Mercury-Resistant Bacteria Isolated from an Industrial Effluent Outlet in Iligan City, Philippines

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Abstract

Water, sediment and soil samples were obtained from an effluent outlet shared by two chemical plants in the western suburb of Iligan City. The samples were screened for mercury-resistant organisms by serial dilution and spread-plating on MS agar medium containing 10 ppm $HgCl_2$. Four bacterial isolates, namely, ***Bacillus sp., Corynebacterium sp., Cytophaga sp.*** *and* ***Klebsiella sp.*** *were obtained. These isolates were able to tolerate up to 40 ppm $HgCl_2$ in the MS agar medium. Comparatively, known cultures from the DBS culture collection tolerated only up to 20 ppm $HgCl_2$ in MS agar medium. Further, the effluent-derived isolates retained their resistance phenotype across several successive colony transfers. This result shows the possession of stable mercury-resistance genes by the isolates. These organisms are therefore, suitable candidates for the biological treatment of mercury-containing wastewater and for genetic research.*

Introduction

The production of toxic or recalcitrant waste effluents by the chemical industry is leading to major problems of their disposal. New biotechnological approaches are now being exploited for the biological treatment of these waste effluents. Such approaches are seen to replace existing methods of effluent treatment(Wyatt, 1988).

Mercury is one of the most toxic and potentially hazardous metals being released into the environment from industrial sources. Organic mercury salts have been known to cause the "Minamata disease" originally reported in Japan. The source of these mercury compounds was a vinyl chloride-producing factory (Hirayama & Takahashi, 1970). Iligan City, being a heavily industrialized city is most likely, a major contributor to mercury pollution in the Iligan Bay area. This metal is commonly removed

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from wastewater by chemical precipitation. However, microbiological methods could be more efficient, safe and economical alternatives for the control of mercury pollution(Hansen *et al.*, 1984).

It is difficult to precisely designate the concentration of heavy metal that is tolerated before an organism is considered resistant(Trevors, 1986). Some authors considered 5 ppm $HgCl_2$ to select candidate mercury-resistant species (Kelly & Reanney, 1984). Several microbial species have been reported to be mercury-resistant tolerating levels of 5 to 10 ppm $HgCl_2$. *Staphylococcus aureus* strains have been mentioned especially those connected with outbreaks of infections in hospitals(Hall, 1970; White et al., 1980). Kelly and Reanney (1984) isolated mercury-resistant soil bacteria belonging to a wide range of taxa (Table 1).

Table 1. Taxonomy of 97 mercury-resistant(Hg^r) soil bacteria isolated by Kelly and Reanney (1984).

TAXON/GROUP	NUMBER OF Hg^r ISOLATES IN TAXON/GROUP
Enterobacteriaceae	30
Pseudomonas	27
Bacillus	24
Alcaligenes	2
Mycobacterium-Nocardia group	6
Flavobacterium-Cytophaga group	5
Gram-positive cocci	3

Possible detoxification of mercury along with other metals was studied by Aïking *et al.*(1985) using a strain of *Klebsiella aerogenes*. In addition, mercury and its compounds may be removed from wastewater by microbial cell uptake(Glombitza *et al.*, 1985).This process is also known as biosorption(Tsezor & Bell, 1989).

This report is the first of three parts of the study on microbiological treatment of industrial effluents. It is the aim of this work to select appropriate mercury-resistant bacteria that could eventually be manipulated for

large-scale application in the detoxification, removal and recycling of mercury from effluents.

Materials and Methods

Isolation of Mercury-Resistant Bacteria

Water, sediment and soil samples, each weighing approximately 500 g were obtained from the banks of an industrial effluent outlet using sterile pre-calibrated one-liter beakers. The samples were protected from direct sunlight and immediately transported to the DBS Research Laboratory, MSU-IIT, for screening.

Duplicate 5 g aliquots of the sediment and soil samples were mixed with 10 ml of 0.1% peptone. The sediment and soil suspensions were then vigorously shaken for 30 minutes to dispense the bacteria and separate the solids. Portions of 0.1 ml volume of this suspension and of the water sample were spread-plated onto MS agar medium supplemented with 10 ppm $HgCl_2$. The plates were covered with dark paper and incubated at 37°C for 48 hours. Isolated colonies were transferred to MS agar stabs and slants with 10 ppm $HgCl_2$ and incubated under the same conditions.

Three successive transfers were made to MS agar plates without $HgCl_2$ and subsequent inoculations to the same medium with 10 ppm $HgCl_2$ to ascertain the stability of the resistance pheno-type. The isolates were then restreaked onto MS agar plates with 10, 20, 30 and 40 ppm $HgCl_2$. The resistance of known cultures from the DBS culture collection was also determined.

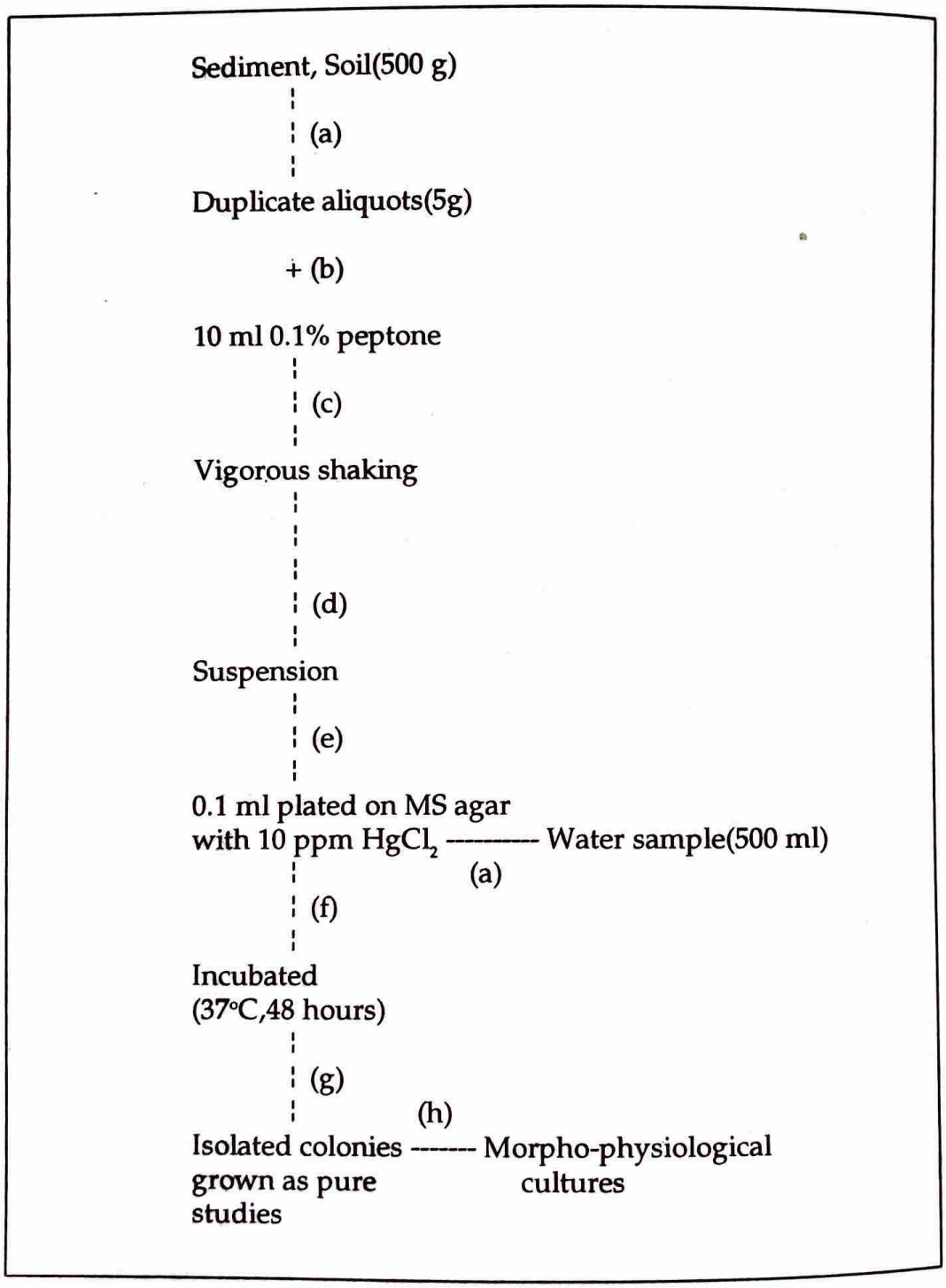
A summary of the isolation procedure is shown schematically in Figure 1.

Medium

Formulation of the MS medium according to Kelly and Reanney (1984) was followed having the following composition: 1.0% trypticase, 0.8% NaCl, 0.1% yeast extract and solidified with 1.0% agar. Sterile glucose was added to a final concentration of 0.1%. Supplementation with 10, 20, 30 and 40 ppm $HgCl_2$ was done for the determination of the tolerance levels of the isolates.

Mercury- resistant cultures were grown and maintained in MS agar and MS broth with 10 ppm $HgCl_2$.

Figure 1. Scheme for the isolation of mercury-resistant bacteria from the effluent outlet.



Taxonomy

Standard schemes for the identification of new isolates were followed according to the eighth edition (1974) of Bergey's Manual of Determinative Bacteriology.

Results and Discussions

Eight colonies designated MS_1 to MS_8 were obtained from the initial screening using plated MS agar supplemented with 10 ppm $HgCl_2$. The growth of these isolates even after three successive alternate transfers to MS agar without and with 10 ppm $HgCl_2$ indicated retention of the resistance phenotype. Morphological and physiological tests revealed these colonies to belong to 4 bacterial genera, namely, *Bacillus*, *Corynebacterium*, *Cytophaga*, and *Klebsiella* (Table 2).

Five known cultures from the DBS culture collection serving as "reference cultures" namely, *Proteus vulgaris*, *Bacillus subtilis*, *Bacillus cereus*,

Table 2. Taxonomy of MS_1 to MS_8 based on the Bergey's Manual of Determinate Bacteriology (eighth edition, 1974).

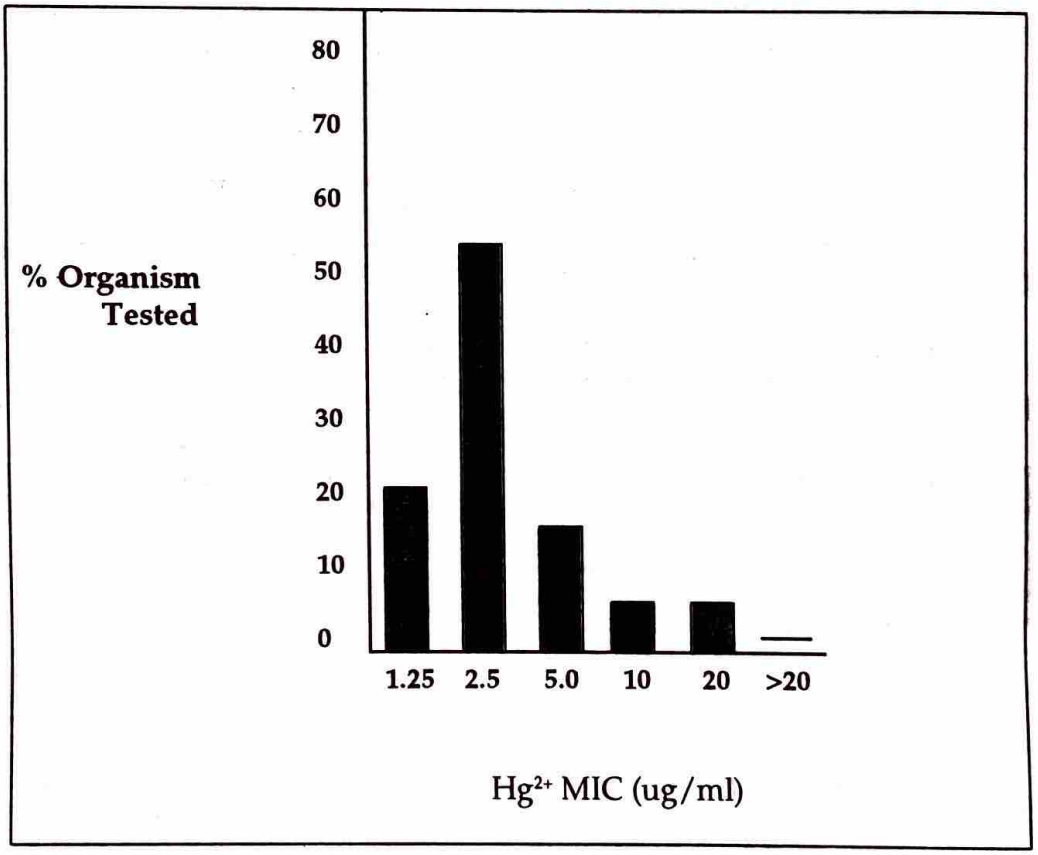
MORPHOLOGICAL AND PHYSIOLOGICAL CHARACTERISTICS	GENERA OF ISOLATED BACTERIA			
	Bacillus (MS_4)	*Corynebacterium* (MS_1,MS_2,MS_3)	*Cytophaga* (MS_5)	*Klebsiella* (MS_6,MS_7,MS_8)
Gram stain	+	+	-	-
Cell morphology	rods	club-shaped	elongated rods	rods
Endospore	+	-	-	-
Oxygen requirement	obligate	facultative aerobes	obligate aerobes	facultative
Catalase	+	+	+	+
Methyl red	+	+	+	-
Gelatinase	+	-	-	-
Motility	+	-	+	-
Metachromatic granules	-	+	-	-
Cellulase	-	-	+	-
Tryptophanase	-	-	-	+
Voges-Proskauer	-	-	-	-

Pseudomonas aeruginosa and *Staphylococcus aureus*, were also shown to grow on MS agar with 10 ppm $HgCl_2$. To assess whether the new isolates and the reference cultures were genuinely mercury-resistant organisms, inocula from all cultures were streaked onto MS agar plates with 20, 30 and 40 ppm $HgCl_2$.

It was reported by Kelly and Reanney(1984) that truly resistant species will tolerate $HgCl_2$ levels of more than 10 ppm whereas most sensitive cells will be inhibited at these levels(Figure 2).

Surprisingly, all cultures grew well at 20 ppm $HgCl_2$ but only the effluent-derived cultures tolerated 30 and 40 ppm $HgCl_2$.

Figure 2. MIC (Minimum Inhibitory Concentration) values with respect to Hg^{2+} obtained from 504 diverse soil bacteria (Kelly & Reanney, 1984).



Note: The effluent-derived isolates obtained in this study belong to the last category(>20 ug/ml).

Why the "reference cultures" were uniformly inhibited at 20 ppm $HgCl_2$ was still not fully understood. Pending the results of follow up studies especially those dealing with the genetic aspects, answers to this question would be highly speculative at the moment. Although it was very probable that the new isolates might still tolerate levels higher than 40 ppm $HgCl_2$, no additional set up was made since their resistance to mercury has already been firmly established in this study. Also, even majority of the pseudomonads, considered to be the most resistant group to mercury, could hardly tolerate 40 ppm $HgCl_2$ in culture media.

The unrelatedness of the four genera isolated confirmed the ubiquitous distribution of the mercury resistance genes among bacterial populations. Their stable Hg^r phenotype makes them ideal for application in the biological treatment of mercury-containing wastewater. The Hg^r marker that is most probably located in plasmids also makes these bacteria suitable materials for genetic experiments.

Sequel reports will concentrate on susceptibility to antibiotics, other heavy metals and organomercurials, plasmid studies including conjugative behavior, optimal growth requirements, and possible biosorptive and detoxifying capabilities by these effluent-derived bacteria.

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