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Effects of acute γ-irradiation on community structure of the aquatic microbial microcosm

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ABSTRACT

To characterise indirect effects of ionising radiation on aquatic microbial communities, effects of acute γ-irradiation were investigated in a microcosm consisting of populations of green algae (*Chlorella* sp. and *Scenedesmus* sp.) and a blue-green alga (*Tolypothrix* sp.) as producer; a ciliate protozoan (*Cyclidium glaucoma*), rotifers (*Lecane* sp. and *Philodina* sp.) and an oligochaete (*Aeolosoma hemprichi*) as consumer; and more than four species of bacteria as decomposers. Population changes in the constituent organisms were observed over 160 days after irradiation. Prokaryotic community structure was also examined by denaturing gradient gel electrophoresis (DGGE) of 16S rDNA. Principle response curve analysis revealed that the populations of the microcosm as a whole were not significantly affected at 100 Gy while they were adversely affected at 500–5000 Gy in a dose-dependent manner. However, some effects on each population, including each bacterial population detected by DGGE, did not depend on radiation doses, and some populations in the irradiated microcosm were larger than those of the control. These unexpected results are regarded as indirect effects through interspecies interactions, and possible mechanisms are proposed originating from population changes in other organisms co-existing in the microcosm. For example, some indirect effects on consumers and decomposers likely arose from interspecies competition within each trophic level. It is also likely that prey–predator relationships between producers and consumers caused some indirect effects on producers.

1. Introduction

Recently, there have been a number of initiatives on national and international scales that are developing frameworks to provide criteria and a methodological approach for the protection of the environment from ionising radiation (Howard and Larsson, 2008; IAEA, 2002; ICRP, 2003; OECD/NEA, 2007; US DOE, 2002). Discussion in such initiatives has pointed out that most studies on ecological effects of radiation have been made at individual or population-levels, but a holistic approach, i.e., evaluation of community-level effects including indirect effects due to interspecies interactions, is also required (Bréchignac, 2002, 2003; Bréchignac and Doi, 2009; Copplestone et al., 2004; IAEA, 1976, 1988, 1992; Monte, 2009; NCRP, 1991; UNSCEAR, 1996). This is because ecosystems consist of various species, which have the wide range of radiosensitivities (Sparrow et al., 1967), and have complex interspecies interactions, e.g., predator–prey and competitive interactions. As a result, after one species is damaged directly by radiation, another radioresistant species may be also affected indirectly by disappearance of an interaction with that damaged species.

Studies on radiation effects at the community level have concentrated on terrestrial ecosystems, and only limited attention has been paid to aquatic microbial communities, which play an important role in material cycles in aquatic ecosystems. According to our knowledge, there has been only one study that detected significant effects of ionising radiation on aquatic microbial communities (Ferens and Beyers, 1972). In this study, radiation effects on biomass, chlorophyll *a* content and gross productivity were detected in the microcosm, i.e., the experimental ecosystem model, consisting of algae, zooplankton and bacteria. Since these effects did not depend on radiation doses, they were regarded as

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indirect effects, which may have been due to interactions between two species of algae (producers). However, in this study, radiation effects on communities of zooplankton (consumers) and bacteria (decomposers) were not investigated, preventing a suitable understanding of how consumers and decomposers respond to ionising radiation in aquatic microbial communities.

In our previous study, we investigated the effects of acute γ-irradiation on an aquatic microcosm consisting of three species of microorganisms, and some indirect effects were observed in the producer and consumer populations (Fuma et al., 1998). However, this microcosm included only one species at each trophic level, i.e., a producer, a consumer and a decomposer, and thus lacked interspecies interactions within each trophic level. It also lacked a prey–predator interaction between a producer and a consumer, which is typical in aquatic microbial communities. To characterise indirect effects due to these interactions, this paper investigated the effects of acute γ-irradiation on a microcosm consisting of three species of algae, four species of microfauna and more than four species of bacteria.

2. Materials and methods

2.1. Microcosm

The microcosm used in this study was developed by Kurihara (1978a) as follows. Microbial communities collected from several aquatic systems such as ponds were mixed, and serially cultured for more than 20 years. As a result of interspecies competition under the culture condition, species fitting the culture condition have been selected, and formed a repeatable ecosystem model. This microcosm is therefore regarded as a self-selected type.

The microcosm consisted of populations of green algae *Chlorella* sp., *Scenedesmus* sp. and a blue-green alga *Tolypothrix* sp. as producer; a ciliate protozoan *Cyclidium glaucoma*, rotifers *Lecane* sp. and *Philodina* sp. and an oligochaete *Aeolosoma hemprichi* as consumer; and the bacteria *Pseudomonas putida*, *Bacillus cereus*, *Acinetobacter* sp., Coryneform bacteria and unidentified other bacteria as decomposers. The culture medium was designed after Taub and Dollar's (1968) salt solution supplemented with 50 mg l^{-1} polypeptone (Nihon Seiyaku, Tokyo, Japan).

Because of allowing for interspecies interactions, the microcosm mimics the essential processes in natural aquatic microbial communities, and it is therefore expected to detect some indirect effects as those which were reported by Kurihara (1978b). At the early stage of the culture, the microcosm is heterotrophically maintained with the energy of polypeptone which decomposers fix. After exhaustion of the polypeptone, the microcosm becomes eutrophically maintained with photoenergy which powers photosynthesis by means of which producers can fix CO_2. Energy flow through decomposers or producers is then distributed to other trophic levels through various interspecies interactions. For example, consumers obtain energy by grazing decomposers or producers, and growth of decomposers or producers is stimulated with metabolites or breakdown products derived from other trophic levels.

This microcosm has been used for ecological studies (Shikano and Kurihara, 1985) and evaluation of ecotoxicity of various chemicals (Inamori et al., 1998; Takagi et al., 1994; Takamatsu et al., 1996).

2.2. Culture and irradiation

Five milliliters of a three-month old microcosm were inoculated to 100 ml of the culture medium in 125-ml polypropylene bottles with screw caps (Nalgene, Rochester, NY, USA), which were loosely closed during culture. The resulting microcosm was statically cultured in an incubator with fluorescent lamps under a photosynthesis photon flux density of 46 μmol m^{-2} s^{-1}, a 12 h light–dark cycle and a temperature of 25 °C. On day 1 after the beginning of the culture, the microcosm was acutely irradiated with a single dose of 100, 500, 1000 and 5000 Gy of ^{60}Co γ-rays at a dose rate of 31 Gy min^{-1}. Then, the microcosm was cultured over 160 days under the conditions mentioned above. There were three replicates for non-irradiated controls and each dose.

2.3. Population measurement

Though the microcosm contained more than four species of bacteria as mentioned in Section 2.1, cell densities of viable bacteria were measured as a whole, regardless of the species. They were measured by the colony counting method using the PY medium (0.5% polypeptone, 0.3% yeast extract, 0.4% NaCl and 1.5% agar in distilled water) after aliquots of the microcosm were sonicated for 10 s using an ultrasonic homogeniser (UH-50; SMT, Tokyo, Japan). To measure populations of the filamentous blue-green alga *Tolypothrix* sp., 1 ml of the sonicated aliquot of the microcosm was put in a counting chamber (20 mm × 50 mm), and six microscopic photographs were taken at different positions. Length of *Tolypothrix* sp. in these photographs was measured using the Image J software, version 1.36b (National Institute of Health, Bethesda, MD, USA). The other organisms were counted under a microscope.

2.4. Analysis of bacterial community structure

The bacterial community structure was analysed by a molecular technique, i.e., denaturing gradient gel electrophoresis (DGGE) targeting 16S rDNA. This technique makes it possible to detect prokaryotes, i.e., bacteria and blue-green algae (cyanobacteria), which cannot be cultured in the laboratory and thus cannot be distinguished by conventional culture-based methods. Since only less than 1% of all bacterial species can be cultured in the laboratory (Hugenholtz, 2002), this technique has been applied to environmental microbiology (Muyzer et al., 1995) and demonstrated as a useful method to assess impacts of chemicals and ionising radiation on bacterial community structures (Becker et al., 2006; McNamara et al., 2007; Sigler and Turco, 2002). The microcosm used in this study originates from natural aquatic microbial communities as described in Section 2.1, and the bacterial species composition was detected only by culture-based methods (Kurihara, 1978a), suggesting the possibility that this microcosm would contain some unidentified bacterial species. It is expected that DGGE analysis detects not only identified bacterial species but also unidentified species in this microcosm.

The DGGE analysis was carried out on days 2 and 15 after beginning of the culture, which corresponded to the growth and mature stages, respectively. At each date, 10 ml of the microcosm was filtered with a 0.2-μm-pore size polycarbonate membrane filter. Total DNA was extracted from the organisms collected onto the filter using an ISOIL for Beads Beating kit (Nippon Gene, Toyama, Japan) as described by the manufacturer.

The V3 region of the 16S rRNA gene of prokaryotes was amplified using forward (341F-GC) and reverse (907R) primers by PCR in accordance with the method of Muyzer et al. (1995).

The DGGE was performed using a Bio-Rad DCode system (Bio-Rad Laboratories, Hercules, CA, USA). The DGGE gel contained 8% polyacrylamide, 30–55% denaturing gradient (The 100% denaturing agent consisted of 40% [vol/vol] formamide and 7 M urea.) and 1×TAE buffer (0.04 M Tris base, 0.02 M sodium acetate and 10 mM EDTA [pH 7.4]). Electrophoresis of the PCR products was conducted initially at 20 V for 10 min and then at 70 V for 20 h at 60 °C. After electrophoresis, the gels were stained with SYBER Gold (Molecular Probes, Eugene, OR, USA), and their images were acquired using a Molecular Imager FX system (Bio-Rad Laboratories).

Nucleotide sequences of some DGGE bands were determined. A piece of gel containing each target DNA band was excised, and the DNA was reamplified with the 341F-GC and 907R primer pair. The PCR products were subjected again to DGGE to ensure that the products contained single bands and showed electrophoretic mobility identical to that of the original bands. For the direct sequencing of DGGE bands, the extracted DNA was reamplified with the 341F (without GC-clamp) and 907R primers after purification using a QIAquick PCR Purification Kit (QIAGEN, Germantown, MD, USA). Sequencing reactions were carried out using the Big Dye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, Foster City, CA, USA). Reaction products were analysed with an ABI Prism 310 Genetic Analyzer (Applied Biosystems). The obtained sequences were compared with EMBL/GeneBank/DDBJ database entries using the BLASTN program 2.2.15 (Altschul et al., 1997) to determine the putative identity of prokaryotic phylotypes.

2.5. Data analysis

Significance of differences in populations between the irradiated and control microcosms were tested at each sampling date by Dunnett's test after the populations were log transformed ($\log_{10}(N+1)$). This test was performed using the StatView software, version 5.0 (SAS Institute Inc., Cary, NC, USA) at the significance level of 0.05.

A trend of holistic population size changes in the microcosm was analysed by the principal response curve (PRC) method using the CANOCO software, version 4.5 (Microcomputer Power, Ithaca, NY, USA). The PRC analysis is a novel multivariate method based on the redundancy analysis adjusted for overall changes in community response over time, and has been used for the analysis of data from microcosm experiments (Ter Braak and Smilauer, 2002; Van den Brink and Ter Braak, 1999). In the PRC method, the abundance of each taxon in the exposed microcosm is modelled at each sampling date by the following equation:

$$y_{d(j)tk} = \bar{y}_{0tk} + b_k c_{dt} + \varepsilon_{d(j)tk} \quad (1)$$

where $y_{d(j)tk}$ = the abundance of taxon k in replicate microcosm j at dose d on day t; $\bar{y}_{0tk}$ = the mean abundance of taxon k in control microcosm ($d = 0$) on day t; c_{dt} = the canonical coefficient, i.e., the principal deviation from controls, at dose d on day t; b_k = the taxonomic group score of the taxon k, i.e., the regression coefficient for taxon k with respect to the canonical coefficient; $\varepsilon_{d(j)tk}$ = an error term of taxon k in replicate microcosm j at dose d on day t.