



# Mercury bioremediation by mercury resistance transposon-mediated in situ molecular breeding

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## Abstract

Mercury-resistant ( $\text{Hg}^{\text{R}}$ ) bacteria occur in various bacterial species from a wide variety of environmental sources. Resistance is conferred by a set of operon genes termed the *mer* operon. Many  $\text{Hg}^{\text{R}}$  bacteria have been isolated from diverse environments and clinical samples, and it is recognized that *mer* operons are often localized on transposons. Previous research reports have suggested that  $\text{Hg}^{\text{R}}$  transposons participate in the horizontal gene transfer of *mer* operons among bacteria. This was confirmed by a study that found that *mer* operons were distributed worldwide in Bacilli with dissemination of Tn*MERII*-like transposons. In this mini review, possible strategies for transposon-mediated in situ molecular breeding (ISMoB) of  $\text{Hg}^{\text{R}}$  bacteria in their natural habitat are discussed. In ISMoB, the target microorganisms for breeding are indigenous bacteria that are not  $\text{Hg}^{\text{R}}$  but that are dominant and robust in their respective environments. Additionally, we propose a new concept of bioremediation technology for environmental mercury pollution by applying transposon-mediated ISMoB for environmental mercury pollution control.

**Keywords** Bacilli · Bioremediation of environmental mercury pollution · Dissemination of mercury resistance genes · Mercury resistance transposon · Transposon-mediated in situ molecular breeding

## Introduction

Elemental mercury and its compounds are distributed widely in the environment as a result of geological and anthropogenic activities (Selin 2009). Microbes convert mercurial compounds and play an important role in the global mercury cycle (Barkay et al. 2003). Although mercury and its compounds are toxic to all living organisms, certain bacteria possess resistance genes to mercurials. Mercury-resistant ( $\text{Hg}^{\text{R}}$ ) bacteria occur in various bacterial species from a wide variety of clinical and environmental sources (Barkay et al. 2003; Osborn et al. 1997). Different resistance mechanisms against mercury compounds have been found in a wide range of bacterial genera that have also been isolated from clinical, intestinal, and

environmental samples (Silver and Phung 1996). The most studied mechanism of bacterial mercury resistance is enzymatic reduction of  $\text{Hg}^{2+}$  to its metallic form,  $\text{Hg}^0$  (Barkay et al. 2003; Misra 1992; Osborn et al. 1997; Silver and Phung 1996). A high vapor pressure and a very low aqueous solubility of  $\text{Hg}^0$  result in its volatilization from the bacterial cytoplasm into the outer atmospheric environment (Barkay et al. 2003).

This underlying resistance mechanism is conferred by a set of operon genes, the *mer* operon. The *mer* operon consists of a cluster of linked genes that encode proteins with functions related to regulation, transport, decomposition, and reduction of mercurial compounds. Although *mer* operons regulating the same mechanisms have been identified from various Gram-negative and Gram-positive bacteria, the component genes of the *mer* operons are not uniform, and genetic variations within each gene exist (Barkay et al. 2003; Silver and Phung 1996). In general, *merR* (regulatory gene), *merT* and *merP* (mercury transport genes), and *merA* (mercury reductase gene) are commonly conserved as core *mer* operon genes, while additional genes such as *merB* (organomercury lyase) (Begley et al. 1986), *merC* (mercury transport) (Liebert et al. 2000), *merD* (regulatory gene) (Nucifora et al. 1989), *merE* and *merF* (mercury transport genes) (Liebert et al. 1999;

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Wilson et al. 2000), and *merG* (phenylmercury resistance gene) (Kiyono and Pan-Hou 1999) are optional.

These *mer* genes are often localized on mobile elements, such as transposons (Hobman and Brown 1997; Mindlin and Petrova 2013; Osborn et al. 1997). Transposons carrying *mer* operons have been identified from both clinical and environmental species. The majority of characterized Hg<sup>R</sup> transposons belong to the Tn3-family of replicative transposons (previously designated class II transposons; DNA without an RNA intermediate) that are generally typified by encoding 35–48 bp terminal inverted repeat (IR) sequences, the *tnpA* gene (transposase), the *tnpR* gene (resolvase), and *res* sites (co-integrated resolution sites) (Grinsted et al. 1990; Liebert et al. 1999; Nicolas et al. 2015). The first investigated Hg<sup>R</sup> transposons were Tn21 and Tn501 (Liebert et al. 2000; Nucifora et al. 1989). The distribution and diversity of transposition genes relating to Tn21 and Tn501 have been studied using environmental bacteria or bacterial community DNA isolated from different environments (Holt et al. 1999; Huang et al. 1999a; Liebert et al. 1999; Mindlin and Petrova 2013).

Environmental mercury contamination, particularly by organomercurial compounds, has caused very serious disasters such as methylmercury poisoning cases in Minamata Bay, Japan, during the 1950s, after the region was polluted with methylmercury compounds from industrial wastewater (Harada 1995; Tsubaki and Irukayayama 1977). Based on reported mercury concentrations in 1959 and the late 1980s, natural processes (largely microbial) had removed between 75 and 90% of the mercury in Minamata Bay sediment (Nakamura and Silver 1994; Silver et al. 1994). The frequency of multiple organomercurial resistant bacteria in isolates from Minamata Bay sediment were at least 20-fold higher than that found in isolates from a nearby unpolluted control site (Nakamura et al. 1990). These records suggest the contribution of bacteria in in situ remediation activity in mercury-polluted environments. In the Minamata Bay sediments, Hg<sup>R</sup> bacteria had been identified from ten different genera (*Bacillus*, *Enterobacter*, *Flavobacterium*, *Moraxella*, *Pseudomonas*, *Vibrio*, *Corynebacterium*, *Micrococcus*, *Staphylococcus*, and *Clostridium*) (Nakamura et al. 1988, 1990; Narita et al. 1999). Among these bacteria, *Bacillus* was the most abundant and was a major contributor of mercury cycling in Minamata Bay sediment (Nakamura et al. 1988, 1990). Similar *mer* operons among isolated Minamata *Bacillus* strains suggest horizontal spread of mercury resistance determinants (Silver et al. 1994).

The first Hg<sup>R</sup> *Bacillus* was isolated from a sediment from a site polluted with heavy metals in Boston Harbor, USA (Mahler et al. 1986), and the genetic properties of the mercury resistance determinant of the isolated strain, *Bacillus cereus* RC607, were characterized (Wang et al. 1989). Subsequently, Huang et al. (1999b) identified a broad-spectrum mercury

resistance Tn3-family replicative transposon, TnMER11, from a Minamata Bay isolate, *Bacillus megaterium* MB1 (collection number: NBRC 110925), which showed resistance to both organomercurials and inorganic mercury salt, and demonstrated the mobile nature of mercury resistance determinants among *Bacillus* organisms. Moreover, the findings of the worldwide distribution of TnMER11-like mercury resistance transposons indicated the ubiquitousness of the catabolic genes (Bogdanova et al. 1998; Matsui et al. 2016; Narita et al. 2004). These studies prompted us to propose a new mercury bioremediation strategy targeting the mercury resistance transposon of *Bacilli*.

In this mini review, we discuss a possible strategy of transposon-mediated in situ molecular breeding (ISMoB) of Hg<sup>R</sup> *Bacilli* in natural habitats. In ISMoB, breeding target microorganisms are indigenous *Bacilli* that are not Hg<sup>R</sup> but are dominant in the environment and have excellent survival. We thus propose a new concept of environmental mercury pollution bioremediation using transposon-mediated ISMoB and ways to apply this concept.

## Diversity of Hg<sup>R</sup> determinants in *Bacilli* isolated from Minamata Bay sediment

Toxic mercurials enhanced the abundance of mercury resistance genes and bacteria in Minamata Bay sediment in Japan (Nakamura et al. 1986, 1990). Previous studies have also suggested the contribution of bacterial in the in situ remediation activity of mercury-polluted environments (Huang et al. 1999b; Narita et al. 2003). *B. megaterium* MB1, a broad-spectrum Hg<sup>R</sup> *Bacillus* strain, was isolated from Minamata Bay sediment. The sediment was collected in June 1984, before the sediment was dredged by authorities to remove mercury contamination, and was stored under air-dry conditions at 4 °C. The detailed mercury resistance genetic module was characterized from *B. megaterium* MB1 chromosomal DNA (Huang et al. 1999a, b). This genetic module is almost identical to the *mer* operon of Gram-positive bacteria previously found from *B. cereus* RC607 (Gupta et al. 1999; Wang et al. 1989), which was isolated in the USA, and an *Exiguobacterium* sp. strain TC38-2b, which was isolated in Ukraine (Bogdanova et al. 1998; Bogdanova and Mindlin 1991). The *mer* genes from *B. megaterium* MB1 are encoded on a Tn3-family replicative transposon designated as TnMER11 (Huang et al. 1999b). This indicates that the transposons are involved in the horizontal dissemination of mercury resistance among Gram-positive bacteria. The previous observation of low genetic diversity in Minamata Bay sediment may reflect a peculiarity of this site (Huang et al. 1999b; Nakamura and Silver 1994), enabling us to further determine the molecular diversity of the Minamata Bay mercury-resistant *Bacillus* population.

In addition to the *B. megaterium* MB1, 30 Hg<sup>R</sup> Bacilli (designated MB2 to MB31) were also isolated from the same Minamata Bay sediment samples, and the mercury resistance genes among these regional Bacilli were characterized in detail (Table 1) (Narita et al. 2003). All isolates were Gram-

positive and grew as rod-shaped spore-forming cells. Eight isolates (MB11, MB12, MB13, MB22, MB24, MB25, MB26, and MB27) were facultative anaerobes while the other 22 were strict aerobes. Their mercury resistance was divided into a narrow spectrum, showing resistance to inorganic

**Table 1** Characteristics of mercury resistance of Minamata Bay Bacilli isolates (strain MB1–MB31) from Narita et al. (2003)

Microbial strain	MIC (μM) <sup>a</sup>		Hybridization with DNA probes <sup>b</sup>				Resistance spectrum
	MC <sup>c</sup>	PMA <sup>d</sup>	merA	merB3	merB2	merB1	
<i>B. subtilis</i> 168 <sup>e</sup>	10	1.0	–	–	–	–	Sensitive
<i>B. megaterium</i> MB1 <sup>f</sup>	80	8.0	++	++	++	++	Broad
MB2	80	1.0	++	–	–	–	Narrow
MB3	80	1.0	–	–	–	–	Narrow
MB4	40	8.0	++	++	++	++	Broad
MB5	80	16.0	++	++	++	++	Broad
MB6	80	8.0	++	++	++	++	Broad
MB7	40	0.5	++	–	–	–	Narrow
MB8	80	2.0	++	–	–	–	Narrow
MB9	160	2.0	++	–	–	–	Narrow
MB10	160	1.0	++	–	–	–	Narrow
MB11	160	1.0	–	–	–	–	Narrow
MB12	80	0.5	–	–	–	–	Narrow
MB13	160	0.5	–	–	–	–	Narrow
MB14	80	0.5	+	–	–	–	Narrow
MB15	80	1.0	++	–	–	–	Narrow
MB16	80	0.5	++	–	–	–	Narrow
MB17	80	0.5	++	–	–	–	Narrow
MB18	80	0.5	++	–	–	–	Narrow
MB19	40	4.0	++	–	–	–	Unidentified
MB20	40	4.0	++	–	–	–	Unidentified
MB21	40	8.0	++	–	–	+	Unidentified
MB22	160	16.0	++	++	++	++	Broad
MB23	80	8.0	++ <sup>g</sup>	++	++	++	Broad
MB24	160	16.0	++	++	++	++	Broad
MB25	160	8.0	++	++	++	++	Broad
MB26	160	16.0	++	++	++	++	Broad
MB27	320	16.0	++	++	++	++	Broad
MB28	320	16.0	++	++	++	++	Broad
MB29	160	8.0	++	++	++	++	Broad
MB30	40	2.0	+	–	–	–	Narrow
MB31	40	2.0	+	–	–	–	Narrow

<sup>a</sup> MIC (minimum inhibitory concentration) of mercurials was determined as the lowest concentration of mercurials at which the bacteria could not grow

<sup>b</sup> ++ positively hybridized, + weakly hybridized, – not hybridized

<sup>c</sup> MC, mercury chloride (inorganic)

<sup>d</sup> PMA, phenylmercury acetate (organic)

<sup>e</sup> Mercury sensitive control strain

<sup>f</sup> Broad-spectrum mercury-resistant (organomercurial-resistant) strain, as described previously (Huang et al. 1999a, b)

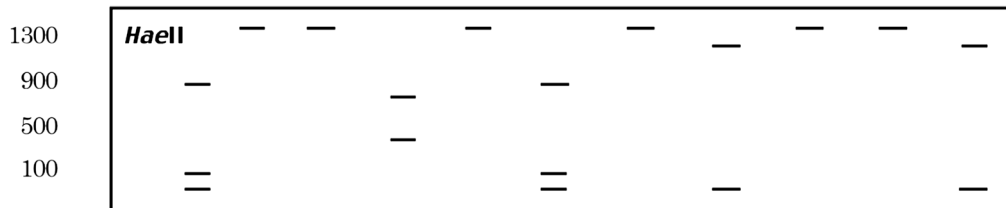
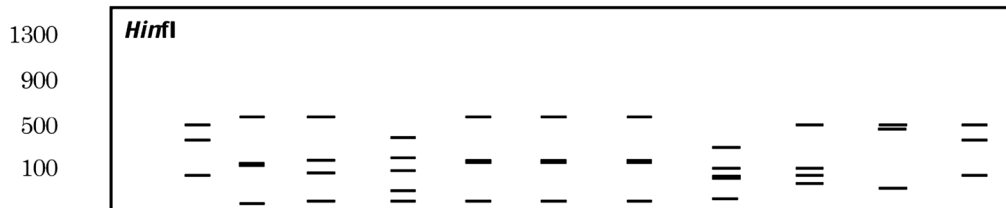
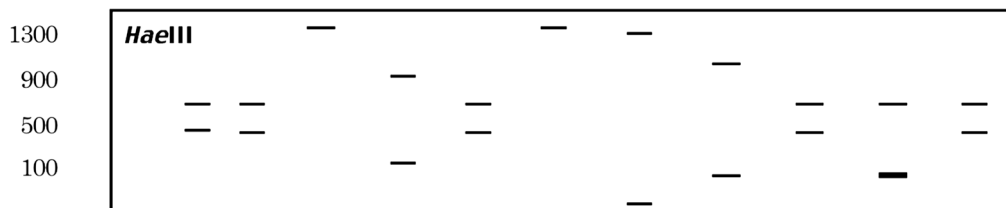
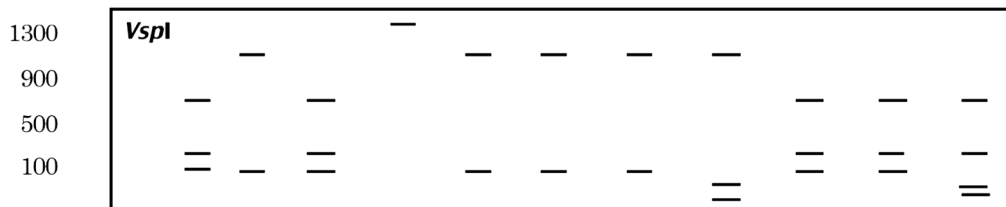
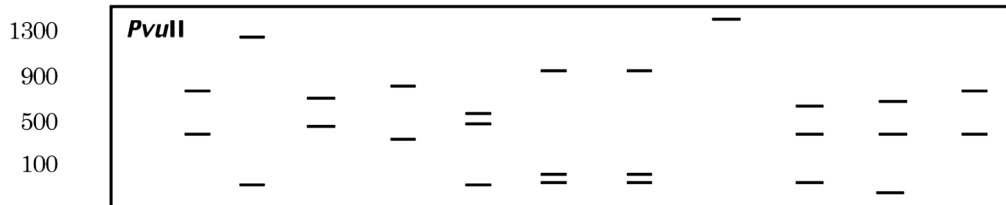
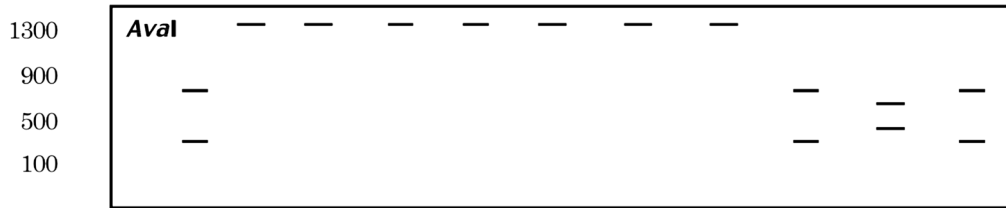
<sup>g</sup> Isolate MB23 produces two hybridization signals with the *merA* probe

RFLP Profile Class: 1 2 3 4 5 6 7 8 9 10 11

Representative Strain of Minamata Bay Bacillus:

MB1, RC607

DNA size (bp)      ↓ MB6 MB23 MB24 MB25 MB26 MB27 MB28 MB29 MB30 MB3



◀ **Fig. 1** Restriction fragment length polymorphism (RFLP) patterns with seven restriction endonucleases of amplified 1.3-kbp *merA* PCR products from Minamata Bay *Bacillus* isolates. Modified from Narita et al. (2003). For *HinfI* digests, doublets are represented as bands of double thickness. The RFLP classes I and VI were previously described in Hart et al. (1998), and classes XXIII to XXVII are new classes identified in our study. The RFLP classes XXVIII to XXXI are also new classes obtained from 1.3-kbp *merA* sequences of Russian and Ukrainian *Bacillus* isolates

mercury salt only and broad-spectrum mercury resistance by their phenotype. Polymerase chain reaction (PCR) amplification analysis targeting different *mer* genes against the 11 broad-spectrum Hg<sup>R</sup> *Bacillus* isolates resulted in the amplification of the PCR fragments of the same size as those from the *B. megaterium* MB1 strain. Southern hybridization analysis showed that these 11 isolates carried the *merA*, *B1*, *B2*, and *B3* genes similar to *B. megaterium* MB1. Eleven of the 30 broad-spectrum Hg<sup>R</sup> *Bacillus* isolate Hg<sup>R</sup> strains possessed *merB* genes; three of 30 unidentified-genotype phenyl-mercury acetate (PMA) resistance *Bacillus* isolates (MB19, MB20, and MB21) did not contain MB1-type *merB* genes. A different PMA resistance mechanism may exist in these bacteria. Furthermore, three isolates (MB11, MB12, and MB13) did not hybridize with the *merA* probes from *B. megaterium* MB1 and did not produce *mer*-related PCR products. As these three isolates showed high minimum inhibition concentrations (MIC) toward mercury chloride, other resistance mechanisms may be employed by these strains.

PCR-restriction fragment length polymorphism (PCR-RFLP) analysis of the entire 6.8 kb *mer* operon encoding *merB3*, *R1*, *E*, *T*, *P*, *A*, *R2*, *B2*, and *B1* in the 11 isolates showed identical results with that of the *B. megaterium* MB1 *mer* operon. Thus, 11 of 30 isolates had the same *mer* operon as that of TnMER11 in *B. megaterium* MB1. Targeting the 1.3 kbp *merA* core region, the diversity of *merA* gene structure configurations was determined using RFLP profiles. The 11 broad-spectrum Hg<sup>R</sup> *Bacillus* isolates showed identical RFLP patterns, whereas the 16 narrow-spectrum isolates and the three unidentified genotype Hg<sup>R</sup> Bacilli were classified into six classes including five new RFLP classes not previously observed (Fig. 1) (Hart et al. 1998). The most abundant *merA* sequences were class 2 type and were shared by eight narrow-spectrum Minamata Bay *Bacillus* isolates and 13 previously identified English *Bacillus* isolates (Hart et al. 1998). Thus, the class 2 *merA* sequences are distributed globally and are shared by different *Bacillus* species in different geographic regions.

A previous study demonstrated that variations of *mer* genes among bacteria from less polluted soils are greater than those in bacteria from mercury-polluted soils (Olson et al. 1991). The employed Minamata Bay sediment sample was highly polluted by Hg<sup>2+</sup> and methylmercury, and a low genetic diversity was observed from the studied broad-spectrum Hg<sup>R</sup> *Bacillus* isolates. These results are consistent with the strong

selective pressure of organomercurials, mainly methylmercury, on the broad-spectrum Hg<sup>R</sup> Bacilli in the studied sediment. However, these results also indicate a weaker selective pressure of inorganic mercury on narrow-spectrum mercury detoxification genes among Bacilli in the same sediment. These differences demonstrate that broad-spectrum Hg<sup>R</sup> *Bacillus* may be responsible for resistance to toxic pollutants in Minamata Bay sediment and that bacteria carrying broad-spectrum Hg<sup>R</sup> were selected. At the same time, horizontal dissemination of *mer* genes may occur under weaker selective pressure of inorganic mercury and may play a key role in the adaptation of bacterial populations to environmental contaminants.

## Worldwide dissemination of mercury resistance transposons in Bacilli

Bacteria are ubiquitous in the atmosphere and can be transported by wind over long distances (Burrows et al. 2009). Generally, the survival of disseminated non-indigenous microorganisms is expected to be low. However, spores of Bacilli show high tolerance against harsh environmental conditions such as heat and desiccation (Nicholson et al. 2000). Previous studies showed that spore-forming *Clostridium* spp. and *Bacillus* spp. were viable after 30 years on the Antarctic Peninsula, while fecal coliforms lost their viability rapidly under the same conditions (Hughes and Nobbs 2004). Thus, spore-forming Bacilli have advantages for long-term survival and transportation over long distances.

Bacilli showing mercury resistance have been described repeatedly in various geographically distinguished regions worldwide (Table 2). Indeed, mercury-resistant Bacilli of different genera and species have been described. Their mercury resistance determinants were identified on mobile genetic elements such as plasmids and transposons. In Gram-negative bacteria, transposons carrying mercury resistance determinants have been well documented including the Tn21-, Tn501-, and -Tn5053 families (Mindlin and Petrova 2013). However, few studies have investigated Bacilli. The linkage between *mer* operons of Gram-positive bacteria and transposition genes was first reported by Bogdanova et al. in 1998. Further studies subsequently confirmed that some mercury resistance determinants of Gram-positive bacteria are located on transposons, similar to Tn3 (Bogdanova et al. 2001; Huang et al. 1999b). TnMER11 was reported in the chromosome of *Bacillus megaterium* MB1, an isolate from mercury polluted sediment in Minamata Bay, Japan (Huang et al. 1999b). TnMER11 carries genes that participate in the resistance to organomercurials and inorganic mercury salts. Three closely related TnMER11-like transposons are Tn5083 from *B. megaterium* MK64-1 in the Kuril Islands, Russia; Tn5084 from *B. cereus* RC607 in Boston Harbor, USA; and Tn5085

**Table 2** Geographical distribution of the identified mercury-resistant Bacilli

Biogeographical region (Ecozones)	Sampling site	Mercury resistance spectrum	References
Palearctic	Woodland, Prague, Czech Republic	B, N	Matsui et al. (2016)
	River Mersey, Fiddlers Ferry, England	N	Hart et al. (1998)
	La Côte Saint André, France	N	Nazaret et al. (2003)
	Aussa river, Marano lagoon, Italy	N	Baldi et al. (2012)
	River Tagliamento, Friuli-Venezia-Giulia, Italy	B, N	Narita et al. (2004)
	Hirose River, Miyagi, Japan	B, N	Matsui et al. (2016)
	Kitakami River, Miyagi, Japan	N	Matsui et al. (2016)
	Lake Izunuma, Miyagi, Japan	B, N	Matsui et al. (2016)
	Minamata Bay, Japan	B, N	Huang et al. (1999b), Nakamura and Silver (1994), Narita et al. (2003)
	Mount Fuji, Japan	N	Matsui et al. (2016)
	Yodo River, Osaka, Japan	N	Matsui et al. (2016)
	Songdo Beach, Busan, Korea	B, N	Narita et al. (2004)
	River Amsterdam, North Holland, Netherlands	B, N	Narita et al. (2004)
	Tagus Estuary, Portugal	B, N	Figueiredo et al. (2016)
	Kamchatka Peninsula, Russia	N	Bogdanova et al. (1998)
	Kuril Islands, Russia	N	Bogdanova et al. (1998)
	Beach by the Citadel near Lund, Sweden	B, N	Narita et al. (2004)
	River Rhein, Graubunden, Switzerland	B, N	Narita et al. (2004)
	Carpathia, Ukraine	B, N	Bogdanova et al. (1998)
	Indomalaya	Calcutta, India	B, N
Odisha coast, Bay of Bengal, India		N	Dash et al. (2014)
Palk Bay, India		N	Nithya et al. (2011)
Pulicat Lake, Tamil Nadu, India		B, N	Kannan et al. (2006)
Rain forest, Sarawak, Malaysia		N	Matsui et al. (2016)
Kasur and Sheikhpura, Pakistan		N	Amin and Latif (2017)
Mount Yushan, Taiwan		B, N	Matsui et al. (2016)
Taichung Harbor, Taichung, Taiwan		B, N	Narita et al. (2004)
Nearctic	Pattaya Beach, Chonburi, Thailand	B, N	Narita et al. (2004)
	Speed River, Guelph, Ontario, Canada	N	Trevors (1987)
	Boston Harbor, Massachusetts, USA	B, N	Mahler et al. (1986)
	Davis Creek Reservoir, California, USA	B, N	Rochelle et al. (1991)
	Little Rock Lake, Wisconsin, USA	N	Rochelle et al. (1991)
	Loon Lake, Michigan, USA	N	Matsui et al. (2016)
Neotropic	Lower East Fork Poplar Creek floodplain, Oak Ridge, Tennessee, USA	B, N	Rochelle et al. (1991), Oregaard and Sorensen (2007)
	Rio de Janeiro, Brasil	N	Silva et al. (2012)
	Boca Del Rio Beach, Veracruz, Mexico	N	Narita et al. (2004)
	Cancun, Quintana Roo, Mexico	N	Matsui et al. (2016)
Australasia	San Joaquin, Queretaro State, Mexico	N	Medina et al. (2013)
	Manly Beach, New South Wales, Australia	N	Narita et al. (2004)
Afrotropic	Dunedin, New Zealand	N	Matsui et al. (2016)
	Lake Malawi, Malawi	N	Matsui et al. (2016)
Oceania	Lagos Lagoon, Nigeria	N	Olukoya et al. (1997)
	Suva, Fiji	B, N	Matsui et al. (2016)



**Table 2** (continued)

Biogeographical region (Ecozones)	Sampling site	Mercury resistance spectrum	References
	Waikiki Beach, Hawaii, USA	B, N	Narita et al. (2004)
Antarctic	East Ongul Island, Antarctica	B, N	Matsui et al. (2016)

*N* narrow- spectrum mercury resistance, *B* broad-spectrum mercury resistance

from *Exiguobacterium* sp. TC38-2b in Carpathian, Ukraine (Bogdanova et al. 2001). Among these, the DNA sequence of Tn5083 has yet to be completely determined. Our laboratory also identified TnMERII, Tn5084, and Tn5085 transposons in 21 of 56 Bacilli isolates from worldwide environmental samples (Narita et al. 2004).

We further investigated 12 of 65 spore-forming Hg<sup>R</sup> bacteria that were isolated from natural environments worldwide to understand the acquisition of additional genes by and dissemination of Hg<sup>R</sup> Tn3-family of replicative transposons across related Bacilli genera via horizontal gene transfer (HGT) (Matsui et al. 2016). The finding of different TnMERII-like transposons, including Tn6294 (a newly identified mercury resistance transposon) and Tn<sup>d</sup>MER3 (a newly identified deleted transposon-like fragment carrying mercury resistance determinants), suggests the diversity of mercury resistance transposons among Bacilli, similar to mercury resistance transposons in Gram-negative bacteria. Moreover, the identification of Tn6294 in *Bacillus* sp. from Taiwan and in *Paenibacillus* sp. from Antarctica is noteworthy. The horizontal dissemination of TnMERII-like transposons across bacterial species and geographical barriers indicates the worldwide distribution of Bacilli carrying mercury resistance transposons in the environment.

### Transposition of Bacilli Tn3-family of replicative transposon

TnMERII-like transposons are generally unitary non-composite structures belonging to the Tn3-family (Nicolas et al. 2015). Tn3-family transposons replicate during integration into the target sequence, and this replicative mode allows the success of proliferation of catabolic genes in bacteria (Nojiri et al. 2004). Although the molecular mechanisms underlying Tn3-family transposons have been well characterized, the triggers that stimulate and attenuate the transposition process are not well understood.

Transposition activity of TnMERII-like transposon (Tn5085) was first revealed experimentally in *Escherichia coli* cells (Bogdanova et al. 2001). The transposition of Tn5085 into the recipient plasmid was shown to

occur with cointegration of plasmid formation in a recombinase A (*recA*)-deficient *E. coli* HB101 strain. These cointegrates were resolved in *E. coli* IF238, which has a complete recombination system. To evaluate the effect of RecA on translocation of the TnMERII-like transposon, we constructed a mini-TnMERII and confirmed the participation of RecA in the resolution of the cointegrated transposon structure (Matsui et al. 2005). Other studies have also shown that the efficiency of cointegrated resolution is low in the *recA*-deficient *E. coli* strain with different Tn3-type transposons from Gram-negative bacteria (e.g., Tn4652 and TnHad2) (Sota et al. 2002; Tsuda and Iino 1987). RecA may contribute to resolution of the cointegrates and facilitate the translocation of transposons to other replicons. However, in the case of TnMERII, cell treatment with stress agents, including UV irradiation doses of up to 3000 J m<sup>-2</sup>, did not alter transposition frequencies, indicating that RecA functions independently from SOS stress responses (Matsui et al. 2005).

Although environmental stress seems to be an important parameter facilitating transposition activity, recent studies have indicated the facilitation of HGT under non-selected conditions. It has been shown that the conjugative mercury resistance plasmid pQBR57 is expanded among *Pseudomonas fluorescens* populations via HGT without mercury selection. Selection with mercury stimulated the clonal expansion of mercury-resistant bacteria but did not stimulate HGT (Stevenson et al. 2017). Similar results were obtained in our previous study on the Minamata Bay *Bacillus* populations, as described in the previous section (Narita et al. 2003). Environmental and cellular parameters that influence transposition activity of TnMERII-like transposons are scarcely characterized; however, the dissemination of transposons may occur without mercury selection. Frequent findings of mercury-resistant Bacilli from non-contaminated regions (Table 2) support these findings. The findings from 74 of 78 *Bacillus* isolates from mercury-polluted Minamata Bay sediment, showing that these carry identical *mer* determinant to *Bacillus* sp. RC607 (Nakamura and Silver 1994), agree with the study by Stevenson et al. (2017). Further characterization of these mobile elements will be valuable to disseminate TnMERII-like transposons for remediation purposes.

## Concept of transposon-mediated ISMoB

In conventional bioremediation methods, purification of contaminated environments by microbes is often achieved using specific microbes that degrade or convert environmental pollutants to non-dangerous substances (El Fantroussi and Agathos 2005). Under a mercury contamination scenario, purifying microbes are isolated from the polluted environment and are grown in pure cultures. They are subsequently used for augmentation of capable microbes in the polluted environment, as shown in Fig. 2. In another case, these bacteria are genetically modified by molecular methods and then introduced into the polluted environment. In the latter method, a selected host microorganism is genetically transformed with the functional genes from an isolated microorganism that possesses pollutant degradation or conversion activity, markedly increasing the microbial purification activity (Singh et al. 2011).

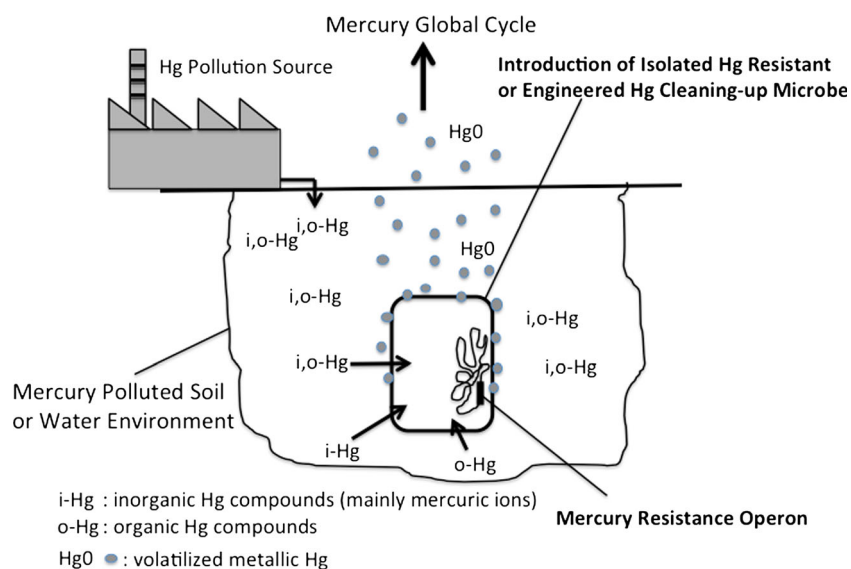
However, host microorganisms for use in artificial molecular-breeding are limited in the environment (El Fantroussi and Agathos 2005; Tyagi et al. 2011). Additionally, genetically modified microorganisms must be assessed before their introduction, and their elimination from the site is sometimes required when the purification process is completed (Keese 2008). Usually, microbes introduced into the environment that are not indigenous and that do not acclimatize are less competitive against wild microbes and are targeted by grazing organisms in the environment (Cunningham et al. 2009; Kota et al. 1999). If we are able to transfer special genetic components for environmental purification from introduced microbes to indigenous and predominant microbes in a habitat, and if the transferred microbes can express these genetic features, naturally bred transformant microbes will be preferable for environmental bioremediation

(Wiedenbeck and Cohan 2011). Indeed, these microbes are more familiar with the environment and have survival advantage over introduced microbes (Ikuma and Gunsch 2013).

As described in the above sections, the same genetic components involved in microbial response to environmental deterioration are common within various microbial species due to transposon transfer. Therefore, transposon-mediated HGT is considered an effective tool for natural breeding of remediating microbes in polluted environments (Shahi et al. 2017). We propose this as “transposon-mediated in situ molecular breeding (ISMoB)”; ISMoB is a fundamentally natural process. Introduced donor microbes are not indigenous but possess transposons for environmental purification, and recipient microbes are indigenous, dominant, and robust in the given environment. However, the ISMoB process can be enhanced by adjusting conditional factors.

Three major factors are involved in transposon-mediated ISMoB. First, effective transposons and mobilization vectors are required. Next, excision of the transposons from vectors and insertion of transposons to the genomic DNA in the recipients must occur. Lastly, there must be mating probability between the donor and recipient microbes. As previously mentioned, useful transposons for environmental purification exist, and some of them are conjugative transposons and self-transmissible (Burrus et al. 2002; Salyers et al. 1995). However, many others are non-conjugative transposons. In the process of intercellular transfer of non-conjugative transposons, lysogenic phages, conjugative plasmids, genomic islands, or other unclassified elements are needed as transferring vectors (Shahi et al. 2017; Tan 1999). The excision and insertion capabilities of a transposon originate from the genetic elements of the transposon itself, while other host cellular components may enhance the excision and insertion of transposons (Bellanger et al. 2014).

**Fig. 2** Schematic diagrams of conventional bioaugmentation for bioremediation of mercury-contaminated environments





Transposon-harboring microbes that contain genetic elements for pollutant removal and for excision/insertion can be found and isolated from natural or polluted environments (Wright et al. 2008). In addition, vectors can be used for intercellular transfer of these transposons from the environment (Wyndham et al. 1994). By combining effective transposons and transfer vectors, two of the three required factors for ISMoB are provided. The third factor requires the development of methodologies to enhance the possibility of mating between donor and recipient microbes. In laboratories, transposon-mediated transfer of genetic elements beyond the border of microbial species has been established. However, in situ mating methods for ISMoB are not yet established. An important first step of transposon-mediated in situ gene transfer is to establish microbial mating with cell-to-cell contact or cell aggregation of both donor and recipient microbes.

Figure 3 shows a schematic diagram depicting ISMoB use for bioremediation of environments polluted with mercury. A facility with biofilm or immobilization technology to enhance the mating of donor and recipient microbes is required to perform ISMoB. Biofilm technology is considered an effective way to provide an adequate mating environment for these microbes. Biofilm formation media can be used to provide adhesion surfaces to increase mating opportunities, and use of entrapping medium is also effective to enable prolonged co-existence of mating microbes. Nutrients are supplied to increase numbers of the introduced donor and indigenous recipient microbes and mating activities of them in the polluted environment.

As mentioned above, we found that *mer* genes were distributed worldwide in Bacilli with the dissemination of TnMER11-like transposons (Matsui et al. 2016). To confirm the mobile nature of *mer* genes and the ways in which Hg<sup>R</sup> Bacilli take root in the environment, we developed a reliable quantitative procedure with a real-time PCR-based method for

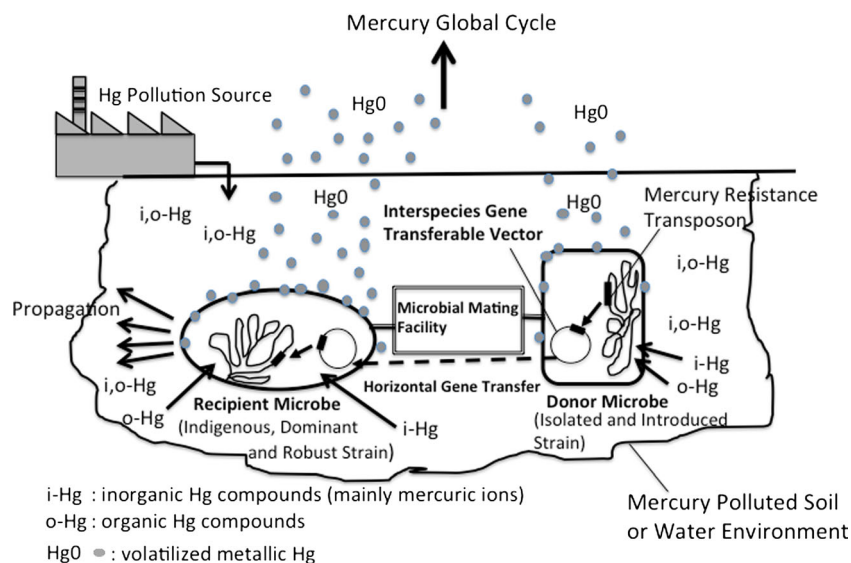
identifying *mer* genes in environmental soil samples. Using the developed method, we could only quantify *mer* genes from 7 out of the 70 soil samples analyzed. The other 63 sites, including the sites augmented with Bacilli harboring known Hg<sup>R</sup> transposons, contained quantities of genetic material that were below the detection limit of the real-time PCR assay. However, further cultivation of the unsterilized soils under nutrient-rich conditions allowed the detection of the *mer* genes from Hg<sup>R</sup> *Bacillus* via PCR (data not published). These results suggest that recently appeared Hg<sup>R</sup> Bacilli could propagate under suitable soil environmental conditions. This finding prompted us to propose transposon-mediated ISMoB of Hg<sup>R</sup> bacteria in the environments. We are now seeking direct evidence of HGT with the Hg<sup>R</sup> transposons in soil samples, and the results of this research will be presented in our next original paper.

Bioreactors are also available for ISMoB. Using bioreactors for removal of contaminants is an ex situ procedure. However, bioreactors can be more effective in enhancing transposon transfers than in situ methods. In bioreactors, microbes that are gel-entrapped or surface-immobilized onto fixed beds or fluidized bed systems can be used to provide adequate room and time for mating between donor and recipient microbes. Propagating conditions for microbes including nutrients, temperature, and pH after mating, as well as genetic transfer, can also be easily delivered in bioreactors.

### New environmental biotechnology for mercury bioremediation using transposon-mediated ISMoB

Recently, Garbisu et al. (2017) proposed plasmid-mediated bioaugmentation and its utilization in bioremediation of contaminated soils. This technology is effective if plasmids with

**Fig. 3** Schematic diagrams of transposon-mediated ISMoB for bioremediation of mercury-contaminated environments



environmental purifying genes are stable in the environment of the recipient microbes. However, possession of genes with plasmids usually renders bacteria less stable than genes integrated in recipient microbe chromosomal DNA. Therefore, in ISMoB, transposon-mediated gene integration into microbial chromosomes is newly proposed.

To remove mercury from contaminated environments by applying transposon-mediated ISMoB, microbes that are highly Hg<sup>R</sup> and can actively reduce and volatilize mercury from the environments are isolated. It is then determined whether the isolates possess Hg<sup>R</sup> operons and their specific location. Possession of vectors for interspecies transfer of Hg<sup>R</sup> transposons is also investigated. If an isolate with a Hg<sup>R</sup> transposon does not possess a vector for intercellular gene transfer, the microbe must be transformed with appropriate conjugative plasmids or phages (Garbisu et al. 2017; Shahi et al. 2017).

Non-indigenous microbes with Hg<sup>R</sup> transposons and conjugative plasmids are introduced into mercury-contaminated sites as donors of Hg<sup>R</sup> transposons. In these sites, procedures to enhance HGT from the donor to the indigenous recipient microbes are applied, which include supplementation with nutrients and mating location to activate ISMoB. After HGT, indigenous recipient microbes survive and propagate, executing more effective remediation activity.

As described above, *B. megaterium* MB1 isolated from Minamata Bay, Japan possesses a Hg<sup>R</sup> transposon, TnMER11; *B. cereus* RC607 isolated from Boston Harbor, USA possesses a Hg<sup>R</sup> transposon, Tn5084; and *Exiguobacterium* sp. Tc38-2b isolated from Carpathian Mountains, Ukraine possesses a Hg<sup>R</sup> transposon, Tn5085 (Bogdanova et al. 2001; Narita et al. 2004). These Hg<sup>R</sup> bacteria can be used as donors for transposon-mediated ISMoB. However, no vector from these Hg<sup>R</sup> bacteria has been identified for interspecies HGT. Effective Hg<sup>R</sup> transposon vehicles must be found for interspecies transfer and use in ISMoB.

A gene transfer module of the Hg<sup>R</sup> transposon TnMER11 from *B. megaterium* MB1 contains *tnpT* and *tnpR* for genetic transposition and a bacterial group II intron named *B.me.II* (Huang et al. 1999b; Chien et al. 2008). However, TnMER11 does not contain the same IR sequences at each end of the transposon. In this case, the IR sequences should be repaired before using this bacterium for ISMoB to increase the possibility of transfer. The use of *B.me.II* as a gene transfer carrier may also be considered since group II bacterial introns can splice themselves from the transcribed intron RNA and home to other genetic regions. Further research on intron-mediated gene transfer between microbes is warranted.

In conclusion, interspecies gene transfer in natural environments actively occurs via transposon mediation and is applicable to ISMoB. To use transposon-mediated ISMoB as a new biotechnology for environmental mercury pollution control, finding and using effective vectors such as conjugative and broad-range transferable plasmids, providing appropriate

mating conditions for both the donor and recipient microbes, and developing augmentation methods for naturally bred microbes are required.

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## Compliance with ethical standards

This article does not contain any studies with human participants or animals performed by any of the authors.

**Conflict of interest** The authors declare that they have no conflict of interest.

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