Volume 234, number 2, 280–282 FEB 06052 July 1988

The diversity of mercury reductases among mercury-resistant bacteria

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Received 19 April 1988

Two immunologically non-cross-reactive types of mercury reductases were found among Gram-negative and two among Gram-positive mercury-resistant environmental bacteria. Mercury reductases were further discriminated by 'spur' formation immunodiffusion tests. Immunologically indistinguishable mercury reductases were found among strains belonging to phylogenetically distant genera. This suggests a horizontal transfer of mercury resistance genes between these strains.

Mercury reductase; Immunological cross-reaction; Horizontal gene transfer; (Environmental bacteria)

1. INTRODUCTION

Mercury resistance operons, which are often found in plasmids and transposons, are a good model for the study of horizontal gene transfers in natural populations of bacteria. To examine the diversity and the possible transfer of *mer*-operons between bacteria, one can use as a specific probe antibodies against the largest protein product of this operon, mercury reductase. There are only brief reports in the literature about the antigenic properties of the mercury reductases studied with sera against the enzymes from Gram-negative bacteria [1,2].

In the present work mercury reductases from different environmental bacteria have been compared using sera against the enzymes of both Gram-negative and Gram-positive bacteria. Mercury reductases proved to be far more diverse than formerly believed. We have obtained indications of horizontal transfers of *mer*-operons among environmental bacteria.

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2. MATERIALS AND METHODS

The mercury resistant bacterial strains were isolated from soil, ore and water samples from the mercury deposits in Central Asia (Kirgizia), the Caucasus, the Carpathians, and the Kamchatka peninsula as well as from the intestines of toads, mice and rats captured in the same areas.

The bacteria were grown overnight in the presence of 1–3 μg/ml $HgCl_2$ and induced in the morning, upon dilution with broth, by twice adding $HgCl_2$.

Cell extracts were obtained by sonication. Mercury reductases were isolated from the extracts essentially according to [3] on columns with Orange A matrex gel for the Tn*501* and coryneform enzymes, and on DEAE-cellulose, Blue Sepharose and Biogel A 0.5 m for the *Bacillus* enzyme.

Mercury reductase activity was determined by spectrophotometry at 340 nm of the mercury-dependent oxidation of NADPH for 30 min at 30°C. The standard reaction mixture contained 50 mM Na-phosphate, pH 7.4, 0.5 mM EDTA, 0.2 mM $MgCl_2$, 0.2 mM NADPH, 1 mM mercaptoethanol and 0.1 mM $HgCl_2$.

Immunodiffusion in agar according to Ouchterlony was carried out at 22–25°C. The gels were photographed, at a magnification of 2×, 24 and 48 h after the beginning of the reaction. The specificity of mercury reductase precipitation was tested with extracts of uninduced bacteria which produced no precipitin bands or faint bands (in the latter case the extracts carried a small reductase activity).

3. RESULTS AND DISCUSSION

We obtained antibodies against mercury reduc-

Published by Elsevier Science Publishers B.V. (Biomedical Division)

tases of Tn*501* and of two Gram-positive bacteria from our collection: *Bacillus sphaericus* FAB-2 and coryneform CHM19-3. The sera were designated I, II and III, respectively.

All mercury reductases of 50 Gram-negative strains with the only exception being *Flavobacterium* sp., formed precipitin bands upon immunodiffusion with sera I, but not II or III.

The *Flavobacterium* strain has an inducible mercury reductase activity, but the enzyme formed no precipitin bands with sera I–III and was not inactivated by these sera. The mercury reductase of the *Flavobacterium* strain seems to be different from the mercury reductase of *Thiobacillus ferrooxidans*, also known to have no immunological cross-reaction with the Tn*501* enzyme [4], for the *Thiobacillus* reductase binds to Orange A matrex gel [5], while the *Flavobacterium* reductase does not.

The mercury reductases of 21 Gram-positive bacterial strains formed precipitin bands exclusively with sera II, while the enzymes of 8 other Gram-positive strains reacted exclusively with sera III (table 1).

The diversity of mercury reductases within the various immunological types was determined by

Table 1

Immunological diversity of mercury reductases

Immunological subtype	Bacteria	Area of isolation or source
I-1	*E. coli* (ColE1::Tn*501*)	laboratory collection
	Acinetobacter lwoffii (3)	Carpathians, Caucasus
I-2	*E. coli* (R831b)	E. Lederberg
	A. calcoaceticus KHP18	Kirgizia
	A. lwoffii (3)	Carpathians, Caucasus, Kamchatka
	Enterobacteriaceae (2)	Kirgizia, Carpathians (from the intestines of a toad and a rat)
I-3	*A. calcoaceticus* KHW14	Kirgizia [7]
	A. lwoffii (5)	Carpathians, Caucasus, Kamchatka, Moscow region
	Xanthomonas ssp. (2)	Kirgizia, Kamchatka
	Pseudomonas ssp. (2)	Kirgizia, Kamchatka
	Aeromonas sp. (1)	Kamchatka
I-4	*E. coli* (Tn*21*)	J. Grinsted
	Enterobacteriaceae (3)	Carpathians (from the intestines of mice and a rat), Kamchatka
I-5	*Pseudomonas mendocina* (2)	Kamchatka
I-6	Enterobacteriaceae (1)	Kamchatka
I-7	*Pseudomonas* sp. KHP41	Kirgizia
	P. alcaligenes (1)	Kamchatka
	P. fluorescens (2)	Kirgizia, Carpathians
	P. aeruginosa (4)	Kamchatka
	P. mendocina (2)	Kamchatka
	Xanthomonas campestris (4)	Kamchatka
I-8	*P. fluorescens* (2)	Kamchatka
I-9	*Erwinia* sp. (1)	Moscow region
II-1	*Bacillus sphaericus* FA8-2	Kamchatka
	Bacillus sp. (1)	Kirgizia (from the intestines of mice)
	B. sphaericus (1)	
	B. polymyxa (1)	Kamchatka
	Rhodococcus sp. (1)	Kamchatka
II-2	*B. licheniformis* (2)	Kamchatka
	Bacillus sp. (1)	
	coryneform (1)	Kamchatka
II-3	*Oerskovia* sp. (1)	Carpathians
II-4	*Oerskovia* sp. (1)	Carpathians
	B. sphaericus (1)	Carpathians, Kamchatka
II-5	*B. stearothermophilus* (3)	Iland Kunashir
II-6	*Staphylococcus saprophyticus* (3)	Moscow
II-7	coryneform (1)	Kamchatka
	S. aureus (pI258)	L. Nesterenko
	S. aureus (pI147)	L. Nesterenko
III-1	coryneform CHM 19–3	Kamchatka
III-2	coryneform (2)	Kamchatka
III-3	coryneform (1)	Kamchatka
	Citreobacterium (3)	Kamchatka
III-4	*Micrococcus roseus* (1)	Moscow

The enzymes that refer to one subtype do not form spurs with one another. The strains are listed in order of diminishing immunological similarity with the prototype mercury reductases (Tn*501*, *B. sphaericus* FA8-2 or coryneform CHM19-3). The numbers in parentheses indicate the number of independently isolated strains. The immunological difference between subtypes I-2 and I-3 is slight: the spurs appear only 40–48 h after the beginning of the reaction and are small in size. The enzymes of II-6 and II-7 subtypes form faint but specific precipitin bands and are not inhibited by any sera

the spur formation test upon immunodiffusion. Using serum I it proved possible to distinguish nine subtypes of mercury reductases among the Gram-negative bacteria (table 1). The order of diminishing similarity between the tested mercury reductases and the Tn*501* enzyme is consistent with the data on the amino acid sequences of three reference reductases for which the complete (Tn*501* and Tn*21*) and partial (KHW14 strain) amino acid sequences are known: the difference from Tn*501* is about 10% amino acid substitutions for Tn*21* [6] and about 5% for KHW14 [7].

Enzyme subtypes were also identified for Gram-positive bacteria (table 1).

The results demonstrate a large diversity of the primary sequences of mercury reductases. At the same time one cannot fail to notice several cases where immunologically indistinguishable mercury reductases are found in bacteria belonging to different species, genera or even families (table 1). Apparently these bacteria have exchanged *mer*-operons in the relatively recent past. Immunological data also show that the exchange of mercury resistance genes is subject to a number of constraints. No exchange of mercury reductase genes between Gram-positive and Gram-negative bacteria has been observed (see also [1,2]). Probably a barrier exists between the ordinary Gram-negative bacteria (Pseudomonadaceae, *Acinetobacter*, Xanthomonadaceae and Enterobacteriaceae) and the phylogenetically and ecologically remote Gram-negative bacteria, such as flavobacteria and acidophilic thiobacilli. It seems that there are some limitations with regard to the exchange of mercury reductase genes among the 'ordinary' Gram-negative bacteria as well: this is indicated by the more narrow spectrum of mercury reductases in *Acinetobacter* as compared with Pseudomonadaceae and Enterobacteriaceae.

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