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"Candidatus Competibacter"-lineage genomes retrieved from metagenomes reveal functional metabolic diversity

Simon Jon McIlroy¹, Mads Albertsen¹, Eva Kammer Andresen¹, Aaron Marc Saunders¹, Rikke Kristiansen¹, Mikkel Stokholm-Bjerregaard^{1,2}, Kåre Lehmann Nielsen¹ & Per Halkjær Nielsen¹

Introduction

The "Candidatus Competibacter"-lineage (henceforth Competibacter) exhibits a glycogen accumulating organism (GAO) phenotype, relying on aerobically stored glycogen to energize anaerobic carbon uptake and storage as polyhydroxyalkanoates (PHA).

This bi-phasic mode of life is important for Competibacter to survive in EBPR systems. However, as they do not contribute to phosphorus removal, but compete for organic resources with the PAOs, they theoretically reduce EBPR efficiency.

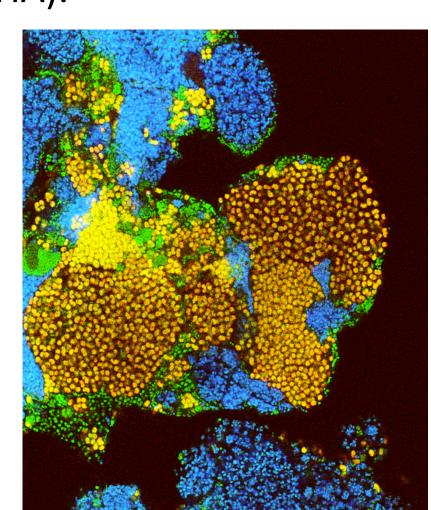


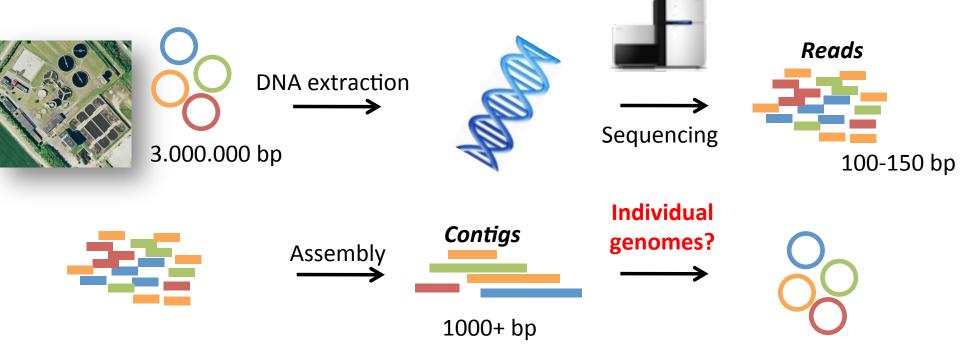
Fig. 1. FISH image of **Competibacter (yellow)**

Aim

To enhance our understanding of the Competibacter-lineage through the construction of genome-based-metabolic models.

Methods

Metagenome



Metagenome datasets generated from 2 labscale enrichments, seeded from a full-scale EBPR waste water treatment plant (WWTP).

Assembly

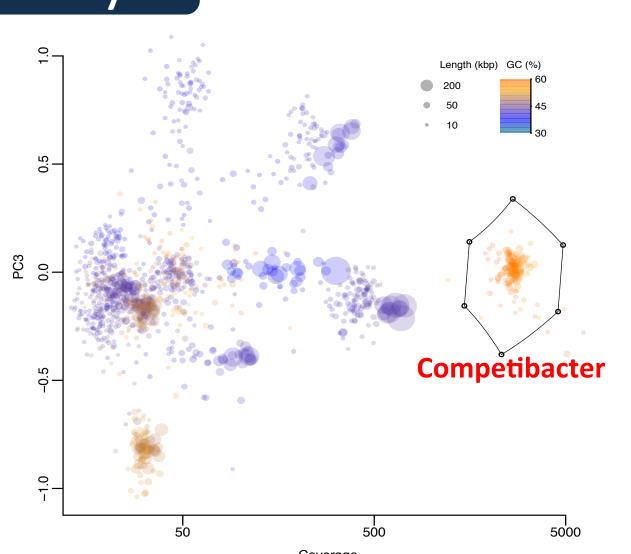


Fig 2. PCA plot for genome extraction.

De novo assembled genome fragments from 2 individual Competibacter strains extracted using tetranucleotide frequency, coverage, and GC content.

Annotation

Both genomes annotated using the 'MicroScope' pipeline (GenoScope) and curated for pathways relevant to metabolic model construction (Fig. 5).



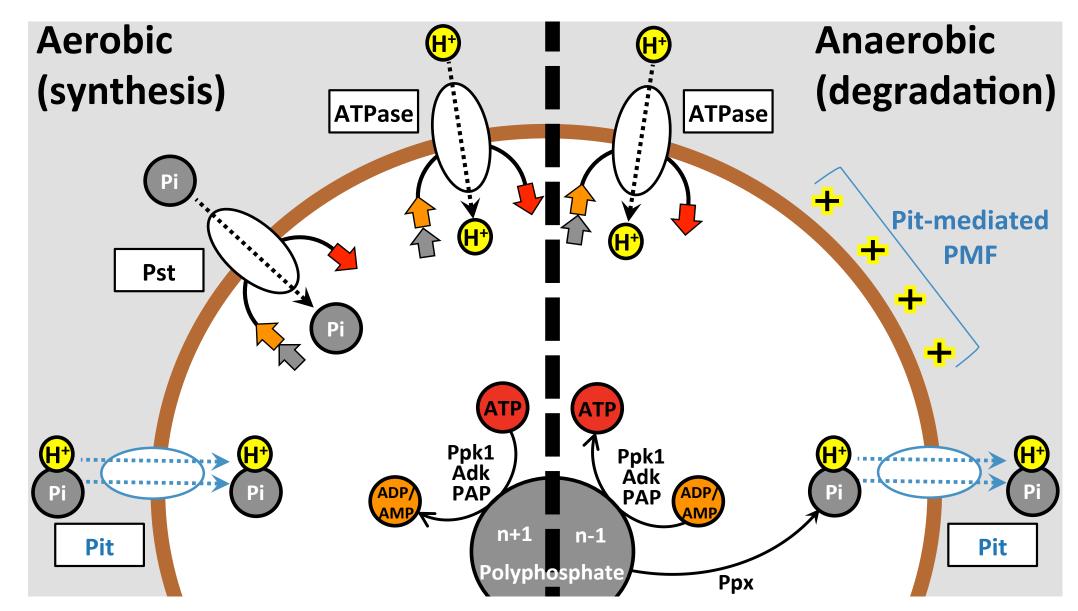
Conclusions

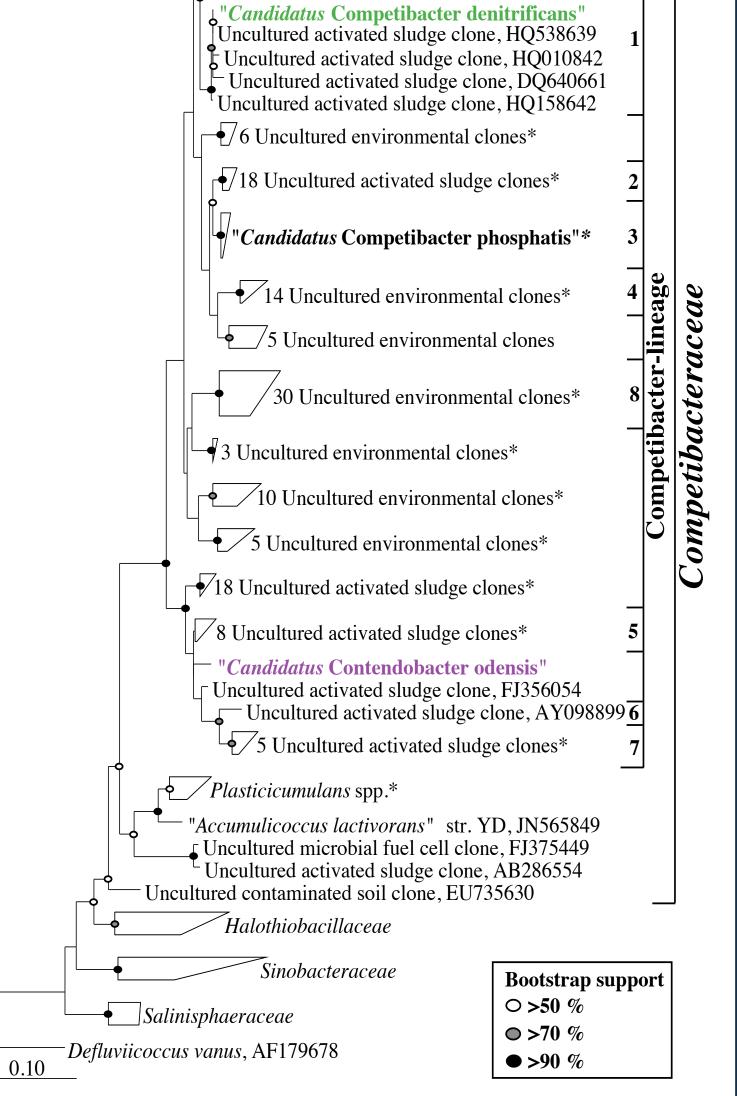
This study is the first of its kind for organisms with the GAO phenotype and provides a solid foundation for future —omic based studies. The revealed potential for phenotypic diversity among these related Competibacteraceae species indicates potential niche partitioning and underlines the complex nature of interaction between the PAO and GAO populations in EBPR systems.

Results

Table 1. Metabolic comparison of pathways in sequenced GAO and PAO

	GAO ^φ		PAO*				
	C. denitrificans	C. odensis	Accumulibacter	T. elongata	T. australiensis	T. jenkinsii	T. japonica
Central metabolism							
TCA cycle	+	+	+	+	+	+	+
Glyoxylate shunt	+	+	+	-	-	_	-
Pentose phosphate (oxidative)	-	-	+	+	+	+	+
Pentose phosphate (non-oxidative)	+	+	+	+	+	+	+
Glycolysis EMP pathway	+	+	+	+	+	+	+
Glycolysis ED pathway	+	-	l - l	-	-	-	-
Fermentation (glucose → lactate)	+	-	-	+	-	-	+
Storage compound metabolism		_		_			
Glycogen synthesis	+	+	+	+	+	+	+
Trehalose synthesis	-	+	-	+	+	+	+
PHA synthesis	+	+	+	-	-	-	+
TAG synthesis	-	+	+	+	+	+	+
Polyphosphate synthesis	+	+	+	+	+	+	+
- Pit transporter	-	-	+	+	+	+	+
Nitrogen metabolism							
Nitrate reduction	+	+	i <u>-</u>	+	+	+	+
Nitrate reduction to nitrogen (denitrification)	+	-	_	-	-	-	-
Nitrite reduction (respiratory)	+	-	+	+	+	+	+
Nitrite reduction to nitrogen	+	-	+	-	-	-	-
Nitrite reduction to ammonia (assimilatory)	-	+	+	-	-	-	+
Nitrogen fixation	-	+	+	-	_	-	-





10 Uncultured activated sludge clones

Phylogenetic analyses reveal genomes are members of Competibacter sub-groups 1 and 5. Diversity among the 'genus' Competibacter is high and thus the group better represents a family – *Competibacteraceae*.

Fig 3. Phylogenetic tree for Competibacter-

lineage sequences. Genome derived

sequences are presented in colour.

Fig. 4 Diagrammatic representation of P metabolism in PAO. Comparison of polyphosphate metabolism genes in available PAO and GAO genomes reveals the absence of the pit gene in the latter. The Pit phosphate transporter is theoretically involved in anaerobic proton motive force generation energising carbon uptake in PAO.

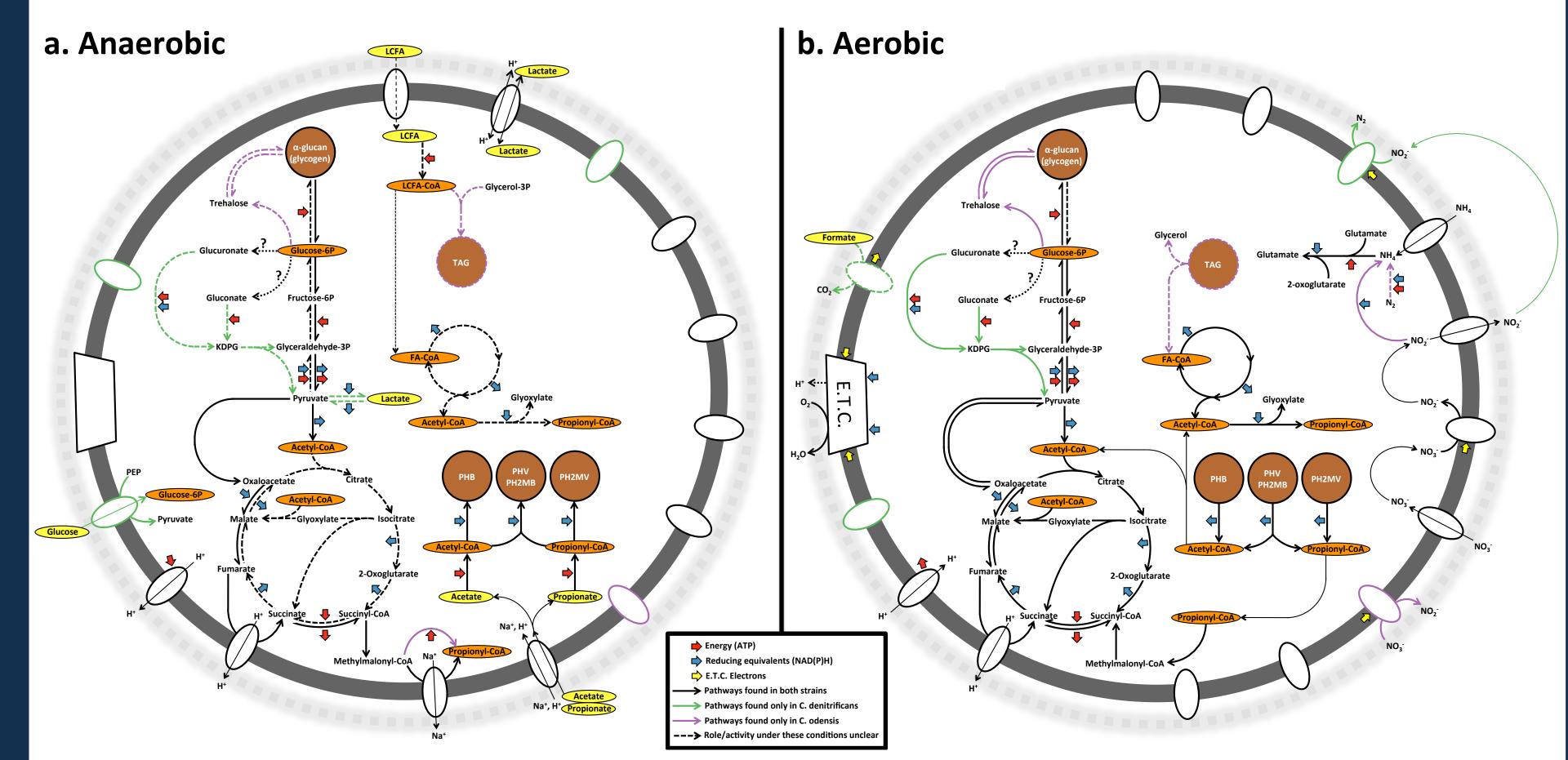


Fig 5. Metabolic model for Competibacter-lineage genomes in EBPR WWTPs a. in the absence of electron acceptors (anaerobic) and b. in the presence of the electron acceptors oxygen (aerobic) or nitrate. E.T.C = electron transport chain; PEP = phosphoenolpyruvate; KDPG = 2-dehydro-3-deoxy-D-gluconate-6-phosphate; TAG = triacylglycerols; PHB = polyhydroxybutyrate; PHV = polyhydroxyvalerate; PH2MB = polyhydroxy-2-methylbutyrate; PH2MV = polyhydroxy-2-methylvalerate. Both genomes contain genes for glycogen and PHA cycling and VFA metabolism. Differences between the genomes relate to storage polymers, nitrogen metabolism and glucose fermentation.











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