Metagenomics of Bacteria in ballast water and implications for ship-mediated dispersal of microbes

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SUMMARY

In contrast to what is known about the diversity of metazoans and protists transported by ships' ballast water, little is understood about corresponding diversity of bacteria and viruses. Instead, studies have emphasized their enumeration, sometimes with a taxonomic focus on selected groups. To our knowledge, no one has undertaken a metagenomics study of bacteria or viruses in a ballast-water context. To that end, we analyzed the Bacteria community composition of ballast tanks water from 17 ships arriving in June and August 2013 to Norfolk, Virginia (USA) following voyages in the North Atlantic Ocean (**Fig. 1**).

DNA was extracted and the V4 hypervariable region of the 16S rRNA gene was sequenced using Illumina MiSeq technology. We processed raw reads using the Mothur pipeline and clustered final aligned sequences into operational taxonomic units (OTUs) at the ≥97% similarity level. Taxonomy was assigned using the Ribosomal Database Project Naïve Bayesian Classifier.

Tank assemblages were dominated (**Fig. 2**) by Alphaproteobacteria (range across all vessels, 12 to 38%), Gammaproteobacteria (10 to 34%), Bacteroidetes (2 to 40%), and unclassified Bacteria (5 to 37%), except for one case, in which Planctomycetes accounted for 32% of total bacterial diversity, higher than all other tanks by 2- to 121-fold. Similarities among communities calculated using different indices (e.g., Jaccard, **Fig. 3**) showed high fidelity among triplicate samples from each ship in nearly all cases (except CB32). August samples (CB36 to CB42) were distinct from those in June (CB18 to CB32), a result driven principally by lower abundance or even absence of many OTUs in August (**Figs. 3,4**). PCA underscored differences in un-exchanged tanks (CB26, CB28, CB29), but showed no clear distinction between 100% and 300% exchange (**Fig. 5**). Two PCs explained only 44% of variance in the data. Bacteria assemblages in Norfolk Harbor waters were distinct from those in ballast water (**Figs. 2-5**). A phylogenetic tree (**Fig. 6**) was constructed to decipher the taxonomic relationships of 19 OTUs representing ca. 23% of the total sequences, but which remained unaffiliated at the Bacteria or Proteobacteria level. The broad array of OTUs reflects the several biogeographic provinces from which these ballast waters, exchanged or not, originated. We assigned the most abundant OTU (OTU1) to the SAR11 clade, a largely uncultured clade typically retrieved from the open ocean. In Norfolk harbor waters, OTU3 (Cyanobacteria) was most abundant, with OTU1 second most.

DNA from all ships was subjected to quantitative PCR to detect potentially pathogenic species such as *Vibrio cholerae, V. vulnificus, V. parahaemolyticus,* and *E. coli*, bacteria of human health significance monitored in marine environments. All samples were negative.

We conclude that 1) whether ballast tanks were exchanged at sea or not, their Bacterial assemblages differed substantially from local (Norfolk, VA) harbor waters; 2) among tanks, there was a diversity not fully explained by at-sea exchange; 3) outlier assemblages were associated with physico-chemically distinct ports. These results demonstrate what long has been suggested, i.e., that ships' ballasting operations inadvertently re-distribute coastal and oceanic Bacteria throughout the world's ports. Whether this anthropogenic mixing of Bacteria, and of all microbes more generally, serves to re-structure or even "homogenize" microbial biogeographies remains to be seen.

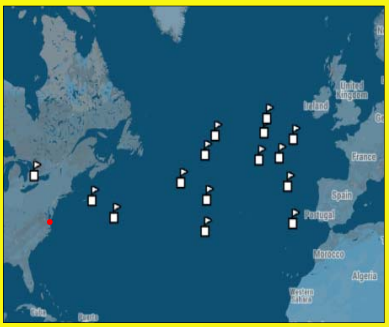


Fig. 1 Location of ballast-tank ocean exchange for the 14 of 17 ships reporting exchange. The port of Norfolk, Virginia (USA) is indicated by a red dot.

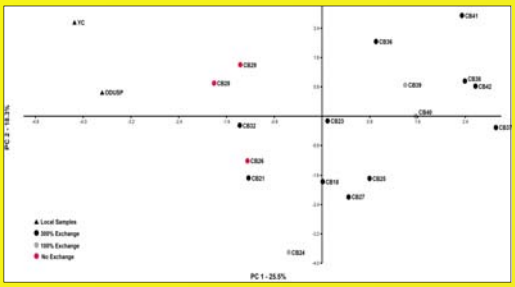


Fig. 5 Principal Components Analysis of ballast-water Bacteria assemblages created using abundances of normalized data of the major phyla and physical variables (salinity, temperature). Symbols correspond to those in Figure 3.

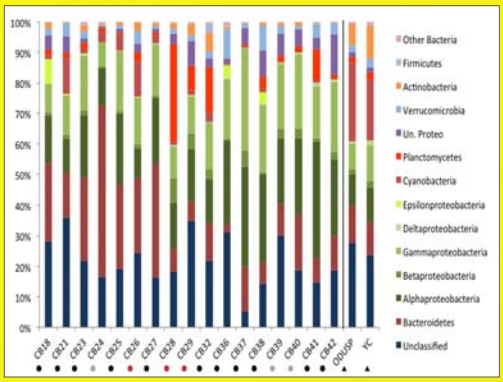
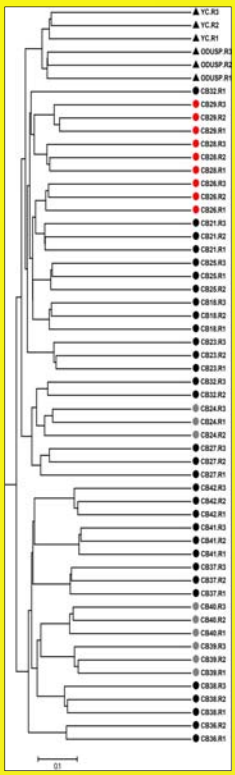


Fig. 2 Relative abundances of partial (ca. 260 bp) sequences of bacterial 16S rRNA gene were estimated by classification at the phylum level, using MOTHUR with a modified 16S rRNA database from the Ribosomal Database Project (RDP). The diverse phylum of Proteobacteria is represented at the class level with different shades of green. A vertical line separates the ballast water samples (CB labels) from those of Norfolk harbor waters (ODUSP and YC).

Fig. 6 Phylogenetic tree of the partial (ca. 260 bp) bacterial 16S rRNA gene of OTUs (**in bold**) from ballast water collected from 17 vessels. Each OTU accounts for ≥0.5% of total abundance or is of special interest (e.g., *Vibrio* spp.). Tree is based on the neighbor-joining method as determined by distance using the Jukes-Cantor model. One thousand bootstrap analyses were conducted, and percentages ≥50% are indicated at nodes. Numbers in brackets are GenBank accession numbers. Scale bar represents 5% estimated distance. *Sulfolobus* sp. was used as an outgroup.

Fig. 3 Distances between communities were calculated with the Jaccard index (similarity in community membership) and clustered using the UPGMA algorithm. Black triangles=local Norfolk Harbor samples; black dots=tanks having a 300% water exchange. Grey and red dots=tanks having a 100% mid-ocean exchange or no exchange, respectively.



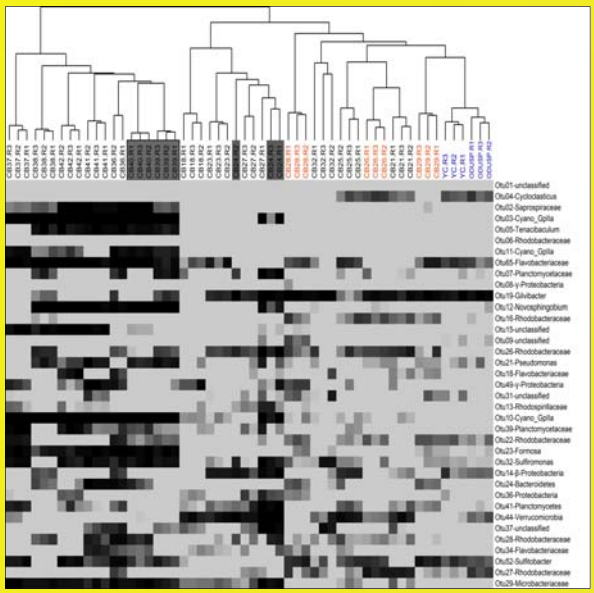


Fig. 4 Average neighbor hierarchical clustering of ballast-water Bacteria assemblages based on the most abundant OTUs (n=37). "Abundant" was defined as an OTU ≥0.5% of total abundance; together these OTUs represent >50% of total abundance (normalized data). Cluster analyses were performed with Kendall's tau nonparametric distance metric. Panel on the right shows the 37 OTUs and their lowest taxonomic affiliation. In the heat map, shading indicates an OTU's abundance in its corresponding sample (light gray=abundant, black=absent). Sample designations: blue font=local Norfolk Harbor; red=tanks not exchanged at sea; grey background=tanks exchanged 100% at sea. All other tanks were exchanged 300% at sea.

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