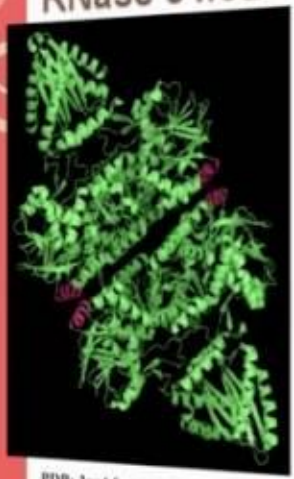
RNase J1/J2 Complex



- Deletion occurs in the stretch of a.a. that separates the metallo-β-lactamase and β-CASP domains.
  - Unique to β-CASP subfamily.

PDB: 3rq4 from:
Newman et al., 2012.*Structure* 19:1241-1251.

1
00:00:10,570 --> 00:00:08,770
good afternoon my name is Sam for those

2
00:00:12,279 --> 00:00:10,580
of you don't know me I work in the

3
00:00:16,299 --> 00:00:12,289
laboratory of Wayne Nicholson at the

4
00:00:18,160 --> 00:00:16,309
kennedy space center in florida and my

5
00:00:19,960 --> 00:00:18,170
project primarily concerns the ability

6
00:00:21,670 --> 00:00:19,970
of a terrestrial microorganism to grow

7
00:00:24,820 --> 00:00:21,680
in an extraterrestrial environment

8
00:00:27,430 --> 00:00:24,830
specifically a low pressure terrestrial

9
00:00:29,800 --> 00:00:27,440
environment so my lab like many of your

10
00:00:32,740 --> 00:00:29,810
labs is primarily an astro biology lab

11
00:00:34,720 --> 00:00:32,750
but outside of astrobiology low pressure

12
00:00:36,639 --> 00:00:34,730
adaptation and microorganisms has

13
00:00:39,190 --> 00:00:36,649

relevance to the field of hyperbaric

14
00:00:42,010 --> 00:00:39,200
food and plant preservation as well as

15
00:00:44,139 --> 00:00:42,020
arrow microbiology it also adds to the

16
00:00:45,940 --> 00:00:44,149
general knowledge of extreme pressure

17
00:00:48,270 --> 00:00:45,950
adaptation and microorganisms and

18
00:00:50,860 --> 00:00:48,280
currently this is only known for

19
00:00:53,170 --> 00:00:50,870
organisms that live in the deep sea or

20
00:00:54,729 --> 00:00:53,180
the PI's of files of the peso's fear now

21
00:00:55,750 --> 00:00:54,739
to give you a reference of the low

22
00:00:58,299 --> 00:00:55,760
pressure i'm going to be talking about

23
00:01:00,310 --> 00:00:58,309
fifty millibars it is approximately one

24
00:01:02,650 --> 00:01:00,320
twentieth of the pressure that is at sea

25
00:01:04,840 --> 00:01:02,660
level on earth it is five times the

26
00:01:07,569 --> 00:01:04,850
highest pressure that is found on the

27
00:01:09,190 --> 00:01:07,579
Martian surface it is also one half of

28
00:01:11,350 --> 00:01:09,200
the lowest pressure that is found in the

29
00:01:12,880 --> 00:01:11,360
troposphere and the troposphere is where

30
00:01:17,170 --> 00:01:12,890
all of the biosphere is currently found

31
00:01:18,880 --> 00:01:17,180
on earth so why do I care about

32
00:01:21,310 --> 00:01:18,890
hyperbaric adaptations in bacillus

33
00:01:23,620 --> 00:01:21,320
subtlest well bacillus seller list is a

34
00:01:25,420 --> 00:01:23,630
very well known species it is probably

35
00:01:27,820 --> 00:01:25,430
behind you coli the most well known

36
00:01:29,590 --> 00:01:27,830
species on this planet its genome is

37
00:01:31,149 --> 00:01:29,600
completely sequenced and it is

38
00:01:33,070 --> 00:01:31,159

genetically tractable which means it is

39
00:01:35,649 --> 00:01:33,080
very easy to manipulate in the lab now

40
00:01:37,780 --> 00:01:35,659
members of the genus of bacillus or

41
00:01:39,749 --> 00:01:37,790
spore formers and spores are extremely

42
00:01:42,399 --> 00:01:39,759
resistant to a wide number of

43
00:01:45,210 --> 00:01:42,409
environmental insults including

44
00:01:48,340 --> 00:01:45,220
long-term duration at space vacuum

45
00:01:49,840 --> 00:01:48,350
spores of bacillus are also common

46
00:01:52,030 --> 00:01:49,850
spacecraft assembly facility

47
00:01:54,130 --> 00:01:52,040
contaminants so with the continued

48
00:01:55,539 --> 00:01:54,140
robotic missions being sent to Mars and

49
00:01:57,730 --> 00:01:55,549
hopefully other extraterrestrial

50
00:01:59,440 --> 00:01:57,740
environments this raises the potential

51
00:02:01,300 --> 00:01:59,450
threat that one of these missions could

52
00:02:04,060 --> 00:02:01,310
carry with it a terrestrial organisms

53
00:02:05,770 --> 00:02:04,070
which could subsequently contaminate the

54
00:02:10,059 --> 00:02:05,780
extra-terrestrial environment and this

55
00:02:12,880 --> 00:02:10,069
is termed forward contamination so in

56
00:02:14,199 --> 00:02:12,890
2006 dr. Andrew sugar another University

57
00:02:16,179 --> 00:02:14,209
of Florida professor at the Kennedy

58
00:02:18,280 --> 00:02:16,189
Space Center in Wayne looked at the

59
00:02:18,750 --> 00:02:18,290
lower pressure limit on growth of a

60
00:02:20,940 --> 00:02:18,760
number of

61
00:02:22,500 --> 00:02:20,950
slow species whose spores are known to

62
00:02:24,569 --> 00:02:22,510
contaminate spacecraft assembly

63
00:02:26,550 --> 00:02:24,579

facilities and what they found is when

64
00:02:29,039 --> 00:02:26,560
you get down to 25 millibars this is

65
00:02:30,809 --> 00:02:29,049
inhibitory to their growth important to

66
00:02:31,979 --> 00:02:30,819
note it is not lethal to their growth

67
00:02:33,690 --> 00:02:31,989
when you bring them back up to a

68
00:02:35,670 --> 00:02:33,700
pressure at which they can grow they do

69
00:02:37,289 --> 00:02:35,680
grow so there's something about low

70
00:02:40,500 --> 00:02:37,299
pressure that is inhibitory to the

71
00:02:43,020 --> 00:02:40,510
cellular functions in bacillus now Wayne

72
00:02:45,300 --> 00:02:43,030
asked could you adapt vasilis subtlest

73
00:02:49,080 --> 00:02:45,310
to grow better at low pressure and by

74
00:02:52,619 --> 00:02:49,090
doing so study low pressure adaptation

75
00:02:54,330 --> 00:02:52,629
and how low pressure affects cells so

76
00:02:56,309 --> 00:02:54,340
along these lines you set up a thousand

77
00:02:59,399 --> 00:02:56,319
generation continuous culture experiment

78
00:03:02,819 --> 00:02:59,409
at fifty millibars 27 degrees C and lb

79
00:03:05,670 --> 00:03:02,829
media the input or the ancestral strain

80
00:03:07,920 --> 00:03:05,680
is our laboratory 624 now 624 doesn't

81
00:03:09,360 --> 00:03:07,930
grow very well at 50 millibars it

82
00:03:12,330 --> 00:03:09,370
reaches an optical density of

83
00:03:14,819 --> 00:03:12,340
approximately 40 klett units during the

84
00:03:16,530 --> 00:03:14,829
thousand generation experiment weekly

85
00:03:18,349 --> 00:03:16,540
average optical densities seem to

86
00:03:21,059 --> 00:03:18,359
indicate periods of punctuated evolution

87
00:03:22,949 --> 00:03:21,069
at the terminus of the experiment a

88
00:03:26,220 --> 00:03:22,959

stream is isolated from the growth media

89
00:03:27,990 --> 00:03:26,230
which we call 1106 now 1106 grows to a

90
00:03:30,059 --> 00:03:28,000
higher optical density at 50 millibars

91
00:03:32,280 --> 00:03:30,069
reaching approximately 80 to 85 light

92
00:03:34,319 --> 00:03:32,290
units it also has a higher relative

93
00:03:36,059 --> 00:03:34,329
fitness than the ancestor meaning that

94
00:03:38,309 --> 00:03:36,069
if you compete them in a flask at 50

95
00:03:42,569 --> 00:03:38,319
millibars the ancestor gets knocked out

96
00:03:43,650 --> 00:03:42,579
of the growth media so the first series

97
00:03:45,390 --> 00:03:43,660
of experiments looking at the

98
00:03:48,449 --> 00:03:45,400
differences between hypobaric evolved

99
00:03:50,550 --> 00:03:48,459
1106 and the ancestral 624 was a series

100
00:03:52,020 --> 00:03:50,560
of transcriptional microarrays now there

101
00:03:53,729 --> 00:03:52,030
were some similarities in their response

102
00:03:55,710 --> 00:03:53,739
to low pressure they're both still very

103
00:03:57,059 --> 00:03:55,720
stressed out at low pressure so they

104
00:03:59,550 --> 00:03:57,069
turn on their general stress response

105
00:04:01,680 --> 00:03:59,560
which is dependent upon the alternative

106
00:04:03,539 --> 00:04:01,690
transcription factor Sigma B they also

107
00:04:05,189 --> 00:04:03,549
due to lowering the pressure in the head

108
00:04:07,530 --> 00:04:05,199
space above media you're lowering the

109
00:04:09,240 --> 00:04:07,540
oxygen available for the bugs so they

110
00:04:11,460 --> 00:04:09,250
both turn on their hypoxic related

111
00:04:14,099 --> 00:04:11,470
signals now there's some dissimilarities

112
00:04:17,129 --> 00:04:14,109
at low pressure in the hyperbaric

113
00:04:19,020 --> 00:04:17,139

evolved strain it regulates a system

114
00:04:20,759 --> 00:04:19,030
which is involved in sulphate metabolism

115
00:04:23,399 --> 00:04:20,769
there's also a difference in their iron

116
00:04:26,040 --> 00:04:23,409
responses at low pressure and in the

117
00:04:27,899 --> 00:04:26,050
ancestral 624 strain it highly induces

118
00:04:30,899 --> 00:04:27,909
its flagella operon and this isn't seen

119
00:04:32,490 --> 00:04:30,909
in 1106 now across all of my pressure

120
00:04:34,590 --> 00:04:32,500
transcriptional micro razor

121
00:04:36,480 --> 00:04:34,600
approximately a thousand a thousand

122
00:04:39,120 --> 00:04:36,490
genes that are differentially expressed

123
00:04:41,580 --> 00:04:39,130
that is about twenty-five percent of

124
00:04:43,380 --> 00:04:41,590
bacillus at alyssas genome and a lot of

125
00:04:47,430 --> 00:04:43,390
hay to sort through for a couple needles

126
00:04:49,470 --> 00:04:47,440
for my dissertation so as I said during

127
00:04:51,390 --> 00:04:49,480
the thousand generations it looks like

128
00:04:53,310 --> 00:04:51,400
there are periods of punctuated

129
00:04:55,500 --> 00:04:53,320
evolutionary changes that leads one to

130
00:04:57,270 --> 00:04:55,510
believe that there were specific changes

131
00:04:59,730 --> 00:04:57,280
to the genome that gave rise to the

132
00:05:04,200 --> 00:04:59,740
phenotype that we see an 1106 at fifty

133
00:05:06,150 --> 00:05:04,210
millibars so I looked for these changes

134
00:05:08,490 --> 00:05:06,160
by conducting whole ginamarie sequencing

135
00:05:10,830 --> 00:05:08,500
and so far in my analysis i have found

136
00:05:13,740 --> 00:05:10,840
ten single nucleotide polymorphisms or

137
00:05:15,630 --> 00:05:13,750
snips now what's interesting is four of

138
00:05:18,390 --> 00:05:15,640

these occurred in a ten nucleotide

139
00:05:20,190 --> 00:05:18,400
stretch of the R&J be coding region now

140
00:05:22,800 --> 00:05:20,200
when I pcr-amplified Andhra sequenced

141
00:05:24,300 --> 00:05:22,810
this region I found that these are not

142
00:05:26,280 --> 00:05:24,310
snips at all but in fact a nine

143
00:05:29,159 --> 00:05:26,290
nucleotide deletion which results in a

144
00:05:30,960 --> 00:05:29,169
three codon in frameshift so what you're

145
00:05:33,030 --> 00:05:30,970
seeing here is the region of interest in

146
00:05:35,250 --> 00:05:33,040
the ancestral strain and then below that

147
00:05:37,680 --> 00:05:35,260
is my hyperbaric evolved protein

148
00:05:40,640 --> 00:05:37,690
sequence and it resulted in the deletion

149
00:05:45,030 --> 00:05:40,650
of an alanine a lysine and an isoleucine

150
00:05:48,810 --> 00:05:45,040
now our NJ b codes for rnas j2 rnase j's

151
00:05:51,090 --> 00:05:48,820
are members of the beta casts members of

152
00:05:53,760 --> 00:05:51,100
the beta cast family which is it within

153
00:05:56,700 --> 00:05:53,770
the superfamily of the metal 0 beta

154
00:05:58,290 --> 00:05:56,710
lactamase family they work on members of

155
00:06:01,170 --> 00:05:58,300
this family work on either RNA or DNA

156
00:06:04,080 --> 00:06:01,180
substrates rnase JS work on RNA

157
00:06:06,600 --> 00:06:04,090
substrates so in bacillus titleist rnas

158
00:06:08,850 --> 00:06:06,610
j2 is involved in global messenger RNA

159
00:06:11,490 --> 00:06:08,860
processing in the cell it is an

160
00:06:12,510 --> 00:06:11,500
endonuclease with extremely low five

161
00:06:15,930 --> 00:06:12,520
prime to three prime exonuclease

162
00:06:18,390 --> 00:06:15,940
activity in the cell it forms

163
00:06:21,659 --> 00:06:18,400

heterodimers with rnas j1 it is believed

164
00:06:24,360 --> 00:06:21,669
that the predominant form of the rnas j1

165
00:06:27,300 --> 00:06:24,370
j2 complex is actually a tetramer now

166
00:06:28,500 --> 00:06:27,310
our NH j 1 is essential it has the the

167
00:06:30,690 --> 00:06:28,510
main five prime to three prime

168
00:06:33,240 --> 00:06:30,700
exonuclease activity in the complex and

169
00:06:36,060 --> 00:06:33,250
it is also an endonuclease activity has

170
00:06:39,719 --> 00:06:36,070
a nucleus activity rnas j2 is

171
00:06:42,300 --> 00:06:39,729
non-essential however when rnas j2 binds

172
00:06:44,380 --> 00:06:42,310
with rnase j1 it affects the substrate

173
00:06:49,150 --> 00:06:44,390
specificity of the endonuclease

174
00:06:51,520 --> 00:06:49,160
of rnase j1 so what are the possible

175
00:06:53,920 --> 00:06:51,530
effects of this mutation on my mutant

176
00:06:55,720 --> 00:06:53,930
protein well the three codon deletion

177
00:06:59,440 --> 00:06:55,730
happened in a very highly conserved

178
00:07:01,810 --> 00:06:59,450
region of rnase JS which forms a the

179
00:07:03,700 --> 00:07:01,820
helix 5 structure so I thought okay if

180
00:07:05,740 --> 00:07:03,710
you delete some amino acids and a helix

181
00:07:08,470 --> 00:07:05,750
you're possibly disrupting the rest of

182
00:07:10,240 --> 00:07:08,480
the helix so I did an in silico analysis

183
00:07:12,970 --> 00:07:10,250
for a secondary structure prediction and

184
00:07:14,890 --> 00:07:12,980
what you're seeing here is the top

185
00:07:16,800 --> 00:07:14,900
sequence are the amino acids in the

186
00:07:19,750 --> 00:07:16,810
wild-type protein that form helix

187
00:07:21,220 --> 00:07:19,760
underneath that or is my mutant the

188
00:07:23,490 --> 00:07:21,230

corresponding amino acids in my mutant

189
00:07:25,900 --> 00:07:23,500
and they no longer have helix hits

190
00:07:28,180 --> 00:07:25,910
underneath that is the corresponding

191
00:07:33,190 --> 00:07:28,190
amino acids that form helix five and

192
00:07:35,530 --> 00:07:33,200
rnase j1 now rnas j1 abyssal subtlest

193
00:07:37,120 --> 00:07:35,540
has recently been crystallized so i did

194
00:07:38,620 --> 00:07:37,130
a tertiary structure prediction which

195
00:07:42,940 --> 00:07:38,630
agrees with the secondary structure

196
00:07:44,950 --> 00:07:42,950
prediction on your left is the mutant

197
00:07:46,660 --> 00:07:44,960
protein and as you can see that aren't

198
00:07:49,150 --> 00:07:46,670
that helix five is no longer forming

199
00:07:53,890 --> 00:07:49,160
this is the corresponding helix five in

200
00:07:56,680 --> 00:07:53,900
RNA sj1 now this is believed to be how

201
00:07:59,320 --> 00:07:56,690
the rnas j1 j2 complex looks like in the

202
00:08:01,720 --> 00:07:59,330
cell I've highlighted the helix five in

203
00:08:04,450 --> 00:08:01,730
pink and as you can see it's on the

204
00:08:05,830 --> 00:08:04,460
tetra more interface it is also occurs

205
00:08:07,690 --> 00:08:05,840
in a stretch of amino acids that

206
00:08:09,790 --> 00:08:07,700
separates the metallic 8a lactamase

207
00:08:11,470 --> 00:08:09,800
domain from the beta calf's domain and

208
00:08:13,630 --> 00:08:11,480
this is unique to the beta cast

209
00:08:16,150 --> 00:08:13,640
subfamily and it ties the two domains

210
00:08:21,250 --> 00:08:16,160
together and could possibly have impacts

211
00:08:23,830 --> 00:08:21,260
on their catalytic efficiency so the

212
00:08:25,660 --> 00:08:23,840
mutations in 1106 is our NJ b may be

213
00:08:29,110 --> 00:08:25,670

causing structural changes that affect

214
00:08:32,290 --> 00:08:29,120
the messenger RNA processing of this RNA

215
00:08:34,570 --> 00:08:32,300
j1 j2 complex this in turn may lead to

216
00:08:36,820 --> 00:08:34,580
an increase in messenger RNA half-life

217
00:08:39,670 --> 00:08:36,830
which at 50 mil of ours may cause an

218
00:08:42,100 --> 00:08:39,680
increase in product there may also yet

219
00:08:44,680 --> 00:08:42,110
be an unknown specific activity of rnase

220
00:08:46,960 --> 00:08:44,690
j 2 and this activity may be disrupted

221
00:08:49,330 --> 00:08:46,970
by these mutations and the disruption of

222
00:08:54,040 --> 00:08:49,340
this activity could be affecting the

223
00:08:56,410 --> 00:08:54,050
essential function of rnase j1 so to

224
00:08:58,120 --> 00:08:56,420
test these hypotheses

225
00:09:00,639 --> 00:08:58,130
I recently received an orange a bee

226
00:09:03,129 --> 00:09:00,649
mutant from dr. stole keys lab a very

227
00:09:06,100 --> 00:09:03,139
generous gift and I'm using this to

228
00:09:07,870 --> 00:09:06,110
transform my hyperbaric evolved 1106 in

229
00:09:09,490 --> 00:09:07,880
my ancestral strain and then I'm going

230
00:09:11,829 --> 00:09:09,500
to compete the mutants and see what

231
00:09:14,470 --> 00:09:11,839
their fitness is at fifty millibars I'd

232
00:09:16,389 --> 00:09:14,480
also like to do a messenger RNA decay

233
00:09:18,610 --> 00:09:16,399
rate to see if any of the substrates of

234
00:09:20,319 --> 00:09:18,620
the RNAs j1 j2 complex have an increase

235
00:09:22,689 --> 00:09:20,329
in half-life or possibly a decrease in

236
00:09:25,810 --> 00:09:22,699
half-life and I would also like to do a

237
00:09:29,680 --> 00:09:25,820
protein analysis of the of the RNA j1 j2

238
00:09:31,629 --> 00:09:29,690

complex in 1106 to see if the tetramer

239
00:09:34,509 --> 00:09:31,639
is being disrupted and possibly maybe

240
00:09:36,460 --> 00:09:34,519
even the heterodimer as well but

241
00:09:38,500 --> 00:09:36,470
ultimately I would like to tie in these

242
00:09:40,540 --> 00:09:38,510
mutations to what we saw on the

243
00:09:43,509 --> 00:09:40,550
continuous culture experiment so I would

244
00:09:45,790 --> 00:09:43,519
like to test the population before an

245
00:09:47,680 --> 00:09:45,800
optical density jump and after an

246
00:09:49,180 --> 00:09:47,690
optical density jump and I could see one

247
00:09:51,040 --> 00:09:49,190
of two things I could see that this 9

248
00:09:53,500 --> 00:09:51,050
nucleotide deletion happened all at once

249
00:09:55,120 --> 00:09:53,510
or it slowly happened and there were

250
00:09:56,560 --> 00:09:55,130
multiple little deletion events that

251
00:10:04,290 --> 00:09:56,570
occurred and ultimately gave rise to

252
00:10:04,300 --> 00:10:22,990
thank you so any questions for Samantha

253
00:10:28,460 --> 00:10:26,420
hey I was just wondering why why is low

254
00:10:33,710 --> 00:10:28,470
pressure bad for bacteria why don't they

255
00:10:39,110 --> 00:10:33,720
like that why is it inhibitory so many

256
00:10:42,199 --> 00:10:39,120
reasons uh-huh well so pressure is

257
00:10:44,870 --> 00:10:42,209
sometimes compared to temperature where

258
00:10:47,269 --> 00:10:44,880
high pressure is compared to cold

259
00:10:48,980 --> 00:10:47,279
temperature extreme cold temperature low

260
00:10:51,079 --> 00:10:48,990
pressure could be compared to high

261
00:10:53,780 --> 00:10:51,089
temperature so you could be having too

262
00:10:56,749 --> 00:10:53,790
much expansion of catalytic lovellette

263
00:10:58,309 --> 00:10:56,759

enzymes the membrane could become too

264
00:11:00,170 --> 00:10:58,319
fluid so you could have leakage across

265
00:11:03,079 --> 00:11:00,180
the membrane of water molecules and

266
00:11:05,629 --> 00:11:03,089
other things but honestly nobody knows

267
00:11:08,990 --> 00:11:05,639
because nobody studies low pressure at

268
00:11:11,689 --> 00:11:09,000
all except for apparently me so the only

269
00:11:13,879 --> 00:11:11,699
thing that I've seen is they will take

270
00:11:17,509 --> 00:11:13,889
plants down to 100 millibars which is

271
00:11:20,120 --> 00:11:17,519
still you know not as low as bacteria

272
00:11:22,610 --> 00:11:20,130
can go obviously to down to 25 millibars

273
00:11:25,550 --> 00:11:22,620
or even lower in some cases to march and

274
00:11:28,550 --> 00:11:25,560
pressure some Mel bars but um there it

275
00:11:30,400 --> 00:11:28,560
could be so many things go wrong and

276
00:11:33,620 --> 00:11:30,410
including in eukaryotes there's a

277
00:11:35,269 --> 00:11:33,630
increase in oxidative stress to you have

278
00:11:37,819 --> 00:11:35,279
an increase of reactive oxygen and

279
00:11:39,559 --> 00:11:37,829
nitrogen species ron's when they expose

280
00:11:52,519 --> 00:11:39,569
mice and other animals to like low

281
00:11:55,670 --> 00:11:52,529
pressure so lots of things is there is

282
00:11:59,720 --> 00:11:55,680
it a homogeneous population at the end

283
00:12:02,090 --> 00:11:59,730
or have you seen any distinct different

284
00:12:05,120 --> 00:12:02,100
populations within the continuous growth

285
00:12:08,090 --> 00:12:05,130
culture for the continuous culture so

286
00:12:09,679 --> 00:12:08,100
one strain was isolated from the growth

287
00:12:12,590 --> 00:12:09,689
culture that doesn't mean that there

288
00:12:15,100 --> 00:12:12,600

aren't more hyperbaric evolved strains

289
00:12:17,420 --> 00:12:15,110
in there but right now I just study 1106

290
00:12:19,040 --> 00:12:17,430
so yes looking at the whole population

291
00:12:35,670 --> 00:12:19,050
is actually something my boss wants to

292
00:12:41,650 --> 00:12:39,700
don't need repeat that question so do

293
00:12:49,440 --> 00:12:41,660
the strains that have been adapted to

294
00:12:55,500 --> 00:12:53,530
so I have not adapted a strain to high

295
00:13:00,250 --> 00:12:55,510
pressure and grown it out low pressure

296
00:13:01,810 --> 00:13:00,260
however there is a genus of bacteria the

297
00:13:04,210 --> 00:13:01,820
first gram positive that was isolated

298
00:13:06,970 --> 00:13:04,220
from the peso's fear its karna bacterium

299
00:13:09,340 --> 00:13:06,980
karna bacterium also grows at marsh and

300
00:13:13,120 --> 00:13:09,350
conditions so extremely low pressure so

301
00:13:15,430 --> 00:13:13,130
it is very plastic genus so it is

302
00:13:18,310 --> 00:13:15,440
possible for an organism to grow both at

303
00:13:21,250 --> 00:13:18,320
high pressure and low pressure I hope I