

Summary of FRC Isolates

	A	B
Phylogenetic affiliation	<i>Agrobacterium/</i> <i>Sinorhizobium,</i>	<i>Pseudomonas</i> <i>Klebsiella</i>
pH range (optimal)	4.5/5.5 - 8.0 (6.5)	5.5/6.0 - 8.0 (8.0)
Max growth rate	0.106 h ⁻¹	0.200 h ⁻¹
Max NO ₃ ⁻ reduction rate	185.4 μM NO ₃ ⁻ * h ⁻¹	293.9 μM NO ₃ ⁻ * h ⁻¹
Nitrite accumulation	Low	High
Nitrate Reductase	Nap only	Nap and Nar
Nitrite Reductase	NirK	NirK

Microbial Community Analysis

Clone Libraries

Purpose: to examine the microbial communities (with focus on denitrifiers) of sites undergoing bioremediation at the FRC

- What denitrifying organisms are stimulated with ethanol addition?
- Do factors such as nitrate concentration and pH affect community composition?

Experiment: Push pull tests: November, 2003
DNA extracted from core material nearby wells

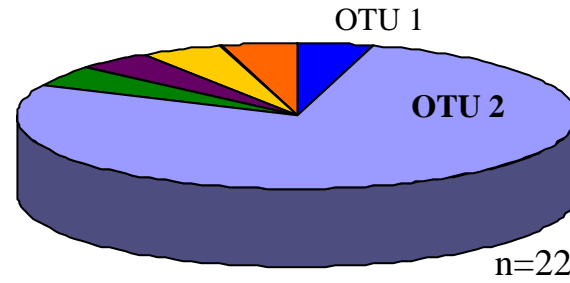
PCR results

Treatment Groups	Well location	16S	nirK	nirS
Area2, stimulated	DP15D	+	+	-
Area2, stimulated	DP06	+	-	+
Area2, unstimulated	FW003	+	-	-
Area1, stimulated	FW028	+	+	+
Area1, stimulated	FW034	+	+	+
Area1, unstimulated	FW031	+	+	-

nirK clone libraries

13 OTU s

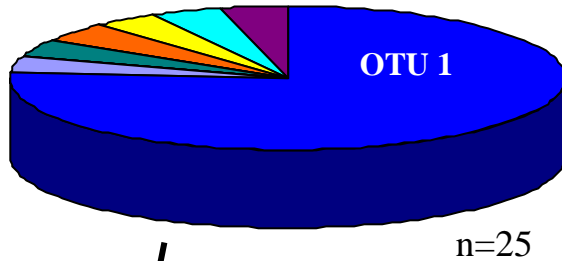
DP-15D



85% similar to
Nitrosomonas sp.

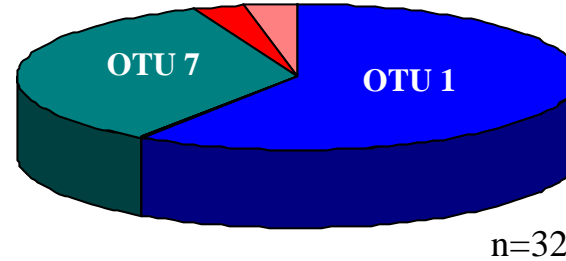
Area 2
EtOH
stimulated

FW028



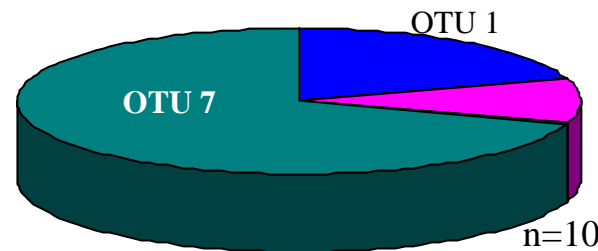
82% similar to
Alcaligenes xylosoxidans

FW034



Area 1
EtOH
stimulated

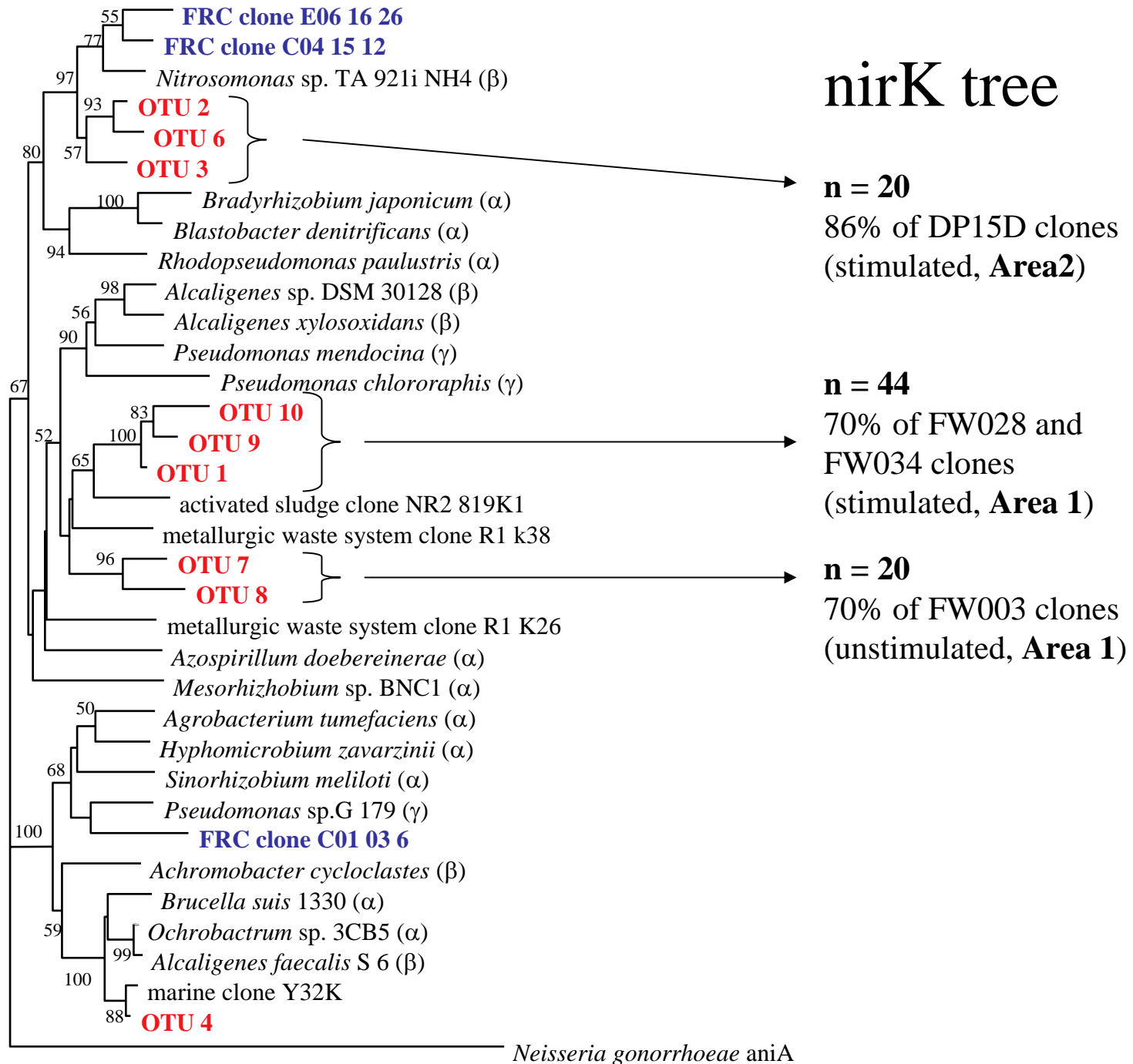
FW031



85% similar to
Rhodopseudomonas
palustris

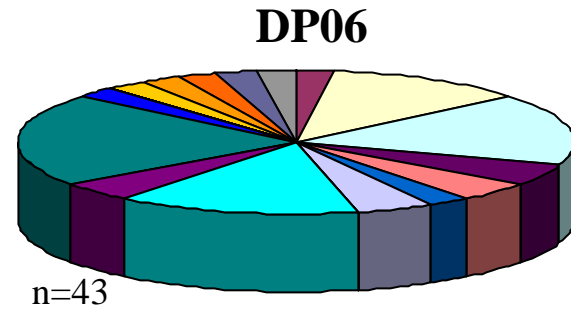
Area 1
(donor
control)

nirK tree

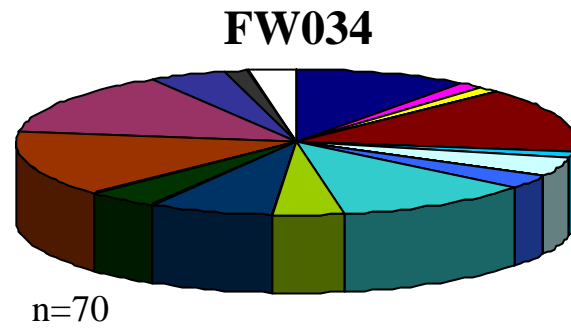
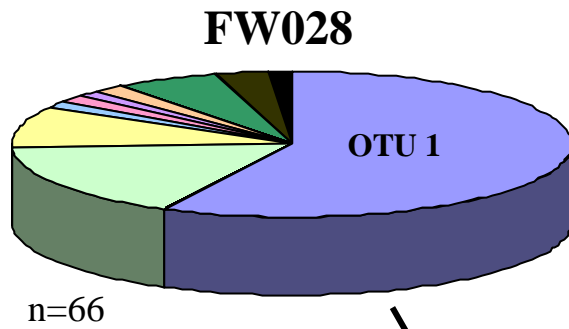


nirS clone libraries

42 OTU s



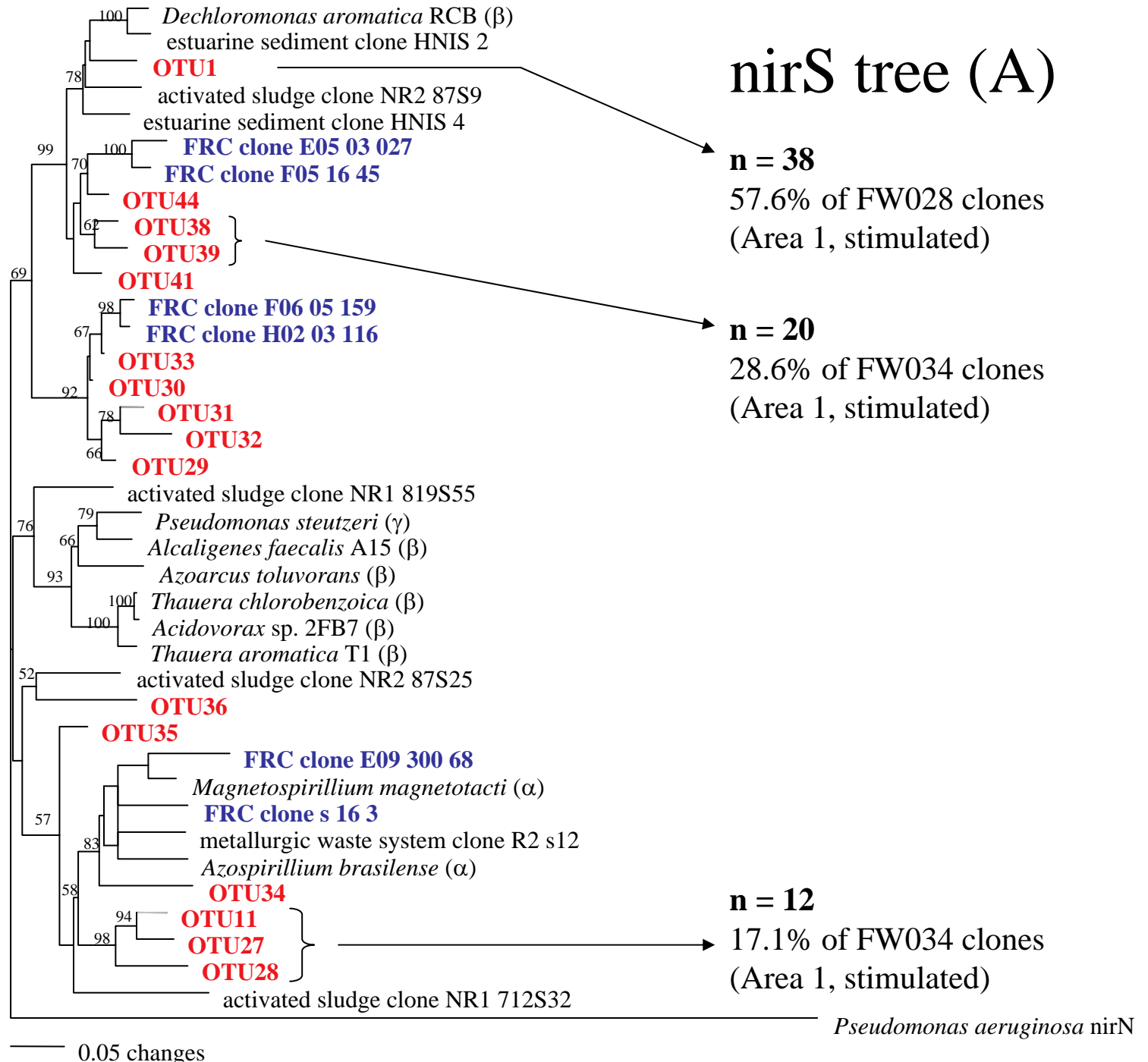
Area 2
EtOH
stimulated



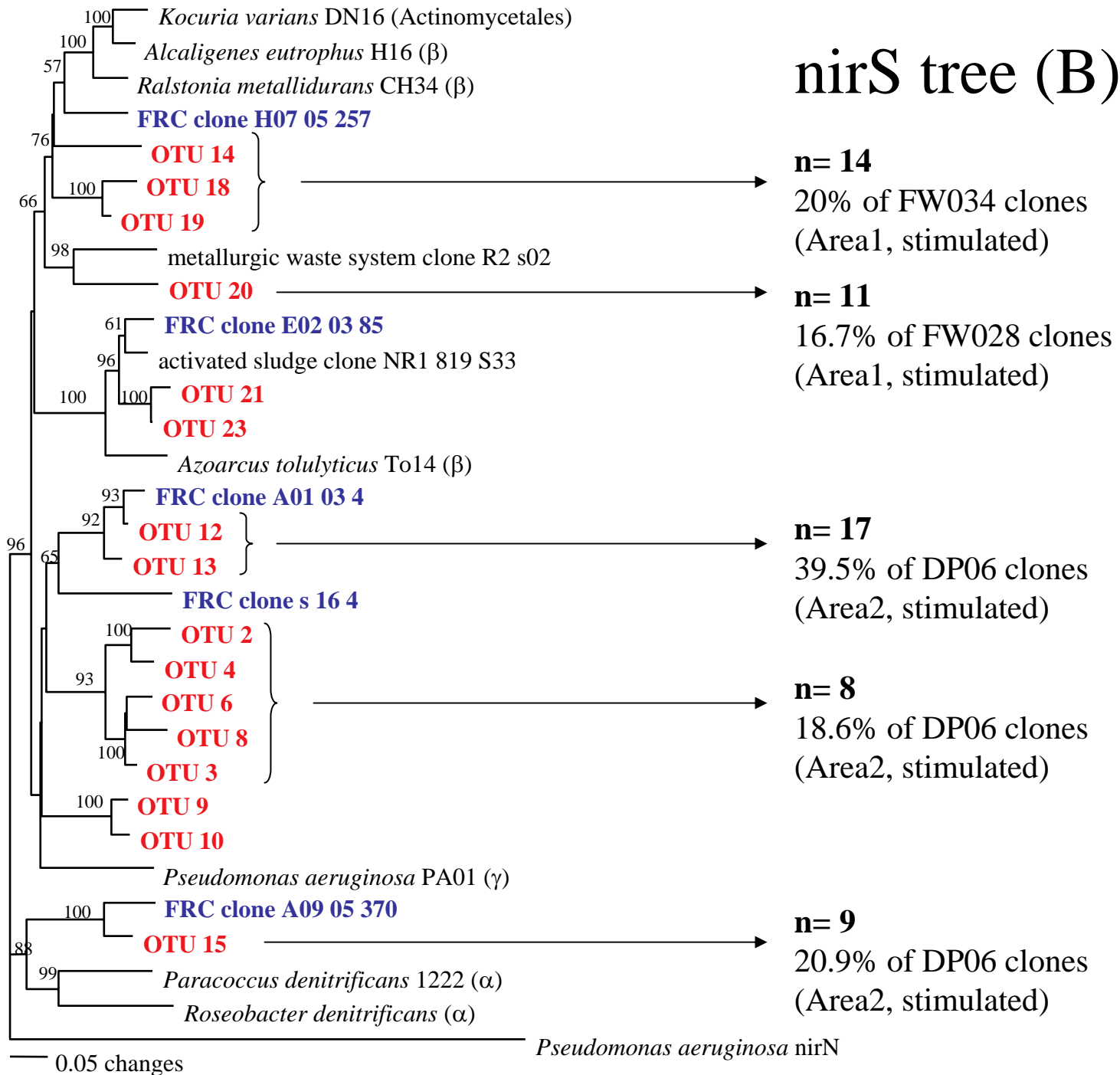
Area 1
EtOH
stimulated

90% similar to
Dechloromonas aromatica

nirS tree (A)

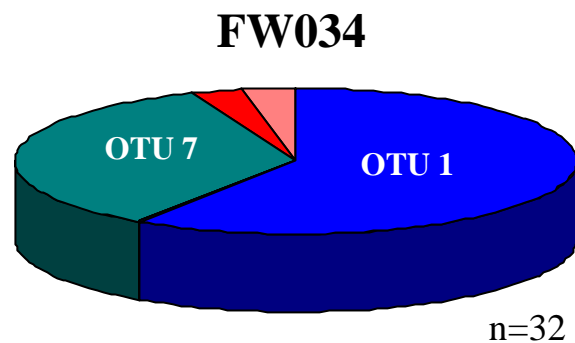
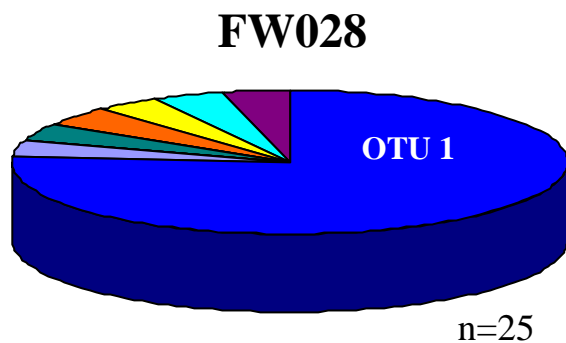


nirS tree (B)

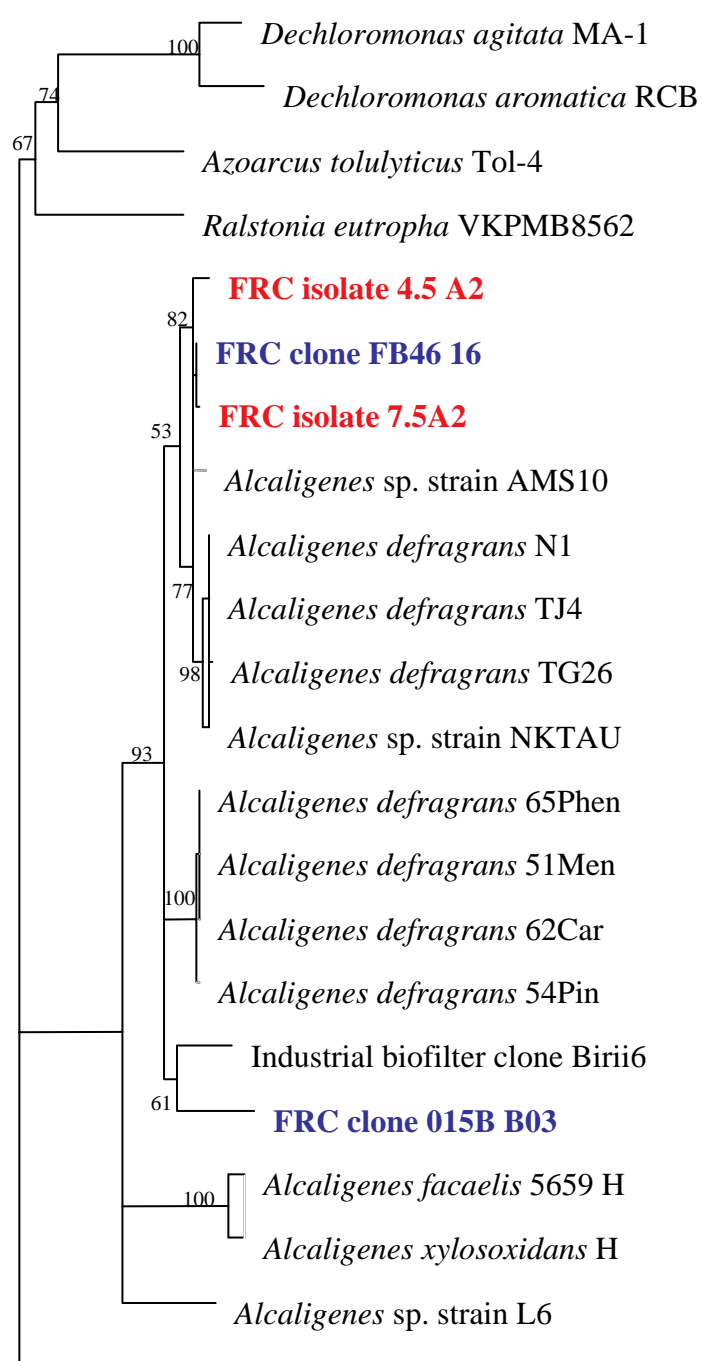


FRC Isolates

- New isolates have nirK gene
- New isolates are closely related to each other (based on both nirK and 16S sequences)
- nirK sequences of isolates are 99% similar to OTU 1 from the nirK clone libraries -- **This is the dominant clone sequence for both ethanol-stimulated Area 1 sites.**



FRC Isolates



β -proteobacteria

— 0.01 substitutions/site

Methanospirillum hungatei

Alcaligenes defragrans strain 54Pin

- First described in 1998
- Isolated from activated sludge on the monoterpene α -pinene and nitrate
 - Belongs to genus *Alcaligenes*, well known for degradation of aromatic and chlorinated compounds
 - Rod-shaped, 1.3-1.8 μm in length
 - Motile
 - pH range 6.3-8.4 (optimum 6.7-7.5)
 - Temperature range 15-40°C (optimum 30°C)
 - Electron acceptors: O_2 , NO_3^- , NO_2^- , N_2O)
 - Carbon sources: monoterpenes, acetate, ethanol, butyrate, propionate, pyruvate, malate, succinate, fumarate, some amino acids.
 - Cannot utilize sugars or aromatic hydrocarbons.