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Preparation of media

Materials:

- Luria Bertani powder
- DI water
- Gentamicin
- Kanamycin

Procedure:

LB agar:

1. Weight 37 g of Luria Bertani powder and add to the 1 L dd water.
2. Mix until powder is dissolved.
3. Autoclave the solution for 15 minutes at 121°C.

LB broth:

1. Weight 20 g of Luria Bertani powder and add to the 1 L of dd water.
2. Mix until powder is dissolved.
3. Autoclave the solution for 15 minutes at 121°C.

Selective media:

LB agar + gentamicin:

1. Prepare LB agar by weighing 37 g of Luria Bertani powder and add to the 1 L dd water.
2. Heat and autoclave.
3. Add gentamicin into LB agar in a ratio of 5 ul of gentamicin per 1 ml LB agar.
4. Pour 90mL LB agar + gentamicin into 3 plates 30mL each. Allow to solidify.

LB broth + gentamicin:

1. Prepare LB broth by weighing 20 g of Luria Bertani powder and add to the 1 L dd water.

2. Heat and autoclave.
3. Add gentamicin into LB agar in a ratio of 5 ul of gentamicin per 1 ml LB broth.

LB agar + kanamycin:

1. Prepare LB agar by weighing 37 g of Luria Bertani powder and add to the 1 L dd water.
2. Heat and autoclave.
3. Add gentamicin into LB agar in a ratio of 1 ul of kanamycin per 1 ml LB agar.
4. Pour 90mL LB agar + kanamycin into 3 plates 30mL each. Allow to solidify.

LB broth + kanamycin:

1. Prepare LB broth by weighing 20 g of Luria Bertani powder and add to the 1 L dd water.
2. Heat and autoclave.
3. Add kanamycin into LB agar in a ratio of 1 ul of gentamicin per 1 ml LB broth.

DNA Extraction

DNA extraction from *P.aeruginosa* was made following the protocol by Dr. O. M. Ajunwa

Materials:

- Cultured cells
- DI water
- TE buffer (10 mM Tris-HCl, pH 8.0 , 0.1 mM EDTA)
- Lysis buffer (50 mM Tris, 1% SDS, pH 8.0)
- Lysis solution
- Phenol/chloroform/isoamyl alcohol
- Sodium Acetate
- Absolute ethanol

Procedure:

1. Mix 1ml of cell suspension with 1ml of DI water in a falcon tube. Centrifuge at 4700 rpm for 1 hour.
2. Discard the supernatant. Mix the pellet with bacteria with a TE buffer (10mM Tris-HCl, pH 8.0, 0.1mM EDTA) of volume 1ml.
3. Thoroughly mix the mixture and centrifuge at 4700 rpm for 20 min.
4. Discard the supernatant leaving 300ml of the solution. Gently resuspend the mixture and divide into new 1.5ml reaction tubes.
5. Treat one 1.5ml reaction tube with the cell suspension with 1ml lysis buffer (50mM Tris, 1% SDS, pH 8.0) (BioRAD).
6. Mix the second tube with a lysis solution from BioRAD Aurum Plasmid Minikit of volume 1ml.
7. Incubate tubes in a thermomixer at 37°C for 1 hour without applying rotations during incubation.
8. Keep the tubes with cell lysates in the fridge (4°C) overnight.

9. Divide the cell lysis solutions into new 2ml reaction tubes, making up in total 4 tubes (2 treated with LB and 2 treated with LS). Each tube should contain 0.5ml of solution.
10. Treat all 4 tubes with 1 volume of phenol/chloroform/isoamyl alcohol (25:24:1 v/v) and thoroughly mix with pipettes.
11. Allow the reaction tubes to stand for 5 minutes and then centrifuge at 10 000 rpm for 15 minutes.
12. Carefully decant the upper aqueous layer into a new 2ml reaction tube (4 tubes in total).
13. Mix each reaction tube with 0.1 volume Sodium Acetate (3M) and 1v absolute ethanol.

Preparation of reaction tubes for DNA extraction

	tube 1	**tube 2**	**tube 3**	**tube 4**
Volume in a tube	0.4ml	0.5ml	0.6ml	0.4ml
0.1v Sodium acetate	40ul	50ul	60ul	40ul
1v absolute EtOH	400ul	50ul	60ul	40ul

14. Keep tubes in the -20°C fridge for 3 days
15. Centrifuge the tubes at 10 000 rpm for 15 minutes.
16. Discard the supernatant.
17. Add 200 microliters of 70% ethanol tube and centrifuge for 15 minute at 10 000 rpm (x3)
18. Decant the ethanol solution and leave to air dry tubes containing pellets.
19. Treat the dried tubes with ultrapure water in volume 100 microliters.

Nanodrop Measurements

Usually are used to be sure in the presence of DNA and to know its exact concentration.

20. Use the ultrapure water to blank the nanodrop 8000 spectrophotometer (Thermo FIsher Scientific)
21. Measure samples at the same time, and record the measurements in Table

Nanodrop measurements of extracted DNA samples

1	50.44 ng/ul	LS	1.55
2	30.14 ng/ul	LS	1.54
3	58.52 ng/ul	LB	1.85
4	150.1 ng/ul	LB	1.60
5	290 ng/ul	LB and LS	1.93

References:

Ajunwa, O. M. (2018). Physiological evaluation and genetic engineering of *Pseudomonas aeruginosa* A4 for enhanced electrogenicity. Ph.D. Thesis, University of Ibadan, Nigeria.

PCR techniques

After several trials with different concentrations of reagents along with different conditions, we adjusted and optimized conditions and parameters for PCR amplification to obtain *nadE*, *rhlA*, and *rhlB* genes from *Pseudomonas aeruginosa*. Following volumes of reagents, PCR step temperatures and times are the most effective ones for amplification of *nadE*, *rhlA*, and *rhlB* genes.

Procedure:

1. Prepare PCR tubes

Preparation of PCR samples

Composition	**Volume, ul**
Q5 buffer	5
Q5 enhancer	5
10 mM DNTP mix	1
10 mM forward primer	1.5
10 mM reverse primer	1.5
Q5 polymerase	0.25
Genomic DNA (70 ng)	-
Nuclease free water	Up to 25

2. Run PCR

PCR conditions for *rhlB*:

Steps	**t (°C)**	**time**	
Initial Denaturation	98	3 min	
Denaturation	98	10 sec	35 cycles
Annealing	72	30 sec	
Elongation	72	93 sec	
Final elongation	72	5 min	
Hold	4		

PCR conditions for *nadE* and *rhlA*:

Steps	**t (°C)**	**time**	
Initial Denaturation	98	3 min	
Denaturation	98	10 sec	35 cycles
Annealing	72	30 sec	
Elongation	72	40 sec	
Final elongation	72	2 min	
Hold	4		

Gel electrophoresis

It was implemented to observe and to prove presence of obtained parts and for the elution of genes of interest.

Materials:

- Agarose
- Nuclease free water
- TAE 50X
- Loading dye (No SDS)
- DNA Ladder 1kb NEB

Procedure:

1. Prepare 1% agarose gel (2mL 50xTAE + 1g agarose + water up to 100mL+10 ul fluorescent DNA staining dye)
2. Sample preparation: 15 ul of DNA sample, 5 ul of loading dye
3. Gel electrophoresis at 90V for 60 minutes
4. Observe the gel under UV

Gel Elution Protocol

Gel Elution was done to purify and isolate genes of interest. It followed standard manufacturer protocol.

Materials:

1. Solubilization Buffer (L3)
2. Wash buffer (W1)
3. Elution Buffer (E5)

Equipment:

- Weighing Balance
- Thermomixer
- Centrifuge

Procedure:

1. Cut minimal area of gel with a Band
2. Weight gel
3. Add Gel Solubilization Buffer (L3) to the gel in a ratio 3:1
4. Place the tube in a thermomixer for 10 min until the gel will dissolve. Each 3 min gently mix by inverting
5. Incubate the tube for additional 5 min
6. Purify DNA using a centrifuge
7. Pipet the dissolved gel into the column in the wash tube. One column per 400 mg capacity is 850ml, so that we took approximately 700 ml
8. Centrifuge the flow through and place column into the wash tube
9. Add 500ul Wash buffer (W1)
10. Centrifuge at 12000 rpm for 1 min, discard the flow through
11. Centrifuge one more time at 14000 rpm for 2 mins, discard the flow through
12. Place column in a new wash tube (recovery tube). Add 50 ul of Elution Buffer (E5) into the centre of the column. Incubate 1 min at RT
13. Centrifuge the tube at 12 000 rpm for 1 min
14. Store for long term usage at -20C

References

PureLink Quick Coel Extraction KIt Protocol

Transformation of *E. coli* via Electroporation

The main reason for choosing electroporation over heat-shock transformation was its higher efficiency in the uptake of plasmid DNA because of the high-voltage current application on cells. Additionally, electroporation is faster and is a less involved production of competent cells.

Materials:

- Equipments
    - Electroporator for bacteria (e.g., Gen Pulser Xcell Electroporation System, Bio-Rad)
    - Refrigerated centrifuge
    - Refrigerated microcentrifuge
    - Shaking incubator (37 C)
    - Incubator (37 C)
    - 15-ml sterile polypropylene snap-cap tubes
    - Electroporation cuvette, 0.1 cm gap
    - 15-ml glass centrifuge tube
    - Rubber adaptors (to fit glass centrifuge tube into floor centrifuge rotor)
    - Pipettes
    - Micropipettors
    - Micropipettor tips
    - 1.5-ml microcentrifuge tubes
    - Kimwipes
- Plasmid DNA (to be transformed)
- LB agar plates (selective plates containing appropriate antibiotic)
- Sterile ddH2O
- Glycerol (optional)

Procedure:

Preparation

Inoculate 5 ml of LB with *E. coli.* Grow overnight at 37 C with shaking. Chill the 15-ml glass centrifuge tube, a 1.5-ml microcentrifuge tube, the electroporation cuvette, and the ddH2O on ice. Run the floor centrifuge for a few minutes to cool the chamber to 4 C.

Create electro-competent *E. coli*

1. Pour the bacterial culture into the prechilled 15-ml glass centrifuge tube on ice.
2. Centrifuge at 7000 rpm at 4 C, for 5 min.
3. Discard the supernatant.
4. Resuspend the pellet in 1 ml of sterile, ice-cold ddH2O and transfer into a chilled 1.5-ml microcentrifuge tube.
5. Spin in a microcentrifuge at 7000 rpm at 4 C, for 5 min.
6. Discard the supernatant.
7. Resuspend the pellet in 1 ml of ice-cold ddH2O.
8. Repeat steps 6-8 three more times.
9. Spin a final time at 7000 rpm at 4 C, for 5 min, discard the supernatant, resuspend the pellet in 50 ml of ice-cold ddH2O, and put the cells on ice.

Electroporation of *E. coli*

10. Add 50–100 ng of supercoiled plasmid to the electro-competent *E. coli* and mix gently (do not pipette up and down).
11. Transfer the bacteria to a chilled electroporation cuvette. Be careful to pipette straight in between the metal plates and avoid introducing any bubbles.
12. Cap the cuvette and tap it lightly on the bench to settle the bacteria DNA mix.
13. Put the cuvette back on ice and carry it to the electroporator.
14. Turn on the electroporator and set it to 1.8 kV, 25 mF, 200 Ω. This is a standard-setting for most *E. coli* strains. Other bacterial strains may require an adjustment of the electroporation conditions.

15. Wipe the cuvette briefly with a Kimwipe to remove any residual water or ice, and then place it in the electroporation chamber.
16. Push the pulse button. The time constant displayed should be around 4 ms.
17. *Immediately* after the pulse has been delivered, add 1 ml of LB (or another growth medium, e.g., SOC) to the cuvette and pipette quickly but gently up and down. Be aware that the transformation efficiency decreases proportionally to the lag time between the electric pulse and the addition of media.
18. Transfer the mixture to a fresh 1.5-ml microcentrifuge tube.
19. Incubate at 37 C for 1 h with shaking.
20. Evenly spread 100 ml of your transformation onto a selective plate. Electroporation is highly efficient and often yields a very large number of colonies. To ensure that individual, medium-sized colonies can be picked the next day, mix 10 ml of the transformation with 90 ml of LB and spread this 1:10 dilution evenly onto another selective plate. Incubate the plates upside down overnight at 37 C.

References

Lessard, J. C. (2013). Transformation of *E. coli* Via Electroporation. *Methods in Enzymology*, 321–327. https://doi.org/10.1016/b978-0-12-418687-3.00027-6

Plasmid Extraction protocol (extraction in small amount)

Materials:

- LB Broth
- Stirrer
- ALS I
- ALS II
- ALS III
- Centrifuge
- 70% Ethanol

Procedure:

1. Transfer 1 colony from a plate into a 1 ml LB broth + 1 ul of antibiotic (selective medium), leave at +37 C stirrer overnight
2. 1 ml of culture tube, centrifuge at 13460 rpm at 4 C for 30 sec, discard the supernatant, leave the pellet as dry as possible.
3. Resuspend the pellet in 100 mcl of ice cold ALS I (has to be pre-autoclaved), mix by pipetting, making sure the cells are dispersed fully
4. Add 200 mcl of ALS II - (does not have to be fresh, stored at room temperature) into the tube. Mix the contents gently by inverting the tube 5 times. Make sure the contents of the tube come in contact with ALS II (!). Store tube n ice for 5 min.
5. Add 150 mcl of ALS III (has to be pre-autoclaved). Mix the contents gently by inverting the tube 5 times. Store the tube on ice for 5 min.
6. Centrifuge at 13460 rpm for 5 min, transfer the supernatant 450 mcl to fresh labelled tube (DNA - supernatant)
7. Precipitate DNA from the supernatant by adding 2 volumes (700 mcl) of 70% Ethanol at room temperature. Gently mix by inverting. Incubate 2 min at room temperature.

8. Collect the precipitated nucleic acids by centrifugation at 13460 rpm for 5 min at 4 C. DNA recovery from tube - visible white pellet. Remove supernatant tubes till dry
9. Add 1 ml of 70% Ethanol mix by inerting. Centrifuge at 13460 rpm for 2 min at 4C. Remove supernatant. Leave tubes on paper towels until ethanol evaporates for 10-15 minutes.
10. Dissolve the nucleic acids in 50 mcl of H2O. Check OD 1 ul of each solution with nanodrop.
11. Gel electrophoresis to confirm the presence of plasmid
12. Unused DNA stored at -20 C.

References:

Soltabayeva, A. (2020). Plasmid Extraction Protocol

Restriction Digestion:

Restriction Digestion was performed in order to prepare pRGPDuo2 plasmid for insertion *nadE* gene into it. Double digestion allowed the cutting of plasmid from two sides by SacI and SalI restriction enzymes at the site of interest. It followed standard manufacturer protocol.

Double digestions of plasmids:

1. Prepare reaction

Preparation of Double Digestion sample

Composition	Volume
CutSmart buffer 10X	5 ul
DNA (500 ng)	-
SacI	1 ul
SalI	1 ul
Nuclease free water	Up to 50 ul

2. Incubate for 1 hour at 37°C
3. Heat inactivation at 65°C for 20 minutes.

DNA Ligation

DNA ligation was applied to join the *nadE* gene with pRGPDuo2 plasmid by T4 DNA Ligase. It followed standard manufacturer protocol.

Materials:

- T4 DNA Ligase Reaction Buffer (10X)
- T4 DNA ligase
- Nuclease free water
- Plasmid Duo2
- *nadE* gene

Procedure:

1. Set up reaction tube in a microcentrifuge tube on ice

Preparation of DNA Ligation sample

Components	**20 ul reaction**
T4 DNA Ligase Reaction Buffer (10X)	2 ul
Plasmid Duo2 (vector DNA)	2 ul (36.12ng)
nadE gene (insert DNA)	2.3 ul diluted (26.85ng)
Nuclease free water	13.8 ul
T4 DNA ligase	1 ul

2. Resuspend gently up and down
3. Incubate at room temperature for 10 minutes.
4. Incubate at 4°C overnight.

References:

Ligation Protocol with T4 DNA Ligase (M0202)

Protocol for transformation of *P. putida*

We developed a new protocol for insertion of plasmid into *Pseudomonas putida* based on our observations, combining previous papers, and by switching different parameters and conditions.

While treatment of bacteria with CaCl2 is not performed before electroporation, we decided to do so because we were unable to conduct transformation of *P. putida* solely via electroporation.

Procedure:

Part 1. Preparation of competent cells

1) Transfer one colony of *P.putida* from plate to 30 ml LB broth and leave in the +30 shaking incubator overnight
2) Measure Optical density (OD) 600 nm. Preferable to use bacteria with OD of 1.0
3) Pour 5 ml of bacterial culture into pre-chilled 15ml centrifuge tube
4) Centrifuge at 4700 rpm at 4 °C, for 10 min
5) Discard the supernatant
6) Resuspend the pellet in 5 ml of 0.1M CaCl2 solution
7) Put on ice for 30 minutes
8) Centrifuge at 4700 rpm at 4 °C, for 10 min. Discard the supernatant
9) Resuspend the pellet in 5 ml of 0.1M CaCl2 20% glycerol solution
10) Put on ice for 10 minutes
11) Centrifuge at 4700 rpm at 4 °C, for 10 min. Discard the supernatant
12) Resuspend the pellet in 1 ml of sterile, ice-cold DI water
13) Centrifuge at 4700 rpm at 4 °C, for 10 min to remove residues of LB broth, CaCl2 solutions. Discard the supernatant
14) Resuspend the pellet in 50 uL of ice-cold DI water

Part 2. Electroporation of *P. putida*

1) Measure concentration of plasmids with NanoDrop. Add 500 ng of plasmid DNA into competent cells. Mix gently
2) Transfer bacteria to chilled electroporation cuvette
3) Cap the cuvette and tap it lightly on the bench to settle the bacteria/ DNA mix.
4) Put the cuvette back on ice and carry it to the electroporator.
5) Turn on the electroporator and set it to 2.5 kV, 25 mF, 200 Ω. This is a standard setting for most *P.putida* strains. Other bacterial strains may require an adjustment of the electroporation conditions.
6) Wipe the cuvette briefly with a Kimwipe to remove any residual water or ice, and then place it in the electroporation chamber.
7) Push the pulse button. The time constant displayed should be around 53 ms.
8) Immediately after the pulse has been delivered, add 1 ml of LB to the cuvette and pipette quickly but gently up and down. Avoid introducing air bubbles.
9) Transfer the mixture to a fresh 1.5-ml microcentrifuge tube.
10) Incubate at 30 °C for 1 h with shaking.
11) Evenly spread 100 uL of your transformation onto a selective plate. Perform positive and negative control
12) Incubate plates upside down overnight at 30 °C

References

This protocol was created by adjusting the following papers:

1) Lessard, J. (2013). Transformation of *E.coli* via electroporation. *Methods in enzymology*, 529, 321-327. https://doi.org/10.1016/B978-0-12-418687-3.00027-6
2) Soltabayeva, A. (2020). Competent cell preparation protocol
3) Gauttam, R., Mukhopadhyay, A., & Singer, S. (2020). Construction of a novel dual-inducible duet-expression system for gene (over)expression in *Pseudomonas putida*. *Plasmid*, 110, p.2. https://doi.org/10.1016/j.plasmid.2020.102514

Transformation of *P. aeruginosa* via Electroporation

Transformation of *P. aeruginosa* was followed protocol for *E.coli* as standard-setting except the setting the current supply. From the experimental trials the most optimal current intensity was chosen which is recorded in this protocol.

Materials:

- Equipments
    - Electroporator for bacteria (e.g., Gen Pulser Xcell Electroporation System, Bio-Rad)
    - Refrigerated centrifuge
    - Refrigerated microcentrifuge
    - Shaking incubator (37 C)
    - Incubator (37 C)
    - 15-ml sterile polypropylene snap-cap tubes
    - Electroporation cuvette, 0.1 cm gap
    - 15-ml glass centrifuge tube
    - Rubber adaptors (to fit glass centrifuge tube into floor centrifuge rotor)
    - Pipettes
    - Micropipettors
    - Micropipettor tips
    - 1.5-ml microcentrifuge tubes
    - Kimwipes
- Plasmid DNA (to be transformed)
- LB agar plates (selective plates containing appropriate antibiotic)
- Sterile ddH2O
- Glycerol (optional)

Procedure:

Preparation

Inoculate 5 ml of LB with *E. coli*. Grow overnight at 37 C with shaking. Chill the 15-ml glass centrifuge tube, a 1.5-ml microcentrifuge tube, the electroporation cuvette, and the ddH2O on ice. Run the floor centrifuge for a few minutes to cool the chamber to 4 C.

Create electro-competent *P. aeruginosa:*

1. Pour the bacterial culture into the prechilled 15-ml glass centrifuge tube on ice.
2. Centrifuge at 7000 rpm at 4 C, for 5 min.
3. Discard the supernatant.
4. Resuspend the pellet in 1 ml of sterile, ice-cold ddH2O and transfer into a chilled 1.5-ml microcentrifuge tube.
5. Spin in a microcentrifuge at 7000 rpm at 4 C, for 5 min.
6. Discard the supernatant.
7. Resuspend the pellet in 1 ml of ice-cold ddH2O.
8. Repeat steps 6-8 three more times.
9. Spin a final time at 7000 rpm at 4 C, for 5 min, discard the supernatant, resuspend the pellet in 50 ml of ice-cold ddH2O, and put the cells on ice.

Electroporation of *P. aeruginosa:*

1. Add 2µl of the DNA sample to 50µl of competent *P.aeruginosa*
2. Electroporate at 1600V for 5 seconds.
3. Immediately add 1mL of LB broth solution after electroporation.
4. Resuspend and transfer the mixture to 2 ml LB broth
5. Incubate 3ml of transformed *P.aeruginosa* solution for 2 hours at 37°C.
6. Plate 100µl of transformed *P.aeruginosa* on 3 plates of LB agar + kanamycin

Conventional fermentation experiments

Conventional fermentation involved the introduction of the *P. aeruginosa* (wild type) in the test medium. Supernatant from rhamnolipid production was used to treat soil contaminated with crude oil.

Procedure:

1. Prepare the minimal salt media (1 g/L $MgSO_4.7H_20$ +2 g/L KH_2PO_4 + 6 g/L $NaNO_3$)
2. Add non-fat dried milk powder to make 1% concentration of milk in media
3. Add ammonium chloride (0.6 g/L) and crude oil (2 %v/v) in media
4. Introduce wild type *P .aeruginosa* into the medium
5. Incubate for 24 hours at 35 °C

Electro fermentation experiments

Bioelectrochemical methods

Bioelectrochemical experiments were conducted using chronoamperometric (CA) method and cyclic voltammetry (CV). Chronoamperometric method was used to determine the stabilization of culture to the medium after inoculation.

Equipment:

- chronoamperometry
- cyclic voltammetry machine
- computer-controlled VSP multichannel potentiostat (Bio-Logic, France)

Procedure:

1. Prepare minimal salt media (1 g/L $MgSO_4.7H_20$ +2 g/L KH_2PO_4 + 6 g/L $NaNO_3$)
2. Add non-fat dried milk powder to make 1% concentration of milk in media
3. Add ammonium chloride (0.6 g/L) and crude oil (2 %v/v) in media
4. Introduce genetically modified *P. aeruginosa* into the medium
5. Incubate for 24 hours at 35 °C
6. After incubation obtain total charge data from the chronoamperometry
7. Measure the initial OD_{600} of wild type and modified type *P. aeruginosa*. It should be ~0.5
8. Grow *P. aeruginosa* cultures (modified and wild type) in 8 mL bio-electrochemical cells with an initial OD_{600} of 0.5 under potentiostatic conditions
9. Run cyclic voltammetry at 10 mV/s
10. Sterilize twice all electrodes in 70% v/v ethanol and twice wash in sterile deionized water

11. Gently blot dry electrodes
12. Set up electrochemical cells in a steel bead dry bath at 35 °C throughout the period of incubation, unless stated otherwise
13. Set up electrode at 400 mV and record current every 60 s for a 24 h incubation time

Extraction and analyses of biosurfactant

Emulsification index test (E_{24})

1. Filter the specific medium for the production of biosurfactants
2. Assess the medium as the water in oil type
3. Add an equal volume of cell- free broth and hydrocarbon (diesel) to test tubes
4. Vortex for 2 min at high speed. Keep at rest for 24 hours
5. Measure the emulsified oil height (cm) and compare with the total height
6. Calculate the emulsification according to

$$E24 = \frac{He}{Ht} \times 100$$

Where E_{24} = emulsification index following 24 h (in %);

He= emulsion height

Ht = total height.

Laboratory-scale bioremediation of soil saturated with crude oil

Soil samples contaminated with crude oil were used to set up a laboratory-controlled bioremediation.

1. Weight about 5 grams of the contaminated soil in a tube
2. Place the crude oil contaminated soils in separate conical flasks
3. Treat flasks as follows:

Setup	Contaminated soil, g	Crude biosurfactant	Sterile distilled water, mL
I	5	-	20

II	5	8 mL from *P. aeruginosa* wild type	12
III	5	8 mL from *P. aeruginosa* pRGPDuo2 + *nadE*	12
IV	5	8 mL from *P. aeruginosa* pRGPDuo2 + *rhlA*	12
V	5	8 mL from *P. aeruginosa* pRGPDuo2 + *rhlB*	12

4. Shake the experimental set ups at regular intervals for 24 h, after which it can be visibly observed.

Biosurfactant extraction

The biosurfactant was extracted using a cold acetone precipitation method:

1. Centrifuge the culture medium at 7500 rpm for 14 minutes at 4°C
2. Add 3 volumes of chilled acetone to the supernatant
3. Allow to stand at 4°C for 10 hours
4. Collect the precipitate by centrifugation
5. Allow to dry to remove residual acetone in a hot air oven
6. Weight the mass of the precipitate
7. Re-dissolve the dried precipitate in sterile water

Analysis of rhamnolipid production:

Perform Fourier Transform InfraRed (FTIR) Spectroscopy analysis on the obtained product in comparison with known standards to determine the presence of rhamnolipids.

1. Drip 3-5 drops of the sample into a Thallium Bromide aperture plate
2. Place another aperture plate into the sample
3. Liquid sample should be between the two aperture plates in a way that bubble formation is avoided
4. Correctly insert the aperture plates into an IR spectroscopy machine
5. Read the IR spectra with Fourier transformation between 400-4000 cm^{-1}

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