Creation of an isolate: PA and FL microbes from whole water

Aubrey Reed[1], Nicole Sunshine[1]

[1]University of Michigan

dx.doi.org/10.17504/protocols.io.ubcesiw

Working

Oct 03, 2018

Environmental Microbiology EEB 401 UMich

Aubrey Reed

PROTOCOL STATUS

Working

We use this protocol in our group and it is working

Collect Water

1 Pre-filter the water with a 20 µm mesh net and fill a clean carboy with several liters of this <20 µm-filtered water. You are filtering 500mL, collect at least 1 liters (~30% more than needed for filtration) at this stage to ensure enough water for system flushing.

Separating particle associated (PA) and free-living microbes (FL)

2 Using a parastolic pump (procedure for usage of this apperatus can be found in Sampling water for microbial and viral counts by Melissa Duhaime.)

Generally we will use the pump with one inline filiter that is 3 micron. We will use our prefiltered whole water, and we will collect about 500mL of the <3 micron (FL sample) water sample in an epandorf tube, and we will take the 3 micron filter (PA sample) and we will roll the filter so the "dirty" side faces out and we suspended it in about 50uL of 1XPBS in an epandorf tube.

Preparing for culture

3 Preferably rapidly after collection you should begin to prepare your samples for culture.

For the PA sample, take the tube with the filter paper and vortex it for 30 seconds to 1 minute. Then using sterlie forecps pull filter paper slightly out of the tube resecure the cap of the tube and then centrifuge sample for 3 minutes. Then remove the filter paper, vortex and pippette up and down to resuspend cell pellet. Plate 50uL of resuspended sample on to four plate types: TSA, BG11, LB and Lake agar

For FL sample, vortex sample for 30 seconds and then plate 50uL of sample on to the four plate types: TSA, BG11, LB and Lake

Isolation of Colonies

4 After plates have grown for 2 or 3 days we should see a lawn of several different colony formations. Isolate unique colony morphologies for each plate and streak them out on to a new plate of the same type. (TSA parent plate -> TSA daughter plate). We collected up to 5 different colony morphologies for each parent plate.

After the streaks had grown out (another 2 to 3 days) we then streaked isolated colonies again (if two colonies where present we then split them and plated one of each).

We restreaked the colonies for 3 generations until we were certain of purity of colony.