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Phylogeny and FISH probe analysis of the “*Candidatus Competibacter*”-lineage in wastewater treatment systems

Tadashi Nittami¹, Simon J. McIlroy², Eri Kanai³, Junji Fukuda¹, Masatoshi Watanabe¹, Per Halkjær Nielsen²



¹ Division of Materials Science and Chemical Engineering, Yokohama National University, Yokohama, Japan

² Centre for Microbial Communities, Aalborg University, Aalborg, Denmark

³ Department of Chemistry, Chemical Engineering and Life Science, Yokohama National University, Yokohama, Japan

Introduction

Members of the family *Competibacteraceae* (McIlroy *et al.*, 2014) have received considerable attention for their perceived negative influence on enhanced biological phosphorus removal wastewater treatment plant (WWTP) efficiency, and their ability to accumulate large amounts of polyhydroxyalkanoates for potential commercial bioplastic production. The *Competibacteraceae* was suggested to include the former *Competibacter*-lineage along with sequences related to the genus *Plasticumulans* (McIlroy *et al.*, 2014). Given the lack of isolates, FISH analysis using available probes facilitate the attainment of important *in situ* information on the morphology, spatial arrangement and metabolism of phylotypes.

The aim of this study is to re-evaluate the taxonomy and FISH probes for the family *Competibacteraceae*.

Phylogenetic analysis

Based on 16S rRNA gene phylogeny the *Competibacteraceae* family is suggested to be made up of the *Competibacter*- and *Plasticumulans*-lineages. We suggest the division of the *Competibacter*-lineage into 13 clades, and have designed seven new FISH probes for coverage of these.

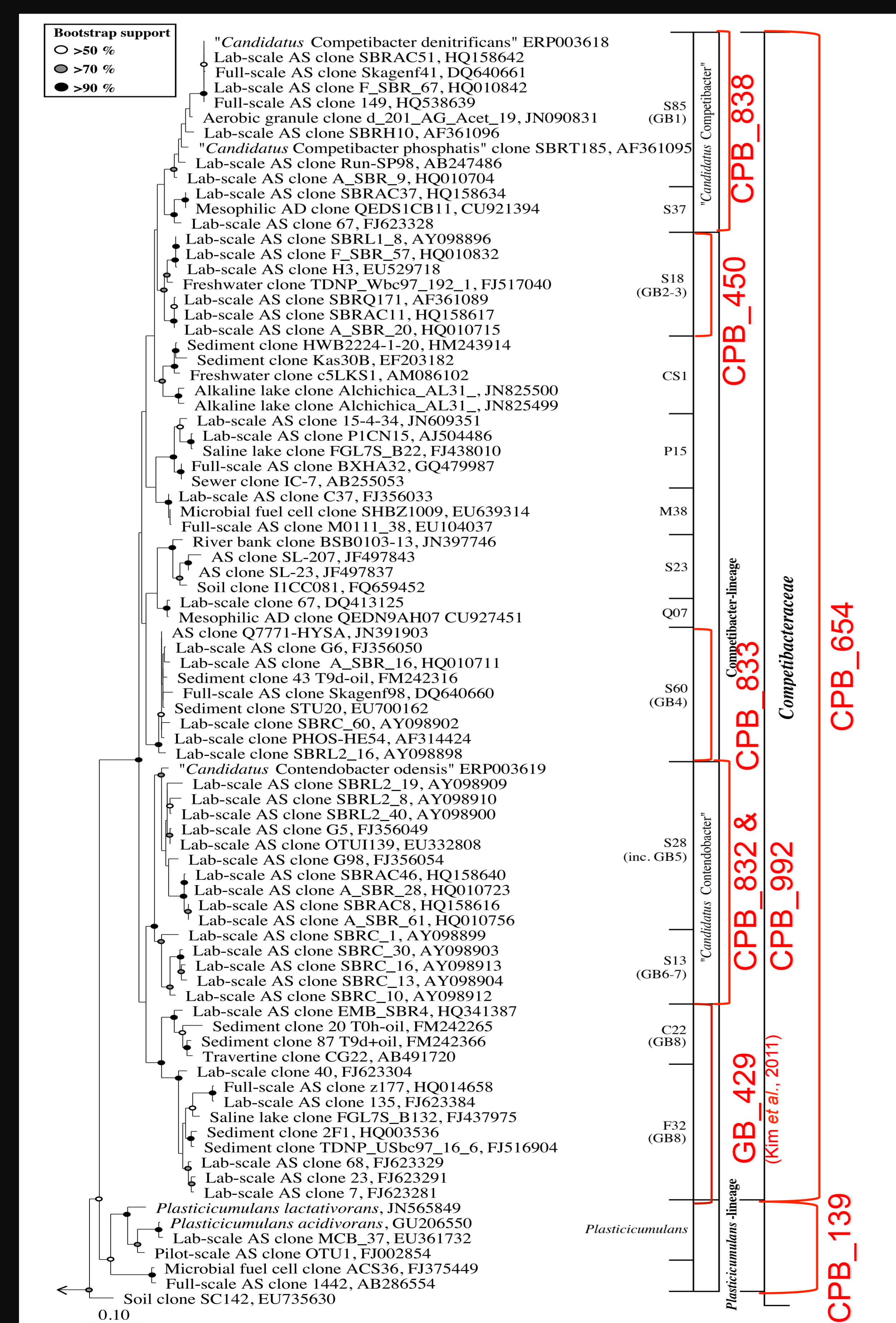


Fig. 1 Maximum-likelihood (PhyML) 16S rRNA gene phylogenetic tree for the family *Competibacteraceae*. Non-parametric bootstrap (from 100 analyses) branch-support values >50% are included. Recommended FISH probes coverage is shown with red brackets.

Results

Probe validation and optimisation was based on generated formamide dissociation curves (Daims *et al.*, 2005).

Table 1 Probes designed to target members of the *Competibacteraceae*.

Probe	Target group	Sequence	FA (%)
CPB_654	Competibacter-lineage	TCC TCT AGC CCA CTC	35
CPB_838	Clades S85 & S37	CGA CAC CGA AAG GTC ACC CC	40
CPB_838_C1	Competitor probe	CGA CAC CGA AA△ GTC ACC CC	—
CPB_838_C2	Competitor probe	CGA CAC CGA C△ GTC ACC CC	—
CPB_450	Clade S18	CTG TTC GCA CGA CGC CGT TC	35
CPB_450_C1	Competitor probe	CTG TTC GC△ CGA CGC CGT TC	—
CPB_450_C2	Competitor probe	CTG TTC GCA CGA CG△ CGT TC	—
CPB_833	Clade S60	ACG GTC ACC CAC CCG A	35
CPB_833_C1	Competitor probe	ACG GTC ACC C△ C CCG A	—
CPB_833_H1	Helper for CPB_833	GCG TTA GCT RCG ACA CCG G	—
CPB_832	Some “Ca. Contendobacter”	GGA CGG TCA CCC GCC CAA C	55–70
CPB_832_C1	Competitor probe	GGG CGG TCA CCC GCC CAA C	—
CPB_832_C2	Competitor probe	GGA CGG TCA CCC GCC C△ A C	—
CPB_992	Some “Ca. Contendobacter” (clade S28)	CTG GAC GTT CCC CAG ATG TCA	55
CPB_992_C1	Competitor probe	CTG GAC GTT CCC C△ G ATG TCA	—
CPB_139	Plasticumulans-lineage	CAT GTT GTC CCC CAC CCG	50
CPB_139_C1	Competitor probe	C△ G GTT GTC CCC CAC CCG	—
CPB_139_C2	Competitor probe	G△ A GTT GTC CCC CAC CCG	—
CPB_139_C3	Competitor probe	G△ T GTT △ TC CCC CAC CCG	—

Table 2 qFISH quantification of the *Competibacteraceae* members in WWTPs.

WWTP	Year	S85 & S37	S18	S60	Contendobacter	C22 & F32	Competibacter	Plasticumulans
Ejby Mølle	2010	2 ± 1	< 1	1 ± 1	3 ± 1	1 ± 1	8 ± 2	< 1
Fredericia	2010	N.D.	< 1	8 ± 5	N.D.	< 1	11 ± 3	< 1
Horsens	2010	3 ± 1	< 1	1 ± 1	< 1	< 1	7 ± 2	N.D.
Odense NE	2009	N.D.	< 1	N.D.	< 1	< 1	2 ± 1	< 1

Values represent the area of cells hybridizing specific probes as a percentage of the total area hybridizing with the universal EUBmix probe set.

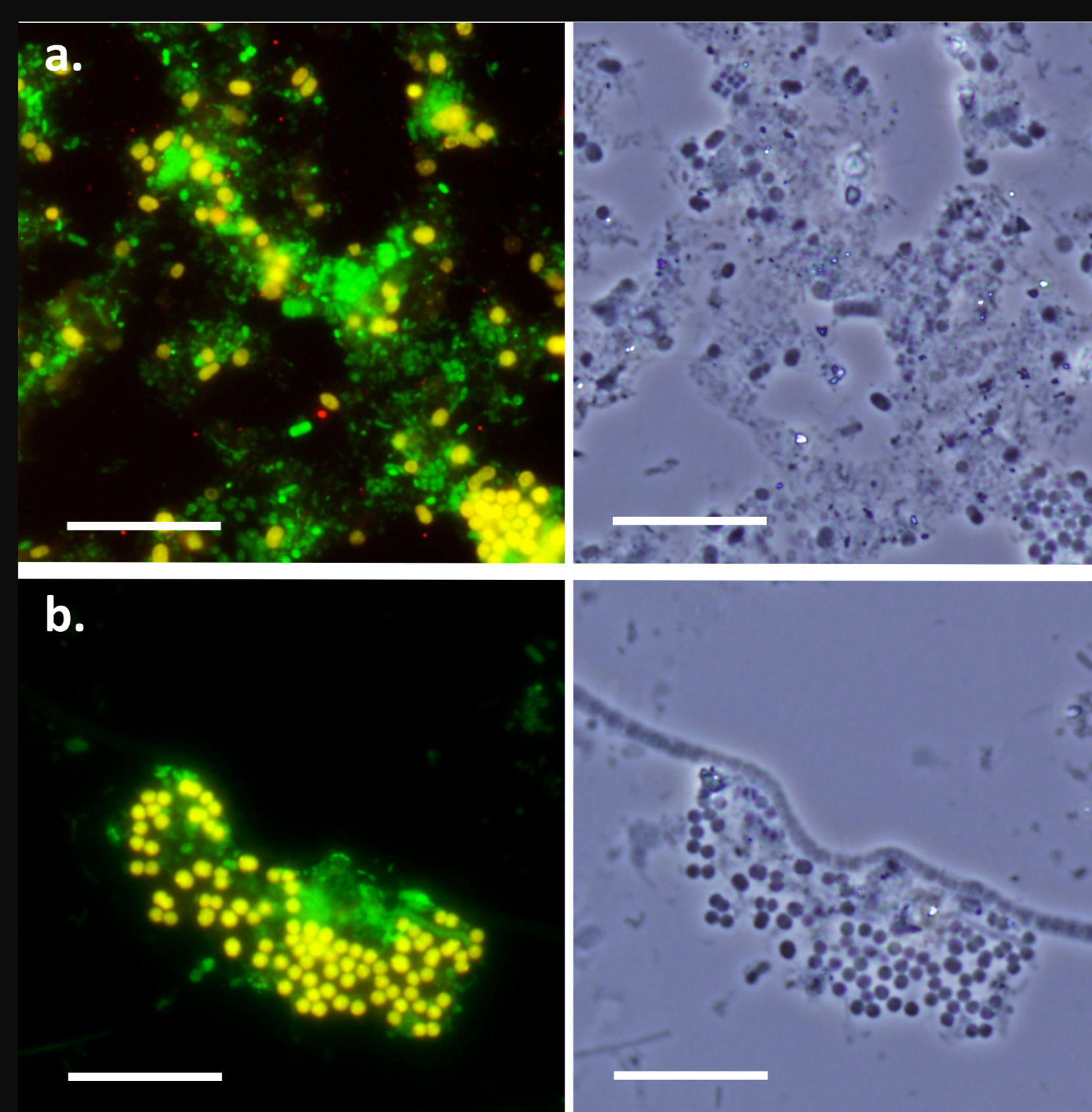


Fig. 2 FISH micrographs of *Competibacteraceae* in full scale WWTPs. a. *Competibacter*-lineage cells (CPB_654); b. *Plasticumulans*-lineage cells (CPB_139); Target cells appear yellow and non-target cells green. Scale bars represent 20 µm.

Conclusions

Surveys of full scale WWTPs based on FISH analysis indicate clades are always found to co-exist, with the composition varying substantially between plants, supporting niche partitioning among members. The proposed FISH probes will allow for *in situ* characterization of the ecophysiology of the different often co-existing clades. The recommendations of this study provide an important framework for future analysis of the ecology of the *Competibacteraceae* members.