Geographical Distribution and Seasonal Dynamics of Polyphosphate Accumulating Organisms and Glycogen Accumulating Organisms in Sewage Treatment Plants

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* Background PAOs – bacteria that accumulate excess phosphorus from wastewater in

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Enhanced biological phosphorus removal (EBPR).
Candidatus Accumulibacter phosphatis (referred to Accumulibacter), Tetrasphaera sp.,
Candidatus Halomonas phosphatis, Gemmatimonas auraniaca.
GAOs - compete for organic carbon sources with PAOs but not contribute to P removal.
Candidatus Competibacter phosphatis (referred to Competibacter),
Defluvicoccus-related tetrad-forming organisms (referred to TFOs)
Research gap:
The geographical distribution of PAOs and GAOs within STPs across a global scale has not
been fully uncovered, and their temporal dynamics is still inconclusive.
Target:
The geographical distribution of PAOs and GAOs among 14 full-scale STPs of
Asia and North America;
The monthly microbial variations of PAOs and GAOs in AS samples of a STP

In this study, we investigated the geographical distribution of EBPR (Enhanced biological phosphorus removal) among 14 full-scale STPs, and their monthly microbial variations in AS samples of a Sewage Treatment Plant (STP).



Activate sludge (AS) samples were collected from 14 STPs located in Asia and North America.

AS samples from Sha-Tin STP (Hong Kong) have been taken monthly for five years.

* Materials and methods

16S rRNA gene pyrosequencing and identification of PAOs and GAOs:

Pyrosequencing reads assigned as PAO-like or GAO-like: 97% similarity 16S rRNA genes in V4 region with 200bp alignment length cutoff.: Identify the bacterial community in the 14 STPs 16S rRNA genes in V3 and V4 regions with 400bp alignment length cutoff.: Identify the bacterial community in <u>Sha</u>-Tin STP.

Statistic analysis:

Principle component analysis (PCA): To evaluate the similarity of the EBPR-related bacterial structures in AS samples. Canonical corresponding analysis (CCA): To reveal the relationship between the EBPR-related bacterial structure to geographic conditions, operational parameters and water characteristics. The overall profile of bacterial communities has been revealed by assigning 16S rRNA gene pyrosequencing reads to well-defined databases.

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	Chy	Chi	S.	S.	CV.8	Sky	Ś	Soc	500	3	5	S.	SW	C.W.Y	C.V.Y
Competibacter subgroup 1	0.0	2.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Competibacter subgroup 2	0.1	0.4	0.0	0.1	0.2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Competibacter subgroup 3	0.0	0.8	0.1	0.1	0.4	0.0	0.0	0.4	0.0	0.1	0.0	0.1	0.0	0.0	0.0
Competibacter subgroup 4	0.2	2.3	0.4	0.9	0.4	0.2	0.1	0.1	0.0	0.0	0.0	0.2	0.1	0.2	0.0
Competibacter subgroup 5	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.5	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Competibacter subgroup 6	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1.6	0.0	0.0	0.0	0.0	0.0	0.1	0.0
Competibacter subgroup 7	0.0	0.0	0.0	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Defluvicoccus-related TFO cluster I	1.1	0.7	0.7	1.4	0.8	1.1	0.6	0.8	0.5	0.4	1.3	0.9	1.5	0.5	0.0
Defluvicoccus-related TFO cluster-II	0.0	0.1	0.0	0.0	0.2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Defluvicoccus-related TFO cluster III	0.0	0.0	0.5	0.7	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.2	0.0
Defluvicoccus-related TFO cluster IV	0.0	1.2	0.3	0.0	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Accumulibacter clade I	0.1	0.4	0.3	0.1	0.1	0.0	0.0	0.0	0.0	0.1	0.0	0.0	0.0	0.0	0.0
Accumulibacter clade IIA	0.4	0.0	0.1	0.1	0.2	0.4	0.0	0.0	0.0	0.1	0.0	0.0	0.0	0.0	0.0
Accumulibacter clade IIB	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Accumulibacter clade IIC&IID	0.5	0.7	0.2	0.3	1.0	0.7	1.1	1.5	0.4	1.5	0.0	0.2	0.0	0.3	0.6
Candidatus Halomonas phosphatis	0.0	0.0	0.1	0.0	0.0	0.0	0.0	0.0	0.7	0.2	0.1	0.0	0.0	0.0	0.0
Gemmatimonas aurantiaca	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.2	0.1	0.1	0.0	0.0	0.1	0.0
Tetrasphaera cluster I	0.1	0.1	0.2	0.1	0.1	0.0	0.0	0.4	0.1	0.1	3.4	0.3	0.3	0.4	0.0
Tetrasphaera cluster II	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.4	0.0	0.0	3.8	0.0	0.0	0.4	0.0
Tetrasphaera cluster III	0.3	0.2	0.1	0.1	0.1	0.0	0.2	0.0	0.3	0.1	0.0	0.8	0.0	0.0	0.0

Accumulibacter and *Tetrasphaera* were most popular PAOs in most STPs. Tetrasphaera clusters were evenly distributed and GAOs presented in almost all full-scale STPs.



No significant geographical difference was found between sludge samples from Asia and North America.



Most AS samples in Asia distributed either along or very close to the TP, TN and COD lines (or their extension lines).



A seasonal variation pattern was observed among abundances of the identified PAO and GAO populations.