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Technical Report

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Contract No.: DE FG-22-91PC-91289

Project Title: Characterization of the Organic-Sulfur-Degrading Enzymes

Principal Investigator: Nancy W.Y. Ho
LORRE
Purdue University
1295 Potter Building
West Lafayette, IN 47907-1295

OBJECTIVES

The immediate objective of this project is to characterize and purify the enzymes involved in degrading organic sulfur in coal from two well characterized organic sulfur degrading strains, IGTS8 and K3B. We believe that characterization and purification of these enzymes may provide valuable information that will lead to developing or isolating better strains for desulfurization of coal. Our original plan was first to characterize these enzymes, followed by purification of these enzymes, particularly the IGTS8 enzymes and cloning and overexpressing the genes encoding these enzymes in IGTS8 or other better organisms.

Our recent results imply that the IGTS8 enzymes are firmly attached to the cell wall, most likely that they are covalently bonded to the cell wall. These results have changed our overall approach in solving the coal desulfurization problem. We believe that for coal desulfurization, it is better to have a microorganism that can secrete its desulfurization enzymes into the medium. The enzymes can penetrate into coal hundreds of times better than a bacterial cell. Thus we have changed the priority of this project.

We believe that the isolation of a mutant of IGTS8 which can release the desulfurization enzymes in the cultural medium will be able to develop a much improved microbial process for coal desulfurization. Furthermore, the isolation of such a mutant will be able to shed some light on how the wild type IGTS8 desulfurization enzyme(s) are attached to the cell wall.

Research Performed

During this period, we carried out more electron microscope analysis of IGTS8 and related species. Specifically, we analyzed *R. rhodochorous* strains ATCC184 and ATCC13808. Both these strains grow very poorly in DBT medium as well as in medium containing high sulfate concentration. The cells used for EM analysis were cultured in rich medium with glucose (the LB medium containing 1% trypton, 1% sodium chloride, 0.5% yeast extract, and 0.1% glucose). We found that there is no protruding structures on the cells of *R. rhodochorous* 184 and 13808 as shown in Figure 1 (see our reports dated March 1 and June 1, 1992). However, we believe that we should also examine the 183 and 13808 cells that have been cultured in the presence of DBT. We are currently investigating whether it is possible to grow enough of these cells using DBT as the sole sulfur source for EM studies.

We also carried out EM analysis of IGTS8 cells that have been replicated on LB plates for numerous generations. Such IGTS8 cells were cultured in rich LB medium and we found that such IGTS8 cells also contained no protruding structures as shown in Figure 1 (see reports dated March 1 and June 1, 1992).

These results, particularly those obtained from EM analysis of IGTS8 cells cultured in rich LB medium, indicate that the protruding structures (see reports dated March 1 and June 1, 1992) is the direct result of culturing the IGTS8 cells in medium relying on organic sulfur compounds such as DBT as the sole sulfur source.

In this period, most of our efforts were devoted to the study of whether it is possible to isolate intact active desulfurization enzymes from large volumes of culture medium of IGTS8 and K3B. Previously we tried to isolate intact active desulfurization enzyme from IGTS8 culture medium by using ultrafiltration with Centroprep. The largest volume can be done in a reasonable time period is about 100 ml. From 100 ml culture medium, we did not obtain detectable active enzymes from either IGTS8 or K3B. We also were not successful in trying to isolate active enzymes by ammonium sulfate precipitation with culture medium as large as 500 ml. The failure to detect active enzyme activity in the above described experiments may be due to too small a volume for Centroprep experiments inactivation of the by ammonium sulfate in the second set of experiments. Thus we decided to carry out large scale ultrafiltration experiments by using Amicon ultrafilters. From 1000 ml of cultural medium, we still cannot isolate sufficient IGTS8 enzymes to obtain meaningful results by using Gibbs assay. However, from 1000 ml of culture medium of K3B, intact active K3B enzymes were detected. The results are shown in Figures 2. These experiments have been repeated four times. Similar results were obtained.

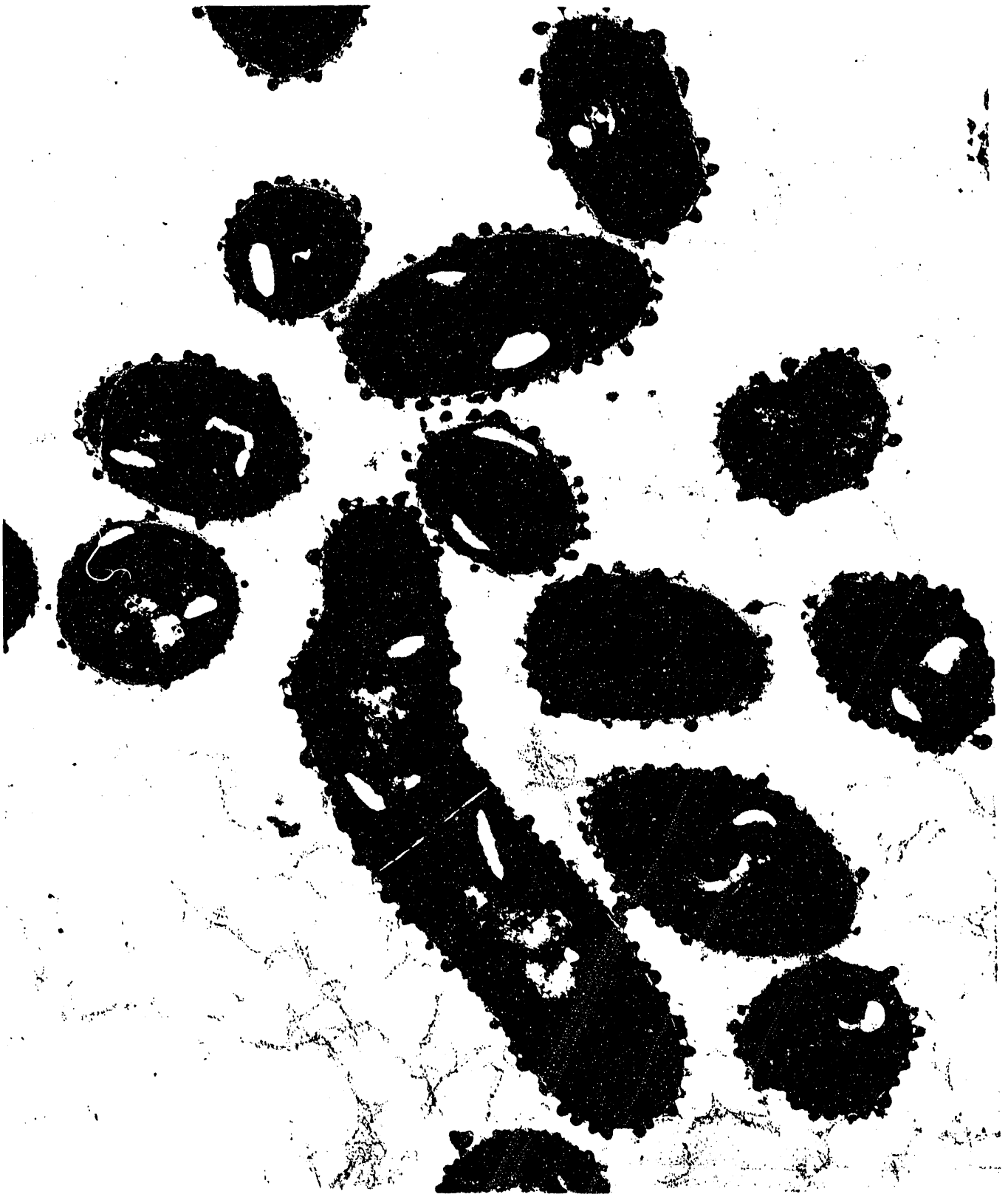

Figure 1. **IGTS8 cultured in DBT**

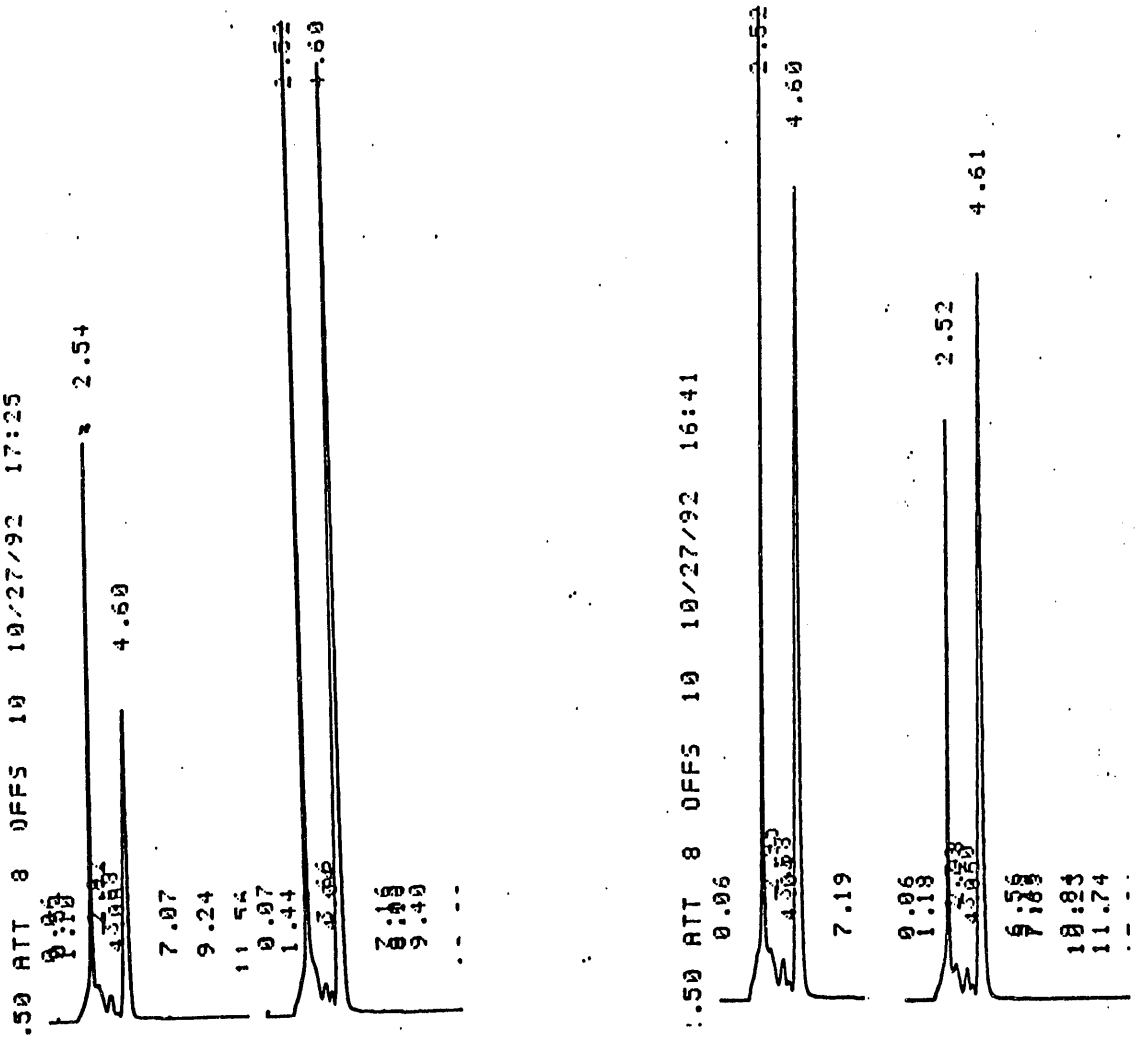

Figure 2 I. K3B cells grew to OD_{600} 0.4, the cells were removed by centrifugation. 100 ml supernatant was concentrated to 4 ml with Centriprep-10 (MW cut off 10,000 Amicon, MA). 2 ml was boiled for 5 min., 2 ml was not boiled. Both were incubated at 30°C in the presence of DBTS for 5 hr. At the end of incubation, excess DBTS was removed and the products were analyzed by HPLC (Col. C18, solvent 45% CH_3CN, DBTS retention time: 4.6).

A. Products from not being boiled concentrates.
B. Products from boiled concentrates.

We interpret these results as in the un-boiled sample, it contains enzymes which convert DBTS to other products. In the boiled sample, the enzyme denatured so that DBTS was not converted to any products.

II. Same experiments except Centriprep 30 was used to concentrate the supernatant.

A. Products from the unboiled concentrates.
B. Products from the boiled concentrates.

We interpret these results as the enzymes probably smaller than 30 K so that they were filtered out. Thus both the boiled and unboiled samples did not contain any enzyme activity. Thus, there were a lot of DBTS present in both reactions.

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