

Sulfate Reducing Bacteria and Mycobacteria Dominate the Biofilm Communities in a Chloraminated Drinking Water Distribution System

C. Kimloi Gomez-Smith^{1,2}, Timothy M. LaPara^{1, 3}, Raymond M. Hozalski^{1,3*}

¹Department of Civil, Environmental, and Geo- Engineering, University of Minnesota, Minneapolis, Minnesota 55455 United States

²Water Resources Sciences Graduate Program, University of Minnesota, St. Paul, Minnesota 55108, United States

³BioTechnology Institute, University of Minnesota, St. Paul, Minnesota 55108, United States

Pages: 9

Figures: 2

Tables: 3

Inquiries to: Raymond M. Hozalski, Department of Civil, Environmental, and Geo-Engineering, 500 Pillsbury Drive SE, Minneapolis, MN 554555, Tel: (612) 626-9650. Fax: (612) 626-7750. E-mail: hozal001@umn.edu

Table S1. Reference sequences used in the newly created alignment and taxonomy databases for *hsp65* Illumina sequencing. Sequences were obtained from the National Center for Biotechnology Information Genbank database.

Organism name	Accession Number	Organism name	Accession Number
<i>Arthrobacter ureafaciens</i>	DQ007457	<i>Mycobacterium koreense</i>	JF271827
<i>Corynebacterium afermentans</i>	EF107157	<i>Mycobacterium kubiae</i>	AY373458
<i>Mycobacterium abscessus</i>	JX154122	<i>Mycobacterium kumamotonense</i>	JX154126
<i>Mycobacterium aemonae</i>	AM902964	<i>Mycobacterium kyorinense</i>	JN974461
<i>Mycobacterium africanum</i>	AF547803	<i>Mycobacterium lacticola</i>	HM030495
<i>Mycobacterium agri</i>	AY438080	<i>Mycobacterium lacticola</i>	HM030495
<i>Mycobacterium aichiense</i>	AJ310218	<i>Mycobacterium lacus</i>	AY438090
<i>Mycobacterium aichiense</i>	AF547804	<i>Mycobacterium lentiflavum</i>	AF547851
<i>Mycobacterium algericum</i>	GU564405	<i>Mycobacterium leprae</i>	AY299192
<i>Mycobacterium alsiensis</i>	DQ381733	<i>Mycobacterium liflandii</i>	AY500839
<i>Mycobacterium alvei</i>	JX154127	<i>Mycobacterium llatzerense</i>	AM421344
<i>Mycobacterium angelicum</i>	AM902962	<i>Mycobacterium llatzerense</i>	JF491330
<i>Mycobacterium aromaticivorans</i>	DQ841182	<i>Mycobacterium longobardum</i>	JN571199
<i>Mycobacterium arupense</i>	DQ168662	<i>Mycobacterium madagascariense</i>	AF547852
<i>Mycobacterium asiaticum</i>	AJ310220	<i>Mycobacterium mageritense</i>	EU732652
<i>Mycobacterium aurum</i>	AY438081	<i>Mycobacterium malmoense</i>	GQ153293
<i>Mycobacterium aurum</i>	FJ172326	<i>Mycobacterium manitobense</i>	DQ350158
<i>Mycobacterium austroafricanum</i>	AM403501	<i>Mycobacterium mantenii</i>	HM602041
<i>Mycobacterium avium</i>	AF126033	<i>Mycobacterium mantenii</i>	FJ426336
<i>Mycobacterium avium</i>	AJ307641	<i>Mycobacterium marinum</i>	FJ868212
<i>Mycobacterium avium</i>	DQ284771	<i>Mycobacterium marseillense</i>	HQ332519
<i>Mycobacterium avium</i>	FJ643451	<i>Mycobacterium massiliense</i>	JQ082095
<i>Mycobacterium avium</i>	HM454220	<i>Mycobacterium montefiorens</i>	AY943204
<i>Mycobacterium avium</i>	U55826	<i>Mycobacterium moriokaense</i>	AY859680
<i>Mycobacterium avium</i>	GQ153289	<i>Mycobacterium moriokaense</i>	AY859680
<i>Mycobacterium avium paratuberculosis</i>	U55827	<i>Mycobacterium mucogenicum</i>	JX154105
<i>Mycobacterium bacteremicum</i>	HM011133	<i>Mycobacterium murale</i>	AF547859
<i>Mycobacterium boenickei</i>	AY943195	<i>Mycobacterium nebraskense</i>	GQ153294
<i>Mycobacterium boemicum</i>	HQ235059	<i>Mycobacterium neglectum</i>	DQ350159
<i>Mycobacterium bolletii</i>	HM454235	<i>Mycobacterium neoaurum</i>	JF491302
<i>Mycobacterium botniense</i>	AF547812	<i>Mycobacterium neoaurum</i>	DQ081188
<i>Mycobacterium bouchedurhonense</i>	HM602039	<i>Mycobacterium neoaurum</i>	FJ515916
<i>Mycobacterium bovis</i>	JF491332	<i>Mycobacterium neworleansense</i>	AY458076
<i>Mycobacterium branderi</i>	AJ310223	<i>Mycobacterium noviomagense</i>	EU600390
<i>Mycobacterium branderi</i>	JX294379	<i>Mycobacterium novocastrense</i>	JX154123
<i>Mycobacterium brasiliensis</i>	EU165539	<i>Mycobacterium obuense</i>	AJ307640
<i>Mycobacterium brisbanense</i>	JF491333	<i>Mycobacterium pallens</i>	DQ533997
<i>Mycobacterium brumae</i>	AJ310224	<i>Mycobacterium palustre</i>	AY943200
<i>Mycobacterium canariasense</i>	JX154104	<i>Mycobacterium paraffinicum</i>	KJ534365
<i>Mycobacterium canettii</i>	AJ749924	<i>Mycobacterium parakoreense</i>	JF271824
<i>Mycobacterium caprae</i>	AF547884	<i>Mycobacterium parascrofulaceum</i>	AY337276
<i>Mycobacterium celatum</i>	AJ310225	<i>Mycobacterium paraseoulense</i>	JF491324
<i>Mycobacterium cheloneae</i>	AJ310226	<i>Mycobacterium parvum</i>	JX154107
<i>Mycobacterium chimaera</i>	GQ153296	<i>Mycobacterium parrafinicum</i>	GQ153287
<i>Mycobacterium chitae</i>	AJ310227	<i>Mycobacterium peregrinum</i>	AY458069
<i>Mycobacterium chlorophenolicum</i>	AF547820	<i>Mycobacterium petroleophilum</i>	DQ350156

<i>Mycobacterium chubuense</i>	AF547821	<i>Mycobacterium phlei</i>	JX294389
<i>Mycobacterium colombiense</i>	JX154106	<i>Mycobacterium phocaicum</i>	KF667480
<i>Mycobacterium conceptionense</i>	AM902958	<i>Mycobacterium piscinum</i>	DQ350155
<i>Mycobacterium confluentis</i>	AJ310228	<i>Mycobacterium porcinum</i>	JF491326
<i>Mycobacterium conspicuum</i>	AF547823	<i>Mycobacterium poriferae</i>	AJ307645
<i>Mycobacterium cookii</i>	AF547824	<i>Mycobacterium pseudoshottsii</i>	AY571788
<i>Mycobacterium cosmeticum</i>	AY449731	<i>Mycobacterium psychrotolerans</i>	HM602035
<i>Mycobacterium crocinum</i>	DQ533998	<i>Mycobacterium pulveris</i>	AJ307646
<i>Mycobacterium diernhoferi</i>	AF547825	<i>Mycobacterium rhodesiae</i>	JF792840
<i>Mycobacterium doricum</i>	AF547826	<i>Mycobacterium riyadhense</i>	JF896099
<i>Mycobacterium duvalii</i>	AJ310229	<i>Mycobacterium rufum</i>	DQ841181
<i>Mycobacterium elephantis</i>	HM229789	<i>Mycobacterium rutilum</i>	EU727188
<i>Mycobacterium engbaekii</i>	JX154109	<i>Mycobacterium salmoniphilum</i>	DQ866777
<i>Mycobacterium europaeum</i>	HM022220	<i>Mycobacterium saskatchewanense</i>	AY208858
<i>Mycobacterium fallax</i>	AJ310230	<i>Mycobacterium scrofulaceum</i>	GQ478700
<i>Mycobacterium farcinogenes</i>	AJ310231	<i>Mycobacterium senuense</i>	JX154125
<i>Mycobacterium florentinum</i>	JF491317	<i>Mycobacterium seoulense</i>	JF491322
<i>Mycobacterium flouranthenivorans</i>	JF491318	<i>Mycobacterium septicum</i>	JF491329
<i>Mycobacterium fortuitum</i>	AJ310232	<i>Mycobacterium setense</i>	EU371505
<i>Mycobacterium fortuitum</i>	JX154098	<i>Mycobacterium sherrisii</i>	AY365190
<i>Mycobacterium franklinii</i>	HQ153092	<i>Mycobacterium shimoidei</i>	JF491305
<i>Mycobacterium fredericksbergense</i>	AF547834	<i>Mycobacterium shinjukuense</i>	AB268505
<i>Mycobacterium fredericksbergense</i>	DQ184963	<i>Mycobacterium shottsii</i>	AY550225
<i>Mycobacterium fuerthensis</i>	AY550238	<i>Mycobacterium siernhoferi</i>	AJ307651
<i>Mycobacterium gadium</i>	AJ310233	<i>Mycobacterium simiae</i>	GQ153292
<i>Mycobacterium gastri</i>	AJ310234	<i>Mycobacterium simulans</i>	FJ786253
<i>Mycobacterium genavense</i>	EU495310	<i>Mycobacterium smegmatis</i>	HM454230
<i>Mycobacterium gilvum</i>	AJ310236	<i>Mycobacterium sphagni</i>	AJ307655
<i>Mycobacterium goodii</i>	AY458071	<i>Mycobacterium stomatopiae</i>	AM902967
<i>Mycobacterium gordonaie</i>	EF546780	<i>Mycobacterium szulgai</i>	KC481266
<i>Mycobacterium gordonaie</i>	EF601222	<i>Mycobacterium terrae</i>	JX154097
<i>Mycobacterium gordonaie</i>	EU486081	<i>Mycobacterium thermoresistibile</i>	AF547880
<i>Mycobacterium gordonaie</i>	FJ384767	<i>Mycobacterium timonense</i>	KJ364654
<i>Mycobacterium gordonaie</i>	FJ643457	<i>Mycobacterium tokaiense</i>	JF491309
<i>Mycobacterium gordonaie</i>	FJ643460	<i>Mycobacterium triplex</i>	GQ153291
<i>Mycobacterium gordonaie</i>	FJ643461	<i>Mycobacterium triviale</i>	JF491310
<i>Mycobacterium gordonaie</i>	FJ643462	<i>Mycobacterium tuberculosis</i>	JF491311
<i>Mycobacterium gordonaie</i>	FJ643463	<i>Mycobacterium tuberculosis</i>	JX294381
<i>Mycobacterium gordonaie</i>	FJ643464	<i>Mycobacterium tusciae</i>	AF547887
<i>Mycobacterium hackensackense</i>	DQ350160	<i>Mycobacterium tusciae</i>	AJ307660
<i>Mycobacterium haemophilum</i>	GQ245967	<i>Mycobacterium ulcerans</i>	DQ985342
<i>Mycobacterium hassiacum</i>	AF547842	<i>Mycobacterium vanbaalenii</i>	AY438091
<i>Mycobacterium heckeshornense</i>	AF547843	<i>Mycobacterium visible</i>	AY550208
<i>Mycobacterium heidelbergense</i>	AF547844	<i>Mycobacterium vulneris</i>	EU834056
<i>Mycobacterium hiberniae</i>	JF491297	<i>Mycobacterium wolinsky</i>	AY458064
<i>Mycobacterium hodleri</i>	AF547845	<i>Mycobacterium xenopi</i>	AY373454
<i>Mycobacterium holsaticum</i>	AJ310469	<i>Mycobacterium yongonense</i>	JN605801
<i>Mycobacterium houstonense</i>	AY458077	<i>Mycobacterium yunnanensis</i>	DQ350161
<i>Mycobacterium immunogenum</i>	AY458081	<i>Mycobacterium sp.</i>	EU619883
<i>Mycobacterium insubricum</i>	EF584489	<i>Mycobacterium sp.</i>	EU619912
<i>Mycobacterium insubricum</i>	JF491319	<i>Nocardia vinacea</i>	AY756539
<i>Mycobacterium interjectum</i>	JF491298	<i>Nocardia veterana</i>	AY756538
<i>Mycobacterium intermedium</i>	AF547847	<i>Nocardia vaccinii</i>	AY756537

<i>Mycobacterium intracellulare</i>	HM454232	<i>Nocardia uniformis</i>	AY756536
<i>Mycobacterium intracellulare</i>	FJ643456	<i>Nocardia cyriacigeorgica</i>	JQ782423
<i>Mycobacterium intracellulare</i>	GG153290	<i>Nocardia asiatica</i>	JQ782424
<i>Mycobacterium iranicum</i>	DQ381734	<i>Nocardia sp.</i>	JQ782425
<i>Mycobacterium jacuzzi</i>	DQ137415	<i>Nocardiopsis</i>	JF264439
<i>Mycobacterium kansasii</i>	AB232365	<i>Streptomyces</i>	EF375995
<i>Mycobacterium komossense</i>	AY438649		

Table S2. Monthly average water chemistry parameters for the Saint Paul Regional Water Services plant finished water during the water main sampling period.

Parameter	Apr	May	Jun	Jul	Aug	Sep
Temperature (°C)	5	11	22	25	24	20
Total Chlorine (mg/L)	3.29	3.43	3.52	3.57	3.68	3.76
Turbidity (NTU)	0.027	0.030	0.042	0.055	0.044	<0.025
Total Organic Carbon (mg/L)	5.75	3.94	3.52	3.65	4.57	4.01
pH	9.10	9.04	9.13	9.06	9.12	9.10
Dissolved Oxygen (mg/L)	11.9	11.7	8.1	6.2	9.0	8.0
Ammonia-N (mg/L)	0.717	0.705	0.763	0.725	0.812	0.835
Nitrate-Nitrite-N (mg/L)	0.870	0.519	0.396	<0.202	<0.202	0.225
Total Nitrogen-N (mg/L)	1.520	1.220	1.370	0.790	1.110	0.965
Total Dissolved Solids	197	181	170	193	166	342
Chloride (mg/L)	36	42	47	46	40	37
Sulfur-S (mg/L)	3.7	10.0	4.3	4.0	4.3	<2.7
Sulfide-S ₂ ⁻ (mg/L)	<0.02	<0.02	<0.02	<0.02	<0.02	<0.02
Total Hardness (mg/L CaCO ₃)	84	79	72	74	73	74
Total Alkalinity (mg/L CaCO ₃)	59	51	47	50	50	48

Table S3. The complete list of percent abundances (arithmetic mean \pm standard deviation) of *Mycobacterium* species represented in each sample type, as determined by *hsp65* Illumina sequencing. ND – Not detected.

<i>Mycobacterium</i> sp.	Percent Abundance by Sample Type (Mean \pm standard deviation)				
	Overall Average	Tuberculated Surface (n=11)	Cement-Lined (n=2)	Non-Tuberculated (n=1)	Under Tubercl (n=5)
<i>Mycobacterium abscessus</i>	0.006 \pm 0.025	0.00 \pm 0.00	0.00 \pm 0.00	0.00	0.02 \pm 0.05
<i>Mycobacterium aemoniae</i>	<0.001	<0.001	ND	ND	ND
<i>Mycobacterium agri</i>	0.0019 \pm 0.0085	ND	ND	ND	0.0074 \pm 0.016
<i>Mycobacterium alsiensis</i>	<0.001	<0.001	ND	ND	ND
<i>Mycobacterium aromaticivorans</i>	0.017 \pm 0.065	ND	ND	0.015	0.062 \pm 0.12
<i>Mycobacterium asiaticum</i>	0.19 \pm 0.71	0.011 \pm 0.026	ND	ND	0.70 \pm 1.3
<i>Mycobacterium aurum</i>	6.5 \pm 12	11 \pm 15	0.10 \pm 0.10	1.72	0.94 \pm 0.67
<i>Mycobacterium austroafricanum</i>	0.011 \pm 0.045	0.018 \pm 0.060	ND	ND	ND
<i>Mycobacterium bacteremicum</i>	0.0018 \pm 0.0039	0.0021 \pm 0.0041	0.0055 \pm 0.0078	ND	ND
<i>Mycobacterium bohemicum</i>	0.0045 \pm 0.019	0.0077 \pm 0.026	ND	ND	ND
<i>Mycobacterium botniense</i>	0.063 \pm 0.20	0.0073 \pm 0.016	0.011 \pm 0.016	0.24	0.17 \pm 0.38
<i>Mycobacterium brasiliensis</i>	0.020 \pm 0.083	0.033 \pm 0.11	ND	0.015	ND
<i>Mycobacterium brumae</i>	0.030 \pm 0.064	0.0043 \pm 0.014	ND	0.21	0.063 \pm 0.080
<i>Mycobacterium chitae</i>	0.0056 \pm 0.011	0.0070 \pm 0.012	ND	ND	0.0057 \pm 0.013
<i>Mycobacterium chlorophenolicum</i>	0.0011 \pm 0.0037	0.0062 \pm 0.0021	ND	0.015	ND
<i>Mycobacterium colombiense</i>	0.0019 \pm 0.0085	ND	ND	ND	0.074 \pm 0.016
<i>Mycobacterium crocinum</i>	0.049 \pm 0.15	ND	ND	0.015	0.18 \pm 0.26
<i>Mycobacterium doricum</i>	0.0016 \pm 0.0068	ND	ND	0.030	ND
<i>Mycobacterium florentinum</i>	0.0087 \pm 0.034	<0.001	ND	ND	0.032 \pm 0.065
<i>Mycobacterium flouranthenivorans</i>	0.0019 \pm 0.0085	ND	ND	ND	0.0074 \pm 0.016
<i>Mycobacterium frederiksbergense</i>	85.67 \pm 14.50	86.03 \pm 15.90	98.87 \pm 1.08	78.92	80.95 \pm 13.67
<i>Mycobacterium gordonaie</i>	0.065 \pm 0.19	0.021 \pm 0.039	ND	ND	0.20 \pm 0.35
<i>Mycobacterium hackensackense</i>	0.044 \pm 0.053	0.032 \pm 0.035	0.016 \pm 0.023	0.10	0.069 \pm 0.083
<i>Mycobacterium haemophilum</i>	1.54 \pm 2.82	1.02 \pm 1.87	0.67 \pm 0.90	11.33	1.07 \pm 1.20
<i>Mycobacterium hassiacum</i>	0.11 \pm 0.42	0.0012 \pm 0.00	ND	1.82	0.044 \pm 0.066
<i>Mycobacterium heckeshornense</i>	<0.001	ND	ND	ND	0.0026 \pm 0.0058
<i>Mycobacterium heidelbergense</i>	<0.001	0.0012 \pm 0.0041	ND	ND	ND
<i>Mycobacterium hodleri</i>	<0.001	ND	ND	ND	0.0026 \pm 0.0058
<i>Mycobacterium holsaticum</i>	0.74 \pm 2.8	0.0069 \pm 0.011	ND	0.53	2.7 \pm 5.3
<i>Mycobacterium iranicum</i>	0.0024 \pm 0.0070	0.0028 \pm 0.0082	ND	0.015	ND
<i>Mycobacterium komossense</i>	0.74 \pm 0.91	0.97 \pm 1.0	0.044 \pm 0.031	0.58	0.52 \pm 0.79
<i>Mycobacterium kyorinense</i>	<0.001	ND	ND	0.015	ND
<i>Mycobacterium lacticola</i>	0.10 \pm 0.18	0.035 \pm 0.023	0.14 \pm 0.039	0.12	0.22 \pm 0.34
<i>Mycobacterium lentiflavum</i>	1.2 \pm 2.5	0.24 \pm 0.78	ND	ND	4.2 \pm 3.3
<i>Mycobacterium liflandii</i>	0.0045 \pm 0.017	0.0077 \pm 0.023	ND	ND	ND
<i>Mycobacterium mageritense</i>	0.0045 \pm 0.017	ND	0.0055 \pm 0.0078	ND	0.015 \pm 0.033
<i>Mycobacterium manitobense</i>	0.0010 \pm 0.0033	<0.001	ND	ND	0.0026 \pm 0.058
<i>Mycobacterium massiliense</i>	0.0058 \pm 0.025	ND	ND	ND	0.022 \pm 0.049
<i>Mycobacterium montefiorens</i>	0.0067 \pm 0.18	0.010 \pm 0.023	0.011 \pm 0.016	ND	0.23 \pm 0.32
<i>Mycobacterium moriokaense</i>	0.0014 \pm 0.0038	0.0011 \pm 0.0025	ND	0.015	ND

<i>Mycobacterium mucogenicum</i>	0.0019±0.0085	ND	ND	ND	0.0074±0.016
<i>Mycobacterium murale</i>	0.017±0.059	<0.001	ND	0.044	0.05±0.11
<i>Mycobacterium nebraskense</i>	0.31±1.0	0.012±0.018	0.022±0.031	ND	1.15±1.83
<i>Mycobacterium neglectum</i>	0.055±0.011	0.0085±0.014	0.0055±0.0078	ND	ND
<i>Mycobacterium novocastrense</i>	0.025±0.11	0.043±0.14	ND	ND	ND
<i>Mycobacterium obuense</i>	0.0017±0.0037	0.0029±0.0045	ND	ND	ND
<i>Mycobacterium parakoreense</i>	0.0014±0.0043	0.0011±0.0038	ND	0.015	ND
<i>Mycobacterium parascrofulaceum</i>	0.15±0.41	0.086±0.15	ND	ND	0.40±0.77
<i>Mycobacterium phlei</i>	<0.001	ND	ND	ND	0.0021±0.0048
<i>Mycobacterium phocaicum</i>	0.056±0.15	0.012±0.035	ND	ND	0.19±0.26
<i>Mycobacterium porcinum</i>	<0.001	ND	ND	0.015	ND
<i>Mycobacterium poriferae</i>	0.0028±0.0074	0.0049±0.0093	ND	ND	ND
<i>Mycobacterium psychrotolerans</i>	1.0±3.0	0.0092±0.019	ND	ND	3.9±5.1
<i>Mycobacterium riyadhense</i>	<0.001	<0.001	ND	ND	ND
<i>Mycobacterium rufum</i>	0.0012±0.0037	ND	ND	ND	0.0047±0.0065
<i>Mycobacterium rutilum</i>	0.098±0.41	0.16±0.54	ND	ND	0.015±0.033
<i>Mycobacterium salmoniphilum</i>	0.064±0.28	<0.001	ND	ND	0.24±0.54
<i>Mycobacterium septicum</i>	0.0011±0.0034	0.0019±0.0044	ND	ND	ND
<i>Mycobacterium shimoidei</i>	<0.001	ND	ND	0.015	ND
<i>Mycobacterium shinjukuense</i>	<0.001	ND	ND	0.015	ND
<i>Mycobacterium shottsii</i>	0.0040±0.013	0.068±0.016	ND	ND	ND
<i>Mycobacterium siernhoferi</i>	<0.001	<0.001	ND	ND	ND
<i>Mycobacterium simulans</i>	0.011±0.029	0.016±0.037	ND	0.030	ND
<i>Mycobacterium stomatopiae</i>	0.0020±0.0085	0.0034±0.011	ND	ND	ND
<i>Mycobacterium timonense</i>	0.011±0.032	0.011±0.037	ND	ND	0.017±0.032
<i>Mycobacterium tokaiense</i>	0.0055±0.016	0.0010±0.0025	ND	0.030	0.013±0.029
<i>Mycobacterium triplex</i>	0.012±0.035	0.0043±0.014	ND	ND	0.038±0.064
<i>Mycobacterium triviale</i>	0.11±0.47	ND	ND	2.0	ND
<i>Mycobacterium visible</i>	<0.001	ND	ND	ND	0.0026±0.0058
<i>Mycobacterium xenopi</i>	0.017±0.046	0.0020±0.0038	ND	ND	0.061±0.078
<i>Mycobacterium unclassified</i>	0.31±0.37	0.26±0.22	0.066±0.031	0.24	0.55±0.62
<i>Mycobacterium sp.</i>	0.025±0.10	0.043±0.13	ND	ND	ND

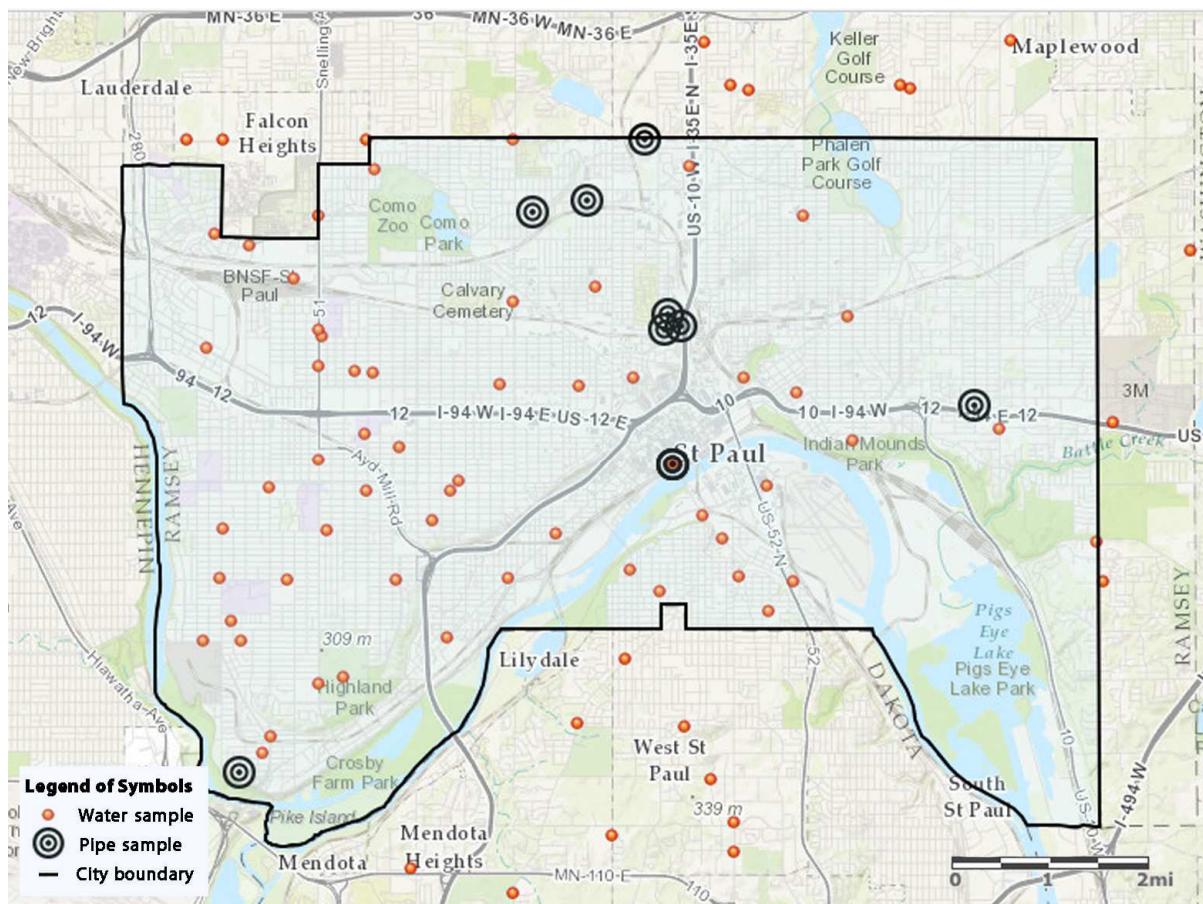


Figure S1. Locations of water main samples ($n = 10$) and tap water samples ($n = 1,090$) in the Saint Paul Regional Water Services (SPRWS) distribution system. Water samples were also taken outside of the Saint Paul city limits (indicated by solid black line) as SPRWS serves some neighboring communities. Samples that were taken from locations in close proximity to each other may appear as one marker in the map.

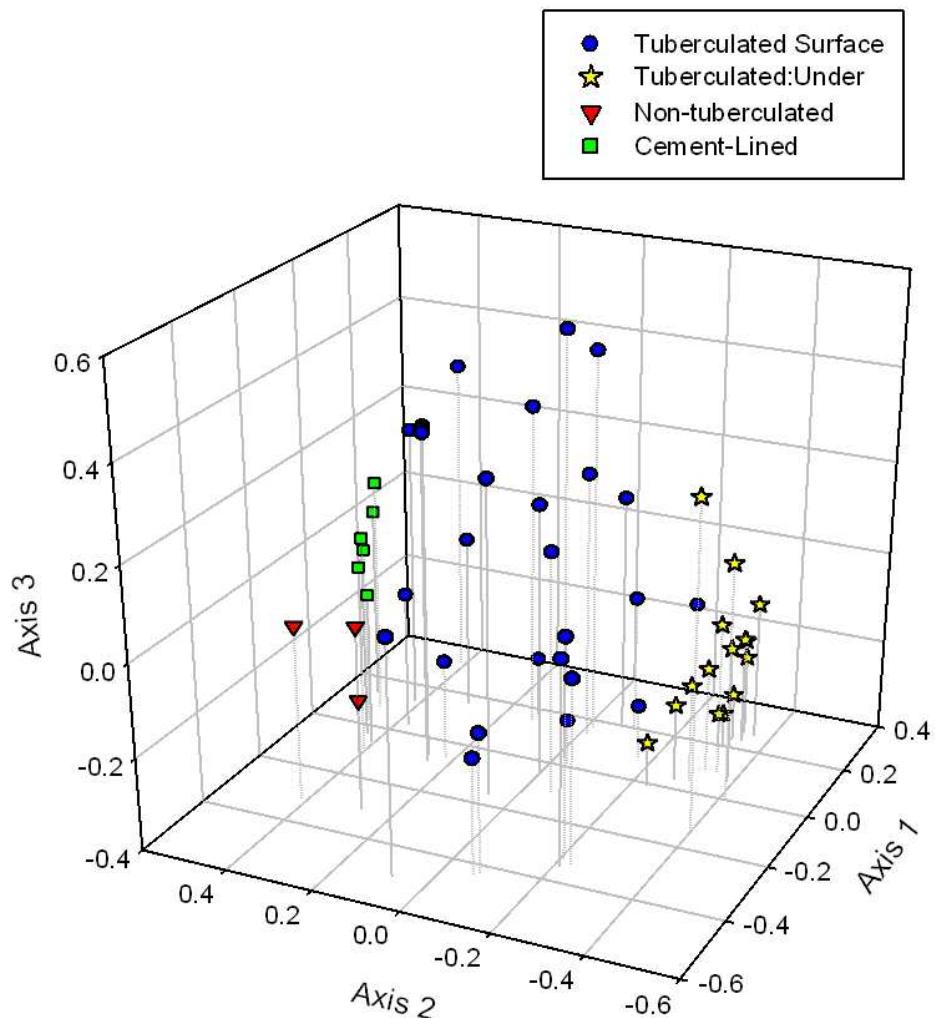


Figure S2. Non-metric multidimensional analysis (nMDS) of water main community samples. nMDS was conducted using weighted unifrac distances for all samples. Stress = 0.15 for 3 dimensions.