Cadmium resistance in bacteria from the Great Sippewissett Marsh

and

Isolation of Anaerobic Bacteria from Fish Intestinal Contents

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Microbial Ecology - Summer 1980
During the past 6 years a project pursued by the Ecosystems Center at MBL and the Boston University Program has involved adding a fertilizer to specified plots in the Great Sippiwissett Marsh. The fertilizer is a commercially-prepared sewage sludge found to contain substantial amounts of heavy metals, including Cd, Cr, Cu, Zn, Pb, Mn and Fe. Unpublished information on amounts found in the fertilizer and in the sediments is available from the BUMP offices. This fertilizer is broadcast during low tide every two weeks from late April to November, to these specified plots. A question which seemed logical to pursue was whether resistance to any heavy metal had occurred among the bacteria in sediments from these sites.

Three plots were sampled: #7-control-no fertilizer, #6-fertilized for 6 yrs. with Milorganite-Wilwaukee source, #13-fertilization begun in April, 1980. For location of these plots, see BUMP people. A 10cc. syringe, adapted for coring was used to obtain 2cm. cores of sediment from each site. 1 g. samples of the top 1 cm. of sediment were taken, diluted in 75%W, dilutions made and plated on 2 kinds of medium: 1. Zobell's (2216E) and 2. diluted Zobell's (2216E/100). 4 types of each of the media were prepared: unsupplemented: Cd, and supplemented: with 10⁻³, 10⁻⁴, and 10⁻⁵M Cd. See Figure 1-flow sheet of plating procedures. Colony counts were made for each type of medium. See Figures 2 and 3 for plotted results. The results indicate a number of things. First, that there are Cd-resistant type bacteria present, found on the fertilized plots, but not on the unfertilized; second that there are more colonies of resistant bacteria found on the plot subjected to
fertilization for the longer period than the plot getting fertilizer only this year; third, that a concentration of $10^{-3}$ M. Cd is a selective concentration for resistant types of bacteria on the concentrated medium. On the diluted medium $10^{-5}$ M. is the selective concentration for resistant types. $10^{-3}$ M. Cd in the diluted medium is lethal. No growth is seen at all.

From this latter observation regarding concentrations of Cd which are selective for resistant bacteria, a question concerning the 2 types of populations—one grown on conc. medium vs. the one grown on dilute medium—was asked as to whether the difference noted was due to a resistance built up by the organism or, was the difference noted due to the medium used?

In order to answer this question, an experiment was designed which would compare growth obtained by isolates from each type of medium and allowed to grow not only on the medium from which they were isolated, but also on the other medium, either diluted or concentrated, whatever the case was. See Figure 1 flow sheet for protocol of plating. 10 clones from the original plates were then selected and dispersed in 1 ml. of 75% sterile SW. Each inoculum was then dropped on the medium from a Pasteur pipette (1 drop), bacteria allowed to grow, and amount of growth recorded as a range from 4 to 1. Figures 4 and 5 summarize the findings. Conclusions which can be made from these graphs would include the following: First, isolates taken from the concentrated medium containing $10^{-3}$ M. Cd were unable to tolerate this concentration on the diluted medium; second, bacteria which were grown on the diluted medium were not only unable to grow on the diluted medium with $10^{-3}$ M. Cd, but also on the concentrated medium with $10^{-5}$ M. Cd. They were able to grow on the diluted medium with
$10^{-4}$ M. Cd, but very poorly. On the concentrated medium with $10^{-4}$ M. Cd, they grew extremely well, at least equal to or almost equal to their growth on diluted medium with no Cd. This suggests that the presence of greater amounts of organic material may have provided a more favorable environment for detoxification or provided nutrients which could be used by the bacteria or merely chemically tied up the Cd.

This is only a preliminary study. Additional replicate samplings should be made, isolation of pure cultures, and characterization of the bacterial population found in these plots is needed. Further work would include a more precise determination of toxic levels of Cd to determine what level is lethal. Laboratory-derived resistant types from controls will be attempted to be grown on Cd-supplemented media to determine length of time for development of resistant types. The mechanism of resistance in both naturally selected and laboratory derived resistant types will then be pursued.
Figure 1. Plating Protocol
Figure 2: Comparison of growth of isolates from 3 plots at different Cd concentrations.
Figure 3. Comparison of growth of isolates from 3 plots at different Cd concentrations.
Clones from Concentrated Medium

OLD  NEW  CONTROL

Amount of Growth

Cd Conc. (M.)

Media used for Growth of Clones
- Concentrated
- Diluted

Figure 4.
Figure 5.
Isolation of Anaerobic Bacteria from Fish Intestinal Contents

0.5 ml. of intestinal contents were obtained from scup and dogfish, immediately after catching and dissecting the fish. The contents were removed with a nitrogen-gassed syringe and put into test tubes containing nitrogen. Dilutions were then made and plated. Media used were brain-heart infusion agar and medium "F" (cf. Postgate: Sulfate-reducing Bacteria). Plates were prepared prior to obtaining samples and left to become anaerobic in the anaerobic-glove-chamber (indicator: resazurin). Transfers of 0.1ml. aliquots of dilutions were made to these plates in the anaerobic chamber and the bacteria allowed to grow. Colony counts were made and the following results were obtained:

<table>
<thead>
<tr>
<th>Medium</th>
<th>Scup</th>
<th>Dogfish</th>
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<tbody>
<tr>
<td>BHI</td>
<td>(5.4 \times 10^8)</td>
<td>(2 \times 10^9)</td>
</tr>
<tr>
<td>&quot;F&quot;</td>
<td>(4.5 \times 10)</td>
<td>(2 \times 10)</td>
</tr>
</tbody>
</table>

Note: medium "F" is specific for sulfate-reducers. Colonies were counted by noting areas of blackness. Colonies found on BHI were transferred aseptically (approximately 15 colonies from each fish sample) to BHI agar and left in the air. All were found to grow also aerobically.

Hence the colonies of bacteria isolated on BHI anaerobically were presumably facultative anaerobes. No colony tested from BHI plates was found to be strictly anaerobic. Obviously, the sulfate-reducers were anaerobes. In addition, aliquots of dilutions from the scup were plated onto sea water complete agar (SWC) with the following results:

Total colony counts/ml of intestinal contents: \(4.5 \times 10^8\), and of this number \(4 \times 10^7\) were found to be luminous bacteria.
Sept. 1, 1980

Dear Holger,

Enclosed find the last page of my report which I couldn't complete on 8/22, due to having already packed the books and notes. It was literally unaccessible unless I unpacked the entire back seat of the car!

I want you to know that I found the course and research session very helpful and profitable intellectually—not only in content, but also in techniques, most of which I hope to use during the academic year.

I have brought back some pure cultures isolated from the marsh and will work with them regarding the Cd resistance. I also have some of the sulfate-reducers and methanogens. Perhaps some work can be organized here in regard to some freshwater fishes.

I again hope to apply for the Steps Towards Independence to continue work. After seeing how far I can work on what I've brought back, perhaps a good followup can occur next summer.

Thank you for everything. Meeting you and your family and working with you has been most enjoyable. Have a good year.

Sincerely,

Sr. M. Francesca Mollura

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