Mercury detoxification genes in river water contaminated by the past mercury mining activity in Idrija, Slovenia

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Abstract: Mercury speciation in water and the presence of mercury resistance genes (*merA*, *merB*, *merC* and *merF*) were examined in river heavily contaminated due to past mercury mining in Idrija, Slovenia. Water samples were collected from few sites of Idrijca River, upstream and downstream the mine. *MerA* and *MerB* genes were detected in all water samples from upstream the mine where water is not contaminated to downstream the smeltery where concentrations of mercury were elevated (up to 54,4 ng/l). The highest percentage of bacteria containing m*erB* genes were detected in uncontaminated water that suggest that emergence of mercury resistant bacteria is not induced only by Hg stress.

Key words: mercury, mercury resistance, *mer* operon, Idrija

INTRODUCTION

The narrow valley of the Idrijca River is highly contaminated with mercury due to 500 years of mercury mining and ore processing (HORVAT ET AL., 2002; HINES ET AL., 2000). Mercury accumulated in the riverbanks is released in the Idrijca where it is biotransformed to different mercury species. Bacterial mechanism of resistance to mercurial compounds is reduction of Hg(II) to the volatile Hg(0). This reaction is catalysed by cytosolic, NADPH-dependent mercury reductase that is encoded by *merA* gene of mercury resistance (*mer)* operon. *Mer* operon also comprises *merT* and *merP* in some loci *merC* and *merF* genes coding proteins involved in Hg(II) uptake. In some operons *merB*, coding organomercurial lyase has been found. Organomercurial lyase has organomercurial-degradation activity: a soluble enzyme, which split the C-Hg bond, releasing a protonated organic moiety and Hg(II) cation. Transcriptional activity of *mer* operon is controlled by regulatory proteins MerR, which acts as repressor in the absence of Hg(II) and as activator in the presence of Hg(II), and MerD antagonist of MerR. Therefore, the degradation of MeHg and reduction of Hg(II) are dependent on the concentration of Hg(II) in the immediate environment.

In this study, the correlation between mercury concentration in water and presence of different mercury detoxification genes was examined.

RESULTS AND DISCUSSION

Hg speciation in water samples. Water samples were collected from four sites (water from the mine (WM), Idrijca by Belca (I1), Idrijca by smeltery (I2) and Idrijca by Kozarska grapa (I3)) and filtered. The following mercury species were determined in water samples: dissolved gaseous Hg (DGM), dissolved monomethylmercury ($MeHg_{diss}$), dissolved total mercury (THg_{diss}), MeHg and THg bound to particulates. Filtration was performed using pre-cleaned Whatman GF/C glass filters (0.75 μm). Validated analytical protocols are described elsewhere (LOGAR ET AL., 2001; HORVAT ET AL., 2002, 2003). Mercury concentrations upstream of the mercury mine at Belca are low. Highest mercury concentrations were measured by the former smelter facilities (19.9 ng/l Hg(0), 54.4 ng/l THg). Due to evaporation of Hg(0) and dilution Hg concentrations decreased downstream (2.5 ng/l Hg(0), 13.7 ng/l THg). Methylmercury (MeHg) concentrations increase from former smelter facilities (<5 pg/l) to Kozarska grapa (67 pg/l). Surprisingly, concentrations of THg and Hg(0) in water from the mine were low, while MeHg concentrations of 44 pg/l was measured.

Bacterial counts. Number of bacteria in samples was determined by plating water samples on LB plates. Mercury resistant counts were determined on plate count agar amended with 10 μM $HgCl_2$ and 100 μM

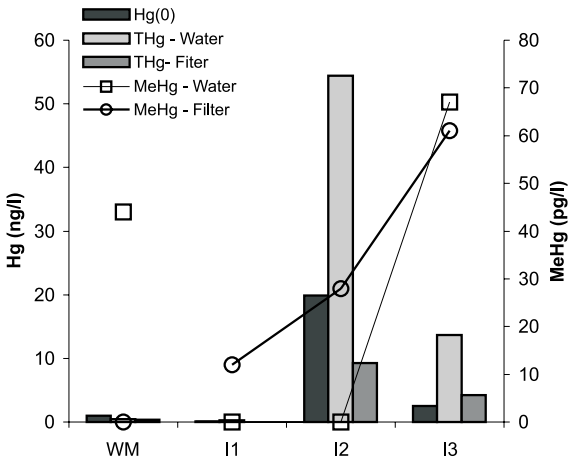

Figure 1. Concentrations of gaseous mercury (Hg(0)) and total (THg) and methyl mercury (MeHg) on filters and in filtered samples from Idrija region.

Table 1: Number of CFU per ml in water samples plated and incubated for 1 day at 30 °C

	WM	I1	I2	I3
LB	**30**	**50**	**300**	**200**
LB 10 μM $HgCl_2$	**20**	**0**	**280**	**90**
LB 100 μM $HgCl_2$	**20**	**40**	**200**	**120**

$HgCl_2$. CFU were counted after 1 day of incubation at 30 °C.

Highest CFU was determined in I2 were concentrations of Hg are highest. This is consequence of largest sewage inflow at that site. Percentage of resistant bacteria is also highest at that site (~80 %). At I3 number of resistant bacteria is significantly lover (~50 %). Sampling site I1 is near spring so small number of bacteria was expected but high number of resistant bacterial is surprising.

Bacterial Hg detoxification genes. Presence of for *mer* gens (*merA*, *merB*, *merC* and *merF*) in mercury resistant bacteria, grown on plate count agar amended with 100 μM $HgCl_2$, was determined with polymerase chain reaction (PCR) (LIEBERT AT AL., 1997). All resistant bacteria poses *merA* gene, coding mercuric reductase. *MerB* gen was determined in different bacteria from all water samples. Surprisingly highest percentage of bacteria possessing gen *merB*, was isolated from I1, where concentrations of MeHg are low. Genes *merC* and *merF* (supplementary transport proteins) are unevenly represented taking into account determined mercury concentrations.

Table 2. Presence of mercury resistant genes in resistant bacterial stains isolated from water samples.

	MerA	*MerB*	*merC*	*merF*
WM	4/4 (100 %)	2/4 (50 %)	0/4 (0 %)	2/4 (50 %)
I1	6/6 (100 %)	5/6 (83 %)	0/6 (0 %)	2/6 (33 %)
I2	10/10 (100 %)	4/10 (40 %)	2/10 (20 %)	0/10 (0 %)
I3	10/10 (100 %)	5/10 (50 %)	1/10 (10 %)	3/10 (30 %)
Total	30/30 (100 %)	16/30 (53 %)	3/30 (10 %)	5/30 (17 %)

CONCLUSION

The preliminary results presented in this study indicate that presence of *mer* genes is not correlated with concentrations of mercury species. If we take into account that *mer* operon is present on plasmids and transposons (HOBMANAT ET AL., 1997), which are easily shifted from one bacteria to another, the appearance of resistant bacteria in mercury uncontaminated site was not surprising.

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