Making heat-killed bacteria for feeding axenic phagotrophic protists

Alle Lie

Abstract

Citation: Alle Lie Making heat-killed bacteria for feeding axenic phagotrophic protists. **protocols.io** dx.doi.org/10.17504/protocols.io.ewkbfcw
Published: 26 Apr 2016

Protocol

Step 1.
Obtaining a clonal bacteria strain (Skip to step 2 if one is already available)

Prepare 1.5.% agar plates with 0.5% yeast extract and 0.5% trypton. Use the salinity that the protist culture in growing in.
i.e. 0.5 g yeast extract+ 0.5 g trypton + 1.5 g agar in 100 ml of MiliQ water (MQ)/Filtered sea water (FSW)
Heat to dissolve.
Autoclave to sterilize.
Pour into medium sized petri dishes
When the agar plates are cooled and set, streak some liquid from a bacterized culture of the protist on the agar plates.
Incubate the agar plates at the same conditions of the protist culture
When bacteria colonies form, use a flame sterilized metal loop to pick one clonal strain and dip the metal loop into a nutrient broth (0.5% yeast extract + 0.5% trypton in MQ/FSW)
Let the bacteria grow in the same incubating conditions

Step 2.
Grow a large amount of the bacteria

Prepare 1 L of bacteria nutrient broth (Or however much depending on how much bacteria you want to grow)
0.5% yeast extract + 0.5% trypton in MQ or FSW and autoclave to sterilize
Transfer 0.1 ml of the bacteria culture into the broth
Let the bacteria grow for 4 days

Step 3.
Heat-killing the bacteria

Filled 250 ml centrifuge bottles entirely with the bacteria culture.
Place in water bath at 70oC for 30 min

Step 4.
Centrifuge and wash the bacteria

Centrifuge the heat-killed bacteria at 10 k g for 20 min.
Decant supernatant.
Add 25 ml of sterilized MQ/FSW (depending on the salinity of your culture) in the bottle.
Vortex/shake to resuspend pellet.
Add MQ/FSW to fill to 200 ml and centrifuge again at 10 k g for 20 min.
Repeat this step until you have washed the bacteria 3 times.
After washing for the last time, centrifuge and decant supernatant.
Add as little MQ/FSW as possible and vortex/shake to resuspend pellet.
You may also sonicate the concentrated bacteria if a sonicator is available.

Step 5.
Test the sterility of the bacteria

Test the sterility of the bacteria by placing a dilute aliquot of the heat-killed bacteria in 5 ml of 0.5% yeast extract + 0.5% trypton broth. Make sure that the liquid is still clear after adding the bacteria so that it will be possible to tell whether the liquid became cloudy if there was bacterial growth.
Incubate the sterility test for several day. The bacteria is sterile if there is no cloudiness in the test after 5 days.