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Gram-negative bacteria responsible for insoluble technetium formation and the fate of insoluble Tc in the water column above flooded paddy soil

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Abstract

We studied the characteristic gram-stain of Tc insolubilizing bacteria using various antibiotics, and the fate of insoluble Tc in a water column above flooded paddy soil to clarify Tc behavior in paddy fields. The formation of insoluble Tc in water column samples was inhibited by the addition of antibiotics, especially reagents against gram-negative bacteria. For a sample without antibiotics, insoluble Tc formation increased with time, and the maximum amount of insoluble Tc was observed on day 4 of incubation with ^{95m}Tc. In contrast, concentrations of ferrous ion decreased with time. These results suggested that gram-negative bacteria were mainly responsible for insoluble Tc formation, and that these bacteria were able to transform soluble Tc to insoluble forms under oxidizing conditions.

Keywords: Gram-negative bacteria; Insoluble technetium; Water column; Paddy field

1. Introduction

There are several known isotopes of technetium with mass number from 92 to 107, and all are radioactive. Among its radioisotopes, ^{99}Tc is the most environmentally significant isotope because of its importance in the long-term storage plans for nuclear waste. This radionuclide is produced mainly by human activities involving fissionable materials and its yield from fission of ^{235}U and ^{239}Pu is about 6%, making ^{99}Tc relatively high in abundance among fission products. In addition to the large quantities which may be present, the half-life of ^{99}Tc is very long (2.1×10^5 years). Thus the total amount of ^{99}Tc worldwide will increase. Release of ^{99}Tc to the environment will also increase in relation to geological disposal of radioactive waste. Once ^{99}Tc is released to the environment, it will persist there for long periods. Therefore, prediction of the behavior and fate of ^{99}Tc in the environment is desirable.

The environmental behavior of ^{99}Tc is probably linked to microbial activity (Landa et al., 1977; Tagami and Uchida, 1996) and the association of Tc with

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anaerobic bacteria is well documented. Henrot (1989) examined the potential for soil bacteria to affect Tc solubility, and showed that anaerobically but not aerobically grown soil bacteria accumulated Tc effectively. Lloyd and Macaskie (1996) demonstrated direct enzymatic reduction of TcO_4^- by resting cells of the dissimilatory metal-reducing bacteria, *Geobacter metallireducens* and *Shewanella putrefaciens*. Sulfate-reducing bacteria can also reduce TcO_4^- enzymatically (Lloyd et al., 1998; De Luca et al., 2001). Although there are detailed studies on Tc reduction mechanisms by those isolated bacteria, reports on the effects of environmental bacteria on Tc behavior are still limited.

Recently, it was reported that soluble TcO_4^- was changed to some insoluble forms in the water column above upland and paddy fields (Ishii et al., 2004a) and the insoluble Tc formation was caused by bacteria (Ishii and Tagami, 2003; Ishii et al., 2004b). The Tc insolubilizing bacteria, however, have not been identified. Insoluble Tc formation influences the transfer of Tc to plants. Technetium is normally present in the heptavalent form as pertechnetate (TcO_4^-), which is soluble and mobile in the environment, and is readily available to plants (Wildung et al., 1977). Especially in the case of paddy fields, the uptake of Tc by rice is a serious issue in the transfer of radioactive Tc to humans through rice. In contrast to soluble TcO_4^-, insoluble Tc such as Tc(IV) is unavailable to rice (Yanagisawa and Muramatsu, 1995).

The physicochemical conditions of flooded paddy fields are not continuously kept constant (Takai, 1984). At first, molecular oxygen is consumed by aerobic microbes. This is followed by aerobic respiration, and nitrates, oxides and hydroxides of manganese and ferric irons are reduced by facultative anaerobes. Finally, hydrogen sulfide and methane are formed by obligate anaerobes such as sulfate-reducing bacteria and methane-forming bacteria. These changes in chemical and biological status may decide the time at which Tc insolubilizing bacteria appear and the rate of insoluble Tc formation in the water column above paddy soil.

Our aims in this study were to determine the characteristic gram-stain of Tc insolubilizing bacteria and to examine both the time of their appearance in the water column above the paddy soil and the time-course of insoluble Tc formation in the water column.

2. Materials and methods

2.1. Materials and flooded paddy soil

Antibiotics listed in Table 1 were used for the determination of the characteristic gram-stain of Tc insolubilizing bacteria. These antibiotics were divided into three groups as follows: (1) effective to gram-positive bacteria (BC and PG), (2) effective to gram-negative bacteria (PL-B and SM), and (3) effective to both gram-negative and positive bacteria (CP). All antibiotics except CP were dissolved in deionized water; CP was dissolved in ethanol. These stock solutions (each $10\ mg\ ml^{-1}$) were sterilized by passing them through a 0.2-μm-pore-size filter. The filtrate was therefore bacteria-free. Sodium molybdate was used as a specific inhibitor of sulfate reducers (Pedrós-Alió et al., 1993). This reagent was dissolved in deionized water and the resulting solution was sterilized by passing it through a 0.2-μm-pore-size filter.

Carrier-free $^{95m}TcO_4^-$ was prepared in deionized water by the method of Sekine et al. (1999). The solution of the radioactive tracer was sterilized by passing it through a 0.2-μm-pore-size filter.

Paddy soil was collected from Koriyama City, Japan and was classified as Fluvisol (FAO, 1990). The soil was air-dried and passed through a 2-mm-mesh-sieve. The air-dried soil was flooded with 0.3% glucose water in a ratio of 1:1.5 (wt/vol) and then incubated statically at 25 °C under 12 h light and dark cycles for 7 days.

2.2. Experiment 1: characteristics of Tc insolubilizing bacteria

The experimental procedure is shown in Fig. 1. A water column sample was collected from the flooded paddy soil on day 7 after the flooding of the paddy soil,

Table 1
Antibiotics and their characteristics

Antibiotics	Abbreviation	Spectrum	Mode of action	Reference
Bacitracin	BC	Gram-positive bacteria	Inhibits bacterial cell wall synthesis	Robert and Adenis (2001)
Penicillin G	PG	Gram-positive bacteria, gram-negative cocci	Inhibits bacterial cell wall synthesis	Nathwani and Wood (1993)
Polymixin B	PL-B	Gram-negative bacteria	Damages cytoplasmic membranes	Robert and Adenis (2001)
Streptomycin	SM	Gram-negative bacteria, gram-positive bacteria	Inhibits protein synthesis	Hawk et al. (1947)
Chloramphenicol	CP	Broad (gram-positive and -negative bacteria)	Inhibits protein synthesis	Hawk et al. (1947)