**Isolation of Sulfate-Reducing Bacteria from Stream-Bank Sediment and Municipal Wastewater**

Sulfate-reducing bacteria (SRB) constitute a group of diverse, primarily chemolithotrophic microorganisms. Sulfur, present in abundance in the anoxic primordial Earth, can assume a wide range of oxidation states from its most oxidized form, sulfate (SO42-), to its most reduced form, hydrogen sulfide (H2S). Due to its redox versatility and availability, early microbes utilized sulfur chemistry to drive metabolic processes. This is exemplified by the SRB, which produce a proton motive force to drive production of ATP through the reduction of sulfate to hydrogen sulfide. This is then released as a waste product of the dissimilatory sulfate reduction pathway. SRB are anaerobic because oxygen is not the final electron acceptor. [[1]](#footnote-1) However, certain species may be tolerant of some levels of oxygen, and hence are aerotolerant. Because of their chemical requirements, SRB are commonly found in freshwater sediments, sewage, and other environments with a high concentration of sulfate and a low concentration of oxygen, including the human oral cavity. [[2]](#footnote-2)

Research on members of the genus *Desulfovibrio* has elucidated many of the key metabolic processes in SRB. An electron transport chain consisting of cytochromes, flavoproteins, and iron-sulfur proteins is used to generate a proton gradient across the bacterial cell membrane. Electrons may be donated from diverse sources that vary greatly among SRB, but commonly include lactate or pyruvate.[[3]](#footnote-3) The terminal electron acceptor is sulfate from environmental sources. Sulfate is first reduced to sulfite (SO32-), a process that requires ATP hydrolysis. Sulfite is next reduced to sulfide (S2-), which is released as hydrogen sulfide, utilizing a second molecule of ATP. This post-electron-transport-chain reductive process incorporates eight electron transfers, use of two molecules of ATP, and is catalyzed by four enzymes.[[4]](#footnote-4) ATP is ultimately synthesized through the dissipation of the proton motive force as protons move down their concentration gradient through an ATP synthase integral protein.

SRB have evolved along with the sulfur-oxidizing bacteria and they contribute greatly to the balance of sulfur, sulfate, and sulfide in the sulfur cycle. [[5]](#footnote-5) In deep layers of sediment, sulfur compounds are present at low concentrations. Therefore, interdependence between SRB and sulfur-oxidizing bacteria evolved to ensure each group has access to enough of its required resource, produced by the other. [[6]](#footnote-6) The study of SRB is thus an examination of the coevolution of two groups. Furthermore, an understanding of the resulting metabolic interdependence is essential for the successful cultivation or reduction of these organisms.

The study of SRB also has numerous industrial applications. SRB are present in hydrothermal vents, and therefore can have impacts on the oil and gas industries. [[7]](#footnote-7) Because one byproduct of sulfate reduction is hydrogen sulfide, which can react with sources of iron in machinery for extracting crude oil to form a precipitate, SRB can cause industrial inefficiencies. [[8]](#footnote-8) They also thrive in industrial wastewater. The contamination of the environment or equipment with a precipitate, color, or hydrogen sulfide smell makes the study and control of SRB a priority for these industries. Although SRB are slow growing, they are able to withstand unfavorable conditions. Some species even form endospores and can therefore exist in a dormant state during anti-SRB treatment.[[9]](#footnote-9) The study of SRB and how to remove them from an environment is both a challenge and a priority for oil industries as well as water treatment plants.

This project centers on isolation of SRB from environmental samples such as stream-bank sediment and wastewater. Although SRB thrive in a multitude of environments, these sources were selected as common representative locations of SRB in the state of Vermont, where SRB contribute a key role to the sulfur cycle as described above. These locations contain abundant sulfates and potentially low concentrations of oxygen, suitable conditions for the growth of SRB. Laboratory culture conditions sought to reproduce these conditions through anaerobic culture and biochemical selections, allowing for SRB to be cultured and isolated from these sources. In closing, this work is significant because an understanding and optimization of SRB cultivation will allow experimentation of inhibitory compounds, which may be applied in both industrial and municipal settings to halt the growth of SRB.

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