Available online at www.sciencedirect.com
ScienceDirect
Harmful Algae 7 (2008) 630–638
HARMFUL ALGAE
www.elsevier.com/locate/hal

Pathology and immune response of the blue mussel (*Mytilus edulis* L.) after an exposure to the harmful dinoflagellate *Prorocentrum minimum*

Eve Galimany [a,*], Inke Sunila [b], Hélene Hégaret [c], Montserrat Ramón [d], Gary H. Wikfors [c]

[a] *IRTA, Crta. Poble Nou s/n, St. Carles de la Ràpita 43540, Spain*
[b] *State of Connecticut, Department of Agriculture, Bureau of Aquaculture, P.O. Box 97, Milford, CT 06460, USA*
[c] *Milford Aquaculture Laboratory - NOAA - NMFS, 212 Rogers Avenue, Milford, CT 06460, USA*
[d] *IEO-Centre Oceanogràfic de Balears, Moll de Ponent s/n, Palma de Mallorca 07015, Spain*

Received 26 November 2007; received in revised form 28 December 2007; accepted 9 January 2008

Abstract

The harmful dinoflagellate *Prorocentrum minimum* has different effects upon various species of grazing bivalves, and these effects also vary with life-history stage. Possible effects of this dinoflagellate upon mussels have not been reported; therefore, experiments exposing adult blue mussels, *Mytilus edulis*, to *P. minimum* were conducted. Mussels were exposed to cultures of toxic *P. minimum* or benign *Rhodomonas* sp. in glass aquaria. After a short period of acclimation, samples were collected on day 0 (before the exposure) and after 3, 6, and 9 days of continuous-exposure experiment. Hemolymph was extracted for flow-cytometric analyses of hemocyte, immune-response functions, and soft tissues were excised for histopathology. Mussels responded to *P. minimum* exposure with diapedesis of hemocytes into the intestine, presumably to isolate *P. minimum* cells within the gut, thereby minimizing damage to other tissues. This immune response appeared to have been sustained throughout the 9-day exposure period, as circulating hemocytes retained hematological and functional properties. Bacteria proliferated in the intestines of the *P. minimum*-exposed mussels. Hemocytes within the intestine appeared to be either overwhelmed by the large number of bacteria or fully occupied in the encapsulating response to *P. minimum* cells; when hemocytes reached the intestine lumina, they underwent apoptosis and bacterial degradation. This experiment demonstrated that *M. edulis* is affected by ingestion of toxic *P. minimum*; however, the specific responses observed in the blue mussel differed from those reported for other bivalve species. This finding highlights the need to study effects of HABs on different bivalve species, rather than inferring that results from one species reflect the exposure responses of all bivalves.

Keywords: Harmful algal blooms; Hemocyte; Histopathology; Immunology; *Mytilus edulis*; *Prorocentrum minimum*

1. Introduction

Mussels (*Mytilus* sp.) are suspension-feeding bivalves that are harvested for human consumption around the world (Figueiras et al., 2002; Mortensen et al., 2006). As mussels, whether from wild fisheries or aquaculture, are grown and harvested under natural conditions, chemical and biological quality of growing waters is very important for survival and growth, hence quantity and quality of the yield. Mussels can be affected by different types of toxins, from both chemical (Dyrynda et al., 2000; Parry and Pipe, 2004) and biological sources (Galimany et al., 2008).

Biological effects of toxic microalgae upon bivalve mollusks can include mortality (Wikfors and Smolowitz, 1993), tissue damage (Pearce et al., 2005), cellular dysfunction (Hégaret and Wikfors, 2005), and reproductive failure (Granmo et al., 1988). In addition, edible tissues can become contaminated with chemical or biological toxins, rendering them unfit for human consumption (EUROHAB, 1998; Heil et al., 2005). Harmful algal blooms (HABs) appear to be increasing in geographic distribution and intensity (Hallegraeff, 2003). This has led to a growing concern about effects of HABs upon shellfish resources, in terms of both seafood safety and production efficiency.

Inimical effects of HABs upon grazing animals have raised questions about the evolutionary and ecological relevance of toxic or noxious properties in these microorganisms. Do toxins

* Corresponding author at: Centre d'Aqüicultura-IRTA, Crta. Poble Nou s/n, St. Carles de la Ràpita 43540, Spain. Tel.: +34 977 745 427/34 932 309 605; fax: +34 977 744 138.
E-mail address: eva.galimany@irta.es (E. Galimany).

1568-9883/$ – see front matter © 2008 Elsevier B.V. All rights reserved.
doi:10.1016/j.hal.2008.01.001

or harmful properties of HABs confer protection from grazing? It is very common for forage species of land plants to protect themselves from grazing by producing noxious properties or toxicity (Agrawal et al., 1999; Agrawal, 2007). In terrestrial ecosystems, it is not uncommon for grazing species to have developed tolerance to toxins from specific forage species (De Mazancourt et al., 2001; Karban and Agrawal, 2002). Recently, growing evidence for similar, grazing-deterrent functions of toxins in marine dinoflagellates have been reported (Selander et al., 2006). As in terrestrial ecosystems, susceptibility of grazers, including bivalve mollusks, to specific grazing-deterrent chemicals produced by HAB taxa appear to be species-specific (Landsberg, 2002).

The microalgal species *Prorocentrum minimum* (Pavillard) Schiller is a dinoflagellate with the capacity to express toxicity (Grzebyk et al., 1997; Wikfors, 2005) that is now considered a "HAB" species (Heil et al., 2005). Toxicity of *P. minimum* varies among different strains studied (Grzebyk et al., 1997; Denardou-Queneherve et al., 1999) and has been found to fluctuate according to growth phase, i.e., stationary-phase populations are more toxic than actively growing populations (Grzebyk et al., 1997). Despite this variability, *P. minimum* has been demonstrated to be toxic to molluscan shellfish, causing a wide diversity of symptoms, including pseudofeces production in several species of oyster and clam (Hégaret et al., 2007), tissue damage and developmental abnormalities in young stages of the Eastern oyster, *Crassostrea virginica* (Wikfors and Smolowitz, 1995a), changes in immune parameters of Eastern oysters and bay scallops, *Argopecten irradians irradians* (Hégaret and Wikfors, 2005) and mortality (Shumway and Cucci, 1987; Shumway, 1990; Luckenbach et al., 1993). Most studies of *P. minimum* effects upon mollusks reported to date have been with oysters and clams, but not with mussels, although it has been demonstrated that mussels can accumulate toxicity (Denardou-Queneherve et al., 1999). Mussels are known to have unusual tolerances to microalgal biotoxins (Wootton et al., 2003), such as saxitoxin, that cause clear biological effects in other molluscan species (Landsberg, 2002; Heil et al., 2005). Thus, possible effects of *P. minimum* upon mussels cannot be deduced from findings with other molluscan species.

The present study, therefore, investigated the effects of *P. minimum* upon adult blue mussels, *Mytilus edulis*, under experimental conditions, focusing on immune functions and histopathology, both of which have been shown to respond to *P. minimum* exposure in other molluscan species.

2. Materials and methods

2.1. Experimental animals

Mussels, *M. edulis* (47.6–73.9 mm shell length) for this experiment were collected from Westcott Cove, Stamford, CT, USA, from an intertidal beach on the north shore of Long Island Sound in June of 2007. Mussels were acclimated for 4 days before the experiment, the first 3 days in the experimental tanks with filtered seawater, and the fourth day fed with *Rhodomonas* sp. (RHODO, see below) at a concentration of 1×10^4 cells ml^{-1}.

2.2. Algal cultures

The *P. minimum* strain used for the experiment was obtained from the Milford Microalgal Culture Collection, strain JA-98-01 (isolated from the Choptank, River, Chesapeake Bay, MD, USA). In addition, the RHODO strain of the cryptophyte *Rhodomonas* sp. was used as a non-toxic, control alga.

The microalgae were cultured in 20-l glass carboy assemblies using aseptic technique (Ukeles, 1973). Cultures were harvested semi-continuously to maintain consistency in culture quality over the course of the study and were harvested in late-log or early-stationary phase. Cultures of *P. minimum* were grown in EDL7 medium, a modified version of the enriched-seawater E-medium (Ukeles, 1973) that contains L-1 trace metals (Guillard and Hargraves, 1993), double the EDTA of the standard E formulation, KNO_3 rather than $NaNO_3$, and 10 ml l^{-1} soil extract. *Rhodomonas* sp. was cultured in E-medium. Both cultures were maintained at 20 °C with 24-h light. Algal cell densities were determined by hemocytometer counts with a light microscope.

The toxicity of the *P. minimum* culture used in the mussel-exposure experiment was tested with a scallop bioassay (Rosetta and McManus, 2003). Five northern bay scallops, *A. irradians irradians*, were placed in each of twelve 1-l beakers; the following 4 treatments were tested in triplicate beakers: (1) filtered seawater, (2) filtered seawater diluted with distilled water to equal salinity of algal treatments, (3) *Rhodomonas* sp. at 1.9×10^5 cells ml^{-1}, and (4) *P. minimum* at the same cell density. Observations of scallop activity and mortality were made periodically for 20 h. Dissolved oxygen, pH and salinity were measured at the beginning and at the end of the exposure.

2.3. Experimental design

The main experiment tested the effects of cultured *P. minimum*, upon the immunology and histopathology of mussels, *M. edulis*. Two-hundred and seventy (270) mussels were distributed randomly into six 20-l glass-aquaria, i.e., 45 mussels per aquarium. Three replicates of each treatment were done in this experiment: *Rhodomonas* sp. or *P. minimum*, each at 1×10^4 cells ml^{-1}, were given with a regime of 16 feedings per day, every 90 min (285 ml per day), using the cover of a rearing-chamber system incorporating computer-automated valves to add microalgal culture as programmed (Smith and Wikfors, 1998).

Samples of mussels were collected on day 0, before exposures, and after 3, 6 and 9 days of exposure to the experimental, microalgal treatments. At each sampling time, mussels were removed from the basins and analyzed for hemocyte parameters, stomach contents, and histopathology. Feces and pseudofeces were also examined microscopically throughout the experiment.