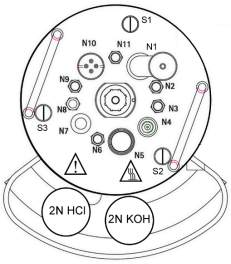
Aug 28, 2020

Fermentor Growth of Streptococcus sanguinis



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Tanya Puccio[1], Todd Kitten[1]

[1]Philips Institute for Oral Health Research, Virginia Commonwealth University

Tanya Puccio

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Protocol status: Working
We use this protocol and it's working

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Keywords: Fermentor, biostat, Streptococcus, sanguinis, manganese depletion, fermentor growth of streptococcus sanguinis streptococcus sanguini, forming bacterium, manganese depletion for various analysis, manganese depletion, fermentor growth, manganese transporter, anaerobic condition, reproducible growth, streptococcus sanguinis streptococcus sanguini, lactic acid,

Abstract

Streptococcus sanguinis is a lactic acid-forming bacterium that can be cultured in both aerobic and anaerobic conditions. It is a primary colonizer of the oral cavity but can also cause a heart disease called infective endocarditis. Our main objective for this protocol was to grow large, controlled cultures before and after manganese depletion for various analyses. In order to obtain large scale, reproducible growth of *S. sanguinis,* a biostat was used to maintain controlled conditions. After optimization, it was determined that traditional chemostat conditions (Burne and Chen, 1998) were not appropriate for these experiments. Thus, we decided on this modified protocol. Using this method, we were able to identify a concentration of the non-specific metal chelator, EDTA, that would lead to a decreased growth rate in a manganese transporter mutant but not in the wild-type strain.

Attachments

20180808_160423.jpg	20180808_160257.jpg	20180808_155550.jpg	20180808_155418.jpg
3.5MB	3.5MB	3.4MB	3.3MB

Guidelines

These intructions are specific to a Sartorius Stedim Biostat® B with a 1.5 L capacity UniVessel® Glass + BioPAT® Fundalux, DO probe, and pH probe. Adjustments may be required if using different equipment.

Materials

MATERIALS

- RNeasy Mini Kit **Qiagen** **Catalog** #74104
- RNase-Free DNase Set **Qiagen** **Catalog** #79254
- Bacto™ Brain Heart Infusion **Thermo Fisher** **Catalog** #237300
- Antifoam 204 **Merck MilliporeSigma (Sigma-Aldrich)** **Catalog** #A8311
- Potassium Hydroxide **Fisher Scientific** **Catalog** #1310-58-3
- Sodium Hydroxide **Fisher Scientific** **Catalog** #1310-73-2
- Hydrochloric Acid **Fisher Scientific** **Catalog** #7647-01-0
- EDTA 0.5 M pH 8.0 **Invitrogen** **Catalog** #AM9261
- RNAprotect Bacteria Reagent **Qiagen** **Catalog** #76506
- DNA-free DNA Removal kit **Invitrogen** **Catalog** #AM1906
- Needleless Injection Site Swabbable Female Luer Lock to Barb Connector **Qosina** **Catalog** #80210

STEP MATERIALS

- RNAprotect Bacteria Reagent **Qiagen** **Catalog** #76506
- RNeasy® Mini Kit **Qiagen** **Catalog** #74104
- RNase-Free DNase Set **Qiagen** **Catalog** #79254
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Troubleshooting

Before start

1. The afternoon before the run, start a 40 mL pre-culture at 37 °C and appropriate oxygen concentration with antibiotics (if appropriate).
2. Make 5 L of BHI with [M] 25 Parts per Million (PPM) antifoam. Autoclave 02:00:00 .
3. Set up fermentor according to manufacturer's specfications/user's needs.

When running an experiment for the first time, test to see how long it takes for the media to flow from the carboy to the vessel at the chosen flow rate (will vary depending on the length of the tubing used).

Inoculation

1 Set up fermentor to experiment specifications: pO_2 set to 5% with 0.03 lpm max air flow; nitrogen set at 0.08 lpm; stirrer set at 250 rpm; 37 °C 7.4 800 mL

Equipment

Biostat® B	NAME
Benchtop Bioreactor	TYPE
Sartorius	BRAND
N/A	SKU

Equipment

UniVessel® Glass 1.5 L capacity	NAME
Vessel	TYPE
Sartorius Stedim	BRAND
N/A	SKU
https://www.sartorius.com/en/products/fermentation-bioreactors/benchtop-bioreactors/univessel-glass	LINK

Equipment

BioPAT® Fundalux

NAME

Optical density probe

TYPE

BioPAT

BRAND

N/A

SKU

Equipment

EasyFerm Plus PHI VP 120 Pt100

NAME

pH probe

TYPE

Hamilton

BRAND

238633-1111

SKU

Equipment

InPro6800/12/160/4112111

NAME

Dissolved oxygen probe

TYPE

Mettler Toledo

BRAND

5230580

SKU

2 Remove 15 mL media from vessel to incubate 37 °C as a check for contamination.

2.1 Remove 500 µL media and store at -80 °C for metabolomics analysis.

3 Take OD_{600} reading using 1 mL of 40-mL overnight pre-culture.

Equipment	
Genesys 150	NAME
UV-VIS Spectrophotometer	TYPE
Thomas Scientific	BRAND
1160V96	SKU

4 Centrifuge remaining volume 39 mL of overnight culture.
4303 x g, 4°C, 00:10:00

4.1 Decant supernatant and resuspend in 15 mL BHI.

4.2 Inoculate into vessel using 20-mL syringe.

Ramping up air flow

5 As the absorbance units (AU) increase, gradually ramp up the air flow.

5.1 When AU reaches 0.10, turn off the DO control and set air flow to 0.03 lpm.

5.2 When AU reached 0.30, set air flow to 0.20 lpm.

Note

If the experiment is meant to be low oxygen, skip this step.

5.3 When AU peaks and begins to drop, set air flow to 0.50 lpm. Turn on media pumps: input at 17% and output at 34%.

Note

For our fermentor, 17% ~ 700 mL h^{-1}

Note

If the experiment is meant to be low oxygen, do not increase the air flow.

Expected result

SK36 (wild type) growth should not be drastically affected by the addition of EDTA. For the primary Mn transporter mutant (Δ*ssaACB*), the OD will increase initially and then should start to drop in OD (by 0.01 AU) ~38 min after EDTA addition.

Experimental Conditions

6 Before proceeding with sample collection, allow culture to adjust to media flow for 01:00:00 .

7 At 1 h post-media flow (T_{-20}), remove sample 40 mL from vessel using a syringe (usually 60 mL capacity) using the sampling port.

8 At the predetermined time, add EDTA to [M] 100 micromolar (µM) to carboy in 5 mL BHI using a syringe into the inoculation port.

EDTA 0.5 M pH 8.0 **Invitrogen Catalog #**AM9261

Note

Using the flow time calculated previously (see *Before Starting*), determine how long it will take for media to flow from carboy to vessel and subtract from 20 min. For example, if it takes 4 min to flow from carboy to vessel, add EDTA 16 min after the first sample was removed.

9 At T_0 (20 min after first sample), add EDTA to [M] 100 micromolar (µM) to vessel in 5 mL BHI using a syringe into the inoculation port.

EDTA 0.5 M pH 8.0 **Invitrogen Catalog #**AM9261

10 Collect the remaining samples at T_{10}, T_{25}, and T_{50}.

Sample Collection Protocols

11 For metabolomics samples, aliquot 30 mL of cell culture into conical tube.

11.1 Swirl immediately in a dry ice/ethanol bath for 00:01:00 .

11.2 Centrifuge sample immediately.

4303 x g, 4°C, 00:05:00

11.3 Aliquot 500 µL of supernatant media and store at -80 °C .

Remaining volume can be decanted or stored at -20 °C for hydrogen peroxide quantification or other analysis.

11.4 Store cell pellet at -80 °C until ready for analysis.

12 For RNA samples, set up tubes for each sample containing 4 mL RNAprotect.
RNAprotect Bacteria Reagent **Qiagen Catalog #**76506

12.1 Add 2 mL of cell culture to RNAprotect tubes. Vortex for 00:00:05 .

12.2 Incubate at Room temperature for at least 00:05:00 but less than 03:00:00 .

12.3 Centrifuge cell culture in RNAprotect 4303 x g, 4°C, 00:10:00 .

12.4 Store at -80 °C until ready to isolate RNA.

12.5 Isolate RNA and remove DNA.

RNeasy® Mini Kit **Qiagen Catalog #**74104

RNase-Free DNase Set **Qiagen Catalog #**79254

DNA-free DNA Removal kit **Invitrogen Catalog #**AM1906

12.6 Store RNA at -80 °C until ready for analysis.