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Volatilization of Mercury Compounds and Utilization of Various Aromatic Compounds by a Broad-Spectrum Mercury Resistant *Bacillus pasteurii* Strain

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Aquatic ecosystems may receive aromatic compounds through various routes. These compounds can cause cancerous diseases in aquatic animals (Mix 1986) and enhance mutagenicity of the sediments (Sato *et al* 1983). The persistence of aromatic compounds deposited in sediments is affected by microbial degradation (Gibson and Subramanian 1984). Degradation, as well as metabolism of different aromatic compounds, have been extensively studied in different bacteria (Kiyohara *et al* 1983; Friello and Chakrabarty 1976; Heitkamp *et al* 1988) including *Bacillus* sp (Buswell 1974; Crawford 1975).

Plasmid-determined mercuric and organomercurial resistance in microorganisms has also been studied by several workers (Summers and Silver 1978; Schottel *et al* 1974). Utilization of various aromatic compounds as sole sources of carbon by any Hg-resistant bacterial strain has not been reported. We have isolated a broad-spectrum Hg-resistant *Bacillus pasteurii* strain DR_2 (Pahan *et al* 1990) which could volatilize different mercury compounds and utilize various aromatic compounds as sole sources of carbon. This strain preferentially utilized benzene in a medium containing both glucose and benzene. To our knowledge, until recently there has been no report on preferential utilization of other compounds, particularly an aromatic compound to glucose in a mixture.

MATERIALS AND METHODS

All chemicals and reagents used in the study were of analytical grade (E. Merck, U.K.).

The broad-spectrum Hg-resistant *Bacillus pasteurii* strain DR_2 was previously isolated from the effluents of Durgapur-Steel Plant, India and identified in our laboratory (Pahan *et al* 1990).

For studies of volatilization of Hg-compounds from liquid media, bacterial cells were grown overnight with 10 μM $HgCl_2$ for

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induction of Hg^{+2} reductase and organomercurial lyase and were diluted 10 times with sterile nutrient broth to a total volume of 200 mL containing 33.6 mg $HgCl_2$, 1.68 mg phenyl mercuric acetate (PMA), 3.36 mg thiomersal, 1.68 mg methoxyethyl mercuric chloride (MEMC) and 1.68 mg methyl mercuric chloride (MMC) in separate flasks. Control flasks contained the same amount of Hg-compounds but no organisms. All the flasks were placed on a rotary shaker (200 rpm) at 32°C. After 24 hrs of growth, cells were harvested by centrifugation at 6,000 x g for 10 mins at -4°C and washed thrice with demineralized water. Wet cells, 1 mL of the supernatant and 1 mL of control medium were separately taken in 50 mL volumetric flasks. Hg-content of each set was determined following the cold-vapor atomic absorption spectrometric technique of Bradenberger and Bader (1967) and Ray *et al* (1989) and, thus, total mercury present in both the supernatant and in the bacterial cells were calculated. The amount of Hg-compounds equivalent to mercury is given in Table 1.

For studies of utilization of different organic compounds as sole sources of carbon, the bacterial cells were grown overnight in a synthetic medium (NH_4Cl - 1.0 g; $MgSO_4.7H_2O$ - 0.13 g; $Na_2HPO_4.2H_2O$ - 6.0 g, KH_2PO_4 - 3.0 g, glucose - 4 g and water - 1 litre) containing 30 µM benzene. The next morning the bacterial culture was aseptically diluted 10 times with sterile glucose-free synthetic media containing 1 mM of an organic compound as the carbon source. Control flasks containing the organisms received neither glucose nor any organic compound. All flasks were placed on a rotary shaker (200 rpm) and bacterial growth was measured at hourly intervals in a Klett-Summerson photoelectric colorimeter using a red # 66 filter. Total viable count was determined by the agar plate method from suitable portions of culture taken out aseptically and diluted serially after 18 hrs of growth. An average of six separate counts was made. Several organic compounds, viz. benzene, toluene, benzyl acetic acid, phenyl acetic acid, benzoic acid, cyclohexane, naphthalene, xylene, petroleum ether, heptane, hexane, diphenylamine, phenol and naphthol were tested separately.

Bacterial cells were grown in media containing either benzene or petroleum ether as sole sources of carbon overnight on a rotary shaker (200 rpm). The cells were harvested and washed thrice with 50 mM sodium phosphate buffer (pH 7.2). The washed cells were suspended in the same buffer and oxygen consumption was measured polarographically by using different organic compounds as sole sources of carbon following the method of Spain and Nishino (1987).

For the glucose-uptake study, the bacterial strain was allowed to grow in synthetic media containing 4 g/L glucose in two different sets of flasks; the first set contained no benzene and the second set contained 5 mM benzene. After various growth periods, cells were harvested and glucose concentration in the

supernatant was measured following the method of Syomogyi (1973). Bacterial growth of these two sets of flasks was also measured in a Klett-Summerson photoelectric colorimeter using a red # 66 filter and expressed graphically.

RESULTS AND DISCUSSION

Bacillus pasteurii strain DR_2 volatilized more than 90% of the mercury from $HgCl_2$, PMA, thimersol and MEMC, whereas volatilization of MMC was 86.9% with bacteria and 24.4% without (Table 1). Abiological volatilization of other Hg-compounds was in the range of 8 to 17%. Mercury bound by bacterial cells was

Table 1. Volatilization of different mercury compounds from 200 mL liquid media by *B. pasteurii* DR_2 after 24 hrs of growth (- without organism; + with organism)

Name of Hg-compounds	Experimental sets	Amount of Hg-compounds in broth			% volatilization of Hg-compounds
		Initial (mg)	Cell bound (µg)	Final (mg)	
$HgCl_2$	-	33.6	-	30.64	8.8
	+	33.6	30	1.20	95.5
PMA	-	1.68	-	1.48	14.3
	+	1.68	6.5	0.008	99.5
Thimersol	-	3.36	-	2.98	11.3
	+	3.36	11	0.25	92.2
MEMC	-	1.68	-	1.40	16.6
	+	1.68	14	0.13	91.6
MMC	-	1.68	-	1.27	24.4
	+	1.68	12	0.21	86.9

very low in each case. This bacterial strain was highly Hg-resistant and removed more than 30 mg of $HgCl_2$ from 200 mL nutrient broth within 24 hrs. Klett readings and the corresponding cell number of bacterial growth after 18 hrs using different aromatic compounds as sole sources of carbon in the growth media are given in Table 2. Higher Klett readings as well as higher cell number indicated higher bacterial growth and more utilization of aromatic compounds as sole sources of carbon. Among the aromatic compounds, only phenol and naphthol were not utilized. The concentration of organic compounds that supported growth of the organism in glucose-free synthetic media was in the range of 0.1-15.0 mM. Suspensions of two differennt types of washed cells pre-exposed to two different

Table 2. Utilization of different organic compounds by *B. pasteurii* DR_2 as sole sources of carbon

Organic compounds	Klett reading after 18 hrs of growth[a]	Cell number after 18 hrs of growth[b]
Benzene	205 ± 10	1.92×10^9
Toluene	200 ± 8	1.84×10^9
Benzyl acetic acid	205 ± 8	1.92×10^9
Phenyl acetic acid	200 ± 10	1.85×10^9
Benzoic acid	195 ± 8	1.75×10^9
Cyclohexane	190 ± 8	1.66×10^9
Naphthalene	165 ± 7	1.36×10^9
Xylene	150 ± 6	1.25×10^9
Petroleum ether	145 ± 5	1.18×10^9
Heptane	110 ± 5	9.05×10^8
Hexane	102 ± 5	8.45×10^8
Diphenyl amine	68 ± 2	5.56×10^8
Phenol	0	0
Naphthol	0	0

1.7×10^8 cells/mL was added initially.

a excluding initial and control readings.

b excluding initial and control cell counts.

organic compounds, namely benzene and petroleum ether, quickly oxidized all the organic compounds tested except phenol and naphthol (Table 3). The rates of oxidation of benzene, toluene, benozic acid, phenyl acetic acid and cyclohexane were remarkably higher than those of naphthalene, xylene, petroleum ether and heptane. This bacterial strain grew poorly in synthetic media supplemented with glucose (Fig. 1) and did not properly utilize glucose when this sugar was the only carbon source (Table 4). When both glucose (4 g/L) and benzene (5 mM) were present in the growth medium, enhanced growth of the organism was observed (Fig. 1). However, growth

Table 3. Oxygen consumption by washed cells of *B. pasteurii* DR_2

Organic compounds in the assay mixture	Rate (nmol/min/mg protein) of oxygen consumption by washed cells after prior growth with	
	Benzene	Petroleum ether
Control	0	0
Benzene	162	152
Toluene	150	144
Benzoic acid	148	140
Phenyl acetic acid	148	142
Naphthalene	95	82
Xylene	90	84
Petroleum ether	86	90
Cyclohexane	145	140
Heptane	68	64
Phenol	0	0
Naphthol	0	0

during the first 10 hours was mainly due to the utilization of benzene as utilization of glucose was insignificant at this stage. Glucose was oxidized quickly by the organism at the end of this growth period when growth was slightly biphasic in nature (Fig. 1). We observed that the presence of low amounts (30 μM) of benzene or other aromatic compounds enhanced utilization of glucose by this bacterial strain (unpublished data) due to facilitated transport (Whiley and Stokes 1963). This organism is a broad-spectrum, Hg-resistant bacterium and with the help of Hg-reductase and organomercurial lyase (Pahan *et al* 1990), it could eliminate different Hg-compounds from its growth medium. Volatilization of Hg-compounds by Hg-resistant bacteria has also been reported by many workers (Schottel *et al* 1974; Ray *et al* 1989). A characteristic feature of this bacterial strain to volatilize mercury from Hg-compounds and utilize various organic compounds widely differing in molecular structures as sole sources of carbon is probably due to the presence of plasmids

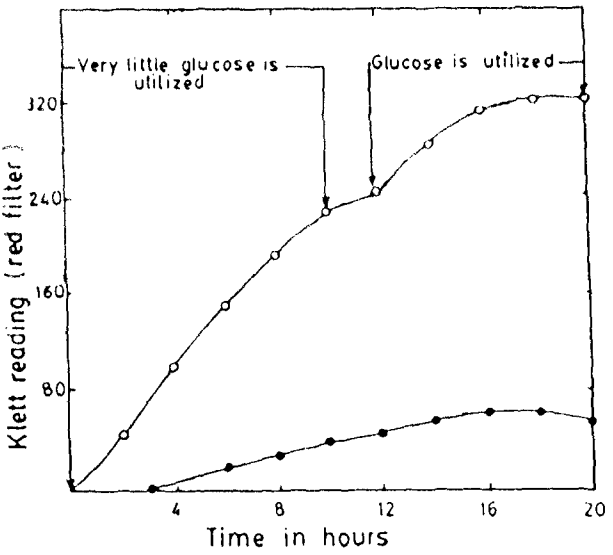

Figure 1 Growth curve of *B. pasteurii* DR_2 in synthetic media with glucose only [●—●] and with glucose and 5 mM benzene [○—○].

Table 4. Glucose utilization by *B. pasteurii* DR_2 in the presence of benzene at different intervals

Time (in hrs)	Amount of glucose (mg) present per ml of broth	
	- Benzene	+ Benzene (5 mM)
0	4	4
4	3.80	3.95
8	3.43	3.88
10	3.10	3.78
12	2.85	3.30
16	2.50	1.80
20	2.28	1.25
24	2.10	0.98

harbored by this organism. Plasmid mediated bacterial degradation of organic compounds has been reported by many workers (Friello and Chakrabarty 1976; Kiyohara *et al* 1983). Ecological importance of this type of bacterium is that it will probably be able to degrade Hg-based pesticides and utilize different aromatic hydrocarbons, and will thereby prevent bioaccumulation of these toxicants in the aquatic environment.

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