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Short Communication

Arsenoriboside degradation in marine systems: The use of bacteria culture incubation experiments as model systems

Elliott G. Duncan [a,*], William A. Maher [a], Simon D. Foster [a], Katarina M. Mikac [b], Frank Krikowa [a], Anthea Florance [a]

[a] Ecochemistry Laboratory, Institute for Applied Ecology, University of Canberra, University Drive, Bruce, ACT 2601, Australia
[b] Institute for Conservation Biology and Environmental Management, University of Wollongong, Northfields Avenue, Wollongong, NSW 2522, Australia

HIGHLIGHTS

- Arsenoriboside degradation never studied in bacteria culture media incubation experiments.
- Arsenoribosides persisted in all bacteria inoculated cultures.
- DMAE and other arsenoriboside degradation products were not detected.
- Properties of culture media influenced composition of bacteria community.
- Traditional algal-tissue decomposition studies more suited experimental approach.

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ABSTRACT

Arsenoribosides (as glycerol; phosphate; sulfate and sulfonate) persisted in all bacteria-inoculated cultures irrespective of the source of bacteria (seawater, macro-algae surface) or the culture media used (DIFCO Marine Broth 2216 or novel blended *Hormosira banksii* tissue-based). This is unlike observations from traditional macro-algae tissue decomposition studies or in nature. In addition known arsenoriboside degradation products such as dimethylarsenoethanol (DMAE), dimethylarsenate (DMA), methylarsenate (MA) and arsenate – As(V) were not detected in any cultures. Consequently, the use of bacterial culture incubation experiments to explain the fate of arsenoribosides in marine systems appears limited as the processes governing arsenoriboside degradation in these experiments appear to be different to those in macro-algae tissue decomposition studies or in nature.

1. Introduction

The fate of arsenoribosides upon the decomposition of macro-algal tissue under controlled laboratory conditions has been studied previously (Edmonds et al., 1982; Pengprecha et al., 2005; Foster and Maher, 2010; Navratilova et al., 2011). From these studies it has been observed that arsenoribosides are degraded to arsenic species such as dimethylarsenoethanol (DMAE), dimethylarsenate (DMA), methylarsenate (MA) and arsenate – As(V).

Culture media incubation studies represent an alternate means to investigate the fate of arsenoribosides in marine systems. Experiments of this nature may be advantageous as they ensure that all observed arsenic cycling processes are of microbial origin. This study thus aims to investigate if bacterial culture media incubation experiments can be used as an alternative to traditional algal-tissue decomposition study arsenoriboside degradation in marine systems.

2. Methods

2.1. Incubation experiment 1 – seawater communities in DIFCO MB 2216

Sterile 1 L Erlenmeyer flasks ($n = 8$) were prepared for the DIFCO MB 2216-based arsenoriboside degradation experiment. All flasks contained 500 mL of sterile 1/5th strength DIFCO MB 2216. Seawater bacteria were collected from Bawley Point, New South Wales, South-Eastern Australia (35.5199°S; 150.3967°E) using an autoclave sterilised 1 L Schott bottle. Half the cultures ($n = 4$) were inoculated with 10 mL of 0.45 μm filtered seawater, with the other cultures ($n = 4$) remaining as non-inoculated controls. All cultures

* Corresponding author. Present address: CSIRO Plant Industry, Centre for Environment and Life Sciences, Underwood Avenue, Floreat, WA 6014, Australia. Tel.: +61 8 93336615.
E-mail address: Elliott.Duncan@csiro.au (E.G. Duncan).

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Table 1
Total phosphorus (TP) and total nitrogen (TN) concentrations in DIFCO Marine Broth 2216, *H. banksii* based media and seawater. Values are means (mg L^{-1}) ± SD (n = 4).

Medium	Total phosphorus (mg L^{-1})	Total nitrogen (mg L^{-1})
DIFCO Marine Broth 2216	7.8 ± 0.4	600 ± 8
H. banksii-based medium	2.9 ± 0.4	15 ± 4
Autoclaved seawater	0.7 ± 0.1	6 ± 2

were wrapped in black plastic and incubated in an environmental chamber (3504 process controller, Eurotherm, Australia) at a temperature regime of 20–25 °C.

After 2 d incubation, all cultures (n = 8) were exposed to a mixed arsenoriboside solution containing approximately 70 μg L^{-1} total arsenic with the four major arsenoriboside species (Gly, PO_4, SO_3^- and OSO_3^-) present in the following proportional ratio Gly/PO_4/SO_3/OSO_3 – 13:15:66:6 (mass/mass/mass/mass). Samples of the culture media for total arsenic and arsenic species analysis were taken after the addition of arsenoribosides and after 8 d incubation.

During the incubation process all cultures inoculated with 0.45 μm filtered seawater were swabbed to determine the dominant bacteria within cultures and thus establish changes in the composition of bacterial communities over time.

2.2. Incubation experiments – Hormosira banksii communities in Hormosira banksii-based media

Full details on the creation of *Hormosira banksii*-based culture media are presented in the Supplementary material.

200 mL of 0.2 μm filter-sterilised *H. banksii*-media was added to sterile 500 mL Erlenmeyer flasks under aseptic conditions (n = 4). Swabs taken from the surfaces of *H. banksii* were added to two cultures as a source of bacteria with the other cultures (n = 2) remaining as non-inoculated controls. Cultures were incubated under the same environmental conditions as described previously for 8 d. As per the previous section all cultures inoculated with bacteria from *H. banksii* surfaces were swabbed to determine the bacterial inhabitants of cultures to establish how the composition of bacterial communities changes over time.

Table 2
Bacteria genera present in seawater, DIFCO MB 2216 cultures exposed to arsenoribosides, *H. banksii* surfaces and within *H. banksii*-based media throughout arsenoriboside degradation experiments. (Further details on GenBank accession numbers, base pairs analysed, similarity (%) and total numbers of sequences analysed can be found in Supplementary Table 3).

Media/environment	Genera present
Seawater	Uncultured β proteobacterium
DIFCO MB 2216 cultures (day 0 – inoculation)	Uncultured bacterium *Vibrio* Uncultured β proteobacterium
DIFCO MB 2216 cultures (day 2 – arsenoriboside addition)	Uncultured bacterium *Vibrio*
DIFCO MB 2216 cultures (day 8 – experiment conclusion)	Uncultured bacterium *Allivibrio*
H. banksii surface	*Cellulophaga* *Octadecobacter* *Pseudoalteromonas* *Maribacter* *Alteromonas* Uncultured bacterium
H. banksii-based media (day 8 – experiment conclusion)	Uncultured bacterium *Marinomonas*

2.3. Analytical methods

Full details on the analytical methods used in this study are presented in the Supplementary material.

In brief, total arsenic concentrations in culture media were determined via ICP–MS as per Maher et al. (2001). Arsenic species in culture media were determined via HPLC–ICP–MS Foster et al. (2007), (2008) and Kirby et al. (2004). Total phosphorus (TP) and total nitrogen (TN) content of the various culture media were determined via alkaline peroxodisulfate digestion and analysed via flow injection analysis as per Maher et al. (2002). Bacteria present in environmental samples and cultures were identified via extraction of DNA from 16S rRNA region using the method published by Smith et al. (2005), with some modifications.

3. Results

3.1. Composition of culture media

The nutrient content of the growth media varied considerably (Table 1). DIFCO MB 2216 media contained far higher TP and TN concentrations than the *H. banksii*-based media (Table 1). In turn the *H. banksii*-based media contained higher TP and TN concentrations than were found in seawater (Table 1).

3.2. Dominant bacteria in cultures

25 Bacterial strains were identified across the different culture media and environments analysed (Supplementary Table 3). In the DIFCO MB 2216 incubation experiment uncultured β proteobacterium were the dominant bacteria present in seawater, whilst uncultured bacterium and *Vibrio* sp. were the dominant in culture media (Table 2).

A range of bacteria genera including *Cellulophaga, Octadecobacter, Pseudoalteromonas, Maribacter, Alteromonas* and uncultured bacterium were present on *H. banksii* surfaces (Table 2). After 8 d incubation in *H. banksii*-based media *Marinomonas* and uncultured bacterium were the only genera present (Table 2).

3.3. Arsenic species in culture media

All four arsenoriboside species (Gly/PO_4/SO_3/OSO_3) were detected in similar concentrations in both sterile and non-sterile DIFCO MB 2216 cultures after 8 d incubation (Fig. 1). These concentrations were very similar to those present at day two when arsenoribosides were added to the culture media (Fig. 1).

In the *H. banksii*-based media Gly^-, SO_3^- and PO_4^- riboside were present as a natural constituent of the media (Fig. 2). In both sterile and bacterially-inoculated cultures all three arsenoriboside species were present in similar concentrations to those present originally (Fig. 2).

4. Discussion and Conclusions

All four arsenoriboside species persisted in DIFCO MB 2216 cultures inoculated with seawater bacteria over the 8 d incubation period (Fig. 1). Although a proportion of arsenic was removed (≈23 μg L^{-1}) in inoculated cultures (presumably by bacteria) (Fig. 1), the persistence of arsenoribosides over this time-frame is unlike in previous work (Pengprecha et al., 2005) or as observed in nature (Francesconi and Kuehnelt, 2002). DMAE the major arsenoriboside degradation product was also not detected in any of the cultures within this experiment (Fig. 1). This suggests that processes occurring within the experiments are not representative