Joshua Leehan

Low-pressure Adapted Bacillus subtilis Exhibit Upregulated Expression of Antibiotic Biosynthesis, Biofilm- & Cell Wall-associated Genes

1
00:00:00,240 --> 00:00:11,110
[Music]

2
00:00:17,510 --> 00:00:14,060
sorry everybody this titles a little

3
00:00:20,560 --> 00:00:17,520
long and a little bit misleading it's

4
00:00:22,790 --> 00:00:20,570
all true but not exactly the point of it

5
00:00:25,300 --> 00:00:22,800
so today I'm going to be talking to you

6
00:00:27,350 --> 00:00:25,310
about the transcriptomics Ponce of

7
00:00:29,930 --> 00:00:27,360
Basilicata list that's been adapted to

8
00:00:32,749 --> 00:00:29,940
grow at low pressure so why do we care

9
00:00:33,770 --> 00:00:32,759
about low pressure we care about it for

10
00:00:36,020 --> 00:00:33,780
a number of different reasons people

11
00:00:38,590 --> 00:00:36,030
that are concerned with preservation of

12
00:00:40,790 --> 00:00:38,600
food are looking at it as a technique

13
00:00:43,099 --> 00:00:40,800

vacuum-sealing food most of you guys are

14
00:00:44,959 --> 00:00:43,109
probably aware of that air microbiology

15
00:00:47,689 --> 00:00:44,969
is another big concern as you guys are

16
00:00:49,939 --> 00:00:47,699
probably aware the pressure of the

17
00:00:52,399 --> 00:00:49,949
atmosphere decreases as you increase in

18
00:00:55,279 --> 00:00:52,409
altitude and microbes actually play a

19
00:00:58,430 --> 00:00:55,289
larger role in cloud formation and stuff

20
00:00:59,899 --> 00:00:58,440
so there that people are looking at how

21
00:01:02,090 --> 00:00:59,909
microbes survive in these upper

22
00:01:04,150 --> 00:01:02,100
atmosphere environments and the reason

23
00:01:06,770 --> 00:01:04,160
we're all here today astrobiology

24
00:01:10,580 --> 00:01:06,780
specifically we're looking at Martian

25
00:01:13,370 --> 00:01:10,590
atmospheric conditions so the atmosphere

26
00:01:16,400 --> 00:01:13,380
on Mars the pressure ranges from 0.1

27
00:01:19,490 --> 00:01:16,410
kilopascal one kilo Pascal which is a

28
00:01:21,680 --> 00:01:19,500
hundredfold less than the 101.3 kilo

29
00:01:25,100 --> 00:01:21,690
Pascal's of Earth's sea level air

30
00:01:27,590 --> 00:01:25,110
pressure so we before we go any further

31
00:01:30,260 --> 00:01:27,600
we won't understand beasts at Alyssa's

32
00:01:32,810 --> 00:01:30,270
relationship with blood pressure and

33
00:01:35,060 --> 00:01:32,820
also talk about bacillus Ellis so we use

34
00:01:37,940 --> 00:01:35,070
the Silla silas because it's extremely

35
00:01:39,380 --> 00:01:37,950
well studied model organism behind

36
00:01:41,300 --> 00:01:39,390
e.coli probably the most well studied

37
00:01:43,190 --> 00:01:41,310
organism in the world it's also very

38
00:01:45,080 --> 00:01:43,200

genetically tractable it's naturally

39
00:01:47,900 --> 00:01:45,090
confident so it has natural mechanisms

40
00:01:50,060 --> 00:01:47,910
to take up DNA and incorporate that into

41
00:01:52,400 --> 00:01:50,070
its genome and for the purposes of

42
00:01:54,800 --> 00:01:52,410
astrobiology it's also a Spore former

43
00:01:57,080 --> 00:01:54,810
these spores are very common spacecraft

44
00:01:59,540 --> 00:01:57,090
contaminants and they're also extremely

45
00:02:03,410 --> 00:01:59,550
resistant to a bunch of extreme

46
00:02:05,510 --> 00:02:03,420
environments so V settles this

47
00:02:07,880 --> 00:02:05,520
relationship with low pressure high boob

48
00:02:10,520 --> 00:02:07,890
area we found that the low pressure

49
00:02:13,520 --> 00:02:10,530
limit for most organ

50
00:02:17,150 --> 00:02:13,530
including the syllabus is about 2.5 kilo

51
00:02:19,970 --> 00:02:17,160
Pascal's which is you know 2.5 times

52
00:02:22,309 --> 00:02:19,980
higher than the highest estimated air

53
00:02:24,590 --> 00:02:22,319
pressure on Mars we also find that it's

54
00:02:26,210 --> 00:02:24,600
growth limiting and non lethal there is

55
00:02:27,920 --> 00:02:26,220
an exception to this two point five kilo

56
00:02:31,309 --> 00:02:27,930
Pascal's there is a genus of bacteria

57
00:02:33,650 --> 00:02:31,319
called karna bacterium that grows at 7 K

58
00:02:34,729 --> 00:02:33,660
or 0.7 kilo Pascal's and if you're

59
00:02:36,440 --> 00:02:34,739
interested in that you should go to my

60
00:02:39,620 --> 00:02:36,450
colleague Kathleen Miller's poster later

61
00:02:40,940 --> 00:02:39,630
today so as we can see in this figure

62
00:02:44,270 --> 00:02:40,950
this kind of explains the relationship

63
00:02:47,120 --> 00:02:44,280

of V settles with low pressure if you

64
00:02:49,490 --> 00:02:47,130
grow them at 5 kilo Pascal's or a

65
00:02:51,890 --> 00:02:49,500
hundred and 1.3 kilo Pascal's you see

66
00:02:54,380 --> 00:02:51,900
that at the low pressure they Plateau

67
00:02:56,660 --> 00:02:54,390
very early and they stay that way for a

68
00:02:58,940 --> 00:02:56,670
long time but if you simply re

69
00:03:00,620 --> 00:02:58,950
pressurize them just add air back into

70
00:03:03,890 --> 00:03:00,630
the container they start growing

71
00:03:08,990 --> 00:03:03,900
immediately within so by 7 hours you're

72
00:03:11,470 --> 00:03:09,000
back up to normal culture density so we

73
00:03:13,820 --> 00:03:11,480
want to understand bacillus analysis

74
00:03:15,800 --> 00:03:13,830
ability to adapt to low pressure so we

75
00:03:19,280 --> 00:03:15,810
did an evolution experiment we grew

76
00:03:22,100 --> 00:03:19,290
bacillus Attalus at 5 kilo Pascal's for

77
00:03:24,740 --> 00:03:22,110
a thousand generations this is about 20

78
00:03:27,890 --> 00:03:24,750
weeks where we changed the media every

79
00:03:30,470 --> 00:03:27,900
day putting them in a new culture so

80
00:03:32,060 --> 00:03:30,480
that they have more nutrients and what

81
00:03:33,410 --> 00:03:32,070
came out the other end was this evolved

82
00:03:35,569 --> 00:03:33,420
strain 11:06

83
00:03:39,080 --> 00:03:35,579
and it exhibits increased relative

84
00:03:42,800 --> 00:03:39,090
fitness to its wild-type ancestor 6:28

85
00:03:44,240 --> 00:03:42,810
at 5 kilo Pascal's so we can see this in

86
00:03:48,520 --> 00:03:44,250
the relative cell number so this is

87
00:03:52,090 --> 00:03:48,530
again over a week weeks worth of growth

88
00:03:54,410 --> 00:03:52,100

we see that the relative cell counts the

89
00:03:56,509 --> 00:03:54,420
ancestral strain keeps dropping and

90
00:03:58,460 --> 00:03:56,519
dropping and dropping dropping whereas

91
00:04:02,060 --> 00:03:58,470
the evolved strain stays pretty

92
00:04:04,520 --> 00:04:02,070
consistent consistent whereas at

93
00:04:07,090 --> 00:04:04,530
atmospheric pressure they're pretty

94
00:04:10,640 --> 00:04:07,100
consistent maybe a slight advantage in

95
00:04:15,259 --> 00:04:10,650
1106 but they're about the same no real

96
00:04:18,440 --> 00:04:15,269
significant difference so to explain the

97
00:04:21,710 --> 00:04:18,450
the evolution experiment a little bit

98
00:04:24,120 --> 00:04:21,720
more what we found was that over the 20

99
00:04:26,010 --> 00:04:24,130
weeks or so the optical density of the

100
00:04:27,870 --> 00:04:26,020
culture is at 24 hours where there you

101
00:04:31,230 --> 00:04:27,880
know have to maximum densely they're

102
00:04:34,770 --> 00:04:31,240
going to be at it kept increasing over

103
00:04:37,110 --> 00:04:34,780
time so as we increase from week to week

104
00:04:39,030 --> 00:04:37,120
to week we see that by the end the

105
00:04:41,880 --> 00:04:39,040
optical density is about twenty or two

106
00:04:44,460 --> 00:04:41,890
times higher than the original optical

107
00:04:47,550 --> 00:04:44,470
original total optical density of the

108
00:04:52,370 --> 00:04:47,560
sample this is correlated with the

109
00:04:55,260 --> 00:04:52,380
accumulation of mutations in the in this

110
00:04:59,400 --> 00:04:55,270
batch so this represents the proportion

111
00:05:03,840 --> 00:04:59,410
of a given snip that we found in these

112
00:05:06,390 --> 00:05:03,850
cultures and the most important gene

113
00:05:09,120 --> 00:05:06,400

that we're interested in is mutations in

114
00:05:11,250 --> 00:05:09,130
this gene rjb so right away there are

115
00:05:13,290 --> 00:05:11,260
two different mutations that pop up even

116
00:05:18,210 --> 00:05:13,300
and it's also the first mutation to pop

117
00:05:22,500 --> 00:05:18,220
up you have a premature stop codon rj bc

118
00:05:26,880 --> 00:05:22,510
177 x and a small 9 base pair in frame

119
00:05:28,950 --> 00:05:26,890
deletion are in jb delta 9 so we want to

120
00:05:30,630 --> 00:05:28,960
compare these mutants a little bit to

121
00:05:32,850 --> 00:05:30,640
potentially understand them because they

122
00:05:36,420 --> 00:05:32,860
do exchange in the population a little

123
00:05:39,630 --> 00:05:36,430
bit to do this instead of having just

124
00:05:42,690 --> 00:05:39,640
the normal premature stop codon we

125
00:05:45,630 --> 00:05:42,700
actually constructed a deletion of the

126
00:05:48,180 --> 00:05:45,640
rj b gene by replacing it with a spec

127
00:05:51,090 --> 00:05:48,190
tina myosin resistance cassette and

128
00:05:53,100 --> 00:05:51,100
we're gonna call this strain 1518 and

129
00:05:57,330 --> 00:05:53,110
our NJ b delta 9 is going to be our

130
00:05:59,250 --> 00:05:57,340
evolved strain 1106 so at 5 kilo pascals

131
00:06:01,080 --> 00:05:59,260
they both exhibit increased fitness

132
00:06:05,370 --> 00:06:01,090
relative to the wild-type which we saw

133
00:06:08,930 --> 00:06:05,380
at which we can see here for 1518 and on

134
00:06:11,880 --> 00:06:08,940
the other slides earlier with 1106 and

135
00:06:14,790 --> 00:06:11,890
at 100 and 1.3 kilo pascals there's

136
00:06:17,390 --> 00:06:14,800
actually a difference between 1518 1106

137
00:06:23,190 --> 00:06:17,400
relative to their ancestral wild type

138
00:06:26,780 --> 00:06:23,200

strain so at 101 kilo pascals

139
00:06:28,470 --> 00:06:26,790
15:18 exhibits significantly increased

140
00:06:31,290 --> 00:06:28,480
fitness relative to the wild type

141
00:06:32,909 --> 00:06:31,300
whereas 1106 had no significant

142
00:06:35,180 --> 00:06:32,919
difference so it tells us that there is

143
00:06:36,999 --> 00:06:35,190
a big difference between the australians

144
00:06:40,119 --> 00:06:37,009
in terms of what their

145
00:06:41,999 --> 00:06:40,129
actually doing but they also exhibit a

146
00:06:44,200 --> 00:06:42,009
no significant difference in fitness

147
00:06:46,839 --> 00:06:44,210
relative to each other at either

148
00:06:50,260 --> 00:06:46,849
pressure so it's not exactly clear what

149
00:06:51,850 --> 00:06:50,270
the mechanism is here so before we go

150
00:06:53,769 --> 00:06:51,860
any further we want to understand R&J be

151
00:06:56,049 --> 00:06:53,779
a little bit better it encodes this

152
00:07:00,159 --> 00:06:56,059
protein RNA sj2 it's an endonuclease

153
00:07:02,769 --> 00:07:00,169
which means it cuts RNA and it degrades

154
00:07:04,209 --> 00:07:02,779
and processes ribosomal and messenger

155
00:07:07,089 --> 00:07:04,219
RNA so it's involved in transcription

156
00:07:08,920 --> 00:07:07,099
and protein translation and it's going

157
00:07:11,499 --> 00:07:08,930
to form hetero tetramers with the

158
00:07:13,179 --> 00:07:11,509
essential RNAs j1 so that means if you

159
00:07:17,139 --> 00:07:13,189
delete this protein the bacteria is not

160
00:07:20,799 --> 00:07:17,149
gonna be able to live and the R&J be

161
00:07:22,689 --> 00:07:20,809
delta-9 deletion interrupts this helix v

162
00:07:25,029 --> 00:07:22,699
which I've highlighted in purple over in

163
00:07:29,139 --> 00:07:25,039

the structure so that might indicate

164
00:07:31,600 --> 00:07:29,149
that if this mutation interferes with

165
00:07:35,980 --> 00:07:31,610
our nhj twos ability to interact with

166
00:07:38,350 --> 00:07:35,990
our niche j1 so to isolate the role of

167
00:07:40,119 --> 00:07:38,360
RNA j2 in this low pressure response we

168
00:07:42,489 --> 00:07:40,129
wanted to look we wanted to construct

169
00:07:46,149 --> 00:07:42,499
strains that were just these mutations

170
00:07:49,239 --> 00:07:46,159
so we have our wild-type back room which

171
00:07:50,739 --> 00:07:49,249
is just replacing an amylase locus which

172
00:07:52,360 --> 00:07:50,749
isn't going to have any impact in the

173
00:07:54,339 --> 00:07:52,370
media we're growing them in with

174
00:07:57,959 --> 00:07:54,349
chloramphenicol resistance cassette and

175
00:08:01,089 --> 00:07:57,969
then in that same background 1589 is

176
00:08:04,119 --> 00:08:01,099
inserting that rjb delta-9 mutation and

177
00:08:06,459 --> 00:08:04,129
in 1602 we are just replacing the entire

178
00:08:09,610 --> 00:08:06,469
orange ad gene with a spectrum of myosin

179
00:08:12,629 --> 00:08:09,620
resistance cassette so then we grew them

180
00:08:15,699 --> 00:08:12,639
all individually at five kilo pascals

181
00:08:18,489 --> 00:08:15,709
101.3 at 27 degrees Celsius because if

182
00:08:20,559 --> 00:08:18,499
you grow them at any higher temperature

183
00:08:24,339 --> 00:08:20,569
at five kilo pascals the media starts to

184
00:08:25,929 --> 00:08:24,349
boil until early log phase we extracted

185
00:08:29,889 --> 00:08:25,939
the RNA and sent it off for RNA

186
00:08:31,360 --> 00:08:29,899
sequencing so the files that we get back

187
00:08:33,279 --> 00:08:31,370
are going to be fast cue files that

188
00:08:34,870 --> 00:08:33,289

gives us the sequence and quality scores

189
00:08:36,879 --> 00:08:34,880
that indicate the quality of the

190
00:08:39,430 --> 00:08:36,889
sequence so we can do some quality

191
00:08:41,079 --> 00:08:39,440
control on that then we map them to the

192
00:08:43,420 --> 00:08:41,089
genome with a burden called bowtie two

193
00:08:45,280 --> 00:08:43,430
we do quality control on the mapping

194
00:08:46,199 --> 00:08:45,290
with samstat and then count the

195
00:08:48,890 --> 00:08:46,209
transcripts

196
00:08:51,710 --> 00:08:48,900
well count the match

197
00:08:54,080 --> 00:08:51,720
Breen's then we do statistical analyses

198
00:08:56,840 --> 00:08:54,090
with our we use two different packages

199
00:08:59,840 --> 00:08:56,850
Lima which is very conservative and Dec

200
00:09:01,160 --> 00:08:59,850
2 which is less conservative and we can

201
00:09:03,290 --> 00:09:01,170
look at the intersection of those two

202
00:09:04,580 --> 00:09:03,300
packages to give us a good idea of what

203
00:09:09,110 --> 00:09:04,590
genes are being differentially expressed

204
00:09:11,180 --> 00:09:09,120
without looking at the biases of one

205
00:09:12,710 --> 00:09:11,190
program or another then with the

206
00:09:14,300 --> 00:09:12,720
differentially expressed genes we looked

207
00:09:15,830 --> 00:09:14,310
at and we look at enrichment and

208
00:09:19,760 --> 00:09:15,840
functional analysis with two different

209
00:09:21,110 --> 00:09:19,770
days of databases david and string so

210
00:09:24,530 --> 00:09:21,120
first we want to look at the impact of

211
00:09:26,270 --> 00:09:24,540
the fall deletion at both five and

212
00:09:29,480 --> 00:09:26,280
hundred 1.3 kilo Pascal's it

213
00:09:31,880 --> 00:09:29,490

differentially expressed over 30% of the

214
00:09:35,390 --> 00:09:31,890
transcriptome so this is having a very

215
00:09:39,560 --> 00:09:35,400
large impact on transcription overall at

216
00:09:40,520 --> 00:09:39,570
five kilo Pascal's the n-word when we're

217
00:09:41,960 --> 00:09:40,530
talking about differentially expressed

218
00:09:44,210 --> 00:09:41,970
genes we're talking about the difference

219
00:09:46,280 --> 00:09:44,220
between the mutant that we're looking at

220
00:09:48,530 --> 00:09:46,290
in the wild-type at its particular

221
00:09:50,360 --> 00:09:48,540
pressure just so we're clear

222
00:09:52,790 --> 00:09:50,370
we found that up regulated genes

223
00:09:54,980 --> 00:09:52,800
included antibiotic biosynthesis

224
00:09:55,970 --> 00:09:54,990
integral membrane components a few

225
00:09:57,590 --> 00:09:55,980
positive positive

226
00:09:59,990 --> 00:09:57,600
I have foam regulators and oxidative

227
00:10:01,340 --> 00:10:00,000
phosphorylation genes and when I'm

228
00:10:03,170 --> 00:10:01,350
talking about antibiotic labs and

229
00:10:05,180 --> 00:10:03,180
physicians I'm not talking about one or

230
00:10:07,190 --> 00:10:05,190
two or three I'm talking about every

231
00:10:08,930 --> 00:10:07,200
antibiotic that bacillus Ellis is

232
00:10:11,060 --> 00:10:08,940
capable of making which is about seven

233
00:10:14,120 --> 00:10:11,070
and all of those genes are being

234
00:10:16,430 --> 00:10:14,130
extremely up regulated then down

235
00:10:18,320 --> 00:10:16,440
regulated genes we see that ribosome and

236
00:10:20,810 --> 00:10:18,330
translation associate genes as well as

237
00:10:23,630 --> 00:10:20,820
RNA biosynthesis genes are all down

238
00:10:27,260 --> 00:10:23,640

regulated which given the role of RNAs

239
00:10:31,160 --> 00:10:27,270
j2a ribosomal messenger RNA degrading

240
00:10:33,830 --> 00:10:31,170
protein it's not entirely surprising so

241
00:10:35,990 --> 00:10:33,840
then at atmospheric pressure under 1.3

242
00:10:38,150 --> 00:10:36,000
kilo pascals the upper regulated genes

243
00:10:39,620 --> 00:10:38,160
we found very little enrichment in terms

244
00:10:40,940 --> 00:10:39,630
of what was functionally going on

245
00:10:42,560 --> 00:10:40,950
despite the fact that the number of

246
00:10:46,010 --> 00:10:42,570
differentially expressed genes was very

247
00:10:48,980 --> 00:10:46,020
similar there were a few transcriptional

248
00:10:51,350 --> 00:10:48,990
regulators but even that didn't form any

249
00:10:53,660 --> 00:10:51,360
significant pattern and in the down

250
00:10:57,740 --> 00:10:53,670
regulated genes we saw the same subset

251
00:10:59,930 --> 00:10:57,750
of genes being down regulated R&J b

252
00:11:02,150 --> 00:10:59,940
delta Nayla on the other hand had a very

253
00:11:04,819 --> 00:11:02,160
subtle impact on

254
00:11:06,939 --> 00:11:04,829
transcription at five kilo pascals only

255
00:11:09,199 --> 00:11:06,949
27 genes were differentially expressed

256
00:11:10,629 --> 00:11:09,209
including a few cell wall associated

257
00:11:13,550 --> 00:11:10,639
genes that haven't been characterized

258
00:11:16,670 --> 00:11:13,560
major biofilm component which is TAS a

259
00:11:18,619 --> 00:11:16,680
but neither of the other neither of the

260
00:11:21,019 --> 00:11:18,629
other genes in its operon so that was a

261
00:11:24,079 --> 00:11:21,029
little confusing and several SP beta

262
00:11:26,929 --> 00:11:24,089
prophase genes also down regulated were

263
00:11:29,389 --> 00:11:26,939

some structural ribosome constituents

264
00:11:31,340 --> 00:11:29,399
and purine biosynthesis genes probably

265
00:11:33,470 --> 00:11:31,350
contributing to the same thing that we

266
00:11:35,150 --> 00:11:33,480
saw earlier and at atmospheric pressure

267
00:11:36,559 --> 00:11:35,160
we actually found no significant

268
00:11:39,199 --> 00:11:36,569
differences in gene expressions so

269
00:11:43,579 --> 00:11:39,209
that's telling that this is only

270
00:11:46,040 --> 00:11:43,589
happening at five kilo pascals so we

271
00:11:48,079 --> 00:11:46,050
want to look at the common genes between

272
00:11:49,910 --> 00:11:48,089
the two mutations because that's going

273
00:11:51,530 --> 00:11:49,920
to give us the best idea of what is

274
00:11:54,590 --> 00:11:51,540
functionally going on with this low

275
00:11:55,939 --> 00:11:54,600
pressure response we had fourteen genes

276
00:11:58,550 --> 00:11:55,949
that were being differentially expressed

277
00:12:01,400 --> 00:11:58,560
between the two groups and these were

278
00:12:04,400 --> 00:12:01,410
all the inosine monophosphate synthesis

279
00:12:07,189 --> 00:12:04,410
genes which is a puring precursor the

280
00:12:09,800 --> 00:12:07,199
purines are going to go into RNA and DNA

281
00:12:11,629 --> 00:12:09,810
but and then we also have a bunch of

282
00:12:13,579 --> 00:12:11,639
structural ribosome constituents all on

283
00:12:17,030 --> 00:12:13,589
the same operon which is again

284
00:12:19,189 --> 00:12:17,040
unsurprising then we want to look at the

285
00:12:20,960 --> 00:12:19,199
common up regulated genes these are the

286
00:12:22,910 --> 00:12:20,970
most likely culprits when we're talking

287
00:12:26,740 --> 00:12:22,920
about the low pressure response and here

288
00:12:29,090 --> 00:12:26,750

we had a very short list of 5 3

289
00:12:30,650 --> 00:12:29,100
transmembrane predicted proteins that

290
00:12:33,530 --> 00:12:30,660
have not been characterized two of which

291
00:12:36,290 --> 00:12:33,540
are SP beta prophase genes and one is

292
00:12:39,079 --> 00:12:36,300
totally uncharacterized then we have a

293
00:12:42,230 --> 00:12:39,089
few other genes wrap F which is a

294
00:12:45,230 --> 00:12:42,240
regulator of Khmer calm a is the master

295
00:12:47,900 --> 00:12:45,240
regulator of confidence which is when

296
00:12:50,299 --> 00:12:47,910
you're taking DNA in front into the cell

297
00:12:54,199 --> 00:12:50,309
from outside in the environment and a

298
00:12:58,309 --> 00:12:54,209
stress response protein associated again

299
00:13:01,460 --> 00:12:58,319
with the ESPE beta perfect so this tells

300
00:13:04,429 --> 00:13:01,470
us a lot and very little at the same

301
00:13:06,139 --> 00:13:04,439
time it tells us that RNA sj2 has a very

302
00:13:08,689 --> 00:13:06,149
large role in the regulation of

303
00:13:10,189 --> 00:13:08,699
transcription globally and that the

304
00:13:12,199 --> 00:13:10,199
transcript tonic response of this

305
00:13:14,749 --> 00:13:12,209
mutation are in JB Delta 9 is very

306
00:13:15,710 --> 00:13:14,759
subtle and fortunately gives us a small

307
00:13:18,920 --> 00:13:15,720
list of gene

308
00:13:21,319 --> 00:13:18,930
to actually examine for future purposes

309
00:13:24,410 --> 00:13:21,329
if NASA wanted to fund it which they

310
00:13:26,660 --> 00:13:24,420
don't so I want to thank all of you guys

311
00:13:28,189 --> 00:13:26,670
for coming to my talk I want to thank

312
00:13:30,259 --> 00:13:28,199
the organizers for putting on a great

313
00:13:32,720 --> 00:13:30,269

committee thank you to my committee and

314
00:13:34,550 --> 00:13:32,730
thank you to my lab members I could not

315
00:13:51,290 --> 00:13:34,560
have done that without literally all of

316
00:13:54,170 --> 00:13:51,300
them so thank you guys thank you

317
00:13:56,900 --> 00:13:54,180
um newbie question when you did your

318
00:13:59,990 --> 00:13:56,910
calculations off of the relative fitness

319
00:14:02,900 --> 00:14:00,000
what metrics did you incorporate so what

320
00:14:06,740 --> 00:14:02,910
we do is just serial dilutions of these

321
00:14:09,170 --> 00:14:06,750
cultures at 24 hours so and that it's

322
00:14:11,509 --> 00:14:09,180
also important to note when we're

323
00:14:15,009 --> 00:14:11,519
looking at all of this data I started my

324
00:14:18,139 --> 00:14:15,019
PhD in 2017 in this lab so this is all

325
00:14:20,949 --> 00:14:18,149
some of you guys know Sam waters this is

326
00:14:23,629 --> 00:14:20,959
a lot of her data from her thesis work

327
00:14:24,920 --> 00:14:23,639
but when we're talking about the

328
00:14:27,290 --> 00:14:24,930
relative cell numbers we're talking

329
00:14:29,179 --> 00:14:27,300
about we take this culture we serial

330
00:14:31,220 --> 00:14:29,189
dilute it and then we played it on two

331
00:14:37,040 --> 00:14:31,230
different antibiotics and that gives us

332
00:14:39,379 --> 00:14:37,050
so 1106 has I think I want to say spec

333
00:14:42,079 --> 00:14:39,389
to know myosin and 628 has

334
00:14:44,420 --> 00:14:42,089
chloramphenicol as the resistance marker

335
00:14:46,639 --> 00:14:44,430
so that allows us to tell the difference

336
00:14:53,769 --> 00:14:46,649
in population size just by plating it on

337
00:14:56,389 --> 00:14:53,779
two different antibiotics hi great doc I

338
00:15:01,819 --> 00:14:56,399

hear I bazoom and I have to like just

339
00:15:05,660 --> 00:15:01,829
say something can you go to the genes

340
00:15:07,910 --> 00:15:05,670
did you yeah there yeah no I don't know

341
00:15:09,650 --> 00:15:07,920
them but gene names but I'll be

342
00:15:12,499 --> 00:15:09,660
interested to sit down with you and look

343
00:15:16,040 --> 00:15:12,509
at them as protein names because that

344
00:15:19,009 --> 00:15:16,050
way because in bacterial transcription

345
00:15:20,869 --> 00:15:19,019
and translation are tightly coupled be a

346
00:15:22,699 --> 00:15:20,879
tightly coupled so you might have

347
00:15:23,900 --> 00:15:22,709
something happening on the structure of

348
00:15:26,419 --> 00:15:23,910
the ribosome

349
00:15:27,000 --> 00:15:26,429
yes you might not have but it's good to

350
00:15:31,950 --> 00:15:27,010
look