The Idaho National Engineering and Environmental Laboratory

IN-TACT BIOFILM PCR OF COLONIZED HEMATITE AS A MEANS TO EXPLORE MICROBIAL DIVERSITY AT THE NABIR FRC

Dave Cummings (INEEL)

Katie Reardon (Montana State University)

Gill Geesey (INEEL/MSU)

Brent Peyton (Washington State University)

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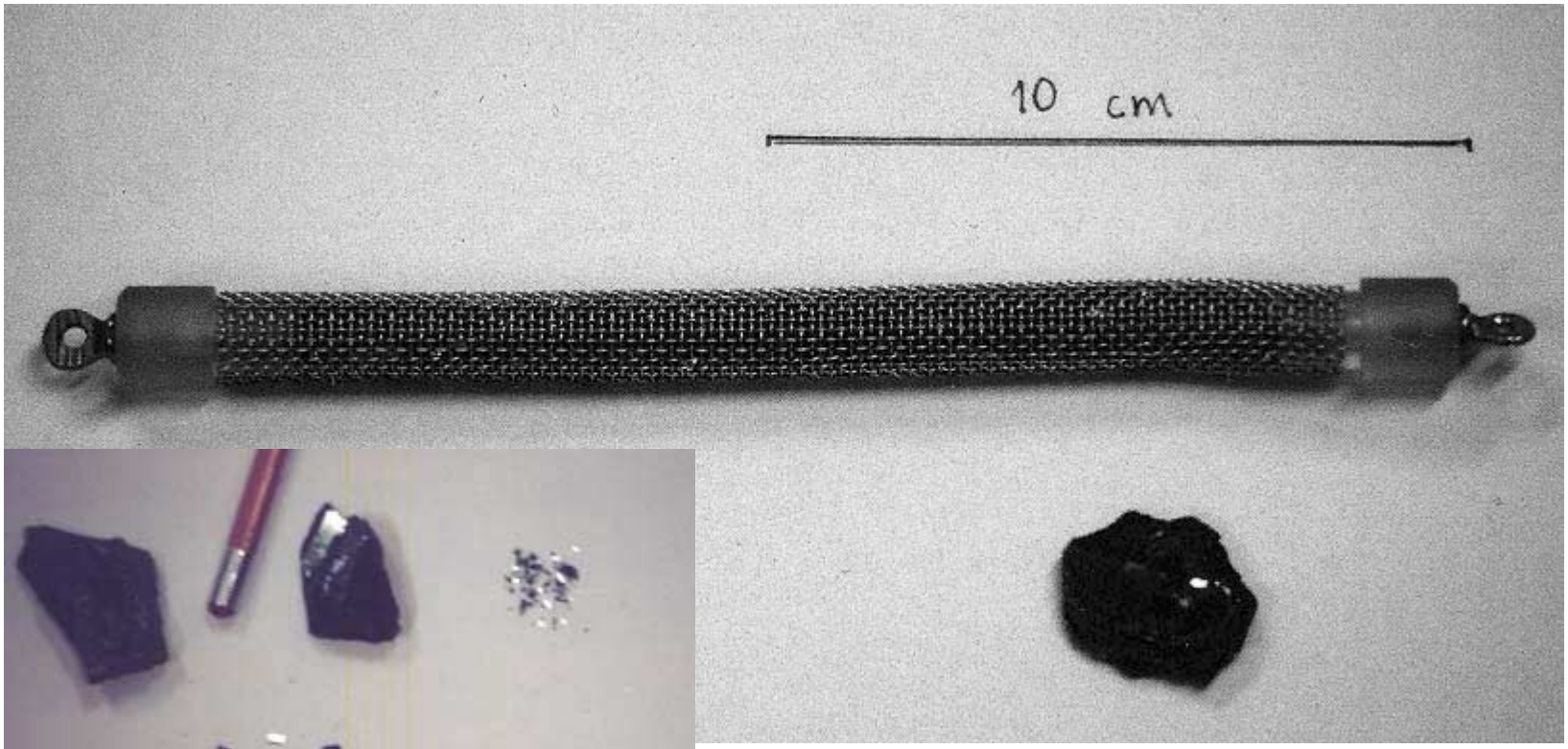
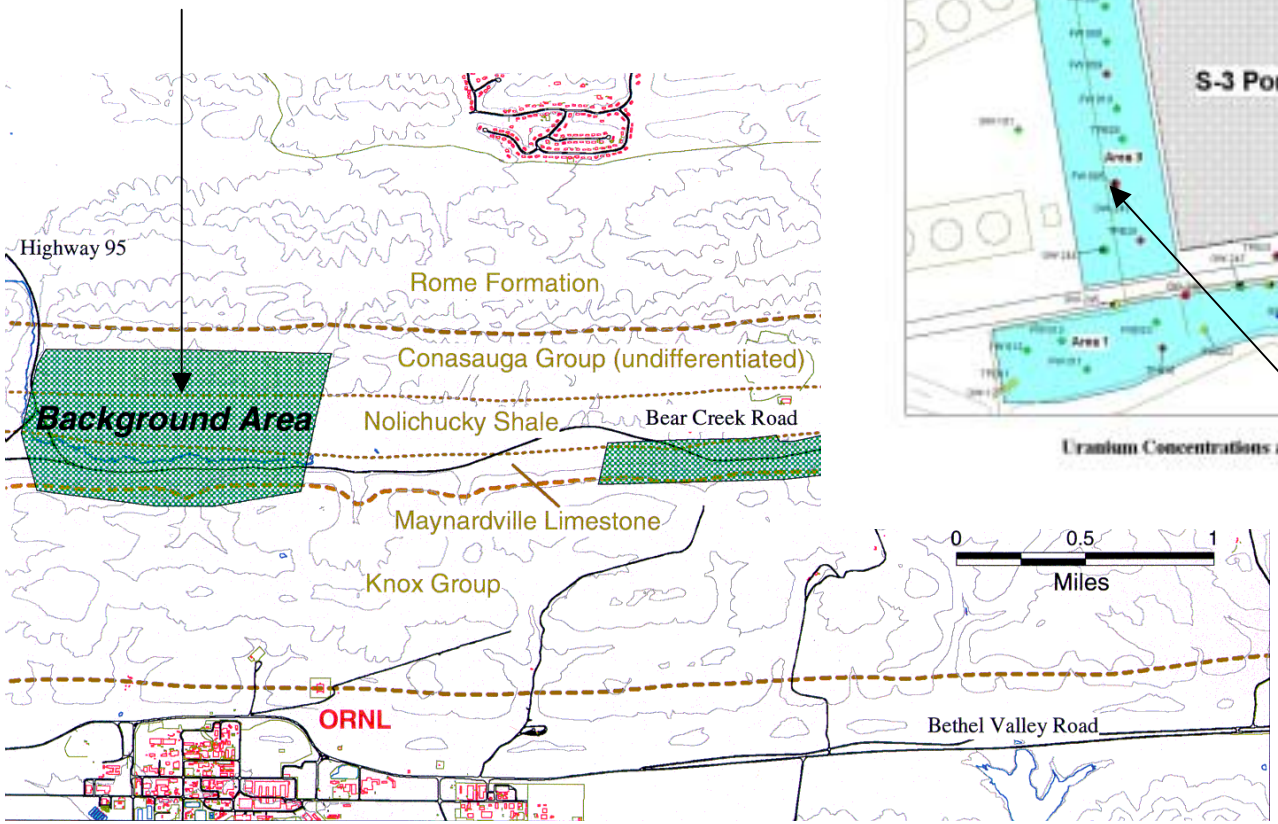
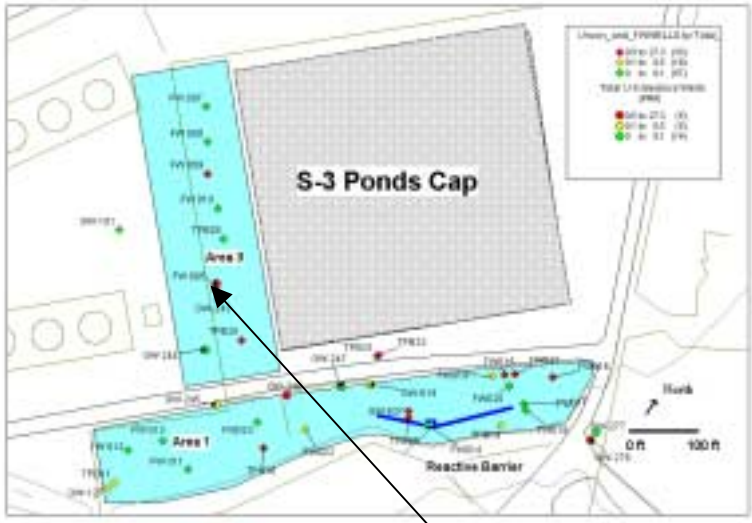
Biofilm Coupon



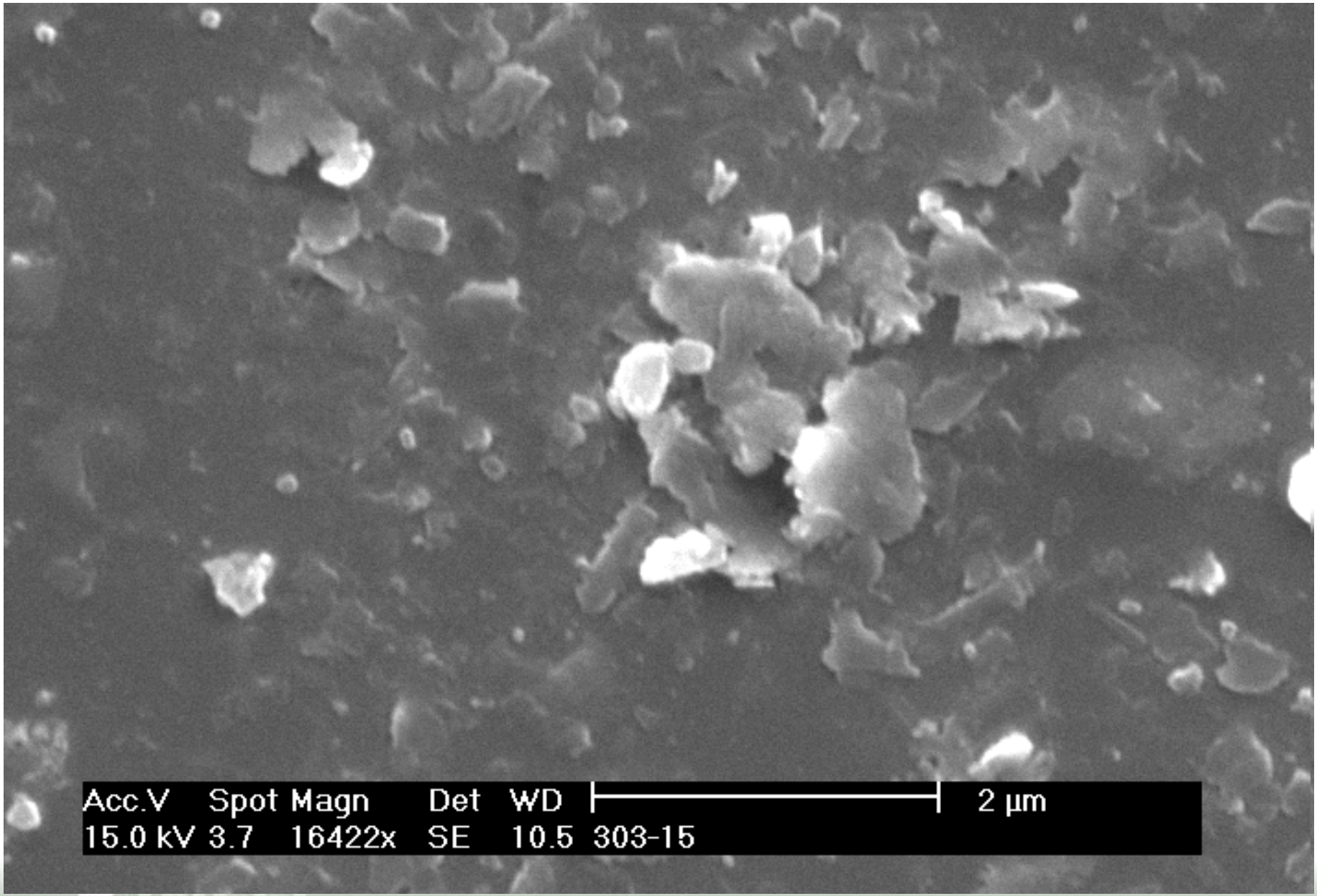
FWB303





Uranium Concentrations at Areas 1 and 3

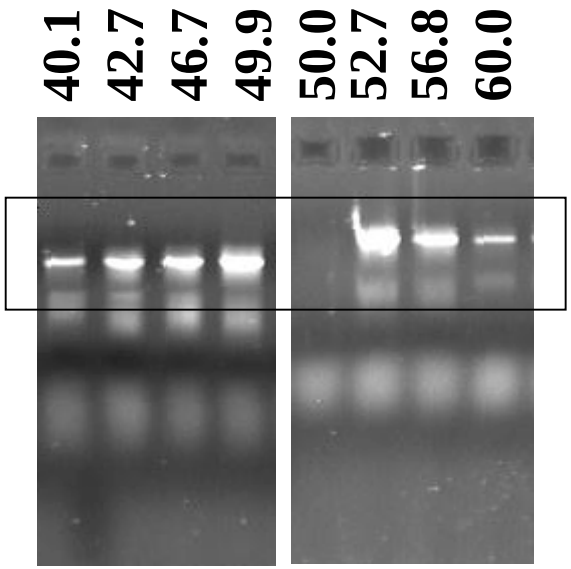
FWB100
FW026


Acc.V Spot Magn Det WD
2 µm
15.0 kV 3.7 16422x SE 10.5 303-15

Intact biofilm PCR reactions

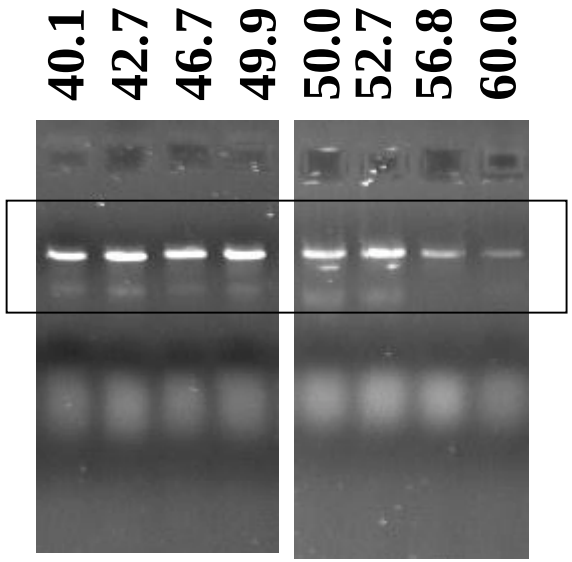
- 99°C, 15 min. in water/buffer alone
- 80°C 10 min., add remaining reaction components
- standard thermal cycling with Bacterial primers 8F/1492R

FWB303 15’



Pooled and cloned

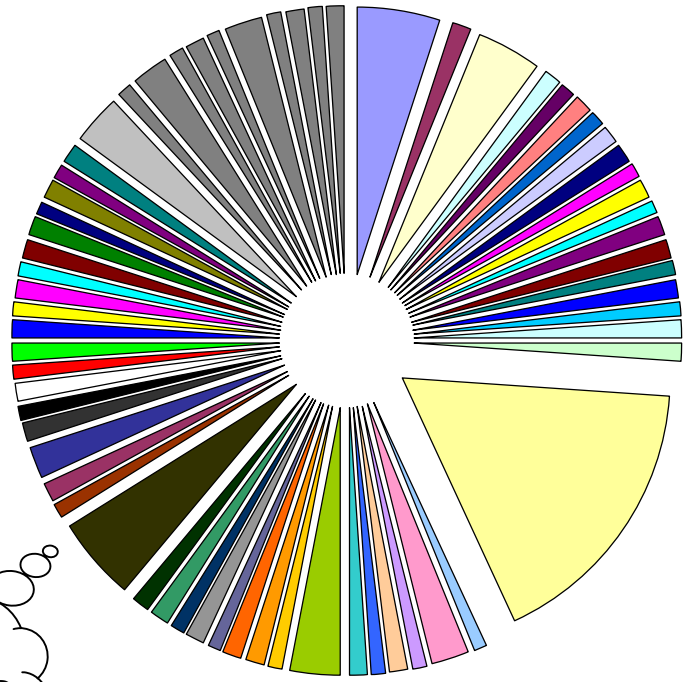
FW026 32’



Pooled and cloned

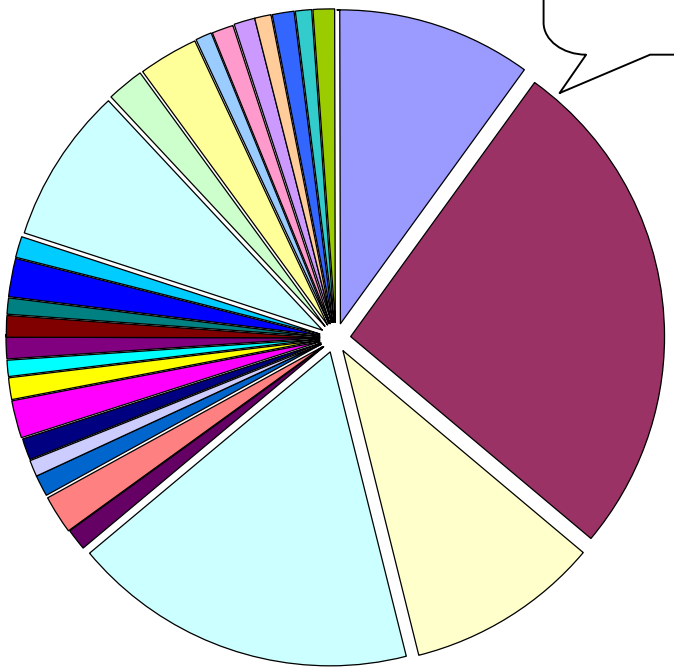
Clone diversity from biofilms

Background Area



65 RFLPs

Area 3



27 RFLPs

*Clone libraries created using in-tact biofilm PCR on hematite chips suspended in groundwater

BACKGROUND AREA

Bacterial 16S rDNA clones from hematite biofilms recovered at the FRC Background Area FWB303-15'

Clone ID	Frequency	Closest GenBank match	Closest identified match	Affiliation
BA15-HemT2	17	clone FBR-E (751/769)	*Rhodoferax antarcticus* (750/790)	β-Proteobacteria
BA15-HemAJ35	5	clone FBR-E (719/734)	*Rhodoferax fermentans* (724/756)	β-Proteobacteria
BA15-HemE7	1	clone FBR-E (653/681)	*Rhodoferax antarcticus* (665/703))	β-Proteobacteria
BA15-HemP23	1	clone FBR-E (754/773)	*Rhodoferax antarcticus* (753/794)	β-Proteobacteria
BA15-HemU28	1	clone PRD01b008B (707/737)	*Rhodoferax antarcticus* (697/740)	β-Proteobacteria
BA15-HemAP59	1	clone FBR-E (418/443)	*Rhodoferax antarcticus* (415/446)	β-Proteobacteria
BA15-HemAR61	1	clone PRD01b008B (947/1006)	*Rhodoferax antarcticus* (928/1004)	β-Proteobacteria
BA15-HemAX74	1	clone FBR-E (722/765)	*Rhodoferax fermentans* (714/764)	β-Proteobacteria
BA15-HemBC80	1	clone FBR-E (460/468)	*Rhodoferax antarcticus* (470/490)	β-Proteobacteria
BA15-HemA1	5	clone Wuba139 (729/750)	*Variovorax* sp. HAB-30 (694/719)	β-Proteobacteria
BA15-HemB3	1	clone GOUTB17 (1448/1459)	*Variovorax paradoxus* (759/774)	β-Proteobacteria
BA15-HemBM98	1	clone GOUTB17 (771/775)	*Variovorax paradoxus* (759/774)	β-Proteobacteria
BA15-HemD5	1	clone PRD01b008B (688/724)	*Variovorax* sp. MN 36.2 (607/628)	β-Proteobacteria
BA15-HemW31	1	clone GOUTB16 (729/750)	*Variovorax* sp. HAB-30 (719/744)	β-Proteobacteria
BA15-HemX32	1	clone GOUTB17 (762/777)	*Variovorax* sp. HAB-30 (752/771)	β-Proteobacteria
BA15-HemY34	1	clone GOUTB17 (733/742)	*Variovorax* sp. HAB-30 (723/736)	β-Proteobacteria
BA15-HemAW71	1	clone PRD01b008B (634/649)	*Variovorax* sp. MN 36.2 (629/650)	β-Proteobacteria
BA15-HemAY76	1	clone GOUTB17 (1215/1238)	*Variovorax* sp. HAB-30 (1184/1223)	β-Proteobacteria
BA15-HemAM55	2	clone GOUTB17 (590/681)	*Acidovorax anthurii* (577/675)	β-Proteobacteria
BA15-HemQ24	1	*Acidovorax* sp. (433/444)	*Acidovorax* sp. (433/444)	β-Proteobacteria
BA15-HemBI94	1	clone GOUTB19 (847/858)	*Acidovorax konjaci* (845/858)	β-Proteobacteria
BA15-HemBG90	1	clone RSC-II-11 (401/412)	*Acidovorax* sp. (308/340)	β-Proteobacteria
BA15-HemBJ95	2	*Pseudomonas* sp. (684/695)	*Pseudomonas* sp. (684/695)	γ-Proteobacteria
BA15-HemH11	1	clone KRA30-07 (438/454)	*Pseudomonas* sp. (440/458)	γ-Proteobacteria
BA15-HemBK96	1	*Pseudomonas* sp. (844/847)	*Pseudomonas* sp. (844/847)	γ-Proteobacteria
BA15-HemAD42	1	clone TRS13 (452/466)	*Pseudomonas* sp. (316/342)	γ-Proteobacteria
BA15-HemM18	1	*Pseudomonas rhodesiae* (632/636)	*Pseudomonas rhodesiae* (632/636)	γ-Proteobacteria
BA15-HemAK53	1	*Pseudomonas rhodesiae* (774/776)	*Pseudomonas rhodesiae* (774/776)	γ-Proteobacteria
BA15-HemAT66	1	*Pseudomonas chlororaphis* (448/451)	*Pseudomonas chlororaphis* (448/451)	γ-Proteobacteria
BA15-HemAU68	1	*Pseudomonas chlororaphis* (764/801)	*Pseudomonas chlororaphis* (764/801)	γ-Proteobacteria
BA15-HemI12	1	*Pseudomonas tolaasii* (638/659)	*Pseudomonas tolaasii* (638/659)	γ-Proteobacteria
BA15-HemO21	1	Clone A0647 (774/784)	*Pseudomonas saccharophila* (768/781)	γ-Proteobacteria
BA15-HemAC40	1	strain BD3-1 (600/643)	*Pseudomonas costantinii* (597/643)	γ-Proteobacteria

BACKGROUND AREA

Bacterial 16S rDNA clones from hematite biofilms recovered at the FRC Background Area FWB303-15'

Clone ID	Frequency	Closest GenBank match	Closest identified match	Affiliation
BA15-HemL17	1	clone DA122 (743/790)	*Beijerinckia indica* (707/762)	α-Proteobacteria
BA15-HemZ36	1	clone 4-Org2-27 (602/648)	*Rhizobium loti* (596/660)	α-Proteobacteria
BA15-HemBF84	2	clone CR98-5-35 (556/601)	*Sphingomonas* sp. MBIC1965 (603/661)	α-Proteobacteria
BA15-HemBA77	1	clone ccspost215 (598/612)	*Agrobacterium albertimagni* (587/603)	α-Proteobacteria
BA15-HemN19	1	*Leptothrix discophora* (634/649)	*Leptothrix discophora* (634/649)	β-Proteobacteria
BA15-HemAI50	1	clone MNE12 (359/360)	*Leptothrix discophora* (355/357)	β-Proteobacteria
BA15-HemAO58	1	clone GOUTA14 (769/779)	*Leptothrix* sp. (770/781)	β-Proteobacteria
BA15-HemR25	1	clone FTL236 (1313/1335)	*Aquaspirillum delicatum* (1300/1345)	β-Proteobacteria
BA15-HemAH48	1	clone Wuba139 (797/829)	*Aquaspirillum delicatum* (778/830)	β-Proteobacteria
BA15-HemAE44	1	clone Rm16 (767/787)	*Alcaligenes* sp. (761/785)	β-Proteobacteria
BA15-HemBO8	1	clone rM17 (388/452)	*Alcaligenes* sp. (316/361)	β-Proteobacteria
BA15-HemAA37	3	*Herbaspirillum seropedicae* (605/640)	*Herbaspirillum seropedicae* (605/640)	β-Proteobacteria
BA15-HemJ13	1	clone ccspost237 (747/796)	*Herbaspirillum* sp. BA161 (722/796)	β-Proteobacteria
BA15-HemV29	2	clone SC-I-50 (760/793)	*Oxalobacter* sp. (746/788)	β-Proteobacteria
BA15-HemBH93	1	clone Wuba68 (545/579)	*Oxalobacter* sp. (709/765)	β-Proteobacteria
BA15-HemAB39	1	clone mle1-7 (605/632)	*Burkholderia* sp. 13SM (584/630)	β-Proteobacteria
BA15-HemAG46	1	clone H20 (798/813)	*Sterolibacterium denitrificans* (766/813)	β-Proteobacteria
BA15-HemAV70	1	clone GOUTB17 (1331/1441)	*Hydrogenophaga taeniospiralis* (1382/1522)	β-Proteobacteria
BA15-HemAL54	1	*Dechloromonas* sp. (480/483)	*Dechloromonas* sp. (480/483)	β-Proteobacteria
BA15-HemAN47	1	clone G12-1429-4 (327/374)	*Aquabacterium* sp. (326/374)	β-Proteobacteria
BA15-HemBE83	1	*Janthinobacterium lividum* (1441/1452)	*Janthinobacterium lividum* (1441/1452)	β-Proteobacteria
BA15-HemBD81	3	*Pseudoxanthomonas* sp. (792/796)	*Pseudoxanthomonas* sp. (792/796)	γ-Proteobacteria
BA15-HemBB79	1	*Pseudoxanthomonas* sp. (858/866)	*Pseudoxanthomonas* sp. (858/866)	γ-Proteobacteria
BA15-HemBL97	1	*Pseudoxanthomonas* sp. (554/573)	*Pseudoxanthomonas* sp. (554/573)	γ-Proteobacteria
BA15-HemK14	1	*Pseudoxanthomonas* sp. (371/403)	*Pseudoxanthomonas* sp. (371/403)	γ-Proteobacteria
BA15-HemAF45	1	*Alcanivorax* sp. (628/699)	*Alcanivorax* sp. (628/699)	γ-Proteobacteria
BA15-HemC4	4	*Stenotrophomonas minatitlanensis* (574/583)	*Stenotrophomonas minatitlanensis* (574/583)	γ-Proteobacteria
BA15-HemS26	1	*Stenotrophomonas minatitlanensis* (752/772)	*Stenotrophomonas minatitlanensis* (752/772)	γ-Proteobacteria
BA15-HemAQ60	1	*Flexibacter columnaris* (630/661)	*Flexibacter columnaris* (630/661)	Bacteroidetes
BA15-HemAS62	1	*Opitutus* sp. VeSm13 (745/776)	*Opitutus* sp. VeSm13 (745/776)	Verrucomicrobia
BA15-HemF9	1	*Arthrobacter* sp. (1420/1436)	*Arthrobacter* sp. (1420/1436)	Actinobacteria

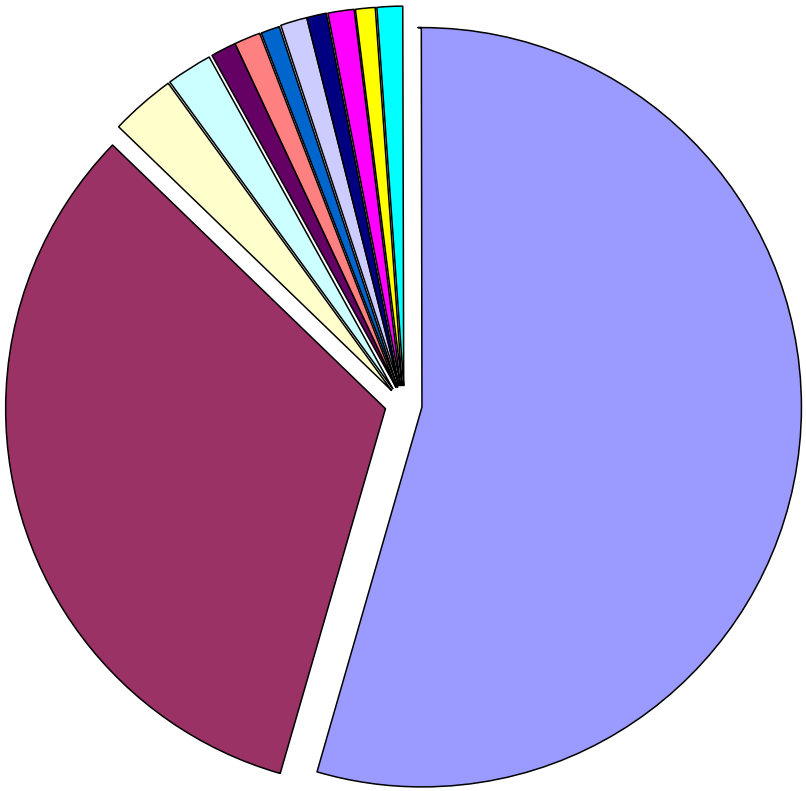
Bacterial 16S rDNA clones from hematite biofilms recovered at the FRC Area 3 FW026-32'

Clone ID	Frequency	Closest GenBank match	Closest identified match	Affiliation
A332-HemCB2	26	strain rM16 (1310/1351)	*Alcaligenes* sp. (1332/1396)	β-Proteobacteria
A332-HemCD52	18	strain rM16 (1343/1393)	*Alcaligenes* sp. R4 (1379/1396)	β-Proteobacteria
A332-HemCC4	10	strain rM16 (1309/1352)	*Pigmentiphaga* sp. (13301396)	β-Proteobacteria
A332-HemCP12	2	strain rM16 (1305/1351)	*Alcaligenes* sp. (1326/1397)	β-Proteobacteria
A332-HemCG	1	strain rM16 (1314/1352)	*Alcaligenes* sp. (1336/1397)	β-Proteobacteria
A332-HemCN47	1	strain rM16 (1305/1351)	*Alcaligenes* sp. (1327/1396)	β-Proteobacteria
A332-HemDB100	1	strain rM16 (768/815)	*Alcaligenes faecalis* (744/794)	β-Proteobacteria
A332-HemCW66	1	strain rM16 (540/567)	*Alcaligenes faecalis* (535/567)	β-Proteobacteria
A332-HemCA1	10	clone BIhiii13 (1411/1471)	*Frateuria* sp. NO-16 (1351/1405)	γ-Proteobacteria
A332-HemCS3	8	clone BIhiii13 (617/635)	*Rhodanobacter lindaniclasticus* (596/612)	γ-Proteobacteria
A332-HemCT61	2	clone BIhiii13 (1409/1469)	*Frateuria* sp. NO-16 (1346/1403)	γ-Proteobacteria
A332-HemCK34	1	clone BIhiii13 (1093/1114)	*Frateuria* sp. NO-16 (1082/1110)	γ-Proteobacteria
A332-HemCI28	1	*Frateuria aurantia* (1422/1467)	*Frateuria aurantia* (1422/1467)	γ-Proteobacteria
A332-HemCE	1	Cimanggu media isolate 88 (540/558)	*Frateuria aurantia* (539/558)	γ-Proteobacteria
A332-HemCF16	2	*Methylobacterium* sp. A1 (1388/1390)	*Methylobacterium* sp. A1 (1388/1390)	α-Proteobacteria
A332-HemCH20	1	*Methylobacterium* sp. G296-5 (716/719)	*Methylobacterium* sp. G296-5 (716/719)	α-Proteobacteria
A332-HemCQ55	1	*Methylobacterium* sp. G296-5 (602/602)	*Methylobacterium* sp. G296-5 (602/602)	α-Proteobacteria
A332-HemCY80	1	clone BPC087 (540/592)	*Ultramicrobacterium* str. D-7 (467/508)	β-Proteobacteria
A332-HemCZ82	1	clone UP9 (292/315)	*Paucimonas lemoignei* (287/310)	β-Proteobacteria
A332-HemDA88	1	*Burkholderia* sp. BPC2 (397/419)	*Burkholderia* sp. BPC2 (397/419)	β-Proteobacteria
A332-HemCU62	3	*Psuedomonas straminea* (1449/1458)	*Psuedomonas straminea* (1449/1458)	γ-Proteobacteria
A332-HemCL	1	*Pseudomonas syr*ingae (1216/1378)	*Pseudomonas syringae* (1216/1378)	γ-Proteobacteria
A332-HemCX74	1	*Acinetobacter* sp. ATCC 9958 (540/589)	*Acinetobacter* sp. ATCC 9958 (540/589)	γ-Proteobacteria
A332-HemCJ32	2	*Beutenbergia cavernosa* (1399/1456)	*Beutenbergia cavernosa* (1399/1456)	Actinobacteria
A332-HemCO51	1	*Microbacterium* sp. (1443/1447)	*Microbacterium* sp. (1443/1447)	Actinobacteria
A332-HemCV63	1	clone GCPF40 (347/393)	*Nocardiodes* sp. C157 (346/394)	Actinobacteria
A332-HemCM	1	clone C026 (1240/1328)[1]	*Phormidium pachydematicum* (270/311)	Cyanobacteria

[1] Unknown affiliation

Clone diversity from sediments

Background Area



12 RFLPs

*Clone library created by extracting DNA with Bio101 kit

BACKGROUND AREA

Bacterial 16S rDNA clones from sediments of the FRC Background Area FWB303-15'

Clone ID	Frequency	Closest GenBank match	Closest identified match	Affiliation
BA15-SedA1	54/100	clone Llangefni 52 (1438/1459)[1]	*Pseudomonas agarici* (1430/1459)	γ-Proteobacteria
BA15-SedC101	33/100	*Pseudomonas rhodesiae* (1448/1459)	*Pseudomonas rhodesiae* (1448/1459)	γ-Proteobacteria
BA15-SedH52	1/100	*Pseudomonas rhodesiae* (1450/1459)	*Pseudomonas rhodesiae* (1450/1459)	γ-Proteobacteria
BA15-SedI107	1/100	*Pseudomonas rhodesiae* (1446/1459)	*Pseudomonas rhodesiae* (1446/1459)	γ-Proteobacteria
BA15-SedK50	1/100	*Pseudomonas rhodesiae* (1450/1459)	*Pseudomonas rhodesiae* (1450/1459)	γ-Proteobacteria
BA15-SedE105	2/100	clone FTL205 (1424/1437)[2]	*Ultramicrobacterium* str. ND5 (1413/1455)	β-Proteobacteria
BA15-SedL121	1/100	clone FTL205 (1429/1437)	*Ultramicrobacterium* str. ND5 (1416/1455)	β-Proteobacteria
BA15-SedG30	1/100	*Duganella zoogloeoides* (1426/1451)	*Duganella zoogloeoides* (1426/1451)	β-Proteobacteria
BA15-SedB2	1/100	clone 32-20 (1411/1479)[3]	*Desulfuromonas palmitatis* (463/518)	δ-Proteobacteria
BA15-SedF26	1/100	clone 3C0d-3 (1289/1372)[4]	*Clostridium sporosphaeroides* (1222/1370)	Clostridia
BA15-SedJ147	1/100	clone CCS37 (1056/1186)[5]	*Thermoleiphilum minutum* (291/322)	Actinobacteria
BA15-SedD13	3/100	*Arthrobacter* sp. JCM 1339 (1420/1436)	*Arthrobacter* sp. JCM 1339 (1420/1436)	Actinobacteria

[1] Sheep mite microflora
[2] TCE-contaminated site undergoing in situ bioremediation
[3] Holphaga/Acidobacterium group
[4] Low G + C Gram positive from cow rumen
[5] Termite gut

Pressing questions about the diversity and activity of microbes at the FRC

Q: How can the use of various solid surfaces improve our understanding of subsurface microbial diversity and activities?

Q: Presented with different solid surfaces, do bacteria display preferential attachment? Can this method be used to infer partitioning of bacteria in the native substrata?

Q: Do anaerobic bacteria (SRB, FeRB) display preferential attachment to and activities on the various substrata that are present in the FRC subsurface?

Acknowledgements

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Katie Reardon (MSU/INEEL)
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DOE-NABIR (Peyton, Geesey)