Determination of Oxygen Respiration Rates in Wetted Developmentally Arrested Spores of *Streptomyces* Species

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[Abstract] *Streptomyces* species produce spores, which, while not as robust as endospores of *Bacillus* or *Clostridium* species, are capable of surviving for months or even years (Hopwood, 2006). During this time these spores remain viable, surviving by slowly degrading internal stores of carbon compounds, such as the carbohydrate trehalose. To enable metabolism to continue they must have access to an electron acceptor that allows the removal of the reducing equivalents that accumulate through metabolic activity. The most commonly used acceptor is oxygen. We describe the quantitative measurement of oxygen respiration rates by developmentally arrested spores of the streptomycete *Streptomyces coelicolor* (Fischer *et al.*, 2013).

Materials and Reagents

1. Freshly harvested *Streptomyces* spores in water
2. Soya flour (from local supermarket)
3. D-mannitol (Sigma-Aldrich, catalog number: 63560)
4. Agar-agar (Kobe I) (Roth North America)
5. 3-(N-morpholino) propanesulfonic acid (MOPS) buffer (Roth North America)
6. Chloramphenicol (Merck KGaA)
7. Sodium hydroxide pellets (Roth North America)
8. MOPS-buffer (see Recipes)
9. SFM agar (see Recipes)

Equipment

1. Cotton wool (standard issue from local Pharmacy) for spore preparation
2. 0.22 µm pore-size filters (PVDF) (Sartorius AG)
3. 30 °C rotary shaker
4. Baffled Erlenmeyer flasks (500 ml) (Glasgerätebau Ochs, Laborfachhandel e. K., catalog number: 100500)

5. Standard-sized plastic Petri dishes for bacterial growth and spore preparation
6. Gas-tight glass Hungate tubes (16 ml) for anaerobic work with butyl rubber septa (Glasgerätebau Ochs, Laborfachhandel e. K., catalog number: 1020471)
7. Cooled table-top centrifuge (*e.g.* Eppendorf)
8. Needles (0.6 mm gauge) (B. Braun Melsungen AG)
9. Water bath or heating block (Biometra)
10. Spectrophotometer capable of measuring absorption in the visible range
11. Optical 'FirestingO_2 oxygen meter' (Pyro Science GmbH)
12. Contactless optical oxygen 'sensor spot' (Pyro Science GmbH)
13. 'Bare fibre' (Pyro Science GmbH)
14. Adjustable mini magnetic stirrer (*e.g.* IKA) (10 mm x 0.3 mm magnetic stirrer bar) (*e.g.* Roth North America)

Software

1. Firesting Logger Software (Pyro Science GmbH)

Procedure

1. *Streptomyces coelicolor* was grown on SFM agar plates at 30 °C until colonies sporulated (Kieser *et al*., 2000). Spores were isolated by using cotton swabs and were washed with water and filtered twice through cotton wool (Kieser *et al.*, 2000). Subsequently they were adjusted to an OD_{450nm} of 10 or 20 (1 ml of an OD_{450} = 1 is equivalent to 3.5 x 10^8 spores/ml or 2.37 x 10^8 cfu).
2. The oxygen reduction rate by spores was measured in 16 ml gas tight Hungate vials in a 30 °C room or chamber.
3. The vials were filled with 5 ml of 50 mM MOPS buffer (pH 7.2), containing chloramphenicol (400 µg/ml) and spore suspension with an OD_{450nm} of 25 (Fischer *et al.*, 2013).
4. The spore suspension was stirred continuously with a magnetic stirrer at 800 rpm upto an oxygen saturation in the spore suspension. Oxygen saturation was determined empirically for each experiment performed and this process generally took approximately 2 min based on a stirring speed of 800 rpm. The vials were closed with a gas-tight rubber plug and simultaneously the pressure was equalized with a needle one time and the on-line measurement cycle was initiated, exactly as indicated in the protocol delivered by the manufacturer. An image of the experimental setup is available at http://www.pyro-science.com/optical_oxygen_meter_firesting_O2.html.

5. The oxygen level in the remaining 11 ml headspace was monitored non-invasively and continuously over a period of 15 h using the oxygen-measuring system of Pyro Science GmbH and documented using the Firesting Logger Software. To do this, oxygen-dependent luminescence sensor spots were affixed to the glass wall of the tube within the headspace and the signals were measured through the vial's glass wall using an optical oxygen meter (FirestingO_2).
6. The oxygen respiration rate was calculated as the moles of oxygen reduced per volume oxygen in the headspace with respect to the dry weight of spores (1 OD/ml = 0.12 mg dry weight). A representative example of data is shown in Fischer *et al*. (2013).

Recipes

1. MOPS-buffer
   Adjusted to pH 7.2 with NaOH
   Sterilized by filtration through 0.22 µm filter
2. SFM agar
   20 g/L soya flour
   20 g/L D-mannitol
   Agar-agar (Kobe I)

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References

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