Occurrence of large fractions of mercury-resistant bacteria in the Bay of Bengal

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As insights from tolerance responses of native microflora are useful in deciphering their involvement in biogeochemical cycling of heavy metals, we enumerated mercury-resistant bacteria (MRB) using sea water nutrient agar medium amended with 10 ppm Hg from oceanic and coastal waters of the Bay of Bengal (BOB) during the summer monsoon (July–August 2001) period. MRB were present in all samples and, intriguingly, the MRB per cent based on total viable counts (TVC) increased significantly \( (r = 0.86; P < 0.001; df = 44) \) with depth. On an average, MRB contributed to over 20% of TVC on the surface, 12% at 100 m, 35% at 500 m and a staggering 49% at 1000 m. The fact that a major portion of the natural, culturable bacterial flora was mercury-resistant from the offshore regions of the BOB might signify the already prevalent adverse impact of heavy metals on the metabolic performance of heterotrophic microflora.

Keywords: Bay of Bengal, extremophiles, mercury-resistant bacteria, mercury pollution, total viable count.

Observations on the occurrence and distribution of native bacteria capable of metal tolerance are of relevance in microbial ecology to understand the extent of metal pollution as well as to reflect on the ability of such native forms to exist and carry on with their metabolic functions. Many human activities have a negative impact on several biological processes and there is no doubt that these will continue to affect the functioning of highly productive coastal ecosystems and beyond. It is therefore of pertinence to obtain information on the abundance of mercury-resistant heterotrophic prokaryotes since their adaptive responses aid in deciphering their involvement in biogeochemical cycling of mercury.

Mercury-resistant bacteria (MRB) are widely distributed and quite ubiquitous in nature, accounting ca. 1–10% of aerobic heterotrophic bacteria. They can be isolated without prior enrichment. However, resistant strains are more abundant in mercury-polluted environments, where up to 50% may grow on nutrient agar media amended with as high as 50 µM (10 ppm) Hg[II], whereas sensitive strains can at best tolerate ca. 1 µM in the growth media.

The presence of MRB is often correlated with the level of mercury contamination in an environment, although they have also been isolated from uncontaminated environments.

Widespread mercury contamination of the coastal zones is increasing at an alarming rate with time and is already affecting marine environments such as the Caribbean region. Many studies on the biota, sediments and water have reported mercury concentrations far above the levels tolerated by humans. As a consequence, mercury-resistance is often seen to be associated with the natural flora. Recently, stringent legislation in the US and European countries has brought down the anthropogenic input and, consequently on a global scale, contamination of natural ecosystems by mercury is decreasing.

Environmental studies on mercury-resistant microflora have focused especially on freshwater ecosystems. In contrast, attention to estuaries and adjacent coastal waters that are major repositories for natural and river-borne/watershed-derived Hg species is scanty. There is a need to increase our knowledge and understanding concerning the biogeochemical cycling of Hg and the impact of anthropogenically related inputs in biologically productive nearshore regions. Further, in an open system like the ocean, mercury vapour released by resistant biota will become part of the local mercury cycle and re-pollute the environment, as has been reported in case of the Amazon River basin. Information on distribution of bacteria tolerating mercury from the offshore waters of the Bay of Bengal (BOB) is lacking. In our continued efforts on documenting the occurrence, distribution and tolerance of marine bacteria to this most toxic heavy metal, abundance and distribution of MRB along 88°E in the open Bay and along the east coast of India (81–85°E) were investigated during the summer monsoon (July–August 2001) period.

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Figure 1. Sampling locations (filled circles) in the Bay of Bengal for enumerating total platable and mercury-resistant bacterial populations.
Large influx of freshwater causing low surface salinities, generally weak winds and almost always warmer sea surface temperature (SST) characterize the northern BOB. Various locations sampled for this study are shown in Figure 1. Sampling was carried out during the ORV Sagar Kanya cruise 166 during the 2001 summer monsoon (6 July–2 August) period. In all, six stations along two transects (Figure 1) were sampled for bacterial abundance and MRB.

Water samples were collected from the upper 1000 m using a Seabird electronics CTD rosette sampling device fitted with 30 l Go Flo bottles that were used for estimating bacterial abundance and for performing various other chemical analyses. The rosette was allowed a 1 min stabilization time before the bottles were closed to ensure sample collection from the desired depths.

Water samples were collected from depths of 1 m (surface), and 10, 20, 40, 60, 80, 100, 120, 200, 300, 400, 500, 600, 800 and 1000 m. Samples from different locations were plated onto sea water nutrient agar (SWNA with composition \( F = 1 \) : peptone [Difco] 5.0 g, yeast extract [Difco] 3.0 g, agar [Difco] 15 g, aged sea water 500 ml; deionized water 500 ml; pH 7.5 ± 0.1) amended with 10 ppm Hg (\( \equiv 50 \mu M \); as HgCl\(_2\)). For enumeration of MRB, 10–15 ml water sample was filtered through 0.22 \( \mu m \) filters depending on the sampling depth (higher volume of water was used for samples from greater depths as bacterial abundance decreases with depth). Plates were incubated at on-board temperature (21 ± 2°C) and final counts of colony forming units (CFUs) taken after 48 h. Total viable counts (TVC) from each sample were also enumerated by plating aliquots in triplicates on SWNA without added Hg.

MRB were present in almost all samples from coastal as well as oceanic waters. In terms of per cent of TVC, MRB occurrence below 100 m was significantly higher \((r = 0.86; P < 0.001, df = 44)\). In case of the two coastal stations located off Orissa (19°N, 85°E; Stn. A), and Chennai (12°N, 82°E; Stn. C), the MRB percentage increased with depth (Figure 2). The counts (cells ml\(^{-1}\)) of MRB ranged from 3.25 to the highest 163.2, forming a maximum of 68% of the TVC at station 19°N, 85°E, whereas at 12°N, 82°E the MRB ranged from nil to 613.3 forming more than 92% of the TVC. Similar trend was found in the other coastal stations off 15°N, 81°E (Stn. B), but the increase in MRB at this location was discernible until 400 m since in contrast to the other two coastal stations, their percentage decreased below 400 m. Interestingly enough, the MRB never exceeded 50% at any depth at the station 15°N, 81°E, although they ranged from 0.4 to 139.2 no ml\(^{-1}\) (Figure 2). Percentage of MRB increased with depth at all oceanic stations along 88°E (Figures 3 and 4), though the bacterial abundance (general bacteria as well as MRB) was lower. The MRB maximum (54.4 cells ml\(^{-1}\)) and minimum (0.9 cells ml\(^{-1}\)) at the southern-most oceanic station 9°N, 88°E (Stn. D) were among the least. The MRB at the northern-most oceanic station (20°N, 88°E; Stn. F) ranged from 2 to 256 cells ml\(^{-1}\), forming more than 92% of CFU (Figure 3). The other oceanic station at 15°N, 88°E (Stn. E) had a maximum MRB ranging from 17.86 to 294 cells ml\(^{-1}\) forming over 53% of the total CFU. In terms of their mean percentages, MRB contributed over 20% of TVC on the surface (1–10 m), 12% at 100 m (below mixed layer), 35% at 500 m and 49% at 1000 m (Figure 5). The TVC values are presented in Table 1.

Use of Hg for industrial and agricultural practices and ultimate disposal of effluents into marine zones continuously increases the concentration of this heavy metal in the marine environment\(^6,8,10\), including the Indian Ocean region\(^20\). The abilities of native microflora to tolerate Hg, its various ionic and molecular forms are of greater interest in microbial ecology. As prokaryotic metabolic pathways dealing with elemental mercury or its many inorganic salts generally lead to production of more toxic forms (e.g. methylmercury), consequences of enhanced atmospheric Hg\(^5\) could bring about highly undesirable environmental changes\(^21\).

India has replaced the US as the biggest consumer of mercury with imports having more than doubled between 1996 and 2002 from 254 tonnes a year to 531 tonnes annually\(^22\). Imports of organomercury compounds (pesticides, biocides, etc.) have jumped 1500 times – from 0.7 to 1312 tonnes during the same period. It consumes 50% of the global production and processes 69% of it. While mapping the ‘mercury hotspots’ in the country, the Center for Science and Environment (CSE) found that coastal areas of Mumbai, Kolkata, Cochin, Karwar and Chennai were severely polluted, contaminating the fish stock\(^22\).

This increased concentration of Hg in marine regimes leads to a natural selection of microbial assemblages that become capable of high tolerance to Hg and thus abundance of MRB in the coastal environments off India has become alarmingly high\(^4\). Since the top layers of water and sediment are best aerated and have the highest concentration of easily degradable carbon sources, it is likely that the energy demanding reduction of mercury is the greatest in these regions, resulting in the lowest selection pressure\(^23\), ensuing survival of Hg-sensitive bacterial population. Photochemical reduction of mercury in the surface waters might also aid in this selection pressure, thus leading to comparatively lower fractions of MRB population in the surface waters of the BOB. High fractions of MRB in the BOB might signify that there is significant Hg contamination in the Bay. This observation from the BOB suggests that the native prokaryote flora capable of dealing with heavy metal toxicity is abundant enough and, intriguingly, its preponderance in the deeper zones (Figure 4 a and b) calls for detailed investigations. One issue that needs to be addressed would be: are the extremophiles in general, versatile enough to deal with diverse types of extreme conditions, including, pressure, temperature, salt and/or toxic metal tolerance? It is also likely, on the other hand, that higher percentages of natural flora in the deep
Figure 2. Vertical profiles of per cent mercury-resistant bacteria in coastal stations in the Bay of Bengal. Total viable counts are presented in Table 1.

Figure 3. Vertical profiles of per cent mercury-resistant bacteria in oceanic stations in the Bay of Bengal. Total viable counts are presented in Table 1.

Figure 4a, b. Vertical profiles of per cent mercury-resistant bacteria in oceanic stations in the Bay of Bengal.
winters might be adversely affected by the prevailing high concentration of heavy metals and experience undue physiological stress.

High correlation of MRB counts ($r = 0.89$, $P < 0.001$; Figure 5) with general plate counts in the oceanic waters is indicative of the certainty of Hg tolerance in open and deep regions of the Bay. Previous studies from the European and North American coasts have reported the occurrence of culturable heterotrophic bacteria capable of tolerating ca. 0.5 ppm (2.5 µM) Hg from locations affected by a variety of anthropogenic activities. Ecological implications of a large fraction of natural bacteria possessing resistance to mercury even at 50 µM, as observed in this study, could mean higher rates of biotransformation of toxic heavy metals; their higher mobilization through the marine food web and increased levels of Hg in the atmosphere. Reyes et al. found 35–55% of bacteria from the marine environments to be resistant to mercury. Thus, large fractions of culturable heterotrophic bacteria resistant to mercury in aquatic ecosystems are attributable to their ability to transform this toxic element.

Long-range atmospheric transport or transport through marine currents to over thousands of kilometres leads to contamination of pristine marine zones. Though no measurements of Hg from the offshore are available, the high percentage of MRB all over the Bay as observed in this study, might suggest the distribution of Hg far and wide in the Bay.

Table 1. Total viable counts (no. ml$^{-1}$) from different locations in the Bay of Bengal sampled during July–August 2001

<table>
<thead>
<tr>
<th>Depth (m)</th>
<th>Oceanic station</th>
<th>Coastal station</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>260.00</td>
<td>154.29</td>
</tr>
<tr>
<td>10</td>
<td>204.29</td>
<td>52.86</td>
</tr>
<tr>
<td>20</td>
<td>10472.14</td>
<td>1414.29</td>
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<td>40</td>
<td>55.71</td>
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</tr>
<tr>
<td>1000</td>
<td>520.00</td>
<td>22.60</td>
</tr>
</tbody>
</table>

*TV (no. ml$^{-1}$; mean of triplicate values); *Average TVC, ‘SD, NS, Not sampled.

Figure 5. Relationship between mercury-resistant bacteria (ml$^{-1}$) and total variable count (ml$^{-1}$) at oceanic stations in the Bay of Bengal.

Production of supernumerary plants from seed fragments in *Garcinia gummi-gutta*: evolutionary implications of mammalian frugivory

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Seeds of *Garcinia gummi-gutta* follow ‘garcinia-type’ of germination in which the primary root and shoot emerge from the opposite ends of the seed. The embryo, which fills up the seed, is an elongated hypocotyl with vasculature connecting the two poles. In a series of experiments we observed that removal of primary root at different stages of development does not affect germination or seedling growth; any seed fragment that contains vasculature produces a root and shoot irrespective of its size and position with precise polarity; and, a seed from which a seedling has germinated is capable of producing another seedling. We propose that the regenerative capacity of seed fragments could be a unique strategy for exploiting mammalian frugivory for seed dispersal.

**Keywords:** Frugivory, *Garcinia gummi-gutta*, germination, root–shoot polarity, seed dispersal, seed fragments.

INTENSE competition in the tropical evergreen forests has led to evolution of varied life-history strategies among different species. In what could probably be another interesting strategy, we report that any fragment of a seed of *Garcinia gummi-gutta*, a tropical evergreen tree, can independently produce root and shoot provided it has some vascular tissue. We discuss the evolution of this feature as a unique way of exploiting mammalian frugivory for seed dispersal.

G. *gummi-gutta* (L.) Robson (family Guttiferae, also called Clusiaceae) is an evergreen tree of medium size. It grows on the humid slopes along the wet evergreen forests of the Western Ghats, India and Sri Lanka1 up to an altitude of 1000 m amsl2. Trees reach approximately 18 m in height and 70 cm in diameter. Like other *Garcinia* species, *G. gummi-gutta* is dioecious3, but has a natural sex ratio4 of 1 : 1. In India, population densities are highest in Uttara Kannada district, Karnataka, which is the northern part of the range of this species5. Fruit is the economically important part of the tree. The pulp of the fruit rind, also known as ‘kokum’, is used in curries as a souring condi-