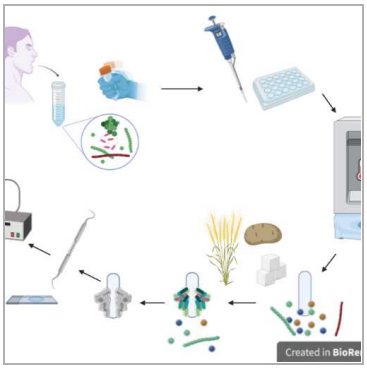

VERSION 2

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

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Biofilm growth with starch treatment V.2

In 1 collection

Bjørn Peare Bartholdy[1], a.g.henry[1]

[1]Leiden University

BYOC

Bjørn Peare Bartholdy
Delft University of Technology, Leiden University

ABSTRACT

This is the main protocol to grow a calcifying oral biofilm model with starch treatments. It uses a 24 deepwell plate with the accompanying lid as substratum (polypropylene). The protocol takes 25 days to run, with daily tasks.

Modified from protocols by Sissons et al. (1991) and Extercate et al. 2010.

IMAGE ATTRIBUTION

Image created by Bjørn Peare Bartholdy using BioRender.com

GUIDELINES

The preferred substratum for inoculation is glass or hydroxyapatite. Plastic substrata can be used but are less effective, so surface treatment of the plastic is recommeded (e.g. heated HCl or acetone).

Substrata should be autoclaved prior to use, both before and during the experiment.

MATERIALS

Solutions
Artificial saliva

Protocol

NAME

Artificial saliva

CREATED BY

Bjørn Peare Bartholdy

PREVIEW

CPMU

Protocol

NAME

CPMU

CREATED BY

Bjørn Peare Bartholdy

PREVIEW

20% (v/v) sterile glycerol in dH_2O

5% (w/v) sucrose in dH_2O

0.25% (w/v) potato starch in dH_2O

0.25% (w/v) wheat starch in dH_2O

0.50% (w/v) equal parts wheat (0.25%) + potato (0.25%) in dH_2O

Equipment
24 deepwell polypropylene plates (w. lid containing pegs suspended from the lid)
Shaking incubator
Powder free nitrile gloves

BEFORE START INSTRUCTIONS

Surfaces in the lab should be cleaned three times. Once with warm water and starch-free detergent, then with 5% NaOH, followed by a final cleaning with distilled water.

Saliva donor criteria

- Must have no/limited history of dental caries
- Must not have used antibiotics in the past 6 months
- Abstain from oral hygiene 24 hours prior to donation
- Refrain from eating and drinking (except water) 2 hours before donation

For experiments involving starches, donors should avoid eating starch-containing foods on the day of donation. To make this more bearable, saliva donation should take place in the morning before breakfast.

Prerequisites

Required solutions should be prepared beforehand.

- 20% (v/v) sterile glycerol in dH_2O
- Artificial saliva (required for day 0)
- Sucrose solution (required for day 0)
- Starch solutions (required for day 9)
- CPMU (required for day 15)

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PREVIEW

Saliva collection

1 Saliva donors rinse their mouth with water for 30 seconds.

2 Stimulate saliva production by chewing tasteless gum or parafilm.

3 Collect the saliva by spitting into 50 ml plastic centrifuge tubes.

> Note
>
> Make sure donors wear gloves to avoid contamination with non-oral bacteria.

4 Make a 2-fold dilution of saliva in **sterile** [M] 20 % (v/v) glycerol and vortex the solution.

Day 0: Inoculation and feeding

5 Before inoculation, vortex the saliva solution again.

6 Pipette the saliva solution into the wells, so approx. 1-2 cm of the substratum is submerged.

7 Place the plate in the incubator at 36°C for 4 hours for static inoculation 04:00:00 4h

8 After inoculation, transfer the samples to a new plate containing the artificial saliva, and place in a shaking incubator at 30 rpm, 36°C for 04:00:00 4h

Protocol

NAME

Artificial saliva

CREATED BY

Bjørn Peare Bartholdy

PREVIEW

8.1 Add 300 mL distilled (or deionized) dH_2O to a 1000 mL beaker, with stirring and heat 60 °C .

8.2 Add:

- 2.5 g Mucin from porcine stomach (Type III) **Sigma Aldrich Catalog #M1778**
- 5 g Trypticase™ Peptone **Thermo Fisher Catalog #211921**
- 10 g Oxoid™ Proteose Peptone **Thermo Fisher Catalog #LP0085B**
- 5 g Bacto Yeast Extract **Becton-Dickinson**

Let the reagents completely dissolve before continuing to the next step

8.3 Add:

- 2.5 g KCl **Contributed by users**
- 0.35 g NaCl **Contributed by users**
- 0.2 g CaCl2 **Contributed by users**
- 0.74 g Sodium phosphate dibasic **Sigma Aldrich Catalog #7558-79-4**

- 0.54 g NaHCO3 **Contributed by users**
- 2.5 mg Hemin **Contributed by users**

8.4 Add the remaining 700 mL distilled (or deionized) dH_2O

8.5 Adjust to pH 7 with NaOH **Contributed by users** and stirring

8.6 Transfer to two 1000 ml bottles, so half of each bottle is filled. 15m

Autoclave at 121 °C , 1 Bar for 00:15:00 minutes

Safety information

Do NOT screw bottle caps on tightly.

Loosely screw the caps on the bottles or cover the tops with foil

8.7 Once the solution has cooled, add:

- 1 mg Menadione **Contributed by users**
- 0.3 g Urea **Contributed by users**
- 0.17 g L-Arginine **Contributed by users Catalog #A5006**

8.8 Store in fridge at ca. 4 °C

Occasionally test the pH to ensure it stays around pH 7

9 Transfer the samples to a plate containing a 5% (m/v) Sucrose **Contributed by users** solution for 00:06:00 , then transfer back to the artificial saliva and leave Overnight . 6m

Plates with lids (substratum) in the incubator. Sucrose treatment plates covered with sterilised foil at the back.

Day 1-2: Feeding

8h 12m

10 First thing in the morning, transfer the samples to a new plate containing a 5% (m/v) Sucrose **Contributed by users** solution for 00:06:00 . While in the sucrose solution, add more artificial saliva to the wells on the original plate that have been partially depleted overnight. 6m

11 After the 6 mins. return the samples to the plate with **artificial saliva**, and cover up the sucrose plate and leave for 08:00:00 . 8h

12 After 8 hours, transfer the samples back to the plate with 5% (m/v) Sucrose **Contributed by users** solution for 00:06:00 . Transfer back to the artificial saliva and leave Overnight . Dispose of the sucrose. 6m

Day 3: Inoculation and feeding

8h

13 Repeat steps from saliva collection and Day 0: Inoculation and feeding. 8h

Expected result

A layer of clear plaque should be visible on the substrata

14 Prepare a new plate with artificial saliva. Transfer the samples from the inoculation plate to the **artificial saliva**.

Day 4: Feeding 8h

15 Repeat steps 10 through 12 8h

Note

Prepare a new plate of artificial saliva every third day throughout the experiment. Every other morning, top up the wells with artificial saliva (so ca. 1-2 mm of the substratum is submerged).

Day 5: Inoculation

16 Repeat steps 1 through 9

Day 6-8: Feeding

17 Repeat steps 10 through 12

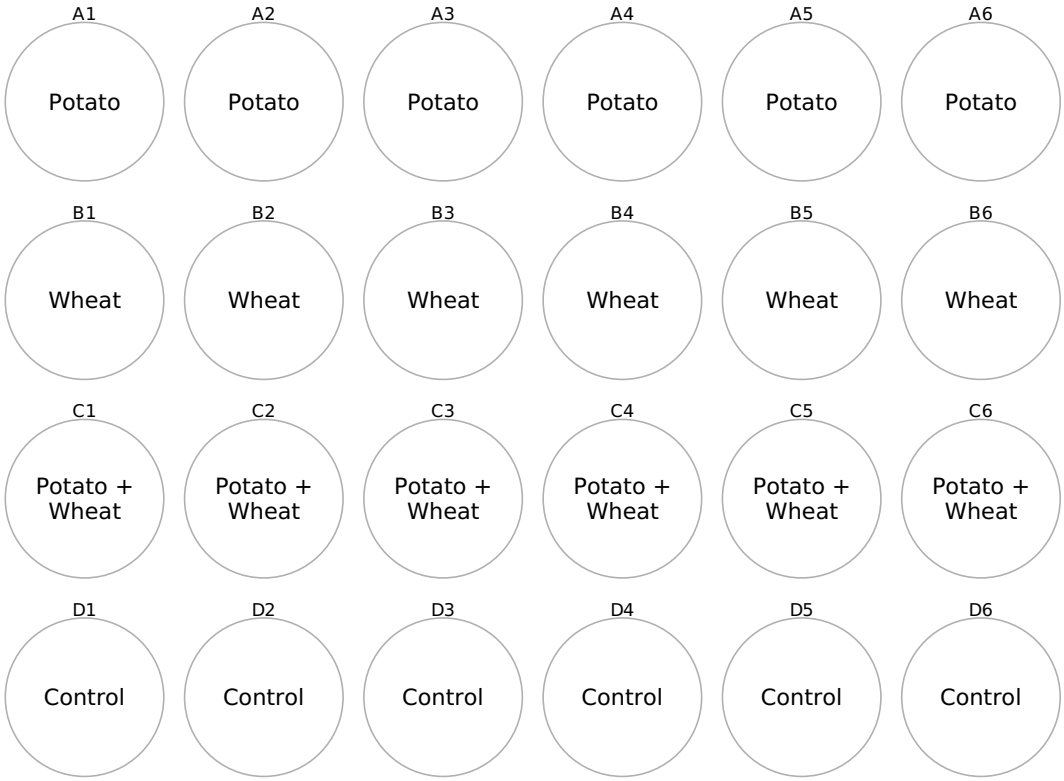
Day 9-14: Starch treatment

8h 6m

18 Transfer the samples to a plate with the starch treatment(s) for 00:06:00 at 60 rpm, 36°C

6m

Example setup:



Safety information

If you are using multiple starch treatments within a single plate, take care to avoid cross-contamination.

19 Transfer the samples back to the artificial saliva plate for 08:00:00 at 30 rpm, 36°C

8h

20 Transfer the samples to a plate with the starch treatment(s) for 00:06:00 at 60 rpm, 36°C

6m

21 Transfer the samples back to the artificial saliva plate at 30 rpm, 36°C and leave Overnight

Day 15-24: Mineralisation

8h 30m

22 Transfer the samples to a plate containing the calcium phosphate monofluorophosphate urea (CPMU) solution for 00:06:00 at 60 rpm, 36°C 6m

Protocol

NAME

CPMU

CREATED BY

Bjørn Peare Bartholdy

PREVIEW

22.1 Add 300 mL distilled (or deionized) dH_2O to a 1000 mL beaker, with stirring and heat 60 °C

22.2 Add:

- 1.55 g Calcium Chloride **Sigma Aldrich**
- 1.44 g Sodium Phosphate monobasic **Sigma Aldrich**
- 0.72 g Sodium Fluorophosphate **Sigma Aldrich Catalog #344443**
- 0.08 g Magnesium Chloride **Sigma Aldrich Catalog #AC223210010**
- 30 g Urea **Sigma Aldrich**

22.3 Add the remaining 700 mL and keep stirring until precipitate has completely dissolved
Store in fridge at 4 °C

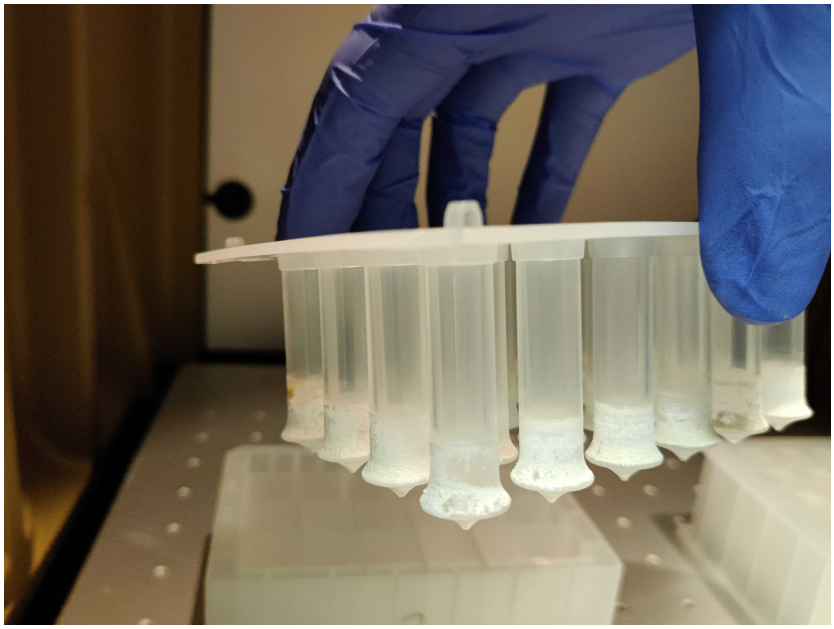
23 Transfer the samples back to the **artificial saliva** for 02:00:00 at 30 rpm, 36°C 2h

Put a lid on the plate with CPMU (or cover with foil) to prevent evaporation.

Repeat step 22 and 23, four more times every two hours.

24 Transfer the samples to a plate with the **starch treatment(s)** for 00:06:00 at 60 rpm, 36°C 6m

25 Transfer the samples back to the **artificial saliva plate** at 30 rpm, 36°C and leave Overnight



Analysis

26 Samples should be dried before sampling.

Transfer to a new plate with no liquid and leave in the incubator.

27 Once dried, samples are processed like archaeological samples.