**Background**

SRB (sulfate-reducing bacteria) (1)

Opportunistic Pathogen Potential

Obligately anaerobic, Slow growing, Fastidious (2)

Dissimilatory reduction of sulfate produces hydrogen sulfide

Numbers of SRB limited by the energy source sulfate.

Require free sulfate for growth.

H2S toxic (3)

SRB require electron accepter for growth, usually sulfate. Sources for anion may be dietary or endogenous. high sulfated food = fermented beverages, dried fruits, white bread. (4)

Obligate anaerobes.

Organic compounds or inorganic ions as terminal e acceptor.

Lack catalase, killed by oxygen. (Lab manual pg 41)

Sulfate (SO4 2- ) is most oxidated form of sulfur.

Reduction to produce H2S requires 8 e.

Step 1: SO4 2- activated using ATP and ATP sulfurylase so that it attaches 🡪adenoside phophosulfate (APS) and elemental PPi

Step 2: Dissimilative: SO4 2- in APS reduced to sulfite (SO3 2-) using APS reductase. (2e in, AMP out)

Step 3: SO3 2- reduced to H2S via sulfite reductase. (6e in)

Provides H2S and free energy to make ATP.

Electron transport chain generates proton motive force.

H+ moves across membrane/down gradient through ATPase.

ATP synthesis conversion of ADP🡪ATP.

Sulfur-Reducers.

Also capable of energy conservation by reducing elementatl S and (source of electrons) 🡪 H2S.

Sulfur reducers lack the capacity to activate sulfate to APS.

Use elemental S instead. Sulfur is the major e acceptor used in nature.

Reduction of oxidized sulfur compounds and the production of H2S that connects the sulfur and sulfate reducing bacteria.

(Book pg 421-423)

Sulfur reducers also reduce e acceptors such as nitrate, ferrous iron, thiosulfate as alternative to elemental S.

Anoxic sulfur cycle. Sulfur reducers + bacteria(H2S 🡪S0) (Book pg 658)

Sulfur used in amino acids.

Any sulfur compound with ox state above sulfide (-2) can be e acceptor for oxidation of carbon substrates.

Dissimilatory: sulphate ion used as oxidant for degradation of organic material

All sulphate reduced is released as sulphide. 🡪H2S.

H2S significant able to inhibit the growth of aerobic org.

Requires inorganic e acceptor.

Type of Carbon source used for reduction of acceptor varies by genus.

Acetate and Hydrogen are essential substrates.

Metabolic end product is sulphide, converted to H2S with exterminal H+

Activity can be detected to odour of H2S

Sulphide is corrosive/binds rapidly to metals. (5)

**Materials/Methods**

Found in periodontal pockets, gastrointestinal tract

Found in sea water, water sediments mud

Belongs in normal microflora of human oral cavity (1)

Ubiquitous in nature

Sewage, industrial effluents, water, soil (2)

Habitats of high sulfate concentration and anaerobiosis

sediments, sewage sludge, and colons. (4)

Samples incubated in anoxic chamber, 37 degrees C

3-7 days until black colonies

Reduced-growth medium for SRB

Iron indicator for sulfide production

DRCA agar

TSN agar (1)

Chocolate agar

5 days, 37 degrees C

Not good in broth medium, better to use solid.

May take 5-7 days for a colony to appear. (2)

Semisynthetic basal medium

pH 7.2

medium made by sterilizing separate solutions that were aseptically combined under anaerobic conditions.

More details in article.

All incubation in anaerobic chamber. (3)

Solution details in article.

Autoclaved at 121 C for 15 minutes.

Anaerobic = oxygen free, nitrogen headspace. (4)

Anaerobic Methods.

Candle Jar. Burning convert oxygen to CO2 and Water.

Gas-Pak system. Chemical packet consumes oxygen when water is added. (Lab Manual pg 41)

Anaerobic chemostat enrichments. Chemostat = continuously add medium.

Sodium acetate as carbon source (2g/l)

Inoculated with 50g freshly collected sediment.

2 day intervals, 10ml samples aseptically removed.

Aliquot volumes transferred to agar shake dilution tubes (same medium)

Substrate Utilization Tests.

25 degrees C, up to 7 days

Gram stain at the end to confirm absence of contaminants.

Chomostat enrichments using acetate as carbon source were successful, target species becoming dominant after 12-14 days

Table 1: morphology of acetate using-sulfate reducing bacteria.

(6)

Found in sediment.

Use enrichment/mixed inocula or chemostat methods.

Batch culture: closed. No need to add. Simpler.

However, might need chemostat.

SRB are slow growing. Unable to compete in enrichment system?

Anaerobic plating do not distinguish bw different genera

Found in marine/estuarine sediments.

Saline ponds.

Especially where habitat contaminated by alternative nutrients from sewage effluent.

Anaerobic, but found in low conc in oxic conditions.

Also polluted environments such as spoiled food, sewage platnts (5)

Identify using respiratory type, morphology, cultural aspects, stain affinity, gram stain

Further test: evidence of motility, production of only H2S, catalase test, sensitivity to kanamycin, colistin, vancomycin. (1)

Tiny, pinpoint, round colonies

Gram negative

Curved/rod-shaped

Single polar flagellum (2)

Presence indicated by strong blackening due to precipitation of FeS.

Morphological criteria and substrate utilization tests to identify (3)

2 categories for those that use lactate for carbon/energy:

spore-forming straight

curved rods

Identify using motility tests, Gram reaction, cell size, morphology

(5)