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Research Article
Identification of Bacterial Species with Nitrogen, Phosphorus and Sulfur Bioremediation Pathways in Wastewater Treatment Plants

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Abstract

Background and Objective: Contamination of water bodies is one of the most impacting anthropogenic activities to the environment, therefore, it is important to understand the biological processes that allow the wastewater bioremediation. The objective of this study was to identify the main bacterial genera present in sewage treatment plants and of which are these species have genes that participate in the degradation or accumulation pathways of nitrogen, phosphorus and sulfur. **Materials and Methods:** Genomes of 158 bacteria species, isolated from sewage treatment plants, were analyzed in search of the following pathways: nitrification, denitrification, dissimilatory nitrate reduction, phosphorus accumulation, assimilatory sulfate reduction and dissimilatory sulfate reduction and oxidation. **Results:** Seventy-nine bacteria species had at least one of the complete pathways, of which 11 had 3 or more complete pathways: *Acidovorax caeni*, *Acidovorax delafieldii*, *Acidovorax temperans*, *Burkholderia vietnamiensis*, *Comamonas thiooxydans*, *Nitrobacter vulgaris*, *Nitrobacter winogradskyi*, *Paracoccus denitrificans*, *Pseudomonas aeruginosa*, *Pseudomonas fluorescens* and *Thiothrixnivea*. *Paracoccus denitrificans* stands out for having the largest number of complete pathways, possessing the genes of denitrification, dissimilatory nitrate reduction, assimilatory sulfate reduction and phosphorus accumulation processes. **Conclusion:** Therefore, the conclusion of this study can be used to improve the optimization of wastewater treatment processes, indicating bacteria that are more adapted for bioremediation: *Paracoccus denitrificans*, *Thiothrixnivea* and *Nitrospiranitrosa*.

Key words: Bioremediation, metagenomics, functional genomics, wastewater treatment, assimilation

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Inadequate disposal of domestic sewage in water bodies can be harmful to the environment, since it has many nutrients and a rich microbial community[1,2]. Depending on the concentrations, these nutrients can become pollutants, such as nitrogen, phosphorous[3] and sulfur. Because they can induce eutrophication and become a risk to aquatic communities and human life. However, Nitrogen (N), Phosphorus (P) and Sulfur (S) are essential elements for all living organisms[4], therefore, their excess should be treated in domestic sewage.

The fundamental reasons for treating wastewater are to prevent water source contamination and to protect public health by safeguarding water supplies against the spread of diseases[5,6]. Municipal wastewater is mainly comprised of water (99.9%), together with relatively small concentrations of suspended and dissolved organic and inorganic solids[7]. Different physical and chemical processes, such as adsorption, incineration, coagulation, precipitation and chemical oxidation, can be applied to treat wastewater[6]. Nevertheless, there are advantages in biological processes such as a reduction in sludge production, low operating cost and suitability for simultaneous removal of different compounds. All biological treatment processes take advantage of the bacteria's ability to use various wastewater constituents as a source of energy for microbial metabolism and as building blocks for cell synthesis. The use of living organisms, primarily microorganisms, to degrade the environmental contaminants into less toxic forms or the process whereby organic wastes are biologically degraded, under controlled conditions, is called bioremediation[7]. The major microorganisms found in wastewater influents are viruses, bacteria, fungi, protozoa and nematodes[5,7]. However, bacteria are typically considered to be the most significant organisms consuming the organic matter in wastewater[5].

New genomic, metabolic and nutritional information from bacteria in biological treatment systems could help in understanding symbiotic relationships in sewage treatment plants[8-10]. In addition, several aspects related to microbial communities should be considered, such as the diversity and interaction between bacteria and the environment[11-13].

High throughput metagenomic sequencing enables the study of the taxonomic and functional diversity of a microbial population[3,14]. Comparative studies of prokaryotic genomes have revealed their complex structure and organization as well as the enormous diversity between these organisms, even among isolates of the same species[15]. Recent works highlight that there are about 1,700-3,600 species of bacteria in wastewater treatment plants[16,17].

With an enhanced understanding of the bioremediation metabolic processes, effluent treatment plants can be enriched with specific microorganisms that enable the development of genetically modified organisms, in turn increases the efficiency of sewage treatment. Moreover, the objective of this study was to identify the main genera of bacteria present in sewage treatment plants and of which are these species have genes that participate in the degradation or accumulation pathways of nitrogen, phosphorus and sulfur. In addition, phylogenetic analyses were performed on the bacteria under this study.

MATERIALS AND METHODS

Identification of the most abundant bacterial generain water treatment plants: Identification of the dominant bacterial genera in wastewater treatment plants was observed. This study considered the genera and species identified among the most abundant and isolated in more than one study. Thousands of bacterial genera were identified in each of the works, however, in all these papers a list of the most abundant species was published. These lists were crossed to identify the most abundant species, even at different treatment plants. Bacterial genomes appearing at least two of these lists were analyzed. This study was conducted in the Bioinformatics and Molecular Analysis Laboratory, "Universidade Federal de Uberlândia", Brazil, from February-August, 2019.

The full genome of these species were downloaded from the National Center for Biotechnology Information database and all of them were bacterial species isolated and sequenced from wastewater treatment plants, activated sludge, or sewage. Downloaded genomes were used to fabricate a database for subsequent analyses.

Identification of genes involved in nitrogen, phosphorus and sulfur metabolic pathways: Protein sequences of key genes in the metabolic pathways of nitrogen, phosphorus and sulfur were downloaded from the Kyoto Encyclopedia of Genes and Genomes database (KEGG). Genes of the following pathways were analyzed: Nitrification, denitrification, dissimilatory nitrate reduction, phosphorus accumulation[18], assimilatory sulfate reduction and dissimilatory sulfate reduction and oxidation. Query protein sequences are listed in Table 1. Protein sequences[19] of these genes were compared with the genome database using command line t Blastn and an e-value cutoff of 1×10^{-20}. Venn diagrams were constructed

Table 1: Query genes for each bioremediation pathway

Gene name	Gene	KEGG entry
Nitrification		
Ammonia monooxygenase subunit C	AmoC	NE2064
Ammonia monooxygenase subunit A	AmoA	NE0944
Ammonia monooxygenase subunit B	AmoB	NE0943
Hydroxylamine dehydrogenase	Hao	NE2339
		Noc_0892
Nitrate reductase/nitrite oxidoreductase, alpha subunit	NxrA	NIDE3237
		N297_4001
Nitrate reductase/nitrite oxidoreductase, beta subunit	NxrB	b1225
		SCV20265_1123
Denitrification		
Nitrate reductase gamma subunit	NarI	N296_3998
Nitrate reductase/nitrite oxidoreductase, alpha subunit	NarG	BN889_04303
		AK36_5148
Nitrate reductase/nitrite oxidoreductase, beta subunit	NarH	b1225
		UIB01_03910
Periplasmic nitrate reductase	NapA	b2206
		UIB01_15470
Cytochrome c-type protein	NapB	PA14_49260 CAP2UW1_3909
Nitrite reductase	NirK	BMA10229_0703
		Neut_1403
Nitrite reductase/ hydroxylamine reductase	NirS	PSE_0898
Nitric oxide reductase subunit B	NorB	NE2004
		BMA0633
Nitric oxide reductase subunit C	NorC	Neut_0521
Nitrous-oxide reductase	NosZ	PA14_20200
DNRA		
Nitrite reductase (NADH) large subunit	NirB	b3365
		PSEEN1418
Nitrite reductase (NADH) small subunit	NirD	Ent638_3794
		Pden_4451
Nitrite reductase (cytochrome c-552)	NrfA	b4070
		Cj1357c
Cytochrome c nitrite reductase small subunit	NrfH	HCBAA847_0636
		Cj1358c
		Desgi_2941
Assimilatory sulfate reduction		
3'-phosphoadenosine 5'-phosphosulfate synthase	PAPSS	sce5751
Sulfate adenylyltransferase	Sat	Tbd_0210
		UZ73_02605
Sulfate adenylyltransferase subunit 1	CysN	b2751
		ECL_04101
		KPN_03113
Sulfate adenylyltransferase subunit 1	CysD	ECL_04100
		KPN_03114
Adenylyl sulfate kinase	CysC	b2750
		ECL_04099
Phosphoadenosine phosphosulfate reductase	CysH	ECL_04104
		PA1756
Sulfite reductase (NADPH) hemoprotein beta-component	CysI	ECL_04105
		CtCNB1_3170
Sulfite reductase (NADPH) flavoprotein alpha-component	CysJ	CtCNB1_3038
		ENC_30120
Sulfite reductase (ferredoxin)	Sir	Abu_2013
		Clopa_4350
DSR		
Adenylyl sulfate reductase, subunit A	AprA	Tbd_0872
		Desaf_0101
		Clopa_4347
		EUBREC_2472

Table 1: Continue

Gene name	Gene	KEGG entry
Adenylyl sulfate reductase, subunit B	AprB	Tbd_0873
		EUBREC_2471
		Desaf_0100
Dissimilatory sulfite reductase alpha subunit	DsrA	Tbd_1309
		Desaf_1370
		Desca_2666
Dissimilatory sulfite reductase beta subunit	DsrB	Desca_2665
		Tbd_2484
		Desaf_1371
Pho		
Acetate kinase	AckA	b2296
		Ent638_2840
Phosphate acetyltransferase	Pta	PST_0690
		BMAA0121
Acetyl-CoA synthetase	Acs	b4069
		AKI40_4606
Polyhydroxyalkanoate synthase	PhaC	PST_0683
		O23A_p1564
Poly(3-hydroxybutyrate) depolymerase	PhaZ	AC233_04595

DNRA: Dissimilatory nitrate reduction, DSR: Dissimilatory sulfate reduction and oxidation, Pho: Phosphorus accumulation

with data generated in the blast to represent the relationships between the bacteria species that have nitrogen, phosphorus, and/or sulfur pathways genes.

Phylogenetic analysis: Phylogenetic analysis was performed on bacterial species that showed at least one of the complete pathways listed in Table 1. Thus, 16S rRNA gene was used to compare all the analyzed bacteria. Sequence alignments were performed using CLUSTALW in the BioEdit Sequence Alignment Editor[20]. Phylogenetic tree construction was performed by the neighbor-joining method using the software MEGA-X[21]. Robustness was paramount and assessed by bootstrap analysis based on 1,000 repetitions.

Detailed analysis of the bacterial species with genes of several pathways: Bacterial species presenting three or more complete pathways for degradation and/or accumulation of nitrogen, phosphorus and sulfur were subjected to a detailed analysis. Reverse BLASTp and InterProScan were used to identify and confirm the best blast hits, which were then analyzed with Blast2GO 5[22] basic software.

RESULTS

Identification of the main bacterial genera in wastewater treatment plants: Identification of the dominant bacterial genera in wastewater treatment plants was based on previous studies data that performed metagenomic analyses in 8 different wastewater treatment plants (Table 2). Studies that conferred information about major genera and species of bacteria were considered as well as the proportion of each group within a sewage treatment plant.

In this study, the genome of 158 bacteria species belonging to 80 genera were scrutinized (Table 4). Genera present among the most abundant and identified in the largest number of analyzed wastewater treatment plants were listed in Table 3.

These 158 species (Table 4) were classified into 22 bacterial classes and one un classified: *Acidimicrobiia*, *Actinobacteria*, *Alphaproteobacteria*, *Anaerolineae*, *Bacilli*, *Bacteroidia*, *Betaproteobacteria*, *Clostridia*, *Coriobacteriia*, *Deltaproteobacteria*, *Epsilonproteobacteria*, *Flavobacteriia*, *Gammaproteobacteria*, *Gemmatimonadetes*, *Negativicutes*, *Nitrospira*, *Oligoflexia*, *Rubrobacteria*, *Saprospiria*, *Sphingobacteriia*, *Spirochaetia* and *Synergistia*.

Identification of genes involved in nitrogen, phosphorus and sulfur metabolic pathways and phylogenetic analysis: After analysis of the 158 bacterial genomes, 79 species conferred at least one of the complete pathways (Table 4). A Venn diagram was constructed to enhance visualization and analyze the relationship between78 bacterial species and pathways. Figure 1 correlates the pathways of phosphorus accumulation, assimilatory sulfate reduction, denitrification, dissimilatory nitrate reduction and dissimilatory sulfate reduction and oxidation. Nitrification pathway were not added to Fig. 1 because it were present in a two bacteria species (Table 4), this made it impossible to construct the Venn diagram with all pathways.

Table 2: Scientific references of main bacterial genera in wastewater treatment plants

Country	pH	Temperature (°C)	References
China	7.3-7.8	35	M.C. Macey *et al.*[26]
Belgium	6.77-7.76	8.3-21.1	K. Meerbergen *et al.*[16]
China	7	34-36	Q. Ma *et al.*[39]
China	Not show	Not show	Y. Yang *et al.*[40]
China	Not show	Not show	L. Cai *et al.*[1]
China	6.4-7.3	Not show	Q. Ma *et al.*[39]
China	6.75-7.5	8.5-13.5	Y. Yang *et al.*[40]
China	Not show	31-32	Q. Huang *et al.*[41]

Table 3: Most abundant genera and the number of identified wastewater treatment plants

Genus	Class	Number of identifications
Clostridium	*Clostridia*	7
Nitrospira	*Nitrospira*	6
Bacteroides	*Bacteroidia*	5
Pseudomonas	*Gammaproteobacteria*	5
Thauera	*Betaproteobacteria*	5
Acidovorax	*Betaproteobacteria*	4
Dechloromonas	*Betaproteobacteria*	4
Dokdonella	*Gammaproteobacteria*	4
Mycobacterium	*Actinobacteria*	4
Streptococcus	*Bacilli*	4
Arcobacter	*Epsilonproteobacteria*	3
Bacillus	*Bacilli*	3
Bifidobacterium	*Actinobacteria*	3
Flavobacterium	*Flavobacteriia*	3
Lactobacillus	*Bacilli*	3
Paracoccus	*Alphaproteobacteria*	3
Rhodobacter	*Alphaproteobacteria*	3
Treponema	*Spirochaetia*	3

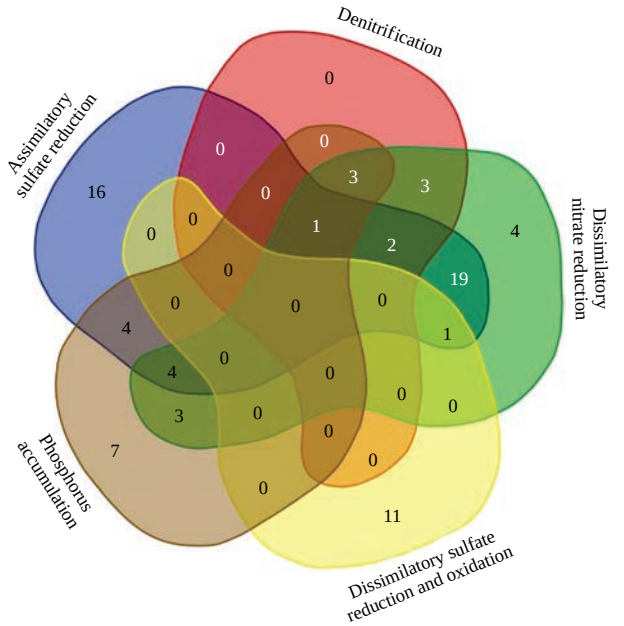

Fig. 1: Venn diagram correlating 78 bacterial species that presented all genes of one or more pathways

Phylogenetic analysis was conducted with all bacteria that presented at least one of the complete pathways (Fig. 2). These 79 bacteria accounted for half of the initial sample and were divided into 9 classes and for majority of them were classified in the phylum Proteobacteria (86%). In addition, the analyses indicated that this phylum has the bacteria species with more genes for bioremediation. Initially, 83 Proteobacteria species were analyzed and 63of them (75.9%) had at least one of the nitrogen's, sulfur or phosphorus pathways.

Nitrification was the least observed pathway, with only two bacteria species presenting the complete pathway: *Candidatus Nitrospiranitrificans* and *Candidatus Nitrospiranitrosa* (Fig. 2). In addition, all *Nitrospira* analyzed are among the most abundant in wastewater treatment plants (Table 3). Otherwise, denitrification was most common and was observed in 9 bacteria species (Fig. 1).

Twelve species conferred with complete dissimilatory sulfate reduction and oxidation pathway (DSR) but only *Thiothrixnivea*, from class Gammaproteobacteria, also has the complete pathways of assimilatory sulphate reduction and dissimilatory nitrate reduction (Fig. 1). Only Eight species of these bacteria belong to the class of Deltaproteobacteria and three species are classified in the *Clostridia* class (Fig. 2).

Forty species of bacteria include at least two of the analyzed pathways (Table 4). Blast experiments showed that 47 bacterial species possess the assimilatory sulfate reduction pathway genes and the other 40 possess dissimilatory nitrate reduction to ammonia (DNRA) pathway genes-also known as nitrate/nitrite ammonification. These two pathways are the most observed in the bacterial genomes. Among these species, 27granted all the genes related to both pathways, assimilatory sulfate reduction and DNRA (Fig. 1). From these 27bacteria species mentioned above, 22of them belong to the class *Gammaproteobacteria* and the others belong to the categories *Alphaproteobacteria* and *Betaproteobacteria* (all belonging to the phylum Proteobacteria) (Fig. 2).

Bacterial species with genes of three or more pathways: Forty species exhibited all genes with more than one pathway (Fig. 1) but 11 of these bacteria had three or more complete

Table 4: Bacteria list indicating complete bioremediation pathways presence

No.	ID - NCBI	Specie	Nitrification	Denitrification	DNRA	ASR	DSR	Phosphor
1	NZ_CP014692	*Acetobacter aceti*						X
2	NZ_CP020917	*Achromobacter denitrificans*						X
3	NZ_CYIG01000001.1	*Acidovorax caeni*		X	X			X
4	NZ_ACQT01000638.1	*Acidovorax delafieldii*		X	X			X
5	NZ_JXYQ01000001.1	*Acidovorax temperans*		X	X			X
6	NC_014259	*Acinetobacter oleivorans*						
7	NC_008570.1	*Aeromonas hydrophila*			X	X		
8	NZ_CP007567.1	*Aeromonas media*			X	X		
9	NZ_CP013119.1	*Alcaligenes faecalis*				X		X
10	NZ_JH370371.1	*Alistipes indistinctus*						
11	NZ_JRGF01000001.1	*Alistipes inops*						
12	NZ_DS499581.1	*Alistipes putredinis*						
13	NC_014011	*Aminobacterium colombiense*						
14	NZ_JAFZ01000001.1	*Aminobacterium mobile*						
15	NC_013171.1	*Anaerococcus prevotii*						
16	NC_014960.1	*Anaerolinea thermophila*						
17	NC_009850.1	*Arcobacter butzleri*						
18	NZ_NWVW01000010.1	*Arcobacter canalis*						
19	NZ_JARS01000021.1	*Arcobacter faecis*						
20	NC_011886.1	*Arthrobacter chlorophenolicus*						
21	NZ_CP018863.1	*Arthrobacter crystallopoietes*						
22	NZ_CP007514.1	*Arthrobacter radiotolerans*						
23	NC_006274	*Bacillus cereus*						
24	NC_000964.3	*Bacillus subtilis*						
25	NC_022873.1	*Bacillus thuringiensis*						
26	NZ_CP011531	*Bacteroides dorei*						
27	NZ_AKBZ01000001.1	*Bacteroides finegoldii*						
28	NC_006347.1	*Bacteroides fragilis*						
29	NZ_CP012938.1	*Bacteroides ovatus*						
30	NC_004663.1	*Bacteroides thetaiotaomicron*						
31	NC_009614.1	*Bacteroides vulgatus*						
32	NC_021017.1	*Bacteroides xylanisolvens*						
33	NC_005363	*Bdellovibrio bacteriovorus*						
34	NZ_CP012373.1	*Beggiatoa leptomitoformis*				X		
35	NC_008618.1	*Bifidobacterium adolescentis*						
36	NC_012815	*Bifidobacterium animalis*						
37	NC_014638.1	*Bifidobacterium bifidum*						
38	NZ_AUAO01000001.1	*Brevundimonas aveniformis*						
39	NZ_JNIX01000001.1	*Brevundimonas bacteroides*						
40	NZ_CP009323.1	*Burkholderia gladioli*				X		
41	NZ_CP013433.1	*Burkholderia vietnamiensis*			X	X		X
42	FLQX01000001.1	*Candidatus Accumulibacter Aalbogensi*			X			
43	NC_013194	*Candidatus Accumulibacter phosphatis*			X			
44	NC_020449.1	*Candidatus Cloacamonas acidaminovorans*						
45	NZ_HG422565.1	*Candidatus Microthrix parvicella*						
46	NC_014355	*Candidatus Nitrospira defluvii*				X		
47	NZ_CZPZ01000001.1	*Candidatus Nitrospira nitrificans*	X					
48	NZ_CZQA01000001.1	*Candidatus Nitrospira nitrosa*	X			X		
49	NZ_JARQ01000001.1	*Chryseobacterium hispalense*						
50	NZ_LFNG01000001.1	*Chryseobacterium koreense*						
51	NZ_CP007557	*Citrobacter freundii*			X	X		
52	NZ_CP019986	*Citrobacter werkmanii*			X	X		
53	NZ_GG730308.1	*Citrobacter youngae*			X	X		
54	NZ_CP013252.1	*Clostridium butyricum*						
55	NZ_CP017603	*Clostridium formicaceticum*						
56	NZ_ACXX02000031.1	*Clostridium papyrosolvens*						
57	NC_021182.1	*Clostridium pasteurianum*						
58	NZ_CP024160.1	*Collinsella aerofaciens*						

Table 4: Bacteria list indicating complete bioremediation pathways presence

No.	ID - NCBI	Specie	Nitrification	Denitrification	DNRA	ASR	DSR	Phosphor
59	NZ_CP016603.1	*Comamonas aquatica*			X			X
60	NZ_AXVM01000001.1	*Comamonas badia*						X
61	NZ_BBJX01000034.1	*Comamonas granuli*						X
62	NZ_CP020121.1	*Comamonas kerstersii*			X			X
63	NZ_CP006704	*Comamonas testosteroni*						X
64	NZ_CYHD01000001.1	*Comamonas thiooxydans*			X	X		X
65	NC_004369.1	*Corynebacterium efficiens*						
66	NC_003450.3	*Corynebacterium glutamicum*						
67	NC_007298.1	*Dechloromonas aromatica*		X	X			
68	NC_013173	*Desulfomicrobium baculatum*					X	
69	NZ_AUAR01000001.1	*Desulfomicrobium escambiense*					X	
70	NC_013216	*Desulfotomaculum acetoxidans*					X	
71	NC_021184.1	*Desulfotomaculum gibsoniae*					X	
72	NC_015565	*Desulfotomaculum nigrificans*					X	
73	NC_016629.1	*Desulfovibrio africanus*					X	
74	NZ_KE383875.1	*Desulfovibrio aminophilus*					X	
75	NZ_KE386885.1	*Desulfovibrio putealis*					X	
76	NC_012881	*Desulfovibrio salexigens*					X	
77	NC_002937	*Desulfovibrio vulgaris*					X	
78	NZ_CP017037.1	*Dialister pneumosintes*						
79	NZ_CP015249.1	*Dokdonella koreensis*				X		
80	CP003026.1	*Enterobacter asburiae*			X	X		
81	CP025225.1	*Enterobacter cancerogenus*			X	X		
82	NC_014121.1	*Enterobacter cloacae*			X	X		
83	NC_020995.1	*Enterococcus casseliflavus*						
84	NC_004668	*Enterococcus faecalis*						
85	NZ_CP016625	*Escherichia coli*			X	X		
86	NC_012778.1	*Eubacterium eligens*						
87	NC_012781.1	*Eubacterium rectale*						
88	NZ_GG688422.1	*Eubacterium saphenum*						
89	NZ_KB907512.1	*Eubacterium siraeum*						
90	NZ_DS264288.1	*Eubacterium ventriosum*						
91	NZ_CP030777.1	*Faecalibacterium prausnitzii*						
92	NC_009441.1	*Flavobacterium johnsoniae*						
93	NC_015321	*Fluviicola taffensis*						
94	NC_012489.1	*Gemmatimonas aurantiaca*						
95	NZ_CP011454.1	*Gemmatimonas phototrophica*						
96	NZ_CP014963.1	*Geobacter anodireducens*						
97	NC_002939	*Geobacter sulfurreducens*						
98	CP009706.1	*Hafnia alvei*			X	X		
99	NZ_LXET01000001.1	*Hafnia paralvei*			X	X		
100	NC_015510.1	*Haliscomenobacter hydrossis*						
101	NZ_CP011636.1	*Klebsiella oxytoca*			X	X		
102	NZ_CP020847	*Klebsiella pneumoniae*			X	X		
103	NZ_CP016766	*Lactobacillus agilis*						
104	NC_004342.2	*Leptospira interrogans*						
105	NC_012803.1	*Micrococcus luteus*						
106	NZ_FPCG01000031.1	*Micrococcus terreus*						
107	NZ_CP007220	*Mycobacterium chelonae*				X		
108	NC_007964	*Nitrobacter hamburgensis*				X		X
109	NZ_MWPQ01000040.1	*Nitrobacter vulgaris*			X	X		X
110	NC_007406	*Nitrobacter winogradskyi*			X	X		X
111	NC_004757	*Nitrosomonas europaea*						
112	NC_008344.1	*Nitrosomonas eutropha*				X		
113	NZ_FODO01000081.1	*Nitrosomonas oligotropha*				X		
114	NZ_CP021106.3	*Nitrosospira lacus*						

Table 4: Bacteria list indicating complete bioremediation pathways presence

No.	ID - NCBI	Specie	Nitrification	Denitrification	DNRA	ASR	DSR	Phosphor
115	NC_007614	*Nitrosospira multiformis*				X		
116	NZ_LT828648.1	*Nitrospira japonica*				X		
117	NZ_CP011801.1	*Nitrospira moscoviensis*						
118	NZ_FUYQ01000044.1	*Parabacteroides chartae*			X			
119	NC_009615	*Parabacteroides distasonis*						
120	NZ_KE159513.1	*Parabacteroides goldsteinii*						
121	NZ_JH976465.1	*Parabacteroides johnsonii*						
122	NZ_JH976452.1	*Parabacteroides merdae*						
123	NC_008686.1	*Paracoccus denitrificans*		X	X	X		X
124	CP025430.1	*Paracoccus zhejiangensis*			X			X
125	NC_013061.1	*Pedobacter heparinus*						
126	NC_007498	*Pelobacter carbinolicus*						
127	NC_008609.1	*Pelobacter propionicus*						
128	CZAM01000001.1	*Prevotella copri*						
129	NZ_CP021852	*Proteus mirabilis*			X	X		
130	GG662004.1	*Proteus penneri*				X		
131	NC_002516.2	*Pseudomonas aeruginosa*		X	X	X		
132	NC_016830	*Pseudomonas fluorescens*		X	X	X		
133	NC_002947.4	*Pseudomonas putida*						
134	NC_014034.1	*Rhodobacter capsulatus*						X
135	NC_009428.1	*Rhodobacter sphaeroides*						X
136	NC_008268.1	*Rhodococcus jostii*				X		
137	NC_003197.2	*Salmonella enterica*			X	X		
138	NZ_CP011642	*Serratia marcescens*			X	X		
139	NZ_AP017655.1	*Sphingobium cloacae*				X		X
140	NZ_CP012900.1	*Stenotrophomonas acidaminiphila*				X		X
141	NC_016826	*Streptococcus infantarius*						
142	NZ_CP007201.1	*Sulfurospirillum multivorans*				X		
143	NC_008554.1	*Syntrophobacter fumaroxidans*					X	
144	NZ_BBCE01000001.1	*Syntrophomonas palmitatica*						
145	NC_008346.1	*Syntrophomonas wolfei*						
146	NZ_CGIH01000001.1	*Syntrophomonas zehnderi*						
147	NC_007759.1	*Syntrophus aciditrophicus*						
148	NZ_CP028339.1	*Thauera aromatica*		X	X			
149	NC_007404	*Thiobacillus denitrificans*		X	X			
150	NZ_CP020046	*Thiomonas intermedia*						X
151	NZ_KB904746.1	*Thiothrix flexilis*			X			
152	NZ_JH651381.1	*Thiothrix nivea*			X	X	X	
153	NC_015732.1	*Treponema caldarium*						
154	NC_014158.1	*Tsukamurella paurometabola*				X		
155	NC_009456.1	*Vibrio cholerae*				X		
156	NZ_JMCG01000001.1	*Vibrio navarrensis*			X	X		
157	NZ_CQBU01000001.1	*Yersinia bercovieri*			X	X		
158	CP009364.1	*Yersinia frederikseni*			X	X		

Bacteria shaded in gray do not have any complete pathway

pathways. *Paracoccus denitrificans* stands out for having the greatest number of complete pathways, possessing the genes of denitrification, dissimilatory nitrate reduction, assimilatory sulfate reduction and phosphorus accumulation processes (Fig. 1).

Thiothrixnivea was the only species that possessed the genes necessary to complete the two routes of sulfur, both assimilatory (ASR) and dissimilatory sulfate reduction (DSR) (Fig. 1).

Burkholderia vietnamiensis, *Comamonas thiooxydans*, *Nitrobacter vulgaris* and *Nitrobacter winogradskyi* have genes that act on the same pathways, i.e., dissimilatory nitrate reduction, assimilatory sulfate reduction and phosphorus accumulation (Fig. 1). *Burkholderia vietnamiensis* and *Comamonas thiooxydans* belong to the Betaproteobacteria class, while *Nitrobacter vulgaris* and *Nitrobacter winogradskyi* belong to the Alphaproteobacteria class (Fig. 2).

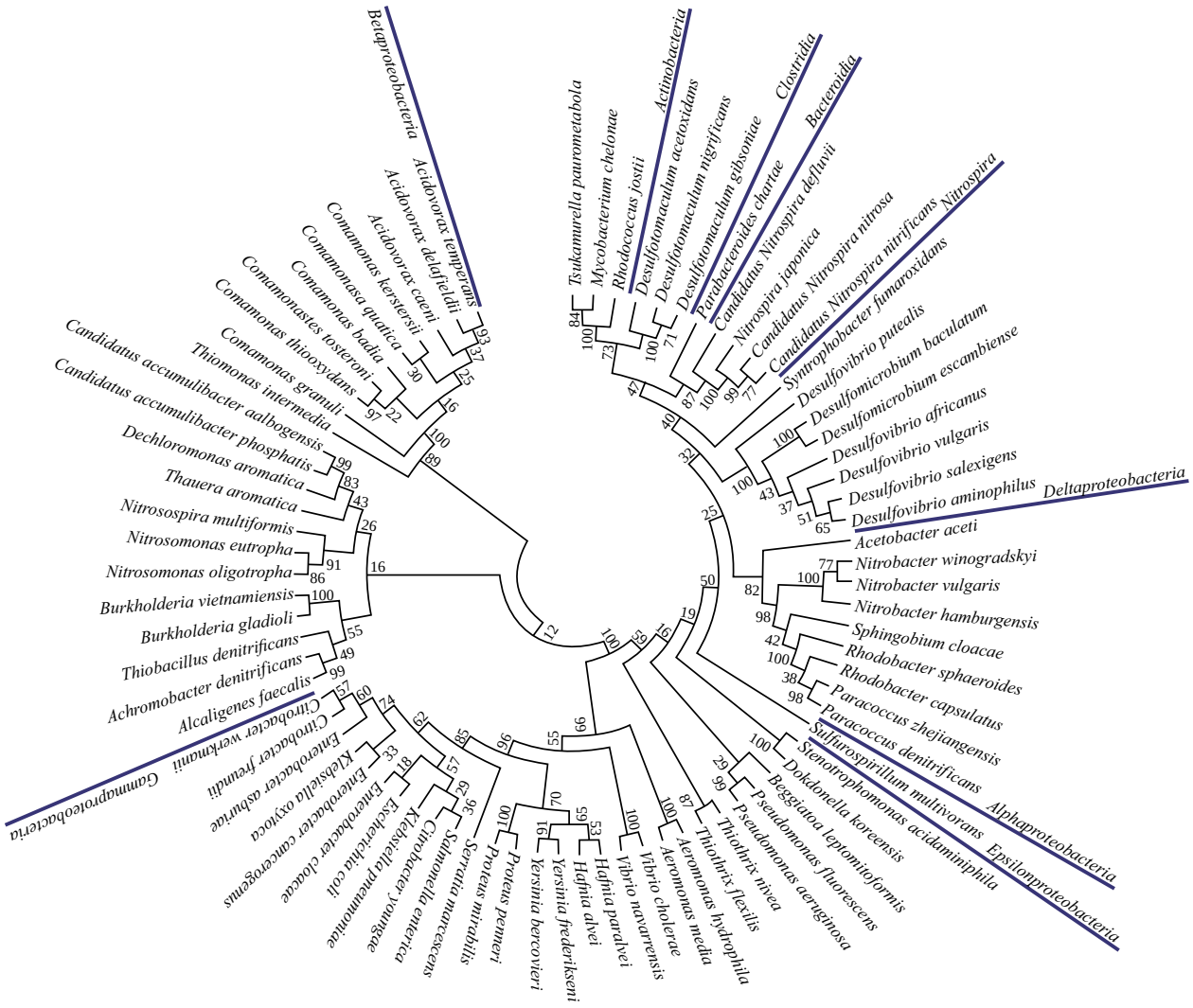

Fig. 2: 16S rRNA phylogeny of bacteria presented at least one of the complete pathways
Class level lineages are indicated on the blue lines

DISCUSSION

In this study, 158 bacterial genomes were analyzed in search of genes that act in six bioremediation processes. Half of the analyzed species (79) have at least one of the complete pathways (Table 4) and 18 of them have 3 or more analyzed pathways (Table 3), which indicate a better metabolic capacity of these species.

Gammaproteo bacteria was the most leading class, with 18.9% of analyzed species, followed by *Betaproteobacteria* (15.1%), *Clostridia* (10.7%), *Bacteroidia* (10.2%), *Deltaproteobacteria* (8.2%), *Actinobacteria* (7.5%) and *Alphaproteobacteria* (6.9%), with predominant species belonging to the phylum *Proteobacteria* (52.5%). This result was similar with other studies[23-26], indicating that this study sampling with the most common bacteria in different sewage treatment plants is significant.

Clostridium was the most identified genus in different wastewater treatment plants analyzed (Table 3) and these bacteria are found in different environments and inhabiting the intestines of several animal species[27,28]. The second most identified genus was *Nitrospire*, that are found in many environments and are responsible for nitrification processes[29]. Remarkably, all bacteria listed in Table 3 belong to the phylum Proteobacteria. These species presented genes that enable them to act in cycles of the three nutrients studied (nitrogen, phosphorus and sulfur). Because of this, it is possible that Proteobacteria is the predominant phylum in practically all sewage treatment plants analyzed[30,31].

Nitrification was the least observed pathway, with two *Nitrospira* bacteria presenting the complete pathway (Fig. 2). Separation of nitrification into two steps led to a cross-feeding interaction between different species of bacteria. On the other hand, those that could catalyze the complete nitrification pathway had growth advantages over the others[32].

Dissimilatory sulfate reduction and oxidation pathway (DSR) are present in 12 bacteria species (Fig. 1). SRB controlled application in wastewater treatment carry several advantages: promotes pathogen and heavy metal removal, reduction of sludge disposal and perform a pre-treatment before anaerobic digestion that results in higher biogas yields[33].

Assimilatory Sulfate Reduction (ASR) and Dissimilatory Nitrate Reduction (DNRA) are the pathways present in a greater number of bacteria (Fig. 1). Recent studies have demonstrated that the nitrogen cycle is also tightly linked to other biogeochemical cycles, such as the sulfur cycle[34,35] and suggest that biogenic sulfide induces DNRA with coproduction of ammonium and nitrite[36].

Denitrification pathway was observed in 9 bacteria species and these bacteria also have genes from other pathways (Fig. 1). Sulfate reduction can indirectly stimulate P release and when sulfate is reduced to sulfide, this molecule can bind its self to Fe(II), leading to more P availability[37]. Curiously, four bacteria species were identified with genes for performing denitrification and phosphorus accumulation (*P. denitrificans* and three bacteria of *Acidovorax* genus) (Fig. 1). Therefore, this combination of genes should make these species efficient nitrate and phosphorus removers.

The bacterium with the greatest capacity for bioremediation is *Paracoccus denitrificans* possessing the genes of 4 pathways: denitrification, dissimilatory nitrate reduction, assimilatory sulfate reduction and phosphorus accumulation processes (Fig. 1). It is a nonmotile coccoid soil organism and is taxonomically part of the Rhodobacteraceae family from a subdivision of the phylum Proteobacteria[38]. *P. denitrificans* can live in oxic and anoxic environments in response to environmental changes, such as oxygen and nitrogenous oxide concentration.

Another interesting bacterium in the bioremediation process is *Thiothrixnivea*, having all genes for assimilatory (ASR) and dissimilatory sulfate reduction (DSR) (Fig. 1). ASR is characterized by sulfate reduction in small amounts required for the synthesis of cellular material, whereas DSR is described as the sulfate reduction in great excess of nutritional requirements, producing massive amounts of sulfide[32].

The main limitation of this study was to have analyzed a fraction of the species in a wastewater treatment plant, which has 1700-3600 species[16,17]. However, the 158 analyzed species are quite representative, being the most abundant in several wastewater treatment plants. Identification of the main species described in the study, such as *Paracoccus denitrificans*, *Thiothrixnivea* and *Nitrospiranitrosa*, would allow to evaluate *in vitro* the metabolic capacities of these species in the wastewater bioremediation.

Therefore, the results of this observation could be used to increase the sewage treatment efficiency, indicating/allocating the most appropriate bacterial species in degradation of nitrogen, phosphorus and sulfur compounds. Additionally, this study could be used in the development of more coherent genetically modified organisms in wastewater bioremediation.

CONCLUSION

Knowledge of the bacterial community composition and how it interacts inside the wastewater treatment plants are essential for better designed bioremediation strategies. Bacteria having genes for the pathways studied here can be introduced into a sewage treatment plant to increase organic matter degradation, for example, *P. denitrificans*, *T.* and *N. nitrosa*. A combination of these three bacteria would have all the genes analyzed in this study.

SIGNIFICANCE STATEMENT

This study identified the main bacterial species that perform wastewater bioremediation process and these results can be used to improve bioremediation processes. The bacteria indicated in the study can be added to treatment plants in a selective enrichment method, increasing nitrogen, sulfur and phosphorus compounds bioremediation.

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