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CARACTERIZAÇÃO FUNCIONAL DE GENES RELACIONADOS À BIORREMEDIAÇÃO EM BACTÉRIAS DE ESTAÇÕES DE TRATAMENTO DE ESGOTO

PATOS DE MINAS-MG

MARÇO DE 2019

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Dissertação de mestrado apresentada ao Programa de Pós-graduação em Biotecnologia como requisito parcial para obtenção do título de mestre.

Orientador: Prof. Dr. Aulus Estevão Anjos de Deus Barbosa

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RESUMO

A contaminação dos corpos d'água, principalmente devido à descarga inadequada de efluentes, é uma das atividades antrópicas mais impactantes ao meio ambiente, e que causa diversos problemas sanitários, econômicos e sociais. Neste sentido, é importante entender os processos biológicos que ocorrem nas estações de tratamento de esgoto que permitem a biorremediação desses resíduos. As bactérias são os microrganismos mais presentes nesses ambientes, promovendo a remoção e/ou degradação de matéria orgânica e do excesso de diversos nutrientes como nitrogênio, fósforo e enxofre. Com o objeto de melhorar a compreensão dos processos de biorremediação, neste trabalho foram identificadas as principais espécies bacterianas presentes em diferentes estações de tratamento de esgoto e, por análise genômica, foi verificado quais dessas bactérias possuem os genes de vias de degradação ou absorção de compostos de nitrogênio, enxofre e fósforo. Os genomas de 158 espécies de bactérias isoladas de estações de tratamento de esgoto foram analisados em busca dos genes dos seguintes processos: nitrificação, desnitrificação, redução dissimilatória de nitrato, acumulação de fósforo, redução assimilatória de sulfato e redução e oxidação dissimilatória de sulfato. 79 espécies de bactérias possuíam pelo menos uma das vias completas, e 11 dessas espécies apresentaram três ou mais vias completas: *Acidovorax caeni*, *Acidovorax delafieldii*, *Acidovorax temperans*, *Burkholderia vietnamiensis*, *Comamonas thiooxydans*, *Nitrobacter vulgaris*, *Nitrobacter winogradskyi*, *Paracoccus denitrificans*, *Pseudomonas aeruginosa*, *Pseudomonas fluorescens* e *Thiothrix nivea*. *Paracoccus denitrificans* destaca-se por possuir o maior número de vias completas, possuindo os genes de desnitrificação, redução dissimilatória de nitrato, redução assimilatória de sulfato e do processo de acumulação de fósforo. A via de nitrificação foi menos frequente, sendo encontrada apenas em duas espécies: *Candidatus Nitrospira nitrificans* e *Candidatus Nitrospira nitrosa*. Portanto, os resultados deste trabalho auxiliam no entendimento dos processos metabólicos realizados por bactérias presentes nas estações de tratamento de esgoto e auxiliam na otimização do processo indicando bactérias mais adaptadas à biorremediação. Além disso, os resultados podem ser utilizados no desenvolvimento de organismos geneticamente modificados mais eficientes no tratamento de esgoto.

ABSTRACT

Contamination of water bodies, mainly due to inadequate discharge of effluents, is one of the most impacting anthropogenic activities to the environment, causing sanitary, economic, and social problems. Therefore, it is necessary to understand the biological processes that occur in the wastewater treatment plants that allow the bioremediation of these wastes. Bacteria are the most present microorganisms in these environments, promoting the removal and/or degradation of organic matter and various nutrients like nitrogen, phosphorus, and sulfur. In this work, the main bacterial species present in different sewage treatment plants were identified and, by genomic analysis, which of these bacteria have the genes of degradation or absorption pathways of nitrogen, sulfur and phosphorus compounds. The 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. 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 *Thiothrix nivea*. *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. Nitrification pathway was less frequent, found only in 2 species: *Candidatus Nitrospira nitrificans* and *Candidatus Nitrospira nitrosa*. Therefore, the results of this work help in understanding the metabolic processes performed by the bacteria in the sewage treatment plants and in optimizing the process that indicate which bacteria are more adapted to bioremediation. In addition, the results can be used in the development of more efficient genetically modified organisms to depollute sewages.

LISTA DE ABREVIATURAS E SIGLAS

AGVs	Ácidos graxos voláteis
AOB	Bactérias oxidantes de amônia
ASR	Redução assimilatória de sulfato
ATP	Adenosina trifosfato
CONAMA	Conselho Nacional do Meio Ambiente
DNRA	Redução dissimilatória de nitrato a amônia
DSR	Redução dissimilatória de sulfato
N	Nitrogênio
NBR	Norma brasileira
NOB	Bactérias oxidantes de nitrito
P	Fósforo
PAB	Bactérias acumuladoras de fósforo
PAO	Organismos acumuladores de fósforo
PHAs	Polihidroxialcanoatos
Poli-P	Polifosfatos
S	Enxofre
SRB	Bactérias redutoras de sulfato

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1. CAPÍTULO 1: INTRODUÇÃO

1.1. Problema de pesquisa

Os problemas ambientais relacionados ao despejo inadequado de efluentes domésticos têm se tornado cada vez mais críticos, principalmente, pelo crescente volume que é descartado em corpos d'água. Além disso, o descarte de esgoto não tratado é uma ameaça à saúde pública, pois sempre é acompanhado por organismos patogênicos (CAI; et al., 2014). De acordo com a NBR 9648, que trata sobre o estudo de concepção de sistemas de esgoto sanitário, o esgoto doméstico é o despejo líquido resultante do uso da água para higiene e necessidades fisiológicas humanas. Dentre os diversos nutrientes presentes, em excesso, nos esgotos domésticos, destacam-se o nitrogênio (ZIELINSKA; et al., 2012, KALLISTOVA; et al., 2016) fósforo (IVANOV; et al., 2004, JU; ZHANG, 2015) e enxofre, (VAN DEN BRAND; et al., 2018). O lançamendo destes efluentes, com altas concentrações desses nutrientes, pode resultar, por exemplo, no processo de eutrofização (WEI; et al., 2008).

A comunidade bacteriana presente nos sistemas de tratamento de efluentes exerce papel fundamental na biodegradação dos nutrientes (ATASHGAHI; et al., 2015, XU; et al., 2018), estabelecendo interações entre grupos de forma a facilitar a sobrevivência num ambiente de grande competição. Diversas técnicas microbiológicas, como isolamento de culturas puras e ensaios bioquímicos, não são capazes de revelar a diversidade microbiana nas estações de tratamento, sendo necessário técnicas mais modernas (HASHIMOTO; et al., 2014) para complementação dos estudos. Assim, utilizando a bioinformática é possível extrair informações relevantes a partir das sequências de DNA e de proteínas, obtidas pelo processo de sequenciamento automático de nucleotídeos e de aminoácidos (ROCHA, 2011).

1.2. Hipótese

Tradicionalmente, os processos de biorremediação são atribuídos a uma população bacteriana muito diversa, e por isso, como hipótese do trabalho, foi avaliado se um número reduzido de bactérias possuiriam genes suficientes para a degradação simultânea de nitrogênio, fósforo e enxofre presentes nos esgotos.

1.3. Objetivos

Os objetivos deste trabalho foram (i) identificar os principais gêneros de bactérias presentes em estações de tratamento de esgoto e quais dessas espécies possuem genes que

participam das vias de degradação ou acumulação de nitrogênio, fósforo e enxofre. Além disso, (ii) realizar análises filogenéticas nas bactérias em estudo e (iii) verificar a existência de genes de diferentes vias em um único organismo.

1.4. Justificativa

Muitos têm sido os avanços em relação a sistemas de tratamento de efluentes, principalmente nos processos biológicos envolvidos na remoção ou degradação de matéria orgânica e nutrientes. Segundo Sanz e Köchling (2007), na maioria das vezes não há informações sobre necessidades nutricionais e físico-químicas dos organismos em estudo, além da complexidade das relações simbióticas aumentarem a dificuldade de se obter culturas puras de grande parte destes organismos. Porém, as informações oriundas dos projetos de sequenciamentos de genomas retirados diretamente de estações de tratamento de esgoto possibilitam o estudo comparativo entre as mais variadas sequências gênicas. Além disso, por meio das relações filogenéticas busca-se a comparação de organismos de modo a estabelecer suas relações evolutivas, utilizando as sequências nucleotídicas de seus genomas.

Portanto, o uso de técnicas de bioinformática permitiu um melhor entendimento do metabolismo bacteriano nas estações de tratamento de efluente, visto que sem a degradação e/ou remoção dos principais nutrientes os corpos receptores podem ser altamente contaminados. Ademais, a compreensão dos processos metabólicos permite otimizar o enriquecimento com microrganismos específicos em estações de tratamento de efluente, além de possibilitar novas pesquisas para criação de organismos geneticamente modificados que consigam degradar mais compostos do efluente.

2. REFERÊNCIAL TEÓRICO

2.1. Tratamento biológico de esgoto

A poluição de ambientes aquáticos tem se destacado como uma das atividades antrópicas mais impactantes ao meio, causando problemas sanitários, econômicos e sociais. A Resolução CONAMA n°430, de 13 de Maio de 2011, estabelece que “os efluentes de qualquer fonte poluidora somente poderão ser lançados, direta ou indiretamente, nos corpos de água, após o devido tratamento e desde que obedeçam as condições, padrões e exigências dispostos nesta Resolução e em outras normas aplicáveis”. Conforme von Sperling (2005), o despejo de

efluentes pode agregar ao corpo d'água receptor diversos agentes transmissores de doenças, podendo afetar o abastecimento de água potável, a irrigação e a balneabilidade.

Esgotos contém microrganismos provenientes de várias fontes como, por exemplo, descargas de fezes, infiltrações no solo e sedimentos (GUO; et al., 2019). Esgotos não são uniformes e estáveis, variam conforme a hora do dia, o mês do ano, a localização geográfica, ou seja, variam significantemente conforme o tempo e o local. A principal razão para essas diferenças deve-se à variação no consumo e na infiltração da água, resultando em diferentes quantidades de substâncias lançadas.

O tratamento de esgotos municipais nos países industrializados evoluiu extraordinariamente durante os últimos anos, porém é um processo que deve ser melhorado para torná-lo mais sustentável (CHAN; et al., 2017). O tratamento de esgoto é um dos processos biotecnológicos mais importantes utilizados mundialmente para o tratamento, principalmente, de esgotos municipais (WAGNER, 2002). Desta forma, cada estação de tratamento de efluente deve adotar a melhor alternativa técnica e econômica, observando questões como disposição do lodo, impactos ambientais, requisitos de área e custos de operação e manutenção.

As estações de tratamento de esgoto possuem etapas para remoção de poluentes e matéria orgânica, utilizando processos físicos, químicos e biológicos. A eficiência de uma estação de tratamento de esgoto depende principalmente da composição e atividade de sua comunidade microbiana (WAGNER, 2002). Os sistemas de tratamento de efluentes, tal como os ambientes naturais, são sistemas abertos, que permitem a convivência de diferentes microrganismos, que estabelecem relações e interações diversas.

O metabolismo bacteriano influencia diretamente na eficácia do tratamento biológico de esgotos, sendo importante definir as relações entre a comunidade de microrganismos e o desempenho das instalações (CYDZIK-KWIATKOWSKA; ZIELIŃSKA, 2016). Estudos mostraram que as diversidades microbianas estavam diretamente associadas à fatores físicos como altitude e temperatura (HU; et al., 2012; FANG; et al., 2018). Porém, o número microrganismos que podem ser cultivados sob condições artificiais é limitado (PANG; et al. 2016).

2.2. Compostos presentes em efluentes

O tratamento biológico de esgotos remove materiais orgânicos utilizando biomassa microbiana que é separada do líquido, por exemplo, via membrana (CHRISTENSEN; et al., 2015). Os nutrientes devem ser removidos dos efluentes, principalmente nitrogênio, fósforo e enxofre, a fim de evitar seu despejo em quantidades inadequadas em corpos d'água. Nitratos, fosfatos e outros nutrientes entram na água através de detergentes, fertilizantes e adubos animais. O enriquecimento abundante da água com nutrientes, chamado eutrofização, leva ao crescimento excessivo de algas e outros vegetais (BLACK, 2013). Segundo von Sperling (1996), a utilização do substrato disponível no meio pelas bactérias se dá em função do tamanho relativo da partícula a ser utilizada.

O nitrogênio é um dos principais nutrientes existentes em sistemas de tratamento de efluentes, presente nas proteínas, nos ácidos nucleicos e em outras moléculas orgânicas que desempenham papéis importantes no metabolismo celular (SANT'ANNA JUNIOR, 2013). Todos os organismos, inclusive os microrganismos, precisam de nitrogênio para sintetizar ácidos nucleicos, enzimas e outras proteínas (BLACK, 2013). Para melhorar a eficiência de remoção de nitrogênio em estações de tratamento de esgoto (ETEs), é essencial compreender o comportamento das comunidades que realizam a ciclagem de nitrogênio (PANG; et al., 2016). Devido à baixa produção de biomassa e à sensibilidade a fatores ambientais, as bactérias nitrificantes representam apenas uma pequena fração da biomassa total (YAO; PENG, 2017). A estrutura de espécies da biomassa determina as vias metabólicas que podem ocorrer no sistema e a eficiência do tratamento (CYDZIK-KWIATKOWSKA; ZIELIŃSKA, 2016).

O processo de nitrificação é definido como a conversão da forma mais reduzida de nitrogênio, NH_3 (amônia) à sua forma mais oxidada, NO_3^- (nitrato), e é realizada em duas etapas que são realizadas por dois grupos diferentes de microrganismos: as bactérias oxidantes de amônia (AOB) e as bactérias oxidantes de nitrito (NOB) (CÁCERES; et al., 2018). Sob condições aeróbias, o íon amônio é oxidado a nitrato, tendo nitrito como produto intermediário. Nos processos de oxidação de compostos nitrogenados reduzidos há presença de diversas enzimas essenciais. Em bactérias oxidantes de amônia, o NH_3 é oxidado pela *amônia mono-oxigenase*, produzindo NH_2OH e H_2O. Uma segunda enzima chave, a *hidroxilamina oxidorredutase*, então oxida NH_2OH a NO_2^- (MADIGAN; et al., 2010).

A desnitrificação, também denominada de respiração do nitrato, pode ser conduzida por um grande grupo de bactérias filogeneticamente não relacionadas entre si (SANT'ANNA

JUNIOR, 2013). A desnitrificação é a redução gradual dos óxidos de nitrogênio com formação de produtos gasosos tais como N_2O ou N_2 sob condições de oxigênio limitado. Os produtos gerados nas etapas do processo de desnitrificação é ilustrado pela equação abaixo:

$$NO_3^- \longrightarrow NO_2^- \longrightarrow NO \longrightarrow N_2O \longrightarrow N_2$$

O requisito de todos os três fatores, fonte de carbono disponível, oxigênio limitado e NO_3 suficiente, deve estar presente para a ocorrência de desnitrificação (MARTENS, 2005). As principais enzimas envolvidas na desnitrificação são: *nitrato redutase*, *nitrito redutase*, *óxido nítrico redutase* e *óxido nitroso redutase*.

Outro processo do ciclo do nitrogênio é a redução dissimilatória de nitrato a amônia (DNRA). A DNRA é outra via de redução de NO_3^-, mas, diferentemente da desnitrificação, o nitrogênio é conservado como NH_4 (BERNARD; et al., 2015). A DNRA reduz o NO_3^- via nitrito (NO_2^-) para NH_4^+, catalisado pela *nitrito redutase* (citocromo c) (FRIEDL; et al., 2018). A fim de controlar a ocorrência de microrganismos atuantes na via DNRA dentro de um sistema de tratamento de esgoto, é essencial reconhecer a sua identidade e diversidade como o primeiro passo para controlar estas populações (CHUTIVISUT; et al. 2018).

Outro importante nutriente presente nos efluentes é o fósforo, sendo encontrado em diferentes formas. Fosfatos, polifosfatos e ortofosfatos são comuns em esgotos domésticos, em decorrência do uso de produtos de limpeza (SANT'ANNA JUNIOR, 2013). Organismos acumuladores de fósforo (PAO) tem a capacidade de absorver ácidos graxos voláteis (AGVs) sob condições anaeróbias, e armazená-los como polihidroxialcanoatos (PHAs) (CHAN; et al., 2017). Sendo que a energia necessária para esta transformação é obtida, principalmente, por meio da hidrólise de polifosfatos (poli-P) e em parte devido à quebra do glicogênio. Polifosfatos podem ser acumulados por uma ampla gama de bactérias (HENZE; et al., 2008).

No estágio aeróbico, P é absorvido e armazenado novamente como poli-P, resultando na remoção líquida de P dos efluentes (CHAN; et al., 2017). A mobilidade de fosfato dentro dos organismos é alta e, portanto, em contraste com as condições ambientais, o fosfato está prontamente disponível nos sistemas biológicos (FILIPPELLI, 2008). As principais enzimas envolvidas são: *acetato quinase*, *fosfato acetiltransferase*, *acetil-CoA sintase* e *polihidroxialcanoato sintase*.

O enxofre também é um nutriente importante presente nos sistemas de tratamento de efluentes. Tecnologias e estratégias têm sido desenvolvidas para lidar com o sulfeto por

décadas, dentre as quais os meios biológicos constituem uma parcela considerável devido às suas baixas exigências de operação e flexibilidade (LIN; et al., 2018). Dentre as principais enzimas envolvidas no ciclo biogeoquímico do enxofre, estão: *sulfato adenililtransferase*, *sulfito redutase* e *sulfito redutase dissimilatória*.

O sulfeto é tóxico, corrosivo e malcheiroso, sendo necessário tomar medidas efetivas para controlar sua emissão ao meio ambiente (CAI; et al, 2017). A enzima ATP sulfurilase catalisa a ligação do íon sulfato a um fosfato do ATP, levando a formação de *adenosina fosfosulfato* (APS) (MADIGAN; et al., 2010). Posteriormente, por intermédio da enzima APS redutase, a APS é reduzida em sulfito (SO_3^{2-}). Logo após, a enzima sulfito redutase vai catalisar a ligação para a redução em sulfeto (H_2S).

Na redução dissimilativa de sulfato o sulfeto é excretado. As bactérias oxidantes de sulfeto incluem bactérias filamentosas e bactérias fotossintéticas, dentre as quais algumas espécies podem oxidar sulfeto (LIU; JIANG; YANG, 2012) e gerar enxofre elementar (CAI; et al, 2017). O ciclo do enxofre se conecta com os ciclos de carbono, nitrogênio e fósforo, assim uma nova plataforma de biotecnologias baseadas em enxofre relacionando tais ciclos pode ser desenvolvida para a remoção de sulfato e outros poluentes (HAO; et al, 2014).

Atualmente, têm sido utilizadas técnicas de biologia molecular para investigar as relações entre os microrganismos responsáveis pela remoção de poluentes dos esgotos (CYDZIK-KWIATKOWSKA; ZIELIŃSKA, 2016). A seleção de tecnologias alternativas eficientes e de baixo custo para o tratamento de águas residuais é significativa, especialmente nas regiões em desenvolvimento (WU; et al., 2015). A relação entre diferentes ciclos biogeoquímicos como enxofre, nitrogênio e fósforo, e o foco contínuo da biorremediação de resíduos pelas bactérias, leva à novas metodologias (LIN, 2018). E o estudo das relações desses organismos entre si e com o meio auxiliam na compreensão de importantes funções relacionadas à remoção de nutrientes no esgoto.

2.3. Bioinformática

A utilização da Bioinfomática envolve a expansão e facilitação do uso de dados biológicos através da capacidade de adquirir, armazenar, organizar, analisar e visualizar tais dados, por meio do desenvolvimento e do uso de ferramentas computacionais (ROCHA, 2011). Estudos mostram que os mecanismos de busca de genes por recursos de bioinformática são de grande importância para direcionar futuros estudos funcionais em busca de novos genes

COUTINHO, 2018). Na bioinformática, os dados, provenientes de problemas reais, se destinam à resolução de problemas biológicos, análises de microarray, genomas e proteomas inteiros e ou sequências de DNA, RNA e proteínas (DIAS; PASCUTTI; SILVA, 2016). Dentre os benefícios da análise computacional, cita-se a possibilidade de esclarecer a estrutura e a função dos genes e proteínas estudados (BAXEVANIS; OUELLETTE, 2005).

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3. CAPÍTULO 2: TRABALHO EXPERIMENTAL

Artigo científico submetido para o periódico Plos One.

Functional genomic characterization of bioremediation genes in the main wastewater treatment bacteria

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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 concentration, 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. Remarkably, there are many advances in wastewater treatment processes, and microorganisms, mainly bacteria, are responsible for removal and degradation of these compounds [5, 6].

Bacteria constitute the most important group in biological treatment systems [5, 6]. However, new genomic, metabolic, and nutritional information from these organisms could help in understanding symbiotic relationships in sewage treatment [7-9]. In addition, several aspects related to microbial communities should be considered, such as the diversity and interaction between bacteria and the environment [10-12].

High-throughput metagenomic sequencing enables the study of the taxonomic and functional diversity of a microbial population [3, 13]. 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 [14]. Recent works highlight that there are about 1,700–3,600 species of bacteria in wastewater treatment plants [15, 16].

Hypothetically, it may be considered that certain species of bacteria, present in wastewater treatment plants, may have suffered advantageous gene loss, resulting in genome reduction and/or increased efficiency of survival processes. Understanding of the bioremediation metabolic processes can be used to optimize the enrichment with specific

microorganisms in effluent treatment plants—also allowing the development of genetically modified organisms that can increase the efficiency of the treatment. Thereby, the objective of this work is to (i) identify the main genera of bacteria present in sewage treatment plants and which of these species have genes that participate in the degradation or accumulation pathways of nitrogen, phosphorus, and sulfur. In addition, (ii) phylogenetic analyses were performed on the bacteria under study and (iii) the existence of genes of different pathways in a single organism. Therefore, the results of this work could be used to increase the sewage treatment efficiency, indicating the most appropriate bacterial species in degradation of nitrogen, phosphorus, and sulfur compounds. Additionally, this study could be used in the development of more efficient genetically modified organisms in wastewater bioremediation.

Materials and Methods

Identification of main bacterial genera in wastewater treatment plants

Identification of the main bacterial genera in wastewater treatment plants was made by analysis of scientific papers. These papers performed metagenomic analyses of bacterial genera in water treatment plants in different geographic locations (Table 1). Papers and databases that presented information about major genera and species of bacteria were considered, as well as the proportion of each group within a sewage treatment plant.

Table 1. List of scientific references that indicated main bacterial genera in wastewater treatment plants. Title and year are show.

Title	Year	Ref.
Dissecting microbial community structure and methane-producing pathways of a full-scale anaerobic reactor digesting activated sludge from wastewater treatment by metagenomic sequencing	2015	[17]
Assessing the composition of microbial communities in textile wastewater treatment plants in comparison with municipal wastewater treatment plants	2017	[15]
A pyrosequencing-based metagenomic study of methane-producing microbial community in solid-state biogas reactor	2013	[18]
Systematic investigation and microbial community profile of indole degradation processes in two aerobic activated sludge	2015	[19]
Tracking human sewage microbiome in a municipal wastewater treatment plant	2014	[1]
Metagenomic analysis of sludge from full-scale anaerobic digesters operated in municipal wastewater treatment plants	2014	[20]
Analysis of Bacterial Community Structure of Activated Sludge from Wastewater Treatment Plants in Winter	2018	[21]
Microbial community dynamics in an ANAMMOX reactor for piggery wastewater treatment with startup, raising nitrogen load, and stable performance	2018	[22]

The most significant genera and species were considered for those identified as the most abundant and that were isolated in more than one scientific article listed in Table 1. The full genome of these species were downloaded at National Center for Biotechnology Information database (NCBI https://www.ncbi.nlm.nih.gov/), and all of them were bacterial species isolated and sequenced from wastewater treatment plants, activated sludge or sewage. Downloaded genomes were used to create a database for subsequent analyzes.

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 at Kyoto Encyclopedia of Genes and Genomes database (KEGG - http://www.kegg.jp). Genes of following pathways were analyzed: nitrification, denitrification, dissimilatory nitrate reduction (https://www.kegg.jp/dbget-bin/www_bget?map00910), phosphorus accumulation [23], assimilatory sulfate reduction and dissimilatory sulfate reduction and oxidation (https://www.kegg.jp/dbget-bin/www_bget?map00920). Query protein sequences are listed in Table 2. Protein sequences of these genes were compared with the genome database using command line tBlastn and an e-value cutoff of 1×10^{-20} [24]. Venn diagrams were constructed with data generated in the blast (http://bioinformatics.psb.ugent.be/webtools/Venn/) to represent the relationships between the bacteria species that have nitrogen, phosphorus, and/or sulfur pathways genes.

Table 2. Query genes selected for each degradation pathway. Nitrification (Nit), denitrification (Den), dissimilatory nitrate reduction (DNRA), assimilatory sulfate reduction (ASR), dissimilatory sulfate reduction and oxidation (DSR), and phosphorus accumulation (Pho) pathways.

	Gene Name	Gene	KEGG Entry
Nit	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
Den	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 CAP 2UW1_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
ASR	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
	Adenylylsulfate 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	Adenylylsulfate reductase, subunit A	**AprA**	Tbd_0872 Desaf_0101 Clopa_4347 EUBREC_2472

	Adenylylsulfate 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

Phylogenetic analysis

Phylogenetic analysis was performed with bacterial species that showed at least one of the complete pathways. Thus, 16S rRNA gene was used to compare all the analyzed bacteria. Sequence alignments were performed using CLUSTALW in the BioEdit Sequence Alignment Editor (http://www.mbio.ncsu.edu/BioEdit/bioedit.html) [25]. Phylogenetic tree construction was performed by the neighbor joining method using the software MEGA-X (https://www.megasoftware.net/) [26]. Robustness was assessed by bootstrap analysis based on 1,000 repetitions.

Detailed analysis of bacteria species with genes of several pathways

Bacterial species presented three or more complete pathways for degradation and/or accumulation of nitrogen, phosphorus and sulfur were conducted to a detailed analysis. Reverse BLASTp and InterProScan were used to identify and confirm best blast hits, and were conducted with Blast2GO 5 [27] basic software (https://www.blast2go.com/).

Results and Discussion

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 [28, 29]. Municipal wastewater is mainly comprised of water (99.9%), together with relatively small concentrations of suspended and dissolved organic and inorganic solids [30]. Different physical and chemical processes, such as adsorption, incineration, coagulation, precipitation, and chemical oxidation, can be applied to treat wastewater [29]. Nevertheless, there are advantages in biological processes such as 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 [31]. 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 [30]. The major microorganisms found in wastewater influents are viruses, bacteria, fungi, protozoa, and nematodes [28, 30, 31]. However, bacteria are typically considered to be the most significant organisms consuming the organic matter in wastewater [28].

Identification of main bacterial genera in wastewater treatment plants

In this work, the genome of 158 bacteria species belonging to 80 genera were analyzed (Table S1). These bacteria were selected because they were the most numerous that were identified in two or more sewage treatment plants, according to consulted literature (Table 1). This number of analyzed bacteria (158) is about 10% of that observed in a sewage treatment plant—a suitable number for a functional analysis of bioremediation related genes [15].

These species were classified in 22 bacteria classes and one unclassified: *Acidimicrobiia*, *Actinobacteria*, *Alphaproteobacteria*, *Anaerolineae*, *Bacilli*, *Bacteroidia*, *Betaproteobacteria*, *Clostridia*, *Coriobacteriia*, *Deltaproteobacteria*, *Epsilonproteobacteria*, *Flavobacteriia*, *Gammaproteobacteria*, *Gemmatimonadetes*, *Negativicutes*, *Nitrospira*, *Oligoflexia*, *Rubrobacteria*, *Saprospiria*, *Sphingobacteriia*, *Spirochaetia*, and *Synergistia*. Gammaproteobacteria 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 [32-34], indicating that our sampling with the most common bacteria in different sewage treatment plants is significant.

Identification of genes involved in nitrogen, phosphorus and sulfur metabolic pathways and phylogenetic analysis

After analysis of the 158 bacterial genomes, 79 of them presented at least one of the complete pathways (Table S1). A Venn diagram was constructed to better visualize and analyze the relationship between bacteria and pathway genes. Fig 1 correlates the pathways of phosphorus accumulation, assimilatory sulfate reduction, denitrification, and dissimilatory nitrate reduction. Nitrification and dissimilatory sulfate reduction and oxidation pathways were not added to Fig 1 because they were present in a few species of bacteria.

Figure 1. Venn diagram correlating bacterial species that presented all the genes of one or more of the pathways. The 68 species of bacteria that have all the genes for phosphorus

accumulation, assimilatory sulfate reduction, denitrification, or dissimilatory nitrate reduction pathways are shown.

A 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, but most of them were classified in the phylum Proteobacteria (86%). Remarkably, Proteobacteria is predominant in practically all sewage treatment plants analyzed [35, 36]. Our analyses indicated that this phylum has bacteria with the most genes for bioremediation. Initially, 83 Proteobacteria species were analyzed and 63 of them (75.9%) had at least one of the nitrogen, sulfur, or phosphorus pathways.

Figure 2. Phylogeny of bacteria that presented at least one of the complete pathways. 16S rRNA gene phylogeny of the 79 bacteria that presented at least one of the complete pathways is shown. Class level lineages are indicated on the blue lines.

Nitrification was the least observed pathway, with only two bacteria species presenting the complete pathway: *Candidatus Nitrospira nitrificans* and *Candidatus Nitrospira nitrosa*, from class Nitrospira (Fig 2). These bacteria were recently discovered to have the complete nitrification pathway. Notably however, most of the bacteria observed, even in the genus *Nitrospira*, do not have this complete pathway [37]. For instance, two other species of the genus *Nitrospira* were analyzed in this work (*Candidatus Nitrospira defluvii* and *Nistrospira japonica*) which do not have the complete nitrification route (Fig 2 and Table S4). The separation of nitrification into two steps led to a cross-feeding interaction between different species of bacteria. However, those that could catalyze the complete nitrification pathway had growth advantages over the others [38]. All *Nitrospira* analyzed here also presented genes that act through the assimilatory sulfate reduction pathway (Fig 1).

Twelve species presented complete dissimilatory sulfate reduction and oxidation pathway (DSR), while only *Thiothrix nivea* of the Gammaproteobacteria class utilized other complete pathways besides DSR (Fig 1). Eight species of these bacteria belong to the class Deltaproteobacteria (*Desulfomicrobium baculatum, Desulfomicrobium escambiense, Desulfovibrio africanus, Desulfovibrio aminophilus, Desulfovibrio putealis, Desulfovibrio salexigens, Desulfovibrio vulgaris,* and *Syntrophobacter fumaroxidans*), and three species are classified in the Clostridia class (*Desulfotomaculum acetoxidans, Desulfotomaculum gibsoniae*, and *Desulfotomaculum nigrificans*) (Fig 2). The genera *Desulfotomaculum, Desulfovibrio and Desulfomicrobium are considered as* sulfate-reducing bacteria *(*SRB) [39]. For energy metabolism, SRB use sulfur compounds as the electron acceptor in wastewater treatment systems [40].

Forty species of bacteria have at least two of the analyzed pathways (Fig 1). Blast experiments showed that 47 bacterial species possess the assimilatory sulfate reduction pathway genes and 40 possess the 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, 27 presented all the genes related to both pathways, assimilatory sulfate reduction and DNRA (Fig 1 and Supplementary Tables). From these 27 bacteria species mentioned above, 22 belong to the class Gammaproteobacteria, and the others belong to the classes Alphaproteobacteria and Betaproteobacteria—all belonging to the phylum Proteobacteria (Fig 2). Recent studies have demonstrated that the nitrogen cycle is also tightly linked to other biogeochemical cycles, such as the sulfur cycle [41, 42], and suggest that biogenic sulfide induces DNRA with coproduction of ammonium and nitrite [43]. If oxygen or nitrate is present, the bacterium will even oxidize sulfur compounds to sulfate [44]. Some bacteria, as sulfate-reducing bacterium, are able to change metabolic pathways from sulfate reduction to nitrate in response to changing environmental conditions [44, 45]. High levels of

nitrate and consequently nitrite, as metabolic intermediates, can inhibit the growth of SRB [40, 46]. Some defense mechanisms, in response to stress by high nitrate concentrations, are developed by SRB, acquiring nitrate/nitrite reducing enzymes for example [47]. Another study shows that the SRB isolate D. YB01 can reduce nitrate and sulfate simultaneously in various nitrate concentration, and this could be the reason for the persistence of this isolate in a high-nitrate environment [48]. This means that nitrogen-transforming microorganisms have to compete with and depend on each other for the substrates [42].

The DNRA process is mostly catalyzed by the NrfAH complex, which includes cytochrome c nitrite reductase (NrfA) and cytochrome c nitrite reductase small subunit (NrfH), or by the NapAB complex, which comprises of periplasmic nitrate reductase (NapA) and diheme cytochrome (NapB). In DNRA, nitrite is reduced to ammonium and eight electrons are transferred ($NO_3^- \rightarrow NO_2^- \rightarrow NH_4^+$) [41, 49]. The processes of denitrification, DNRA, and assimilatory sulfate reduction occur in an anoxic environment, while the process of biological removal of phosphorus occurs in anaerobic and aerobic environments. Notably, *Paracoccus denitrificans* has the genes to perform these 4 pathways (Fig 1). Studies in *Escherichia coli* that have both the NapA and NarG suggest that the Nap system allows bacteria to eliminate nitrate when it is in low concentrations, while the NarG system is utilized in high nitrate concentrations [50]. In this work, it could be observed that the NarG and NapA genes are also present in *Acidovorax delafieldii*, *Acidovorax temperans*, *Arthrobacter radiotolerans*, *Bacillus cereus*, *Bacillus subtilis*, *Bacillus thuringiensis*, *Dechloromonas aromatica*, *Paracoccus denitrificans*, *Pseudomonas aeruginosa*, *Pseudomonas fluorescens*, *Thauera aromatic*, and *Thiobacillus denitrificans*. In comparison to denitrification ($NO_3^- \rightarrow NO_2^- \rightarrow NO \rightarrow N_2O \rightarrow N_2$), the DNRA process consumes NO_x, but it does not produce N^2 or N_2O; therefore, it does not contribute to N-loss, but leads to N-recycling [51]. Generally, there may be competition mainly for nitrate, and under nitrate limiting conditions, DNRA pathway is promoted over denitrification [52].

Moreover, aerobic and anaerobic growth of microbial communities can be inhibited by nitrite in equilibrium with free nitrous acid, resulting in control of microbial growth in a range of water systems [53].

Denitrification pathway was observed in 9 bacteria species (Fig 1), and these bacteria also have genes from other pathways. Denitrifying bacteria also contributes to the biological phosphorus removal process [54]. Sulfate reduction can indirectly stimulate P release, and when sulfate is reduced to sulfide, this molecule can bind to Fe(II), leading to more P availability [55]. Processes contributing to the dissolution of phosphate bound to metal oxides are microbial dissimilatory Fe(III) reduction and chemical Fe(III) reduction by hydrogen sulfide [56]. Denitrifying enhanced biological phosphorus removal (EBPR) systems can be an efficient means of removing phosphate (P) and nitrate (NO_3^-) with low carbon source and oxygen requirements [57]. For example, Tetrasphaera appears to provide sufficient energy to achieve either anoxic denitrification or aerobic P removal rather than both denitrification and P removal simultaneously [57]. Curiously, four bacteria species were identified with genes for performing denitrification and phosphorus accumulation (*Paracoccus denitrificans* and three bacteria of *Acidovorax* genus) (Fig. 1). Therefore, this combination of genes should make these species efficient nitrate and phosphorus removers.

However, 79 analyzed species only have incomplete pathways and therefore must work together to degrade and absorb the compounds present in sewage treatment plants. For example, dissimilatory sulfate reduction and oxidation pathway is listed with Sat (sulfate ↔ APS), AprAB (APS ↔ sulfite), and DsrAB (sulfite ↔ sulfide) genes. *Syntrophomonas zehnderi* has the DsrAB gene, while *Clostridium formicaceticum* and *Clostridium pasteurianum* have Sat and AprAB genes. In the nitrification pathway (ammonia to nitrate), the genes AmoCAB (ammonia ↔ hidroxylamine), Hao (hidroxylamine ↔ nitrite), and NxrAB (Nitrite ↔ Nitrate) were listed. *Nitrosospira lacus* and *Nitrosomonas europaea* have AmoCAB and Hao,

while *Nitrospira moscoviensis*, *Arthrobacter radiotolerans*, and *Brevundimonas aveniformis* have the NxrAB gene.

Bacteria species with genes of 3 or more pathways

Forty species exhibited all genes for more than one pathway (Fig 1), but 11 of these bacteria had three or more complete pathways (Table 3). *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 (Fig 1 and Table 3). Furthermore, it is a nonmotile coccoid soil organism and is taxonomically part of the Rhodobacteraceae family from α-subdivision of the phylum Proteobacteria [58]. Studies have shown that *Paracoccus* resembles a mitochondrion, because it has many attributes in common with its bacterial ancestor [59]. It is based on the oxidative phosphorylation and the constitutive respiratory chain in *P. denitrificans*, but there is differences that could be explained as adaptations to the different environments of the free-living bacterium, such as the presence of a respiratory nitrate reductase [60]. Some species, like *Paracoccus denitrificans*, can live in oxic and anoxic environments in response to environmental changes, such as oxygen and nitrogenous oxide concentration. This species can grow on methanol or methylamine as the sole carbon source [61], and can use either oxygen or nitrite as an electron acceptor for respiration [62]. Therefore, this fact is one of the reasons why *P. denitrificans* survives in such different environments. In this study, *P. denitrificans* also presented the NxrAB of the nitrification pathway that makes possible the transformation of nitrite to nitrate, and Sat genes of the dissimilatory sulfate reduction and oxidation pathway that allows the transformation of sulfate to adenylyl sulfate (APS) in both ways.

Table 3. Bacteria species that showed all genes for three or more analyzed pathways. X indicates the presence of all genes in each pathway: denitrification (Den), dissimilatory nitrate reduction (DNR), assimilatory sulfate reduction (ASR), dissimilatory sulfate reduction and oxidation (DSR), and phosphorus (Pho) accumulation.

	Pathways				
Bacteria	**Den**	**DNR**	**ASR**	**DSR**	**Pho**
Acidovorax caeni	X	X			X
Acidovorax delafieldii	X	X			X
Acidovorax temperans	X	X			X
Burkholderia vietnamiensis		X	X		X
Comamonas thiooxydans		X	X		X
Nitrobacter vulgaris		X	X		X
Nitrobacter winogradskyi		X	X		X
Paracoccus denitrificans	X	X	X		X
Pseudomonas aeruginosa	X	X	X		
Pseudomonas fluorescens	X	X	X		
Thiothrix nivea		X	X	X	

Thiothrix nivea 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). The 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 [38]. Sulfate-reducing prokaryotes are found in different phylum in the Bacteria domain; however, they do not compose a phylogenetically coherent group (Fig 1 and 2) [63]. *Thiothrix* belongs to filamentous sulfur bacteria found in activated sludge [64]. Studies with *T. nivea* have shown that an alteration can occur between sulfate reduction and sulfide oxidation in anoxic and oxic tanks [64] and deposit sulfur internally in the presence of sulfide [65]. There are interactions among filamentous sulfur bacteria, such as *Thiothrix nivea*, sulfate-reducing bacteria, and poly-P-accumulating bacteria. When sulfate reduction rates are high, there is a tendency for the maximum release of phosphate, and the sulfide oxidizing rate also tends to be high, suggesting

that poly-P-accumulating bacteria (PAB) utilize the acetate produced by the sulfate-reducing bacteria (SRB) [66]. In this study, *T. nivea* also exhibited AckA, Acs, and PhaC genes acting on phosphorus biogeochemical cycle. Acetate can be activated to form acetyl-CoA irreversibly through the high-affinity (Acs) or reversibly with low-affinity (AckA/Pta) pathways [67].

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 (Table 3 and 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). 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 [35, 36].

Knowledge of the bacterial community composition and how it interacts inside the wastewater treatment plants are essential to better design strategies for bioremediation. Different species of bacteria have genes that allow them to act in several biogeochemical cycles stimulated by the environment, either simultaneously or individually. Understanding how bacterial communities work can be used to improve the efficiency and timing of sewage treatment, as well as lower operating and maintenance costs. Bacteria having the genes for the pathways studied here can be introduced into a sewage treatment plant to increase the efficiency in the degradation of organic matter, for example, *Paracoccus denitrificans*, *Thiothrix nivea*, and *Candidatus Nitrospira nitrosa*. A combination of these bacteria would have all the genes analyzed in this work. Furthermore, functional genomic characterization can be used to improve

the performance of bacteria in degradation of compounds and, in the future, with creation of genetically modified organisms to upgrade bioremediation.

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Supporting information captions

Table S1. List of bacteria indicating which have some of the complete bioremediation pathways. Bacteria shaded in yellow do not have any complete pathway.

Table S2. tBlastn results using denitrificarion genes as a query.

Table S3. tBlastn results using dissimilatory nitrate reduction genes as a query.

Table S4. tBlastn results using nitrification genes as a query.

Table S5. tBlastn results using assimilatory sulfate reduction genes as a query.

Table S6. tBlastn results using dissimilatory sulfate reduction genes as a query.

Table S7. tBlastn results using Phosphorus accumulation genes as a query.

Table S8. BLAST and InterProScan results of the genomes and genes from *Burkholderia vietnamiensis*, *Comamonas thiooxydans*, *Nitrobacter vulgaris*, *Nitrobacter winogradskyi*, and *Paracoccus denitrificans*.

Figures

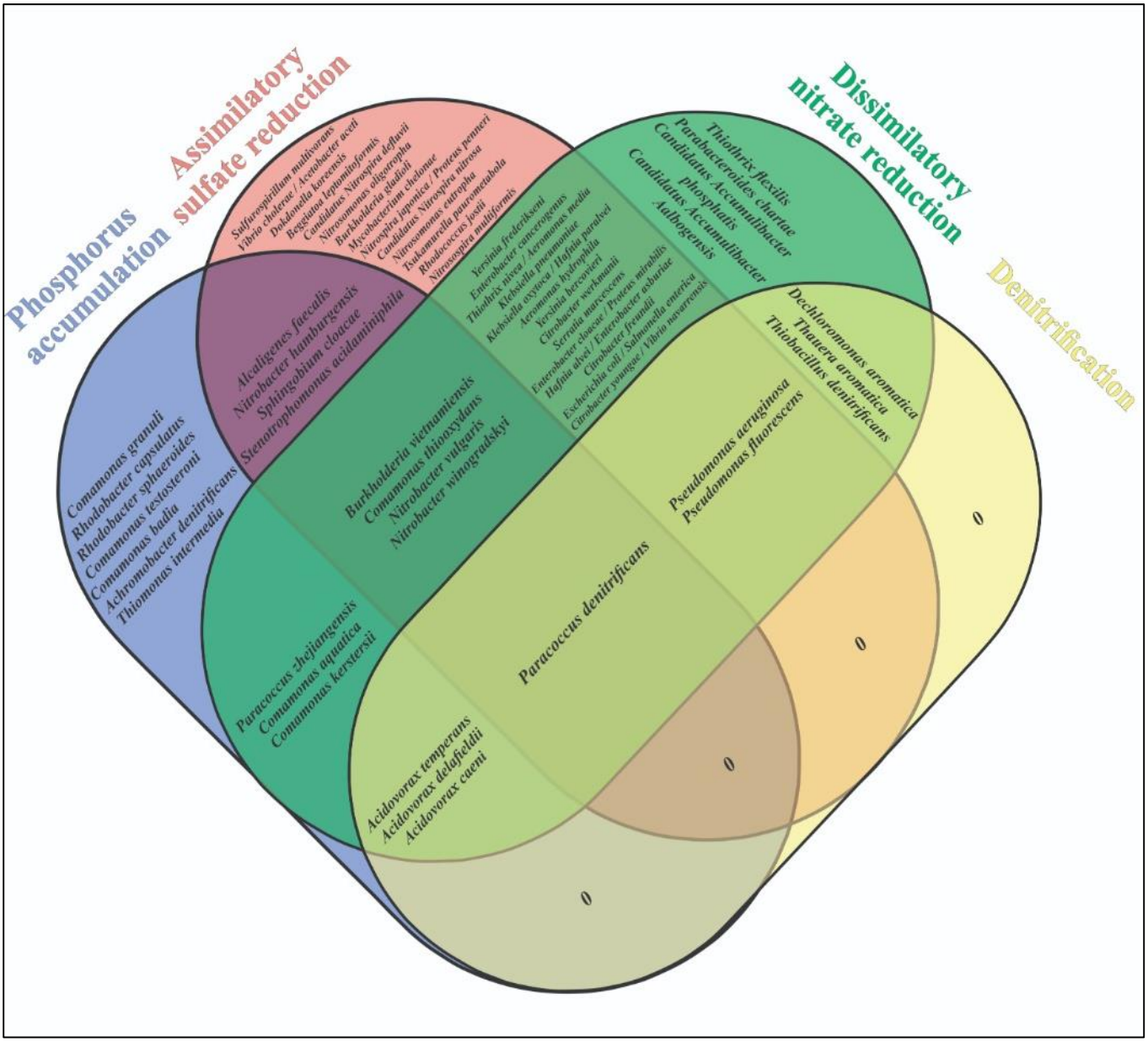

Figure 1. Venn diagram correlating bacterial species that presented all the genes of one or more of the pathways. The 68 species of bacteria that have all the genes for phosphorus accumulation, assimilatory sulfate reduction, denitrification or dissimilatory nitrate reduction pathways.

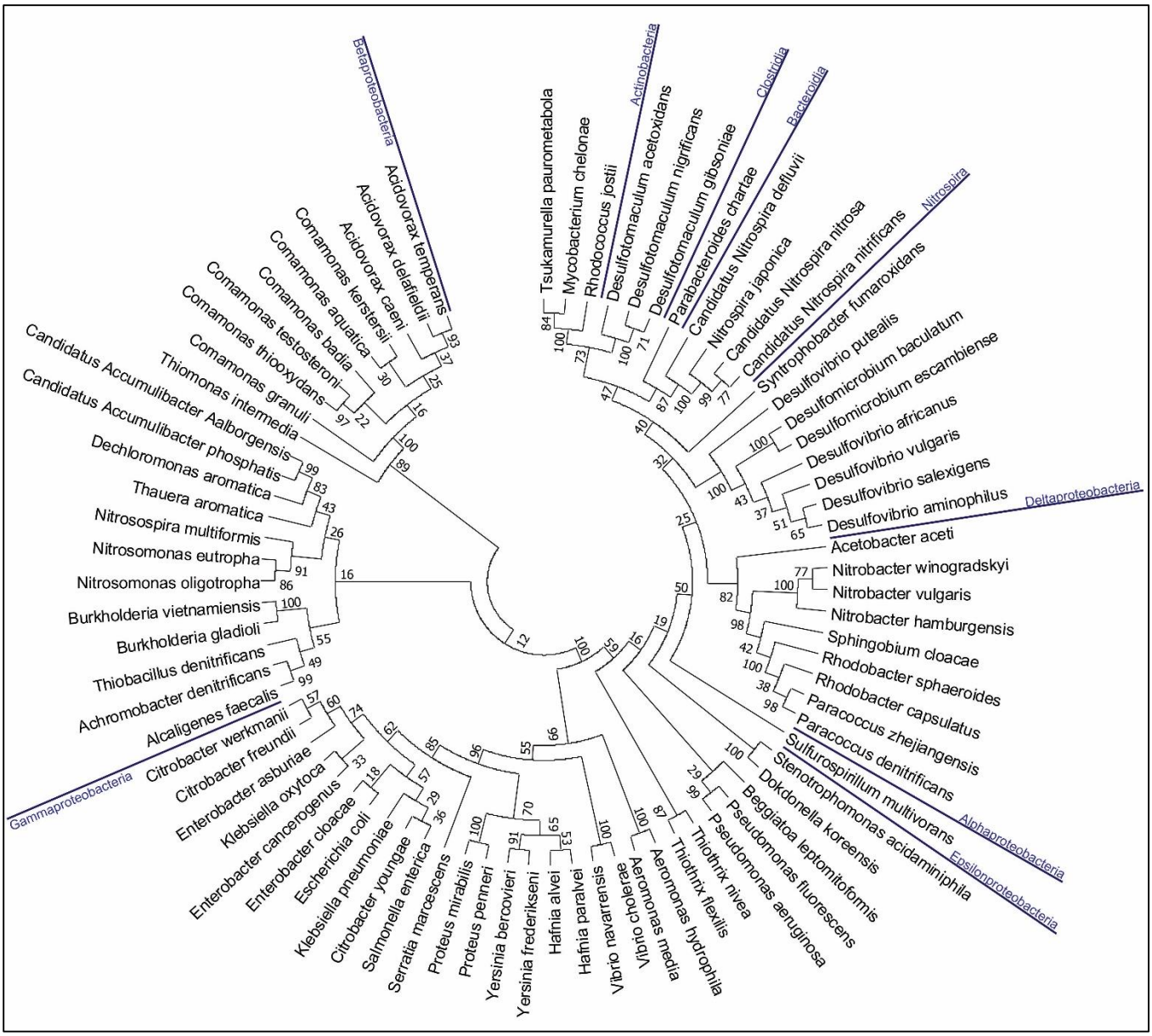

Figure 2. Phylogeny of bacteria that presented at least one of the complete pathways. 16S rRNA gene phylogeny of the 79 bacteria that presented at least one of the complete pathways. Class level lineages are indicated on the blue lines.

4. CONCLUSÃO

O entendimento sobre a composição da comunidade bacteriana e como ela interage dentro das estações de tratamento de esgoto é essencial para melhor desenhar estratégias para a biorremediação. Diferentes espécies de bactérias possuem genes que lhes permitem atuar em vários ciclos biogeoquímicos, simultaneamente ou individualmente, conforme estimulado pelo meio. O ambiente inconstante das estações de tratamento faz com que os organismos criem mecanismos de defesa em resposta aos estresses devido às variações nas concentrações de nutrientes e oxigênio. Compreender como as comunidades bacterianas funcionam pode ser usado para melhorar a eficiência e o tempo do tratamento de esgoto, bem como reduzir os custos de operação e manutenção. Bactérias com os genes das vias aqui estudadas podem ser introduzidas em uma estação de tratamento para aumentar a eficiência na degradação da matéria orgânica, como por exemplo: *Paracoccus denitrificans*, *Thiothrix nivea* e *Candidatus Nitrospira nitrosa*. Uma combinação dessas bactérias teria todos os genes analisados neste trabalho. Além disso, a caracterização genômica funcional pode ser usada para melhorar o desempenho das bactérias na degradação de compostos e, no futuro, possibilitar a criação de organismos geneticamente modificados para a melhoria da biorremediação.

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Books	Bates B. Bargaining for life: A social history of tuberculosis. 1st ed. Philadelphia: University of Pennsylvania Press; 1992.
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Deposited articles	Krick T, Shub DA, Verstraete N, Ferreiro DU, Alonso LG, Shub M, et al. Amino acid metabolism conflicts with protein diversity; 1991. Preprint. Available from: arXiv:1403.3301v1. Cited 17 March 2014.
Published media	Fountain H. For Already Vulnerable Penguins, Study Finds Climate Change Is Another Danger. The New York Times. 29 Jan 2014. Available from:

	http://www.nytimes.com/2014/01/30/science/earth/climate-change-taking-toll-on-penguins-study-finds.html Cited 17 March 2014.
New media	Allen L. Announcing PLOS Blogs. 2010 Sep 1 [cited 17 March 2014]. In: PLOS Blogs [Internet]. San Francisco: PLOS 2006 - . [about 2 screens]. Available from: http://blogs.plos.org/plos/2010/09/announcing-plos-blogs/.
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