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Date: 10/3/19

Goal:

1. To figure out the adequate resuspend procedure for S. microadriaticum and O.marina.

Protocol:

1. Put 2mL samples (S. microadriaticum and O.marina) into each centrifuge tube.
2. Harvested S. microadriaticum and O.marina by centrifugation at 800g for 5min at 4°C.
3. Added separately 200μL 0.1M EDTA or 10% Glycerol or ASP-8A three different solutions to the cells.
4. Used three different ways pipetting or swirling or light vortexing to resuspend these cells.
5. Observed the status of the cells by microscope.
6. Harvested S. microadriaticum and O.marina by centrifugation at 3000xg for 5min at 4°C.
7. Added separately 2μL 0.1M EDTA or 10% Glycerol or ASP-8A three different solutions to the cells.
8. Used three different ways pipetting or swirling or light vortexing to resuspend these cells.
9. Observed the status of the cells by microscope.

Results:

Symbio+800xg	Pipetting	Swirling	Light Vortexing
10% Glycerol	Some of them stayed still, most of them didn't be broken	Some of them stayed still, others were broken	no cell was swimming but just stayed there
0.1 M EDTA	no cell was swimming but just staying there	no cell was swimming but just staying there	Most of them were broken, a few stayed still
ASP-8A	no cell was swimming but just staying there	no cell was swimming but just staying there	no cell was swimming but just stayed there
Symbio+3000xg	Pipetting	Swirling	Light Vortexing
10% Glycerol	Most of them stayed still, a number of cells were large and gathering together.	Most of them stayed still, the number of cells was small	Most of them stayed still
0.1 M EDTA	Some of them stayed still, others were broken	Some of them stayed still, others were broken, numbers of cells were small	Most of them stayed still
ASP-8A	Most of them stayed still.	Half of them stayed still, others stayed still	Most of them stayed still, the number of cells was small

The supernatant of symbio+800*g: there were living cells swimming	
The supernatant of symbio+3000*g: there was no living cell	

O.marina+800g	Pipetting	Swirling	Light Vortexing
10% Glycerol	Most of them were	Most of them were	Most of them swam happily

	broken, a few stayed still	swimming, some stayed still	
0.1 M EDTA	Half of them were broken, others stayed still	No cell was swimming, some stayed still, some's membrane looks like dissolved	Only 1 cell was swimming, others stayed still
ASP-8A	Some swimming around slowly, others staying still	A few of them were swimming, others stayed still	Most of them were broken, some standstill
O.marina+3000g	Pipetting	Swirling	Light Vortexing
10% Glycerol	Most of them stayed still, a few were swimming, the number of cells was small	Half of them were swimming, others stayed still, the number of cells was small	Half of them were swimming, others stayed still, the number of cells was small, better than O.marina(3000g, Glycerol, Swirling)
0.1 M EDTA	Most of them stayed still, a few were swimming	Number of cells was large, most of them were swimming, others stayed still	Some of them were swimming, others stayed still
ASP-8A	Most of them stayed still, 1 cell was trembling, the number of cells was small	No cell was swimming, most of them were broken	Most of them were broken, some standstill

Conclusion:

For S. microadriaticum, both 800xg and 3000xg are not good because there is no cell swimming in the pellet. But most cells were not burst out and stayed the cellular shape. Those cells could be dead or alive but less energetic.

For O.marina, 800xg looks like better than 3000xg because there are more living cells swimming and more activate.

But it is hard to say which resuspend solution is better as well as the solution mixing way.