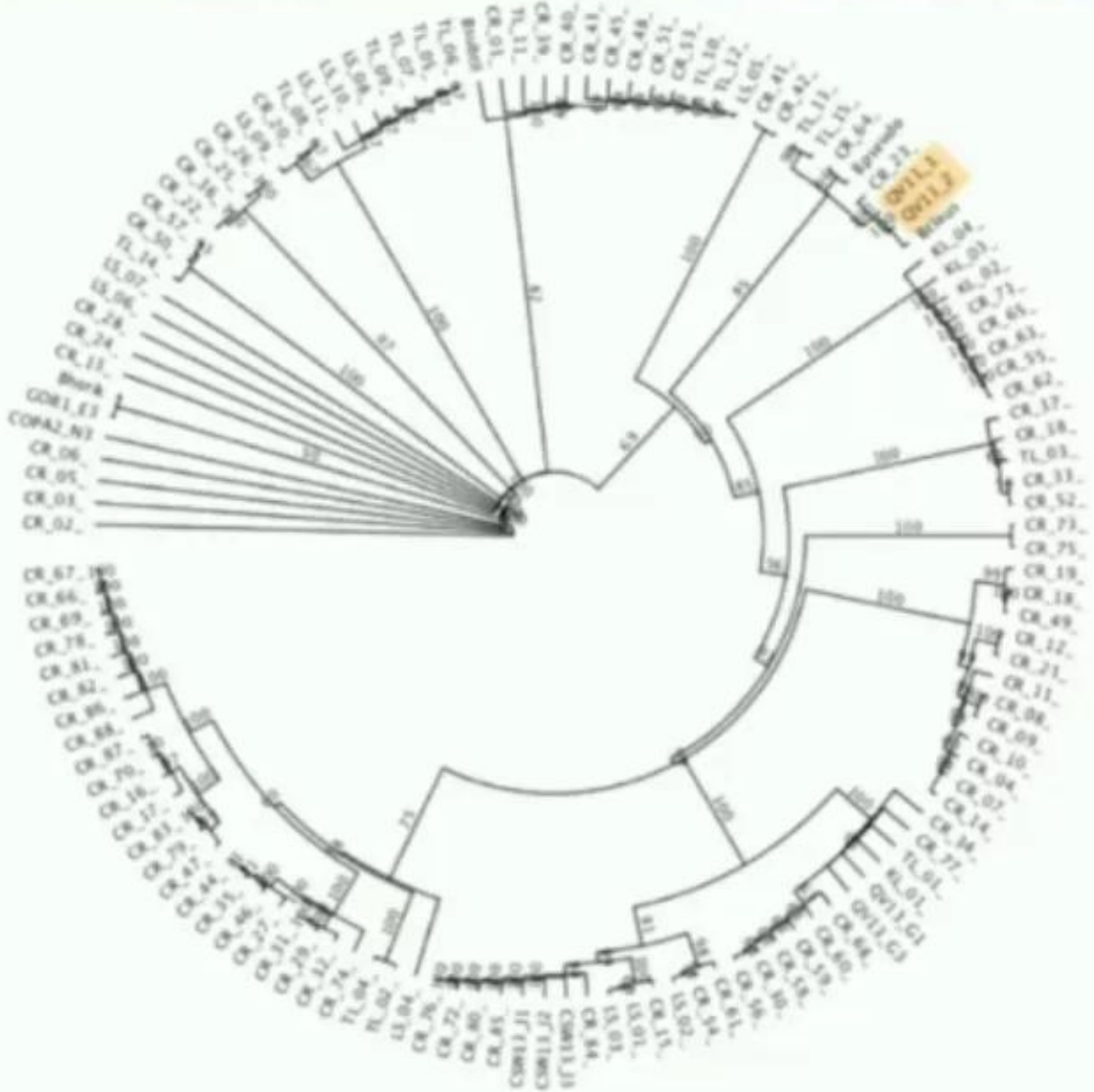
# Isolates from CROMO



1
00:00:11,240 --> 00:00:09,379
hi so I'm Lawrence Tyler I am just

2
00:00:13,190 --> 00:00:11,250
beginning my second year as a postdoc at

3
00:00:15,499 --> 00:00:13,200
Michigan State University working with

4
00:00:18,260 --> 00:00:15,509
Matt shrink and I'd like to talk to you

5
00:00:20,390 --> 00:00:18,270
today about using metabolomics to look

6
00:00:22,099 --> 00:00:20,400
at metabolic processes and surprising

7
00:00:24,740 --> 00:00:22,109
environments I'm very excited to be

8
00:00:26,390 --> 00:00:24,750
kicking off the rocks session and also

9
00:00:27,830 --> 00:00:26,400
to be introducing you to surprising

10
00:00:29,089 --> 00:00:27,840
environment a couple of people in this

11
00:00:31,099 --> 00:00:29,099
session are going to be talking about

12
00:00:32,749 --> 00:00:31,109
them so it's pretty cool that I get to

13
00:00:36,110 --> 00:00:32,759

introduce the topic to you if you're not

14
00:00:37,790 --> 00:00:36,120
very familiar with it so serpenton

15
00:00:40,010 --> 00:00:37,800
ization is the process by which this

16
00:00:43,700 --> 00:00:40,020
really pretty green rock turns into this

17
00:00:45,590 --> 00:00:43,710
really ugly black rock basically what

18
00:00:47,959 --> 00:00:45,600
that means is that the mineral olivene

19
00:00:51,740 --> 00:00:47,969
reacts with water and produces

20
00:00:53,060 --> 00:00:51,750
serpentine minerals as well as a high

21
00:00:55,580 --> 00:00:53,070
amounts of hydrogen so it creates a very

22
00:00:58,250 --> 00:00:55,590
high ph environment energy in the form

23
00:01:01,849 --> 00:00:58,260
of methane and also these small organics

24
00:01:04,100 --> 00:01:01,859
which can be food for microorganisms so

25
00:01:06,020 --> 00:01:04,110
it creates this really incredible

26
00:01:08,240 --> 00:01:06,030
environment where it's very extreme

27
00:01:10,039 --> 00:01:08,250
there's very low amount of dissolved

28
00:01:13,130 --> 00:01:10,049
inorganic carbon available because it

29
00:01:14,510 --> 00:01:13,140
all precipitates as calcite and very

30
00:01:16,100 --> 00:01:14,520
high p age but there's all these

31
00:01:18,080 --> 00:01:16,110
organics and there's all this energy

32
00:01:19,609 --> 00:01:18,090
available for microbes to harness so

33
00:01:23,630 --> 00:01:19,619
it's a very extreme environment but with

34
00:01:25,700 --> 00:01:23,640
a lot of opportunities for microbes also

35
00:01:28,969 --> 00:01:25,710
it is interesting because not only was

36
00:01:31,130 --> 00:01:28,979
it very prevalent on early Earth but

37
00:01:32,630 --> 00:01:31,140
it's also very likely that surprising

38
00:01:34,640 --> 00:01:32,640

environments exist in the subsurface of

39
00:01:37,010 --> 00:01:34,650
Mars and fuel hydrothermal environments

40
00:01:40,910 --> 00:01:37,020
on icy worlds like Europa and Enceladus

41
00:01:42,560 --> 00:01:40,920
and there are a number of origin of life

42
00:01:44,749 --> 00:01:42,570
theories that implicates or penalizing

43
00:01:47,450 --> 00:01:44,759
environments because they create these

44
00:01:52,389 --> 00:01:47,460
high-energy environments with gradients

45
00:01:54,560 --> 00:01:52,399
that can be harnessed by early life so

46
00:01:57,590 --> 00:01:54,570
there are a number of different

47
00:01:59,270 --> 00:01:57,600
serpenton izing environments all over

48
00:02:01,190 --> 00:01:59,280
the globe that our lab studies but I'm

49
00:02:03,770 --> 00:02:01,200
going to focus on the Coast Range

50
00:02:05,149 --> 00:02:03,780
ophiolite microbial observatory it's a

51
00:02:09,979 --> 00:02:05,159
nice place to work because it's just

52
00:02:11,240 --> 00:02:09,989
north of napa so you know drunk science

53
00:02:13,550 --> 00:02:11,250
but anyway

54
00:02:15,890 --> 00:02:13,560
there's two sites that we work at here

55
00:02:18,260 --> 00:02:15,900
at chromel the quarry valley and the

56
00:02:20,300 --> 00:02:18,270
core shed well and I'm going to be

57
00:02:23,600 --> 00:02:20,310
talking about some microbes isolated

58
00:02:25,790 --> 00:02:23,610
from quarry valley we have six wells dug

59
00:02:27,740 --> 00:02:25,800
at quarry valley and another six tug at

60
00:02:33,170 --> 00:02:27,750
the core shed well and they are highly

61
00:02:35,360 --> 00:02:33,180
alkaline and that's the drill rig that

62
00:02:39,620 --> 00:02:35,370
was used to drill these wells with just

63
00:02:41,480 --> 00:02:39,630

a few years ago so we have a ton of 16s

64
00:02:43,370 --> 00:02:41,490
data from these wells we have a pretty

65
00:02:44,930 --> 00:02:43,380
good idea of what kind of microbes are

66
00:02:46,940 --> 00:02:44,940
living in these highly alkaline

67
00:02:49,070 --> 00:02:46,950
environments and as you might suspect

68
00:02:52,190 --> 00:02:49,080
they're not very diverse because it is a

69
00:02:54,290 --> 00:02:52,200
very extreme environment um but we do

70
00:02:56,210 --> 00:02:54,300
see some trends associated with pH

71
00:02:59,300 --> 00:02:56,220
certain groups show up in the higher pH

72
00:03:01,100 --> 00:02:59,310
wells and versus the lower pH 12 I'm not

73
00:03:03,080 --> 00:03:01,110
going to focus on any of that today at

74
00:03:04,580 --> 00:03:03,090
all I'm not interested in who's there

75
00:03:06,890 --> 00:03:04,590
I'm interested in what they're doing and

76
00:03:09,800 --> 00:03:06,900
in an environment like this it's very

77
00:03:12,229 --> 00:03:09,810
difficult to distinguish between biotic

78
00:03:13,640 --> 00:03:12,239
and abiotic processes these reactions

79
00:03:15,560 --> 00:03:13,650
occurring between minerals and water

80
00:03:18,259 --> 00:03:15,570
producing organics producing methane

81
00:03:20,750 --> 00:03:18,269
microbes produce organics and methane so

82
00:03:22,940 --> 00:03:20,760
it's difficult for us to figure out

83
00:03:24,320 --> 00:03:22,950
what's going on at the geological level

84
00:03:26,509 --> 00:03:24,330
and what's going on at the biological

85
00:03:27,949 --> 00:03:26,519
level and it's very likely that microbes

86
00:03:29,449 --> 00:03:27,959
are feeding off of these geologic

87
00:03:34,789 --> 00:03:29,459
processes so there's a very gray area

88
00:03:36,410 --> 00:03:34,799

here so that's where a technique like

89
00:03:39,140 --> 00:03:36,420
metabolomics comes into play

90
00:03:41,569 --> 00:03:39,150
metabolomics basically is a snapshot of

91
00:03:43,789 --> 00:03:41,579
the metabolic activity of a microbe it

92
00:03:45,890 --> 00:03:43,799
takes all of the small organics that

93
00:03:47,509 --> 00:03:45,900
microbes are producing and looks at them

94
00:03:49,430 --> 00:03:47,519
at the chemical level to try to figure

95
00:03:53,390 --> 00:03:49,440
out what metabolic processes are

96
00:03:55,009 --> 00:03:53,400
occurring in the cell and this technique

97
00:03:57,340 --> 00:03:55,019
can be used for a number of different

98
00:04:00,110 --> 00:03:57,350
applications we can figure out whoops

99
00:04:02,120 --> 00:04:00,120
got ahead of myself we can figure out

100
00:04:05,420 --> 00:04:02,130
what chemicals are being produced by the

101
00:04:08,539 --> 00:04:05,430
cells obviously um we can annotate

102
00:04:12,319 --> 00:04:08,549
enzymes based on what is being produced

103
00:04:13,880 --> 00:04:12,329
we can look at pathways we can figure

104
00:04:16,670 --> 00:04:13,890
out what metabolic processes are

105
00:04:18,860 --> 00:04:16,680
occurring we can even phenotype

106
00:04:19,789 --> 00:04:18,870
different cells that we can't different

107
00:04:21,770 --> 00:04:19,799
organisms that we can't necessarily

108
00:04:24,430 --> 00:04:21,780
culture in the lab so this is all very

109
00:04:25,900 --> 00:04:24,440
useful right

110
00:04:27,910 --> 00:04:25,910
but there are some caveats of course

111
00:04:29,740 --> 00:04:27,920
just like with every technique first of

112
00:04:30,700 --> 00:04:29,750
all no extraction method is ideal so

113
00:04:31,990 --> 00:04:30,710

especially if you're doing a nun

114
00:04:33,910 --> 00:04:32,000
targeted approach and you want to look

115
00:04:37,900 --> 00:04:33,920
at all of the metabolites being produced

116
00:04:39,520 --> 00:04:37,910
by microbes now no extraction method is

117
00:04:40,780 --> 00:04:39,530
going to get every single metabolite

118
00:04:43,780 --> 00:04:40,790
there's always going to be some bias

119
00:04:46,180 --> 00:04:43,790
involved there's a bunch of different

120
00:04:48,460 --> 00:04:46,190
ways to analyze the metabolites once

121
00:04:50,380 --> 00:04:48,470
you've extracted them we use a liquid

122
00:04:53,200 --> 00:04:50,390
chromatography mass spec but you can

123
00:04:55,960 --> 00:04:53,210
also use GC you can use NMR and each one

124
00:04:57,190 --> 00:04:55,970
of those methods has its own perks and

125
00:05:01,180 --> 00:04:57,200
drawbacks which I'm not going to get

126
00:05:03,130 --> 00:05:01,190
into right now so the way that we

127
00:05:05,470 --> 00:05:03,140
characterize each of these compounds

128
00:05:07,200 --> 00:05:05,480
once we've extracted them is looking at

129
00:05:11,410 --> 00:05:07,210
the mass-to-charge ratio and the

130
00:05:13,660 --> 00:05:11,420
retention time in the column but that

131
00:05:15,220 --> 00:05:13,670
can vary depending on your methodology

132
00:05:16,780 --> 00:05:15,230
right so if you're trying to

133
00:05:18,400 --> 00:05:16,790
characterize a compound based on these

134
00:05:20,230 --> 00:05:18,410
things and it varies from method to

135
00:05:21,790 --> 00:05:20,240
method then one person will get

136
00:05:25,480 --> 00:05:21,800
completely different results from yours

137
00:05:27,520 --> 00:05:25,490
in another lab it only provides

138
00:05:28,870 --> 00:05:27,530

potential chemical formula and is this

139
00:05:31,240 --> 00:05:28,880
is especially a problem if you're

140
00:05:34,540 --> 00:05:31,250
looking at really big molecules because

141
00:05:36,220 --> 00:05:34,550
you get these results where you have a

142
00:05:37,659 --> 00:05:36,230
code cake and have so many carbon atoms

143
00:05:39,730 --> 00:05:37,669
and it's so many nitrogen atoms and it's

144
00:05:42,130 --> 00:05:39,740
so many oxygen atoms the bigger molecule

145
00:05:44,320 --> 00:05:42,140
is the more potential possibilities

146
00:05:46,480 --> 00:05:44,330
there are for that molecules identity up

147
00:05:50,230 --> 00:05:46,490
to thousands of potential identities

148
00:05:52,570 --> 00:05:50,240
right stable isotope waving labeling can

149
00:05:55,510 --> 00:05:52,580
help constrain that so if you feed

150
00:05:57,400 --> 00:05:55,520
microbes with saying 14c that will help

151
00:05:59,140 --> 00:05:57,410
you constrain it at least how many

152
00:06:02,440 --> 00:05:59,150
carbon atoms are in the molecule which

153
00:06:03,850 --> 00:06:02,450
narrows things down quite a bit and

154
00:06:06,430 --> 00:06:03,860
obviously we haven't identified every

155
00:06:09,190 --> 00:06:06,440
single metabolites that exists there's a

156
00:06:10,990 --> 00:06:09,200
lot of them um and there's a lot of what

157
00:06:12,760 --> 00:06:11,000
we call unknown unknowns we don't know

158
00:06:16,110 --> 00:06:12,770
that it's there and we don't know what

159
00:06:18,100 --> 00:06:16,120
it is so that's also an issue but

160
00:06:19,930 --> 00:06:18,110
databases are currently being developed

161
00:06:23,260 --> 00:06:19,940
and they're constantly being added to

162
00:06:26,560 --> 00:06:23,270
and work like this helps enhance the

163
00:06:27,700 --> 00:06:26,570

development of these databases so a

164
00:06:29,530 --> 00:06:27,710
technique like this is a lot more

165
00:06:31,870 --> 00:06:29,540
powerful when you combine it with other

166
00:06:33,850 --> 00:06:31,880
molecular techniques like genomics or

167
00:06:35,770 --> 00:06:33,860
transcriptomics if you know what genes

168
00:06:38,380 --> 00:06:35,780
microbes have or what genes that

169
00:06:40,510 --> 00:06:38,390
currently have turned on then you can

170
00:06:42,430 --> 00:06:40,520
kind of guess it at least what metabolic

171
00:06:44,800 --> 00:06:42,440
processes are occurring and then use

172
00:06:46,870 --> 00:06:44,810
that to inform your identification of

173
00:06:48,790 --> 00:06:46,880
all the metabolites and vice versa if

174
00:06:50,410 --> 00:06:48,800
you see certain metabolites that are

175
00:06:52,150 --> 00:06:50,420
being produced you can say okay well I

176
00:06:54,850 --> 00:06:52,160
know certain metabolic processes are

177
00:06:56,890 --> 00:06:54,860
currently occurring so metabolomics can

178
00:06:59,620 --> 00:06:56,900
also help you annotate a genome or a

179
00:07:01,210 --> 00:06:59,630
transcript on so this is what the data

180
00:07:04,090 --> 00:07:01,220
looks like coming out the other side of

181
00:07:05,710 --> 00:07:04,100
the LCMS we use tandem liquid

182
00:07:08,170 --> 00:07:05,720
chromatography mass spec can bind with

183
00:07:09,520 --> 00:07:08,180
quadrupole time-of-flight if you don't

184
00:07:11,050 --> 00:07:09,530
know what that is don't worry about it

185
00:07:13,570 --> 00:07:11,060
but this is what the data looks like and

186
00:07:16,270 --> 00:07:13,580
each one of these Peaks represents an

187
00:07:20,080 --> 00:07:16,280
individual metabolites that we can pull

188
00:07:21,870 --> 00:07:20,090

out and try to identify um it's not very

189
00:07:27,280 --> 00:07:21,880
informative to look at this though right

190
00:07:30,400 --> 00:07:27,290
so this is a tree of a number of

191
00:07:32,980 --> 00:07:30,410
isolates that we have from chrome oh and

192
00:07:34,270 --> 00:07:32,990
I know every time I see a phylogenetic

193
00:07:35,470 --> 00:07:34,280
tree and a talk my eyes start to glaze

194
00:07:36,970 --> 00:07:35,480
over because this is a lot of

195
00:07:39,310 --> 00:07:36,980
information and it doesn't really mean

196
00:07:41,050 --> 00:07:39,320
much but I want you to focus on these

197
00:07:43,780 --> 00:07:41,060
two isolates over here but we're

198
00:07:47,920 --> 00:07:43,790
isolated from quarry valley 11 well

199
00:07:49,060 --> 00:07:47,930
which has a pH of about 11.5 and the

200
00:07:51,370 --> 00:07:49,070
reason why it's important is because

201
00:07:52,990 --> 00:07:51,380
it's they're both very closely related

202
00:07:55,240 --> 00:07:53,000
to each other but they're also closely

203
00:07:57,850 --> 00:07:55,250
related to bacillus su de formas which

204
00:07:59,800 --> 00:07:57,860
is a very well-known alkyl o file that's

205
00:08:02,110 --> 00:07:59,810
pretty well characterized so we decided

206
00:08:04,390 --> 00:08:02,120
to focus on these two isolates for now

207
00:08:06,850 --> 00:08:04,400
because it they're so closely related to

208
00:08:08,950 --> 00:08:06,860
a microbe that's genome has been a

209
00:08:11,020 --> 00:08:08,960
sequenced that we know quite a bit about

210
00:08:13,750 --> 00:08:11,030
and what its preferences are when we're

211
00:08:16,690 --> 00:08:13,760
trying to grow it in the lab so I grew

212
00:08:18,430 --> 00:08:16,700
these two isolates and as you can see by

213
00:08:19,780 --> 00:08:18,440

the growth curves it's a pretty standard

214
00:08:21,250 --> 00:08:19,790
growth curve we have your exponential

215
00:08:23,350 --> 00:08:21,260
phase here where the cells are very

216
00:08:24,880 --> 00:08:23,360
actively growing and dividing and then

217
00:08:26,860 --> 00:08:24,890
they just kind of plateau off here at

218
00:08:28,930 --> 00:08:26,870
the stationary phase where you have the

219
00:08:32,800 --> 00:08:28,940
same number of cells being produced as

220
00:08:37,300 --> 00:08:32,810
are dying so I what's interesting here

221
00:08:39,070 --> 00:08:37,310
is that the number one isolate takes off

222
00:08:42,070 --> 00:08:39,080
pretty rapidly but then number two even

223
00:08:43,630 --> 00:08:42,080
though it's very simple costly related

224
00:08:44,770 --> 00:08:43,640
to number one has kind of a different

225
00:08:46,570 --> 00:08:44,780
growth pattern here so it has this

226
00:08:49,120 --> 00:08:46,580
little dip in the beginning and then it

227
00:08:51,570 --> 00:08:49,130
goes in an exponential phase so we

228
00:08:53,370 --> 00:08:51,580
decided to look at this

229
00:08:54,540 --> 00:08:53,380
or if our the exponential phase on the

230
00:08:56,580 --> 00:08:54,550
stationary phase of both of these

231
00:08:58,230 --> 00:08:56,590
isolates and compare the metabolites

232
00:09:00,330 --> 00:08:58,240
being produced by both of these isolates

233
00:09:03,090 --> 00:09:00,340
to see if they're using different

234
00:09:06,870 --> 00:09:03,100
strategies to adapt to this highly

235
00:09:09,480 --> 00:09:06,880
alkaline environment and what we found

236
00:09:11,390 --> 00:09:09,490
is that this is a PCA plot of the total

237
00:09:15,960 --> 00:09:11,400
metabolites of each one of these

238
00:09:17,310 --> 00:09:15,970

cultures and you can see that the

239
00:09:18,900 --> 00:09:17,320
stationary phase of both of these

240
00:09:21,540 --> 00:09:18,910
isolates clumps pretty closely together

241
00:09:23,910 --> 00:09:21,550
but they are distinct from each other I

242
00:09:25,170 --> 00:09:23,920
likewise for the exponential phase and

243
00:09:28,920 --> 00:09:25,180
then our blank is all the way over here

244
00:09:33,360 --> 00:09:28,930
which is always nice so we know I have

245
00:09:35,490 --> 00:09:33,370
good clean controls here so the cool

246
00:09:36,930 --> 00:09:35,500
thing is that we can look at individual

247
00:09:38,640 --> 00:09:36,940
peaks and look at their relative

248
00:09:40,320 --> 00:09:38,650
abundance between all of our different

249
00:09:43,110 --> 00:09:40,330
samples and you can see that there's

250
00:09:45,030 --> 00:09:43,120
certain compounds that are present only

251
00:09:47,070 --> 00:09:45,040
in the exponential phase of both of

252
00:09:49,230 --> 00:09:47,080
these isolates or only in the stationary

253
00:09:51,690 --> 00:09:49,240
phase and then there's ones that show up

254
00:09:53,850 --> 00:09:51,700
only for example in the number two

255
00:09:56,280 --> 00:09:53,860
culture but not in number one at either

256
00:09:57,960 --> 00:09:56,290
phase of growth so that's kind of neat

257
00:10:00,270 --> 00:09:57,970
and then there's also compounds that

258
00:10:04,170 --> 00:10:00,280
only show up during one phase of growth

259
00:10:06,330 --> 00:10:04,180
in one isolate so all of these compounds

260
00:10:07,500 --> 00:10:06,340
are distinguishing between these

261
00:10:10,320 --> 00:10:07,510
different isolates at their different

262
00:10:12,630 --> 00:10:10,330
phases of growth we can use them to look

263
00:10:15,690 --> 00:10:12,640

at what metabolic strategies are being

264
00:10:17,460 --> 00:10:15,700
used in these isolates we haven't

265
00:10:19,560 --> 00:10:17,470
identified these compounds yet and

266
00:10:21,930 --> 00:10:19,570
that's going to be an interesting

267
00:10:24,360 --> 00:10:21,940
exercise because again they're very

268
00:10:25,830 --> 00:10:24,370
likely unknown unknowns if they were

269
00:10:28,320 --> 00:10:25,840
very well known metabolites they'd

270
00:10:29,730 --> 00:10:28,330
probably be seen in all of the cells

271
00:10:32,880 --> 00:10:29,740
because they're extremely common and

272
00:10:34,860 --> 00:10:32,890
very readily identified so we're going

273
00:10:36,270 --> 00:10:34,870
to start searching the databases and see

274
00:10:38,100 --> 00:10:36,280
what we can come up with but we also

275
00:10:40,260 --> 00:10:38,110
have transcriptomic data that we need to

276
00:10:42,240 --> 00:10:40,270
analyze from these cultures and so we

277
00:10:44,280 --> 00:10:42,250
can combine that with the metabolomic

278
00:10:46,500 --> 00:10:44,290
data to try to get it exactly what

279
00:10:48,330 --> 00:10:46,510
strategy these isolates are using to

280
00:10:52,350 --> 00:10:48,340
survive in this extremely alkyl and

281
00:10:53,910 --> 00:10:52,360
environment the thing about these

282
00:10:55,140 --> 00:10:53,920
bacillus cultures though is that they're

283
00:10:56,880 --> 00:10:55,150
not really going to tell us a whole lot

284
00:10:59,390 --> 00:10:56,890
about what goes on in a serpentine izing

285
00:11:02,770 --> 00:10:59,400
environment because they're arif illic

286
00:11:05,050 --> 00:11:02,780
and because bacillus is a pretty

287
00:11:07,630 --> 00:11:05,060
an organism and its found basically all

288
00:11:10,720 --> 00:11:07,640

over the place so we can start to

289
00:11:13,000 --> 00:11:10,730
understand things about like alkyl alkyl

290
00:11:14,020 --> 00:11:13,010
0 tolerance tolerance of high pH but

291
00:11:15,670 --> 00:11:14,030
understanding some of the other

292
00:11:18,040 --> 00:11:15,680
processes that occur in surprising

293
00:11:20,410 --> 00:11:18,050
environments looking at the bacillus is

294
00:11:23,500 --> 00:11:20,420
not going to be particularly useful so

295
00:11:25,480 --> 00:11:23,510
we have this isolate mehsana bacterium

296
00:11:27,100 --> 00:11:25,490
subterranea which is not from a

297
00:11:28,990 --> 00:11:27,110
surprising environment but it's very

298
00:11:31,060 --> 00:11:29,000
closely related to organisms that we

299
00:11:33,310 --> 00:11:31,070
have identified in surprising

300
00:11:35,470 --> 00:11:33,320
environments including chroma this was

301
00:11:40,180 --> 00:11:35,480
actually isolated from a subterranean

302
00:11:41,800 --> 00:11:40,190
aquifer I think in Sweden in 1998 it is

303
00:11:44,290 --> 00:11:41,810
alkyl Oh Phillip just like the bacillus

304
00:11:46,990 --> 00:11:44,300
cultures and halo halo tolerant it will

305
00:11:50,080 --> 00:11:47,000
grow at a wide range of temperatures but

306
00:11:54,480 --> 00:11:50,090
it's also a Miss Hannigan and it might

307
00:11:57,690 --> 00:11:54,490
convert formate co2 or both into methane

308
00:12:01,870 --> 00:11:57,700
so we started growing this in the lab

309
00:12:03,610 --> 00:12:01,880
anaerobically and there's a couple of

310
00:12:05,440 --> 00:12:03,620
different carbon sources available in

311
00:12:06,910 --> 00:12:05,450
the medium I focus on for me and

312
00:12:08,950 --> 00:12:06,920
bicarbonate because they're implicated

313
00:12:12,940 --> 00:12:08,960

in the production of methane and i added

314
00:12:14,620 --> 00:12:12,950
a 13 c label bicarbonate and formate so

315
00:12:16,540 --> 00:12:14,630
I set up cultures where either the

316
00:12:18,520 --> 00:12:16,550
bicarbonate or the formate was labeled

317
00:12:20,860 --> 00:12:18,530
with see 13 in the hopes of tracking

318
00:12:22,960 --> 00:12:20,870
that into the metabolites and these were

319
00:12:25,030 --> 00:12:22,970
harvested during the exponential phase I

320
00:12:29,320 --> 00:12:25,040
put a question mark here whoops because

321
00:12:30,430 --> 00:12:29,330
I'm not really sure if this was the

322
00:12:31,960 --> 00:12:30,440
exponential phase we haven't established

323
00:12:34,510 --> 00:12:31,970
a very good growth curve for these

324
00:12:38,830 --> 00:12:34,520
because they clump a lot and it makes it

325
00:12:40,840 --> 00:12:38,840
difficult to sell counts unfortunately

326
00:12:43,300 --> 00:12:40,850
we didn't get as meat of a distribution

327
00:12:46,120 --> 00:12:43,310
here so the but the media looks an awful

328
00:12:48,220 --> 00:12:46,130
lot like blank media which means that if

329
00:12:50,080 --> 00:12:48,230
these cells are producing extracellular

330
00:12:51,520 --> 00:12:50,090
metabolites they're not as easily

331
00:12:54,520 --> 00:12:51,530
distinguished from the rest of the media

332
00:12:57,250 --> 00:12:54,530
and also we couldn't really tell much of

333
00:12:58,930 --> 00:12:57,260
a difference between the cultures that

334
00:13:00,100 --> 00:12:58,940
were fed 13 c-labeled for meat and the

335
00:13:02,440 --> 00:13:00,110
cultures that were fed 13 c-labeled

336
00:13:05,020 --> 00:13:02,450
acetate and this makes sense if you look

337
00:13:06,760 --> 00:13:05,030
at the piece because if 13c label was

338
00:13:09,400 --> 00:13:06,770

being incorporated into the metabolites

339
00:13:12,220 --> 00:13:09,410
you would see a distribution of various

340
00:13:15,300 --> 00:13:12,230
Peaks here like a spread where there's

341
00:13:16,710 --> 00:13:15,310
car compounds that kick that

342
00:13:18,480 --> 00:13:16,720
incorporated some of the 13 c and

343
00:13:19,740 --> 00:13:18,490
there's ones that still have 12 see it

344
00:13:22,050 --> 00:13:19,750
doesn't look like any label has been

345
00:13:23,850 --> 00:13:22,060
incorporated here so all of the 13 c is

346
00:13:25,560 --> 00:13:23,860
probably going into the methane and not

347
00:13:27,450 --> 00:13:25,570
into the metabolites or at least most of

348
00:13:30,750 --> 00:13:27,460
it but again we can still see there's

349
00:13:34,590 --> 00:13:30,760
certain compounds that occur only in say

350
00:13:36,180 --> 00:13:34,600
the palate or only in the media so our

351
00:13:38,490 --> 00:13:36,190
next steps are to grow the bacillus in

352
00:13:40,860 --> 00:13:38,500
more defined media sequence the genome

353
00:13:42,750 --> 00:13:40,870
and also look at the transcriptome to

354
00:13:46,170 --> 00:13:42,760
try to tease apart what exactly is going

355
00:13:48,079 --> 00:13:46,180
on metabolically in those isolates but

356
00:13:50,760 --> 00:13:48,089
also to take the Madonna bacterium and

357
00:13:51,990 --> 00:13:50,770
try feeding at 13 C acetate to see if

358
00:13:54,120 --> 00:13:52,000
that is incorporated into the

359
00:13:55,800 --> 00:13:54,130
metabolites trace the 13 c into the

360
00:13:57,570 --> 00:13:55,810
headspace gases to see if it's going

361
00:13:59,430 --> 00:13:57,580
into the methane from the older cultures

362
00:14:01,620 --> 00:13:59,440
it should be it's got to be going

363
00:14:03,870 --> 00:14:01,630

somewhere and also look at the

364
00:14:05,370 --> 00:14:03,880
transcriptome and hopefully develop a

365
00:14:07,530 --> 00:14:05,380
better growth curve we've been looking

366
00:14:09,660 --> 00:14:07,540
at protein assays as a substitute for

367
00:14:11,730 --> 00:14:09,670
doing direct cell counts and we're also

368
00:14:14,700 --> 00:14:11,740
working on doing field metabolomics on

369
00:14:16,230 --> 00:14:14,710
environmental samples from chroma so

370
00:14:18,000 --> 00:14:16,240
with that I'm going to thank you for

371
00:14:24,530 --> 00:14:18,010
your attention and I'll take your

372
00:14:36,140 --> 00:14:32,850
questions for Lauren yep had a feeling

373
00:14:38,700 --> 00:14:36,150
you'd have one so I thought you have a

374
00:14:40,170 --> 00:14:38,710
abundant khammam honest and recycle Asia

375
00:14:42,630 --> 00:14:40,180
at the beginning when you sampled and

376
00:14:44,460 --> 00:14:42,640
look for the 16s mm-hmm did you also get

377
00:14:48,390 --> 00:14:44,470
to isolate those are in what type of

378
00:14:50,310 --> 00:14:48,400
media um in which in the some of the

379
00:15:00,540 --> 00:14:50,320
earlier the 60s Taylor slice you showed

380
00:15:03,150 --> 00:15:00,550
we're all the 16s of abundance they're

381
00:15:08,850 --> 00:15:03,160
up there okay you have so that pretty

382
00:15:11,760 --> 00:15:08,860
much the Red Army yeah so um I believe

383
00:15:14,130 --> 00:15:11,770
some of these have been isolated I don't

384
00:15:15,660 --> 00:15:14,140
know what media we're currently growing

385
00:15:21,410 --> 00:15:15,670
them in so I've been focusing on the

386
00:15:24,360 --> 00:15:21,420
bacillus but um if you look at the tree

387
00:15:26,400 --> 00:15:24,370
we have a ton of isolates right now that

388
00:15:29,790 --> 00:15:26,410

we just haven't actively culture because

389
00:15:36,510 --> 00:15:29,800
there's just so many of them but that's