Journal of Experimental Marine Biology and Ecology 486 (2017) 22–31

Contents lists available at ScienceDirect

Journal of Experimental Marine Biology and Ecology

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

Effects of salinity on the immune system cells of the tropical sea urchin *Echinometra lucunter*

Thaís Bezerra Mangeon Honorato [a], Raianna Boni [a], Patricia Mirella da Silva [b], Luis Fernando Marques-Santos [a,*]

[a] Laboratório de Biologia Celular e do Desenvolvimento (LABID), Departamento de Biologia Molecular, Universidade Federal da Paraíba, João Pessoa, Paraíba, Brazil
[b] Laboratório de Imunologia e Patologia de Invertebrados (LABIPI), Departamento de Biologia Molecular, Universidade Federal da Paraíba, João Pessoa, Paraíba, Brazil

ARTICLE INFO

Article history:
Received 28 February 2016
Received in revised form 20 September 2016
Accepted 23 September 2016
Available online xxxx

Keywords:
Salinity
Sea urchin
Immune system
Phagocytosis
ROS
ABC transporter

ABSTRACT

Human activities have caused climate changes and altered the salinity of the oceans. The reduction of the salinity is one of the factors that may limit the distribution and the survival of marine organisms. Coelomocytes are the immune system cells of the echinoderms and have been studied as biomarkers of stress. The aim of the present study was to investigate the effect of the salinity on the immune system cells of the tropical sea urchin *Echinometra lucunter*. Animals were collected in João Pessoa coast (Brazilian Northeast). Animals or coelomocytes were exposed to different salinities (25, 35, 45) from 4 to 24 h. Phagocytic parameters, production of reactive oxygen species (ROS), mitochondrial activity and ABC transporter activity were analyzed. The phagocytic parameters did not change when animals or cells were exposed to a salinity of 25 or 45 in any time intervals monitored. However, the coelomocytes concentration increased when animals were exposed to the lower salinity. The levels of ROS were higher when cells were incubated at a salinity of 25 but lower when cells were kept at a salinity of 45. It was observed the loss of the mitochondrial inner membrane potential when coelomocytes were incubated at a salinity of 45. The activity of ABC transporters decreased when cells were incubated at the lowest salinity and increased when cells were incubated at the highest salinity tested. The present work shows that the immune system of the tropical sea urchins *Ei lucunter* tolerates salinity changes from 25 to 45, and suggests two cellular parameters (ROS levels and ABC transporters activity) as potential biomarkers for the monitoring of the impact of environmental salinity changes.

1. Introduction

Deuterostome invertebrates have developed a variety of immune responses against foreign pathogens and molecules (Gross et al., 1999). The genomic sequencing of the sea urchin *Strongylocentrotus purpuratus* revealed that these animals have a robust immune system which is comprised by a vast repertoire of genes involved in the innate recognition of pathogen molecules (Sodergren et al., 2006). The main mechanisms of immune defense against infectious agents used by the most of the invertebrates are: a) synthesis and secretion of proteins/molecules with recognition, neutralizing or lytic activity, which participate in nodule formation; b) encapsulation, phagocytosis of foreign particles and cell lysis (Cervello et al., 1996; Gerardi et al., 1990; Li et al., 2014; Loker et al., 2004; Majeske et al., 2013; Stabili et al., 1996; Tahseen, 2009).

In echinoderms, the immune response is divided into humoral (mediated by molecules present in the body fluids) and cellular (cell mediated). The cells responsible for the innate immunity are known as coelomocytes. The coelomocytes are found in the coelomic perivisceral cavity, vascular water system, circulatory system, connective tissue and the tissues of various organs (Tahseen, 2009); and consist of four subpopulations: phagocytes; vibratile cells; red spherule cells and colorless spherule cells (Johnson, 1969). The phagocytes are involved in cell migration and phagocytosis, and represent the major subpopulation of coelomocytes. Phagocytosis is a mechanism in which immune cells migrate to the infected site, recognize, ingest and destroy the foreign particle (inert or alive) and is the main immune defense mechanism of the marine invertebrates (Ellis et al., 2011). Several phagocytic receptors, such as Toll-like receptors and scavengers receptors, are responsible for the phagocytic process in the specialized cells (Aderem and Underhill, 1999). It is estimated that about 4 to 5% of the genes in the genome of *S. purpuratus* are directly involved with the immune system (Hibino et al., 2006).

The production of reactive oxygen species (ROS) is a cellular process associated with phagocytosis and the response to the stress (Buggé et al., 2007; Coteur, 2004; Forman and Torres, 2002; Lesser, 2006). The reactive oxygen species are produced during phagocytosis to destroy the internalized particles (Forman and Torres, 2002). The process begins

Abbreviations: ROS, reactive oxygen species; FSW, filtered seawater; ASW, artificial seawater; M.F.I., mean of fluorescence intensity; LB, latex beads; MK, MK-571; Rev, reversin 205; $\Delta\Psi_m$, mitochondrial inner membrane potential.

* Corresponding author at: Departamento de Biologia Molecular, Universidade Federal da Paraíba, Campus I, Cidade Universitária, CEP: 58051-900, João Pessoa, Paraíba, Brazil.
E-mail address: marques@ltf.ufpb.br (L.F. Marques-Santos).

http://dx.doi.org/10.1016/j.jembe.2016.09.012
0022-0981/

on the cell or phagosome membranes by activating NADPH oxidase, followed by strong oxygen consumption, in a mechanism known as *oxidative burst*. This process leads to the reduction of molecular oxygen to superoxide anion (O_2^-); which can be spontaneously or enzymatically (superoxide dismutase) converted into hydrogen peroxide (H_2O_2). Other reactive radicals, such as hydroxyl radical (•OH) or singlet oxygen ($^1O_2$), are also produced (Dupré-crochet et al., 2013). Furthermore, ROS are also produced by the mitochondrial electron transport chain under physiological or stress conditions (Banh et al., 2016, 2015; Figueira et al., 2013; Kandola et al., 2015; Orrenius et al., 2007). When an imbalance between ROS production and ROS scavenger occurs, the cell enters in a state named oxidative stress (Costantini and Verhulst, 2009). Several works have been using the ROS levels of sea urchins immune system cells as biomarker of stress from different sources, such as: UV radiation, acute heat shock, pH reduction or heavy metals exposure (Coteur et al., 2005; Lu and Wu, 2005; Matranga et al., 2000).

Another important defense mechanism present in deuterostome invertebrates which acts against physical and chemical stressors is the activity of the ABC transporters (Bonaventura et al., 2011, 2005; Dean, 2001; Miller, 2010; Russo et al., 2010). The ABC transporters constitute a large group of integral membrane proteins that promote the active transport of a substrate across the membrane. In eukaryotes, ABC transporters are found in the plasma membrane, but also in the membranes that constitute the endomembrane system (Babakhanian et al., 2007; Burke and Ardehali, 2007; Gibbons et al., 2003; Higgins and Gottesman, 1992; ter Beek et al., 2014; Zutz et al., 2009). The ABC proteins are widely distributed - from microorganisms to human - and their structures are highly conserved (Dean et al., 2001). These transporters were firstly associated with the multidrug resistance phenomenon in cancer cells (Gottesman et al., 2002; Rumjanek et al., 2001). Recently, ABC transporters have been linked to cellular detoxification and associated with the protection of marine organisms against xenobiotics (multixenobiotic resistance, also known as MXR) (Ferreira et al., 2014; Kurelec and Pivčević, 1991, 1989; Kurelec, 1992). The sequenced genome of the sea urchins *S. purpuratus* revealed a wide range of genes encoding ABC transporters (Sodergren et al., 2006). The expression of ABC transporters is regulated in response to xenobiotics, stress and diseases (Bonaventura et al., 2005; de Araujo Leite et al., 2014; Felix and Barrand, 2002; Miller, 2010). Recently, it has been reported that ABC transporters may also play an important role in immune system processes, such as phagocytosis and cell migration (Hinz and Tampé, 2012; Seyffer and Tampé, 2014; van de Ven et al., 2009).

Marine ecosystems play a key role in the ecology of the planet. Human activities have caused climate changes which have altered the hydrological cycle of the planet, including: increase in the incidence of ultraviolet radiation, rise of the sea surface temperature, acceleration in the global rainfall, changes in the rate of evaporation and changes in the salinity of the oceans (Haerter et al., 2010; Semenov et al., 2012; Talley et al., 2002; Trenberth, 1998; Williamson et al., 2014). The reduction of the salinity is one of the main factors that limits the survival and the distribution of marine species (Kaiser, 2011; Li et al., 2013; Russell, 2013; Tomanek et al., 2012). Curry et al. (2003) reported that global warming and changes in the hydrological cycle have altered the distribution of water in the oceans all over the world (Curry et al., 2003). These changes have effects on the physiology and survival of several organisms (Allen and Pechenik, 2010; Carballeira et al., 2011; Choi et al., 2013; Kumar et al., 2010; Luo and Liu, 2011). Some works have reported that fertilization rate, embryo cleavage and polyspermy occurrence - in echinoderms - are sensitive to salinity variations (Allen and Pechenik, 2010; Allen et al., 2015; Carballeira et al., 2011). In spite of being an osmoconformer, echinoderms are stenohaline animals and do not tolerate large variations in the salinity of the environment (Freire et al., 2011). For the sea urchins, fluctuations in environmental salinity may be reflected in the coelomic fluid and impact the physiology of the coelomocytes. However, some studies have shown that Echinoidea species are able to tolerate moderate salinity changes (Drouin et al., 1985; Stickle and Denoux, 1976; Wolff, 1968).

Benthic marine organisms can act as excellent biosensors on the monitoring of the effects of stress on the marine ecosystem. Several studies have demonstrated that sea urchin immune cells respond to environmental stressors, such as: temperature (Borges et al., 2002; Pinsino et al., 2008), UV radiation (Matranga et al., 2006) and pollutants (Pinsino et al., 2008). It has also been described that salinity can affect marine invertebrate immune system, reducing the immune response to foreign agents (Fisher et al., 1987). The tropical sea urchin *Echinometra lucunter* inhabits intertidal areas and is subject to environmental changes such as temperature and salinity (Lima et al., 2009). The investigation of the status of sea urchin immune system cells under different salinity conditions may contribute to the understanding of the effects of environmental changes in the marine ecosystem. Adding to this, the sea urchin reproductive process plays a relevant role in the marine ecosystem as an important component of the marine food chain. So, the aim of the present study was to investigate the effect of salinity on immune system cells of the tropical sea urchin *E. lucunter*, contributing to the knowledge about the effects of climate change on the physiology of marine invertebrates.

2. Material and methods

2.1. Drugs

Calcein-AM (C/AM), carbonyl cyanide *m*-chlorophenyl hydrazone (CCCP), 2′,7′-dichlorodihydrofluorescein diacetate (H2DCFDA), MK571, reversin 205, hydrogen peroxide (H_2O_2) and DiOC6(3) (3,3′-Dihexyloxacarbocyanine,iodide) were purchased from Sigma-Aldrich (St. Louis USA). Fluorescent latex beads were purchased from Polysciences, Inc. (Pennsylvania, USA) and NaCl, KCl, $CaCl_2 2H_2O$, $MgCl_2 6H_2O$, $MgSO_4 7H_2O$ and $NaHCO_3$ were purchased from VETEC Química Fina (Rio de Janeiro, Brazil).

2.2. Animals capture and maintenance

Adult sea urchins *E. lucunter* (Linnaeus, 1758) were sampled at Ponta do Seixas, João Pessoa, Paraíba, Brazil (7°08′54.1″S; 34°47′43.2″W). Animals were transported to the laboratory in plastic containers filled with local seawater and extensively washed with filtered local seawater (FSW, 80 μm) and disposed in a glass tank (80 L FWS; 4 L per animal) under constant aeration. Animals capture was authorized by ICMBio (Instituto Chico Mendes de Conservação da Biodiversidade/Authorization code number: 32105-1).

2.3. In vivo exposure to different salinities

Before the assay, sea urchins were acclimatized in glass tank containing FSW (ambient salinity, 35) at 25 °C and under constant aeration.

For the assay, animals were distributed into plastic tanks (20 L; 4 L per animal; $N = 2$ sea urchin per tank), containing FSW with different salinities (2 replicates per condition): 25 (low salinity), 35 (ambient salinity; control group), and 45 (high salinity). To adjust the salinities, synthetic sea salt (Tetra Marine Salt Pro) was added to FSW. To prepare the ambient salinity and low salinity, FSW was firstly diluted with distilled water. All tanks were kept under constant aeration and the water temperature was 25 °C. The experiment was repeated twice.

The analyses of coelomocyte phagocytosis activity and total and differential coelomocytes concentration were performed 6 h and 24 h after salinity challenge.

2.4. Coelomocytes sampling and preparation

Firstly, the coelomic fluid was withdrawn through a puncture in the peristomial membrane by inserting a needle (21 gauge) coupled to a