



Physicochemical surface properties of microalgae and their combined effects on particle selection by suspension-feeding bivalve molluscs



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ABSTRACT

The capabilities of bivalve molluscs to feed selectively have been well documented, and physicochemical properties of particles have been implicated as possible factors in the selection process. In this study, the surface-property profiles of nine different microalgal species were determined by characterizing the surface charge, wettability (= contact angle), and surface carbohydrate moieties. Three fluorescein isothiocyanate (FITC) conjugated lectins were used to characterize carbohydrate moieties, including concanavalin A (ConA), *Pisum sativum* agglutinin (PEA), and wheat germ agglutinin (WGA). Distinct surface-property profiles were identified using linear discriminant analysis (DA) and used to design mixed-algal feeding experiments to assess particle selection by the blue mussel *Mytilus edulis* and the eastern oyster *Crassostrea virginica*. Results demonstrated preferential ingestion of some algal species over others, with strong rejection of some species (e.g. *Pavlova lutheri* and *Prasinocladus marinus*). These data were then used to develop DA and multiple linear regression models that considered the quantified surface properties and microalgal fates (rejected, ingested, or no selection) to examine determinants of selection. The DA model correctly classified 58% of the selection outcomes in mussels and 57% in oysters. Wettability was the most important factor in predicting selection in mussels, and surface charge was most important for oysters. In the multiple linear regression analyses, lectin affinity and wettability were the strongest predictors of particle selection, explaining ca. 90% of the variability in electivity index for mussels and 94% of the variability for oysters. By characterizing both physical and chemical surface properties of several microalgae used as food for suspension-feeding bivalves, we demonstrate that multiple surface-property characteristics need to be considered in order to develop meaningful models of particle selection in bivalves. Future research also should take into account species-specific differences in selection.

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1. Introduction

The capabilities of suspension-feeding bivalve molluscs to select some particles over others for ingestion have been well documented (see Shumway et al., 1985; Cognie et al., 2003; Ward and Shumway, 2004). Prior studies have demonstrated that chemical substances on particle surfaces can mediate selection in these organisms (e.g., Newell and Jordan, 1983; Ward and Targett, 1989; Ward et al., 1997; Pales Espinosa et al., 2007), and physicochemical properties, such as electrostatic charge and wettability, also have been suggested to play roles in selection (Newell et al., 1989; Beninger, 1991; Hernroth et al., 2000; Rosa et al., 2013). Charged particles, for example, have been shown to be more readily captured than particles with a neutral charge by both the brittle star *Ophiopholis aculeata* (LaBarbera, 1978) and by larvae of the northern quahog (= hard clam) *Mercenaria mercenaria* (Solow and Gallager, 1990). Hernroth et al. (2000) examined the influence of the surface charge of radioactive-labeled *Salmonella*

typhimurium cells upon particle selection by the blue mussel *M. edulis* and found that cells with a lower net-negative charge were more likely to be captured than cells with a higher negative charge. Wettability, a weak force dependent upon hydrophobic-hydrophilic interactions between a surface and a liquid, can also act to mediate feeding in invertebrates (Mihm and Loeb, 1992; Conova, 1999). In the crustacean *Daphnia magna*, particle capture was related to wettability, with wettable (= hydrophilic) particles being retained at a higher proportion than non-wettable (= hydrophobic) particles (Gerritsen and Porter, 1982). In a more recent study, Rosa et al. (2013) demonstrated that the eastern oyster, *Crassostrea virginica*, and the blue mussel, *Mytilus edulis*, can discriminate between synthetic particles of the same size based upon the surface charge and wettability of particles. These findings suggested that non-specific physicochemical interactions play a role in mediating a passive selection mechanism. Most of the studies examining effects of physicochemical properties upon selection, however, have used synthetic particles (e.g., polystyrene and silica). To understand fully how physicochemical characteristics of particles mediate the feeding process, surface properties of natural food particles (e.g., microalgae) need to be characterized and assessed in selection experiments.

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More specific chemical interactions (e.g., lectin-sugar) also contribute to particle discrimination. Scientists have long recognized the role of lectins as signaling molecules in marine bivalves (e.g. Renwrautz and Stahmer, 1983; Gauthier et al., 2004), and research by Pales Espinosa et al. (2009) provided some of the first evidence that lectins are involved in selective feeding. Working with mucus isolated from the pallial organs of oysters, *Crassostrea virginica*, and mussels, *Mytilus edulis*, commercially available lectins, and neoglycoproteins covalently attached to synthetic microspheres, Pales Espinosa et al. (2009, 2010a, 2010b) demonstrated that 1) the cell surfaces of four different microalgal species contain several different sugars that vary in abundance, 2) lectins present in pallial-organ mucus interact with sugars on the surface of microalgal cells, and 3) sugar-lectin interactions are involved in mediating particle sorting in both *C. virginica* and *M. edulis*. These findings suggest that sugars act as recognition molecules for mucosal lectins produced by the feeding organs to mediate particle discrimination.

Different chemical substances (e.g., proteins, carbohydrates, humics) likely change the surface characteristics of organic and inorganic particles, affecting the aforementioned physicochemical characteristics (charge and wettability), as well as the adhesive interactions with mucus on the feeding structures. Waite et al. (1995) measured sugar-containing compounds that accumulated on the cell surface of two microalgal species. By binding FITC-labeled Concanavalin A (Con A) lectins to the cell surfaces of the microalga *Thalassiosira pseudonana* and *Chaetoceros neogracile*, the authors showed that the accumulation of sugars increased with growth phase and varied between the two microalgae. The red microalga *Porphyridium* sp. was found to have an overall negative surface charge, attributed to the presence of sulfate groups and glucuronic acid associated with the cell wall (Shrestha et al., 2004). Dam and Drapeau (1995) used a mesocosm study to examine the dynamics of a phytoplankton bloom and reported a strong correlation between the presence of cell surface carbohydrates and aggregation rates (= cell stickiness). Results of these studies suggest a correlation between cell-surface carbohydrates and physicochemical properties that can vary between species and among living and non-living particles (Waite et al., 1995). Understanding how different carbohydrate moieties (= groups) affect the surface properties of microalgal cells can help determine which properties serve to mediate selection in different bivalve species.

To understand more clearly the mechanisms mediating selection, an examination of the effects of all measurable particle characteristics, and how they form a profile for different microalgal species, is necessary. Even when suspension-feeders exhibit a strong preference for one particular particle type over another, calculated selection indices are not 100% (e.g. Shumway et al., 1985; MacDonald and Ward, 1994; Ward et al., 1997), indicating discrimination is not an absolute process. Therefore, it is necessary to determine which characteristics are more important in determining what makes one particle more likely to be ingested compared to another. This project was designed to quantify the differences in surface properties of microalgae that have often been used in aquaculture settings and in prior studies with suspension-feeding bivalves. Understanding differences in surface properties will help to

determine if certain surface characteristics typically lead to rejection or ingestion, and if general rules for particle discrimination can be developed. Finally, results of this study will be used to explore whether statistical models can be developed to predict selection by bivalve molluscs fed different algal species. This study is the first to examine the influence a range of physicochemical surface properties of microalgae (e.g. charge, wettability, carbohydrate moieties) has on particle selection by suspension-feeding bivalve molluscs.

2. Methods

2.1. Microalgal characterization – surface charge and wettability

To develop surface-property profiles for the types of algae that typically are rejected versus preferentially ingested by bivalves, the surface charge and wettability of nine different microalgae species were determined (Table 1). Algal species were chosen based on their previous use in selection studies (e.g., Møhlenberg and Riisgård, 1978; Newell and Jordan, 1983; Shumway et al., 1985) and as feed for commercially-important bivalve species (Wikfors, 2000). Each species was grown in biological triplicate, and were obtained from the NOAA NMFS, Northeast Fisheries Science Center Milford Marine Microalgal Culture Collection. All triplicate cultures were grown aseptically using enriched, sterilized seawater from Milford Harbor (7.8 pH, salinity of 15), under a 24-h light regime, and were harvested in early stationary-phase.

Characterization of microalgal surface properties was carried out using previously described methods (Rosa et al., 2013). Briefly, zeta potential was determined for each microalgal species using a Zetasizer Nano ZS© (Malvern Instruments Inc., UK). The instrument measures electrophoretic mobility using laser-Doppler electrophoresis (Pashley et al., 1985), and zeta potential was calculated by means of the Smoluchowski equation (Sze et al., 2003) using values for viscosity and dielectric constant of the solution, as well as the measured electrophoretic mobility. In a standard solution such as that used in this analysis (i.e., pH 8.0, salinity 15) zeta potential is an indication of surface charge and henceforth is designated as such.

Wettability of the algae was determined by measuring contact angle of dried cells (Hiemenz, 1986). Microalgal cultures were vacuum-filtered through 3- μ m polycarbonate filters to form a pad of cells. Pads were rinsed with isotonic ammonium formate to remove salts and dried overnight at 70 °C. Control pads also were produced by passing filtered seawater (0.2 μ m) through the polycarbonate filters, rinsing with ammonium formate, and drying overnight to test whether or not the contact angles of the microalgae changed significantly during the washing treatment. A drop of MQ water (4 μ l) was then placed on the dried pad and photographed using a digital camera attached to a side-mounted dissecting microscope (i.e., goniometer; Mohammadi et al., 2003). Image J was used to measure the contact angle formed between the water droplet and the microalgal surface. By convention, surfaces with a contact angle >90° are classified as non-wettable (= hydrophobic), and conversely, surfaces with a contact angle <90° are classified

Table 1
Physical surface properties of the characterized microalgal species and cordgrass. Significant differences in surface properties were found among species, but no significant correlation was found between surface charge and contact angle across species. Data presented as means (SD), $n = 5-6$. Algal sizes are based on the peak obtained using a Coulter Multisizer IIe© instrument. * denotes significant differences in surface properties (DA, $0.01 < P < 0.05$).

Species	Cell surface	Strain	Mean size (μ m)	Surface charge (mV)	Contact angle (°)
<i>Tetraselmis chui</i>	Scales	PLY429	13	-7.5 (1.2)*	99.9 (1.8)
<i>Rhodomonas lens</i>	Membrane	Rhodo	12	-13.9 (0.6)	93.5 (2.4)
<i>Chlamydomonas</i> sp.	Glycoprotein wall	11/35	9	-13.6 (0.7)	102.9 (2.4)*
<i>Cricosphaera carterae</i>	Coccoliths	961	11	-13.8 (0.1)	106.0 (2.1)*
<i>Prorocentrum minimum</i>	Membrane	Exuv	21	-12.4 (1.1)	98.9 (7.8)
<i>Dunaliella salina</i>	Membrane	LB200	10	-11.0 (1.4)*	91.5 (2.8)
<i>Pavlova lutheri</i>	Membrane	MONO	3	-16.5 (0.7)*	66.4 (3.6)*
<i>Prasinocladus marinus</i>	Scales	163/1B	10	-24.1 (2.4)*	91.3 (2.5)
<i>Rhodomonas salina</i>	Membrane	F-3C	8	-13.2 (1.0)	93.7 (2.8)
<i>Spartina alterniflora</i>	Cellulose wall	N/A	2-6	-11.4 (0.5)	92.9 (6.2)

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