Date: December 1, 2014 **PROPOSAL**
To: James Bode, St. Paul Regional Water Services (SPRWS)
From: Raymond M. Hozalski, Timothy M. LaPara, and Kimi Gomez-Smith
Re: Research Proposal: *Investigation of Nitrifying and Sulfate-Reducing Bacterial Communities in the SPRWS Distribution System and Their Roles in Water Quality and Infrastructure Deterioration*

Introduction

Despite the harsh, nutrient-limited environment of the drinking water distribution system (DWDS), most surfaces within the DWDS support the growth of biofilms (LeChevalier *et al.,*1987). DWDS biofilms are of concern for several reasons. First, these biofilms could harbor pathogens and opportunistic pathogens and thus represent a potential health risk to water consumers (Wingender and Flemming, 2011). DWDS biofilms also could contain bacteria that accelerate corrosion rates and thus degrade drinking water infrastructure. Furthermore, biofilms could harbor nitrifying bacteria that can facilitate the rapid destruction of residual chloramine during nitrification episodes. Consequently, water utilities have historically employed "broad spectrum" control methods, such as the use of a disinfectant residual, to eliminate pathogens and limit microbial growth within the distribution system. The majority of bacteria in the DWDS, however, reside in biofilms on the pipe walls where they are protected from residual disinfectant (Flemming *et al,* 2002).

Despite a relatively high and stable chloramine residual of 2 – 3 mg/L as Cl_2 in the St. Paul Regional Water Services (SPRWS) distribution system, we recently demonstrated that bacteria are ubiquitous on the pipe walls (Figure 1). Furthermore, we elucidated the community composition and structure of biofilm samples from 10 full-scale SPRWS water mains (both unlined and cement-lined iron) using high-throughput Illumina sequencing of 16S rRNA gene fragments (Figure 2). The study indicated that pipe wall biofilms were dominated by bacteria from two genera: *Desulfovibrio* and *Mycobacteria. Desulfovibrio* are anaerobic sulfate-reducing bacteria that dominated the bacterial community underneath corrosion tubercles. *Desulfovibrio* are of concern because they have been shown to accelerate corrosion (Javaherdashti, 2011). *Mycobacteria* were prevalent at the pipe-water interface and are of concern because this genus contains some species that are opportunistic pathogens (Vaerewijck *et al.*, 2005). Further differentiation of the *Mycobacteria* in the SPRWS system demonstrated that the two dominant species present, *M. frederiksbergense* (83.3%) and *M. aurum* (11.4%), are generally considered to be non-pathogenic. The dominance of these benign, disinfectant-resistant *Mycobacteria* in the biofilms, as well as the general lack of pathogenic bacteria and nitrifying bacteria, suggests that the conditions in the SPRWS DWDS promote a "healthy" DWDS community in the surface biofilms. Conversely, these DWDS conditions also appear to promote the growth of undesirable bacteria underneath tubercles that may exacerbate corrosion. In addition, although not a problem in free-flowing water mains, nitrification episodes occur in some parts of the DWDS (e.g., storage tanks). The particular DWDS conditions that are driving factors for biofilm community composition are yet to be identified, and the impact of *Desulfovibrio* on DWDS infrastructure degradation is still unknown.

Further research is needed to identify the main drivers of DWDS community composition. This should enable more targeted biofilm control methods in which DWDS conditions are

manipulated to select for desirable communities and suppress pathogenic and nuisance populations, such as corrosion-causing bacteria. Consequently, it is necessary to compare "healthy" chloraminated DWDS communities to communities in DWDS areas that are considered problematic (e.g. experience nitrification episodes, contamination issues), as well as to communities from other DWDSs that maintain different conditions (e.g., free chlorine residual, no residual). As complete eradication of DWDS biofilms is not feasible, it may be beneficial to pursue more targeted methods for controlling the growth of pathogenic and nuisance organisms in these biofilms.

The overarching goal of this study is to elucidate some of the key drivers of DWDS community composition. The main objectives for this study are to: (1) investigate the effects of disinfectant type and concentration on DWDS community composition and (2) investigate the impact of microbial-influenced corrosion in SPRWS distribution system.

Research Approach

The proposed research involves the sampling of tap water and pipe biofilms from three distribution systems employing different disinfection regimes: chloramine, chlorine, and negligible residual. Samples will be obtained from full-scale water mains as well as from various points within the treatment process and the distribution system, such as water storage towers. The metagenome and microbial communities of biofilms and drinking water will be analyzed using next-generation, high-throughput Illumina sequencing in conjunction with quantitative real-time polymerase chain reaction (qPCR). The impact of microbial induced corrosion in SPRWS drinking water will be investigated using lab-scale bioreactors and electrochemical noise analysis.

Objective 1. Investigate the effect of disinfection type and concentration on DWDS biofilm and tap water community composition.

Hypothesis 1: The disinfection regime is a driving factor for biofilm community composition in the distribution system. The maintenance of a consistent chloramine residual selects for the predominant growth of disinfectant-resistant *Mycobacteria*. Thus, *Mycobacteria* will not comprise a major portion of the chlorinated and non-chlorinated DWDS communities.

Significance. This will provide insight into the effect of disinfectant type and concentration on microbial community composition. The presence of a disinfectant residual in drinking water can provide a selective advantage to disinfectant-resistant bacteria. Studies have indicated that *Mycobacteria* outcompete many other bacteria in the presence of a disinfectant residual due to their increased disinfectant resistance (Lin *et al*., 2013; Pryor *et al*., 2004; Chiao *et al*., 2014). Additionally, there is evidence to suggest that *Mycobacteria* are more resistant to chloramine than to free chlorine (Pryor *et al.*, 2004; Gomez-Alvarez *et al.*, 2013). The identification of the major drivers of DWDS biofilm community composition is necessary for developing methods to manipulate community composition. Also, DWDS biofilms dominated by *Mycobacteria* might be a good indicator of a stable chloramine residual. If so, quantification of of non-pathogenic *Mycobacteria* may be a viable DWDS monitoring strategy.

Hypothesis 2: Ammonia-oxidizing bacteria and archaea will be present at higher concentrations in SPRWS biofilms obtained from areas with long water residence times and more variable

chloramine residuals (such as water storage tanks), in comparison to biofilms in mains with free-flowing water and stable water quality.

Significance. Routine SPRWS nitrification sampling reports have indicated certain areas within the DWDS, including water storage tanks (i.e. Highland reservoir, Highwood tank, Mendota Heights tank) that experience nitrification events. Characterization of water storage tank biofilm communities and tank water quality over a year will provide information about temporal variation of community composition and links with water quality parameters. Thus, these data will allow us to elucidate the conditions that select for ammonia-oxidizing bacteria and archaea, i.e., organisms that can cause nitrification events. An understanding of the conditions that select for these undesirable organisms will help utilities to better prevent nitrification events and thereby maintain acceptable water quality.

Experimental Design: Full-Scale Distribution System Sampling

Biofilm communities will be compared from three distribution systems employing different disinfection regimes: (1) the chloraminated SPRWS distribution system, (2) a chlorinated distribution system, and (3) the Trondheim, Norway distribution system, which does not maintain a disinfectant residual in the DWDS.

Six full-scale SPRWS water mains will be collected during routine main replacement activities. Cement-lined, non-tuberculated, and tuberculated unlined cast-iron pipes will be sampled in triplicate. Pipe samples (1 foot sections) will be extracted using a hinged reed cutter, the ends sealed with sterile plastic sheeting and transported to the laboratory in a cooler. Pipe biofilm and communities underneath tubercles will immediately be sampled using a sterile scraper. Each sample will be homogenized then split into two 1.5-mL microcentrifuge tubes. One tube will be set aside for immediate RNA extraction (Objective 2), while the second tube will be set aside and stored at -20^{o} C until DNA is extracted. Water samples will be obtained in conjunction with pipe sampling. Temperature, pH, total chlorine concentration, and free chlorine concentration in the water will be measured immediately at the sampling site. Water for assimilable organic carbon (AOC) testing will be collected in organic carbon-free glass bottles, while water samples for other testing parameters will be collected in 5-L sterile plastic containers, placed on ice, and transported to the laboratory in a cooler for immediate processing. Bacteria will be concentrated from water samples via filtration (1 L per filter in triplicate). DNA will be extracted from the biofilm samples or filters with the FastDNA Spin Kit (MP Biomedicals, Solon, GA) and then stored at -20 ^{o} C until further processing. The remaining water will be used for analysis of water quality parameters, including ammonia, nitrite, nitrate, sulfate, sulfite and heterotrophic plate counts.

A grant from the Norwegian Department of Education funded a recent sampling campaign (October 26th -November 2nd, 2014) of the Trondheim, Norway DWDS that was performed by another graduate student in our group. Biofilm samples were collected from three ductile iron water mains in one location in the DWDS. An additional sampling campaign will be conducted in June 2015 to collect more samples that cover a broader geographic area of the system as well as different pipe materials (e.g., cast iron, cement-lined ductile iron). The extracted DNA extracted will be shipped to our laboratory for further processing. Water and pipe sampling of a chlorinated DWDS (i.e., free chlorine) will occur during the summer of 2015, according to the

aforementioned sampling protocol. We have a contact at the Boulder, CO water utility, but we will try to find a system in Minnesota to minimize travel costs and associated issues.

Additional SPRWS biofilm and water samples will be obtained quarterly (April 2015-April 2016) from up to six water storage tanks, half of which have experienced nitrification problems in the past and the other half 'control' tanks with no history of nitrification episodes. We will also collect water samples from the SPRWS raw water, filter effluent, and finished water. Water samples will be obtained according to aforementioned protocol. Biofilm samples will be obtained onsite using a sterile scraper. The scrapings will be placed into sterile microcentrifuge tubes, immediately placed on ice and transported to the laboratory. The DNA will be extracted from samples using the FastDNA Spin Kit for Soil (MP Biomedicals, Solon, GA) and then stored at -20^{o}C until further processing.

High-throughput Illumina sequencing will be conducted on all water and biofilm DNA samples to enable detailed characterization of the DWDS metagenome. Additionally, the community composition and structure will be characterized. Diversity measurements and community analyses will be conducted using mothur (Schloss *et al*, 2009). Quantitative real-time PCR will be used to quantify microbial populations of interest. These include total bacteria, sulfate-reducing bacteria, bacteria from the *Mycobacterium avium* complex (*M. avium, M. intracellulare*), and ammonia-oxidizing bacteria and archaea.

Objective 2. Investigate the impact of microbial-influenced corrosion on SPRWS DWDS pipes.

Hypothesis 1. Genes associated with microbial-influenced corrosion (i.e. dissimilatory sulfate reduction genes, dissimilatory metal reduction genes, etc) will be abundant in communities underneath corrosion tubercles in comparison to pipe surface communities.

Significance. Bacterial function can vary significantly from environment to environment, even for bacteria of the same taxonomic species. Consequently, it is often inappropriate to assume bacterial function (such as corrosion-enhancement) merely from the presence of a given organism as indicated by the 16S rRNA gene. Characterization of the DWDS metagenome (i.e., all genes present, including functional genes) is thus necessary, as it will elucidate the potential functions and roles of bacterial strains that are present in the DWDS. This will provide stronger evidence regarding the corrosion-enhancing abilities of DWDS bacteria living in pipes with corrosion tubercles. This information, in conjunction with data from Hypothesis 2, can be used to optimize water treatment and corrosion mitigation practices. If microbial-influenced corrosion is a significant contributor to infrastructure degradation, an important corrosion-mitigation practice would be to decrease levels of corrosion-causing bacteria, such as sulfate-reducing bacteria. One way this might be achieved is by reducing concentrations of sulfate in the water (e.g., by using ferric chloride as a coagulant during water treatment, rather than aluminum sulfate or alum).

Hypothesis 2: Pitting corrosion will be initiated more rapidly in lab-scale bioreactors that mimic SPRWS distribution conditions in comparison to control bioreactors in which microbial growth is inhibited by UV irradiation. Additionally, pitting propagation will be directly proportional to the amount of microbial growth on the coupon, and specifically the surface density of *Desulfovibrio* bacteria.

Significance. The analysis of the biofilm community in conjunction with real-time pitting initiation will enable the direct association of bacterial community composition with corrosion events. Additionally, the measurement and comparison of corrosion rates in sterile and non-sterile bioreactors fed with SPRWS drinking water will enable the quantification of corrosion due to microbial activity. The impact of microbial-influenced corrosion on DWDS infrastructure has not been quantified, in part due to the difficulty of measuring localized corrosion - the typical form of microbial-influenced corrosion. This will provide valuable information as to whether microbial-influenced corrosion is a main contributor of corrosion within the DWDS.

Experimental Design: SPRWS DWDS Metagenomic and Gene Expression Analyses
Metagenomic analysis will be conducted on SPRWS DWDS pipe samples obtained in Objective 1. The metagenomes of 3 communities obtained from underneath corrosion tubercles and 3 communities obtained from pipe surface samples will be compared. Sample DNA will be sheared and subjected to random whole-genome amplification, using K9-DNA primers and the polymerase chain reaction (PCR) protocol developed by Shanks *et al.* (2006), in order to generate sufficient DNA for metagenome analysis. Purified PCR products for each sample will be sequenced on the Illumina MiSeq platform, using an entire lane per sample. Metagenome data for each sample will be assembled and filtered for quality using a pipeline such as MG-RAST.

Additionally, we will use reverse-transcriptase qPCR (RT-qPCR) to target the expression of dissimilatory sulfate reduction genes. In the production of enzymes to perform a certain function, such as sulfate reduction, the first step is transcription of the gene (DNA) to the corresponsing messenger RNA (mRNA) strand. This short-lived mRNA strand is then used as a template to produce the protein (i.e., enzyme) via the process of translation. The RT-qPCR method will allow us to quantify the mRNA used to make a key enzyme needed for sulfate reduction. *Hence, RT-qPCR will enable us to determine whether bacterial sulfate-reduction is occurring in the under-tubercle environment.* Identifying or quantifying the equivalent sulfate reducing gene using conventional PCR or qPCR merely tells us that there is the potential for sulfate reduction. To perform RT-qPCR, RNA will be extracted immediately after sampling pipe biofilms (described in Objective 1). After RNA extraction, a reverse transcription step will be conducted immediately to produce the corresponding DNA molecule. Following reverse transcription, the sample will be stored at -20 ^{o}C until qPCR is conducted. *Thus, with our molecular toolbox we can determine which bacteria are present (Illumina MiSeq sequencing targeting 16S rRNA gene), what the bacteria are capable of doing (metagenomics), and what they are actually doing (RT-qPCR).*

Another approach for determining whether sulfate reduction is occurring or has occurred is to identify sulfide underneath tubercles, which can be done through the use of a simple qualitative test. A drop of dilute hydrochloric acid will be placed on material obtained from underneath a tubercle, and the material exposed to lead acetate test paper. The presence of sulfide will be indicated by a change in the lead acetate test paper color from white to brown and/or the production of a rotten egg smell (Little and Lee, 2007).

Experimental Design: Lab-Scale Bioreactors.
The effect of microbial growth on iron corrosion rates in SPRWS tap water will be monitored in three bioreactors, each representing different microbial growth conditions. Three continuous

stirred tank bioreactors will be set up using CDC bioreactors (Biosurface Technologies, Inc.), containing 24 unlined cast-iron coupons and a PTFE coated stir bar. The bioreactors will be fed continuously from the same tap with finished SPRWS water. Bioreactors will represent three different microbial growth conditions: (1) inhibited microbial growth, (2) neither inhibited nor promoted microbial growth, and (3) promoted microbial growth. Growth will be inhibited in the first bioreactor by filtering influent water with two 0.2-μm in-line membrane filters in conjunction with sterilization via an immersion UV-c lamp located within the bioreactor. The second bioreactor will be fed SPRWS water with no other manipulation. Growth will be promoted in the third bioreactor by a constant feed of 1% R_2A, a growth medium that is particularly suitable for enhancing the growth of bacteria found in potable water. All bioreactors will be covered with foil to keep out ambient light. At each sampling event, the coupons will be sampled in triplicate. Sampling will occur during at least three pitting initiation events in each bioreactor, as well as at significant pitting propagation events as indicated by electrochemical noise analysis. A simple, preliminary experiment will be conducted to determine the corrosivity of 1% R_2A in SPRWS water in comparison to only SPRWS water.

Electrochemical noise analysis will be used to monitor real-time initiation and propagation of pitting corrosion for 8 coupons in each bioreactor. Electrochemical noise analysis will be conducted by coupling two identical cast-iron coupons (working and counter electrodes) through a zero-resistance ammeter, in conjunction with a silver/silver chloride reference electrode. Characteristic fluctuations of current and potential occur in the metal coupon during the initiation and propagation of pitting corrosion, which can be measured and identified in real-time with the zero-resistance ammeter. Maximum pit depth measurements will be used in conjunction with electrochemical noise to obtain pitting corrosion rates. Uniform corrosion rates, which generally have abiotic causes, will be determined using coupon weight loss measurements. Dry, polished coupons will be weighed before placement into the bioreactors. After coupons are removed from the bioreactors, and sampling of the biofilm communities has concluded, coupons will be immediately placed in a weakly acidic solution for 5 seconds to remove corrosion deposits from the surface. The coupons will then be dried and weighed again to determine weight loss.

Illumina sequencing of the 16S rRNA gene will be used to characterize the community composition of coupons at time points corresponding to the initiation of pitting and other significant corrosion events observed by electrochemical noise analysis. Dissimilatory sulfate-reduction genes will be quantified using qPCR. The total biomass in coupon biofilms will be measured using qPCR of the 16S rRNA gene.

Budget and Budget Justification

The proposed budget for this 2-year project (Total = ~$179,874) is attached.

Labor. The budget includes 1.25 months per year of Professor Hozalski's time (1 month in summer and 0.25 months during the academic year) and 0.75 months of Professor LaPara's time (0.5 months in summer and 0.25 months during the academic year) for project design and planning, student guidance and supervision, data analyses and interpretation, attending meetings with SPRWS staff, and final report preparation. A graduate student research assistant (Kimi-Gomez-Smith) will work on the project at 50% time for 12 months per year for two years. The

cost of a 50% time graduate student research assistant is fixed by the University of Minnesota and includes full tuition, stipend, and health insurance.

Supplies. An amount of $6,500 per year is requested for research supplies including but not limited to the following: glassware, chemicals, DNA extraction/purification kits, PCR primers, and a laptop computer with software for data entry and analysis.

Lab Services. An amount of $6,000 per year is requested for use of the BioMedical Genomics Center (BMGC) at the University of Minnesota, including the Illumina MiSeq 2000 sequencer.

Travel. Funds are requested for:

1. Mileage for trips to and from the field sampling sites ($250 per year) and for trips to the plant for meetings.
2. Funds ($1,750 per year) for the graduate student and/or adviser(s) to attend the state drinking water conference (i.e., MN AWWA) and a national/international conference (e.g., AWWA WQTC) to present and discuss the results of the work.

Costs not included. Note that the costs associated with sampling the water mains and storage tanks are not included in the budget. The water main sampling costs include site excavation, labor for main extraction, main replacement, backfill, etc. It is assumed that these costs will be covered by SPRWS as part of routine main replacement activities or otherwise. Similarly, it is assumed that the costs associated with sampling water storage tanks, such as disposable sterile inflatable rafts, staff or contractor time to aid in accessing the inside of the tanks and collecting water and biofilm from the tanks, will be covered by SPRWS.

References

1. Chiao, Tzu-Hsin, Tara M Clancy, Ameet Pinto, Chuanwu Xi, and Lutgarde Raskin. 2014. Differential Resistance of Drinking Water Bacterial Populations to Monochloramine Disinfection. *Environmental Science & Technology* **48** (7) (April 1): 4038–47.
2. Gomez-Alvarez, Vicente, Randy P Revetta, and Jorge W Santo Domingo. 2012. Metagenomic Analyses of Drinking Water Receiving Different Disinfection Treatments. *Applied & Environmental Microbiology* **78** (17): 6095–102.
3. Javaherdashti, Reza. 2011. Impact of Sulphate-Reducing Bacteria on the Performance of Engineering Materials. *Applied Microbiology and Biotechnology* **91** (6) : 1507–17.
4. Lin, Wenfang, Zhisheng Yu, Hongxun Zhang, and Ian P Thompson. 2013. Diversity and Dynamics of Microbial Communities at Each Step of Treatment Plant for Potable Water Generation. *Water Research* **52** (November 7): 218–230.
5. LeChevallier, M W, T M Babcock, and R G Lee. 1987. Examination and Characterization of Distribution System Biofilms. *Applied & Environmental Microbiology* **53** (12): 2714–24.
6. Pryor, M, S Springthorpe, S Riffard, T Brooks, Y Huo, G Davis, and S Sattar**.** 2004. Investigation of Opportunistic Pathogens in Municipal Drinking Water under Different Supply and Treatment Regimes. *Water Science and Technology*: A Journal of the International Association on Water Pollution Research **50** (1) (January): 83–90.
7. Schloss, Patrick D, Sarah L Westcott, Thomas Ryabin, Justine R Hall, Martin Hartmann, Emily B Hollister, Ryan a Lesniewski, et al. 2009. Introducing Mothur: Open-Source,

Platform-Independent, Community-Supported Software for Describing and Comparing Microbial Communities. *Applied and Environmental Microbiology* 75 (23) : 7537–41.
8. Shanks, Orin C, Jorge W Santo Domingo, James E Graham. 2006. Use of competitive DNA hybridization to identify differences in the genomes of bacteria. *Journal of Microbiological Methods* **66**(2): 321-330.
9. Wingender, Jost, and Hans-Curt Flemming. 2011. Biofilms in Drinking Water and Their Role as Reservoir for Pathogens. *International Journal of Hygiene and Environmental Health* 214 (6): 417–23.

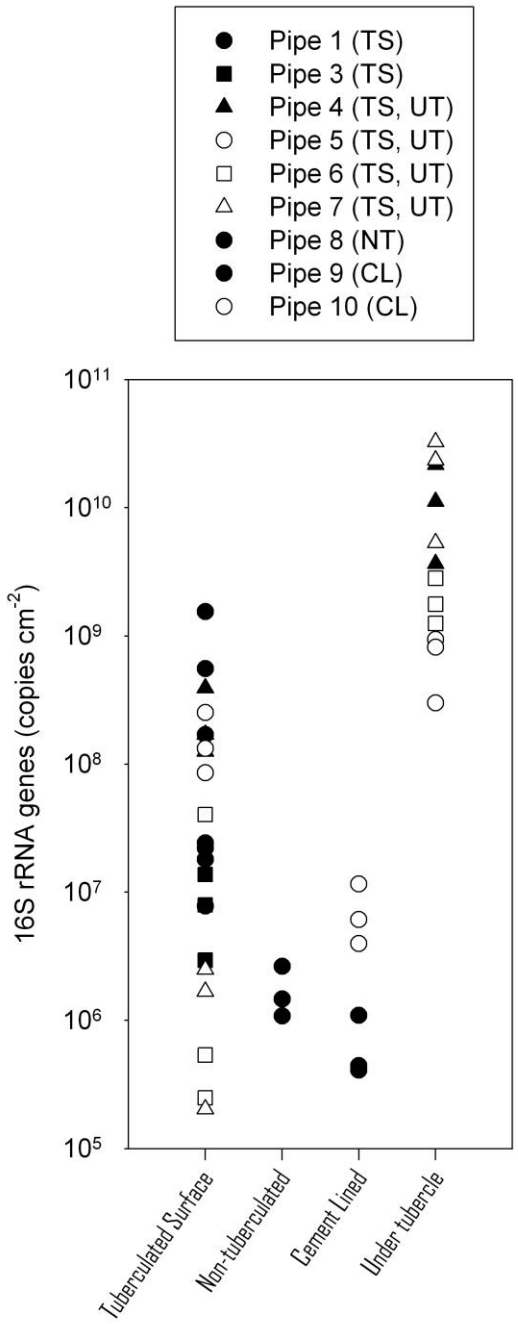

Figure 1. Total biomass of pipe community samples as indicated by quantitative real-time PCR of the 16S rRNA gene per cm^2 of scraped biofilm. Abbreviations refer to sample type and pipe type: TS-Tuberculated surface, UT-Under tubercle, NT- Non-tuberculated, CL-Cement lined.

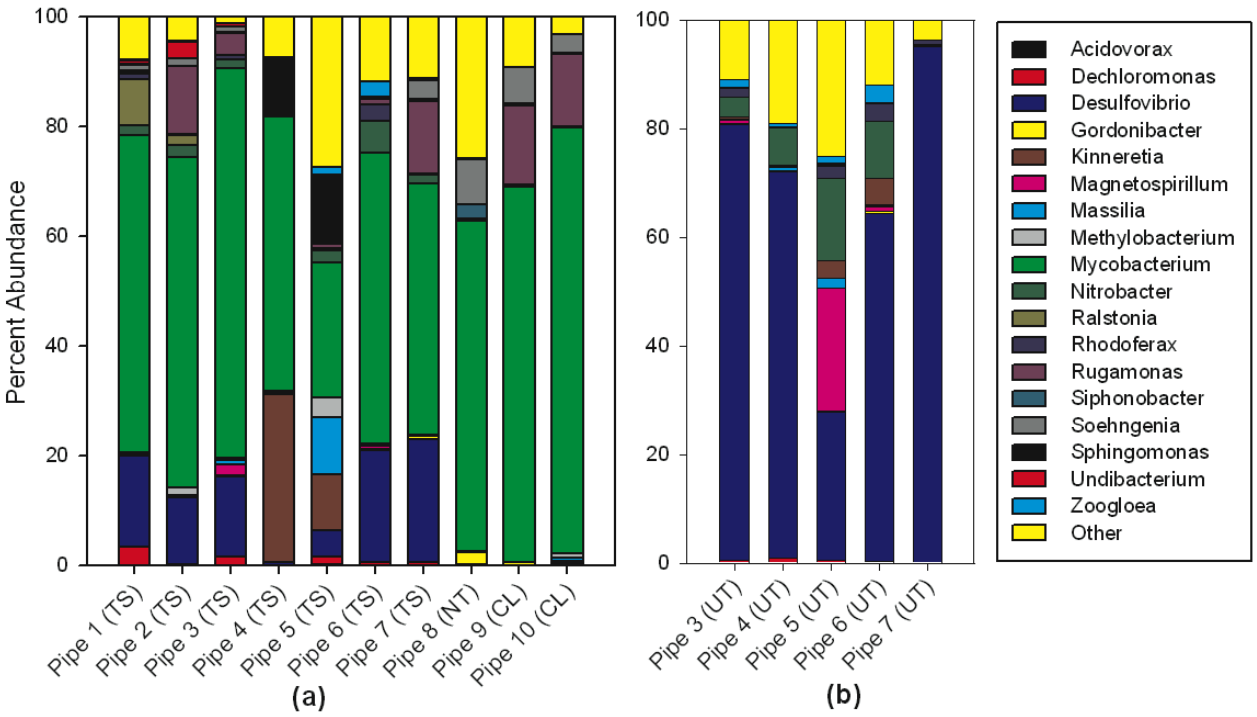

Figure 2. Miseq distribution profiles of the 18 most abundant genera represented in (a) pipe surface samples, and (b) under tubercles samples. Abundant genera are those that comprised greater than 0.5% of total sequences for all samples combined (2,079,805 total sequences). Genera that represented less than 0.5% of total sequences are grouped in "Other". Distribution profiles represent the arithmetic mean of triplicate communities obtained from each pipe, with the exception of Pipe 1, which had 7 replicates. Abbreviations refer to sample type and pipe type: TS-Tuberculated surface, UT-Under tubercle, NT- Non-tuberculated, CL-Cement lined.

		ORGANIZATION Regents of the University of Minnesota PRINCIPAL INVESTIGATOR	Final Budget		YEAR 1 5/1/2015 TO 4/30/16	YEAR 2 5/1/16 TO 4/30/17	Summary 5/1/2015 TO 4/30/17
A.		SENIOR PERSONNEL					
	1.	Ray Hozalski 4 wks Summer + 1 wk AY per year		15,685	16,313	31,998
	2.	Tim LaPara 2 wks Summer + 1 wk AY per year		8,684	9,031	17,715
	3.				0	0
	4.			0	0	0
		TOTAL SENIOR PERSONNEL		24,369	25,344	49,713
B.		OTHER PERSONNEL				
	1.					0
	2.	1 grad student 50% with 2.5 % raise each year		17,956	18,404	36,360
	3.	1 grad student 50% summer with2.5% raise each year		5,985	6,135	12,120
	4.					
	5.					
		TOTAL SALARIES AND WAGES(A+B)		48,310	49,883	98,193
		FRINGE BENEFITS	0.92857			
C.	1.	Academic: B-term Faculty A1 @	33.80%	8,237	8,566	16,803
	2.	Post Doc B1 @	21.40%	0	0	0
	3.	B3 @				
	4.	Grad Student Acedemic Year: Tution	$17.84	13,915	13,915	27,830
		Graduate Health Acedemic Year	16.60%	2981	3055	6,036
		Graduate Health Summer	16.60%	994	1018	2,012
	5.	Undergraduate & Grad Student: FICA		0	0	0
		TOTAL FRINGE BENEFITS		26,126	26,555	52,681
		TOTAL SALARIES, WAGES, AND FRINGE BENEFITS(A+B+C)		74,436	76,438	150,874
D.		PERMANENT EQUIPMENT: To be explained in budget justification. ($2500+)				
		168100 - Capital Equipment ($5000 & over)				0
				0	0	0
		(Exempt from indirect cost)				
		TOTAL PERMANENT EQUIPMENT		0	0	0
E.		TRAVEL				
	1.	720600 - Domestic:		2,000	2,000	4,000
	2.	720604 - Foreign:		0	0	0
	3.	720607 - Non-Empl		0	0	0
		TOTAL TRAVEL		2,000	2,000	4,000
F.		PARTICIPATE				
	1.	Stipends:		0	0	0
	2.	Travel:		0	0	0
	3.	Subsistence		0	0	0
	4.	Other		0	0	0
		TOTAL PARTICIPANT EXPENSES		0	0	0
G.		OTHER DIRECT COSTS				
		Materials and Supplies				
	1.	Lab Supplies		6,500	6,500	13,000
				0	0	0
	3.	720400 Lab Servies		6,000	6,000	12,000
	4.			0	0	0
	5.			0	0	0
	6.					0
	7.			0	0	0
	8.			0	0	0
	9.			0	0	0
		TOTAL OTHER DIRECT COSTS		12,500	12,500	25,000
H.		TOTAL DIRECT COSTS (A THROUGH G)		88,936	90,938	179,874
I.		INDIRECT COSTS (H-Graduate Fringe) @ 52%		0	0	0
J.		TOTAL PROJECT COST		88,936	90,938	179,874

NOTES: If you intend to use computer supplies: lab computer, cables, zip disks or other peripheral computing equipment, these items must be justified in your proposal.