Supplementary Material

A novel in-vivo phagocytosis assay to gain cellular insights on sponge-microbe interactions

Angela M. Marulanda-Gomez, Kristina Bayer , Lucia Pita\*, Ute Hentschel\*

**\* Correspondence:**Lucia Pita  
[luciapita@cmima.csic.es](mailto:luciapita@cmima.csic.es)

Ute Hentschel   
[uhentschel@geomar.de](mailto:uhentschel@geomar.de)

Chart, scatter chart

Description automatically generated

**Supplementary Figure 1.** Relation of sponge cells recovered per g (wet weight) sponge after the cell dissociation of the individuals used for the phagocytosis assay.

Graphical user interface, application

Description automatically generated

**Supplementary Figure 2.** Estimates of *Nannochloropsis* sp. cells found inside the sponge tissue or incorporated in *H. panicea* cells **(A)** Representative FACS cytograms showing the population of intact, free algae (dashed oval). **(B)**  Percentage of free algal cells after the pulse-chase experiment. All sponges were incubated for 30 min (pulse-period) with *Nannochloropsis* sp.(initial concentration of approx. x106 algae mL-1) and sampled after 0 min, 30 min, and 150 min chase period.

Chart, scatter chart

Description automatically generated

**Supplementary Figure 3.** Relation between phagocytosis and particle uptake. The percentage of *H. panicea* cells phagocytizing algae (*Nannochloropsis* sp.), bacteria (*Vibrio* sp.), and fluorescent latex beads tends to increase linearly (n = 3. More data points are needed to validate a linear regression fit) with a higher number of particles removed by the sponge during the 30 min incubations. Dotted gray line: extension of the slope calculated with the linear regression equation.

**Supplementary Table 1.** Fluorescent microscopy observations of phagocytic cells of *H. panicea* from the assays with algae (*Nannochloropsis* sp.), TAMRA-stained bacteria (*Vibrio* sp.), and fluorescent latex beads (1 µm). The total number of cells observed and percentages are reported. A two-tailed z-test was performed to compare the proportion of different cell types between treatments. \*Significant values.

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| --- | --- | --- | --- | --- | --- |
| **Assay** | **Visible flagellum (5 µm)** | **No visible flagellum (5 µm)** | **Medium (6 to 10 µm)** | **Big (10 to 12 µm)** | **Total** |
| Algae (+ 0 min) | 11 (39.3 %) | 4 (14.3 %) | 5 (17.9 %) | 8 (28.6 %) | 28 |
| Algae (+ 30 min) | 2 (11.8 %) | 3 (17.6 %) | 7 (41.2 %) | 5 (29.4 %) | 17 |
| Algae (+ 150 min) | 1 (4.0 %) | 4 (16.0%) | 14 (56.0 %) | 6 (24.0 %) | 25 |
| Bacteria | 11 (45.8 %) | 9 (37.5%) | 3 (12.5 %) | 1 (4.2 %) | 24 |
| Beads | 15 (51.7 %) | 10 (34.5 %) | 3 (10.3 %) | 1 (3.4 %) | 29 |
|  |  |  |  |  |  |
|  | **Visible flagellum (5 µm)** | | **Med-Big (6 to 12 µm)** | |  |
| **Pairwise comparison** | **z-value** | **p-value** | **z-value** | **p-value** |  |
| Algae + 0 min vs. + 30 min | 1.97 | 0.049\* | -1.58 | 0.114 |  |
| Algae + 0 min vs. + 150 min | 3.13 | 0.002\* | -2.30 | 0.021\* |  |
| Algae + 30 min vs. + 150 min | 1.00 | 0.317 | -0.47 | 0.638 |  |
| Algae + 0 min vs. Bacteria | 0.476 | 0.631 | -2.28 | 0.023\* |  |
| Algae + 0 min vs. Beads | 0.400 | 0.689 | -2.69 | 0.007\* |  |