NOTEBOOK

1. **Wetwork**

JUNE

18 – Our first day lab! Agar nutrient medium for bacteria was prepared by Marcos and Maria.

Luna reactivated Shewanella in liquid culture, 28°C. After 1 hour in liquid LB culture, Maria plated Shewanella in solid LB agar nutrient medium at 28°C.

19 – The bacteria's growth was observed and transferred to a plate with LB solid medium (28°C) and liquid culture at 37°C.

20 – The resistance testing for Shewanella to antibiotics was performed. Kanamicin and Ampicilin were tested in concentration range of 50 to 250µg/ml.

21 – We extracted DNA from Shewanella.

22 -- PCR 16S from Shewanella was performed.

24 – The sequencing reaction was performed.

Luna and Jennifer performed celular's growth medition (OD) from Shewanella.

We made quimiocompetent *E. coli* S17 cells this week for growing our suicides plasmids (pnpt and pknock) and biobricks, therefore, *E. coli* S17 was inoculated on fresh medium today.

25 – We made quimiocompetent *E. coli* S17 cells and put them in the -80°C freezer.

E.coli colonies with the suicide plasmid pknock were inoculated in LB liquid culture.

26 – We performed suicide plasmid pknock isolation through Plasmid Prep Mini Spin Kit (GE Healtcare) in *S. putrefaciens* to investigate if had some original plasmid.

27 - DNA sequencing gives *Chromobacterium violaceum*.

28 – We asked for a new *Shewanella Putrefaciens* in ATCC.

The -suicide plasmid pknock was precipitated.

We transformed plasmid suicide pnpt into commercial eletrocompetent cells. It got growing over the weekend.

29 – Marcos prepared LB medium and Jennifer prepared LB medium with oil.

30 – Colonies with suicide plasmid pknock was inoculated for plasmid extraction tomorrow.

JULY

1 – Marcos made again the pknock plasmid extraction of new bacteria colonies (the extraction performed before results showed up very very very weak DNA)

2 – Transformation with suicide plasmid pnpt was succesful and transferred to solid LB medium with 25µg/mL of Kanamicin.

3 - We made quimiocompetent *E. Coli* S17 cells and the cells were transformed with both GFP gene and gene of Ampicillin resistance.

4 – We had a meeting about the chronogram methodologies to increase the FadL expression and to interrupt FadR expression.

5 – *S. Putrefaciens* finally came to us!!!!!

We made the transformation of pGSM in S17 quimiocompetent, plasmid resistant to Ampicillin.

S. putrefaciens was plated in LB medium and in growing culture.

The RFP coding device biobricks (BBa_J04450) was transformed in *E. coli* DH5α for transformation testing.

6 – Luna and Maria prepared LB culture medium. Then

they performed the antibiotics resistence testing in *S. putrefaciens* with Ampicilin, Kanamicin and Chloramphenicol in concentration range of 34 to 200µg/ml.

7 – *S. putrefaciens* and *E. coli* DH10B were inoculated in LB medium.

Also transformants with pnpt, pknock and RFP coding device biobricks (BBa_J04450) were inoculated for plasmid isolation of them.

8 – We extracted DNA from the transformants colonies of *S. putrefaciens*, and also from pnpt and RFP coding device biobricks (BBa_J04450).

Lais prepared competent cells with DH10B and Jennifer made the *S. putrefaciens*` inoculum for optic density (OD) observation and growing curve making afterwards.

MFC

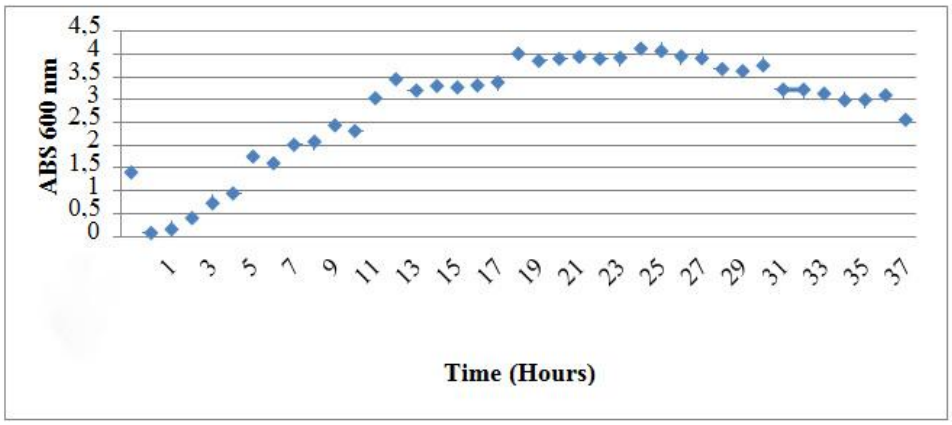
For this month we searched in the literature about the MFC – fuel cell microbian (the materials that we need to development and how it works)

9 – It was observed the cellular growing in *Shewanella putrefaciens*.

We transformed plasmid puc in- quimiocompetent cells DH10B.

DNA and plasmids were quantified using nanodrop.

10 – The cellular growth of *Shewanella putrefaciens* had been observed.



Cell growth curve of *Shewanella putrefaciens* in LB medium at 30°C and 150 rpm for 37 hours.

11 – *Shewanella putrefaciens* was stored at -80°C, and it was inoculated for competent preparation. *E.coli* S17 and cells with pknock and pnpt were also were inoculated.

The *S. putrefaciens* testing of different pH, medium and temperature are started!

12 – We made eletrocompetents cells with *S. putrefaciens*.

S. Putrefaciens was inoculated in media with pH 4 in both 30°C and 37°C temperatures

13 - We transformed RFP coding device biobrick with pSB1C3 plasmid (BBa_J04450) in *S. putrefaciens* competent cells.

The *S. putrefaciens*' growth in culture with oil at either 30°C and 37°C with pH 4 was analyzed.

15 – *S. putrefaciens* transformed with RFP coding device biobrick (BBa_J04450) did not grow. The transformation of *S. putrefaciens* with another biobrick it had been repeated and it has the p15A replication origin in the pSB3C5 plasmid (BBa_I50032).

16 – Transformation with RFP coding device biobrick (BBa_J04450) to test *E. coli* DH10B, *E. coli* DH5α and *E. coli* S17 quimiocompetent cells.

17 – RFP coding device (BBa_J04450) and PNPT plasmid isolation from the transformants were performed.

The *S. Putrefaciens* transformation didn't work, so it was made a new transformation in *S. putrefaciens* with biobrick (BBa_I50032) in pSB3C5 plasmid.

Transformation of new biobricks for *S. putrefaciens* (pSB3K3 [BBa_J04450] and pSB3C5 [BBa_I50032]) were made in DH10B to obtain clones.

18 – We analyzed all the plasmids in our little box using agarose gel in order to know what was functional or not.

S. putrefaciens was prepared to be eletrocompetent and transformed with suicide plasmid pnpt and pknock, and with biobricks with p15A replication oringin (BBa_J04450 and BBa_I50032), compatible with *Shewanella putrefaciens*).

Jennifer observed the *S. putrefaciens*` growth in culture with oil.

19 – -The eletrotransformation of *S. putrefaciens* haven't worked out in a long time, so we prepared quimiocompetents *S. putrefaciens* and transformed with suicide plasmid pnpt and pknock; and with plasmids biobricks pSB3K3 and pSB3C5.

– The transformation in *S. putrefaciens* didn't work once again.

S. putrefaciens was inoculated in culture growing with different concentrations of 5, 10 and 15% of oil either with and without Triton X-100.

23 – *S. Putrefaciens* was inoculated in media with pH 5 at both 30°C and 37°C.

24 – The transformation of *S. putrefaciens* was made again with a new transformation protocol (fast transformation with sorbitol).

The plasmids properly isolated were quantified.

25 – The *S. putrefaciens*` growth in culture with oil at either 30°C and 37°C in pH 5 was analyzed.

26 – The biobricks (pSB3K3 and pSB3C5) were transformed in *S. putrefaciens*.

AUGUST

6 – We performed a biobrick searching to figure out which plasmids should replicate in *S. putrefaciens* (p15A replication origin), e.g. pSB3K3 (BBa_J04450), pSB3C5 (BBa_I50032) and pSB3T5 (BBa_I50032).

The *S. putrefaciens*` growth had been observed and compared with the previous results.

7 – The biobricks with p15A replication origin were transformed in competent commercial cells.

- We made solutions to Genomic DNA extration:

Solution 1: Buffer oral A (0,3 M sucrose; 50Mm Tris-Hcl pH 8.5 ; 5 Mm EDTA)

Solution 2 : Proteinase K (1mg/50μl)

Solution 3 : Lysozyme (2mg/50μl)

Solution 4 : SDS 10%

TE Buffer(50mM Tris-HCl; 1mM EDTA)

MFC

For this month we made the logistic to buy the MFC's materials: electrode, membrane, tygon tubes, threaded rod, wingnut, peristaltic bomb, acrylic and extras materials like silicone, silicone paste, copper wires, etc.

8 -Transformation of biobricks pSB3K3 (BBa_J04450), pSB3C5 (BBa_I50032) and pSB3T5 (BBa_I50032) were successful done and it was transferred to LB medium for overnight growth (they also were extracted from colonies).

It was performed genomic DNA extration of *E. coli* S17 and *Shewanella putrefaciens* (hopefully).

S. putrefaciens was inoculated in media with pH 6 at both 30°C and 37°C.

9 – The biobricks transformants pSB3K3 (BBa_J04450), pSB3C5 (BBa_I50032) and pSB3T5 (BBa_I50032) were extracted with Plasmid Prep Mini Spin Kit, (GE Healthcare) and quantified using nanodrop.

The plasmid pSB3K3 showed a very low concentration, so it was inoculated again to make a new plasmid isolation from the transformants.

PCR was performed to amplify 16S rDNA of *E. coli* S17 and *S. Putrefaciens* to choose the best dilution of DNA (1X or 10X).

Resuspetion of FadR gene primers in TE buffer was made.

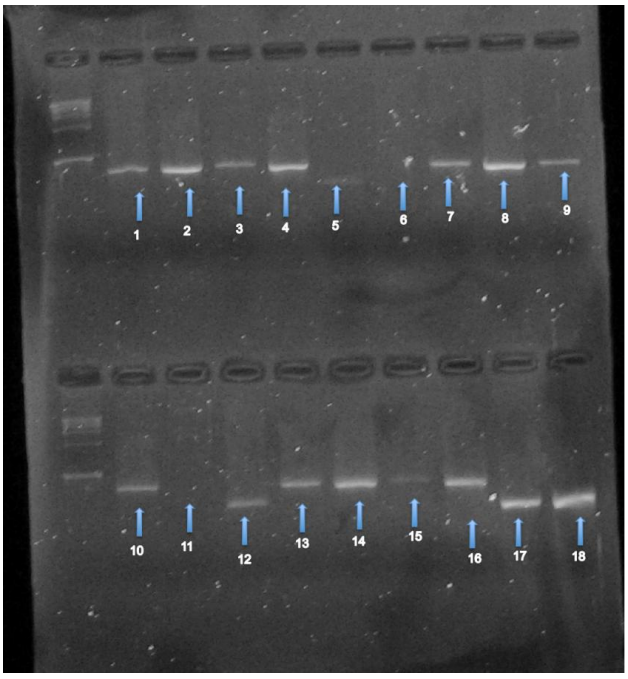
10 – The plasmids pSB3K3 were isolated and quantified.

The plasmids were put into a microtube and and it was concentrated to 100 ng/µL in lyophilizer.

12 - PCR to amplify the FadR gene out of BBa_K1076000 and the upstream and dowstream regions of the gene FadR out of BBa_K1076001.

Mix :

DNA	1µl
10X Buffer	2,5 µl
50 mM $MgCl_2$	0,75 µl
2,5 mM dNTPs	2,5 µl
10 pmol/µl Primer Forward	0,5µl
10 pmol/µl Primer Reverse	0,5 µl
5u/µl Taq polymerase	0,3µl
Milli-Q water	16,95µl
Total	25 µl



1-6 The anelling tempeture was 56°C (1 and 2 are amplification of upstream gene, 3 and 4 are amplification of downstream gene and 5 and 6 are amplification of internal gene); 7-12 The anelling tempeture was 57°C (7,8 – up/ 9,10 – down/ 11,12- int); 13 – 18 The anelling tempeture was 57 °C (13,14 – up/ 15,16 - down/17,18- int).

The *S. putrefaciens* growth in culture with oil in pH 6 at both 30°C and 37°C was analyzed.

The inoculum of *S. putrefaciens* growth in culture with was analyzed once more again and again and again!!

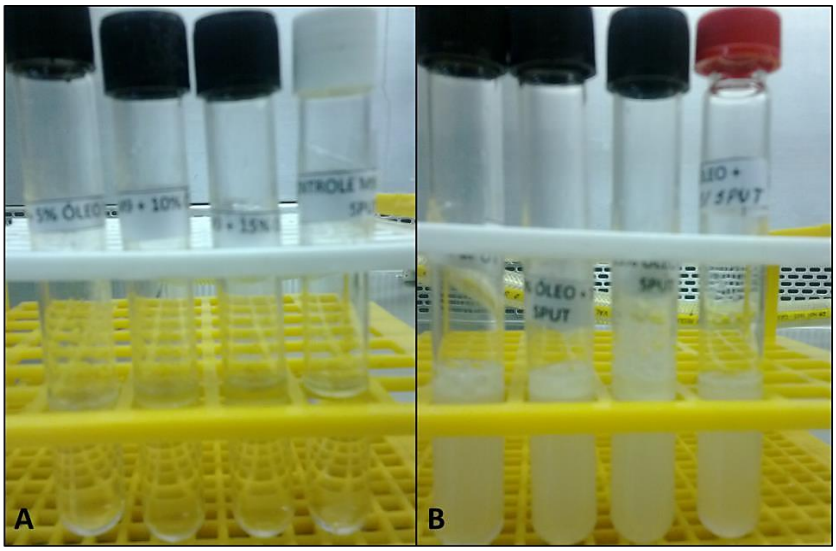


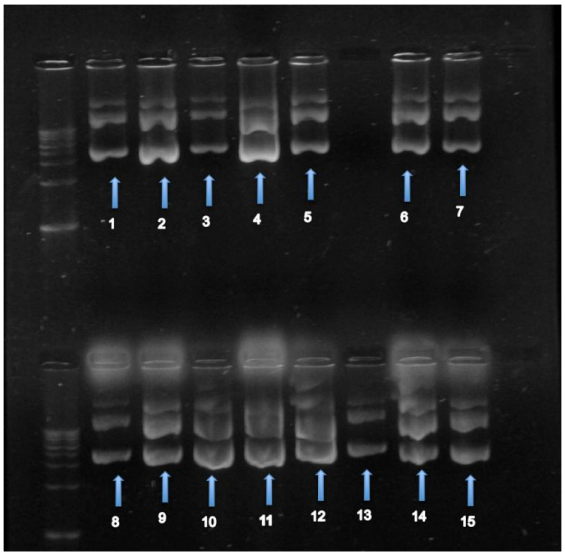
Figure: (A) Test of soybean oil without triton; (B) Tests of soybean oil emulsified with triton.

13 - Ligation of PCR products in TOPO 2.1 and then plasmids transformation into *E. coli* DH10B.

14 It was performed isolation and inoculation of white colonies for transformation to do plasmid extration later on.

It was also performed pNPTS138 and pKNOCK purification with SDS 1% and potassium acetate.

15 - Miniprep of 15 colonies (5 - upstream/ 5- internal (FadR)/ 5- downstream):

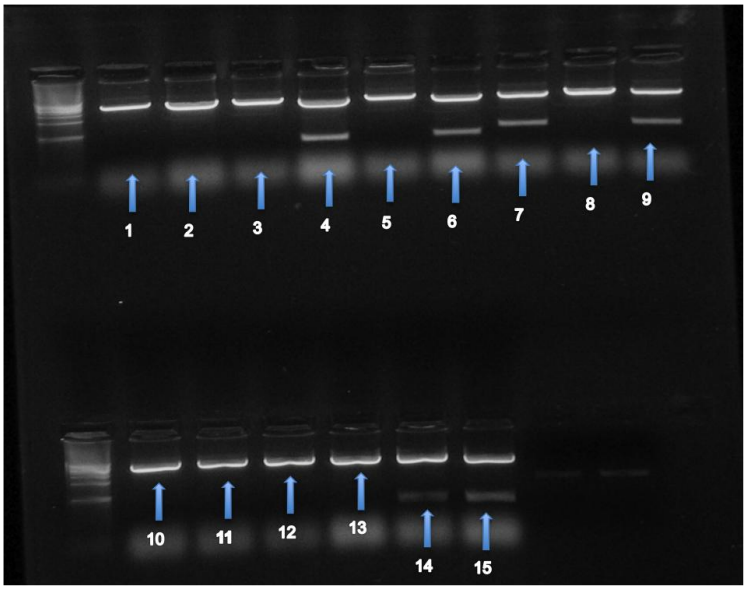


1-5 : topo with amplicon of upstream gene; 6-10: topo with amplicon of downstream gene; 11-15 : topo with amplicon of internal gene.

16 - Digestion of miniprep plasmid with EcoRI was done to check if our plasmids could been inserted.

We didn't get the upstream insert. Because of that, Luna remade the product ligation of upstream gene PCR in TOPO vector 2.1.

Digestion gel



4,5,14,15 TOPO with a confirmed insert of internal gene/ 7,9 TOPO with a confirmed insert of downstream gene.

17 - To insert these genes (Internal [FadR] / downstream) in pKNOCK and pNPTS138 we need set up a double digestion of the TOPO vector with the internal insert with BamHI and APAI and to the TOPO vector with downstream insert we used BamHI and NdeI. After that Luna gel purified the insert of the digestion product.

And then, also made the sequencing of these cloning vectors with the inserts

In parallel, we did the isolation of colonies appeared from last night.

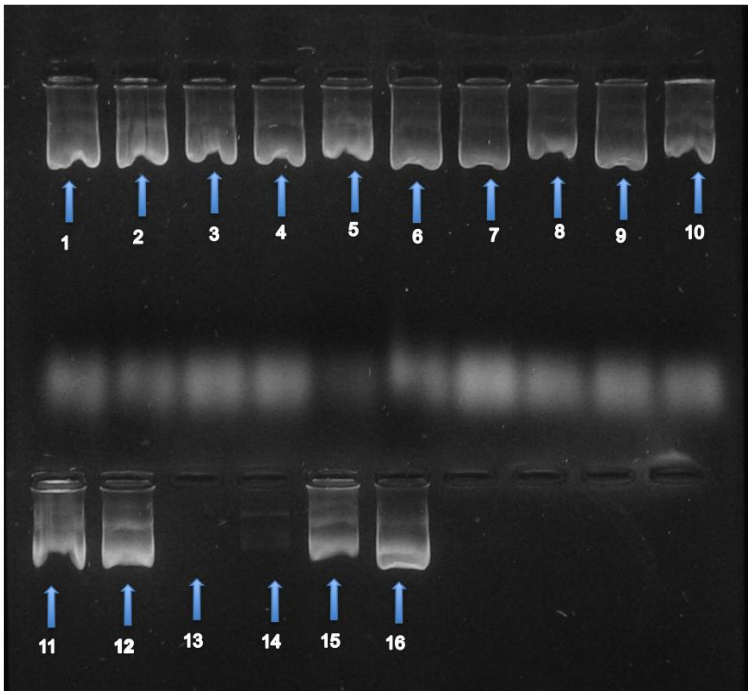
18 - Inoculation of the isolated colonies to make miniprep tomorrow

Jennifer prepared LB media with oil

19 - Miniprep of overnight cultures, then set up a new digestion with EcoRI, but the Upstream insert didn't appear again.

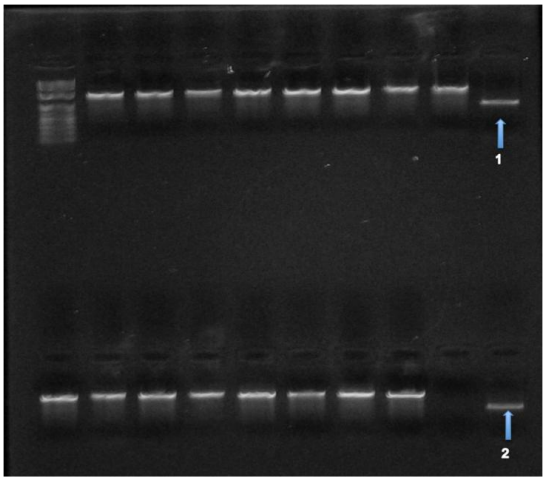
So we decided did a PCR from plasmids with upstream region again, but this PCR didn't work, neither the positive control.

Up plasmids miniprep gel



1- 18 Plasmids extration from upstream gene ligation with TOPO

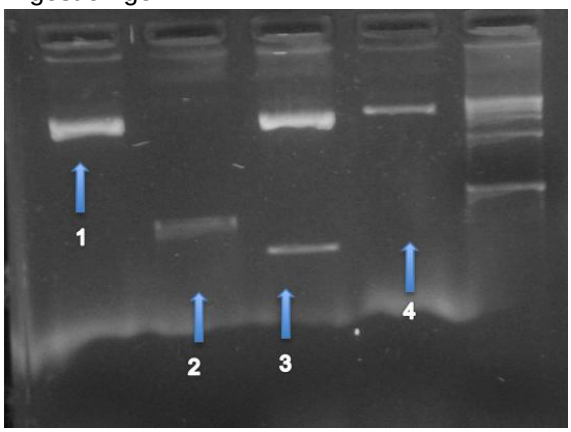
Plasmids PCR



1-2 were old PCR of upstream gene to confirm a amplification, but no plasmid had amplification.

20 – In this morning with a very nice humor, we remake the PCR of plasmids with upstream gene and appear 3 results not to clear, so we decided digest the plasmid with another enzymes BamHI and NotI but had no released insert, so we one more time remade de PCR of plasmids and the digestion and one more time the PCR work and the digestion doesn't.

Digestion gel



1 and 4 are negative result for plasmid digest ; 2 is a PCR amplification of upstream gene; 3 – Positive control(plasmid with internal gene digest);

21– So, with all this trouble we decided start again, we remake a PCR to the Upstream gene from genomic DNA of S. putrefaciens and did the ligation in TOPO vector.

Resuspention of primers to FadL and FadD genes in TE buffer.

S. Putrefaciens was inoculated in media with pH 7 in 30 and 37°C

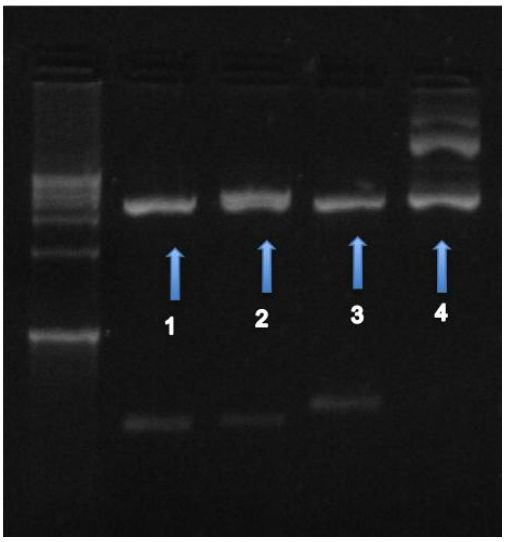
22 - Today with the gratest patience in the world, Luna isolated ther grown colonies of the TOPO ligation with upstream gene.

We also did a test of restriction enzymes that we suspect that were not working.

23 – Inoculation of the isolated colonies to do miniprep.

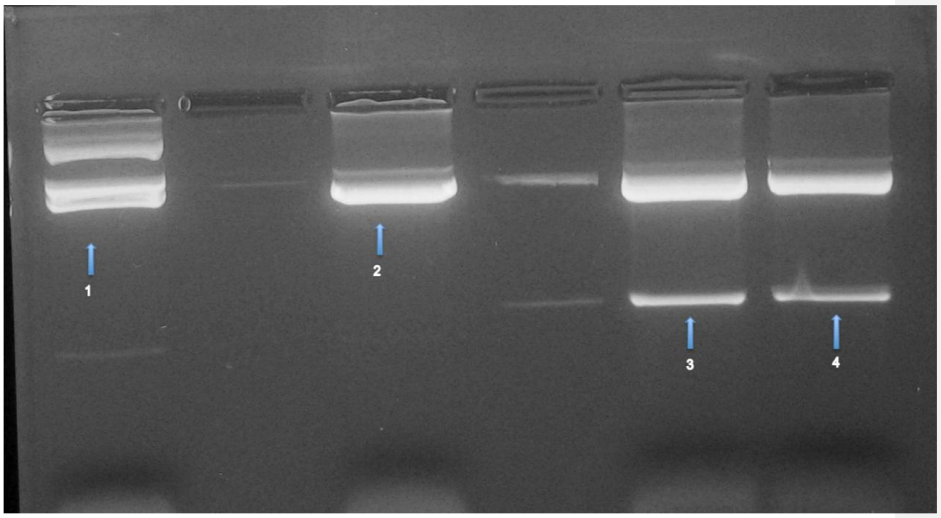
Luna also purified the PCR product and make another ligation into TOPO again.

Digest of internal fragment with ApaI and BamHI



1 and 2 are plasmid with internal gene digest with ApaI and BamHI; 3 and 4 plasmids with internal gene digest with ApaI, the insert liberation on number 3 confirm that this insert was in a invert positon in vector

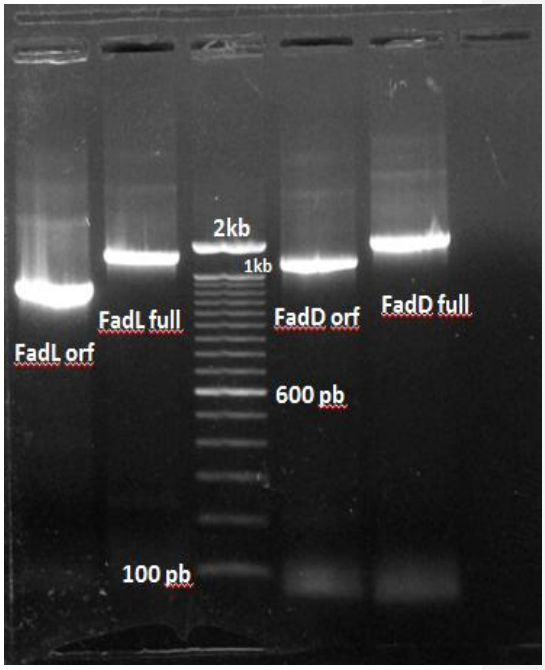
Digest bam internal / nde e bam down



1- Plasmid with a internal gene parcial digest with BamHI; 2- Plasmid with a internal gene digested with BamHI; 3 and 4 - Plasmid with a downstream gene digest with NdeI and BamHI.

PCR from S. putrefaciens' genomic DNA with primers to amplification FadL's orf and full lengh (BBa_K1076005 and BBa_K1076002) and FadD's orf and full lengh (BBa_K1076003 and BBa_K1076004) genes:

Reagents	**Final concentration**
MgCl2	1.5Mm
Tp	1x
Dntp's	0.2Mm
Primer forward	0.2pmol
Primer reverse	0.2pmol
Taq polymerase	1,5U
DNA	Xxx
Final volume	15 L



The S. putrefaciens' growth in culture with oil in pH 7 and temperature of 30 and 37°C was analyzed.

24 – The PCR of FadL and and FadD amplicons were analyzed, purified with gel band purification kit, and ligated in a cloning vector (TOPO 4.0), soon the ligation was transformed in E. coli DH10B.

Protocol to ligation:

PCR product - 2 µL

Salt Solution - 1 µL

Topo - 1 µL

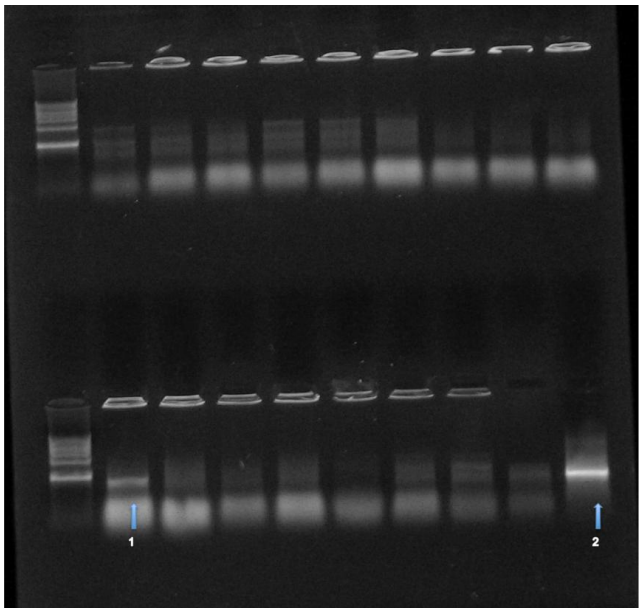
Water – 2 µL

23°C for 5MIN

26 –

FadR gene

We did PCR of the colonies with (TOPO+ upstream) and from 17 , and just 1 amplified.



1- colonie pcr amplification of upstream gene ; 2- positive control (known pcr amplification of upstream gene)

FadL and FadD genes

The transformants appear with blue color, showing that our amplicon didn't ligate with our clonage vetor. Repeated ligation with TOPO 4.0 and transformation of E. coli.

FadR gene

Colonies PCR for upstream region 2

FadL and FadD genes

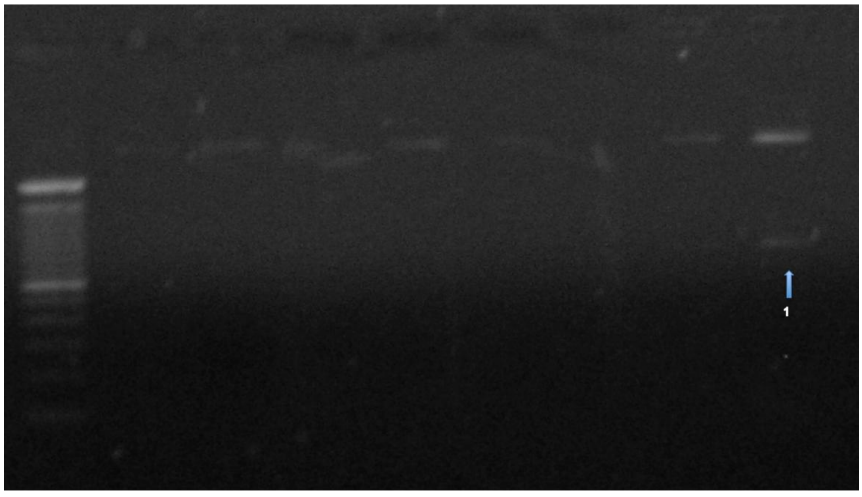
Transformants with FadL and FadD genes grow with the insert, brought to liquid culture.

All the tests' results of S. putrefaciens growth are being observed and quantified–

27 –

FadR gene

We did miniprep and than set up a digestion with ApaI e NdeI. And FINALLY two insert appear.



1- very weak insert (upstream gene) liberation

FadL and FadD genes

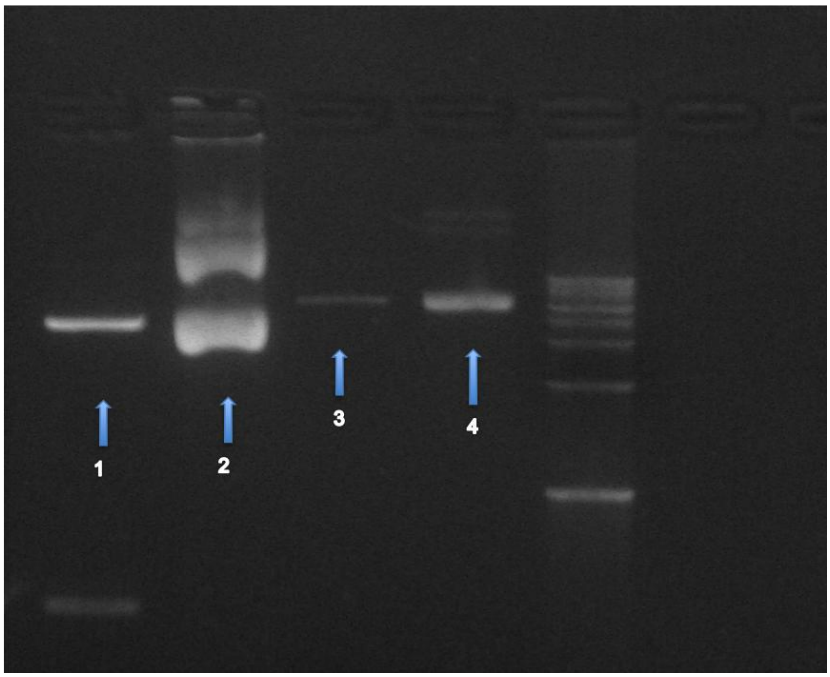
The plasmids (TOPO 4.0 + Fads) were isolated from the transformants and the digestion was performed with ECORI, and the plasmids were senquencing.

28 –

FadR gene

We did a digestion of our require plasmids (pNPTS138 and pKNOCK) with ApaI and BamHI using a plasmid with internal insert (FadR) as a control.

Digest ApaI and BamHI



1- Positive control digest; 2-positive control no digest; 3- pNPTS138 digest ; And 4 pNPTS138 no digest.

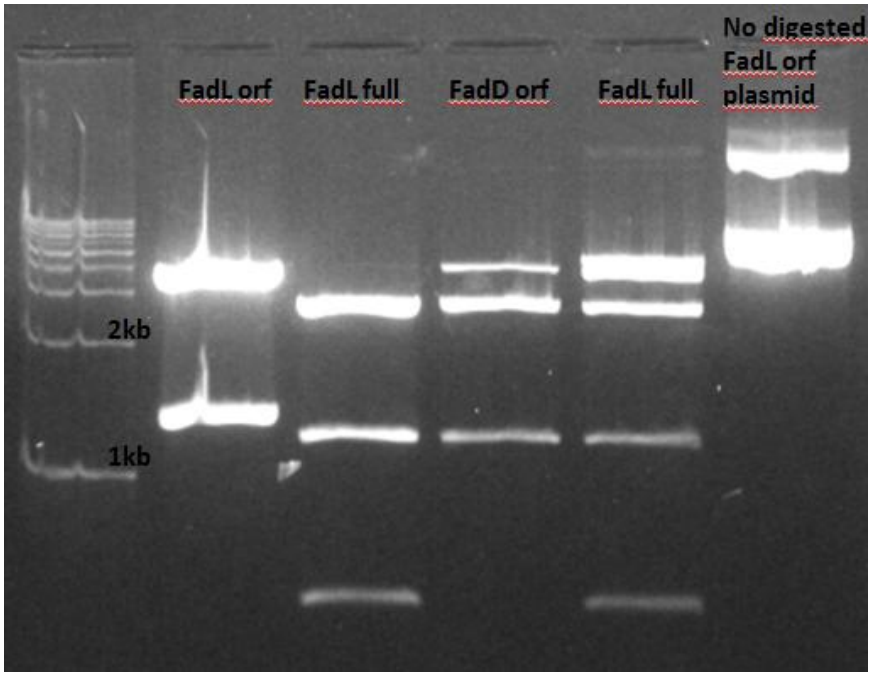
29 –

FadR gene

Large extration of plasmids pNPTS138 and pKNOCK

FadL and FadD genes

Plasmids' digestion (topo + FadL/FadD) with EcoRI and PSTI.



S. putrefaciens transformation

The transformation to test the suicides plasmids PNPT and PKNOCK was made in E. Coli DH10B. And also transformation with pSB3K3 (BBa_J04450), pSB3C5 (BBa_I50032), and a plasmid called PCV, that transform in a bacteria that have similar behaviors with S. putrefaciens.

30 -

FadL and FadD genes

We saw that TOPO 4.0 vector has inconvenient restriction sites of ECORI and PSTI to our genes, so we decide digest from the PCR product.

The purified amplicons (FadL/FadD) and pSB1C3 were digested with ECORI and PSTI. They were purified using the kit to gel band purification kit,

GE Healthcare. And the amplicons were ligated in the plasmid pSB1C3, and transformated in E. coli.

S. putrefaciens transformation

E. coli S17 and *S. putrefaciens* were inoculated in solid media with Ampicilin (50 – 100 – 150 µg/ml).

E.coli S17 was inoculated in liquid culture to make quimiocompetent cells.

SEPTEMBER

1 –

FadL and FadD genes

The transformants did grow, brought to liquid culture

S. putrefaciens transformation

It was made quimiocompetent cells with E. coli S17 and transformation with pnpt, pknock, pSB3C5, pSB3K3 and PCV plasmids to conjugate in S. putrefaciens.

In parallel, we did again the eletrotransformation in S. putrefaciens with the PCV, pSB3C5, pSB3K3, pnpt and pknock plasmids.

MFC

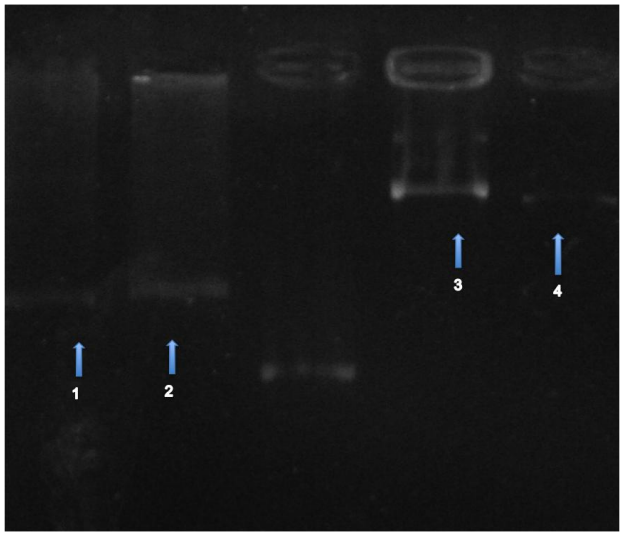
In this month we made de MFC shopping: electrode, membrane, tygon tubes, threaded rod, wingnut, peristaltic bomb, acrylic and extras materials like silicone, silicone paste, copper wires, etc.

2 –

FadR gene

Large disgest of pKNOCK with ApaI and BamHI.

Miniprep gel



1 and 2 pKNOCK miniprep; 3 and 4 pNPTS138 miniprep – both result demontrated low eficience of this extration.

FadL and FadD genes

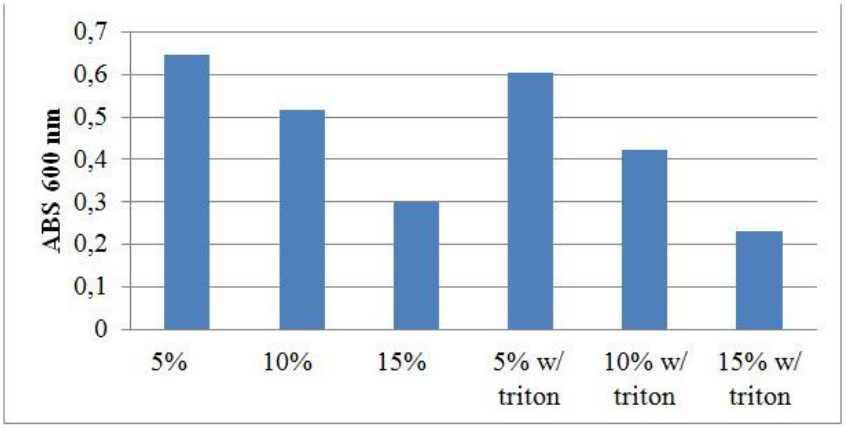
The plasmids “pSB1C3 + FadL/FadD” were isolated from the transformants and it was made the PCR, but didn’t work.

S. putrefaciens transformation

Transformants from *S. putrefaciens* didn’t grow up, so we made inocules of *E. coli* S17’s transformants and *S. putrefaciens* to conjugate in liquid culture with their antibiotics respectives.

We made electrotransformation in *S. putrefaciens* with the PCV, pSB3C5, pSB3K3 again.

All the tests of *S. putrefaciens* growth in different concentrations of oil with and without triton can be observed in this graphic:



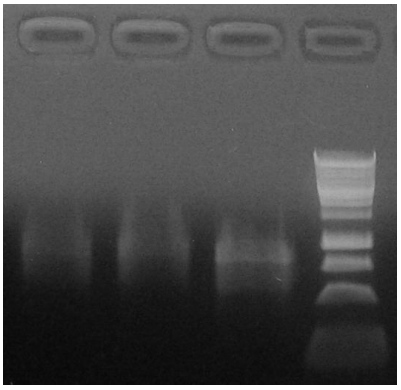
Cell growth of *S. putrefaciens* in M9 medium in different concentrations (5, 10 and 15%) of Oil with and without Triton X-100.

3 –

FadR gene

We tried Amplification of the products of PCR from Downstream gene and upsetram gene by PCR using the Fw upstream primer and the rev downstream primer, but this test didn't work . The fragments weren't at the required length;

PCR gel



FadL and FadD genes

Digestion was performed with ECORI and PSTI. This experiment didn't work.

Digestion:

BSA – 0,5 µL
TP – 1,5 µL
ECORI – 0,5 µL
PST1 – 0,5 µL
WATER – 2,0 µL
DNA - 10 µL

37°C for 3 hours.

S. putrefaciens transformation

Transformants of E. coli S17 were plated in LB media to conjugation.

4 –

FadR gene

To do the ligation, we did a digestion with a good quantities of plasmid DNA to plasmid with upstream gene we need did two diferent digest because the enzymes are not compatibles, so first the digestion with NdeI , did the purification and then the digestion with ApaI and to plasmid with downstream gene we did a double digest with NdeI and BamHI.

FadL and FadD genes

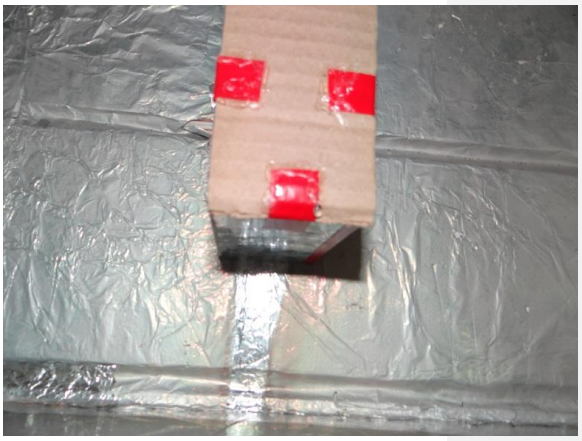
The things weren't working so transformants were brought to liquid culture again.

S. putrefaciens transformation

It was made inocule of E. coli S17 and *S. putrefaciens* after conjugation in solid media with Ampicilin 200µg/ml and Kanamicin 50 µg/ml or Chloramphenicol 34 µg/ml to avoid the *E. coli* S17's growing and let only conjugated transformants of S. putrefaciens

MFC

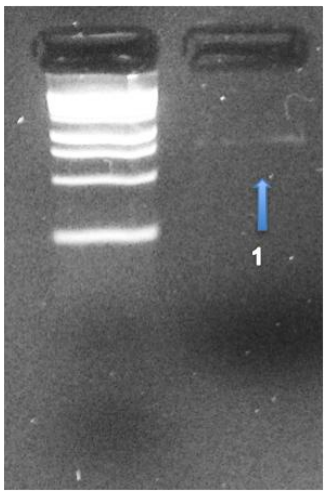
Today the home BOD was assemblied to make the experiments with S. putrefaciens in MFC.



5 –

FadR gene

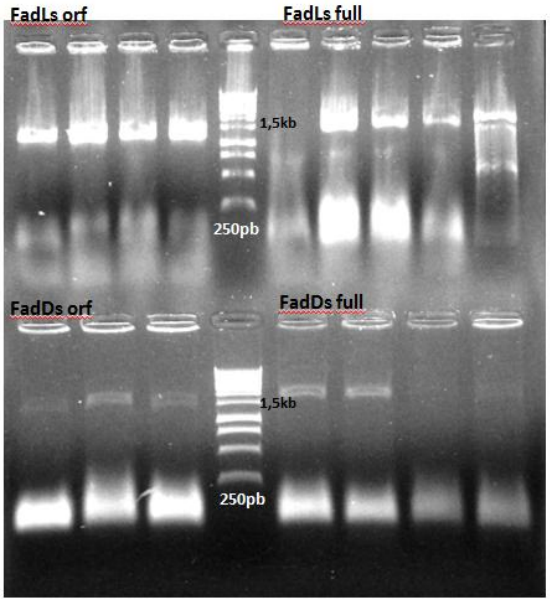
Today we purified the inserts from digest reaction and make a ligation reaction overnight.



1. Upstream gene gel purified

FadL and FadD genes

PCR from colonies and plasmids extraction.



S. putrefaciens transformation

The colonies grow up, so we purified these colonies in LB solid media with Ampicilin + Kanamicin or Chloramphenicol after conjugation between E. coli S17 and *S. putrefaciens*.

The transformation in *S. putrefaciens* using the fast protocol with sorbitol was performed again with the pSB3K3 and pSB3C5 plasmids.

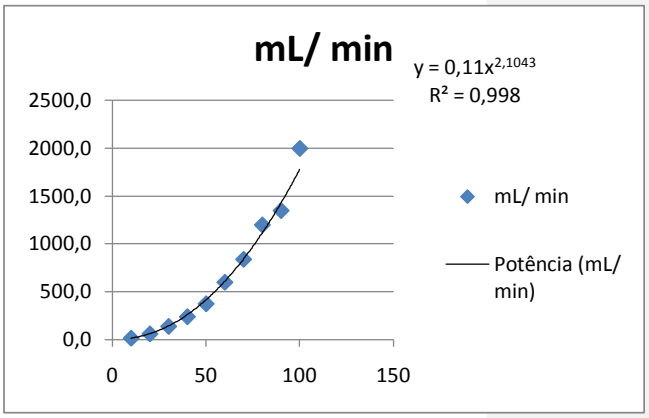
Agarose gel electrophoresis got done to analyze the PCV, PNPT and PKNOCK plasmids

MFC

The peristaltics bombs flow velocity to MFC were tested.

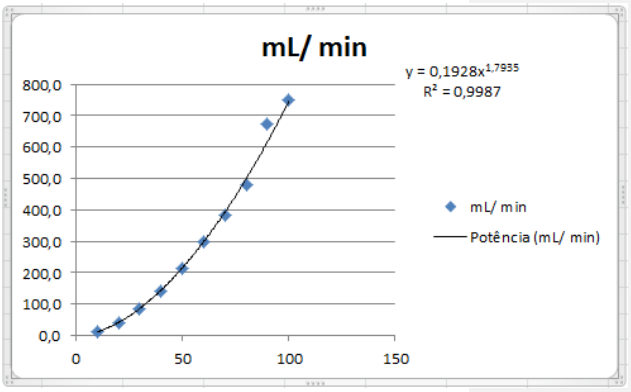
White bomb

Tempo 1	Tempo 2	mL/ min	mL/ min
39	40	15,4	15,0
21	20	57,1	60,0
14	13	128,6	138,5
12	10	200,0	240,0
11	8	272,7	375,0
7	6	514,3	600,0
5	5	840,0	840,0
5	4	960,0	1200,0
4	4	1350,0	1350,0
3	3	2000,0	2000,0



The blue bomb

Tempo 1	Tempo 2	mL/ min	mL/ min
51	47	11,8	12,8
31	31	38,7	38,7
21	21	85,7	85,7
17	17	141,2	141,2
14	14	214,3	214,3
12	12	300,0	300,0
13	11	323,1	381,8
10	10	480,0	480,0
8	8	675,0	675,0
7	8	857,1	750,0



6 –

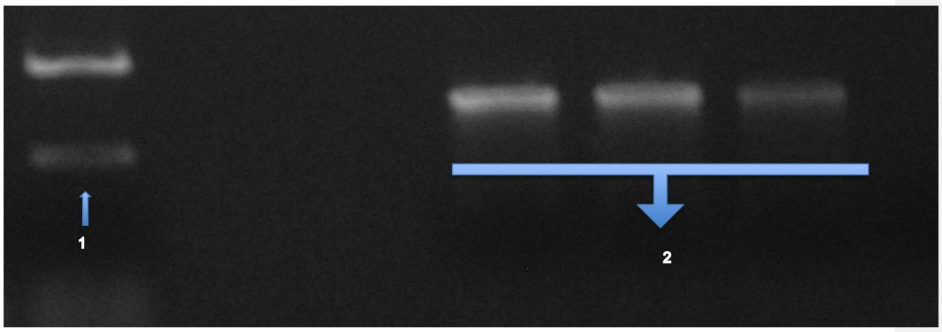
FadR gene

At first I did a electrophoresis gel, to see the ligation, but no band appeared, but if the both bands disappeared , we expected that had a ligation in a very low concentration, so we did a PCR with the rest of the reaction, and, THANKS GOD, the ligation of upstream and downstream gene were amplified and ligated in TOPO vector.

And also digested this amplicon with ApaI and BamHI to insert in pNPTS138.

And to send our biobricks, I started digest the internal gene in TOPO vector and the linearized pBS1C3 with SpeI and XbaI, then purified the insert and the pSB1C3.

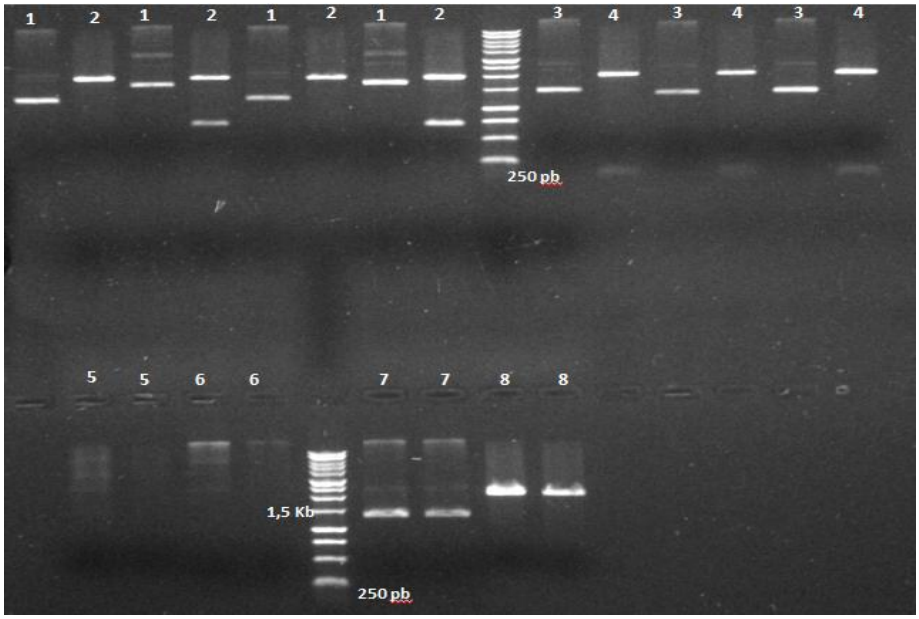
Product of Ligation (upstream and downstream gene) PCR.



1 Control: plasmid with downstream gene digestin ; 2- PCR amplification of upstream+downstream gene (length correct).

FadL and FadD genes

Digestion of the pBS1C3 + FadS with EcoRI and PSTI :



1 – FadL orf insert in pSB1C3 no digested; 2 – "FadL orf insert in pSB1C3 digested"; 3 - FadL full lengh insert in pSB1C3 no digested; 4 – "FadL full lengh insert in pSB1C3 digested"; 5 - FadD orf insert in pSB1C3 no digested; 6 - "FadD orf insert in pSB1C3 digested"; 7 - FadD full lengh insert in pSB1C3 no digested; 8- "FadL full lengh insert in pSB1C3 no digested

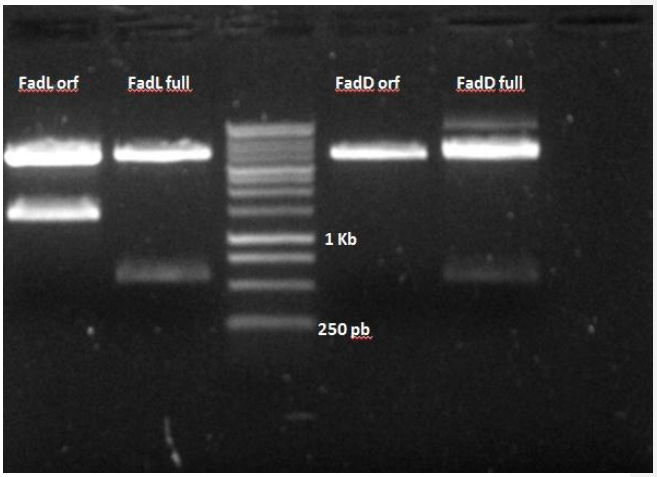
Conclusion: The cloning didn't work and we see that the FadL has a PSTI restriction site inside his gene.

It was made the digestion of TOPO 4.0 vector + inserts with EcoRI:

Digestion:

Water - 2 µL
TpEcoRI – 1,5 µL
EcoRI - 1 µL
PSTI – 0,5 µL
DNA - 8 µL (strong)
DNA - 10 µL (weak)

37°C for 2 hours



It was made the ligation of PCR fragments with TOPO 4.0 and transformated in E. coli DH10B.

Ligation:

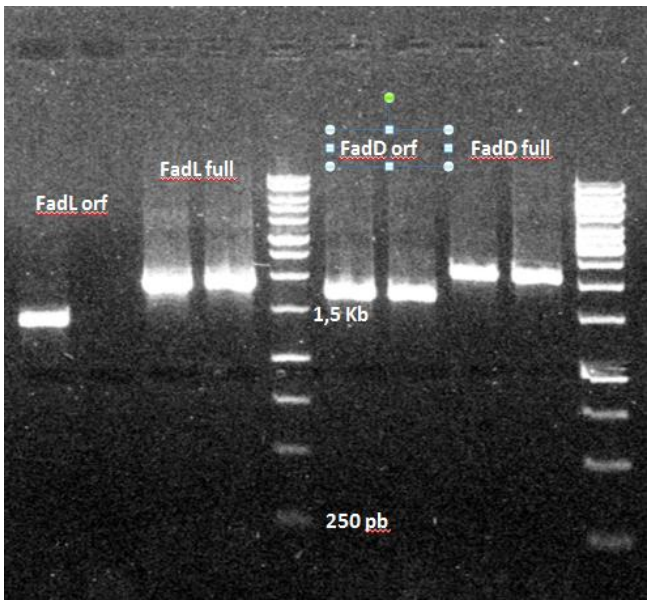
PCR - 4 µL

Salt solution - 1 µL

Water - 1 µL

Topo - 1 µL

New PCR from S. putrefaciens' genomic DNA with primers to amplification FadL and FadD genes:



S. putrefaciens transformation

Transformants of S. putrefaciens didn't grow.

It was made purification of the colonies that grew with Ampicilin + Kanamicin or Chloramphenicol again, but now using different antibiotics' concentration.

Formatado: Cor da fonte: Automática, Inglês (Estados Unidos)

In Parallel, it was made again conjugation between E. coli S17 and S. putrefaciens with psB3K3, psB3C5, pNPT, pKNOCK and pCV.

7 –

FadR gene

We did five ligations reaction :

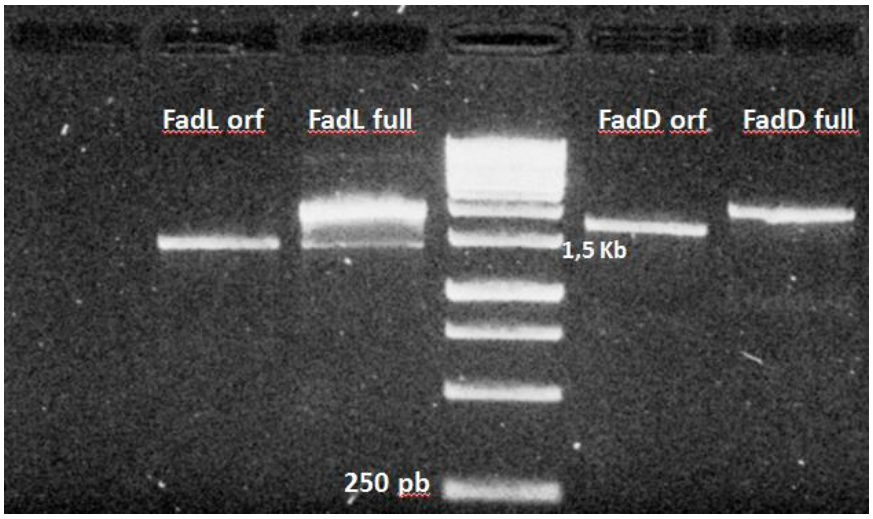
1- Internal gene(FadR) + pSB1C3 – 3: 1
2- Internal gene + pSB1C3 – 3:3
3- Internal gene + pNPTS138 – 3:1
4- Internal gene + pKNOCK – 3:1
5- Digested Ligation (Upstream+downstream) + pNPTS138

Then we cloned that ligations in E.coli DH10B

FadL and FadD genes

The transformation with TOPO 4.0 plasmids didn't grow.

The PCR amplicon from *S. putrefaciens* were purified and ligated in another vector (pGEM):



It was transformated.

S. putrefaciens transformation

E. coli S17 with psB3K3, psB3C5, PCV, PNPT e pknock plasmids were inoculated in solid LB media with Ampicilin 100-150µg/ml to test if it would grow.

Also were made test with *S. putrefaciens* in solid LB media with Ampicilin 100-150µg/ml.

8 –

FadR gene

The cloning of ligation with pSB1C3 didn't work, not colonies appeared. So we tried another way, digested the pSB1C3+RFP generator(BBa_ J04450) with SpeI and Xba, gel purified, then we did another 4 reactions ligation, overnight.

1- Internal gene(FadR) + pSB1C3 – 2:1
2- Internal gene(FadR) + pSB1C3 – 5 :1
3- Internal gene(FadR) + pSB1C3 – 3: 1
4- Internal gene(FadR) + pSB1C3 – 1: 1

We also isolated the grown colonies from ligations (3,4) and inoculed in LB liquid .

And the ligation of amplicon digested Ligation (Upstream+downstream) + pNPTS138 didn't work too.

FadL and FadD genes

Transformants did grow, brought to liquid culture.

S. putrefaciens transformation

Transformants of E. coli S17 and S. putrefaciens were inoculated in liquid culture with their respective antibiotics to make a new conjugation.

MFC

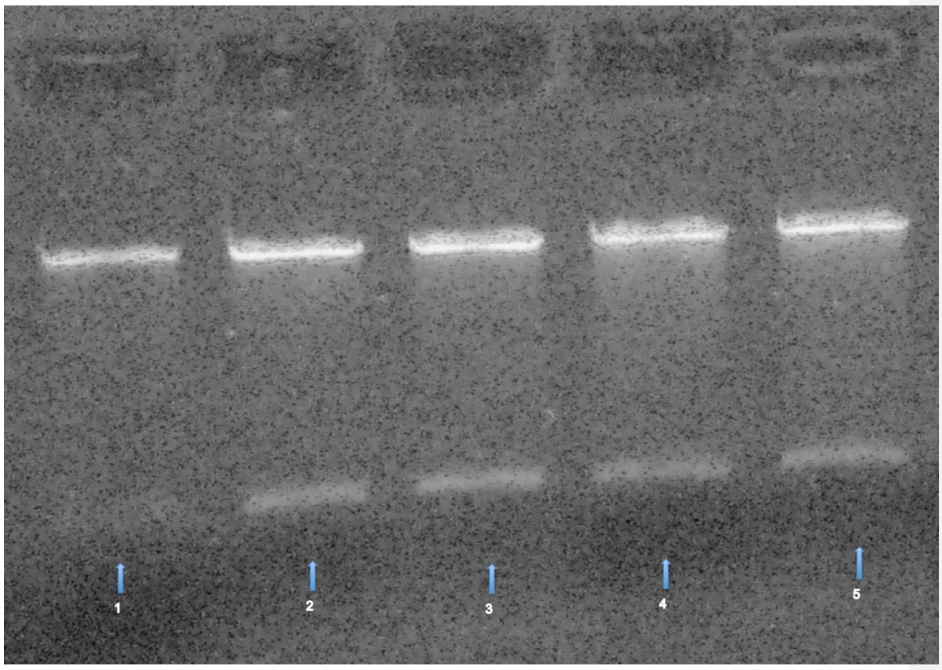
It was made the acrylic's cut to assembly the MFC.

9 –

FadR gene

At morning we cloning the reaction ligation internal gene(FadR) + pSB1C3 [BBa_K1076000] in E. coli RR1.

And we miniprep of overnight cultures (Internal in pNPTS138), and did a digest with ApaI and BamHI to confirm the insert .



1-5 are pNPTS138 with internal gene digestion

And internal in pKNOCK, because the low replication we did a PCR to confirm.

FadL and FadD genes

Plasmidial extraction of pGEM inocules.

Digestion with EcoRI, and we made colonies' PCR (Nothing was working in this day!!!). So we transformated the longer ligation in pGEM.

S. putrefaciens transformation

Transformants of *E.coli* S17 were plated in solid LB to conjugation.

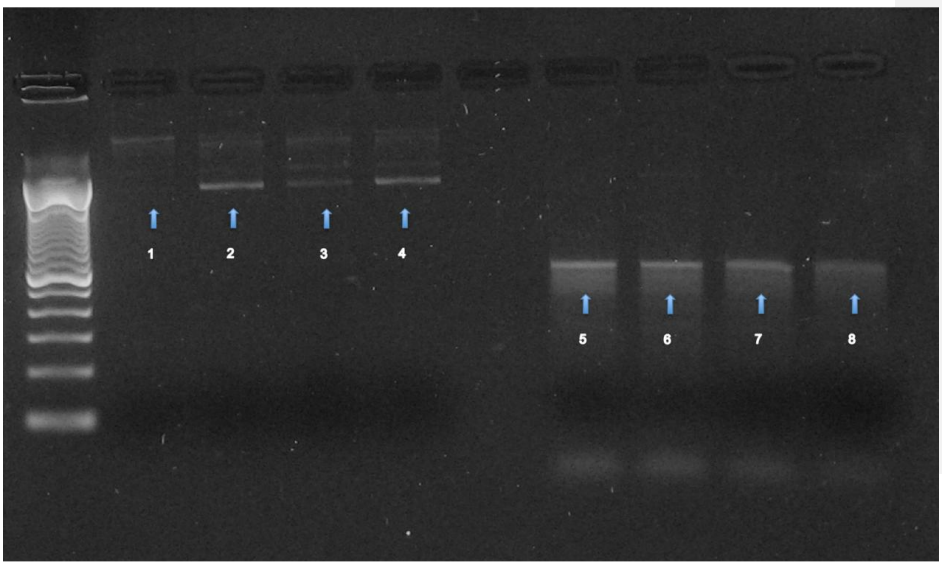
MFC

The acrylics were pasted to assembly the MFC structure. And this keeps pasting about 24 hours.

10 –

FadR gene

Today, is a lovely day, the colonies of cloning from reaction ligation Internal gene(FadR) + pSB1C3 [BBa_K1076000] grown up! So we inoculated this colonies early morning and at night we did miniprep of these plasmids and did a PCR to confirm the insert.



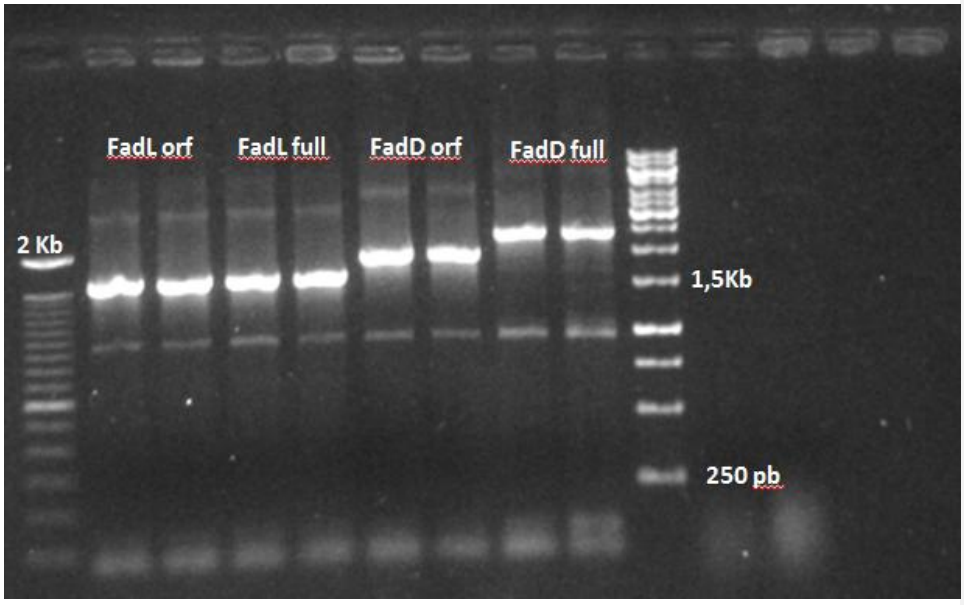
1-4 PSB1C3 with FadR gene no digest; 5 and 8 are these plasmid amplification to confirm the gene insert.

We also did the ligation reaction of amplicon Ligation (Upstream+downstream) in TOPO vector, because the last one didn't work and transforming in *E.coli* RR1, early morning, at night we inoculated some grown colonies.

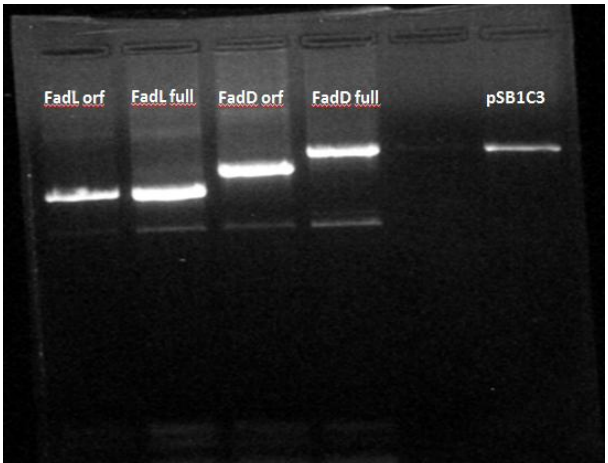
FadL and FadD genes

Transformants did grow, brought to liquid culture.

We made colonies' PCR:



The amplicons and pSB1C3 were digested wit EcoRI and SpeI, and were purified.



Soon, the inserts were ligated with pSB1C3 and a part were transformated while another keeps linking. We made more digestion of pSB1C3.

Digestion of 20 µL of pSB1C3:

DNA - 20 µL

TPECO - 20 µL

BSA - 2 µL

ECORI - 2 µL

SPE - 4 µL

WATER - 152 µL

37°C for 1hour.

Calculations to know how much inserts use in a ligation:

$$\frac{[\text{vetor}] \times \text{kb insert}}{\text{Kb vetor}} \times \frac{3}{1}$$

Ligation in pSB1C3:

Vetor: 6 µL

Insert: 1,3 – 2,1 µL

Buffer: 2 µL

T4 ligase: 1 µL

Water:

Vf: 20 µL

16°C for 1 hour.

S. putrefaciens transformation

E. coli S17 and S. putrefaciens were inoculated after the conjugation in solid LB media with Ampicilin 200µg/ml + Kanamicin 50 µg/ml or Chloramphenicol 34 µg/ml, to avoid the *E. coli*'s presence, and let only the transformated S. putrefaciens on the plate.

11 –

FadR gene

At morning, we minipreped of overnight cultures (Up and down ligation in TOPO vector), and double digest that with XbaI and SpeI, then gel purified that and did two ligation reaction with a digested pSB1C3 to do the BBa_K1076001

1- Up+down ligation + pSB1C3 - 2,5:1
2- Up+down ligation + pSB1C3- 3:1

And transform in E. coli RR1

FadL and FadD genes

We made plasmids pGEM isolation and digestion.

The plasmids pGEM with the inserts were sequencing.

We transformed the longer ligation and in the end of the day the transformants with inserts in pSB1C3 did grow, brought to liquid culture.

We made more ligation of inserts in pSB1C3.

S. putrefaciens transformation

The colonies that appeared were purified in solid media with Ampicilin + Kanamicin or Chloramphenicol after the conjugation between *E. coli* S17 and *S. putrefaciens*.

12 –

FadR gene

We inoculated this colonies early morning and at night we did miniprep of these plasmids and did a PCR to confirm the insert.

FadL and FadD genes

Plasmidial extraction of transformants colonies PCR and digestion; and inocules de another colonies.

The PCR didn't amplificated.

S. putrefaciens transformation

It was made again, one more time, a new purification of the colonies in LB media with Ampicilin + Kanamicin or Chloramphenicol with different antibiotics' concentrations after the conjugation between transformants of E. coli S17 with psB3K3 – psB3C5 – PNPT – pKnock e pCV plasmids, and S. putrefaciens.

Transformants of *E. coli* S17 and *E. coli* DH10B were inoculated to test the conjugation between both, and compare the results with the conjugation between *E. coli* and *S. putrefaciens*.

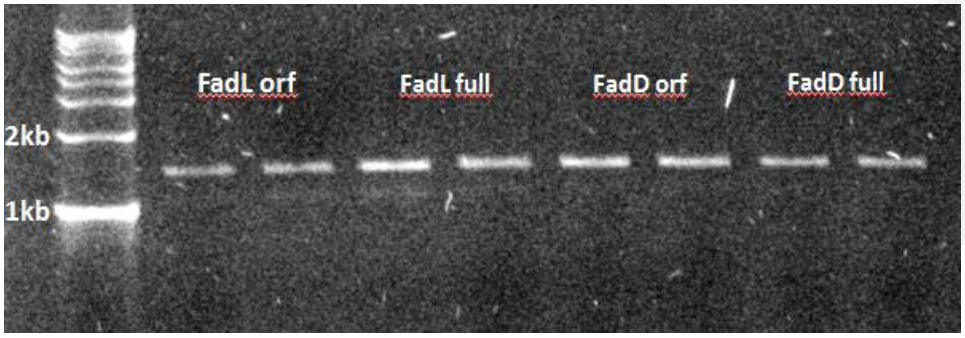
13 –

FadR gene

We sequencing these plasmids

FadL and FadD genes

PCR from plasmids and all amplificated



Preparation of biobricks to send to competition.

S. putrefaciens transformation

Transformants of *E. coli* S17 with psB3C5 and psB3K3, and *E. coli* DH10B were plated in LB solid media to conjugate and compare the results with the conjugation between *E. Coli* S17 and *S. putrefaciens*.

14 –

FadR gene

Concetring theses plasmid to sending parts.

S. putrefaciens transformation

Transformants of E. coli S17 and E. coli DH10B were inoculated in solid media with Ampicilin + Kanamicin or Chloramphenicol after the conjugation to compare results of the conjugation of E. coli + E. coli and E. coli + S. putrefaciens.

We looked for others bacteria with the sexual peeling on the laboratory's bacteria library of the Amazonas Federal University.

15 –

S. putrefaciens transformation

The colonies of *E. coli* S17 and *E. coli* DH10B that grew were purified in solid media with Ampicilin + Kanamicin or Chloramphenicol after conjugation.

16 –

S. putrefaciens transformation

New bacteria with the sexual peeling were found on the University bacteria' laboratory: *E. coli* DH5α f' Iq and *E. coli* JM109.

It was made again the purification of *E. coli* S17 and *E. coli* DH10B colonies in solid media with Ampicilin + Kanamicin or Chloramphenicol after conjugation.

FadL and FadD genes

We made the digestion of pSB1C3 + inserts with EcoRI; and another with EcoRI and PSTI.

Digestion with ECORI:

Water: 4,5 µL

Buffer eco: 1,5 µL

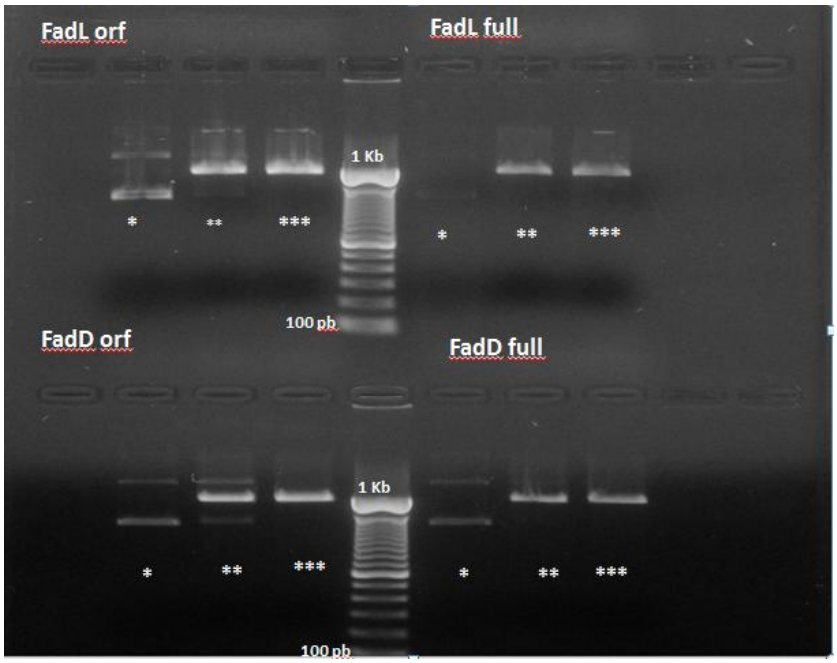
ECORI: 1 µL

DNA: 8 µL

Vf: 15 µL

Digestion with ECORI and PSTI:

Water: 4 µL
Buffer eco: 1,5 µL
ECORI: 1 µL
PSTI: 0,5 µL
DNA: 8 µL
Vf: 15 µL



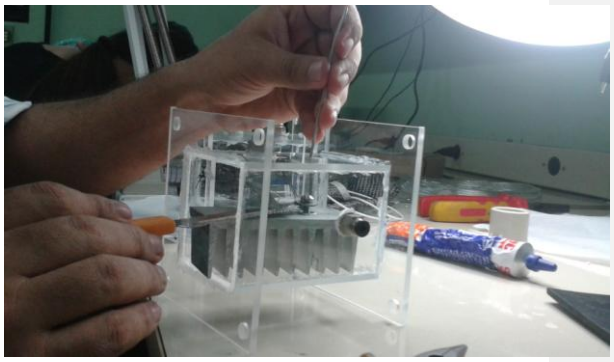
* -Plasmid not digested, ** - Plasmid digested only with EcoRI, *** - Plasmid digested with EcoRI and PSTI.

After we noted that the FadL full lengh was cloned with the wrong band, and this biobrick had been rejected because of the incompatible RFC.

20

MFC

The MFCs structure were assemblied.



23 – **FadR gene**

Transformation of S. putrefaciens with pNPTS138 with FadR

24 –

FadL and FadD genes

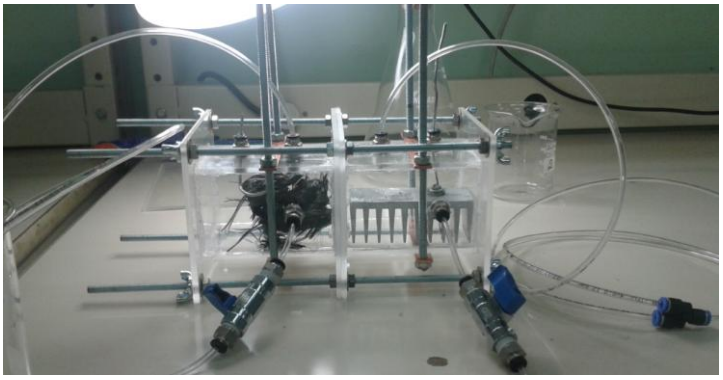
We digest FadL and FadD amplicons

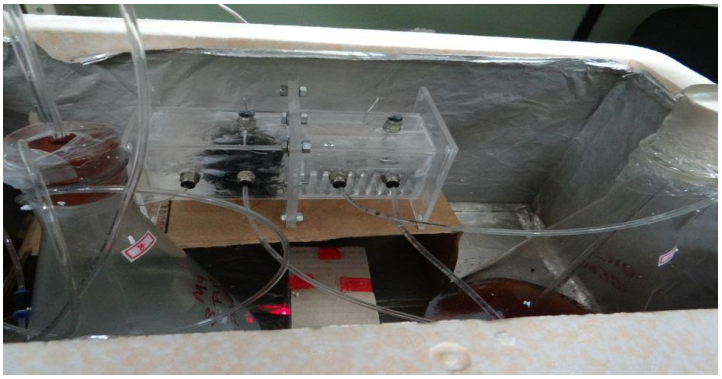
25- FadR gene

Tranformants of S. putrefaciens with pNPTS138 + FadR were inoculated in LB liquid culture.

25 – MFC

It was assembled the MFC system (the MFC's structure were connected on the bacteria through a tube that pass the culture medium with *S. putrefaciens* and oil, and another tubes connected on peristaltic bombs and multimeter.). This were put inside the home BOD, and turned on.





When the MFC was turned on, it was made tests with the bacteria and it was analyzed or 15 and 15 minutes for a total of 7:30.

HUMAN PRACTICE

June, 12

The iGEM's competition and the Electrobacter project were presented in a university's event to the new biotechnology's students class.

JULY

4 – Apresentation about the igem's competition, the electrobacter project and molecular biology to students from public high school in a oficine called "cool science".

30 (July) – 3 (August) – Travel to Manicoré (interior of Amazonas) to spread and teach about synthetic biology to high school and collegiate students.

August, 16

We were to the restaurants in the Amazonas federal university and we have a talk with the cookers about the correct oil discard. We also introduced some bottle to store the fried oils and use in our project.