**Materials and Methods**

Soil Collection

To isolate SRB from soil, organic soil samples were collected from two locations along Otter Creek, with moist or wet sites selected. The first sample was from a drainage pond close to the Addison County Transit building in Middlebury, VT (“ACTR location”), and the other farther south along the bank of Otter Creek (“East Middlebury location”). Both sediment and the water above it were obtained from both sites. Ten-fold serial dilutions were performed in sterile water.

Winogradsky Column samples

SRB were also isolated from Winogradsky columns prepared earlier in the semester at Middlebury College. Columns were generated using pond water, mud, and supplementary compounds as described by Allen and Spatafora, 1 and were grown for eight weeks with abundant sunlight prior to the cultivation of SRB. SRB are thought to grow primarily in the anoxic sediment of the column. Samples of this sediment were removed from the column, and ten-fold serial dilutions were performed in sterile saline.

Selection of SRB

Samples from environmental soil and Winogradsky columns were plated on TSA (or LB) media supplemented with filter sterilized 150 (or 75) mM FeSO4**·**7H2O. This compound was necessary for SRB cultivation as a sulfate source for accepting electrons, and also provided a visible marker of SRB since iron reacts with the H2S waste product of sulfate reduction to produce a black precipitate. Cultures were grown anaerobically for (5-10) days at 37˚ (or room temperature), within the optimum temperature range for the growth of *Desulfovibrio* sp.2 and other SRB. Anaerobic culture conditions were generated with a GasPak (BC) in a sealed jar. Following incubation, black colonies were selected from plates and used to inoculate thioglycollate broth supplemented with 150 (or 75) mM FeSO4**·**7H2O. Additionally, broth cultures were supplemented with a 3% solution of NaSO3**·**7H2O, which has been used previously to inhibit the growth of competing and/or contaminating bacteria. 3

Confirmation of SRB Isolation

Various biochemical tests were performed to determine whether the cultivated microbes were sulfate-reducers, which produce characteristic black iron precipitates and a distinctive rotten eggs smell due to the production of H2S. SRB are gram negative organisms, and further tests, summarized in the tables below, were used to support the identity of the isolated organisms as SRB. The experimentally produced SRB were compared to positive and negative controls, enumerated below in Table 1.

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| --- | --- | --- | --- |
| Biochemical Test | SRB(*Desulfovibrio* sp.) | Positive control | Negative control |
| Gram Stain | Gram Negative | *E. coli* | *Staphylococcus aureus* |
| Triple Sugar Iron Agar | H2S/Gas produced | *Proteus vulgaris* or *Salmonella typhimurium* | *E. coli* |
| Sulfide, Indole, Motility Test | H2S | *Proteus vulgaris* | *E. coli* |

Table 1: Biochemical tests and controls to verify SRB.

|  |  |  |  |
| --- | --- | --- | --- |
| Biochemical Test | *Desulfovibrio* | Positive control | Negative control |
| Oxygen Profile: anaerobic jars and thioglycolate broth | Aerotolerant | *Pseudomonas aeruginosa*: aerobe;  *Escherichia coli*: facultative aerobe | *Clostridium beijerinckii*: strict anaerobe |
| Catalase Test | Positive | *Staphylococcus aureus* | *E. coli* |
| Motility | Positive | *P. aeruginosa* | *S. aureus* |
| Motility: Flagella stain | Positive | *Pseudomonas fluorescens* | *E. coli* |
| Antibiotic Resistance/Sensitivity | Sensitive to Penicillin4 | *S. aureus* | *E. coli* |

Table 2: Biochemical tests and controls to distinguish *Desulfovibrio* species, a characteristic SRB.

1. Allen & Spatafora. 2017. BIOL310: General Microbiology Laboratory Manual. Twelfth Edition.

2. Gilmour CC, Elias DA, Kucken AM, Brown SD, Palumbo A V., Schadt CW, Wall JD. 2011. Sulfate-reducing bacterium Desulfovibrio desulfuricans ND132 as a model for understanding bacterial mercury methylation. Appl Environ Microbiol 77:3938–3951.

3. Butlin KR, Adams ME, Thomas M. 1949. The isolation and cultivation of sulphate-reducing bacteria. J Gen Microbiol 3:46–59.

4. Warren YA, Citron DM, Merriam CV, Goldstein EJC. 2005. Biochemical Differentiation and Comparison of *Desulfovibrio* Species and Other Phenotypically Similar Genera. J Clin Microbiol 43:4041–4045.