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Triterpene extraction protocol from Synechocystis sp. PCC6803 and R. capsulatus V.1

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We use this protocol and it's working

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Abstract

In this protocol, the extraction of naturally occurring as well as heterologously synthesized triterpenes in *Synechocystis* and *R. capsulatus* are described

Materials

MATERIALS

- Chloroform
- 100% methanol
- n-hexane
- Acetone
- Beta-Sitosterol
- NaCl

Before start

Prepare high-quality solvents (opt. LC-MS grade) and add 0.05% BHT (butylated hydroxytoluene) to all solvents (acetone, n-hexan, chloroform/methanol [2:1])

1 Collect the volumetric equivalent of $OD_{[750]}$ 20 cellculture, pellet cells and discard the medium supernatant.

Store die pellet in freezer at -80°C in lobind reaction tubes.

Also prepare a solvent control without pellet (mimick pellet with equivalent amount of water (or medium), and proceed exactly as with the “real” samples

2 Add a total of 10 nmol β-sitosterol (internal standard) to acetone stock and resuspend frozen pellet in 1 ml Acetone and incubate 15 min at 50 °C under gentle agitation

Note

- do not let it thaw, add acetone directly to frozen Pellet after taking it from the freezer and place immediately to 50°C)

1 µL Acetone

50 °C gentle agitation

00:15:00

3 Centrifuge to separate pellet from solvent extract at 1780 rcf for 3 min (flexible), and transfer solvent to new 5 ml reaction tubes

If the pellet is not discolored (white in case of *Rhodobacter*, blue in case of *Synechocystis)*, **definitely repeat extraction step with Acetone** for quantitative analysis and and combine both solutions in the same tube

00:03:00 1780 rcf

go to step #2

4 Add 1 ml 1 M NaCl to the combined acetone solution and mix

1 µL 1M NaCl

5 Add 0.9 ml n-Hexane and vortex 30 sec to extract non-polar components to hexane-phase

0.9 µL n-hexan

00:00:30 Vortex

Note

make sure the phases are vigorously mixed, therefore alternate between vortexing the tube vertically and horizontally, take care to prevent the tube from opening accidentally

6 Centrifuge shortly for clean phase separation (e.g. 1', 1780 g)

00:01:00 1780 rcf

7 Transfer hexane phase to 2ml reaction tubes.

Repeat the extraction of the pellet with 0.9 ml n-Hexane in order to increase the yield and combine both in the same reaction tube

Note

take care to transfer the complete hexane phase for quantitative analysis, however avoid contamination with the NaCl-containing lower phase, if you accidentally take too much, you may observe phase separation within your pipet tip, you can carefully let go of the lower few drops (aqueous phase with NaCl) back to the original tube.

go to step #5

8 Speed vac hexan phase to dryness, as briefly as possible, (at 30°C fast program, about 20 min)

Alternatively use rotation evaporator or N_2-Stream

30 °C

00:20:00 Speed vac

9 Resuspend in 150 µl Chloroform/MeOH (2:1)

Note

Prepare a stock of Chloroform/MeOH (2:1) including BHT, do not add MeOH and Chloroform separately

150 µL Chloroform/MeOH (2:1)

10 Transfer into HPLC vial with insert

Note

Store extracts at -20 °C