**Materials and Methods**

**DNA extraction, PCR and high-throughput amplicon sequencing**

Total DNA was extracted from 20 samples (five column reactors) and three controls (influent) samples as previously described (ref). DNA samples were stored at -20°C until used for next generation sequencing. The DNA was used as templates to generate independent libraries targeting bacterial 16S ribosomal RNA genes. We used barcoded 16S rRNA gene targeting primers (i.e., 515F and 806R) described in Caporaso et al. (2011) and sequenced the targeted product (i.e., 291 bp) in both directions using an Illumina MiSeq PE250 sequencing kit. Sequencing was performed at the Cincinnati Children’s Hospital Medical DNA Sequencing and Genotyping Core facility.

**Next generation sequencing data preprocessing and analysis**

Sequence reads were processed and analyzed using Mothur software (Schloss et al., 2009). Sequence reads that did not fit the following criteria were discarded from further analyses: did not form contigs, deviated considerably from the expected PCR size product, had ambiguous bases, and had homopolymers greater than 8 bases long. Sequence reads were grouped at a 97 % similarity and the consensus sequences were then identified using Mothur and the Silva (Quast et al., 2013) database as a reference. Prior to the classification analysis, a prescreening step was performed with a randomly selected subset of all the sequences generated per sample to further filter out chimeras and difficult to align sequences. Excel was used to determine the overall relative abundance of representative sequences at different taxonomic levels (e.g., class, order, family, genus). Sequences were analyzed using Blast (<http://www.ncbi.nlm.nih.gov/BLAST/>) and RDP classifier (Wang et al., 2007) to further confirm their phylogenetic affiliation and to classify sequences at a low taxonomic level (genus and species) whenever possible.

**References**

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TABLE 1-1. Distribution of bacterial 16S rRNA gene (Sample 1-12)

|  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Class | Genus | T1  (n=13009) | T2  (n=11337) | T3  (n=16547) | T4  (n=12641) | T5  (n=15413) | T6  (n=10585) | T7  (n=14514) | T8  (n=10712) | T9  (n=14056) | T10  (n=14074) | T11  (n=20135) | T12  (n=12002) |
| Actinobacteria | *ACK-M1\**  *Arthrobacter* | -  - | -  - | -  - | -  - | -  - | -  - | -  - | -  - | -  - | -  - | -  - | -  - |
| Chloracidobacteria | *Ellin6075\** | **567** | 296 | **699** | 431 | **752** | **677** | **884** | **647** | 388 | 472 | **2334** | **1809** |
| Fimbriimonadia | *Fimbriimonas* | 219 | **1085** | **1547** | 824 | 42 | 125 | **1078** | **1595** | 17 | 12 | 24 | 15 |
| Cytophagia | *Cytophagaceae\** | 27 | - | 15 | 29 | 54 | 40 | 18 | 16 | 47 | 46 | 59 | 26 |
|  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| Saprospirae | *Chitinophagaceae\** | 53 | 28 | 59 | 42 | 142 | 65 | 51 | 87 | 77 | 52 | 82 | 86 |
| *Saprospiraceae\** | 60 | 26 | 28 | 120 | **612** | 90 | 82 | 144 | **674** | 256 | 245 | **718** |
| Chlamydiia | *Candidatus* Rhabdochlamydia | 47 | 79 | 44 | 81 | - | 16 | 41 | 51 | - | 39 | 38 | 214 |
| Unclassified | 153 | 327 | 221 | 257 | 64 | 206 | 339 | 324 | 25 | 167 | 360 | 377 |
| SJA-28 | Unclassified | 61 | 48 | 62 | 24 | 46 | 52 | 31 | 26 | - | 61 | 189 | 393 |
| Chloroplast | *Stramenopiles\*\** | 23 | 33 | 36 | 28 | - | 10 | - | - | - | - | 14 | 11 |
| Nitrospira | *Nitrospira* | 151 | 284 | 64 | 124 | 61 | 79 | 84 | 75 | 30 | 61 | 108 | 95 |
| Planctomycetia | *Gemmataceae\** | 46 | 37 | 60 | 243 | 58 | 45 | 40 | 54 | 26 | 46 | 305 | 53 |
| *Pirellulaceae\** | 128 | 157 | 247 | 469 | 50 | 126 | 119 | 233 | 74 | 151 | **946** | 165 |
| *Planctomyces* | 51 | 53 | 146 | **678** | 19 | 64 | 84 | 131 | 12 | 41 | 327 | 94 |
| Alpha-Proteobacteria | *Mycoplana* | 28 | 13 | 22 | 47 | 16 | - | - | - | - | - | 23 | - |
| *Rhizobiales\*\** | **634** | 375 | **1142** | **715** | 205 | 198 | 344 | 282 | 142 | 200 | **1976** | 258 |
| *Hyphomicrobium* | 126 | 50 | 111 | 107 | 54 | 48 | 38 | 28 | 40 | 68 | **941** | 28 |
| *Rhodoplanes* | 87 | 36 | 154 | 139 | - | 14 | 37 | 13 | - | - | 131 | 12 |
| *Hypohmonadaceae\** | 77 | 77 | 87 | 77 | 55 | 66 | 54 | 51 | 80 | 96 | 242 | 53 |
| *Rhodobacter* | 97 | 56 | 281 | 38 | 141 | 73 | 119 | 17 | 87 | 59 | 136 | 13 |
| *Acetobacteraceae\** | 195 | 255 | 149 | 157 | 34 | 66 | 74 | 23 | 30 | 65 | 76 | 45 |
| *Rhodospirillaceae\** | 181 | 235 | 232 | 266 | 47 | 116 | 118 | 103 | 29 | 110 | 371 | 53 |
| *Pelagibacteraceae\** | - | - | - | - | - | - | - | - | - | - | - | - |
| *Erythromicrobium* | - | - | - | - | - | - | - | - | - | - | - | - |
| *Sphingomonadacea\** | 71 | 47 | 127 | 85 | 105 | 79 | 67 | 28 | 120 | 90 | 186 | 50 |
| Unclassified | **2745** | **1887** | **1627** | **1298** | 283 | **529** | 325 | 331 | 177 | 333 | **641** | 289 |
| Beta-Proteobacteria | *Comamonadaceae\**  *-Hydrogenophaga* | **826**  335 | 310  126 | 448  229 | **544**  223 | **5069**  429 | **1758**  213 | **1879**  166 | **706**  73 | **5192**  378 | **3607**  313 | **4010**  **702** | **576**  61 |
| *Methylotenera* | 669 | 133 | 79 | 327 | 10 | - | - | - | 20 | - | - | - |
| *Dechloromonas* | - | - | - | - | - | 27 | 203 | 16 | - | 40 | 73 | 20 |
| *Rhodocyclaceae\**  *-Methyloversatilis* | 86 | 34 | 30 | 68 | **4284** | **2553** | **3255** | **2253** | **4442** | **4461** | 569 | **2759** |
| Unclassified | **786** | **677** | **725** | **815** | 102 | 184 | 349 | 209 | 95 | 183 | 405 | 244 |
| Delta-Proteobacteria | *Polyangiaceae\** | - | - | - | 35 | 237 | 44 | 38 | 66 | 113 | 107 | 77 | 53 |
| *Syntrophobacteraceae\** | 18 | 11 | 15 | 65 | 54 | 58 | 45 | 87 | 17 | 74 | 237 | 139 |
| Gamma-Proteobacteria | *Rheinheimera* | - | - | - | - | - | - | - | - | - | - | - | - |
| *E. coli* | - | - | 30 | - | - | - | - | - | - | - | - | - |
| *Salmonella* | - | - | - | - | - | - | - | - | - | - | 47 | - |
| *Aquicella* | 94 | 216 | 323 | 209 | 10 | 26 | 51 | 33 | - | - | 20 | - |
| *Pseudomonas* | - | - | - | - | - | - | - | - | - | - | - | - |
| *Silanimonas* | - | - | - | - | - | - | - | - | - | - | - | - |
| Unclassified | **2143** | **1429** | **5179** | 200 | 260 | 434 | **1694** | 157 | 128 | 244 | **1373** | 170 |
| Opitutae | *Opitutaceae\** | 154 | 221 | 60 | 245 | - | 68 | 151 | 109 | - | 48 | 17 | 47 |
| Verrucomicrobiae | *Prosthecobacter* | - | - | - | - | **621** | 270 | 146 | 190 | 256 | 185 | 290 | 74 |

\* Family, \*\* Phylum, - less than 10 sequences

TABLE 1-2. Distribution of bacterial 16S rRNA gene (Sample 13-20, Control 1-3)

|  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Class | Genus | T13  (n=12087) | T14  (n=12159) | T15  (n=13366) | T16  (n=15546) | T17  (n=28769) | T18  (n=22090) | T19  (n=4522) | T20  (n=15523) | Control 1  (n=26001) | Control 2  (n=20592) | Control 3  (n=21571) |
| Actinobacteria | *ACK-M1\**  *Arthrobacter* | -  - | -  - | -  - | -  - | -  - | -  - | -  - | -  - | 21  144 | **3307**  108 | **8270**  - |
| Chloracidobacteria | *Ellin6075\** | 209 | 525 | 459 | 493 | 12 | 10 | - | 20 | - | 22 | - |
| Fimbriimonadia | *Fimbriimonas* | 180 | 297 | 269 | 10 | - | - | - | - | - | - | - |
| Cytophagia | *Cytophagaceae\** | 74 | 38 | 18 | 33 | 245 | 342 | - | 21 | 22 | 154 | 21 |
| Saprospirae | *Chitinophagaceae\** | 168 | 202 | 88 | 75 | 60 | 57 | - | 327 | **1611** | **919** | **1131** |
| *Saprospiraceae\** | - | 34 | 45 | - | 776 | 374 | - | - | - | 198 | 53 |
| Chlamydiia | *Candidatus* Rhabdochlamydia | 31 | 82 | 71 | 236 | - | - | - | - | - | 25 | - |
| Unclassified | 124 | 66 | 91 | 463 | - | - | - | - | - | - | - |
| SJA-28 | Unclassified | **871** | **1785** | **1814** | **2108** | - | - | - | - | - | - | - |
| Chloroplast | *Stramenopiles\*\** | **2120** | 350 | 105 | 229 | - | 19 | - | 22 | - | 10 | 46 |
| Nitrospira | *Nitrospira* | **1599** | 475 | 318 | **865** | - | - | - | - | - | - | 18 |
| Planctomycetia | *Gemmataceae\** | 12 | 14 | 12 | 104 | - | - | - | - | - | - | - |
| *Pirellulaceae\** | 107 | 401 | 281 | **1743** | - | - | - | - | - | - | - |
| *Planctomyces* | 24 | 122 | 221 | **668** | - | - | - | - | - | - | - |
| Alpha-Proteobacteria | *Mycoplana* | 58 | 90 | 85 | 32 | 36 | 118 | - | 190 | 231 | 431 | 23 |
| *Rhizobiales\*\** | 278 | **682** | **1405** | **986** | 281 | 352 | - | 188 | 116 | 144 | 79 |
| *Hyphomicrobium* | 59 | 49 | 73 | 107 | - | - | - | - | - | 13 | - |
| *Rhodoplanes* | 18 | 57 | 161 | 325 | - | - | - | - | - | - | - |
| *Hypohmonadaceae\** | 102 | 90 | 75 | 199 | - | - | - | - | 15 | - | - |
| *Rhodobacter* | 78 | 98 | 96 | 58 | 62 | 65 | - | 181 | **815** | 293 | **702** |
| *Acetobacteraceae\** | 214 | 294 | 216 | 221 | - | - | - | - | 120 | 91 | 37 |
| *Rhodospirillaceae\** | 122 | 114 | 139 | 177 | - | - | - | - | 14 | - | - |
| *Pelagibacteraceae\** | - | - | - | - | - | - | - | - | 13 | **2004** | 453 |
| *Erythromicrobium* | 28 | 12 | - | 43 | - | - | - | - | 384 | 86 | 59 |
| *Sphingomonadacea\** | 76 | 77 | 61 | 51 | 149 | 199 | - | 355 | **1014** | **1697** | **1297** |
| Unclassified | 37 | 43 | 28 | 27 | 87 | 108 | - | 136 | 213 | 435 | 1133 |
| Beta-Proteobacteria | *Comamonadaceae\**  *-Hydrogenophaga* | **510**  173 | **525**  194 | 401  136 | 274  71 | **12551**  **2871** | **10000**  **2471** | 90  - | **5739**  **1792** | **5051**  **3207** | **5400**  **2616** | **2445**  **642** |
| *Methylotenera* | 563 | 133 | 62 | 15 | 587 | 578 | - | 247 | **14159** | **1699** | 108 |
| *Dechloromonas* | - | - | - | - | - | - | - | - | - | - | - |
| *Rhodocyclaceae\**  *-Methyloversatilis* | 95 | 74 | 35 | 19 | **2653** | **3003** | - | **2807** | 140 | 27 | - |
| Unclassified | **495** | **1198** | **1945** | **1600** | 65 | 101 | - | 47 | 130 | 130 | 259 |
| Delta-Proteobacteria | *Polyangiaceae\** | - | - | - | - | - | - | - | - | - | - | - |
| *Syntrophobacteraceae\** | - | - | - | 154 | - | - | - | - | - | - | - |
| Gamma-Proteobacteria | *Rheinheimera* | 13 | 16 | - | 14 | **8330** | **4999** | - | **3106** | 51 | 209 | 214 |
| *E. coli* | - | - | - | - | - | - | 143 | - | - | - | - |
| *Salmonella* | - | - | - | - | - | - | 2633 | - | - | 71 | 10 |
| *Aquicella* | - | 64 | 74 | 66 | - | - | - | - | - | - | - |
| *Pseudomonas* | 11 | - | - | - | **2195** | **915** | 106 | **925** | 12 | 90 | 328 |
| *Silanimonas* | 24 | 17 | 10 | 14 | - | - | - | - | 240 | 53 | **2264** |
| Unclassified | **721** | **1244** | **2939** | **642** | - | - | - | 11 | 17 | 16 | 44 |
| Opitutae | *Opitutaceae\** | 138 | 95 | 50 | 270 | - | - | - | - | - | - | - |
| Verrucomicrobiae | *Prosthecobacter* | - | - | - | - | - | - | - | 12 | - | - | - |

\* Family, \*\* Phylum, - less than 10 sequences