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Ballast Tank Biofilms are Protected Reservoirs of Microspecies that Challenge Native Biodiversity

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INTRODUCTION

The introduction of exotic microbial species into new ecosystems is a probable pathway for the establishment of non-native species that may have pathogenic effects or disturb a system's natural biodiversity. This research evaluated the natural biodiversity and possible threats of exotic species introductions, using ship ballast tank water, ballast tank biofilms and a subset of microorganisms associated with the biofilms. Previous studies documenting the routine survival of organisms in ballast tanks' water columns are extended here to the ballast tank wall and sediment surface-attached biofilms. **This study detected, enumerated, and documented microstructural patterns of attachment of five "benchmark" species of marine bacteria associated with the biofilms of ballast tanks in ships traveling the world's oceans**.

METHODS & MATERIALS

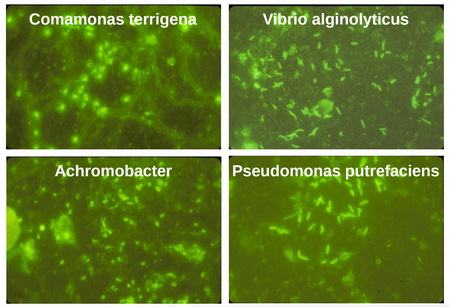
Rabbit antisera were prepared for the following organisms and converted to immunofluorescent reagents:

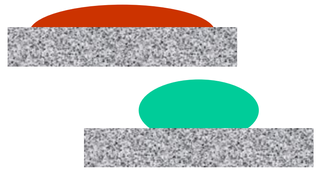
- *Pseudomonas putrefaciens*
- *Pseudomonas sp.*
- *Comamonas terrigena*
- *Achromobacter spp.*
- *Vibrio alginolyticus*

The reagents were used to identify and quantify the five species of bacteria in **biofilms from four ships, three on trans-oceanic routes and one entering the Great Lakes system**.

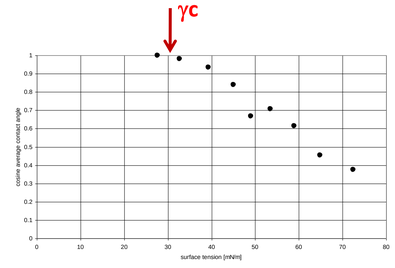
Non-toxic materials and coatings, with pre-characterized and different surface properties, were deployed in the ballast tanks of these ships as a means of acquiring biofilms. **A "ballast organic biofilm" (BOB) sampler and a "portable biofouling unit" (PBU), harbored the test surfaces and coatings inside the actual ballast tanks or as sampled waters from ballast tanks,** providing viable biofilms within which the immunofluorescence staining techniques identified specific bacteria species.

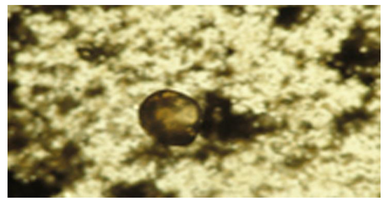
Analysis of test coupons also included multiple attenuated internal reflection infrared spectroscopy and scanning electron microscopy.





Above & Right: Contact angle (theta) measurements of a series of purified diagnostic fluids on test coatings and uncoated controls are used to determine the "Critical Surface Tensions" (γ^c) of the test materials. Contact angle measurements are sensitive to the outermost 0.1nm of the surface chemistry. When plotted as shown to the right, the critical surface tension is determined from linear extrapolation of the data to the top axis, where the contact angle = 0° (cosine = 1). The slope of the line is an indication of surface polarity.

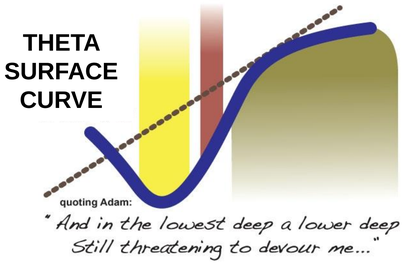


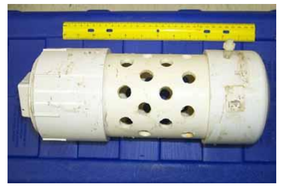


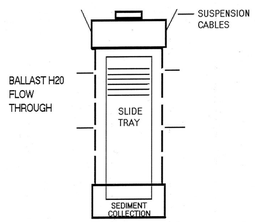
Please visit our website to see a video of active protozoa moving in, under, and above biofilms seeded from ballast water biofilms collected during the study reported here.

www.wings.buffalo.edu/iucb/video.html

Right: The **Theta Surface Curve** summarizes the frequently observed relationship between the critical surface tension of a material and the relative strength of biological adhesion to that material. The dip in the curve (yellow bar) is from 20-30 mN/m; methylsilicones are in this range. Highly fluorinated materials are below 20 mN/m. Very clean metals and ceramics are above 50 mN/m. The **dashed line** represents the relationship between critical surface tension and the adhesive strength of synthetic adhesives.

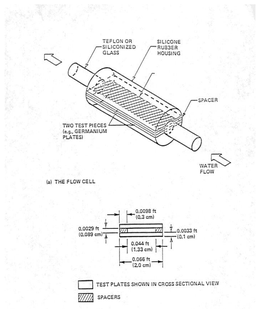






Above & Left: The Ballast Organic Biofilm [BOB] sampler These samplers were suspended in the ballast tanks of the ships that participated in this study. Each of the two trays in each sampler held multiple replicates of the test plates, including different surface coatings, and uncoated controls.

Above: The Portable Biofouling Unit [PBU] This unit was positioned on deck of one of the ships that participated in the study. The tubing is connected to individual parallel plate flow cells through which water sampled from the ballast tank was pumped at a controlled flow rate and shear stress. **Right:** Schematic of the parallel plate flow cell; each flow cell contains 2 test plates *[U.S. Patent No. 4,175,233]*



RESULTS & CONCLUSIONS

Concurrent analysis of test coupons revealed that…

❑ Coatings with Critical Surface Tensions in the range of 20 - 30 mN/m, typically **methylsilicone polymers, changed the normally tightly bound, thin biofilms into looser biofilms (and associated particles), having clustered and more easily detachable patches**.

❑ All five "benchmark" bacteria species were present on all materials and coated materials installed aboard all vessels, **indicating high species persistence with respect to these bacteria over time and geography.**

❑ Ballast tank **biofilms from different vessels revealed different associated small particle compositions, a result of sediment re-suspension during ballast exchange events and at-sea ship motions**.

❑ Ballast tank **biofilm-coated specimens taken from a cross-ocean BOB sampler did release bacteria and other biota into laboratory tanks of particle-free surrounding waters, seeding new biofilms on the tank walls and demonstrating a likely path for suppression of global biodiversity**.

Current studies seek to determine whether wall shear rates associated with ballasting/deballasting are sufficient to release these accumulating biofilms into the ballast water volume where mechanochemical control methods may be effective at much lower doses than required for disinfection of microorganisms in the biofilm state.

QUESTIONS?

Please contact baier@buffalo.edu

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