Volatilization of mercury from natural water by a broad-spectrum Hg-resistant *Bacillus pasteurii* strain DR2


Summary

A broad-spectrum mercury-resistant bacterial strain was isolated from contaminated water and was identified as *Bacillus pasteurii* strain DR2. It could volatilize Hg-compounds including organo-mercurials from its growth media. It utilized several aromatic compounds as a sole source of carbon. The bacterial strain eliminated HgCl$_2$ from sterile river water and the presence of benzene, toluene, naphthalene and nitrobenzene at 1 mM concentration in the system increased the rate of mercury volatilization, the volatilization rate being highest with benzene. When $1.7 \times 10^7$ cells of this bacterial strain were added per ml of non-sterile water the bacterial strain volatilized more than 90 percent of mercury from mercuric chloride and organo-mercurials like PMA, thiomersol and methoxy ethyl mercuric chloride (MEMC). In the absence of this bacterial strain the volatilization of PMA and MEMC due to the presence of other Hg-resistant organisms in non-sterile polluted water ranged between 20-25 percent and of HgCl$_2$ was about 40 percent. However, in the presence of *B. pasteurii* DR2 volatilization of these Hg-compounds from non-sterile water increased by 20-40 percent. In the presence of 1 mM benzene the rate of mercury volatilization was even higher. In all the cases the rate of volatilization was higher in the first seven days than in the next seven days.

Introduction

The presence of mercury in the fat of penguins and in glacial ice from Antarctica indicates that pollution by this heavy metal is a global phenomenon. Mercury compounds are often used in cultivation as seed-dressers and pesticides, and in hospitals as disinfectants (Summers and Silver, 1978). Mercury finds applications in the paper, pulp and chloro-alkali industries. Mercury is also released into the environment during burning of fossil fuels, mining, painting and sewage treatment (Mitra, 1986). Its alloys are used for several chemical purposes. Several thousand million tones of mercury are dispersed in the environment every year (Summers and Silver, 1978). Rainwater washes mercury from the environment and also from soils and rocks. Associated with water bodies, sediments are the richest depot of mercury compounds (Summers and Silver, 1978; Mitra, 1986). These compounds are mutagenic and teratogenic (Summers and Silver, 1978; Mix, 1986).

In recent years, it has been widely accepted that hydrophobic organic chemicals have contaminated numerous aquifers. Gasoline from underground storage tanks, petroleum products from refineries, and chlorinated solvents from cleaning processes in various industries are examples of groundwater contamination (Hwang et al., 1993). Use, transportation, storage, and disposal of pesticides and hazardous chemicals are all increasing this chance of contamination. Additionally, there is always the risk of new contamination from accidental spillage and discharges. These compounds are often carcinogenic and enhance mutagenicity (Sato et al., 1983; Mix, 1986). It is apparent that both mercury compounds and hydrophobic aromatic compounds have already contaminated several aquatic bodies threatening the existence of aquatic animals (Cocchieri et al., 1993). All these pollutants are affecting survival, growth and reproduction of aquatic organisms.
Volutilization of Mercury from natural water

The current and potential damage to water bodies demands that attention be directed to developing methods for removing these contaminants from aquifers.

Several gram-positive and gram-negative bacteria can effectively detoxify mercury compounds through the sequential action of organo-mercurial lyase and mercuric reductase as reviewed by Summers and Silver (1978). In many cases bacterial resistance to mercury compounds is plasmid mediated (Schottel et al., 1974; Silver and Misra, 1988). Aromatic compounds are also degraded and utilized as sole sources of carbon by different plasmid-bearing strains (Friello and Chakrabarty, 1976; Kiyohara et al., 1983; Dong et al., 1992). Exploitation of microorganisms to scavenge these pollutants has been suggested in many studies (Leshniowsky et al., 1970; Summers and Silver, 1978; Bury and Miller, 1993). Bio-remediation may involve the ability of the in situ microorganisms to utilize any particular waste or the application of microorganisms adapted to degrade typical combinations of wastes including organic pollutants and mercury contaminants.

A broad-spectrum Hg-resistant Bacillus pasteurii strain DR2 that volatilized Hg-compounds including organo-mercurials from its growth media and utilized different aromatic compounds as a sole source of carbon has been isolated (Pahan et al., 1990a; Pahan et al., 1991a). This strain can also degrade and utilize fluorescein, mercuric acetate and merbromine (Pahan et al., 1992). Its growth was also stimulated in the presence of benzene and phenylmercuric acetate (Pahan et al., 1993a). The aim of the present study was to utilize the dual characteristics of the organism, elimination of Hg compounds and utilization of aromatic compounds, in natural conditions. Here, the increased rate of biodegradation and elimination of mercury compounds by this strain from natural water in the presence of organic compounds is reported.

Materials and methods

All chemicals and reagents used in this study were of analytical grade (E. Merck, UK). Phenylmercuric acetate (PMA) was from Sigma Chemical Co., St. Louis, USA. Methoxyethyl mercuric chloride (MEMC) was purchased from a local market as Emisan-6, a mercury based pesticide containing 6 percent (w/w) mercury as MEMC. Water samples were collected from Kalighat Nala, flowing through the heart of Calcutta, India. This water body is highly contaminated as is evident from its high BOD and COD values and reported to contain 0.26 ± 0.02 ppm mercury (Pahan et al., 1990b).

Bacterial strain B. pasteurii DR2 was grown in nutrient broth media containing 10 μM HgCl₂ and 30 μM benzene. HgCl₂ was added to induce mercuric reductase and organo-mercurial lyase (Pahan et al., 1990a) and benzene was added to stimulate its growth (Pahan et al., 1993a). Flasks were kept overnight in a rotary shaker at 200 rpm at 32°C. This bacterial culture was diluted 1:100 with sterile river water to a total volume of 200 ml to maintain approximately 1.7 × 10⁷ bacterial cells/ml. After sterilization to the river water samples, solutions of benzene and petroleum ether (boiling range 60–80°C) were added to separate flasks to a final concentration of 1 mM. Solutions of these aromatic compounds were made in 90 percent ethanol and these solutions were not sterilized. The water samples were sterilized in an autoclave after cotton plugging. Control flasks containing only the organisms were also run. To some flasks HgCl₂ was added to a concentration of 100 μM and PMA to 30 μM. Similar experiments were also run in distilled water using only benzene. All the flasks were kept in a BOD incubator at 20°C. At intervals of several days suitable portions of these water samples were taken out aseptically and diluted serially with sterile distilled water. Bacterial counts were determined by spreading the definite volumes of the diluted bacterial suspension onto nutrient agar. An average of six separate determinations was taken in each case.

To study the volatilization of mercury compounds, bacterial cultures induced with 10 μM HgCl₂ were diluted 1:100 similarly with sterile river water, and HgCl₂ was added to a concentration of 100 μM. Benzene, toluene, naphthalene and nitrobenzene were added separately to each sterile flask at 1 mM concentration. Control flasks were also set up; one flask contained only the organism and HgCl₂ and another to which only HgCl₂ was added. In similar experiments, HgCl₂-induced bacterial cultures were diluted 1:100 with non-sterile river water containing 1 mM benzene and 100 μM HgCl₂ or 30 μM PMA or 30 μM MEMC. Respective control flasks were also run. All the flasks were incubated at 20°C and mercury content was measured following the cold vapor atomic absorption spectrometry (Bradenberger and Bader, 1967; Ra et al., 1989). The volatilization rate was measured after 7 days and 15 days, calculating the initial and final amount of mercury present in 200 ml of water.

Results and Discussion

The survival of the organism in natural water is a prerequisite for its ability to remove any toxins from the system. In earlier studies, it was found that Hg-detoxifying enzymes of Hg-resistant bacterial strains isolated from different aqueous en-