Fish & Shellfish Immunology 51 (2016) 17–25

Contents lists available at ScienceDirect

Fish & Shellfish Immunology

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

Full length article

Modulating effects of orally supplied *Euglena gracilis* on the physiological responses of the freshwater mussel *Diplodon chilensis*, exposed to sewage water pollution in a Patagonian river (Argentina)

Virginia A. Bianchi [a, *], Juan M. Castro [b], Iara Rocchetta [b, c, d], Visitación Conforti [c, e, f], Mariano Pascual [b], Carlos M. Luquet [b]

[a] *Laboratorio de Investigaciones Bioquímicas, Químicas y de Medio Ambiente (LIBIQUIMA-CITAAC), Universidad Nacional del Comahue, Buenos Aires 1400, CP: 8300, Neuquén, Argentina*
[b] *Laboratorio de Ecotoxicología Acuática, INIBIOMA(CONICET-UNCo) – CEAN, ruta provincial N° 61, km 3, CCP 7, Junín de los Andes, 8371, Neuquén, Argentina*
[c] *Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Av. Rivadavia 1917, Buenos Aires, Argentina*
[d] *Departamento de Química Biológica, Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires, Pab. II, Ciudad Universitaria, 1428, Buenos Aires, Argentina*
[e] *IBBEA, Instituto de CONICET, Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires, Pab. II, Ciudad Universitaria, 1428, Buenos Aires, Argentina*
[f] *Departamento de Biodiversidad y Biología Experimental, Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires, Pab. II, Ciudad Universitaria, 1428, Buenos Aires, Argentina*

ARTICLE INFO

Article history:
Received 28 September 2015
Received in revised form
6 January 2016
Accepted 11 February 2016
Available online 15 February 2016

Keywords:
Bivalve
Paramylon
Immune response
Oxidative stress
Metabolic condition
Urban pollution
Bioremediation

ABSTRACT

In order to test if orally supplied *Euglena* sp. cells modulate the physiological status of bivalves during bioremediation procedures, we evaluated the effect of *Euglena gracilis* diet on the immune response, oxidative balance and metabolic condition of *Diplodon chilensis* exposed to sewage water pollution. Mussels were fed for 90 days with *E. gracilis* (EG) or *Scenedesmus vacuolatus* (SV, control diet), and then exposed for 10 days at three sites along the Pocahullo river basin: 1) an unpolluted site, upstream of the city (control, C); 2) upstream (UpS) and 3) downstream (DoS) from the main tertiary-treated sewage discharge, in the city of San Martín de los Andes, Northwest Patagonia, Argentina. Our results show that the total hemocyte number decreases while pollution load increases along the river course for both, EG and SV mussels. Phagocytic activity is higher in EG mussels than in SV ones under all conditions. Reactive oxygen species (ROS) production in hemocytes increases with the increase in the pollution load, being significantly higher for EG mussels than for SV ones at DoS; no changes are observed for total oxyradical scavenging capacity (TOSC). Hemocytes' viability is increased for *E. gracilis* diet at C and remains unchanged in this group of mussels when exposed at the polluted sites. Lysosomal membrane stability is higher in EG mussels than in SV ones for all conditions, although it is decreased at polluted sites compared with that at C. Antioxidant (catalase) and detoxifying (gluthatione S-transferase) defenses are generally lower in gills and digestive gland of EG mussels than in SV ones. Lipid peroxidation (TBARS) is evident in gills of EG mussels at C, and in digestive gland of the same group, at all the sites. Gill mass factor (GF) is affected by the *E. gracilis* diet; it is increased at C and decreased at polluted sites when compared with that of SV ones. Digestive gland mass factor (DGF) is higher in EG mussels than in SV ones. In *D. chilensis*, continuous and long term feeding with *E. gracilis* cells favors immune response and reduces the damage caused by sewage pollution exposure on hemocytes. Nevertheless, diet and

Abbreviations: EG, Fed with *Euglena gracilis* cells; SV, Fed with *Scenedesmus vacuolatus* cells; C, River control site; UpS, Polluted site, upstream from the tertiary-treated sewage discharge; DoS, Polluted site, downstream from the tertiary-treated sewage discharge; WTM, Wet tissue mass; GF, Gill factor; DGF, Digestive gland factor.

* Corresponding author.
E-mail address: vabianchi@comahue-conicet.gob.ar (V.A. Bianchi).

http://dx.doi.org/10.1016/j.fsi.2016.02.016

transplantation procedures may produce negative effects on the oxidative balance of gills and digestive gland and should be taken into account for bioremediation strategies.

1. Introduction

Euglena spp. cells are being considered as a promising dietary complement to be used in aquaculture activities [1,2]. These unicellular flagellates are able to synthesize and accumulate high amounts of paramylon, a β-1,3 glucan, which has been reported as an efficient immunostimulant for the rainbow trout [3,4] and for fingerlings of the fish *Labeo rohita* [5]. Orally supplied paramylon extracted from *Euglena gracilis*, enhances antioxidant responses in rat liver after carbon tetrachloride injection [6]. In addition, the high content of proteins in *Euglena* spp. cells promotes growth rates in farmed crustaceans and fish [3,7–9], while polyphenols, flavonoids, tannins, β-carotene, vitamin C and E may be present in these cells as a source of vitamins and antioxidant compounds, contributing to a healthy general status [2]. In hemocytes of bivalves, exposure to β-glucans increases nitric oxide production, peroxidase and antibacterial activity and phagocytosis during *in vitro* and injection-based experiments [10–13]. New evidence has been recently published for the immunostimulant effect of orally supplied *E. gracilis* cells on bivalves exposed to *Escherichia coli* [14].

Filtering bivalves are proposed as useful bioremediation tools against anthropogenic pollution, containing metals, organic matter, bacteria, algae and nutrients, eg. Refs. [15–17]. In particular, the freshwater mussel *Diplodon chilensis* is able to filter high amounts of particulate organic matter, coliform bacteria and algae, reducing microorganism and nutrient loads from eutrophicated and bacteria polluted water bodies [18–22]. Regarding this, Sabatini et al. [21] have found that *D. chilensis* may clear *Escherichia coli*, a typical bacterium found in sewage water, at a rate of 0.510 ± 0.036 L h^{-1} per gram of dry soft tissue mass (DTM), while Bianchi et al. [22] report that enteric bacteria are efficiently removed from sewage polluted water at a rate of 0.155 ± 0.01 L h^{-1} per gram of DTM. However, it has been shown that exposure to *E. coli* may cause hemocyte damage [14] and increased lipid peroxidation in gills and digestive gland of this bivalve [14,21].

In our previous work [14], feeding with *E. gracilis* cells has been evaluated in *D. chilensis* in order to improve its physiological responses against *E. coli*. The cited work brought promising results concluding that *E. gracilis* can be used as a nutritional and immune protective diet complement, suitable for filtering bivalves. Nevertheless, the variety of pollutants found in sewage water may have a different effect compared with those of the isolated bacteria. It has been reported that organic and inorganic pollutants contained in domestic effluents may cause alterations in the physiological status of bivalves, modifying cellular immune responses and viability [23–26] and causing genotoxic effects [27]. In addition, oxidative stress and detoxification mechanisms are increased while growth rate is altered in bivalves exposed to sewage polluted aquatic environments [26]. Similar results were obtained for wild and caged *D. chilensis* exposed to sewage water pollution in the field [21,22,28]. Thus, in order to test whether orally supplied *Euglena* sp. cells modulate the physiological status of bivalves during bioremediation procedures, we evaluated the effect of *E. gracilis* diet on the immune response, oxidative balance and metabolic condition of *D. chilensis*, after field exposure to different concentrations of sewage water pollution in a North-Patagonian river.

2. Materials and methods

2.1. Mussel collection and handling

Mussel collection, experimental feeding and field exposure were performed during the non-reproductive season of *D. chilensis* (May to August, 2012) in order to avoid physiological changes due to reproductive status [29]. Adult *D. chilensis* (n = 60; 68.13 ± 0.66 mm shell length) were collected by a diver from 1.5 m depth at an unpolluted area in the north coast of Lacar lake (Yuco, 40° 10′ S, 71° 31′ 30″ W). Mussels were immediately transported to the laboratory and placed in aerated tanks (150 individuals per m^2) containing dechlorinated tap water.

2.2. Strains

Lyophilized cells of *E. gracilis* (UTEX 753 strain, from the Culture Collection of Algae of Texas University, USA) and of the green algae *Scenedesmus vacuolatus* (BAFC CA4 strain from Laboratory of Phycology, Departamento de Biodiversidad y Biología Experimental, Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires) used in this work correspond to the same cultures previously used by Bianchi et al. [14]. Both experimental diets were set at 0.133 mg of lyophilized cells per mussel per feeding event, and each mussel received a ration of 0.128 mg paramylon per feeding, contained in lyophilized *E. gracilis* cells [14].

2.3. Experimental feeding

During 21 days of acclimation in laboratory, individuals were fed three times a week with *S. vacuolatus*. After acclimation, mussels were sorted into two groups: SV (n = 30), fed with *S. vacuolatus* (control diet) and EG (n = 30), fed with *E. gracilis*. Experimental diets were supplied three times a week for 90 days, performing water changes before each feeding. During this period, temperature was kept at 11.5 ± 1.0 °C.

2.4. Field exposure

After experimental feeding, both SV and EG mussels were sorted into six groups (n = 5 per group), which were placed into six cages (iron structure covered with plastic mesh) at a final density of 87 individuals per m^2. Two cages with SV or EG mussels were placed at each of three sites along the Pocahullo river basin, which crosses suburban and urban areas of San Martín de los Andes city, Northwest Patagonia, Argentina (40°09′24″S; 71°21′09″W). The sites were set as follows: C: upstream control, at a site with no significant sewage pollution (40° 7′ 7,4″ S; 71° 14′ 14.2″ W); UpS: 40 m upstream from the main tertiary-treated sewage discharge but downstream from diffuse discharge of untreated effluents (septic tank infiltrations and horse-cattle farming) and point source discharges of suburban primary treated sewage (40°09′32,1″S; 71°21′40.9″W); and DoS: 20 m downstream from the main tertiary-treated sewage discharge (40°10′S; 71°20′60″W). After 10 days of exposure, individuals were collected and transported in the cold to the laboratory, for immediate processing.