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Interrelationship of salinity shift with oxidative stress and lipid metabolism in the monogonont rotifer *Brachionus koreanus*

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ABSTRACT

Salinity is a critical key abiotic factor affecting biological processes such as lipid metabolism, yet the relationship between salinity and lipid metabolism has not been studied in the rotifer. To understand the effects of salinity on the monogonont rotifer *B. koreanus*, we examined high saline (25 and 35 psu) conditions compared to the control (15 psu). In vivo life cycle parameters (e.g. cumulative offspring and life span) were observed in response to 25 and 35 psu compared to 15 psu. In addition, to investigate whether high salinity induces oxidative stress, the level of reactive oxygen species (ROS) and glutathione *S*-transferase activity (GST) were measured in a salinity- (15, 25, and 35 psu; 24 h) and time-dependent manner (3, 6, 12, 24 h; 35 psu). Furthermore composition of fatty acid (FA) and lipid metabolism-related genes (e.g. *elongases* and *desaturases*) were examined in response to different salinity conditions. As a result, retardation in cumulative offspring and significant increase in life span were demonstrated in the 35 psu treatment group compared to the control (15 psu). Furthermore, ROS level and GST activity have both demonstrated a significant increase ($P < 0.05$) in the 35 psu treatment. In general, the quantity of FA and mRNA expression of the lipid metabolism-related genes was significantly decreased ($P < 0.05$) in response to high saline condition with exceptions for both *GST-S4* and *S5* demonstrated a significant increase in their mRNA expression. This study demonstrates that high salinity induces oxidative stress, leading to a negative impact on lipid metabolism in the monogonont rotifer, *B. koreanus*.

1. Introduction

Rotifers (phylum Rotifera), which are microzooplankton, are widely distributed throughout aquatic ecosystems and play a key role as a bridge between producers and higher-level consumers in aquatic food chains (Hutchinson, 1957). Thus, rotifers have been used as a biological indicator for monitoring and evaluating aquatic ecosystem condition. In particular, monogonont rotifers such as *Brachionus plicatilis*, and *B. rotundiformis* have been considered as suitable model species and are extensively used in aquaculture, ecology, gerontology, and ecotoxicology research (Dahms et al., 2011; Won et al., 2017). Thus, influences of the abiotic factors (e.g. temperature and salinity) for the growth and reproduction of rotifers (e.g. *B. plicatilis* and *B. rotundiformis*), including new species, have been widely studied (Miracle and Serra, 1989; Fielder et al., 2000; Bosque et al., 2001; Chigbu and Suchar, 2006).

In general, salinity is a key external factor affecting biological processes and can lead to changes in the life cycle parameters (e.g. growth and fecundity) of rotifers (Cervetto et al., 1999; Yin and Zhao, 2008). In the aquatic environment, changes in salinity affect biological processes at organism, population, community, and ecosystem levels (Cervetto et al., 1999; Yin and Zhao, 2008). An understanding of eco-physiological responses to salinity-induced oxidative stress is essential for evaluating the physiological requirements of each species throughout the aquatic food chain (Verity and Smetacek, 1996). In addition, analysis of lipid content and fatty acid (FA) composition has been used to determine physiological state of aquatic animals (e.g. shrimp and fish) including live food organisms (e.g. *Artemia* and copepod) in the aqua-ecosystems (Chakraborty et al., 2007; Ouraji et al., 2011).

Changes in lipid content and FA composition were associated with different salinities. For example, in juvenile American shad *Alosa sapidissima*, exposed to various salinity condition (7, 12, 21 and 28 psu), increase in the polyunsaturated fatty acids (PUFA) were demonstrated

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with significant enrichment of omega 3 fatty acids (e.g. eicosapentaenoic acid [EPA] and docosahexaenoic acid [DHA]), while reduction in the monounsaturated fatty acids (MUFA) were shown (Liu et al., 2017). In the razor clam *Sinonovacula constricta*, a significant increase in the proportion of PUFAs was shown after exposure to high salinity (20–25 psu), despite an optimum salinity of 10–15 psu (Ran et al., 2017). Also, in vivo (e.g. growth and reproduction) analysis of FA composition and the transcriptional regulation of heat shock proteins (*hsps*) in response to salinity stress was performed to determine physiological condition in the copepod *Paracyclopina nana* (Lee et al., 2017). Although rotifers (e.g. *B. plicatilis* and *B. rotundiformis*) are important food source for higher-level consumers in the aqua-ecosystem, both molecular and biochemical parameters have not been studied easily, as genomic information is not yet available.

The monogonont rotifer *B. koreanus* is a suitable model species for eco-toxicological and eco-physiological studies to examine the effects of marine environmental stressors (e.g. UV-B, gamma radiation, and BDE-47) (Kim et al., 2011; Han et al., 2014; Park et al., 2017), due to rapid reproductive rate, high fecundity, and easy maintenance (Dahms et al., 2011). Furthermore, extensive RNA-seq information of *B. koreanus* provides a better means to investigate effects of environmental stressors at molecular and cellular levels.

In this study, we observed life cycle parameters (e.g. cumulative offspring and life span) and measured the level and activity of oxidative stress markers (e.g. reactive oxygen species [ROS] and glutathione *S*-transferase [GST]) in high salinity. In addition, we investigated the effects of high salinity on fatty acid composition and examined transcriptional regulation of the lipid metabolism-related genes (e.g. *elongases* and *desaturases*).

2. Materials and methods

2.1. Culture and maintenance of Brachionus koreanus

The rotifer *B. koreanus* was collected from a hatchery of East Sea Fisheries Research Institute, Uljin (36°58′43.01″N, 129°24′28.40″E), maintained at the Department of Biological Science, Sungkyunkwan University, Suwon, South Korea, and used for this study. Rotifers were fed with the green marine microalgae *Tetraselmis suecica* (6×10^4 cells/mL) every 24 h and maintained in 15-psu filtered artificial seawater (ASW) (TetraMarine Salt Pro, Tetra™, Cincinnati, OH, USA) with a 12:12 h (light:dark) photoperiod at 25 °C. Species identification was confirmed by morphological analysis (Hwang et al., 2013; Mills et al., 2017) and sequencing of the mitochondrial DNA gene *CO1* (Hwang et al., 2014).

2.2. Assessment of cumulative offspring and life span in response to different salinity condition

To examine life cycle parameters, we recorded the number of offspring and life span. Adult *B. koreanus* was transferred into a new 24-well cell culture plate (SPL Life Science Co. Ltd., Seoul, South Korea) containing 1 mL ASW with *T. suecica* (6×10^4 cells/mL) at different salinities (15 [control], 25, and 35 psu) every 24 h. The number of newborn rotifers was counted every 12 h to examine fecundity, while the death of mature rotifers was counted to quantify life span. All experiments were performed in triplicate and stereomicroscopy (M205-A, Leica Microsystems, Wetzlar, Germany) was used to observe *B. koreanus*.

2.3. Measurement of the reactive oxygen species and the enzyme activity of glutathione S-transferase

Approximately 5000 healthy individuals were exposed to different salinities in salinity- (15 [control], 25, and 35 psu; 24 h) and time-dependent (3, 6, 12, 24 h; 35 psu only) manner. The samples were collected to perform the whole experiments. *B. koreanus* were homogenized in 0.32 mM sucrose containing 20 mM HEPES, 1 mM $MgCl_2$, and 0.5 mM PMSF (pH 7.4). Then, after centrifugation (10,000 × *g* for 20 min at 4 °C) the supernatant were allowed to react with H_2DCFDA and the fluorescence was measured at 485 nm (emission) and 520 nm (excitation) using a multi-channel plate reader (Thermo Scientific Co., Varioscan Flash, Waltham, MA, USA). Intracellular GST activities were measured by following Regoli et al. (1997). Briefly, samples were homogenized in homogenization buffer (2 mM Tris–HCl, containing 20% glycerol, 2 mM mercaptoethanol, and 0.5 mM PMSF [pH 8]), centrifuged at 13,000 × *g* for 20 min at 4 °C, the supernatants were used for further analysis. For GST activity, the increasing absorbance at 340 nm was measured for the conjugation of GSH with 1-chloro-2,4-dinitrobenzene (extinction coefficient of CDNB is 9.6 mM^{-1} cm^{-1}) using a spectrophotometer at 25 °C. The total protein content of the supernatant for each experiment was determined by the dye-binding method (Bradford, 1976) using bovine serum albumin standard (0–200 μg BSA/mL PBS).

2.4. Effect of salinity on fatty acid composition

Variations in FA composition in response to different salinity (15 [control], 25, and 35 psu) were analyzed in *B. koreanus* as described in Hama and Handa (1987) with minor modifications. Briefly, the lipids were extracted with dichloromethane/methanol 2:1 (v/v). Nonadecanoic acid (C19:0) was added to the extracts as an internal standard. Extraction procedures were repeated thrice with sonication. Lipid fractions were separated from the water-methanol phase and converted into fatty acid methyl esters (FAMEs) by saponification using 0.5 M KOH-methanol, followed by methylation with BF3-methanol. Concentrations and compositions of formed FAMEs were analyzed in a gas chromatograph (GC-2010, Shimadzu, Kyoto, Japan) with a flame ionization detector (FID) using a fused silica capillary column (DB-5, 30 m × 0.25 mm i.d., 0.25-μm film thickness). Helium was used as a carrier gas. Samples were injected in splitless mode at an initial oven temperature of 40 °C, raised to 200 °C at 10 °C/min and, then to 300 °C at 2 °C/min. FAs were identified from the retention times (RT) of standards and from mass spectra from gas chromatograph-mass spectrometer (GCMS-QP2010 Plus; Shimadzu, Kyoto, Japan). All experiments were performed in triplicate.

2.5. Identification of the glutathione S-transferase isoforms and lipid metabolism-related genes

To obtain the glutathione *S*-transferase (*GST*) and lipid metabolism-related genes, in silico analysis of *B. koreanus* RNA-seq information was performed (Lee et al., 2015). Genes were subjected to BLAST analysis in the GenBank non-redundant (NR; including all GenBank, EMBL, DDBJ, and PDB sequence except EST, STS, GSS, and HTGS) amino acid sequence database (http://blast.ncbi.nlm.nih.gov/). The amplicons were sequenced on the ABI PRISM 3700 DNA analyzer and putative transcription factor-binding sites were screened using Geneious (v.10.0.7; Biomatters Ltd., Auckland, New Zealand) (Kearse et al., 2012). To investigate the salinity stress-induced modulation of *GST* isoforms and lipid metabolism-related genes, we measured mRNA expression levels over 24 h (control, 3, 6, 12 and 24 h) in response to 35 psu. Total RNAs were extracted with TRIZOL® reagent (Invitrogen, Paisley, Scotland, UK) according to the manufacturer's instructions. The quantity and quality were analyzed spectrometrically at 230, 260, and 280 nm (QIAxpert, Qiagen, Hilden, Germany). To synthesize cDNA for a quantitative real-time reverse transcription-polymerase chain reaction (qRT-PCR), two μg of total RNA and oligo$(dT)_{20}$ primer were used for reverse transcription (SuperScript™ II RT kit, Invitrogen, Carlsbad, CA, USA). qRT-PCR was conducted under the following conditions: 95 °C/4 min; 40 cycles of 95 °C/30 s, 55 °C/30 s, 72 °C/30 s, and 72 °C/10 min using SYBR Green as a probe (Molecular Probes Inc., Eugene, OR, USA)