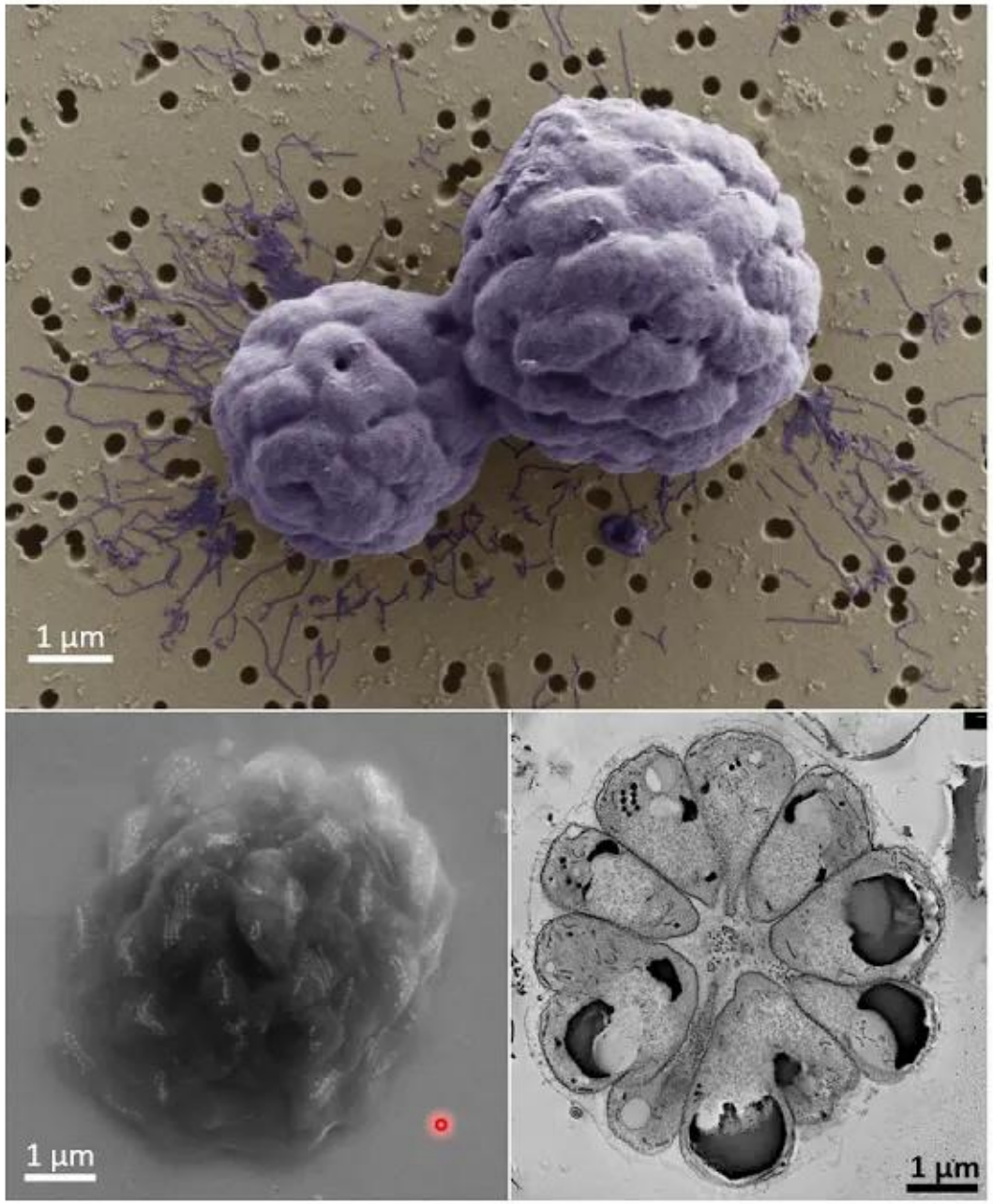

Multicellular Magnetotactic Bacteria (MMB)

- Multicellular = Composed of several cells from one species
- Magnetotactic = Capable of sensing Earths geomagnetic field for navigation
- Bacteria = A Gram negative Desulfobacterota bacterium

1
00:00:04,950 --> 00:00:03,750
hello my name is george shibal and today

2
00:00:08,070 --> 00:00:04,960
i'm going to talk about

3
00:00:09,669 --> 00:00:08,080
biological complexity specifically the

4
00:00:12,470 --> 00:00:09,679
cellular differentiation within

5
00:00:14,150 --> 00:00:12,480
multicellular magnetotactic bacteria

6
00:00:17,750 --> 00:00:14,160
and the implications in the evolution of

7
00:00:19,590 --> 00:00:17,760
complex life on earth

8
00:00:21,670 --> 00:00:19,600
to begin i would like to introduce the

9
00:00:23,429 --> 00:00:21,680
idea complexity in regards to the

10
00:00:26,950 --> 00:00:23,439
evolution of life on earth using a

11
00:00:29,269 --> 00:00:26,960
simple cartesian coordinate system

12
00:00:31,429 --> 00:00:29,279
for the first cell to evolve simple

13
00:00:32,950 --> 00:00:31,439

molecules had to interact to form more

14
00:00:36,709 --> 00:00:32,960
complex molecules

15
00:00:38,549 --> 00:00:36,719
that eventually yielded the first cell

16
00:00:39,990 --> 00:00:38,559
there are many great talks at

17
00:00:42,389 --> 00:00:40,000
appgrad.com that

18
00:00:43,110 --> 00:00:42,399
discuss this first step in the evolution

19
00:00:44,950 --> 00:00:43,120
of life

20
00:00:46,869 --> 00:00:44,960
but i'm going to focus on the next step

21
00:00:48,950 --> 00:00:46,879
which was when that simple cell

22
00:00:49,990 --> 00:00:48,960
continued to evolve into a multicellular

23
00:00:51,990 --> 00:00:50,000
organism

24
00:00:54,229 --> 00:00:52,000
that eventually gave rise to life that

25
00:00:56,869 --> 00:00:54,239
we see on earth today

26
00:00:58,310 --> 00:00:56,879
of course at certain steps in this

27
00:01:00,310 --> 00:00:58,320
evolution of life in regards to

28
00:01:02,310 --> 00:01:00,320
complexity some cells plateaued and

29
00:01:04,950 --> 00:01:02,320
remain the same giving the diversity we

30
00:01:07,230 --> 00:01:04,960
see on earth today

31
00:01:08,550 --> 00:01:07,240
it helps to have criteria for

32
00:01:11,310 --> 00:01:08,560
multicellularity

33
00:01:12,469 --> 00:01:11,320
to describe what we observe as far as

34
00:01:15,030 --> 00:01:12,479
multicellularity

35
00:01:15,590 --> 00:01:15,040
organisms the first criteria being that

36
00:01:17,510 --> 00:01:15,600
the

37
00:01:19,429 --> 00:01:17,520
organism is built from several cells of

38
00:01:21,910 --> 00:01:19,439

the same species

39
00:01:23,270 --> 00:01:21,920
as a specific shape and organization as

40
00:01:26,390 --> 00:01:23,280
well as synchronized growth

41
00:01:28,070 --> 00:01:26,400
so this would exclude things like cancer

42
00:01:29,429 --> 00:01:28,080
there would be no competition between

43
00:01:31,510 --> 00:01:29,439
cells and they would

44
00:01:33,429 --> 00:01:31,520
exhibit a coordinated behavior in

45
00:01:34,789 --> 00:01:33,439
response to internal and external

46
00:01:36,390 --> 00:01:34,799
stimuli

47
00:01:37,910 --> 00:01:36,400
and they would do this using cell to

48
00:01:39,670 --> 00:01:37,920
cell signaling

49
00:01:41,030 --> 00:01:39,680
and finally there would be an existence

50
00:01:43,830 --> 00:01:41,040
of a division of

51
00:01:45,749 --> 00:01:43,840
labor something like the cells in our

52
00:01:47,910 --> 00:01:45,759
lungs and stomach are from the same

53
00:01:48,550 --> 00:01:47,920
organism but they're performing a unique

54
00:01:53,030 --> 00:01:48,560
and different

55
00:01:54,630 --> 00:01:53,040
function for the entire organism

56
00:01:57,109 --> 00:01:54,640
the organism that i study is

57
00:02:00,550 --> 00:01:57,119
multicellular magnetotactic bacteria

58
00:02:02,389 --> 00:02:00,560
or mmb for short and this organism is

59
00:02:05,510 --> 00:02:02,399
multicellular in that it's composed of

60
00:02:07,749 --> 00:02:05,520
several cells from one species

61
00:02:09,990 --> 00:02:07,759
it's magnetotactic which means it's

62
00:02:12,630 --> 00:02:10,000
capable of sensing earth's geomagnetic

63
00:02:14,150 --> 00:02:12,640

poles that it uses for navigation and

64
00:02:17,110 --> 00:02:14,160
it's a bacteria belonging to the

65
00:02:20,390 --> 00:02:17,120
diesulfo bacteriota

66
00:02:24,070 --> 00:02:20,400
in this scm image on the upper left

67
00:02:26,070 --> 00:02:24,080
i show uh two mmb that have potentially

68
00:02:26,949 --> 00:02:26,080
just undergone division and you see that

69
00:02:29,750 --> 00:02:26,959
it's a

70
00:02:31,270 --> 00:02:29,760
small consortia of cells that are

71
00:02:34,869 --> 00:02:31,280
tightly packed together

72
00:02:37,670 --> 00:02:34,879
forming this ball shaped structure

73
00:02:39,110 --> 00:02:37,680
and the uh backscatter electron image in

74
00:02:42,229 --> 00:02:39,120
the lower left

75
00:02:44,710 --> 00:02:42,239
you'll see it's uh mmv and you'll see

76
00:02:46,869 --> 00:02:44,720
these white lines within each cells

77
00:02:49,229 --> 00:02:46,879
and these are the magnetosomes a

78
00:02:52,630 --> 00:02:49,239
bacterial organelle responsible for

79
00:02:56,470 --> 00:02:52,640
magnetotaxis of the organism

80
00:02:57,910 --> 00:02:56,480
the tem is showing a slice of the mmb

81
00:03:01,670 --> 00:02:57,920
where you see individual

82
00:03:03,670 --> 00:03:01,680
cells organized around a cellular center

83
00:03:05,270 --> 00:03:03,680
in which you see these black dots in

84
00:03:07,750 --> 00:03:05,280
this cell in the upper left

85
00:03:09,190 --> 00:03:07,760
and these are the same as the white dots

86
00:03:11,830 --> 00:03:09,200
you see in this backstab

87
00:03:13,990 --> 00:03:11,840
electron image the magnetosomes and

88
00:03:17,589 --> 00:03:14,000

these are

89
00:03:20,309 --> 00:03:17,599
synthesized by the mmb it's a

90
00:03:21,350 --> 00:03:20,319
mineral called gright that has a

91
00:03:23,750 --> 00:03:21,360
paramagnetic

92
00:03:24,390 --> 00:03:23,760
dipole and they form them in these

93
00:03:26,149 --> 00:03:24,400
chains that

94
00:03:28,149 --> 00:03:26,159
essentially act as a compass needle

95
00:03:29,509 --> 00:03:28,159
allowing them to sense or geomagnetic

96
00:03:31,589 --> 00:03:29,519
poles that they use for

97
00:03:35,430 --> 00:03:31,599
taxes in the water column to find their

98
00:03:38,550 --> 00:03:35,440
desired redox state for survival

99
00:03:40,550 --> 00:03:38,560
this organism was discovered in 1983

100
00:03:42,309 --> 00:03:40,560
and we still have not yet been able to

101
00:03:44,470 --> 00:03:42,319
bring it into culture which complicates

102
00:03:47,190 --> 00:03:44,480
all the studies so you can't simply

103
00:03:49,030 --> 00:03:47,200
throw genetics at it to test really

104
00:03:51,190 --> 00:03:49,040
interesting questions

105
00:03:53,429 --> 00:03:51,200
because we cannot culture them yet so

106
00:03:57,350 --> 00:03:53,439
all the techniques used in this study

107
00:03:59,910 --> 00:03:57,360
are culture independent techniques

108
00:04:00,869 --> 00:03:59,920
the mmb are an obligate multicellular

109
00:04:03,110 --> 00:04:00,879
organism

110
00:04:05,030 --> 00:04:03,120
that have a putative life cycle shown

111
00:04:07,030 --> 00:04:05,040
here in this panel sems

112
00:04:08,949 --> 00:04:07,040
where the organism grows in size and

113
00:04:11,350 --> 00:04:08,959

then eventually divides

114
00:04:14,630 --> 00:04:11,360
and similar observations have been used

115
00:04:17,110 --> 00:04:14,640
made using microscopy

116
00:04:17,909 --> 00:04:17,120
mmb cannot survive as single cells

117
00:04:19,990 --> 00:04:17,919
either

118
00:04:21,670 --> 00:04:20,000
and here in this lower left panel you

119
00:04:24,230 --> 00:04:21,680
see a live dead stain

120
00:04:26,150 --> 00:04:24,240
where cells that are stained red have

121
00:04:27,030 --> 00:04:26,160
lost their membrane integrity and have

122
00:04:29,590 --> 00:04:27,040
died

123
00:04:30,469 --> 00:04:29,600
and so here you see two dislodged cells

124
00:04:32,390 --> 00:04:30,479
that die

125
00:04:34,710 --> 00:04:32,400
and eventually this loss of cells

126
00:04:38,710 --> 00:04:34,720
compromises the integrity of the entire

127
00:04:41,189 --> 00:04:38,720
consortium and the whole consortium dies

128
00:04:43,270 --> 00:04:41,199
in the sem panels on the right we look

129
00:04:45,830 --> 00:04:43,280
at a a healthy mmb

130
00:04:47,030 --> 00:04:45,840
and then b c and d some different

131
00:04:48,629 --> 00:04:47,040
scenarios where

132
00:04:50,870 --> 00:04:48,639
cells have become dislodged and the

133
00:04:55,189 --> 00:04:50,880
organism the entire consortia has

134
00:04:58,790 --> 00:04:57,670
the mmbi study are found at little sip

135
00:05:01,830 --> 00:04:58,800
wisdom salt marsh

136
00:05:03,830 --> 00:05:01,840
located on cape cod in massachusetts in

137
00:05:04,790 --> 00:05:03,840
here i show an aerial view of what this

138
00:05:07,029 --> 00:05:04,800

site looks like

139
00:05:07,909 --> 00:05:07,039
and it's a brackish site so there's a

140
00:05:11,830 --> 00:05:07,919
influx of

141
00:05:13,670 --> 00:05:11,840
sea water from the the bay there and

142
00:05:14,310 --> 00:05:13,680
then fresh water coming out towards the

143
00:05:15,909 --> 00:05:14,320
bay

144
00:05:17,749 --> 00:05:15,919
here's a ground level view of what the

145
00:05:20,629 --> 00:05:17,759
site looks like it's just a small

146
00:05:22,150 --> 00:05:20,639
marshy pool and the bacteria that i

147
00:05:23,909 --> 00:05:22,160
study

148
00:05:26,390 --> 00:05:23,919
are just found in the sediment of this

149
00:05:27,990 --> 00:05:26,400
pool now the video i'm going to show you

150
00:05:29,029 --> 00:05:28,000
is the mmb are in the bottom of this

151
00:05:30,950 --> 00:05:29,039
eppendorf tube

152
00:05:32,790 --> 00:05:30,960
and they're swimming up towards this

153
00:05:35,189 --> 00:05:32,800
magnet that you see here

154
00:05:37,430 --> 00:05:35,199
you see them slowly taxing in this 20

155
00:05:39,590 --> 00:05:37,440
minute time lapse video

156
00:05:41,189 --> 00:05:39,600
in the next video it's a hanging water

157
00:05:42,550 --> 00:05:41,199
droplet with the mmb

158
00:05:44,469 --> 00:05:42,560
swimming towards the edge of this

159
00:05:46,469 --> 00:05:44,479
droplet where i show a cartoon of the

160
00:05:49,189 --> 00:05:46,479
magnet that is present there

161
00:05:50,550 --> 00:05:49,199
i then flip this magnet and the

162
00:05:51,990 --> 00:05:50,560
individual mmd

163
00:05:53,909 --> 00:05:52,000

all start swimming in the opposite

164
00:05:57,110 --> 00:05:53,919
direction now thinking that the magnetic

165
00:05:59,510 --> 00:05:57,120
north is in the opposite direction

166
00:06:00,710 --> 00:05:59,520
just so to know each little dot you see

167
00:06:04,230 --> 00:06:00,720
here is a single

168
00:06:07,430 --> 00:06:04,240
mmv consortium that's swimming

169
00:06:09,510 --> 00:06:07,440
now next i switch the magnet again

170
00:06:10,550 --> 00:06:09,520
and you see the mmd switch their

171
00:06:11,990 --> 00:06:10,560
swinging direction

172
00:06:17,189 --> 00:06:12,000
moving back towards the edge of this

173
00:06:22,469 --> 00:06:19,469
now when we return to the criteria for

174
00:06:24,150 --> 00:06:22,479
multicellularity in regards to mmb

175
00:06:26,309 --> 00:06:24,160
we see that several criteria are

176
00:06:28,309 --> 00:06:26,319
actually met in that they're built

177
00:06:29,430 --> 00:06:28,319
from several cells of the same species

178
00:06:30,790 --> 00:06:29,440
they have a specific shape and

179
00:06:32,629 --> 00:06:30,800
organization

180
00:06:34,070 --> 00:06:32,639
there's no observed competition between

181
00:06:37,270 --> 00:06:34,080
cells and they exhibit

182
00:06:38,309 --> 00:06:37,280
a coordinated response as we see by an

183
00:06:40,790 --> 00:06:38,319
external stimuli

184
00:06:43,510 --> 00:06:40,800
of a magnetic field that coordinates

185
00:06:45,830 --> 00:06:43,520
their swimming

186
00:06:48,309 --> 00:06:45,840
we still are investigating cell to cell

187
00:06:49,830 --> 00:06:48,319
signaling that we're using genomics for

188
00:06:52,070 --> 00:06:49,840

and we want to know more about a

189
00:06:54,550 --> 00:06:52,080
division of labor within mmb

190
00:06:56,550 --> 00:06:54,560
where we may potentially observe

191
00:06:58,309 --> 00:06:56,560
differences between the function of

192
00:07:05,270 --> 00:06:58,319
cells within the consortia

193
00:07:08,469 --> 00:07:07,510
so to begin we want to do whole genome

194
00:07:10,790 --> 00:07:08,479
sequencing

195
00:07:11,670 --> 00:07:10,800
so we sampled sediment from the marsh

196
00:07:16,309 --> 00:07:11,680
and

197
00:07:19,270 --> 00:07:16,319
magnet next to it

198
00:07:19,670 --> 00:07:19,280
for about 60 minutes and the mmd will

199
00:07:21,670 --> 00:07:19,680
swim

200
00:07:23,350 --> 00:07:21,680
up out of the sediment towards the

201
00:07:25,670 --> 00:07:23,360
magnetic magnet and

202
00:07:27,510 --> 00:07:25,680
form this small cell pellet you can see

203
00:07:31,110 --> 00:07:27,520
here that we can then simply just

204
00:07:33,029 --> 00:07:31,120
remove by pipetting we then took that

205
00:07:34,870 --> 00:07:33,039
sample of mmb and sorted them using

206
00:07:37,430 --> 00:07:34,880
fluorescent activated cell sorting to

207
00:07:40,309 --> 00:07:37,440
isolate the population of mmb

208
00:07:42,070 --> 00:07:40,319
and we sorted them into a 96 well plate

209
00:07:43,110 --> 00:07:42,080
where we did multiple displacement

210
00:07:46,070 --> 00:07:43,120
amplification

211
00:07:46,790 --> 00:07:46,080
in short read illumina sequencing to get

212
00:07:49,909 --> 00:07:46,800
22

213
00:07:52,230 --> 00:07:49,919

mmv genomes

214
00:07:53,189 --> 00:07:52,240
we then use the mmb genomes to address

215
00:07:55,909 --> 00:07:53,199
whether or not the

216
00:07:57,830 --> 00:07:55,919
consortium is clonal and then is the

217
00:07:59,830 --> 00:07:57,840
genome in one cell the same as the

218
00:08:01,430 --> 00:07:59,840
genome in its neighboring cell

219
00:08:02,869 --> 00:08:01,440
addressing this division of labor

220
00:08:04,710 --> 00:08:02,879
question

221
00:08:06,550 --> 00:08:04,720
and what we found where they were not

222
00:08:09,110 --> 00:08:06,560
entirely clonal so

223
00:08:09,909 --> 00:08:09,120
what this sequencing project did is we

224
00:08:13,909 --> 00:08:09,919
generated

225
00:08:16,469 --> 00:08:13,919
a several libraries from a single mmb

226
00:08:18,309 --> 00:08:16,479
in a well that was sequenced and we

227
00:08:20,469 --> 00:08:18,319
mapped those libraries back to the best

228
00:08:22,869 --> 00:08:20,479
assembly to identify

229
00:08:23,589 --> 00:08:22,879
single nucleotide polymorphism

230
00:08:26,790 --> 00:08:23,599
differences

231
00:08:28,790 --> 00:08:26,800
within a single mmb consortia

232
00:08:29,990 --> 00:08:28,800
and this work done by frederick schultz

233
00:08:32,310 --> 00:08:30,000
at jgi

234
00:08:33,269 --> 00:08:32,320
found that mmp have a higher rate of

235
00:08:35,750 --> 00:08:33,279
variance

236
00:08:37,029 --> 00:08:35,760
uh snips per genome as compared to the

237
00:08:39,750 --> 00:08:37,039
pseudomonas control

238
00:08:42,550 --> 00:08:39,760

we use as well as other environmental

239
00:08:45,670 --> 00:08:42,560
co-sorts that we had

240
00:08:48,870 --> 00:08:45,680
so what we ended up with was roughly

241
00:08:50,470 --> 00:08:48,880
50 to 110 sniff differences per genome

242
00:08:52,870 --> 00:08:50,480
within the consortia

243
00:08:54,070 --> 00:08:52,880
so between individual cells there was

244
00:08:56,070 --> 00:08:54,080
this difference

245
00:08:57,829 --> 00:08:56,080
well we're still interpreting this

246
00:08:59,269 --> 00:08:57,839
result we do know it's going to raise

247
00:09:02,470 --> 00:08:59,279
some very interesting questions and

248
00:09:07,430 --> 00:09:05,750
using the full-length 16s rna

249
00:09:09,110 --> 00:09:07,440
gene that we obtained from these genomes

250
00:09:11,990 --> 00:09:09,120
we found that there are

251
00:09:12,710 --> 00:09:12,000
five distinct populations or groups of

252
00:09:15,269 --> 00:09:12,720
energy

253
00:09:16,710 --> 00:09:15,279
existing in our site so we built this

254
00:09:17,750 --> 00:09:16,720
phylogenetic tree to show their

255
00:09:20,470 --> 00:09:17,760
relatedness

256
00:09:21,750 --> 00:09:20,480
and we compared them to other known and

257
00:09:25,030 --> 00:09:21,760
published on

258
00:09:25,590 --> 00:09:25,040
mmb existing across the world here you

259
00:09:27,670 --> 00:09:25,600
see a

260
00:09:29,910 --> 00:09:27,680
b and c and the top of this tree have

261
00:09:33,829 --> 00:09:29,920
this morphology of a

262
00:09:36,949 --> 00:09:33,839
spherical shape with these soft edge

263
00:09:39,030 --> 00:09:36,959

shaped cells within the aggregate

264
00:09:41,670 --> 00:09:39,040
and in the bottom of the tree in d e and

265
00:09:44,230 --> 00:09:41,680
f of course finding these micrographs

266
00:09:45,670 --> 00:09:44,240
you see that the mmv have an oblong

267
00:09:49,430 --> 00:09:45,680
shape to them and these this

268
00:09:52,550 --> 00:09:49,440
uh unique h4 shaped shell

269
00:09:54,710 --> 00:09:52,560
cells so we then have the question well

270
00:09:56,310 --> 00:09:54,720
do we observe these unique morphologies

271
00:09:58,870 --> 00:09:56,320
in the samples from our site

272
00:09:59,990 --> 00:09:58,880
that are shown in these colored groups

273
00:10:00,949 --> 00:10:00,000
and when we go to the fluorescent

274
00:10:03,590 --> 00:10:00,959
microscope

275
00:10:05,269 --> 00:10:03,600
just using a dna stain we see you know

276
00:10:06,630 --> 00:10:05,279
basically that the morphology

277
00:10:09,110 --> 00:10:06,640
is uniform across the different

278
00:10:10,870 --> 00:10:09,120
populations so we needed to develop a

279
00:10:13,509 --> 00:10:10,880
method to look at the

280
00:10:14,310 --> 00:10:13,519
distinct groups or populations of mmb

281
00:10:17,590 --> 00:10:14,320
from our site

282
00:10:19,910 --> 00:10:17,600
and their morphology so we developed a

283
00:10:22,389 --> 00:10:19,920
protocol for correlative fluorescent

284
00:10:25,190 --> 00:10:22,399
in-situ hybridization or fish

285
00:10:26,790 --> 00:10:25,200
scanning electron microscopy shown here

286
00:10:28,870 --> 00:10:26,800
we're going to take a sample to a

287
00:10:29,670 --> 00:10:28,880
scanning electron microscope obtain an

288
00:10:32,230 --> 00:10:29,680

image

289
00:10:33,430 --> 00:10:32,240
and then use fish to identify specific

290
00:10:36,630 --> 00:10:33,440
populations here

291
00:10:38,949 --> 00:10:36,640
group 1 and group 4 from our sample site

292
00:10:41,269 --> 00:10:38,959
and then map that back to the sem image

293
00:10:43,269 --> 00:10:41,279
to look at their morphology

294
00:10:45,110 --> 00:10:43,279
i'll discuss a little bit more of where

295
00:10:48,870 --> 00:10:45,120
this project is going at the

296
00:10:49,829 --> 00:10:48,880
in my last slide we also wanted to look

297
00:10:51,829 --> 00:10:49,839
at the

298
00:10:53,670 --> 00:10:51,839
division of labor and to do this we use

299
00:10:56,150 --> 00:10:53,680
substrate analog probing

300
00:10:57,350 --> 00:10:56,160
where you'll incubate the mmb in the

301
00:11:00,310 --> 00:10:57,360
presence of a

302
00:11:01,670 --> 00:11:00,320
methionine analog or an amino acid

303
00:11:04,949 --> 00:11:01,680
analog that has the

304
00:11:06,710 --> 00:11:04,959
azide functional group and so when new

305
00:11:08,710 --> 00:11:06,720
proteins are synthesized these new

306
00:11:09,430 --> 00:11:08,720
proteins will have an azide functional

307
00:11:12,150 --> 00:11:09,440
group

308
00:11:13,110 --> 00:11:12,160
they can then do azide alkyne click

309
00:11:15,430 --> 00:11:13,120
chemistry and

310
00:11:17,110 --> 00:11:15,440
covalently attach a fluorescent dye to

311
00:11:19,430 --> 00:11:17,120
the proteins

312
00:11:20,470 --> 00:11:19,440
so what we hypothesized was that the mmb

313
00:11:23,110 --> 00:11:20,480

consortia

314
00:11:25,350 --> 00:11:23,120
would either not incorporate this aha

315
00:11:27,829 --> 00:11:25,360
and see no fluorescent in the contortion

316
00:11:29,430 --> 00:11:27,839
or every cell in the consortia would

317
00:11:33,030 --> 00:11:29,440
incorporate this uh

318
00:11:35,670 --> 00:11:33,040
methionine analog dha or alternatively

319
00:11:37,110 --> 00:11:35,680
we'd see a division of labor where some

320
00:11:39,350 --> 00:11:37,120
cells are incorporating

321
00:11:40,790 --> 00:11:39,360
the aha and newly synthesized protein

322
00:11:42,470 --> 00:11:40,800
and would be highly active and other

323
00:11:45,910 --> 00:11:42,480
cells may not be making

324
00:11:48,710 --> 00:11:45,920
as much protein we can also use other

325
00:11:49,430 --> 00:11:48,720
substrate analogues for peptidoglycan

326
00:11:51,750 --> 00:11:49,440
synthesis

327
00:11:53,430 --> 00:11:51,760
and as well as a fatty acid for membrane

328
00:11:55,590 --> 00:11:53,440
synthesis

329
00:11:56,629 --> 00:11:55,600
we then use structured illumination

330
00:12:00,470 --> 00:11:56,639
microscopy

331
00:12:03,030 --> 00:12:00,480
the incorporation

332
00:12:04,069 --> 00:12:03,040
or the synthesis of protein and

333
00:12:06,790 --> 00:12:04,079
peptidoglycan

334
00:12:08,790 --> 00:12:06,800
between cells within the consortia and

335
00:12:10,870 --> 00:12:08,800
we did this with the peptidoglycan and

336
00:12:13,509 --> 00:12:10,880
the membrane and didn't observe any

337
00:12:14,230 --> 00:12:13,519
major differences the dark spots you see

338
00:12:17,389 --> 00:12:14,240

here are

339
00:12:18,790 --> 00:12:17,399
uh just the light block from the

340
00:12:21,030 --> 00:12:18,800
polyhydroxybutyrate uh

341
00:12:22,470 --> 00:12:21,040
granules or energy storage granules

342
00:12:26,069 --> 00:12:22,480
within the cells

343
00:12:27,829 --> 00:12:26,079
so this investigation is ongoing

344
00:12:29,350 --> 00:12:27,839
to conclude the current and future work

345
00:12:31,350 --> 00:12:29,360
of this project is to

346
00:12:34,310 --> 00:12:31,360
continue with the correlated microscopy

347
00:12:36,870 --> 00:12:34,320
where we'll use stable isotope probing

348
00:12:38,389 --> 00:12:36,880
and link that with our scanning electron

349
00:12:40,389 --> 00:12:38,399
microscopy

350
00:12:42,710 --> 00:12:40,399
to get morphology elemental composition

351
00:12:45,190 --> 00:12:42,720
as well as raman microspectroscopic

352
00:12:46,870 --> 00:12:45,200
micro spectroscopy to look at the

353
00:12:49,190 --> 00:12:46,880
chemical composition

354
00:12:51,110 --> 00:12:49,200
and link that with fish and then finally

355
00:12:51,750 --> 00:12:51,120
use nanosims to look at the localization

356
00:12:53,750 --> 00:12:51,760
of these

357
00:12:55,269 --> 00:12:53,760
substrates in individual cells within

358
00:12:57,190 --> 00:12:55,279
the consortium

359
00:13:00,150 --> 00:12:57,200
to link taxonomy physiology and

360
00:13:03,509 --> 00:13:01,990
of course i couldn't do this without all

361
00:13:05,670 --> 00:13:03,519
my collaborators in my lab as well as

362
00:13:07,750 --> 00:13:05,680
those at jgi and emsl