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Arsenobetaine-decomposing Ability of Marine Microorganisms Occurring in Particles Collected at Depths of 1100 and 3500 Meters

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The arsenobetaine-decomposing ability of microorganisms occurring in sinking particles, which play a main role in the vertical transport of organic substances produced in the photic zone, was investigated. The microorganisms in particles collected in the deep sea, 1100 and 3500 m in depth, clearly showed decomposing ability. With the particles from 1100 m, the degradation products were the same as those produced by microorganisms occurring in sources in the photic zone, i.e. trimethylarsine oxide (TMAO), dimethylarsinic acid (DMA) and inorganic arsenic(V). At 3500 m, the degradation activity was diminished, smalls amount of DMA and TMAO being produced. These results suggest that arsenobetaine contained in the animals starts to degrade immediately after the death of the animals and their transformation to particles. The degradation of arsenobetaine to inorganic arsenic in our tentative arsenic cycle in marine ecosystems (inorganic arsenic to inorganic arsenic via the biosynthesis of arsenobetaine) may apply to the deep sea as well as to the photic zone. © 1997 by John Wiley & Sons, Ltd.

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INTRODUCTION

Arsenobetaine is an arsenic compound isolated from the tail muscle of the western rock lobster, *Panulirus cygnus*, and identified as a naturally occurring organoarsenic compound.[1] This was a very important finding and prompted many workers to research in this field: various arsenic compounds and biotransformations were investigated in various marine organisms.[2, 3] A route for the synthesis of arsenobetaine from inorganic arsenic in seawater through the food chain has been suggested.[2] Recently the occurrence of arsenobetaine was reported in mushrooms.[4] This is very interesting, because the occurrence of arsenobetaine has been considered to be characteristic of marine animals; whether or not the presence of arsenobetaine in mushrooms is exceptional in terrestrial organisms will be determined in the future.

After the confirmation of the ubiquitous occurrence of arsenobetaine in marine animals, the fate of arsenobetaine in marine ecosystems aroused our interest. Thus, the degradation of arsenobetaine by microorganisms occurring in various sources, i.e. sediments,[5–9] macro-algae,[10] suspended substances[11] and mollusk intestine,[12] was investigated. Arsenobetaine was degraded in each of the sources, some of which completely or partly degraded it to inorganic arsenic. These results led us to the conclusion that there is an arsenic cycle in marine ecosystems:[3, 13] it begins with the methylation of inorganic arsenic and terminates with complete degradation, to inorganic arsenic via the biosynthesis of arsenobetaine. However, because all the microbial sources investigated so far were from photic

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zones, it is insufficient to apply this degradation or cycle to the whole ocean. In order to apply the degradation behavior of arsenobetaine to the whole ocean including the deep sea, it is necessary to consider sinking particles or settling matter as the source of the microorganisms, because they play the main role in the vertical transport of organic substances produced through the marine food chain in the photic zone.

In this study, we investigated the degradation of arsenobetaine by microorganisms occurring in particles collected by sediment traps moored at depths of 1 100 and 3 500 m: the particles were collected periodically at both depths, arsenobetaine-decomposing ability being evaluated for each period.

MATERIALS AND METHODS

Particles

The particles were collected with a sediment trap of the double-cone type, SMD9W-6000 (Nichiyu Gigen Co.), for two successive years. The trap was moored from June 1991 to May 1992 at a station about 50 km off Kushiro, Hokkaido, Japan, at a depth of 1100 m (42° 35–40′ N, 144° 55–65′ E) and from May 1992 to May 1993 at about 100 km off Kushiro at a depth of 3500 m (42° 01–05′ N, 145° 20–25′ E). It was set for eight (1100 m, 40 days each) or nine (3500 m, 39 days each) collecting periods, except for each of the last periods which were shortened to 26 (1100 m) or 35 days (3500 m). The collected particles were sent aseptically to our laboratory and subjected to the experiments.

Cultivation

Cultivation was performed using two culture media that were also used in our previous conversion experiments with arsenicals. These were 1/5 ZoBell 2216E ($\mathrm{g\,dm^{-3}}$ filtered seawater: peptone 1.0; yeast extracts 0.2, pH 7.5) and an aqueous solution of inorganic salts at pH 7.5 [$\mathrm{g\,dm^{-3}}$: sodium chloride (NaCl) 30.0; calcium chloride ($CaCl_2{\cdot}2H_2O$) 0.2; potassium chloride (KCl) 0.3; iron(II) chloride ($FeCl_2{\cdot}nH_2O$) 0.01; phosphates (KH_2PO_4) 0.5, (K_2HPO_4) 1.0; magnesium sulfate ($MgSO_4{\cdot}7H_2O$) 0.5; ammonium chloride (NH_4Cl) 1.0]. The latter has been used to force the microorganisms to use arsenobetaine as the only carbon source except for trace amounts of sources introduced by addition of the particles. Synthetic arsenobetaine (50 mg) and the particles (about 10 mg as dry matter) from each collecting period were added to each medium (20 $\mathrm{cm^3}$) and the mixtures were shaken at 25 °C for 130 days in the dark under an atmosphere of air. A ZoBell mixture and an inorganic salt mixture, autoclaved at 120 °C for 20 min after addition of arsenobetaine and the particles, served as control for these experiments. An aliquot (0.1 $\mathrm{cm^3}$) of each mixture was withdrawn at intervals of several days of incubation and diluted with 2.0 $\mathrm{cm^3}$ of water.

High-performance liquid chromatography

The arsenic compounds in the diluted media were fractionated by high-performance liquid chromatography (HPLC) (Tosoh Co. Ltd, CCP8000 series) using an ODS 120T column (4.6 mm × 250 mm; Tosoh Co. Ltd) together with authentic arsenic compounds with a mobile phase of 11.2 $\mathrm{mmol\,dm^{-3}}$ of sodium heptanesulfonate in water–acetonitrile–acetic acid (95:5:6, vol.; flow rate, 0.8 $\mathrm{cm^3\,min^{-1}}$; sample size, 5 $\mathrm{mm^3}$.[14] A portion of 20 or 50 $\mathrm{mm^3}$ of each eluate collected for every 25 s was injected into a graphite furnace atomic absorption spectrometer (GFAA) and analyzed as described previously.[7] Mixtures of the authentic arsenic compounds, which have been detected in previous *in vitro* degradation experiments of arsenobetaine, were also fractionated [retention times, s: inorganic arsenic(III), 225–300; inorganic arsenic(V), 150–225; methanearsonic acid (MMA), 225–300; dimethylarsinic acid (DMA), 325–400; arsenobetaine, 525–625; trimethylarsine oxide (TMAO), 725–850; tetramethylarsonium iodide, 1125–1275].

Extraction and purification of arsenic compounds from microorganisms

Each medium containing the arsenicals as microbial conversion products from arsenobetaine was centrifuged for 15 min at 4000 rpm, and the supernatant was subjected to column chromatography to purify each metabolite: it was applied to a cation-exchange resin, Dowex 50W–×8 (50–100 mesh, H^+ form) column (2.2 cm × 18.5 cm), and eluted with 600 $\mathrm{cm^3}$ of water, 600 $\mathrm{cm^3}$ of 2.0 $\mathrm{mol\,dm^{-3}}$ pyridine and

600 cm^3 of 1.0 mol dm^{-3} HCl, successively. The arsenic-containing fraction was further applied to a cation-exchange resin, Dowex 50W-2 (200–400 mesh, pyridinium form) column (1 cm × 50 cm) equilibrated with 0.1 mol dm^{-3} pyridine–formic acid buffer (pH 3.1) and eluted with the same buffer (200 cm^3) and 0.1 mol dm^{-3} pyridine (200 cm^3).

Confirmation of the metabolite

Thin-layer chromatography (TLC) was performed on cellulose thin layers (Avicel SF, thickness 0.1 mm, Funakoshi Yakuhin Co. Ltd). Iodine vapor, $SnCl_2$–KI reagent[15] and Dragendorff reagent were used to indicate the position of the metabolites or the authentic arsenic compounds. Fast atom bombardment (FAB) mass spectrometry was performed with a JEOL JMS DX-300 mass spectrometer equipped with an FAB ion source and xenon atoms at 6 keV.

RESULTS

Particles from 1100 m

Although no metabolite was detected in the inorganic salt medium over any of the periods, a few species of metabolites were derived in the ZoBell medium from five collecting periods out of nine. As shown in Fig. 1, the relative extents of degradation particles collected during these periods were, in decreasing order, as follows (dates are abbreviated in the US style, e.g. [9/29–11/7] denotes 29 September to 7 November.

$$[9/29\text{–}11/7] > [12/18\text{–}1/26] \gg [11/8\text{–}12/17] > [4/15\text{–}5/11] > [7/11\text{–}8/19]$$

Arsenobetaine was most degraded by the particles collected in [9/29–11/7]. In this period, about 80% of the arsenobetaine was degraded, the detected metabolites being TMAO and a small amount of DMA. In [12/18–1/26], in which the activity of the particles follows that from [9/29–11/7], inorganic arsenic(V) was detected as a complete degradation product of arsenobetaine besides TMAO and DMA. In the other three periods, the degradation of arsenobetaine was low compared with the two periods mentioned above; only about 20% of the arsenobetaine was degraded in them. DMA was detected in each of three periods and TMAO was also detected in [11/8–12/17] and [4/15–5/11]. Thus, DMA was derived by the microorganisms occurring in the particles from every period during which the arsenobetaine-decomposing ability was demonstrated.

Particles from 3500 m

Although the extent of production of metabolites was much smaller than that with particles from 1100 m, degradation of arsenobetaine was also observed with particles from a total of five collecting periods at 3500 m (Fig. 2). They are [5/22–6/29] in the ZoBell medium and [8/8–9/22], [9/23–10/31], [11/1–12/9] and [12/10–1/17] in the inorganic salt medium. The relative extents of their degradation were in the following order:

$$\begin{aligned}[5/22\text{–}6/29] > [8/8\text{–}9/22] &= [9/23\text{–}10/31]\\ &= [11/1\text{–}12/9]\\ &= [12/10\text{–}1/17]\end{aligned}$$

During the period [5/22–6/29], in which the highest degradation activity was shown, 10–20% of the arsenobetaine was degraded to DMA as sole metabolite. The formation of DMA here was rather an exception as, for the particles from 3500 m, TMAO was derived as the sole metabolite in small amounts in four other periods.

Confirmation of the metabolites

The structures of the metabolites formed in the ZoBell media amended with the particles from 1100 m were confirmed as stated below. Confirmation of the metabolites formed by microorganisms in the particles from 3500 m was omitted because their amounts were small and the specifications were in common with the metabolites from 1100 m. One metabolite whose retention time agreed with TMAO was purified from [9/29–11/7]; two metabolites agreeing with DMA and inorganic arsenic(V) were purified from [12/18–1/26]. The three purified metabolites were chromatographed on cellulose thin layers together with authentic arsenic compounds. The R_f value of each purified metabolite agreed with that of the corresponding authentic arsenic compound in five solvent systems. Figure 3 shows the FAB mass spectra of synthetic TMAO and the metabolite whose HPLC retention time agreed with TMAO. Both are essentially the same, showing peaks at $m/z = 137$

$(M+1)^+$, $m/z=273$ $(2M+1)^+$, and $m/z=409$ $(3M+1)^+$. Besides these peaks, adduct ions, $m/z=159$ $(M+\mathrm{Na})^+$, $m/z=175$ $(M+\mathrm{K})^+$, $m/z=295$ $(2M+\mathrm{Na})^+$, $m/z=311$ $(2M+\mathrm{K})^+$, and $m/z=431$ $(3M+\mathrm{Na})^+$, were also present in the spectrum of the purified metabolite.

DISCUSSION

In the particles collected in the deep sea (1100 and 3500 m depths), the occurrence of microorganisms that have the ability to degrade arsenobetaine was clearly demonstrated in this study. In some mixtures amended with particles from 1100 m, the metabolites derived from arsenobetaine were the same as those produced by microorganisms occurring in various sources in the photic zone or the surface area of the sea, i.e. sediments, suspended substances and so on: TMAO, DMA and inorganic arsenic(V) were confirmed as the metabolites. Thus, we can postulate in the sea the ubiquitous occurrence of arsenobetaine-decomposing microorganisms that show analogous degradation behavior. The degradation of arsenobetaine to inorganic arsenic in

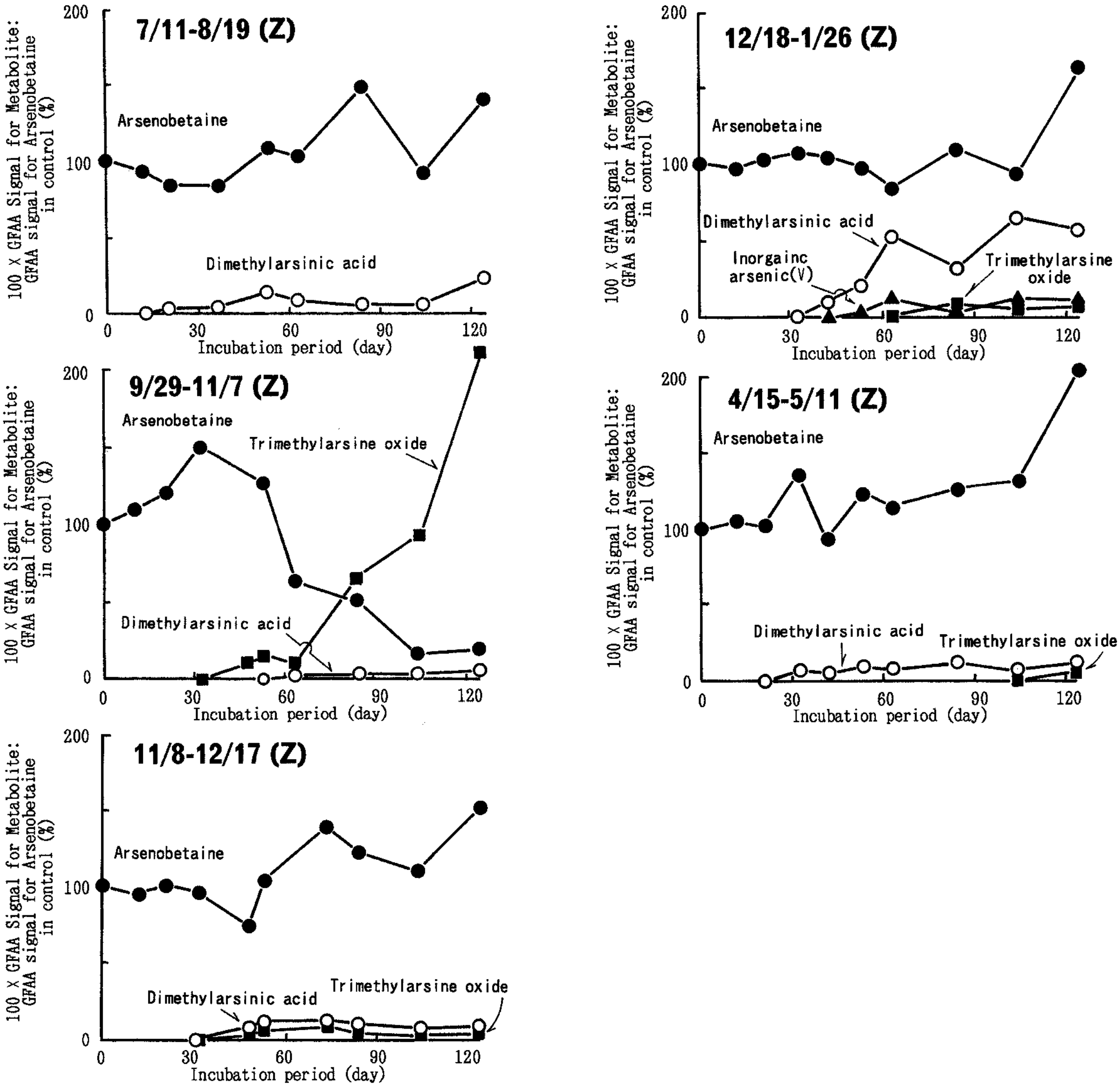

Figure 1 The degradation of arsenobetaine by microorganisms occurring in particles collected at a depth of 1100 m.

our tentative arsenic cycle in marine ecosystems (from inorganic arsenic to arsenobetaine and arsenobetaine to inorganic arsenic)[3, 13] is now suggested to be applicable to the deep sea as well as to the photic zone.

Taking into account that the particles used in this study were so-called sinking particles from the photic zone, their degradation ability suggested that if arsenobetaine remains in the particles, it is degraded to inorganic arsenic before and/or after they reach the sea bottom. Arsenobetaine, or at least a part of it, contained in the animals may be subjected to degradation on the route to inorganic arsenic. The degradation is considered to start immediately after the death of the animals and their transformation to particles.

The activity, however, was diminished with an increase in depth. In [5/22–6/29] from 3500 m, the period that showed the highest degradation activity at 3500 m, DMA was detected as sole metabolite. This result is the same as that with the two strains of bacteria isolated as arsenobetaine-decomposing bacteria. They aerobically degrade a part of the arsenobetaine to DMA in both the media (to a much greater extent in the ZoBell medium than in the inorganic salt medium).[16] From this limited degradation behav-

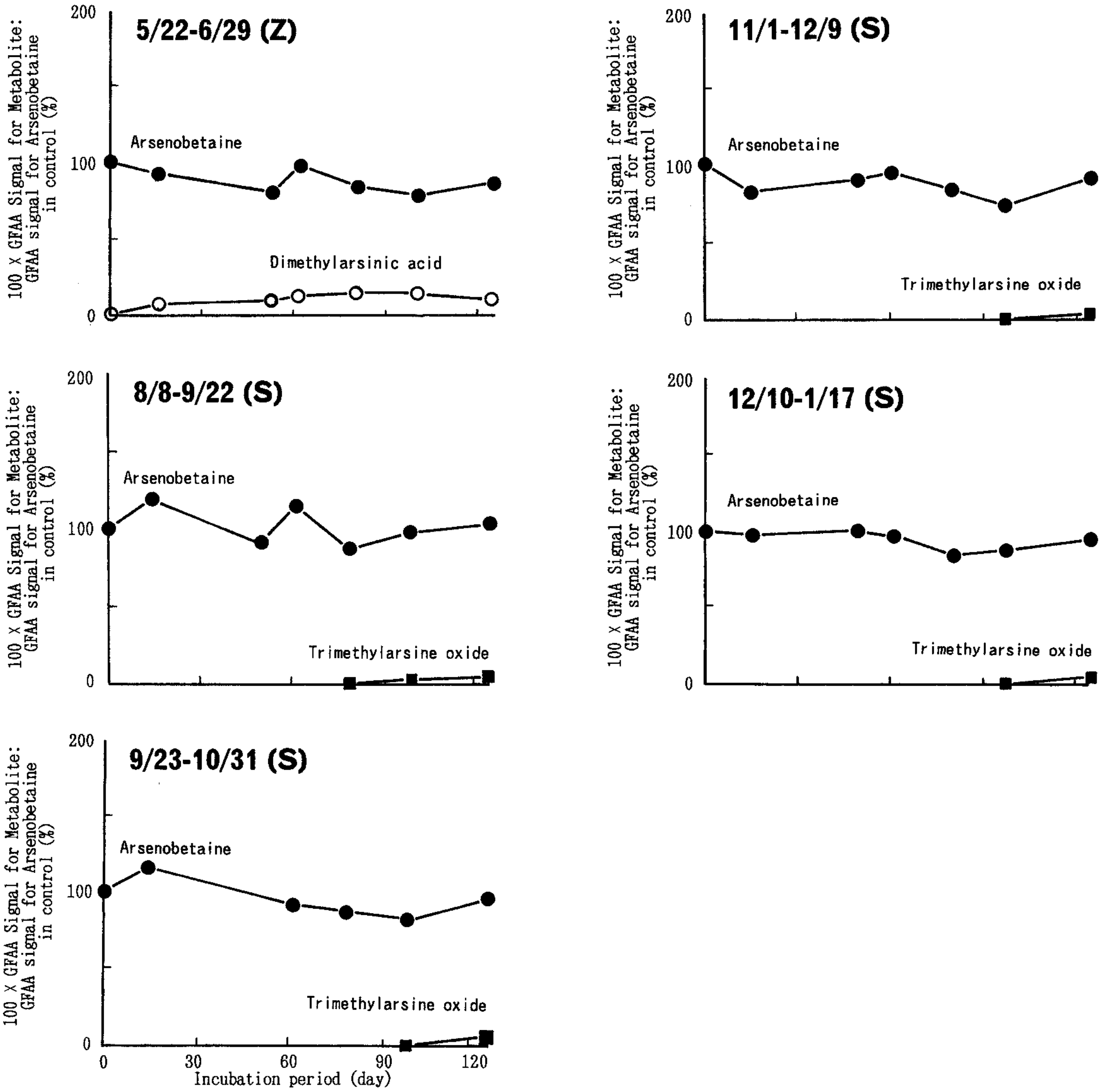

Figure 2 The degradation of arsenobetaine by microorganisms occurring in particles collected at a depth of 3500 m.

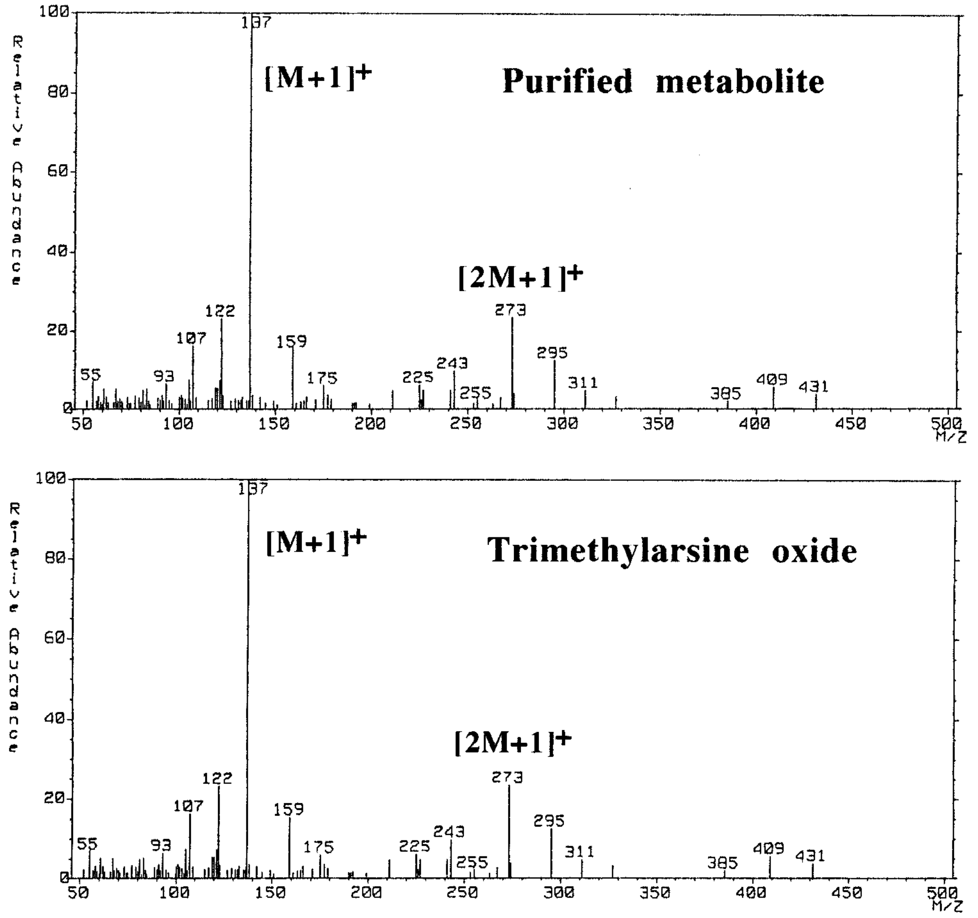

Figure 3 FAB mass spectra of the purified metabolite (upper panel) and synthetic trimethylarsine oxide (lower panel).

ior, we attribute the complete degradation of arsenobetaine to inorganic arsenic to the cooperative action of various microorganisms, including the bacteria isolated by us. From this point of view, the presence of DMA as sole metabolite in the [5/22–6.29] sample from 3500 m may be explained by the scarcity of the species of microorganisms that could participate in the degradation of arsenobetaine. Although the microbial degradation of arsenobetaine is ubiquitous, its rate seems to be considerably lower in the deeper sea.

In the inorganic salt media, no conversion occurred with particles from 1100 m. This indicates that the microorganisms require organic matter in order to show activity to degrade arsenobetaine. Further discussion, however, is very difficult at this stage, because although the activity was very low, the microorganisms from 3500 m degraded arsenobetaine in the inorganic salt medium from four collecting periods out of the five periods that showed the activity. Whether or not the occurrence of abundant organic matter is favorable for the microorganisms to degrade arsenobetaine may be depend on the flora of the microorganisms occurring in the particles.

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