Journal of Thermal Biology 59 (2016) 1–12

Contents lists available at ScienceDirect

Journal of Thermal Biology

journal homepage: www.elsevier.com/locate/jtherbio

Immunomodulatory effects of temperature and pH of water in an Indian freshwater sponge

Soumalya Mukherjee, Anindya Sundar Bhunia, Niladri Sekhar Bhunia, Mitali Ray, Sajal Ray*

Aquatic Toxicology Laboratory, Department of Zoology, University of Calcutta, 35 Ballygunge Circular Road, Kolkata 700019, West Bengal, India

ARTICLE INFO

Article history:
Received 2 June 2015
Received in revised form
16 April 2016
Accepted 17 April 2016
Available online 26 April 2016

Keywords:
Eunapius carteri
Immunological resilience
Nitric oxide
Phagocytosis
Phenoloxidase
Superoxide anion
Water temperature and pH

ABSTRACT

Eunapius carteri, a freshwater sponge of India, inhabits the ponds and lakes and experiences variations of temperature and pH of water throughout the year. Sponges bear evolutionary and ecological importance with limited information on their immunological attribute and adaptational resilience in a changing environment. This paper reports temperature and pH specific responses of immune related parameters in sponge maintained in the experimental conditions of laboratory. Innate immunological parameters like phagocytosis and generation of cytotoxic molecules like superoxide anion, nitric oxide and phenoloxidase activity were estimated in *E. carteri* at different environmentally realistic water temperatures (10, 20, 30 and 40 °C) and pH (6.4, 7.4 and 8.4). Phagocytosis and cytotoxicity are established as important immune parameters of invertebrates. Calalase, an antioxidant enzyme and phosphatases are involved in pathogen destruction and are considered as components of innate immunity. Activities of catalase, acid and alkaline phosphatases were estimated in *E. carteri* at different thermal regimes and pH. Modulation of phagocytic and cytotoxic responses and the activities of catalase and phosphatases at different water temperatures and pH indicated temperature and pH specific immunological status of *E. carteri*. Present investigation deals with the effects of selected hydrological parameters on the fundamental immune related parameters in sponge indicating its adaptational plasticity. Immunological resilience of this species in the face of variation of water temperature and pH is thought to be a special adaptive feature of sponge, a reported "living fossil".

1. Introduction

Sponges, belonging to the phylum Porifera, survived the environmental severity of Varanger–Marinoan ice age around 605–585 million years ago (Müller et al., 2007). During this period, many of the species faced extinction (Hoffman et al., 1998) due to the harsh and stressful condition of the environment. Sponges are reported to overcome the diverse environmental challenges and stresses and existed till recent times (Saby et al., 2009). For their evolutionary primitiveness, poriferans are often considered as candidate species to study the evolution of defense mechanism in the metazoans (Funayama, 2008). Being a poikilotherm, sponge presents a wide range of physiological tolerance against varied thermal regimes. Goodwin et al. (2014) reported water acidification related stress in marine sponges, *Phorbas tenacior*, *Petrosia ficiformis*, *Chondrilla nucula* and *Hemimycale columella*. Freshwater poriferans are relatively a less studied group with limited scientific information regarding their cellular spectrum and immunological attribute in the face of temperature and pH variation of ambient water. *Eunapius carteri* (Porifera: Demospongiae: Spongillidae) is a common variety of freshwater sponge of India distributed in the perennial and seasonal ponds and lakes. They are considered as potential biological resource (Mukherjee et al., 2015c) and are reported as a source of bioactive and biomimetic molecules (Manconi et al., 2013). Sponges evolved a highly developed canal system which ensures flow of ambient water into their body following a specific route (Elliott and Leys, 2007).

Sponges, in general, depend on innate immune system (Wiens et al., 2007) to combat pathogen invasion and toxin exposure under different environmental conditions. Phagocytosis is a classical and pivotal innate immunological response reported in majority of invertebrate phyla. However, in Porifera, phagocytosis is functionally involved in feeding (Leys and Eerkes–Medrano, 2006) and cell mediated defense activity under specific environmental challenge (Saby et al., 2009). The ability of immunocompetent cells of sponges to generate cytotoxic molecules like superoxide anion,

* Corresponding author.
E-mail addresses: mukherjee.soumalya259@gmail.com (S. Mukherjee), anindyasbhunia@gmail.com (A.S. Bhunia), niladri80.bhunia@gmail.com (N.S. Bhunia), mitalirayin@yahoo.co.in (M. Ray), raysnailmail@rediffmail.com (S. Ray).

http://dx.doi.org/10.1016/j.jtherbio.2016.04.005
0306-4565/

nitric oxide and activity of phenoloxidase are proposed to play a significant role in their immunity (Mukherjee et al., 2015b). In invertebrates, superoxide anion and nitric oxide are reported as potential "killing agents" (Nappi and Ottaviani, 2000) of the phagocytosed pathogens by generating respiratory burst in the selected immunocytes. Peskin et al. (1998) reported a high rate of generation of superoxide anion in the cells of sponge, *Sycon* sp. during phagocytosis. According to them, the generation of superoxide anion by resting phagocytes of sponge was negligible. Phenoloxidase is an established enzyme associated with the immunological activities in invertebrates including melanization (Müller et al., 1999a) and cytotoxicity (Nappi and Christensen, 2005). The enzyme catalase is an antioxidant enzyme which is functionally associated with immune defense in sponge (Marques et al., 2008). Phosphatases are lysosomal enzymes involved in pathogen destruction and are reported as markers of environmental stress (Murti et al., 1984; Yin et al., 2014).

In this paper, we report differential immunological responses of the dissociated cells of *E. carteri* at 4 experimental temperatures of 10, 20, 30 and 40 °C. These experimental temperatures fall within the range of natural environmental temperatures of the habitat of *E. carteri* and thus appeared to be rational and environmentally realistic ones for experimentation. In India, the environmental temperature of the natural habitat of *E. carteri* ranges from 8 to 45 °C throughout the year (http://www.imdkolkata.gov.in). This wide range of temperature tolerance of *E. carteri* is assumed to be a characteristic adaptive feature of this group. The natural habitat of *E. carteri* experiences a wide range of fluctuation of pH of ambient water ranging from 6 to 8. In India, the aquaculturists apply different kinds of chemical compounds for the purposes of aquaculture and fishery related practices. These often result in the variation of pH of water of freshwater ponds and lakes. Additionally, various effluent discharges containing environmental toxins like washing soda and mineral acids may also alter the pH of pond water. Present paper reports the effects of different pH (6.4, 7.4 and 8.4) and temperatures (10, 20, 30 and 40 °C) of water in generation of superoxide anion, nitric oxide and activities of phenoloxidase, catalase, acid and alkaline phosphatases and phagocytic response of the cells of *E. carteri*. Current investigation would provide an important information of the immunological plasticity of *E. carteri* at different thermal regimes and pH of water.

2. Materials and methods

2.1. Collection and laboratory acclimation of E. carteri

Live *E. carteri* was manually collected from the selected waterbodies (22° 86′ N, 88° 40′ E) of the state of West Bengal of India without the history of pisciculture and toxin contamination. The sponges were acclimated in the controlled static water environment for 7 d in glass aquaria of the laboratory fitted with electrically operated aerators. During acclimation, the temperature, pH and dissolved oxygen of the water were maintained at 30 ± 1 °C, 7.4 ± 0.2 and 14.5 ± 1.5 mg/l respectively (Mukherjee et al., 2015b). A uniform illumination with 12:12 h dark–light cycle was maintained throughout the acclimation period. The water of the experimental glass aquaria was replenished at every 24 h with freshly collected pond water procured from their natural habitat to supplement the suspended food and for avoidance of toxicity due to accumulation of excretory products and metabolites (Mukherjee et al., 2015c). The entire experiment on *E. carteri* was designed in accordance to the guidelines of the institutional norms of animal handling and maintenance of the University of Calcutta.

2.2. Experimental design

Body mass of *E. carteri* was dissected into pieces with approximate size of 8 cm^3 bearing at least 1 osculum (Hansen et al., 1995). The acclimation of the sponge specimens was carried out in aerated glass aquaria in controlled laboratory conditions for 7 d to minimize the physiological stress and reorganize their canal system (Duckworth and Pomponi, 2005). For the temperature experiment, the sponges were maintained in pond water at temperatures of 10, 20, 30 and 40 °C at a pH of 7.4. The experimental temperature above 30 °C was maintained by immersion of thermostatic heater in the glass aquarium, whereas, temperatures below 30 °C were maintained in glass aquaria placed in insulated ice water recirculating chambers (Wang et al., 2008). Sponge specimens, for pH experiment, were maintained in pond water with pH of 6.4, 7.4 and 8.4 with a fixed temperature of 30 °C. The pH of pond water, below and above 7.4, were adjusted by addition of 0.05 N hydrochloric acid and 0.2 N sodium hydroxide respectively (Vidal et al., 2002). Each experimental set consisted of 5 replicates of live *E. carteri* (Mukherjee et al., 2015b). Each of the 5 experimental replicates was immersed in a volume of 10 l of pond water taken in different glass aquaria. Body masses of *E. carteri* with an approximate dimension of 0.5 cm^3 were surgically excised from the experimental specimens with sterile scalpel for analyses of immune related parameters.

2.3. Preparation of sponge cell suspension and screening of cell viability

The dissected body masses of *E. carteri* were rinsed thrice with sterile phosphate buffered saline (PBS, pH–7.4) to remove clay, sand and other adhered particles. Excised body parts of experimental sponge were suspended in sterile mineral medium (M–medium: 1 mM $CaCl_2 \cdot H_2O$, 0.5 mM $MgSO_4 \cdot 7\ H_2O$, 0.5 mM $NaHCO_3$, 0.05 mM KCl, 0.25 mM $Na_2SiO_3 \cdot 9H_2O$; pH adjusted to 7.5) (Funayama et al., 2005; Mukherjee et al., 2015a). Suspended pieces of excised sponge were shaken mildly in a rotary shaker (Sun et al., 2007) for 30 min for cell dissociation. The resultant cell suspension was filtered through a 50 μm nylon mesh to eliminate spicules and other undissociated masses (Chernogor et al., 2011). The filtrate with suspended cells, was centrifuged (Hermle Z323 K; Hermle Labortechnik, Wehingen, Germany) at 650*g* for 10 min, pellets were resuspended in sterile M–medium.

The viability of *E. carteri* cells was routinely screened by staining the cells with 0.4% trypan blue (HiMedia, India) following the principle of vital dye exclusion (Mussino et al., 2013; Mukherjee et al., 2015b).

2.4. Phagocytic index

Aliquot of cell suspension from each experimental set was smeared on individual glass slide and incubated in a sterile humid chamber for 30 min for cell adherence. The phagocytic efficiency of *E. carteri* cells was determined by challenging the adherent sponge cells with yeast particles (*Saccharomyces cerevisiae*) *in vitro* in a prestandardized ratio of 1:10 (Mukherjee et al., 2015a) and was incubated for 1 h. Postincubated monolayer was washed with M–medium, air dried, fixed in methanol, stained with Giemsa (HiMedia, India) and observed under light microscope (Axiostar Plus; Zeiss Microscopy, Jena, Germany) for phagocytosis of yeast. At least 200 fields were examined in each slide and the phagocytic index (PI) was determined by estimating the percent engulfment of yeast particles by sponge cells (Di et al., 2013).