

Potential Indicators of Stress Response Identified by Expressed Sequence Tag Analysis of Hemocytes and Embryos from the American Oyster, *Crassostrea virginica*

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Abstract: A pilot program was initiated to identify genes from the American oyster, *Crassostrea virginica*, that are potentially involved in the stress response for use as bioindicators of exposure to environmental pollutants and to toxic and infectious agents. A PCR-based method was used to construct cDNA libraries from pooled embryos and the hemocytes of a single individual. A total of 998 randomly selected clones (expressed sequence tags, ESTs) were sequenced. Approximately 40% of the ESTs are novel sequences. Several potential biomarkers identified include an antimicrobial peptide, recognition molecules (lectin receptors), proteinases and proteinase inhibitors, and a novel metallothionein. Diversity analysis shows that 363 and 286 unique genes were identified from the hemocyte and embryo libraries, respectively, indicating that full-scale EST collection is a valuable approach for the discovery of new genes of potential significance in the molluscan stress response.

Key words: oyster, biomarker, expressed sequence tags, immunity, development.

INTRODUCTION

Increasing land use and development in coastal zones over the past 50 years have placed additional stresses on resident estuarine organisms. These stressors include such factors as increased nutrient loads, organic and inorganic contaminants, increased runoff, increased sediment loads, and reductions in habitat. The impacts of these stressors on estuarine ecosystems are often not fully realized until long

after the damage has been done. Over the past two decades molecular tools have been developed that can provide more sensitive indicators of stress on organism health. While advances have been made in linking particular stressors to individual biochemical pathways, much remains to be learned. This is due to the difficulty of assessing the actions of more than a handful of biochemical traits, the complex interactions between metabolic pathways, the diversity of stressors that may simultaneously assault the organism, and the myriad interactions between species and their environment.

A major recent advance has been “transcript profiling” (Shimkets et al., 1999; Ishii et al., 2000; Nelson et al., 2000), which allows the simultaneous assessment of the levels

of expression of many genes, up to and including the whole genome. This approach will greatly expand our understanding of physiology at the molecular level and can revolutionize our understanding of the impacts of anthropogenic modifications on the marine ecosystem. Before transcript profiling can be accomplished, however, an appropriate number of genes must be cloned, sequenced, and identified to permit the assembly of informative suites of genes in microarrays.

In this paper, we present the second in a series of studies (Gross et al., 2001) aimed at creating the necessary tools to evaluate the health of aquatic ecosystems. We have chosen for this investigation the American oyster, *Crassostrea virginica*, because it is an important member of the benthic community in estuarine systems along the Atlantic Coast of the United States and the Gulf of Mexico. Oyster reefs can have a pronounced effect on estuarine ecosystem structure and function by (1) providing physical substrates for colonization and aggregation; (2) filtering particulates from the water, which can impact water quality and plankton composition; and (3) excreting nutrients, which can provide a positive feedback mechanism for supporting plankton production. In addition, *C. virginica* is a commercially important species for aquaculture and harvesting of native populations. The past century has seen a major decline of natural populations for a variety of reasons including overharvesting, loss of habitat, degradation of water quality and increasing susceptibility to disease, including parasitism (Hofmann et al., 1995; Bushek and Allen, 1996; MacKenzie et al., 1997; White et al., 1998; Ford et al., 1999).

Industrial and urbanization activities leading to the contamination of sediment and water with multiple pollutants results in potential threats to human health and the loss of natural resources. Estuarine sediments act as a repository for environmental contaminants. Oysters are particularly susceptible to contaminant exposure due to their association with sediments, sessile nature, and potential for high filtration activity. In addition, oyster bioaccumulation of chemical pollutants, biotoxins, or infectious agents can adversely affect other ecosystem components or humans through trophic transfer.

Harmful algal blooms (HABs), defined as the sudden abundance of toxic or harmful algae within a localized region, can also have detrimental effects on estuarine environments (Hallegraeff, 1993; Anderson, 1995). Because of their filter feeding behavior, bivalves are capable of bioaccumulating harmful toxins by ingestion of these algae. Human consumption of these bivalves can result in paralytic

shellfish poisoning (PSP), diarrhetic shellfish poisoning (DSP), amnesic shellfish poisoning (ASP), and neurotoxic shellfish poisoning (NSP) (Anderson, 1995; Lipp and Rose, 1997; Sierra-Beltran et al., 1998; Steidinger et al., 1998; Poli et al., 2000). In addition, bivalve shellfish are capable of acting as a vector for human pathogens such as *Vibrio* spp., *Salmonella* spp., *Campylobacter jejuni*, hepatitis A, and Norwalk and other enteric viruses (Lipp and Rose, 1997; Linkous and Oliver, 1999; Lee, 2000).

Both cultured and natural populations of oysters are susceptible to infectious agents. Three of the most notable diseases are dermo disease (*Perkinsus marinus*), MSX disease (*Haplosporidium nelsoni*) and juvenile oyster disease (JOD, etiology unknown). *Perkinsus* infections occur in *C. virginica* all along the eastern United States and in the Gulf of Mexico, from south Florida to Mexico (Hofmann et al., 1995). This protozoan is transmitted from oyster to oyster and can contribute to high mortalities throughout its distribution. MSX disease, also responsible for high mortalities along the Atlantic coast, has been reported from Maine to Florida (Bureson and Calvo, 1996; Barber et al., 1997; Ford et al., 1999). Finally, JOD is responsible for significant mortalities in hatchery-reared oysters cultured in the northeastern United States (Lewis et al., 1996). Although the etiological agent is unknown, JOD has been associated with *Vibrio* spp., as well as a novel marine species of α -proteobacteria belonging to the Roseobacter group (Lee et al., 1996; Boettcher et al., 1999, 2000).

C. virginica, because of its close relationship to aquatic sediments, susceptibility to chemical contamination, and ecological and economic relevance, is an important indicator species of estuarine health and a potentially valuable model for evaluating relationships between ecosystem and human health. In addition, the success of future restoration and management practices will rely on a greater understanding of the effects of environmental pressures, both natural and anthropogenic in origin, on organism health. Therefore, a functional genomics approach has been initiated to increase our understanding of stress-related biomarkers associated with oyster responses to chemical pollutants, pathogens, and toxic agents, including those endemic to oysters as well as humans.

MATERIALS AND METHODS

Animal Handling

Adult *C. virginica* were collected from Lighthouse Creek, Charleston, S.C. and maintained in aerated natural seawater