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Effect of chemical stress and ultraviolet radiation in the bacterial communities of zebrafish embryos

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ABSTRACT

This study aimed to assess the effect of ultraviolet radiation (UVR) and chemical stress (triclosan-TCS; potassium dichromate-PD; prochloraz-PCZ) on bacterial communities of zebrafish (*Danio rerio*) embryos (ZEBC). Embryos were exposed to two UVR intensities and two chemical concentrations not causing mortality or any developmental effect (equivalent to the No-Observed-Effect Concentration-NOEC; NOEC diluted by 10-NOEC/10). Effects on ZEBC were evaluated using denaturing gradient gel electrophoresis (DGGE) and interpreted considering structure, richness and diversity. ZEBC were affected by both stressors even at concentrations/doses not affecting the host-organism (survival/development). Yet, some stress-tolerant bacterial groups were revealed. The structure of the ZEBC was always affected, mainly due to xenobiotic presence. Richness and diversity decreased after exposure to NOEC of PD. Interactive effects occurred for TCS and UVR. Aquatic microbiota imbalance might have repercussions for the host/aquatic system, particularly in a realistic scenario/climate change perspective therefore, future ecotoxicological models should consider xenobiotics interactions with UVR.

1. Introduction

The microbial community plays a crucial role in organism life. In fish's early stages of development, in particular, it provides various services, e.g. (i) augmenting protection via different strategies (Brunvold et al., 2007; Gil-Turnes and Fenical, 1992; Hansen and Olafsen, 1999; Heikkinen et al., 2014; Olafsen, 2001) and (ii) promoting early nutrient metabolism or complementing nutrition (Hansen and Olafsen, 1999; Li et al., 2012). Depending on the developmental stage of the fish and the nutritional and environmental situation, the associated bacterial community constantly adapts, tending to the microbial balance, essential to maintain overall fish health and function (Gómez and Balcázar, 2008). However it is well known that factors inducing stress such as environmental stressors [e.g. temperature, ultraviolet radiation (UVR), oxygen levels and pH] or anthropogenic stressors (e.g. chemical pollutants), might determine abundance, structure and diversity shifts in microbial communities jeopardizing microbiota-host stability and further development or survival of the organism (Hansen and Olafsen, 1999; Li et al., 2012). Because the development of the fish and of the immune system is not complete, these impacts are particularly relevant during early developmental stages (Giatsis et al., 2014). To our knowledge, the bacterial communities of early life stages of various fish was already studied by several methods (e.g. Brunvold et al., 2007; Griffiths et al., 2001; Jensen et al., 2004; Romero and Navarrete, 2006) but the bacterial communities of zebrafish, *Danio rerio*, (for the different stages of development) remains unknown.

Zebrafish inhabits low depth (15–103 cm) water of relatively high clarity and unshaded, where spawning usually occurs (Lawrence, 2007; Spence et al., 2008). The penetration of ultraviolet radiation (UVR) in the aquatic environment is dependent on different factors (Tedetti and Sempéré, 2006). Despite of the lack of information of the UV penetration in these habitats, in locations with close-related characteristics, the penetration of UV is enough to be potentially biologically damaging to water microorganisms

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(e.g. 10% of UV-B may penetrate 3 m or between 0.5 and 4 m in coastal eutrophic and estuarine waters, respectively) (Speekmann, 2000). Thus we can infer that the microbiota of zebrafish eggs, laying in depths as low as 15–103 cm, may be affected by UVR. Indeed, research showed that exposure to UVR caused inactivation and decrease of fish eggshell adherent bacteria (Heikkinen et al., 2014, 2013) enabling proliferation of opportunistic bacteria (Olafsen, 2001), resulting in adverse effects to the eggs and leading, ultimately, to larval mortality (Hansen and Olafsen, 1999; Hansen et al., 1992). Since UVR (UV-B and UV-A) has a decisive role on the bacterial community structure of aquatic organisms, particularly in early stages of development, further investigations regarding this issue must be conducted.

Triclosan (TCS), potassium dichromate (PD) and prochloraz (PCZ) are widely used in industry and agriculture practices (Barnhart, 1997; Dann and Hontela, 2011; Ohlsson et al., 2009) leading to their guaranteed appearance in the aquatic environment (Chalew and Halden, 2009; Mohan and Pittman, 2006). The effects of these chemicals in the aquatic organisms are well-documented (Domingues et al., 2013, 2010; Oliveira et al., 2009; Orvos et al., 2002; Prabakaran et al., 2006; Saglio et al., 2003). Chemical pressure (depending on the type and level of chemical contamination) might also result in strongly altered microbial communities, as bacterial species will be differently affected depending on their chemical-tolerance. Recent works showed that TCS induced changes in the structure of bacterial communities from anaerobic digesters (Mcnamara et al., 2014), river biofilms (Lawrence et al., 2009), soil (Harrow et al., 2011) and sediments (Huang et al., 2015) revealing also the occurrence of TCS resistance among environmental microorganisms (Mcnamara et al., 2014; Yazdankhah et al., 2006). For Cr (VI), distinct bacterial susceptibility has been reported (Branco et al., 2005; Camargo et al., 2003; Dhal et al., 2013; Francisco et al., 2002; Shakoori et al., 2000). On the other hand, PCZ was shown not to affect negatively soil bacterial biodiversity, but rather cause stimulation of community growth (Tejada et al., 2011), possibly because bacteria might use PCZ as a source of carbon and energy (Karn and Balda, 2013).

In natural environments, organisms-microorganisms systems are simultaneously exposed to several environmental and anthropogenic stressors supporting the need for a more realistic approach by analysing the combined effects of stressors. Indeed, synergistic effects of other chemicals and UV treatments showed inactivation of enteric bacteria (Koivunen and Heinonen-Tanski, 2005). These premises underline the hypothesis that TCS, PD or PCZ contamination and UVR exposure may act synergistically towards a higher stressor pressure inducing selective shifts on zebrafish embryos bacterial community (ZEBC). However, although literature proved that PCZ, PD, TCS or UVR affect distinct bacterial communities, evidence of their potential effects on the ZEBC has not been explored, neither in a single nor a combined approach. Thus, here our objective was to investigate the effect of distinct UVR regimes and different concentrations of TCS, PD or PCZ on the overall bacterial communities of *D. rerio* embryos. In line with this goal, molecular [PCR and denaturing gradient gel electrophoresis (DGGE) approach], ecotoxicological and statistical tools were used to evaluate the potential effects imposed by each treatment on bacterial communities of zebrafish embryos.

2. Materials and methods

2.1. Test organisms

Zebrafish (*D. rerio*) eggs were provided by the facility established at the Biology Department from University of Aveiro (Portugal) where adult fish were maintained as described by Domingues and collaborators (Domingues et al., 2013).

Zebrafish eggs were collected within 30 min after natural mating, rinsed in fish system water and screened using a stereomicroscope (Stereoscopic Zoom Microscope-SMZ 1500, Nikon Corporation) to exclude unfertilized eggs, injured embryos or embryos with irregularities during cleavage.

2.2. Zebrafish embryos' combined assay

2.2.1. Test chemicals

Zebrafish embryos were exposed to TCS (Irgasan, 5-chloro-2-(2,4-dichlorophenoxy)phenol; Sigma–Aldrich, Co., St. Louis, MO), PD ($K_2Cr_2O_7$; CAS Number: 7778-50-9; Merck KGaA, Darmstadt, Germany) used as a source of hexavalent chromium (Cr(VI)) or PCZ (1-[N-propyl-N-2-(2,4,6-trichlorophenoxy) ethylcarbamoyl] imidazole I: C15H16Cl3N3O2; PESTANAL®; CAS Number: 67747-09-05; Sigma–Aldrich, Co., St. Louis, MO). Besides the control exposure, two concentrations were used: the No-Observed-Effect Concentration (NOEC) and the 10 times dilution of the NOEC (NOEC/10) of each chemical (Table 1). These concentrations were chosen based on previous works showing no mortality neither developmental effects on the organism (Domingues et al., 2013, 2010; Oliveira et al., 2009). As PD does not penetrate the chorion, no effects on embryo development or mortality were achieved within 48 h, thus NOEC value was impossible to be determined; therefore, the first concentration leading to developmental effects at 72 h but not at 48 h was chosen reflecting a compromise between time of exposure, dose and effects. For uniformity purposes, all concentrations will be herein after referred to as NOEC and NOEC/10.

For TCS and PCZ, the stock solutions were prepared by dissolving each chemical separately in acetone (<100 μl. L^{-1}) and filling up with ultrapure water. For PD, the stock solution was prepared by dissolving PD in water from the fish system. Tested concentrations were obtained by diluting the stock solutions of each chemical in water from the fish system.

2.2.2. Ultraviolet radiation

UVR levels were used according to previous studies carried out (Almeida et al., 2015) revealing that neither survival nor development of the organism was affected. UVR (UV–B (280–320 nm) and UV-A (320–400 nm) radiations) was provided by UV lamp (Spectroline XX15F/B, Spectronics Corporation, NY, USA, peak emission at 312 nm) coupled with a cellulose acetate filter (previously UV irradiated for 12 h to cut off UVR-C radiation and to minimize the variations in UVR intensity). Two UVR intensities were used (Table 1) by varying the distances between the petri dishes and the UV lamp (Low radiation – R_L; High radiation – R_H), plus a control with no UVR incidence (referred as R_0).

Intensities of emitted UVR were measured, before and after each exposure, with a spectro-radiometer, connected to a monochromator that provided information on energy per nanometer. Spectral irradiance was obtained by the BenWin + software (Benthan Instruments, Reading, UK). The mean UVR dose (kJ cm^{-2}) was obtained according to the weighting factor of the CIE (International Commission on Illumination) reference action spectrum for erythema in human skin (McKinlay and Diffey, 1987). The biologically effective doses of UVR (UVD_{eff}) were calculated taking into account the time of exposure and the biologically effective UV irradiance (I_{eff}) (Equation (1)) (Morgado et al., 2013).

2.2.3. Exposure conditions

Three tests were performed where TCS, PD or PCZ were combined with UVR. The experimental set up included nine treatments in a full factorial design using three chemical concentrations and three levels of UVR, and including a control treatment with no