Developing new model organisms among marine holozoans, the closest unicellular relatives of animals

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INTRODUCTION

The Holozoa includes, probably the eukaryotic clade with the most complex body plans, as well as several protists lineages, some of which seem to be quite abundant in the oceans. These holozoan unicellular lineages include Choanoglagellata, the Filastera, the Ichthyosporea, and the Corallochytrea, comprising an incredible diversity of morphologies and cell behaviors. However, our knowledge of their biology and ecology remains quite poor. More importantly, given their key phylogenetic position as closely related to animals and their potential ecological importance, development of experimentally tractable holozoans will allow deeper understanding of the evolution of animals, the origin of those cell morphologies and the ecological role of those marine taxa. There is not yet an unicellular holozoan that can be considered a model organism. To fill this gap, we are currently screening for transormability five representative marine taxa from the less known holozoan lineages, *Ministeria vibrans* (Filasterea), *Corallochytrium limaci*sporium (Corallochytrea) and *Abeoforma whisleri, Pirum gemmata* and *Sphaeroforma arctica* included in the Ichthyosporea lineage (**Fig 1**). We here present our work to elucidate their life cycles and our systematic screening for a transformation protocol of those taxa.

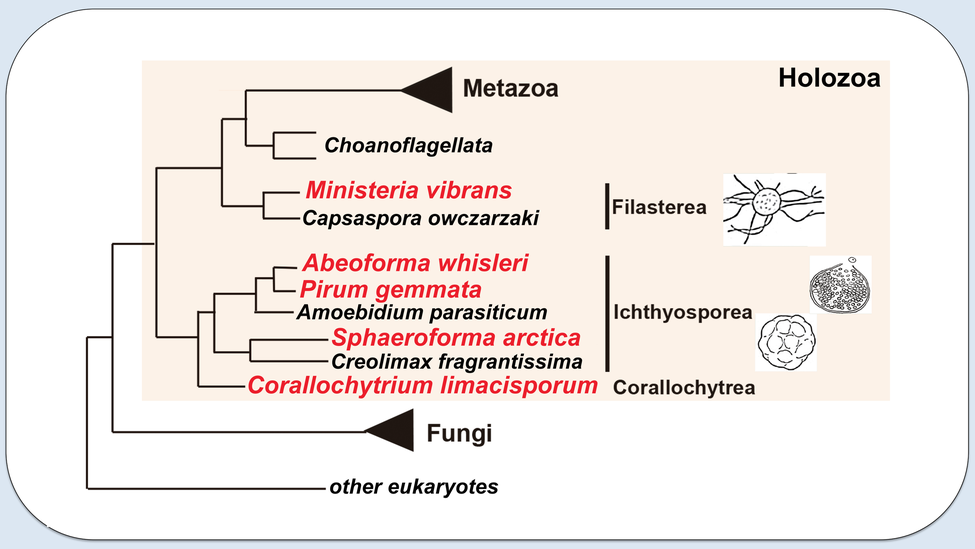

Figure 1. Schematic phylogenetic tree of the Holozoa. Taxa to be screened in red.

Sphaeroforma artica

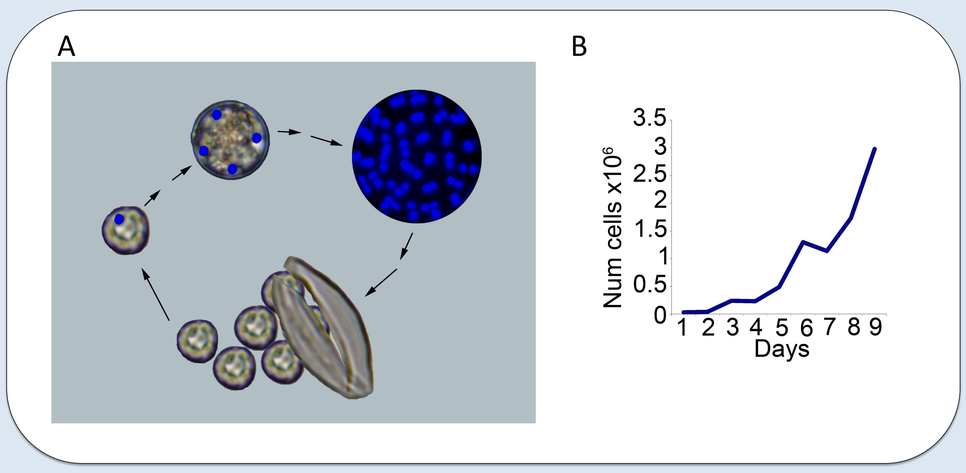

Figure 2. Cell cycle of *S. arctica*. **A.** S. arctica grows by syncytial division from a mononuclear cell forming more than 100 daughters cells. Then the wall collapses and cells are dispersed. Multinuclear cell is stained with DAPI. **B**. Grafic representation of the growth curve of *S. arctica*. Aproximately every 48h increase the number of cells.

Abeoforma whisleri

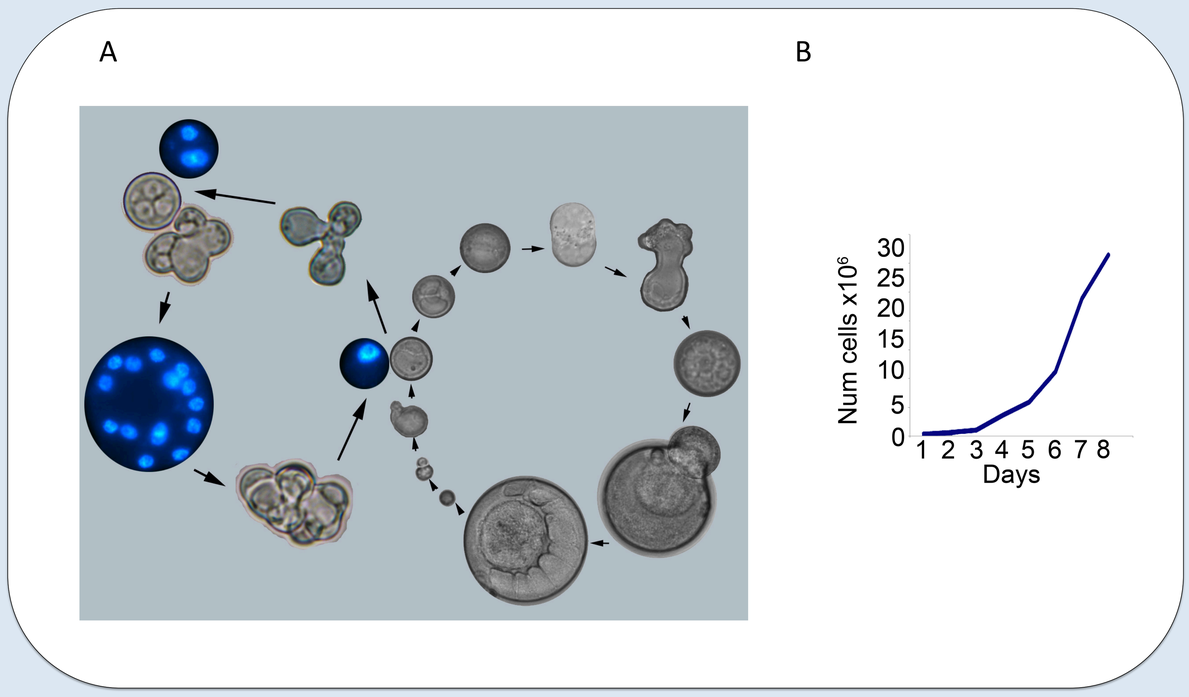

Figure 3. Cell cycle of *A. whisleri.* **A.** Examples of various cell shapes of *A.whisleri*. A preliminar time-lapse experiment using cells stained with Hoechst shows that *A. whisleri* when divides changes shape to form daughter cells and once different nuclei are formed they circularize again to form another spherical cell. This kind of division keeps continuing until the number of nucleus are large enough and daughter's cells spread individually. It is common to observe spherical and irregular forms with prominent vacuoles that occupied a large proportion of the cell volume. Some cells are stained with DAPI (blue nuclei) **B.** Grafic representation of the growth curve of *A. whisleri.*

Corallochytrium limacisparum

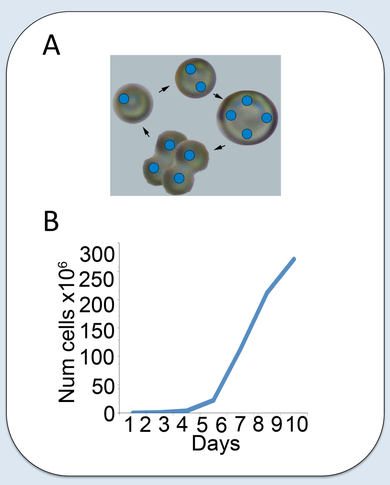

Figure 4. Cell cycle of *C. Limasciparum*. **A.** Single, diad and tetrad spherical vegetative cells of *C. limascisparum*. **B.** Grafic representation of the growth curve of *C. limascisparum.*

Pirum gemmata

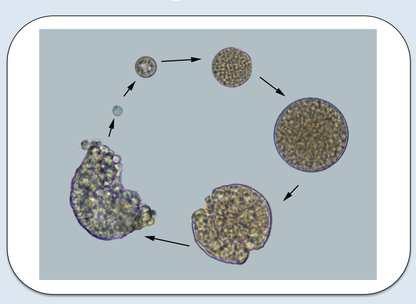

Figure 5. Cell cycle of *P gemmata*. The number of nucli increases by synchronous division. Older cells had nuclei in the hundreds. Nuclear size gradually decreased as the number of nuclei increased. The parental cell wall open and release individual endospore.

TRANSFORMATION by ELECTROPORATION

Electroporetion Neon System (Invitrogen)

-Harvest cells from a one o two days new culture and pelleted by centrifugation 5min x1000g.

-Wash cells with PBS+1MSorbitol

-Take 10^4-10^5 cells in PBS+1M Sorbitol (in an volum of 10uL) and add 500ng of Dextran-FITC (should be 1 uL)

-Prepare 3mL buffer E (Neon System) at RT in the electroporator cuvette.

-Proceed with the electroporation:

A. whisleri 1000V	S. arctica 1500V	C.limascisporum 1500V
20ms	30ms	30ms
1 pulse	2 pulse	1 pulse

-After the pulse put the cells in the dish with 1mL medium and observing to the fluorescent microscope. Cells must be washed once to clean the Dextran remained.

Results: All of the them suvives but none of the cells were FITC positive.

GENERAL SCREENING ANTIBIOTICS

We have tested the susceptibility of *A. whisleri*, *P. gemmata*, *C. limacisporum* and *S. arctica* to several antibiotics.

G418	Puromycine	Hygromycine	Tetracycline
Zeocine	Streptomycin	Ampicilline	Spectinomycine
Rapamycin	Paromomicina	Paclitaxel	

None of them affect the viability of the cells.

Antifungal FUNGIZONE (Amphotericin B) is toxic to *Abeoforma whisleri* at 300ug/mL. The drug acts by binding to sterols in the cell membrane of susceptible fungi with a resultant change in membrane permeability allowing leakage of intracellular components.

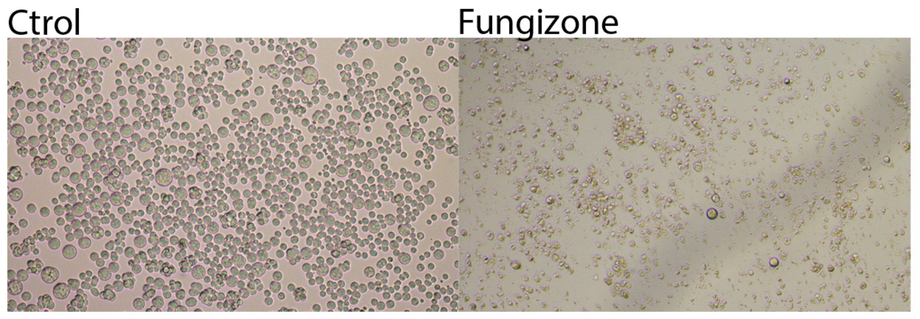

Figure 6. *Abeoforma whisleri* dies in presence of fungizone (right panel) compared to control cells in absence of fungizone (left panel).