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Conditions for growth of Rhodobacter sphaeroides in different media

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We use this protocol and it's working

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Abstract

This protocol provides a method for culturing *Rhodobacter sphaeroides* wildtype 2.4.1 and its KO strains in shake flask and BioTek Epoch 2, 48-well microplates. A baffled side arm flask is used but other flasks can be used with appropriate modifications.

Materials

- Incubator at 33^{0}C
- ^{G}YCC agar plate and liquid medium
- Centrifuge
- Sterile tooth pick
- Colorimeter/spectrophotometer

Conditions for growth of *Rhodobacter sphaeroides* in GYCC medium in shake flasks

1 Inoculate a GYCC agar plate from a glycerol stock containing appropriate antibiotic (if necessary) and incubate at 33 °C in an incubator in dark.

2 After 72:00:00 pick one colony from the plate and inoculate 80 ml fresh GYCC medium with appropriate antibiotic (if necessary) in 125 mL baffled side arm flask. 3d

3 Incubate at 33 °C shaking at 125 rpm for 72 -96 hours.

4 Monitor the growth of the culture with regular measurement of turbidity. Side-arm flasks are especially convenient for the determination of culture turbidity with a Klett-Summerson colorimeter. The equivalent OD_{600} may also be used if side arm flasks are not used.

Conditions for growth of *R. sphaeroides* in GYCC or MR26 medium in Microplate

5 Inoculate GYCC or MR26 agar plates from a glycerol stock containing appropriate antibiotic if necessary and incubate at 33 °C .

6 After 72:00:00 s pick one colony from the plate and inoculate 25 ml fresh medium of GYCC or MR26 containing appropriate antibiotic (if necessary) in 50 mL flask 3d

7 Incubate at 33 °C with shaking at 125 rpm for 72 -96 hours.

8 Monitor the growth of the culture with measurement of turbidity. Once the culture reaches to the log phase (OD_{600}~0.7), the starter culture is ready for inoculation of the microplate.

9 For each well 600, µl of fresh GYCC or MR26 medium was added. It is recommended to use Microplate Greiner BioOne 48 well (Item no. 677180) for compatibility with a BioTek Epoch2 microplate reader

Procedure Details in microplate reader

9.1 Plate Type: Greiner BioOne 48-wellv2 (Use plate lid)
Eject plate on completion
Set Temperature: Setpoint 33°C, Gradient 2 °C
Start Kinetic: Runtime 96:00:00 (HH:MM:SS), Interval 0:15:00, 385 Reads
Shake Double Orbital: Continuous
Frequency: 548 cpm (2 mm)
Read:A600
Absorbance Endpoint
Full Plate
Wavelengths:600
Read Speed: Normal, Delay: 200 msec, Measurements/Data Point: 8
End Kinetic

10 Once the run is complete, export the data in a .txt or .xls file and save in an appropriate location. Analyze and visualize the data by any software.