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Bacterial Communities in Activated Sludge

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Activated sludge



Photo by Ivan Bandura on Unsplash

More than 300 km³ of wastewater is produced globally each year. This volume equals one-seventh of the global river volume. About 60% of this wastewater is treated before release, and biological processes such as **activated sludge (AS)** are widely used in wastewater treatment plants (**WWTPs**).

Wu et al. *Nat Microbiol.* 2019

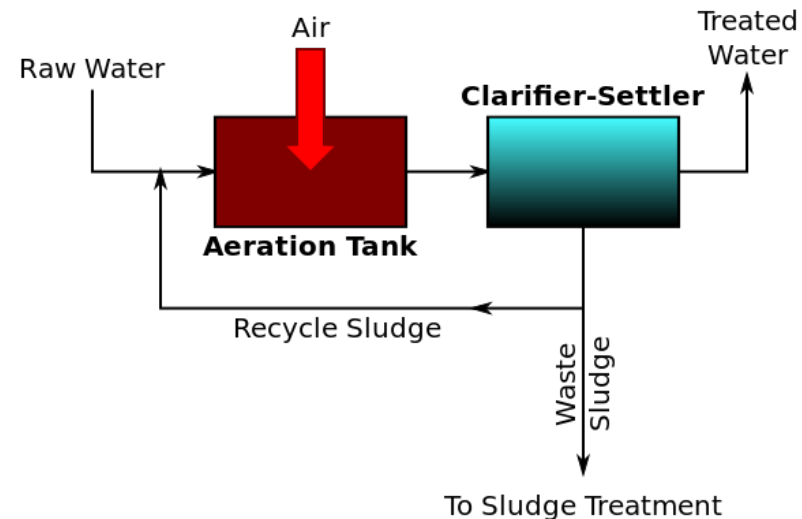




Activated sludge



- Biological treatment is carried out in aeration tanks where compressed air is continuously delivered and provides the oxygen taken by microorganisms.
- Activated sludge employs microbial flocs or granules to remove organic carbon (C), nitrogen (N), phosphorus (P), micropollutants, etc.



https://commons.wikimedia.org/wiki/File:Activated_Sludge_1.svg

van Loosdrecht et al. *Science*. 2014
Wu et al. *Nat Microbiol*. 2019






GLOBAL WATER MICROBIOME CONSORTIUM

HOME GENERAL PARTICIPATION INITIATIVES STANDARDS PROTOCOLS RESOURCES MEMBERS MEETINGS

GWMC members

LATEST MEETINGS

9 DEC **AGU Fall Meeting 2019**
San Francisco, CA, USA.
Dec 9-13, 2019

11 AUG **ESA 2019 annual meeting**
Louisville, Kentucky, USA.
Aug 11-16, 2019

Till Jul 22 2018, GWMC has a total of **113** investigators from **23** countries.

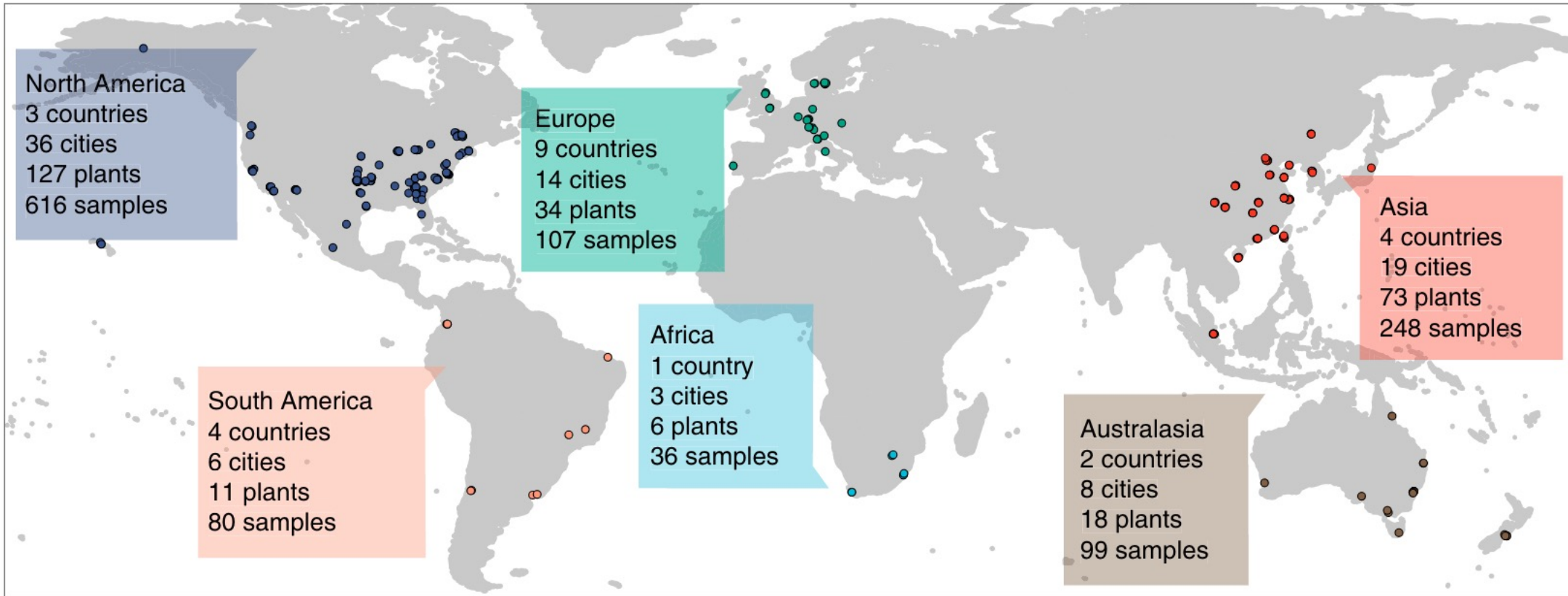
Last names in alphabet order.

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To facilitate international collaboration and communication on global water microbiome studies, **Global Water Microbiome Consortium (GWMC)** was formed in 2014. They conducted a global campaign to **systematically** collect and analyze activated sludge microbiomes.

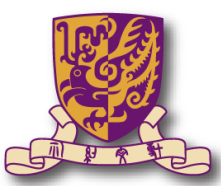
<http://gwmc.ou.edu/>



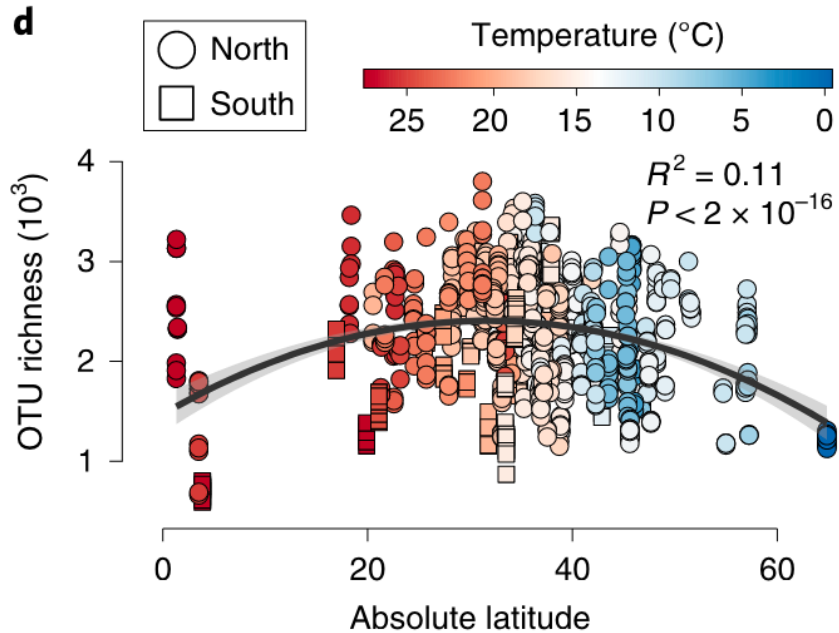


Researchers analysed the 16S ribosomal RNA gene sequences from ~1,200 activated sludge samples taken from 269 WWTPs in 23 countries on 6 continents.

Wu et al. *Nat Microbiol.* 2019



Latitudinal diversity pattern



In contrast to macroorganism diversity, activated sludge bacterial communities **show no latitudinal gradient.**

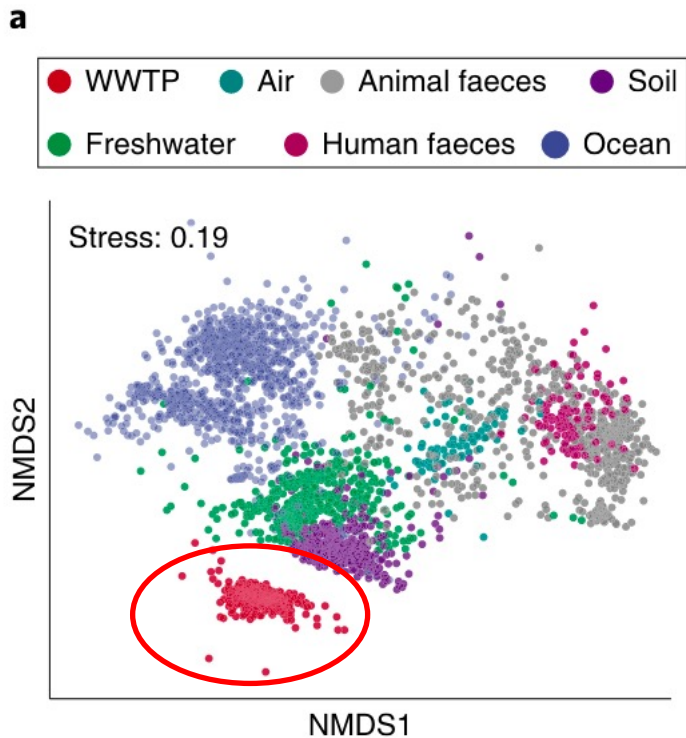
OTU: operational taxonomic unit

Wu et al. *Nat Microbiol.* 2019





Comparing bacterial community with other habitats



NMDS analysis showed that activated sludge of WWTPs harbour a **unique microbiome** compared with other habitats.

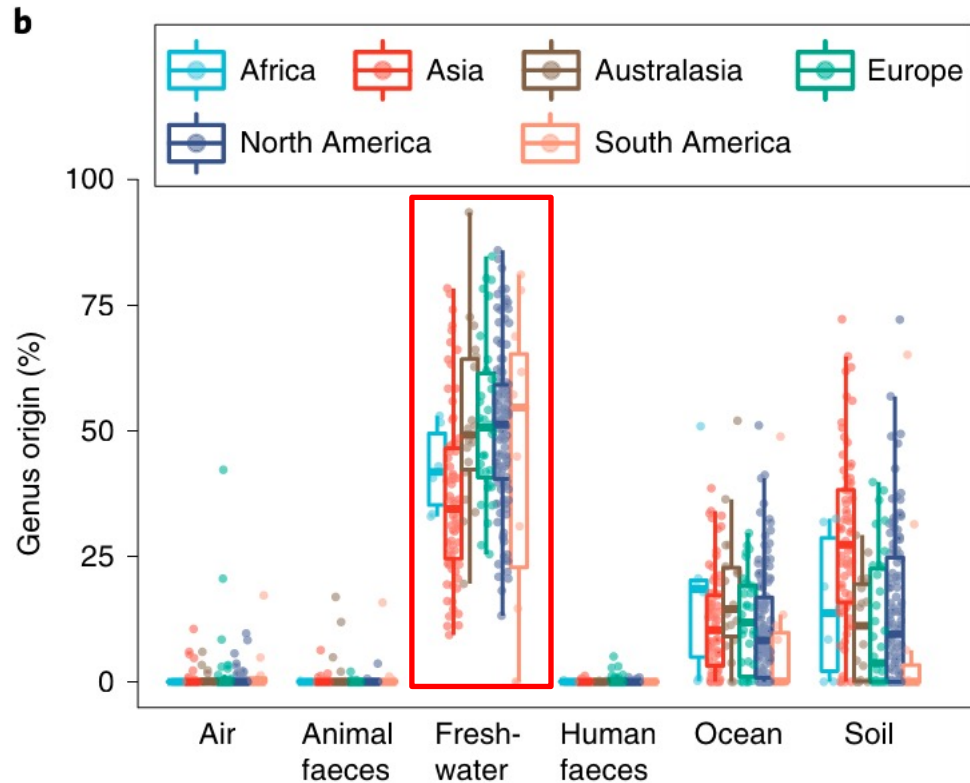
NMDS: Nonmetric Multidimensional Scaling

Wu et al. *Nat Microbiol.* 2019





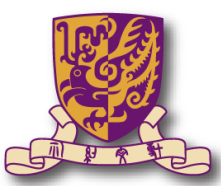
Comparing bacterial community compositions across continents and with other habitats



The most dominant potential source of activated sludge bacterial communities was **fresh water**, attributing on average 46% of genera, followed by soil (17% on average) and ocean (12% on average).

Wu et al. *Nat Microbiol.* 2019

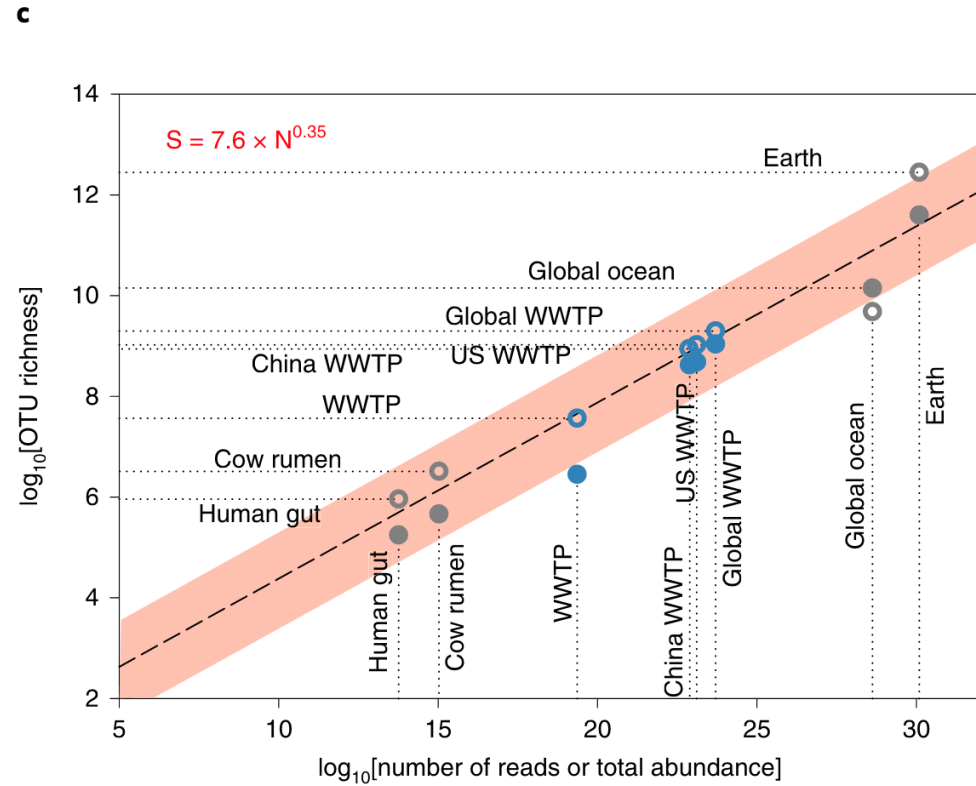




Extent of global microbial diversity



The lognormal model predicts $1.1 \pm 0.07 \times 10^9$ (~1 billion) bacterial phylotypes in activated sludge systems globally.

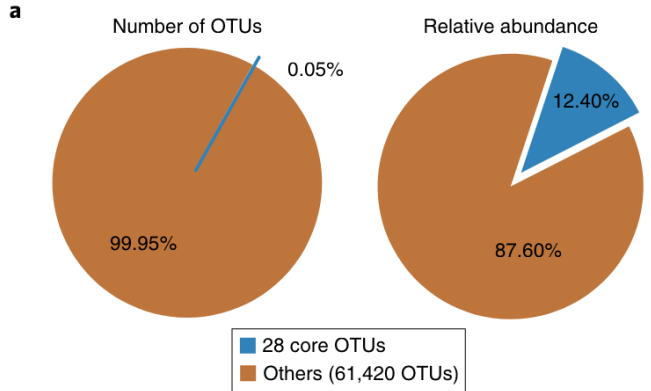


Wu et al. *Nat Microbiol.* 2019

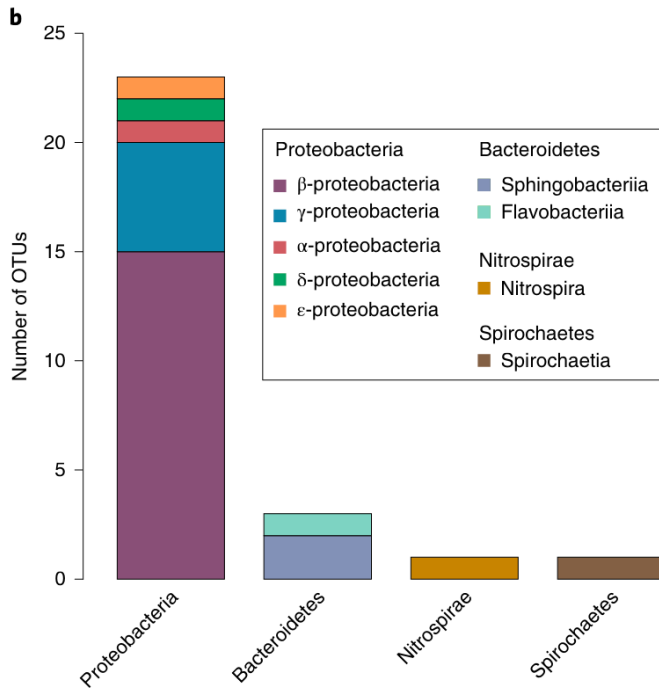




Global core bacterial community



Despite the high diversity, activated sludge has a small, global core bacterial community ($n = 28$ OTUs) that is strongly linked to activated sludge performance.



Most (82%) of the core community members belonged to *Proteobacteria*, with 15 OTUs classified as β -*Proteobacteria*. Other main members belonged to *Bacteroidetes*, *Nitrospirae*, and *Spirochaetes*.

a, Percentage and relative abundance of the global core OTUs versus the remaining microbial OTUs.
b, The taxonomic composition of the global core OTUs at the phylum and class level.

Wu et al. *Nat Microbiol.* 2019

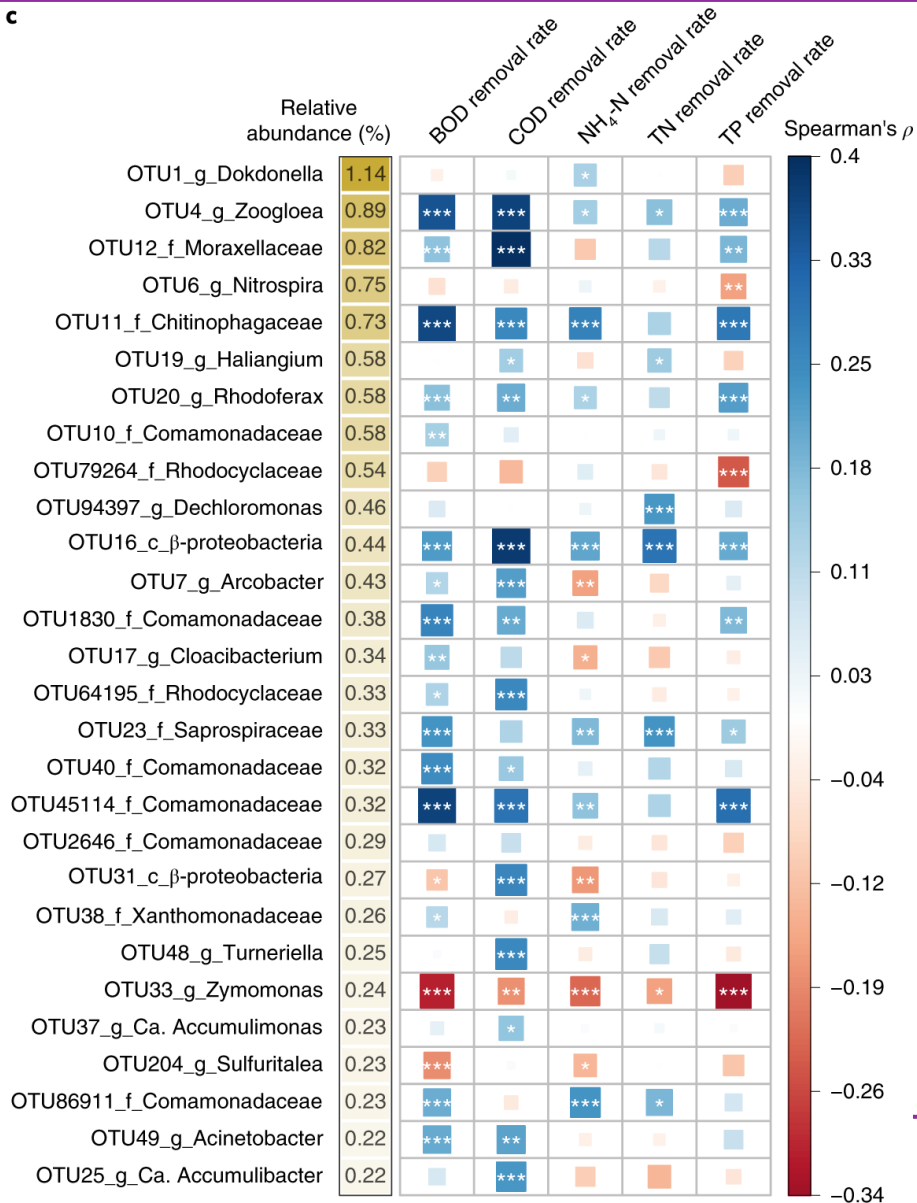




Functional importance of the global core OTUs



c



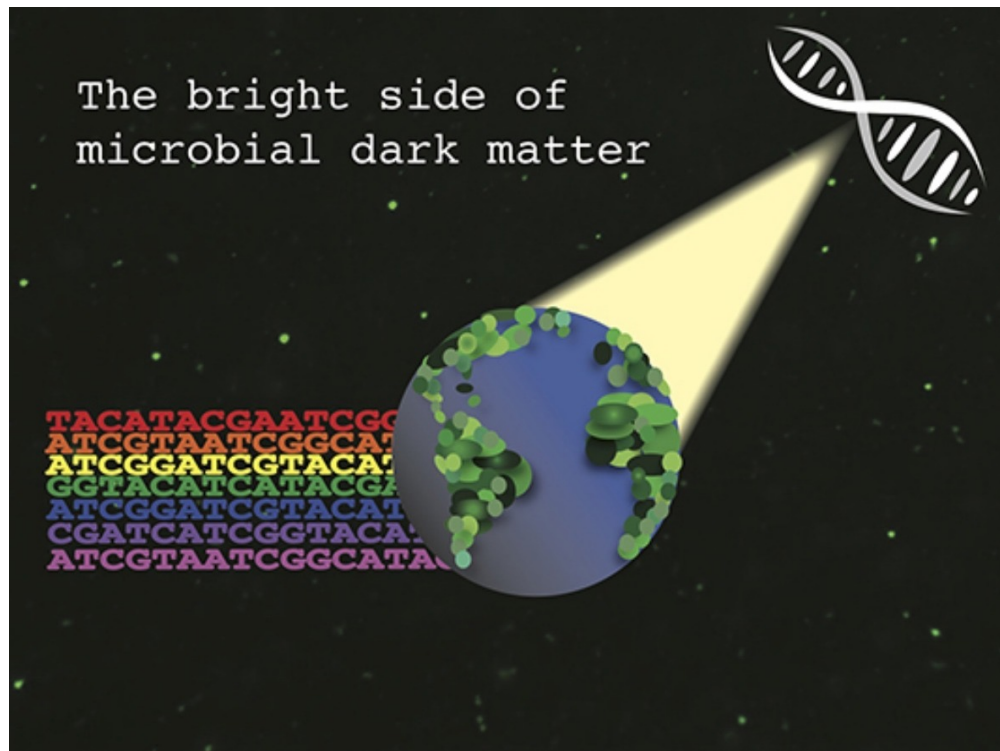
Activated sludge **functions** were calculated as the removal rate of organic carbon (BOD removal, COD removal), nutrients (total nitrogen (TN) and total phosphorus (TP) removal) and ammonia nitrogen (NH₄-N removal).

Of the 28 global core OTUs, **27** were significantly correlated ($P < 0.05$) with at least one out of the five functions examined.

Wu et al. *Nat Microbiol.* 2019



Uncultured microbial species



Solden et al. *Curr Opin Microbiol.* 2016
Wu et al. *Nat Microbiol.* 2019
Song et al. *Appl Environ Microbiol.* 2020

- Based on experimental and theoretical analyses, researchers estimate that activated sludge systems are globally inhabited by $\sim 10^9$ different species. However, fewer than 10^5 microbial species have been cultivated and studied in detail.
- If we assume that all cultivated species are present in activated sludge, potentially 99.99% of activated sludge microbial taxa remain uncultured.





Most wanted taxa in WWTPs



Table 1 (cont.) | Key targets for cultivation

Target microorganism or group ^a	Common environments	Superphylum or phylum	Reasons they are of interest for cultivation
<i>Bacteria (cont.)</i>			
SAR324	Marine	Deltaproteobacteria	They are metabolically diverse and globally distributed throughout the deeper layers of the oceans ¹⁴⁴ .
SAR86	Marine	Gammaproteobacteria	They are abundant in the surface layers of oceans and widespread globally ¹⁴⁵ .
Most wanted taxa in soil ¹⁴⁶	Soil	Assorted	These bacteria are thought to be crucial for accurately forecasting the ecological consequences of ongoing global environmental change, and are important for better understanding soil bacterial communities ¹⁴⁶ . The most ubiquitous and globally abundant of these include bacteria belonging to the phyla Alphaproteobacteria, Betaproteobacteria, Actinobacteria, Acidobacteria and Planctomycetes.
Candidate Phyla Radiation	Assorted	Candidate Phyla Radiation	This is a major group in Bacteria, currently thought to consist of at least 74 different phyla, for which there are an extremely limited number of cultured representatives.
Most wanted taxa in wastewater treatment plants ¹⁴⁷	Wastewater treatments	Assorted	They are essential for municipal and industrial wastewater purification, by removal of pollutants, to protect public and environmental health and have importance for improving the performance of wastewater treatment plants ¹⁴⁷ . The most globally abundant and ubiquitous of these include bacteria belonging to the phyla Betaproteobacteria, Gammaproteobacteria and Bacteroidetes.

Lewis et al. *Nat Rev Microbiol.* 2021



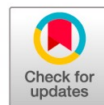


Strain SJ-1



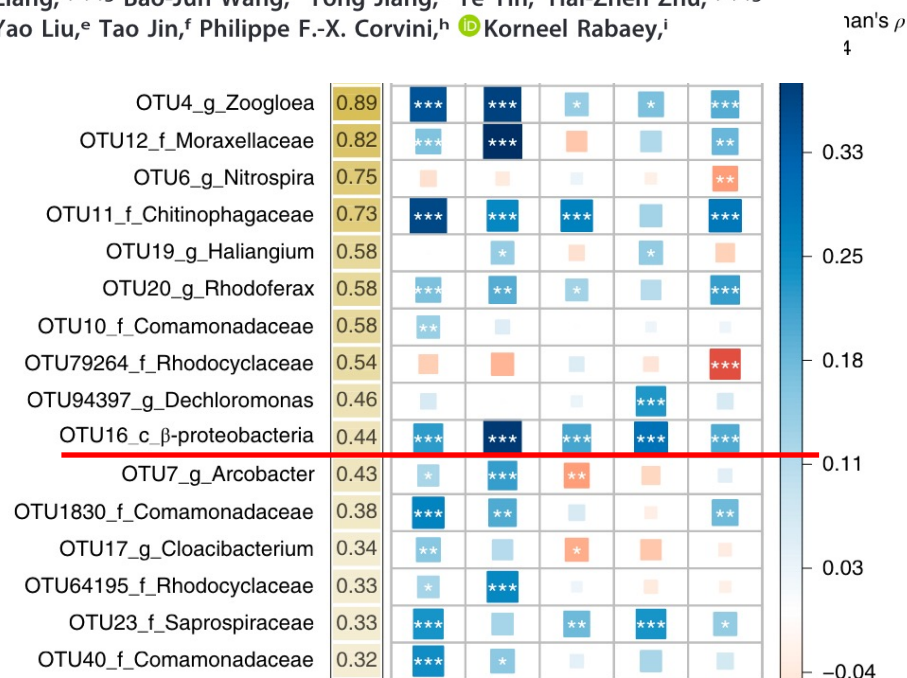
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Casimicrobium huifangae gen. nov., sp. nov., a Ubiquitous “Most-Wanted” Core Bacterial Taxon from Municipal Wastewater Treatment Plants

Yang Song,^{a,b,c,d,g} Cheng-Ying Jiang,^{a,b,c,d} Zong-Lin Liang,^{a,b,c,g} Bao-Jun Wang,^a Yong Jiang,^e Ye Yin,^f Hai-Zhen Zhu,^{a,b,c,g} Ya-Ling Qin,^{a,b,c,g} Rui-Xue Cheng,^a Zhi-Pei Liu,^{a,b,c,d} Yao Liu,^e Tao Jin,^f Philippe F.-X. Corvini,^h Korneel Rabaey,ⁱ Ai-Jie Wang,^{a,d,g} Shuang-Jiang Liu^{a,b,c,d,g}



In 2020, a bacterial strain, designated **SJ-1**, that represents a novel cluster within *β-proteobacteria* and corresponds to **OTU_16** within the 28 core taxa in the “most-wanted” list.

Song et al. *Appl Environ Microbiol.* 2020
Wu et al. *Nat Microbiol.* 2019



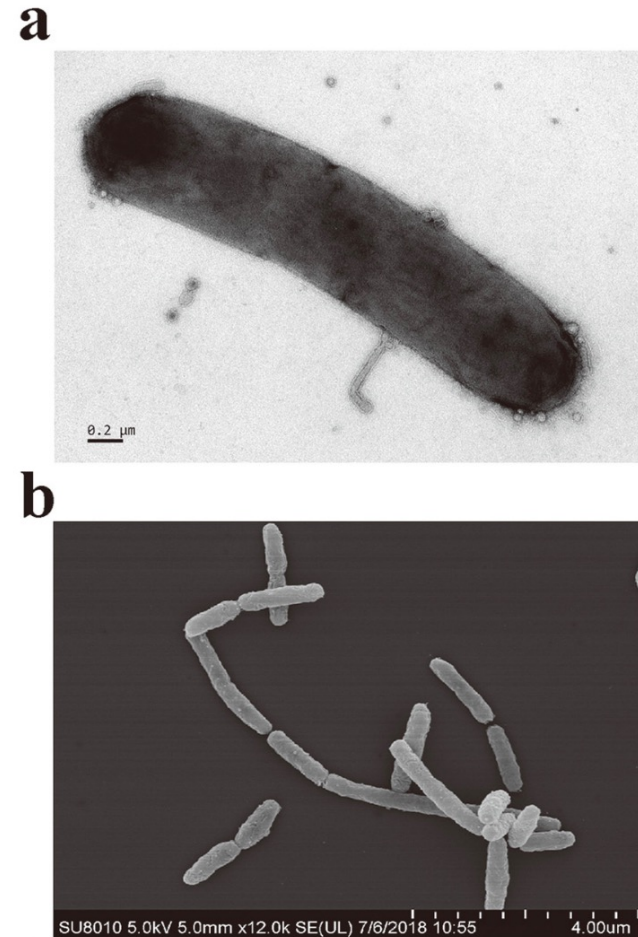


Strain SJ-1



Strain SJ-1 was first isolated with 10-fold-diluted R2A agar, after extended cultivation for 3 weeks. It was isolated from activated sludge of the Qinghe WWTP, Beijing, China.

Phylogenetic, genomic, and phenotypic characterization of strain SJ-1 revealed its standing as a novel species (namely *Casimicrobium huifangae*) of a new genus (*Casimicrobium*) and a new family (*Casimicrobiaceae*) within the β -proteobacteria class.



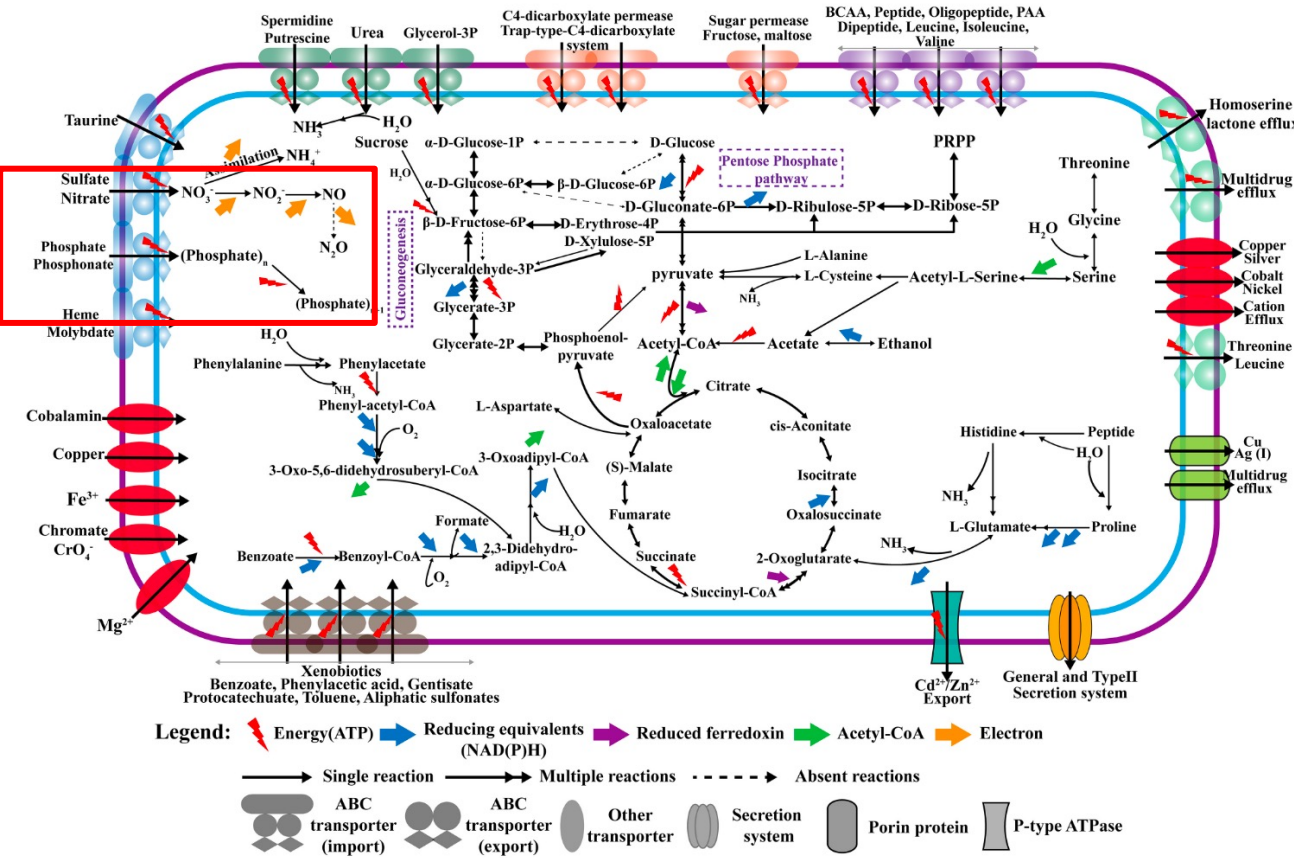
a,b, the cell morphology of strain SJ-1

Song et al. *Appl Environ Microbiol.* 2020





Strain SJ-1



Based on genome annotation, *C. huifangae* is predicted to be functionally important for **nitrogen and phosphorus removal** from wastewater.

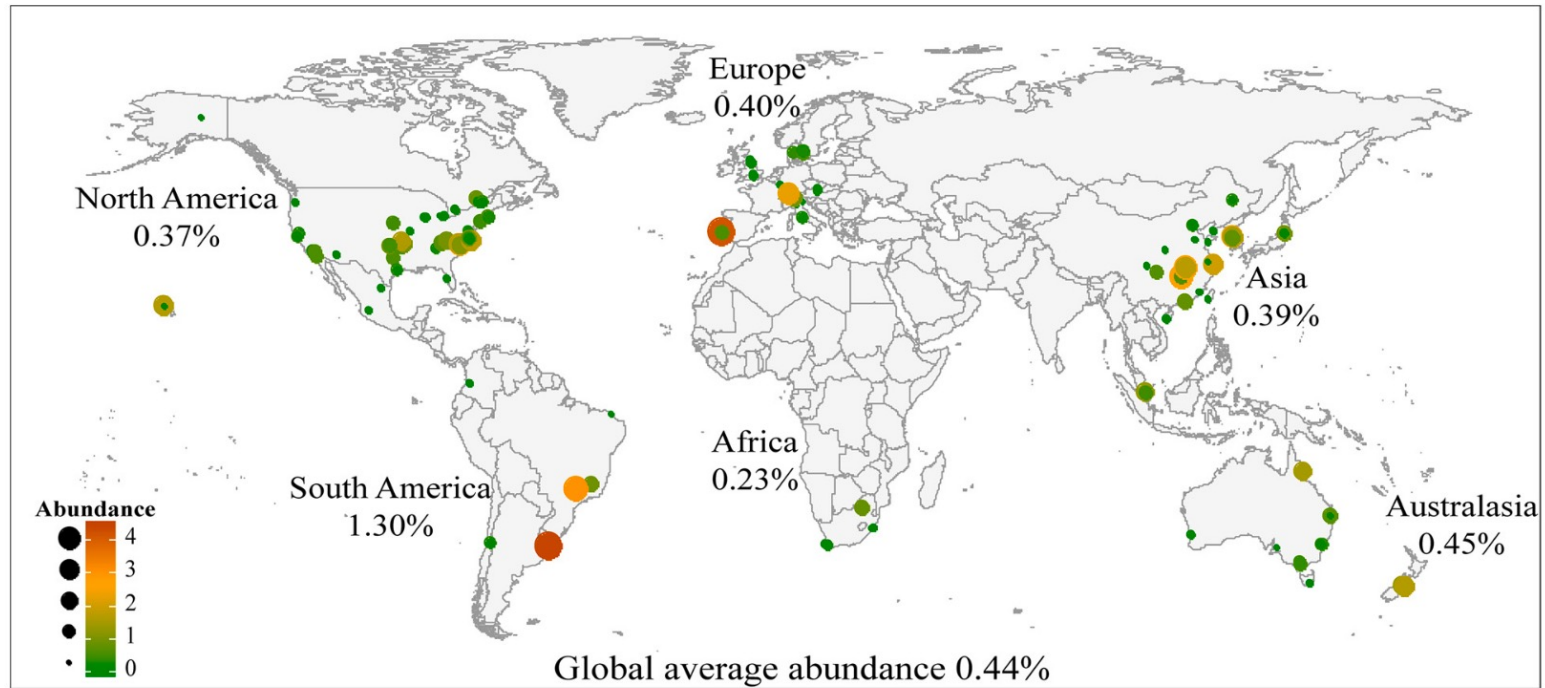
Based on phenotypic experiments, *C. huifangae* is capable of nitrate reduction and phosphorus accumulation.

FIG 2 Predicted physiology and metabolic pathways of *C. huifangae* strain SJ-1 based on genome sequences

Song et al. *Appl Environ Microbiol.* 2020



Strain SJ-1



Strain SJ-1 represents a group of previously uncultured bacteria of the *β-proteobacteria* and is **widely distributed** in global WWTPs.

Song et al. *Appl Environ Microbiol.* 2020





Summary and Expectations



- Systematic sampling efforts enhance our understanding of **microbial ecology** in activated sludge, setting the stage for various future analyses of WWTP microbiomes.
- Such information is important for identifying key players in the process and for providing a basis for **targeted manipulation** of activated sludge microbiomes.
- Although we know a lot of microbial species through sequencing technology, we still have to **culture them** to explore some of their physiological, biochemical, and other traits.
- Bringing microorganisms from the environment into culture represents a formidable task for microbiologists.





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Thank you for your time!
Q&A

