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DNA-Based Diversity Analysis of Microorganisms in Industrial Cooling Towers

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DNA-Based Diversity Analysis of Microorganisms in Industrial Cooling Towers

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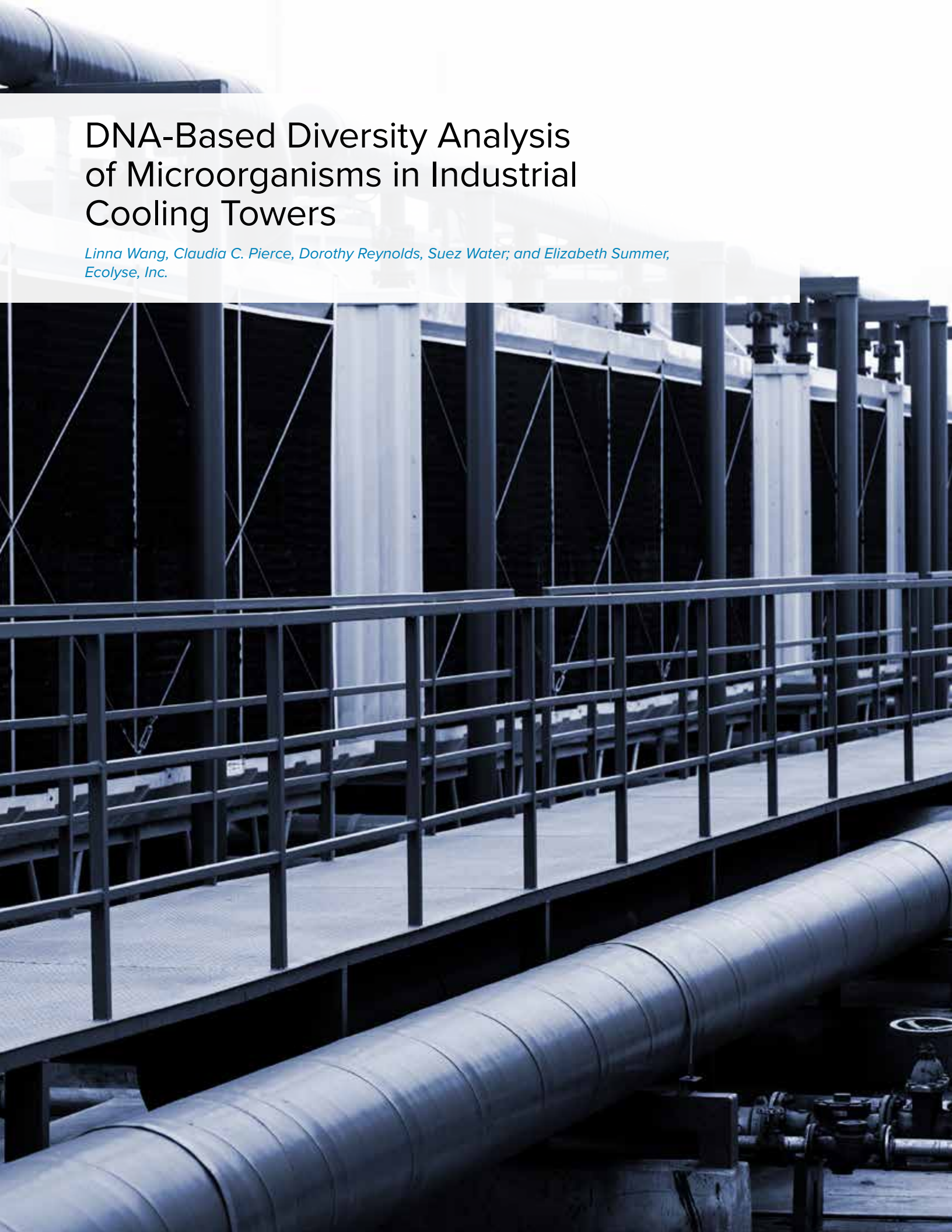


Introduction

Background on WCT Microbial Issues

Cooling processes use water as a heat sink, flowing over and through pipes and surfaces with many areas exposed to ambient conditions and environmental nutrients, not to mention nutrients stemming from industrial processes themselves. This creates an environment where microorganisms thrive and will take advantage of systems that are not maintained with proper chemical treatments.[1] Microbiological activity causes a number of issues, with some particular problem areas in a cooling tower, including fouling, corrosion, and the spread of aerosolized pathogens. One of the major issues caused by organism accumulation is a reduction of heat exchanger efficiency due to the insulating characteristics of biofilm growth, which also can restrict water flow or lead to full plugging of these tubes.[1] Microbial influenced corrosion and pitting are more likely to occur in tubes and pipes containing biofilm due to the creation of chemical gradients and anaerobic conditions.[2] Algal mats can form on open deck systems, restricting water flow, and in water lines, creating clogs. Tower fill encompasses a lot of surface area, is open to ambient air, and, with drift water contacting contaminated surfaces, creates potential health concerns. Organisms will also contribute to biodeterioration of wooden components in a cooling tower. There are also potentially pathogenic organisms, which, if captured in tower drift, may become aerosolized.

In industry, a typical approach to microbial control is through the generic application of chemical biocides. However, bacteria simply do not always respond in a uniform, predictable fashion to chemicals. In medicine, identification of the problem bacteria is sometimes needed to identify an effective antibiotic; similarly, biocides do not always work effectively in every application. Biocide sensitivity impacts many industries and sectors of society, including oil and gas, water treatment, food, personal hygiene, and health.[3-9] The root cause of response variation can be complex. In some cases, the cause is due to incompatibility of the biocide with the system chemistries. Additionally, intrinsic structural and physiological differences between organisms give rise to various susceptibilities. The sensitivity to biocides is also impacted by growth in a biofilm, and changes are observed depending on the species composition of the biofilm. Certain bacteria are more likely to produce the extracellular components of the biofilm (e.g. exopolysaccharides, which form a protective gelatinous film over organism). Others might produce acidic byproducts and inhabit areas under biofilm where they are protected and closest to metal surfaces, making damage likely. In other situations, the toxic nature of chemical biocides is contrary to their application (e.g., waters that may re-enter the environment).

The primary concern of industries is the efficient removal and control of potentially problem-causing organisms, as opposed to details on the types of organisms. However, control and knowledge of the problem organisms cannot always be separated. Growing evidence suggests that different types of bacteria respond very differently to chemical control agents. Therefore, in the interest of developing better microbial control methods, knowledge of the types of organisms causing the problem is paramount. Application of microbial population analysis tools based on amplification metagenomics has revolutionized understanding of bacteria in health, ecology, and other industries.[2,10] This understanding includes information on the types of water cooling tower (WCT) associated bacteria, the relative abundance of each different type of bacteria (i.e., which types of bacteria dominate a given population of a WCT systems), the absolute abundance (i.e., number of cells per milliliter of water, gram of solids, or centimeter of surface area), and how similar the different facilities are. A systematic, broad analysis of microbial populations in WCT using an amplicon metagenomics approach has, to the best of our knowledge, not been conducted. This will better allow for predictive tools to follow the specific types of bacteria that are responsible for microbial-associated issues.

The purpose of this study was to gain a broad perspective on microbial population structures across a spectrum of WCTs, as well as multiple samples within a single WCT. This information can be used to gain a real understanding of the targets of microbial control in WCT. Toward this end, an amplicon metagenomics approach was employed on 40 samples isolated across broad geographical and WCT types. This analysis provides a framework with which to plan the types of microbial control strategy that can be applied across a wide range of WCT, as well as to better understand the potential values of species targeted antimicrobials.

Experimental Procedure

Sample Descriptions

Sample Collection and Storage

WCT liquid samples were collected into clean, new polypropylene bottles, filled to the brim. Sessile samples included solids debrided from the sides of structures, sediments from the bottom of pools, or biofilms formed on corrosion coupons. Solid samples were packed in liquids obtained from the same location. All samples were kept at 4 oC and processed immediately upon arrival at the lab.

DNA Isolation and 16S Amplicon Metagenomics

Total environmental DNA was isolated from each sample. To do this, the bacteria were concentrated by centrifugation or filtration. DNA was isolated using a bead-beating approach, during which chemical and physical disruption of the cells is accomplished by incubation under strong denaturing conditions and maceration by vortexing in the presence of ceramic beads. The isolated DNA was column purified, and subjected to bacterial tag-encoded FLX amplicon pyrosequencing (bTEFAP).10 Resulting sequences were trimmed and quality scored. All sequences passing the quality score were compared using BLASTn to a ribosomal database to make a classification. Identity values were used to make assignments to the appropriate taxonomic levels based on the following cutoffs: Sequences with identity scores, to known or well characterized 16s sequences, greater than 97% identity (<3% divergence) were resolved at the species level, between 95% and 97% at the genus level, between 90% and 95% at the family level and between 85% and 90% at the order level, 80 and 85% at the class level and 77% to 80% at phyla level.

Physiological Annotations

Physiological assignments were made based on analysis of the data available for the most closely related organisms.

Results

A total of 40 samples, including 25 planktonic and 15 sessile samples, were collected from 12 WCT facilities (Table 1). Sample collecting and sequencing occurred between 2012 and 2014. The selection of WCT varied in geographical location, design, volume, and type of facility serviced. Geographically, the WCT samples included single facilities located in Ontario, Canada; Rhein, Germany; the states of Kansas, Maryland, New Jersey, Ohio, Pennsylvania, and South Carolina; and four cities in Texas. About half (19) were collected from WCT servicing industrial plants, primarily refineries. The remaining samples include eight collected at five different WCT within a large academic institution and 13 samples collected from a WCT servicing large commercial buildings. Within each location, samples were taken from collection ponds, makeup waters, and recirculation or sump water, as well as from sessile samples growing on coupons or solids from the location. For 11 of the sessile samples, 1-cm stainless steel biostuds were installed onto Robbins devices connected to recirculation waters at each site and allowed to circulate for varying lengths of time prior to analysis. For sampling, bacteria and DNA were isolated from the Robbins device coupons as well as associated fluids. The four sediments, including sludge and sand, were collected from the bottom of the collection pond for three of the samples. Planktonic samples included any flocculant in the materials recirculating water.

Table 1. Water Cooling Tower Sample Summary

WCT	Location	Type	# Samples	Sessile	Planktonic
A	Strathroy, ON, Canada	Industrial	2	1B	1
B	Germany, Rhein	Industrial	5	1B, 1S	3
C	USA, McPherson, KS	Industrial	1	0	1
D	USA, MD	Industrial	2	1B	1
E	USA, NJ	Industrial	2	0	2
F	USA, Racine, OH	Industrial	2	1B	1
G	USA, Trevose, PA	Commercial	2	1S	1
H	USA, Greenville, SC	Industrial	2	1B	1
I	USA, College Station, TX	Institutional	8	2B	6
J	USA, Sunray, TX	Industrial	2	1B	1
K	USA, Texas City, TX	Industrial	1	1S	0
L	USA, Woodlands, TX	Commercial	11	3B, 1S	7
12 Locations	***total number samples***		***40***	***15***	***25***
	Number Samples Facility Type	Industrial	19	*B, biostud*	
		Commercial	13	*S, sediments*	
		Institutional	8		

Bacterial Diversity in WCT

DNA was isolated from each of the WCT samples. The DNA yields ranged from 0.7 ng/ml to over 5 mg/ml of sample. When converted to bacterial cells per ml, using a conversion factor of 3.3 X 105 bacterial cells per ng DNA, these DNA yields corresponded to between 105 to 109 bacterial cells per ml. Note that this calculation does not take into account that DNA recovery is not 100% and also that at least some of the DNA could originate from nonbacterial sources, such as algae. Both of these factors impact the accuracy of using DNA yields to calculate bacterial load. The isolated DNA was subject to 16S amplicon metagenomic analysis. Following quality scoring and trimming, between 1,058 and 21,362 sequences were generated per sample. These were compared to a RNA sequence database and assigned species annotations. A total of 1,755 bacterial taxa were annotated. On a per sample basis, the number of taxa clusters ranged from 15 in sample I.1 to 519 in sample I.7 (Table 2). The ratio of the number of sequences to the number of taxa generated ranged from 0.007 to 0.08 (Table 2, data not shown). Table 2 provides a summary of the number of sequences and the number of unique taxa obtained from each sample.

Table 2. Number of Bacterial Taxa Identified in 40 Water Cooling Tower Samples

Sample	A.1	A.2	B.1	B.2	B.3	B.4	B.5	C.1	D.1	D.2	E.1	E.2	F.1	F.2
Type	P	B	P	P	S	P	B	P	P	B	P	P	P	B
# Seq	5141	13247	1745	6876	2648	10551	2383	8248	2945	3713	9095	4273	11829	3035
# Taxa	105	195	145	240	131	281	192	95	45	67	518	413	291	158

Sample	G.1	G.2	H.1	H.2	I.1	I.2	I.3	I.4	I.5	I.6	I.7	I.8	J.1	J.2
Type	S	P	P	B	P	P	P	P	P	B	B	P	P	B
# Seq	1969	1665	14610	8337	2142	3592	2308	2453	4598	3665	21088	17559	9833	6630
# Taxa	20	138	119	94	15	24	17	34	45	185	519	324	237	205

Sample	K.1	L.1	L.2	L.3	L.4	L.5	L.6	L.7	L.8	L.9	L.10	L.11	Total	Per
Type	S	P	P	P	P	P	P	S	B	B	B	P	Sum	Avg
# Seq	3938	4521	3390	18733	21326	4136	1937	1058	3282	1283	12075	20187	3E+05	7051
# Taxa	116	29	98	134	215	41	50	58	140	101	189	174	1755	155

Type is the type of sample (P, planktonic; S, sediments; B, biostud), # Seq = number of sequences, # Taxa = number of bacterial taxa. Total is the total for all samples. Note that the total number of unique taxa is not additive because some taxa are present in more than one sample. "Per Avg" is average number of sequences and number of taxa per sample.

Of the 1,755 bacterial taxa, 566 were given genus designations but not species designations because the similarity to known species was not high enough. These organisms are indicated as *Genus sp.* The four most widespread bacteria were annotated as *Psuedomonas sp., Acidobacterium sp., Flavobacterium sp.,* and *Hydrogenophaga sp* (Table 3). Each of these contains subclusters of sequences that are distinct enough to be different species. This indicates that bacterial diversity is even higher than what is indicated here. In all, 823 genera and 57 bacterial classes were identified in the samples. More than 93% of all species identified in the 40 samples were members of one of 16 different classes, including numerous representatives of Gammaproteobacteria, Betaproteobacteria, Alphaproteobacteria, Deltaproteobacteria, Cytophagia, Flavobacteria, Clostridia, Sphingobacteria, and Verrucomicrobia (Table 3, data not shown). Bacteria identified in the sample are predicted to be involved in widespread metabolic and physiological processes, including nitrogen cycling, degradation of xenobiotics or atypical substrates such as cell wall materials, photosynthesis, biofilm formation, iron reduction, sulfidogenesis, and acid production (Table 3).

Table 3. Bacteria Identified Present in at Least 15 of 40 WCT Samples

Class	Species	# WCT	Avg %	Characteristics of Interest
Gammaproteobacteria	*Pseudomonas sp*	36	7.34	Various, i.e., GHB, Biofilm, etc.
Acidobacteriia	*Acidobacterium sp*	34	14.94	APB, unknown
Flavobacteriia	*Flavobacterium sp*	31	8.01	Biodegradation
Betaproteobacteria	*Hydrogenophaga sp*	31	2.99	Biodegradation
Betaproteobacteria	*Acidovorax sp*	28	0.65	NRB, denitrifying
Betaproteobacteria	*Methylophilus sp*	28	8.00	Methylotroph
Alphaproteobacteria	*Sphingomonas sp*	27	1.14	Biodegradation, GHB
Cytophagia	*Flexibacter sp*	26	0.46	Biodegradation
Alphaproteobacteria	*Rhodobacter sp*	25	0.98	Photoautotroph
Gammaproteobacteria	*Legionella sp*	24	0.63	Unknown
Deltaproteobacteria	*Bdellovibrio sp*	23	0.26	Bacterial predator
Alphaproteobacteria	*Bradyrhizobium sp*	23	0.35	Nitrogen fixing
Alphaproteobacteria	*Caulobacter sp*	23	0.59	GHB
Clostridia	*Eubacterium sp*	23	2.30	Fermentative acetogen
Alphaproteobacteria	*Porphyrobacter sp*	23	3.60	Photoautotroph,biodegradation
Gammaproteobacteria	*Thermomonas sp*	23	0.60	NRB, denitrifying
Unclassified	*Aquabacterium sp*	22	0.85	Biofilm Steel
Betaproteobacteria	*Comamonas sp*	22	0.48	Biodegradation PAH denitrifying
Alphaproteobacteria	*Hyphomicrobium sp*	22	0.65	GHB
Betaproteobacteria	*Methylobacillus sp*	22	0.24	Methylotroph
Cytophagia	*Cytophaga sp*	21	1.51	Biodegradation
Gammaproteobacteria	*Pseudomonas aeruginosa*	21	5.67	GHB, biofilm
Gammaproteobacteria	*Acinetobacter sp*	20	1.86	Biodegradation
Gammaproteobacteria	*Aquimonas sp*	20	0.76	GHB
Clostridia	*Clostridium sp*	20	0.82	Diverse, fermentative
Unclassified	*Leptothrix sp*	20	0.24	Filamentous, Mn(II)OX MIC
Alphaproteobacteria	*Mesorhizobium sp*	20	0.17	Nitrogen fixing
Alphaproteobacteria	*Parvibaculum sp*	20	0.33	Biodegradation, alkanes
Alphaproteobacteria	*Porphyrobacter tepidarius*	20	5.25	Photoautotroph, biodegradation
Alphaproteobacteria	*Rhizobium sp*	20	0.21	Nitrogen fixing
Betaproteobacteria	*Methylovorus mays*	19	0.65	Methylotroph
Alphaproteobacteria	*Novosphingobium sp*	19	3.85	Biodegradation
Sphingobacteriia	*Sphingobacterium sp*	19	0.27	GHB aerobe
Verrucomicrobiae	*Verrucomicrobium sp*	19	0.45	Fermentative
Deltaproteobacteria	*Geobacter sp*	18	0.33	IRB
Betaproteobacteria	*Ralstonia sp*	18	0.64	Biodegradation
Unclassified	*Reyranella massiliensis*	18	0.55	Unknown
Gammaproteobacteria	*Stenotrophomonas sp*	18	0.28	Biofilm
Unknown	*Uncultured bacterium*	18	1.61	Unknown
Gammaproteobacteria	*Aeromonas sp*	17	4.44	Biofilm
Gammaproteobacteria	*Alcanivorax sp*	17	0.51	Biodegradation
Bacilli	*Bacillus sp*	17	0.13	Diverse, GHB, biodegradation
Gammaproteobacteria	*P. pseudoalcaligenes*	17	0.75	GHB, biofilm
Betaproteobacteria	*Sterolibacterium sp*	17	0.15	Biodegradation denitrifying
TM7 (class)	*TM7 uncultured*	17	1.12	Unknown
Betaproteobacteria	*Acidovorax delafieldii*	16	1.00	Biodegradation
Betaproteobacteria	*Alcaligenes sp*	16	0.39	NRB

Alphaproteobacteria	*Blastomonas sp*	16	0.29	CT
Gammaproteobacteria	*Cellvibrio sp*	16	1.18	Unknown
Flavobacteriia	*Chryseobacterium sp*	16	5.05	Biodegradation
Betaproteobacteria	*Denitratisoma sp*	16	0.30	NRB, denitrifying
Gemmatimonadetes	*Gemmatimonas sp*	16	0.22	GHB, oligotroph
Unclassified	*Methylibium sp*	16	0.38	Methylotroph
Gammaproteobacteria	*Methylophaga sp*	16	0.25	Methylotroph
Betaproteobacteria	*Methyloversatilis universalis*	16	5.30	Methylotroph
Gammaproteobacteria	*Rheinheimera sp*	16	2.04	Unknown
Betaproteobacteria	*Variovorax sp*	16	0.67	Biodegradation, phenol, TCE
Cytophagia	*Algoriphagus sp*	15	0.37	GHB, biodegradation
Gammaproteobacteria	*Alishewanella sp*	15	0.26	Sulfidogen
Gammaproteobacteria	*Aquimonas voraii*	15	0.22	Biodegradation
Betaproteobacteria	*Azoarcus sp*	15	0.45	Biodegradation, PAH, denitrifying
Deltaproteobacteria	*Bdellovibrio bacteriovorus*	15	0.31	Bacterial predator
Alphaproteobacteria	*Brevundimonas sp*	15	0.32	Biodegradation
Chlamydiia	*Chlamydia sp*	15	0.67	Endosymbiont
Betaproteobacteria	*Delftia sp*	15	0.74	Biodegradation, phenanthrene
Chlamydiia	*Parachlamydia acanthamoebae*	15	0.11	Endosymbiont
Chlamydiia	*Rhabdochlamydia crassificans*	15	0.42	Endosymbiont
Gammaproteobacteria	*Shewanella sp*	15	0.30	IRB, sulfidogen, MIC
Betaproteobacteria	*Thauera sp*	15	0.32	NRB, biodegradation
Betaproteobacteria	*Thiobacillus sp*	15	1.66	APB, SOB

Table 3 abbreviations: # WCT is the number of WCT samples that the indicated organism was identified in. Avg % is the average percent abundance in any samples. Characteristics of Interest are select characteristics of that type of bacteria. APB, acid producing bacteria; GHB, general heterotrophic bacteria, MIC, microbial influenced corrosion; NRB, nitrate reducing bacteria; SOB, sulfur oxidizing bacteria; IRB, iron reducing bacteria.

The majority of bacterial species were present in only a few samples. Bacteria were categorized by select physiological or metabolic traits to determine the distribution and abundance of potentially problematic bacteria (Table 4). Bacterial physiologies associated with corrosion include acid production, sulfidogenesis, and iron reduction. Sulfidogenic bacteria, capable of sulfide generation either from sulfate reduction or other pathways, were detected in 35 of the samples. Over 70 species of sulfidogenic bacteria, including 41 true SRB, were identified in 35 of the 40 samples tested. Representative SRB genera included Desulfovibrio, Desulfomicrobium, Desulfobacter, Desulfobotulus, and Desulfoglaeba (Table 4). Since true SRB are strict anaerobes, it is likely that these bacteria were associated with anaerobic microenvironments, such as in biofilms or floc. IRB are associated with metal corrosion. There were 43 species of IRB present, primarily different Geobacter and Pelobacter isolates. IRB were found in 27 of the 43 samples. Inorganic acid production, usually through sulfur oxidation, is associated with concrete degradation. Important inorganic acid producing bacterial genera, including Thiobacillus strains, were found in 26 of the 40 samples. When present, sulfidogenic, IRB, and inorganic acid producing bacteria did not constitute a significant proportion of the bacterial population, each usually comprising less than 1% of the total population of bacteria (Table 4). Data on these organisms in oil and gas systems, where their impact is most well studied, suggests that even these low concentrations might be enough to pose a threat to structures (2, 13). Organic acid bacteria typically produce acids as a result of fermentation. When present, organic acid bacteria constituted, on average, 7.6% of a given population. Organic acids are a common product of microbial fermentation under reductive conditions, which can be found in bottom layers of a biofilm, decreasing pH near metal surfaces and causing metals to dissolve. Formation of organic acids also supplies rich nutrients into deep layers of biofilm, which can be readily utilized by SRBs to create more aggressive corrosion.

Table 4. Distribution and Relative Abundance of Potentially Problematic Bacterial Types

Corrosion Associated Phenotype	Number of Species	Number of Samples	Average Relative Abundance	Representative Genera
Sulfidogen – all types	74	35	0.3%	Desulfuromonas, Dethiobacter, Alishewanella
Sulfidogen – SRB only	41	25	0.2%	Desulfovibrio, Desulfomicrobium, Desulfobacter, Desulfobotulus, Desulfoglaeba
Iron Reducing Bacteria	43	27	0.4%	Geobacter, Pelobacter, Desulfuromonas
Inorganic Acid Producing Bacteria	20	26	0.6%	Acidithiobacillus, Acidiphilium, Paracoccus
Organic Acid Producing Bacteria	59	36	7.6%	Lactobacillus, Acetobacter, Acidaminococcus

Bacterial Relative Abundance and Distribution

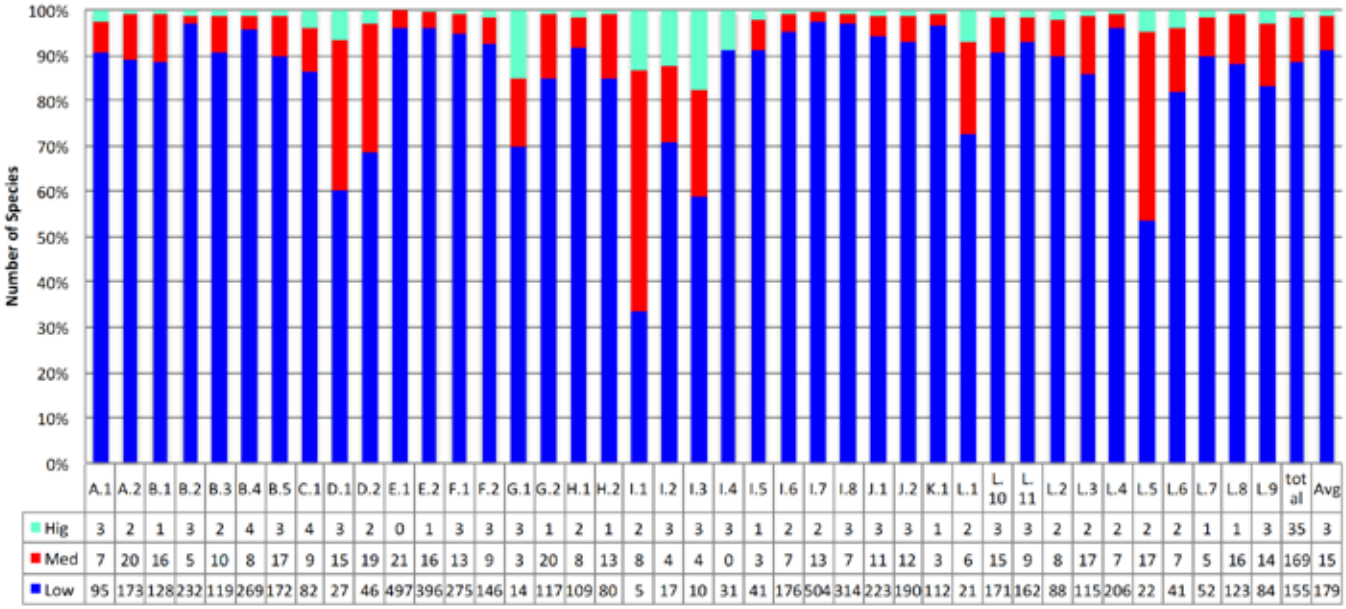
To better understand the impact of bacterial diversity on WCT functioning, the basic parameters of bacterial population structure needs to be determined. Two basic pieces of information about bacterial population structure are 1) the number of different types of unique species in a given sample (absolute diversity) and 2) the relative abundance (e.g., the relative proportion of each bacteria to each other, as a % abundance in the population). For the 40 WCT populations tested here, the number of species identified in each sample varied from 15 to 519, with an average number of species per sample of 155. Not every species was present in every sample. On average, each species was found in 3.5 of the 40 samples. The distribution of species across all samples was found to vary considerably (Table 5). Of 1,755 species, 697 were found in only one of the 40 samples, and 1,619 were found in fewer than 10 samples. Conversely, only 4 species were found in more than 30 samples, with one species of *Pseudomonas* that was present in 36 samples being the most widely distributed. The four most widely distributed species were identified as *Pseudomonas sp., Acidobacterium sp., Flavobacterium sp.,* and *Hydrogenophaga sp.,* which were present in 36, 34, 31, and 31 samples, respectively.

Table 5. Distribution of 1,755 Species Across 40 WCT Samples

Number of samples in which each species is present	Number of Species	% of Species
30 to 40 samples	4	0.23
20 to 29 samples	26	1.48
10 to 19 samples	106	6.04
1 to 9 samples	1619	92.25
40 samples	*1,755 species total*	
Average Species/Sample: 155 species/sample		
Average Samples/Species: 3.5 samples/species		

Most of the different types of bacteria in each sample were present as only a small fraction of the total biomass of bacterial cells (Figure 1). Each of these "low abundance" types of bacteria constituted less than 1% of the population, meaning for every 1,000 bacterial cells in the sample, less than 10 of them were the indicated species. The "medium abundance" types of bacteria each contributed 1% to 10% of the biomass of bacteria, and "high abundance" types of bacteria made up more than 10% of the bacterial biomass. When each bacterium in a sample was classified as "low abundance," "medium abundance," and "high abundance," it was found that most of the types of bacteria in each sample were considered "low abundance," with sample 1.1 being the only exception (Figure 1). Taken together, the information on distribution and relative abundance suggests that most of the genetic diversity in WCT samples is present in "low abundance" organisms; that is, those making up less than 1% of the total biomass of the population. Because they are low abundance, the sequence coverage for most samples is not sufficient to robustly identify them in every sample. It is possible that the species are more widespread than indicated by these data, but much higher sequence coverage is needed to detect them.

Figure 1. The Relative Abundance of Each Species in Each Sample



	A.1	A.2	B.1	B.2	B.3	B.4	B.5	C.1	D.1	D.2	E.1	E.2	F.1	F.2	G.1	G.2	H.1	H.2	I.1	I.2	I.3	I.4	I.5	I.6	I.7	I.8	J.1	J.2	K.1	L.1	L 10	L 11	L.2	L.3	L.4	L.5	L.6	L.7	L.8	L.9	total	Avg
Hig	3	2	1	3	2	4	3	4	3	2	0	1	3	3	3	1	2	1	2	3	3	3	1	2	2	3	3	3	1	2	3	3	2	2	2	2	2	1	1	3	35	3
Med	7	20	16	5	10	8	17	9	15	19	21	16	13	9	3	20	8	13	8	4	4	0	3	7	13	7	11	12	3	6	15	9	8	17	7	17	7	5	16	14	169	15
Low	95	173	128	232	119	269	172	82	27	46	497	396	275	146	14	117	109	80	5	17	10	31	41	176	504	314	223	190	112	21	171	162	88	115	206	22	41	52	123	84	155	179

Comparison of Population Structures Between Different Samples

The next basic question on population structures is to ask how similar each population is to one another. One indicator of population similarity is how similar the two populations are in terms of how many shared organisms they have.[11] The population consortia of the different samples were also compared using the Dice's coefficient, Equation 1, where C is the number of species shared between the two samples, and A and B are the number of species in samples A and B.[9, 10]

$$D=[2C]/[A+B] \quad (1)$$

The assumption is that populations that share a greater percentage of species in common are more similar to each other. A limitation of this calculation is that it is not weighted for relative abundance of the shared organisms. Dice coefficient values range between zero and 1, where a value of 1 indicates the two samples are identical, such as when a sample is compared to itself.

The 40 WCT samples were compared to each other using the Dice coefficient (Figure 2). When all organisms, regardless of percent abundance in the sample, were compared, the overall pair-wise sample similarities were low, with an average value of 0.2, indicating that on average each sample shared less than 20% of potentially shared species in common with any other sample (data not shown). To reduce the impact of low sequence coverage, the values were recalculated using bacteria present in at least 1% of a sample. This resulted in an average pair-wise similarity value of 0.4 (Figure 2). Overall, samples from within a single WCT, or geographical location, were more similar to each other then they were to WCT from other geographical locations, with an average intra WCT value of 0.5. There were nine paired planktonic/sessile samples: A.1p/A.2s; B.1p/B.2s; B.4p/B.5s; D.1p/D.2s; F.1p/F.2s; H.1p/H.2s; I.8p/I.6s, I.7s; J.1p/J.2s; L.11p/L.8s, L.9s, L.10s. The average value for paired coupon and sessile samples was 0.7, indicating that the most similar populations of bacteria analyzed were the cognate biofilm and planktonic samples from a single location (Figure 2). Sample sets I and L included multiple samples from different WCT within a single geographical location. Paired sessile/biofilm samples were more similar to each other than they were to samples collected from different WCT within the same geographical location.

Figure 2. Pair-Wise Comparison of Bacteria in 40 WCT Samples

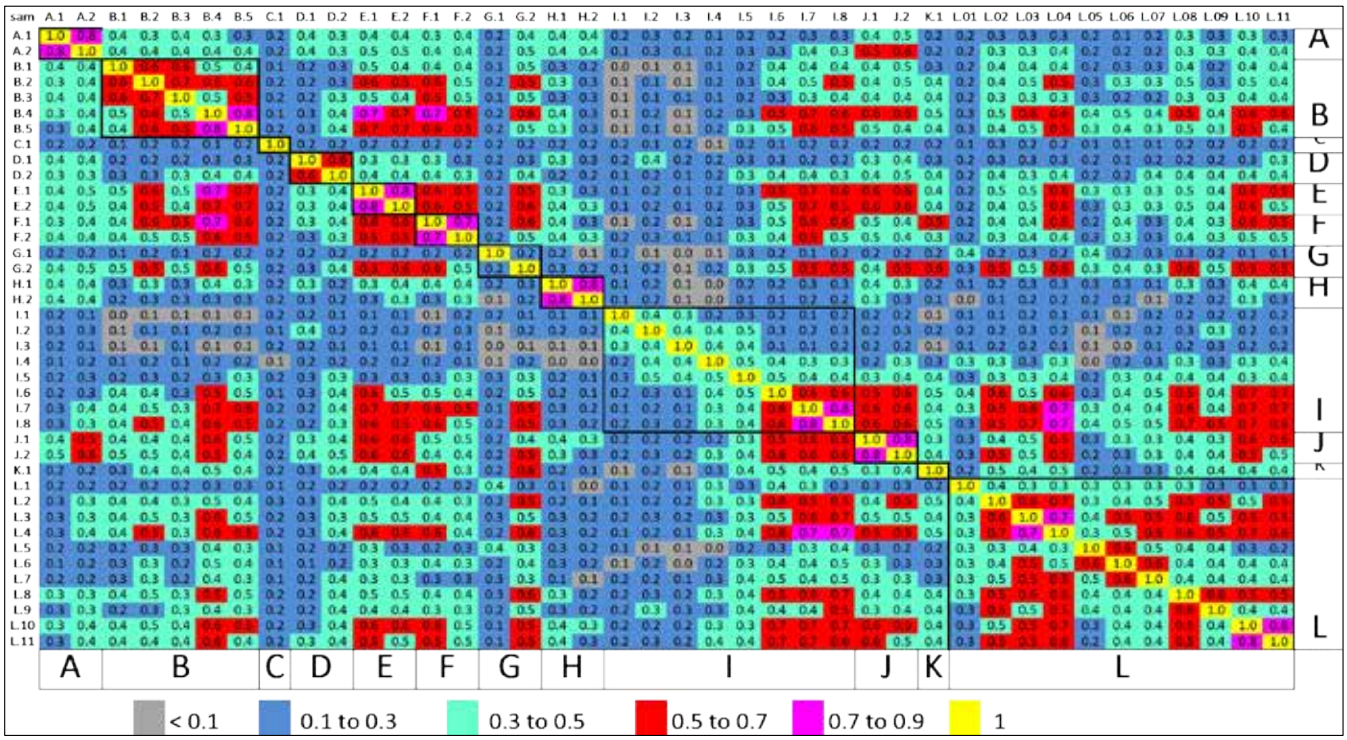


Figure 2 shows a 40 by 40 grid, with the Dice coefficient value for each population compared against every other population. The yellow diagonal indicates samples compared to themselves, which results in a value of 1 (100% identical). The remaining values are duplicated above and below the diagonal. Values are color coded as indicated. Boxed sample labels in the header row indicate the paired planktonic and sessile samples. Boxed values are the values for all samples collected from a single WCT, also indicated by the X-axis label (A through L).

Conclusions

This study included the analysis of a total of 40 samples comprising 12 different geographical locations. Having such diversity of locations proved to be a benefit but also had some downsides. The benefits include having a comparison of diversity from different states and even different countries. It could be seen if even a few organisms were abundant on a global or regional basis. It is much easier to make larger generalizations about particular organisms and their treatments if there is information related to prevalence. On the downside, it is more difficult to conduct a complete and thorough analysis of so many locations, making the data not as complete. Still, much information was gained from the study, and some interesting observations were made.

When just the comparison of the overall population structure in terms of distribution and relative abundance of bacteria in the samples is considered, results here parallel previous findings on bacterial populations from natural and industrial systems. Two generalities supported by independent analysis of multiple bacterial population data sets are that 1) the majority of different types of bacteria in a given sample are scarce (i.e., they constitute less than 1% of all bacteria in that sample), and 2) most of the bacterial types in any one sample are very limited in distribution (i.e., they are not likely to be detected in any other samples, even samples from the same field). However, the latter point might be an artifact of sequence depth, because as sequence coverage increases, the likelihood that low abundance speciess will be detected increases. These patterns were also evident in this study and suggests a generality that among organisms present in WCT, the bulk of genetic diversity is found in comparatively uncommon organisms, both in terms of absolute abundance as well as geographical distribution.

Of the 1,755 different species types detected in the study, 35 are considered to be in high abundance (over 1%), demonstrating the point above that the majority of organisms are in low abundance. This type of population, in which a few types of organisms dominate the system but there is a large diversity among low abundance organisms, has been seen in analysis of water samples taken from other industries, such as the oil and gas segment.[2, 12, 13] This makes detection via standard plate count of the majority of organism types near impossible, as the most abundant organisms will always dominate the cultures. Organisms even at low abundance may act as a reservoir to take advantage of shifting conditions that become more favorable for proliferation and survival. It should be noted from historical data taken from the oil and gas industry that organisms need only be present at low levels ranging from 1% to 10% to become an issue, with an example being SRBs.[2] Thus, detection of even low levels of bacteria is crucial to properly applying treatment to avoid the impact of, in this case, corrosion.

Such high diversity with organisms even in low abundance is a lingering threat to systems that are not optimally treated due to both the intrinsic morphological variances in response to biocidal actives and the unique adaptive tolerance potential of each species. There were, however, four species found in more than 30 samples, with the top two in both abundance and prevalence across locations being acid producing bacteria and bacteria likely to lead to biofilm formation.

When considering the organisms that have potential to cause issues in a cooling tower, biofilm formers and acid producers are two of the major culprits. Biofilm formers are less susceptible to biocide penetration due to their EPS generation potential. Biofilms also combine organisms with collective EPS barriers, which leads to a more protective environment for other organisms through the generation of anaerobic conditions and chemical gradients. Biofilm's intrinsic resistance to biocides is due to the exopolysaccharide glycocalyx polymers, which allow adhesion to surfaces. Under these conditions biocides are forced through a diffusion gradient before reaching the organisms. Some Pseudomonas species are facultative anaerobes and can adjust from aerobic to anaerobic conditions and thrive above and below.

Biocide mechanisms of actions vary, and there is not a one-size fits all, particularly in light of the dynamic nature of organism tolerances. Of the general mechanism classes, there are oxidants, non-oxidant electrophiles, and membrane active chemicals. The oxidants include chlorine and peroxide and work from the outside in through free radical mediated oxidation. Electrophiles such as isothiazolones and glutaraldehyde react with intracellular nucleophiles on enzymes and cellular respiration. Some surface-acting mechanism biocides cationically react with anionic membranes to destabilize and cause leaking followed by cell death. Others act on surfaces through proton destabilization, such as what occurs with pyrithiones.

Organisms react to varying degrees, depending on the ability to adapt to such stressors. Membrane protein alterations, efflux pumps, exclusion, and catalase and oxidase production are all examples. A common example is seen in the waterborne organism Pseudomonas making quats more difficult to penetrate the surface. Oxidants are susceptible to catalase- and oxidase-producing bacteria causing inactivation. Common practice is to combine free radical oxidizers with metabolic inhibitors or surfacing acting agents, the thought being that the organisms are not as likely to have multiple phenotypic changes at once. Testing for susceptibility to a variety of biocides at both the onset of a biocidal program as well as periodically is key to keeping the most optimized and cost-effective solution.

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