwater bacteria also fall into this phyla. Actinobacteria are known for producing secondary metabolites (compounds not directly necessary for the cell's survival) such as antibiotics and they are also known for degrading complex organics as part of the natural decay process. *Streptomyces* is one example which is famous for producing the majority of antibiotics used in medicine. In drinking water treatment, these bacteria are mostly known for causing problems such as filamentous bulking and foaming. *Microthrix* and *Gordonia* (aka Nocardia) are the two most-well known problematic Actinobacteria. The Actinobacteria are known for creating filamentous forms that can cause settling issues in drinking water treatment plants.

Coliforms

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

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

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

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)

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.

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Nitrogen Fixing Bacteria

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

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

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

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

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

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

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

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.

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