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EXPERIMENTAL ARTICLES

Evolution of Mercuric Reductase (*merA*) Gene: A Case of Horizontal Gene Transfer[1]

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Abstract—In the present study the role of horizontal gene transfer events in providing the mercury resistance is depicted. *merA* gene is key gene in *mer* operon and has been used for this swtudy. Phylogenetic analysis of aligned *merA* gene sequences shows broad similarities to the established 16S rRNA gene phylogeny. But there is no separation of bacterial *merA* gene from archael *merA* gene which suggests that *merA* gene in both these groups share considerable sequence homology. However, inconsistencies between *merA* gene and 16S rRNA gene phylogenetic trees are apparent for some taxa. These discrepancies in the phylogenetic trees for *merA* gene and 16S rRNA gene have lead to the suggestion that horizontal gene transfer (HGT) is a major contributor for its evolution. The close association among members of different groups in *merA* gene tree, as supported by high bootstrap values, deviations in GC content and codon usage pattern indicate the possibility that horizontal gene transfer events might have taken place during the evolution of this gene.

Key words: codon adaptation index, horizontal gene transfer, mercuric reductase, *mer* operon, GC content.

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INTRODUCTION

Mercury is a toxic element and exists in several forms: as ionic salts in either the mercurous (I) or mercuric (II) states, as organometallic compounds such as methyl mercury or as elemental mercury Hg (0) in either liquid or vapor phase. Microorganisms have evolved various mechanisms for coping mercury toxicity like efflux pumps removing Hg ions, enzymatic reduction of ionic Hg to elemental Hg(0), chelation by molecules such as metallothioneins, binding of Hg to cell surfaces, precipitation of insoluble mercury sulfides and oxides, and biomethylation and subsequent diffusion out of the cell membrane [1]. However the most well characterized microbial mercuric detoxification pathway involves *mer* operon (Fig. 1). *mer* operon consists of a group of linked genes like *merA* gene coding mercuric reductase, *merB* coding alkylmercuric lyase which cleaves C–Hg bond of organomercurials, *merD* coding HTH-type transcriptional regulator, *merP, T, C* coding mercuric transport protein and *merR* coding probable repressor [2–4]. *mer* operon is plasmid-encoded and in some cases is found on transposable elements. Genome sequencing of a number of microorganisms revealed presence of *merA* gene like sequences which suggests the possibility of presence of *mer* operon in the genome. The flavoprotein mercuric reductase *merA* gene is a key component of an organomercurial detoxification system found in many bacteria as well as archaea. The enzyme catalyzes the reduction of inorganic mercuric ions to elemental mercury which is volatile.

$$Hg + NADP^+ + H^+ \longrightarrow Hg^{2+} + NADPH.$$

merR in Gram negative bacteria is separated from other genes by operator-promotor region transcribed in opposite direction, while Gram positive bacteria have *merR* transcribed in the same direction as other genes [5, 6]. The genes of *mer* operon are classified as essential and accessory. The essential genes include *merR*, *T*, *P* and *A* and are always present in the operon. While accessory genes include *merB*, *C*, *D*, *E*, *F*, *G* and *urf* [5–11], *mer* operons have also been classified as broad or narrow depending on the presence of *merB* [2, 12–14]. *mer* operons have been isolated and sequenced. A high divergence has been seen for *mer* genes *mer* operons have been identified [6]. Mercury resistant strains have been isolated from water and soil polluted with mercury compounds and the adjacent areas. Intestinal bacteria are also found to contain same *mer* operons comparable to those reported in many environmental bacteria [5, 6, 15].

Here we study the role of horizontal gene transfer events in widespread evolution of this gene in microorganisms. The methods for detecting HGT are: deviant composition, anomalous phylogenetic distribution, abnormal sequence similarity (greatest similarity with a gene from a distantly related species) and incongru-

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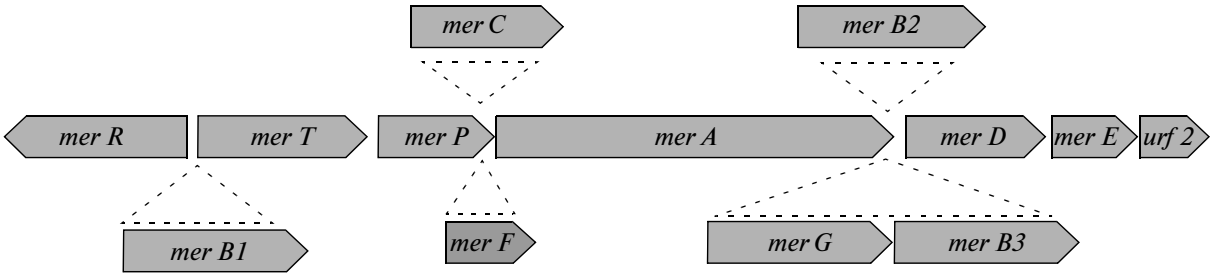

Fig. 1. Genetic structure of *mer* operon in Gram-negative bacteria. (Adapted from [5, 6]).

ent phylogenetic trees [16–18]. These parameters have been exploited in the present stusy to support the idea of HGT of *merA* gene. Phylogenetic analysis of *merA* gene is given and is being compared to the phylogeny one derived from 16S rRNA. Codon usage pattern, GC content analysis and incongruency in 16S rRNA and *merA* gene trees have been used as a tool to depict HGT among the members of different groups.

MATERIALS AND METHODS

Sequence Analysis of Mercuric Reductase merA Gene

Screening for mercuric reductase (*merA*) EC 1.16.1.1 gene was done in KEGG (Kyoto Encyclopedia of Genes and Genomes) (http://www.genome.ad.jp) database [19]. 16S rRNA gene sequences of all organisms were obtained from Ribosomal Database Project Hierarchical Viewer (http://rdp.cme.msu.edu/index.jsp) [20].

Phylogenetic Methods

Prior to phylogenetic analysis, all the selected sequences were aligned via the CLUSTAL_X program [21]. Organisms with available data for complete genome sequences were used for this study and such 43 bacterial and archael sequences were selected for the phylogenetic analysis of *merA*. Tree construction was done using MEGA4 software [22]. Evolutionary tree was constructed by using the Neighbour-Joining method of Saitou and Nei (1987) [23]. Maximum Parsimony method [24] was used to infer tree topology for *merA* gene. In order to know the horizontal gene transfer events T-REX software [25] was used. The gene transfer tree was constructed using Robinson and Foulds topological distance [26].

GC Content

GC content of *merA* gene of each organism was calculated using Gene Runner software which is available free online (http://www.generunner.com). GC content for whole genome was obtained from NCBI database (http://www.ncbi.nlm.nih.gov).

Codon Bias

The Codon Adaptation Index (CAI) of *merA* gene and all the ORF of the genome of the organisms considered in this study was calculated using online CAI calculator (http://www.evolvingcode.net/codon/CAI_Calculator.php).

RESULTS AND DISCUSSION

The 16S rRNA gene based phylogenetic tree (Fig. 2) showed clear separation of different group of bacteria and archaea. Some minor inconsistences are due to limited data set used for the study. Otherwise the major divisions and subdivisions of the bacteria (e.g. the various Proteobacteria, Actinobacteria, and Firmicutes) and archaea formed expected patterns. *merA* gene phylogeny obtained by neighbor-joining (Fig. 3) showed some similarities to the 16S rRNA gene tree. But unlike 16S rRNA gene tree there is no separation of bacteria from archaea which suggests that the bacterial and archaeal *merA* gene share considerable sequence homology. *merA* gene in archaeal groups Euryarchaeota and Crenarchaeota also share sequence homology *merA* gene from both these groups as pair up in the phylogenetic tree. A number of taxa showed *merA* gene phylogenies that are found to be inconsistent with the established 16S rRNA gene phylogene like the pairing of *Kineococcus radiotolerans* (Actinobacteria) and *Aeromonas hydrophila* (γ-proteobacteria), *Rhizobium leguminosarum* (α-proteobacteria), *Mycobacterium avium* (actinobacteria) and *Ralstonia solanacearum* (β-proteobacteria), *Pelobacter carbin-*

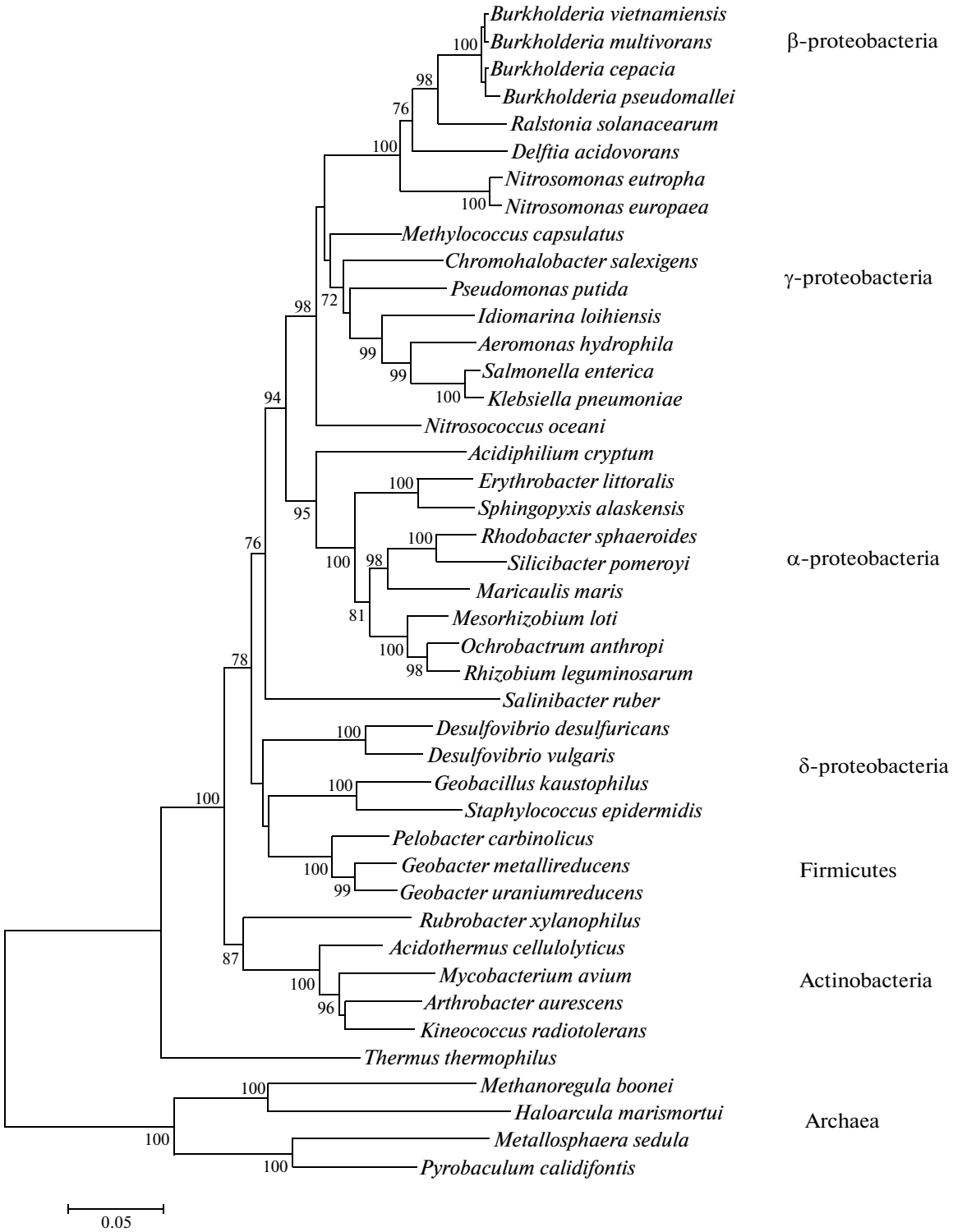

Fig. 2. Phylogenetic tree based on 16S rRNA gene sequences. The tree was constructed by neighbor joining method using 1295 informative positions. The numbers at node represent bootstrap values (based on 100 resampling). Only Bootstrap values >70% are shown.

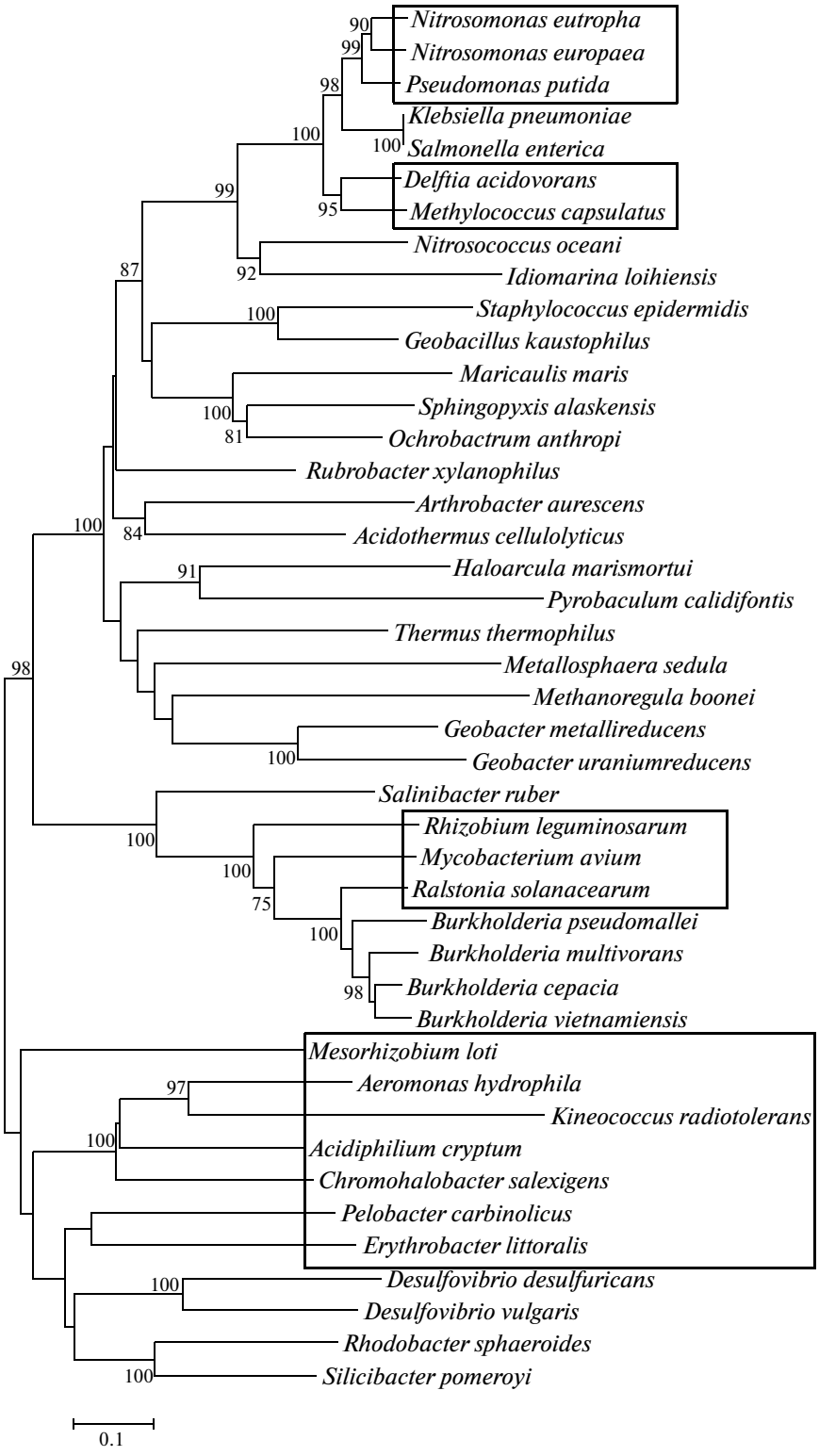

Fig. 3. Neighbour-Joining tree based on *merA* gene nucleotide sequences using 1573 informative positions. The numbers at node represent bootstrap values (based on 100 resampling). Name in boxes represent the branches that are inconsistent with the 16S rRNA tree. Only Bootstrap values >70% are shown.

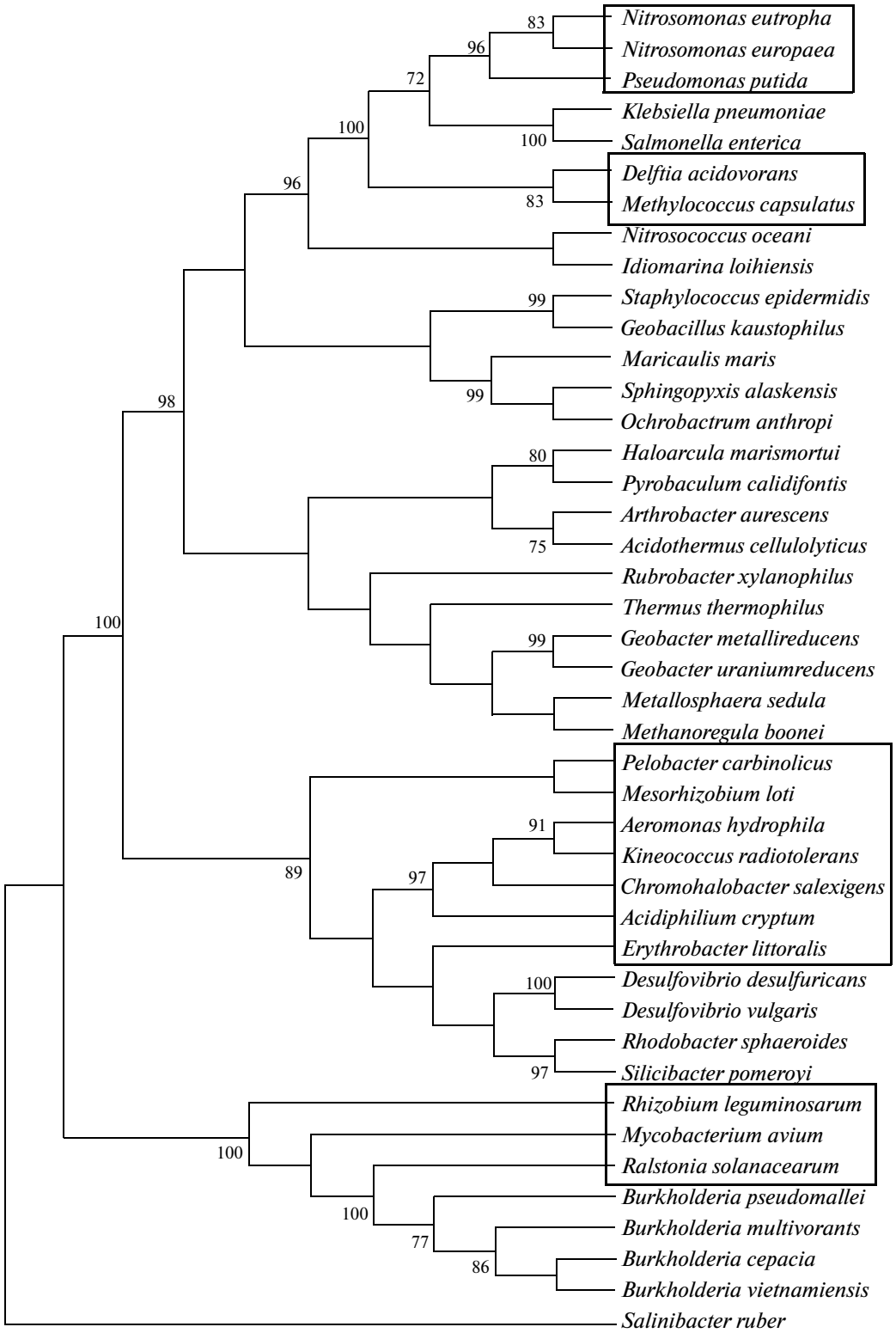

Fig. 4. Maximum-Parsimony tree based on *merA* gene nucleotide sequences using 1573 informative positions. Names in boxes represent the branches that are inconsistent with 16S rRNA gene trees. The numbers at node represent bootstrap values (based on 100 resampling). Only Bootstrap values >70% are shown.

olicus (δ-proteobacteria) with *Erythrobacter litoralis* (α-proteobacteria), *Delftia acidovorans* (β-proteobacteria) with *Methylococcus capsulatus* (γ-proteobacteria), *Acidiphilium cryptum* (α-proteobacteria) with *Chromohalobacter salexigens* (γ-proteobacteria) and pairing of *Nitrosomonas eutropha* and *Nitrosomonas europaea* (both β-proteobacteria) with *Pseudomonas putida* (γ-proteobacteria). These strong inconsistencies are supported by high bootstrap values, deviations in GC content and codon usage pattern. The maximum-parsimony tree (Fig. 4) based on *merA* gene sequence shares broad similarities to the neighbor-joining tree except some anomalies like pairing of *Pelobacter carbinolicus* (δ-proteobacteria) to *Mesorhizobium loti* (α-proteobacteria) [in neighbor-joining tree *Pelobacter carbinolicus* pairs up with *Erythrobacter littoralis* (α-proteobacteria)]. The comparison of GC content of *merA* gene and whole genome provides an important insight into the phylogeny of this gene. GC content of newly acquisited gene is found to differ from GC content of whole genome. This provides an important conclusion in finding whether the gene is transferred horizontally and is acquired recently. There are studies which suggest that HGT events may have been much more widespread during prokaryotic evolution, with genetic exchange even occurring between bacteria and archaea [27–29]. The inconsistencies observed between *merA* gene and 16S rRNA gene phylogenetic tree reflects the possibilities of HGT of *merA* gene among organisms of diverse taxonomic groups. T-REX software [25] exploits this incongruity between the species and gene trees by mapping the gene tree into the species tree and then estimates the prospect of a horizontal gene transfer for each pair of branches of the species tree. It gives an ordered list of the horizontal gene transfer between branches of the species tree. Horizontal transfers of the considered gene are shown by arrows in the species phylogeny. The gene transfer events depicted by T-REX software are shown in supplementary file.

The analysis of GC content and CAI values of these organisms further support the idea of HGT of this gene. The difference in the GC content of *merA* gene and whole genome is evident for α-proteobacteria (*Ochrobactrum anthropi*), β-proteobacteria (*Nitrosomonas eutropha*, *Nitrosomonas europaea*, *Burkholderia cepacia*), γ-proteobacteria (*Salmonella enterica*, *Nitrosococcus oceani*), δ-proteobacteria (*Pelobacter carbinolicus*) and Firmicutes (*Staphylococcus epidermidis*). Similar differences can be observed in the CAI values. The close association among members of different groups in *merA* gene tree, as supported by high bootstrap values, indicate the possibility that horizontal gene transfer events might have taken place. *merA* gene tree shows a number of associations between different proteobacteria suggesting the possibility that HGT events could be more prevailing among the members of this group. The association of *N. eutropha* and *N. europaea* both β-proteobacteria with *P. putida* (γ-proteobacteria) suggests the possibility of gene transfer events between them. There is significant difference in GC content of *merA* gene and their respective genomes (Table 1). The associated organism *P. putida* however showed little difference in GC content as well as CAI values. GC content of *merA* gene in all the three organisms is similar suggesting possible gene transfer events. But these β-proteobacteria appear to acquire these genes recently as depicted by large difference in GC content of the gene and whole genome. The analysis of GC content of *merA* gene and whole genomes in *P. carbinolicus* (δ-proteobacteria) and *E. littoralis* (α-proteobacteria) suggests the possibility of gene movement between them. GC content of the *merA* gene from both the organisms is same (table). But in *P. carbinolicus* there is significant difference in GC content of whole genome and *merA* gene. The association of *M. capsulatus* (γ-proteobacteria) and *D. acidovorans* (β-proteobacteria) is supported by high bootstrap value and GC content. *merA* gene in both these organisms seems to be adapted to their respective genomes as indicated by GC content and codon usage. Similar association is observed between *C. salexigens* (γ-proteobacteria) and *A. cryptum* (α-proteobacteria) is supported by GC content analysis. The most important finding from *merA* gene tree is the association of members of diverse groups other than proteobacteria. Association between *K. radiotolerans* (Actinobacteria) and *A. hydrophila* (γ-proteobacteria) is supported by high bootstrap value. *merA* gene in both these organisms is found to be well adapted to their respective genomes as depicted by GC content and CAI values suggesting the possibility of an ancient gene transfer event. This association also suggests the prospects of HGT among Gram positive and Gram negative bacteria. Another such association is found between archaea (*M. boonei* and *M. sedula*) and δ-proteobacteria (*G. metallireducens* and *G. uraniumreducens*). This pairing again establishes the possibility of gene transfer between bacteria and archaea [28]. *R. leguminosarum* (α-proteobacteria), *R. solanacearum* (β-proteobacteria) and *M. avium* (Actinobacteria) are found to pair up in *merA* gene tree supported by good bootstrap values and the GC content as well as CAI values. *S. epidermis* seems to have acquired this gene recently as depicted by large difference between GC content of the gene and whole genome. From these analyses it is clear that various horizontal gene transfer events would have taken place during the evolution of this gene. Gene transfer between different proteobacteria (β-proteobacteria and γ-proteobacteria, α-proteobacteria and δ-proteobacteria, α-proteobacteria and γ-proteobacteria), members of diverse groups (actinobacteria and γ-proteobacteria); and archaea and bacteria would have taken place leading to spread of

GC content of *merA* gene, whole genome and CAI of *merA* gene and whole genome

S.No.	Name	Group	%GC whole genome	%GC gene	CAI of gene	Avg. CAI whole genome
1.	*Ochrobactrum anthropi*	α-proteobacteria	56.1	65.9	0.252	0.315
2.	*Sphingopyxis alaskensis*	α-proteobacteria	65.5	63.8	0.318	0.257
3.	*Maricaulis maris*	α-proteobacteria	62.7	59.8	0.343	0.263
4.	*Rhizobium leguminosarum*	α-proteobacteria	61.1	62.4	0.293	0.277
5.	*Silicibacter pomeroyi*	α-proteobacteria	64.2	66.8	0.212	0.248
6.	*Rhodobacter sphaeroides*	α-proteobacteria	69	71.7	0.221	0.227
7.	*Erythrobacter littoralis*	α-proteobacteria	63.1	61.1	0.255	0.274
8.	*Acidiphilium cryptum*	α-proteobacteria	67	71.9	0.225	0.25
9.	*Mesorhizobium loti*	α-proteobacteria	62.7	65.5	0.243	0.273
10.	*Nitrosomonas eutropha*	β-proteobacteria	48.5	65.06	0.305	0.347
11.	*Nitrosomonas europaea*	β-proteobacteria	50.7	65.9	0.301	0.33
12.	*Delftia acidovorans*	β-proteobacteria	66.5	68.8	0.289	0.268
13.	*Burkholderia vietnamiensis*	β-proteobacteria	66	69.3	0.277	0.286
14.	*Burkholderia cepacia*	β-proteobacteria	60	68.9	0.276	0.282
15.	*Burkholderia multivorans*	β-proteobacteria	65	67.6	0.264	0.283
16.	*Burkholderia pseudomallei*	β-proteobacteria	67	69.8	0.303	0.28
17.	*Ralstonia solanacearum*	β-proteobacteria	67	69.7	0.294	0.272
18.	*Pseudomonas putida*	γ-proteobacteria	61.9	65.6	0.295	0.298
19.	*Klebsiella pneumoniae*	γ-proteobacteria	57.5	65.1	0.3	0.325
20.	*Salmonella enterica*	γ-proteobacteria	52.2	65.1	0.3	0.357
21.	*Methylococcus capsulatus*	γ-proteobacteria	63.6	67.7	0.276	0.259
22.	*Nitrosococcus oceani*	γ-proteobacteria	50.3	63.9	0.28	0.323
23.	*Idiomarina loihiensis*	γ-proteobacteria	47	49.5	0.313	0.388
24.	*Chromohalobacter salexigens*	γ-proteobacteria	63.9	66.4	0.234	0.216
25.	*Aeromonas hydrophila*	γ-proteobacteria	61	67.1	0.287	0.27
26.	*Desulfovibrio vulgaris*	δ-proteobacteria	63.1	65.1	0.241	0.277
27.	*Desulfovibrio desulfuricans*	δ-proteobacteria	57.8	59.8	0.284	0.301
28.	*Pelobacter carbinolicus*	δ-proteobacteria	55.1	61.1	0.271	0.295
29.	*Geobacter metallireducens*	δ-proteobacteria	59.5	66.9	0.246	0.259
30.	*Geobacter uraniumreducens*	δ-proteobacteria	54.2	62.9	0.257	0.291
31.	*Staphylococcus epidermidis*	Firmicutes	32.2	46.2	0.368	0.477
32.	*Geobacillus kaustophilus*	Firmicutes	52.1	58.5	0.272	0.334
33.	*Arthrobacter aurescens*	Actinobacteria	62.7	64.1	0.334	0.3
34.	*Acidothermus cellulolyticus*	Actinobacteria	66	70	0.291	0.256
35.	*Mycobacterium avium*	Actinobacteria	69	69.1	0.266	0.245
36.	*Kineococcus radiotolerans*	Actinobacteria	74.4	77.9	0.202	0.214
37.	*Rubrobacter xylanophilus*	Actinobacteria	70.5	73.5	0.266	0.216
38.	*Salinibacter ruber*	Sphingobacteria	66.2	70.2	0.232	0.247
39.	*Thermus thermophilus*	Deinococcus	69.4	73.4	0.191	0.214
40.	*Metanoregula boonei*	Archaea	54.5	57.6	0.267	0.284
41.	*Metallosphaera sedula*	Archaea	46.2	52.4	0.321	0.322
42.	*Pyrobaculum calidifontis*	Archaea	57.2	65.4	0.215	0.286
43.	*Haloarcula marismortui*	Archaea	62	63.7	0.258	0.281

this gene resulting in mercury resistant bacterial population. However the use of GC content and Codon Adaptation Index has their own biases.

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