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SHORT PAPER

Conversion of arsenobetaine to dimethylarsinic acid by arsenobetaine-decomposing bacteria isolated from coastal sediment

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Arsenobetain [$(CH_3)_3As^+CH_2COO^-$]-containing growth media (1/5 ZoBell 2216E and a solution of inorganic salts) were inoculated with two bacterial strains, which were isolated from a coastal sediment and identified as members of the *Vibrio–Aeromonas* group, and incubated under aerobic and anaerobic conditions. Arsenobetaine was converted to a metabolite only under aerobic conditions. This arsenic metabolite was identified as dimethylarsinic acid [$(CH_3)_2AsOOH$] by hydride generation/cold trap/GC MS/SIM analysis and high-performance liquid-chromatographic behaviour. The conversion pattern shown by these arsenobetaine-decomposing bacteria (that is, arsenobetaine → dimethylarsinic acid) was fairly different from that shown by the addition of sediment itself as the source of arsenobetaine-decomposing micro-organisms (that is, arsenobetaine → trimethylarsine oxide → inorganic arsenic). This result suggests to us that various micro-organisms, including the arsenobetaine-decomposing bacteria isolated in this study, participate in the degradation of arsenobetaine in marine environments.

Keywords: Arsonobetaine, dimethylarsinic acid, degradation, bacteria, micro-organisms, sediment

INTRODUCTION

In recent years, we have dealt with the microbial degradation of arsenobetaine, an organoarsenic compound, which was reported by Edmonds *et al.* for the first time in 1977,[1] and which is ubiquitous in marine animals. Coastal sediments[2–5] and marine macro algae[6] have been investigated as a possible source of arsenobetaine-decomposing micro-organisms so far. As for the results, we have tentatively concluded that there is a marine arsenic cycle that begins with the methylation of inorganic arsenic on the way to arsenobetaine and terminates with the complete microbial degradation of arsenobetaine to inorganic arsenic.[4,7] Higher degradation activity was shown by sedimentary micro-organisms. To clarify further the role of microbial degradation of arsenobetaine in a sediment, it is essential to examine the degradation of this compound by isolated micro-organisms. We isolated arsenobetaine-decomposing bacteria using enrichment culture methods and confirm their ability to degrade arsenobetaine. Evidence for the presence of bacteria which decompose arsenobetaine is presented for the first time in this paper.

MATERIALS AND METHODS

Culture media

Two culture media which have been used previously in degradation experiments[2–7] were used also in this study. These are 1/5 ZoBell 2216E [as $g\,dm^{-3}$ filtered seawater: peptone 1.0; yeast extract 0.2, pH 7.5] and an aqueous solution of inorganic salts at pH 7.5 [as $g\,dm^{-3}$: sodium chloride (NaCl) 30.0; calcium chloride ($CaCl_2.2H_2O$) 0.2; potassium chloride (KCl) 0.3; iron(II) chloride ($FeCl_2.nH_2O$) 0.01; phosphates (KH_2PO_4) 0.5 and (K_2HPO_4) 1.0; magnesium sulphate ($MgSO_4.7H_2O$) 0.5; and ammonium chloride (NH_4Cl) 1.0].

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Isolation of arsenobetaine-decomposing micro-organisms

Each medium (25 cm^3) containing synthetic arsenobetaine [$(CH_3)_3As^+CH_2COO^-$, 50 mg] was mixed with sediment (1 g) collected from coastal waters of Yoshimi, Japan, in a 50-cm^3 Erlenmeyer flask. The flasks were shaken aerobically in the dark at 25 °C for several days.

Two bacterial strains were isolated from the inorganic medium and several from the ZoBell medium using the enrichment culture method. The two strains from the inorganic medium (which contained no organic carbon except arsenobetaine) were identified as members of the *Vibrio–Aeromonas* group[8] by means of biochemical reactions and morphological characteristics.

Conversion of arsenobetaine by the arsenobetaine-decomposing bacteria

Each medium (25 cm^3) containing synthetic arsenobetaine (50 mg) was inoculated with the isolated bacterial strains in the flask. The flasks were shaken at 25 °C in the same way as above. For anaerobic incubation, abut 5 cm^3 of liquid paraffin was placed on the surface of each mixture. In order to examine the effect of trace amounts of nutrients occurring in the sediment, a mixture with added sterilized sediment was also incubated. Mixtures autoclaved at 120 °C for 20 min served as controls. Filtered aliquots from the mixtures in the flasks were withdrawn over intervals of several days and diluted with distilled water to 20 times their volumes. The arsenic compounds in the diluted aliquots were fractionated by high-performance liquid chromatography (HPLC).

High-performance liquid chromatography

The arsenic compounds in the diluted media samples were separated on a high-performance liquid chromatograph (TOSOH Co., CCP 8000 series, TSK Gel ODS-120T column, 4.6 mm × 250 mm) with a 11.2 mmol dm^{-3} solution of sodium heptanesulphonate in water/acetonitrile/acetic acid (95 : 5 : 6 by volume)[9] as the mobile phase at a flow rate of 0.8 cm^3 min^{-1}. Fractions were collected and an aliquot (20 mm^3) of each fraction was injected into the graphite furnace atomic absorption spectrometer (GF AA) as described previously.[4]

Purification and identification of the metabolite

The mixture (consisting of ZoBell medium, arsenobetaine and isolated bacterial strains) incubated for 111 days was centrifuged and the supernatant was applied to a Dowex 50W-X2 (200–400 mesh) 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. The purified metabolite was characterized by a combination of gas-chromatographic separation with hydride generation followed by a cold-trap technique and selected ion monitor mass spectrometric analysis (hydride generation/cold trap/GC MS/SIM).[10]

RESULTS

Conversion of arsenobetaine by arsenobetaine-decomposing bacteria

Figure 1 shows the time-course pattern of arsenobetaine and its metabolite in the mixtures. Throughout the experiments, only one kind of metabolite was detected, of which the retention time was the same as that of dimethylarsinic acid. In the ZoBell medium, this metabolite appeared after seven days of incubation independently of the presence of sterilized sediment [Fig. 1(c) and (d)], although the recovery of the metabolite was slightly greater in the presence than in the absence of the sediment. In its presence 22–24% of arsonobetaine, and in its absence 10–20% of arsenobetaine, was converted to the metabolite. The metabolite, however, showed little increase throughout the incubation period after 20 days either in the presence or in the absence of the sediment. On the other hand, in the inorganic medium, only a little metabolite appeared after 96 days and only in the presence of sterilized sediment [Fig. 1(a)].

Under anaerobic conditions, only a trace amount of the metabolite, of which the retention time also agreed with that of dimethylarsinic acid, was derived after 40 days (inorganic salt medium with or without the sterilized sediment) or 60 days

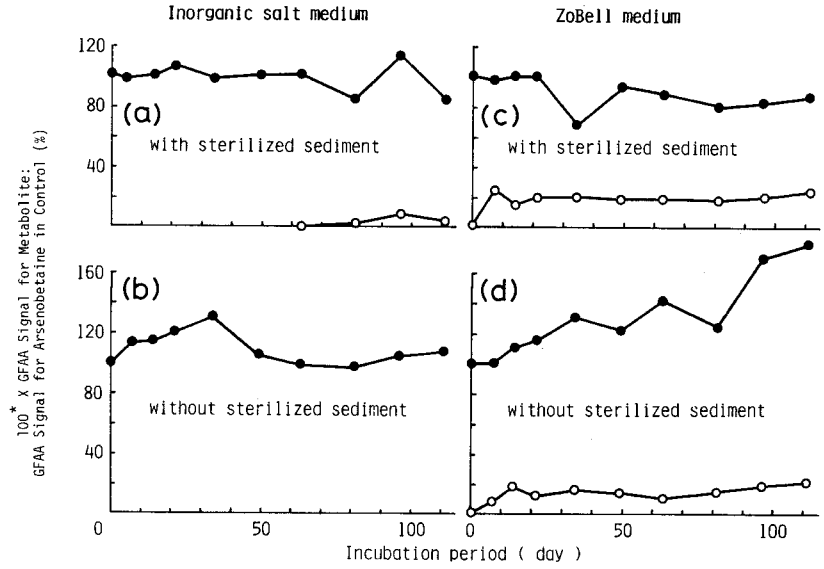

Figure 1 The conversion of arsenobetaine, under aerobic conditions, to a metabolite whose HPLC-retention time agreed with that of dimethylarsinic acid, in a ZoBell medium and in an inorganic salts medium inoculated with two isolated bacterial strains. In (d) the level of arsenobetaine itself increased considerably from its initial value probably as a result of a larger extent of evaporation of this medium in a flask compared with a control. ●, Arsenobetaine; ○, metabolite.

(inorganic salt medium with the sediment, and ZoBell medium with or without the sediment) of incubation.

Isolation of the metabolite

The supernatant from the ZoBell medium containing the sterilized sediment was applied to cation-exchange chromatography (Dowex 50W-X2) after 111 days of aerobic incubation. The metabolite was eluted with 0.1 mol dm^{-3} pyridine–formic acid buffer with a slightly smaller retention time than that of arsenobetaine. In order to separate the metabolite from arsenobetaine or the salts added to the medium, this chromatography was used repeatedly. The arsenic-containing fractions were pooled and freeze-dried to yield a white crystalline powder.

Identification of the metabolite

The purified metabolite from the ZoBell medium was subjected to the hydride generation/cold-trap/GC MS/SIM analysis. Only dimethylarsine was detected with this analysis. On the basis of this result and the high-performance liquid-chromatographic behaviour, this metabolite was confirmed as dimethylarsinic acid.

DISCUSSION

Arsenobetaine was shown to be decomposed by two isolates of the arsenobetaine-decomposing bacteria in aerobic conditions in the present study. We reported previously[3] that the isolated bacterial strains did not metabolize arsenobetaine. The lower concentration of the metabolite misled us as above about their ability to decompose arsenobetain. The derived arsenical however, was dimethylarsinic acid alone. This result was contrary to our expectations. We had intended to isolate bacteria which have the ability to convert arsenobetaine to fully degraded compounds such as inorganic arsenic. Actually, we recently proved the complete degradation of arsenobetaine to inorganic arsenic by sedimentary micro-organisms.[7] The usual conversion pattern by sedimentary micro-organisms may be arsenobetaine → trimethylarsine oxide → inorganic arsenic.[7] With micro-organisms associated with marine macro-algae, trimethylarsine oxide was derived as well as dimethylarsinic acid.[6] We conclude that various micro-organisms, including the isolated bacteria in this study, participate in the conversion of arsenobetaine to inorganic arsenic.

The conversion of arsenobetaine to dimethylarsinic acid was significant in ZoBell medium but

little observed in the inorganic salt medium. That is, the arsenobetaine-decomposing bacteria in this study needed a carbon source other than arsenobetaine to show their activity. However, this is not always the case in those experiments performed with sediments as a source of micro-organisms.[2–5] As for the rate or extent of the conversion of arsenobetaine, we have considered the situation as follows: it depends on the flora or the number of micro-organisms introduced by addition of materials such as sediments rather than the presence of abundant carbon sources other than arsenobetaine in the medium, as previously pointed out.[6] There may be not only arsenobetaine-decomposing micro-organisms which need a carbon source other than arsenobetaine, but also those which do not need it. The effect of a trace amount of nutrients occurring in the sterilized sediment was also shown in both media, even if it was relatively small. This fact suggests that there is a factor in the sediment to increase the degradation activity besides those mentioned above. Little is known about it at the present stage, however.

Under anaerobic conditions, only a trace amount of demethylarsinic acid was derived. This minor conversion of arseobetaine probably depended on a small amount of residual oxygen which was unavoidable under the anaerobic conditions used in this study. The present result that the decomposition of arsenobetaine is shown only in aerobic conditions agreed with that obtained from the degradation experiment performed with sediment.[5] It is reasonable that the carboxymethyl moiety in arsenobetaine is utilized under aerobic conditions when one takes account of its possible utilization in aerobic pathways such as the tricarboxylic acid (TCA) cycle.

CONCLUSION

Arsenobetaine was degraded to dimethylarsinic acid by two arsenobetaine-decomposing bacterial strains isolated from the sediment. They were identified as members of the *Vibrio–Aeromonas* group. This degradation was observed only under aerobic conditions in the 1/5 ZoBell 2216E medium, little degradation being observed under anaerobic conditions. The present conversion pattern, i.e. arsenobetaine → dimethylarsinic acid, was fairly different from that shown by the addition of the sediment as the source of micro-organisms, suggesting that various micro-organisms participate in the degradation of arsenobetaine.

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