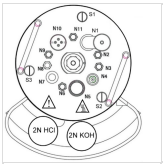

Aug 28, 2020

Fermentor Growth of Streptococcus sanguinis

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1 Works for me dx.doi.org/10.17504/protocols.io.bkayksfw

Tanya Puccio

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.

THIS PROTOCOL ACCOMPANIES THE FOLLOWING PUBLICATION

Puccio, T., Kunka, K.S., Zhu, B., Xu, P., and Kitten, T. (2020). Manganese depletion leads to multisystem changes in the transcriptome of the opportunistic pathogen *Streptococcus sanguinis*. bioRxiv. doi: 10.1101/2020.08.06.240218

ATTACHMENTS

20180808_160423.jpg 20180808_160257.jpg 20180808_155550.jpg 20180808_155418.jpg

DOI

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PROTOCOL CITATION

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MANUSCRIPT CITATION please remember to cite the following publication along with this protocol

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KEYWORDS

Fermentor, biostat, Streptococcus, sanguinis, manganese depletion

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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

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

STEPS MATERIALS

NAME	CATALOG #	VENDOR
RNAprotect Bacteria Reagent	76506	Qiagen
RNeasy® Mini Kit	74104	Qiagen
RNase-Free DNase Set	79254	Qiagen
DNA-free DNA Removal kit	AM1906	Invitrogen
EDTA 0.5 M pH 8.0	AM9261	Invitrogen

EQUIPMENT

NAME	CATALOG #	VENDOR
Genesys 150	1160V96	Thomas Scientific
InPro6800/12/160/4112111	5230580	
Biostat® B	N/A	Sartorius
UniVessel® Glass 1.5 L capacity	N/A	Sartorius
BioPAT® Fundalux	N/A	Sartorius
EasyFerm Plus PHI VP 120 Pt100	238633-1111	Hamilton Company

BEFORE STARTING

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 **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** pH **7.4** **800 mL**

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Biostat® B
Benchtop Bioreactor
Sartorius **N/A**

UniVessel® Glass 1.5 L capacity
Vessel
Sartorius Stedim **N/A**

BioPAT® Fundalux
Optical density probe
BioPAT **N/A**

EasyFerm Plus PHI VP 120 Pt100
pH probe
Hamilton **238633-1111**

InPro6800/12/160/4112111
Dissolved oxygen probe
Mettler Toledo **5230580**

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.

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

Genesys 150
UV-VIS Spectrophotometer
Thomas Scientific **1160V96**

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.

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%.

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

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

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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 **100 Micromolar (µM)** to carboy in **5 mL** BHI using a syringe into the inoculation port.

EDTA 0.5 M pH 8.0
by Invitrogen
Catalog #: AM9261

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 **100 Micromolar (µM)** to vessel in **5 mL** BHI using a syringe into the inoculation port.

EDTA 0.5 M pH 8.0
by Invitrogen
Catalog #: AM9261

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

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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
by 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.

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RNeasy® Mini Kit
by Qiagen
Catalog #: 74104

RNase-Free DNase Set
by Qiagen
Catalog #: 79254

DNA-free DNA Removal kit
by Invitrogen
Catalog #: AM1906

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

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