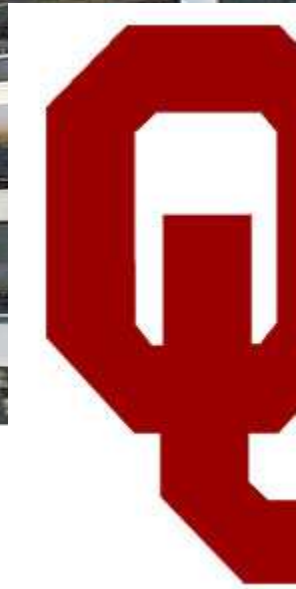


Investigating Pathways of Sulfidogenesis in Sulfate-Reducing *Halanaerobium* Species



Annette De Capite

Department of Microbiology, University of Oklahoma

Sulfidogenic Bacteria

Sulfate-reducing bacteria

Sulfate (SO_4^{2-})

Sulfite (SO_3^{2-})

Sulfur-reducing bacteria

Elemental sulfur (S_8)

Thiosulfate-reducing bacteria

Thiosulfate ($\text{S}_2\text{O}_3^{2-}$)



Observations

-4 M Cl⁻, 49°C

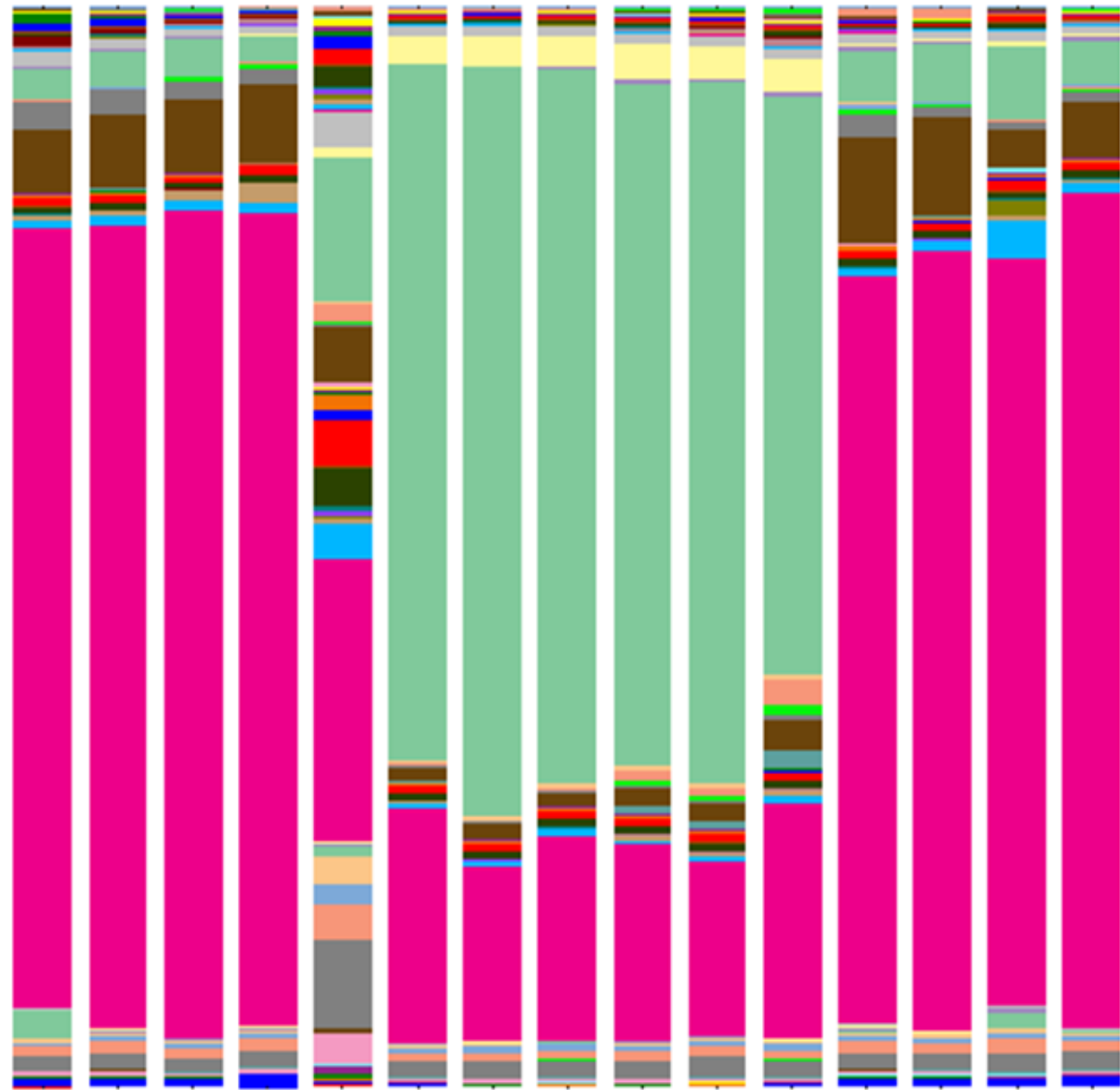
Halanaerobium!

SRB?

anaerobic fermenters

common to high salt, high temperature oil production

US.



Middle East Field 16S Results (OU Biocorrosion)

Materials

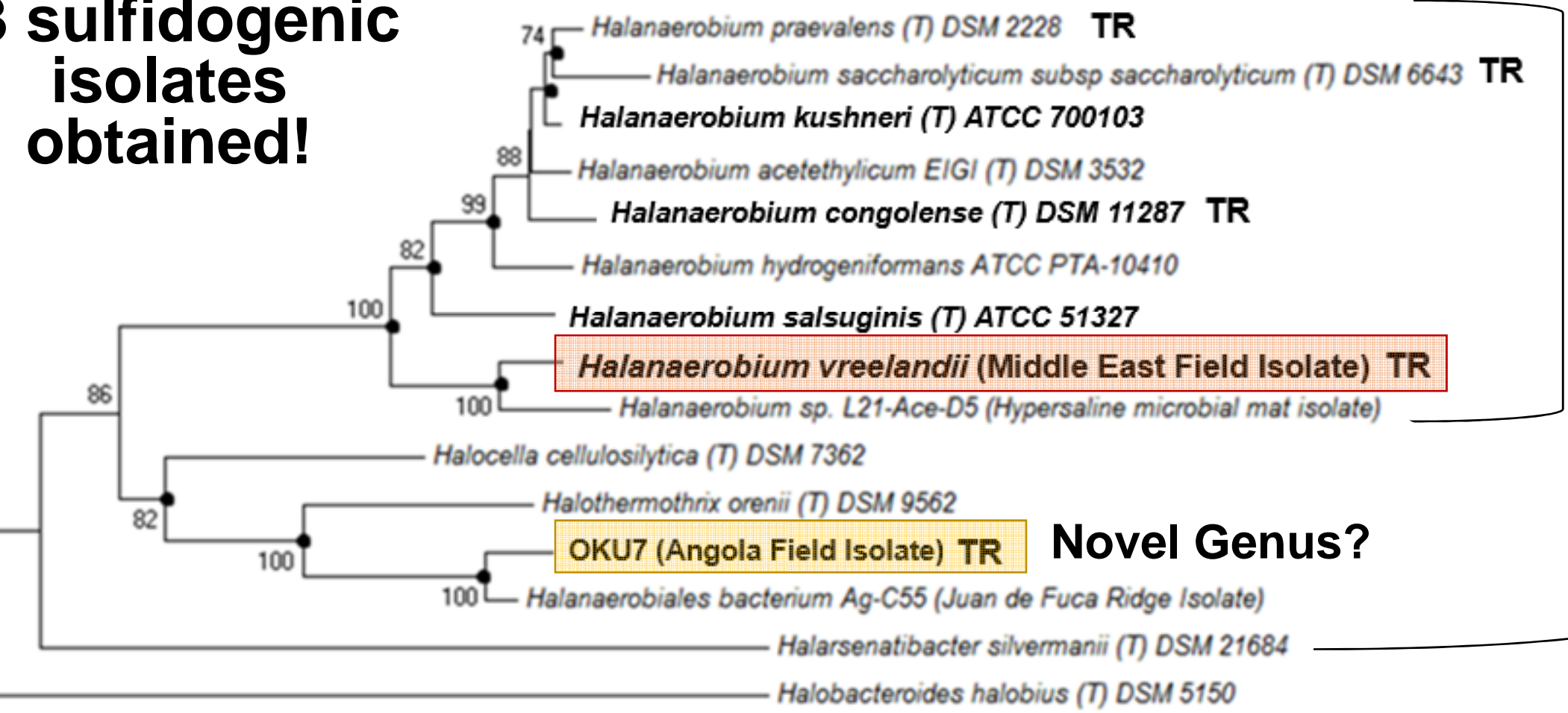
Sample	[Cl ⁻] (Molar)	Temperature
Angola production water	1.5	49°C
Middle East Field production water	2.5	49°C
European Oil Reservoir	2.5	37°C

High Temperature, High Salt Production Water

Environments established under fermentative and thiosulfate-reducing conditions

Halanaerobiaceae Isolation Results

3 sulfidogenic isolates obtained!



Novel Genus?

TR denotes species confirmed for thiosulfate re...
Bold strains represent species used in this ...

Genus *Halanaerobium*

Thiobacillales

Obligate Anaerobes

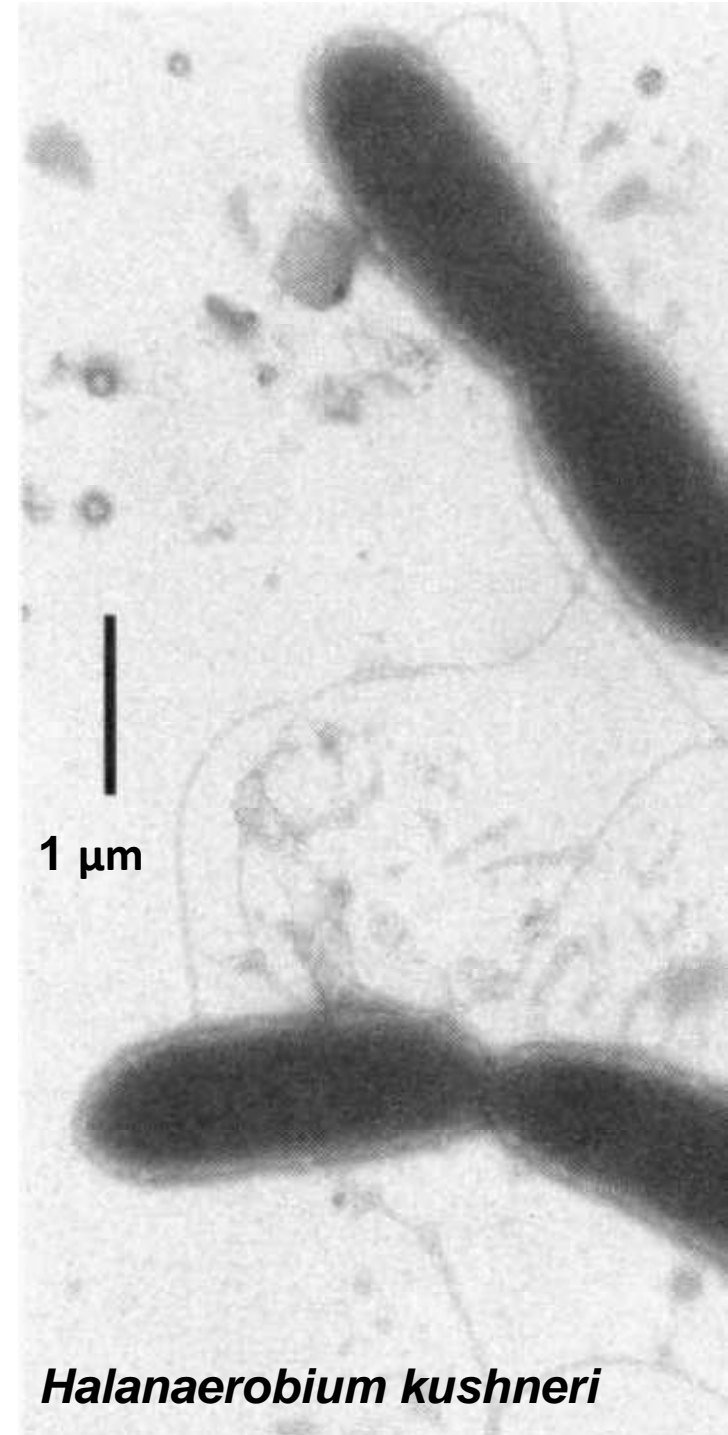
Obligate halophiles

Isolated from saline production waters, brine lakes, and microbial mats

fermentative metabolism

Do not reduce sulfate!

Some species produce sulfide from the reduction of thiosulfate and sulfur.



Sources of Sulfate and Thiosulfate

ate

duction waters

water

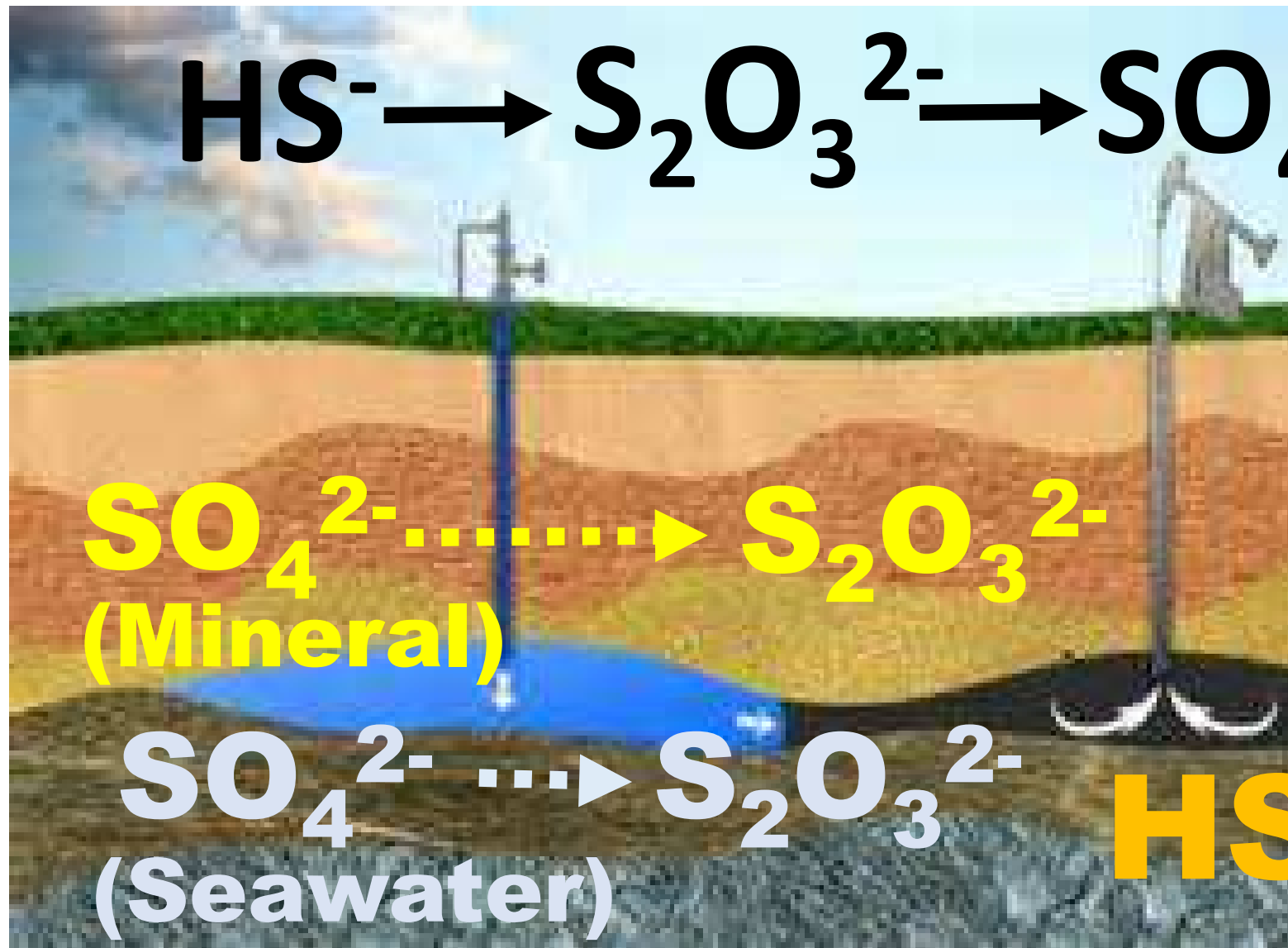
conversion to
er sulfur anions

sulfate

duction waters

water

ation of
ides



Molecular Detection of Sulfidogenic Bacteria



16S rRNA gene
Functional gene

Applications

Assess if microbial community has the genetic potential for sulfidogenesis.

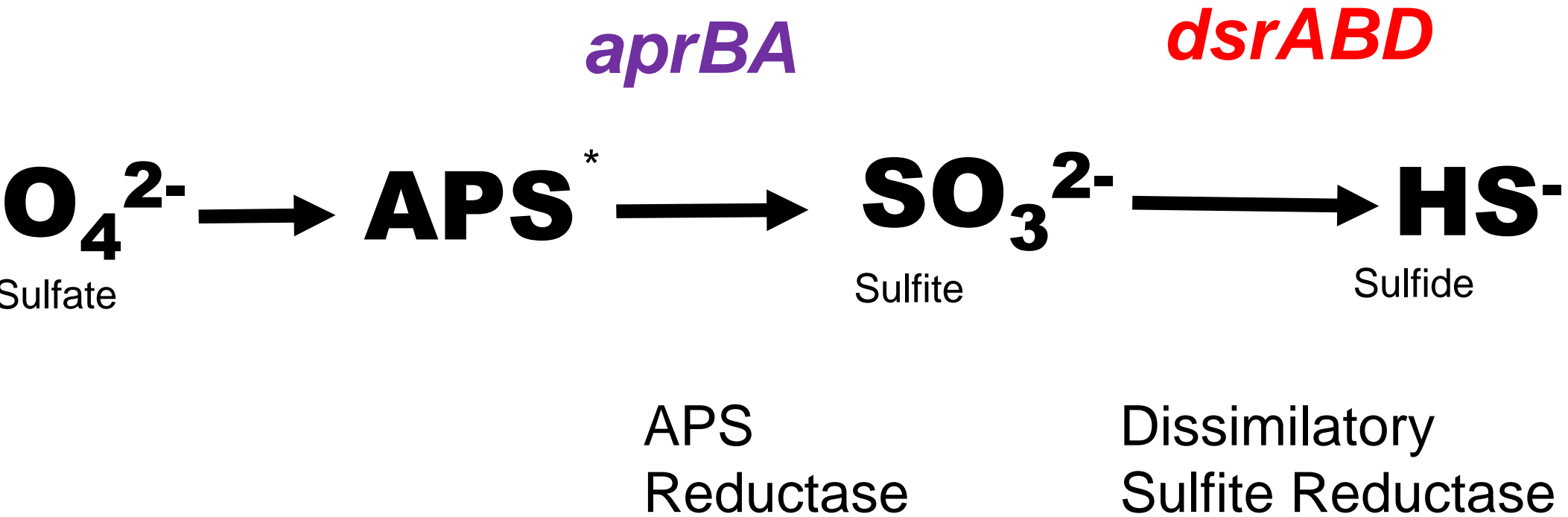
Caveats

Does not confirm viability of bacteria or activity of sulfidogenic enzymes.

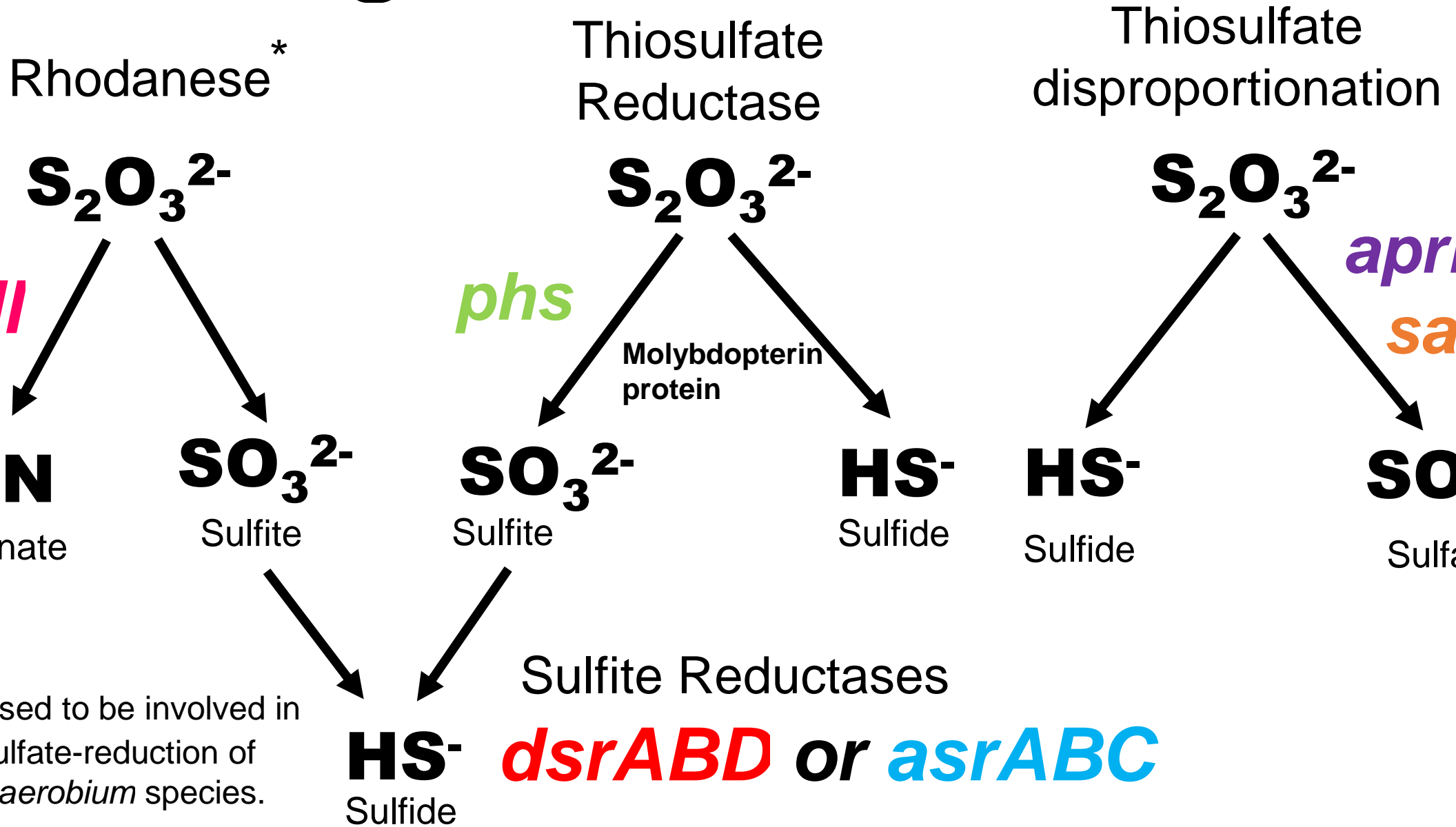
Cultivation and physiology

Gene Targets for SRB

Amplification \neq Enzymatic Activity



ene Targets for TRB



Investigating Enzymatic Pathways

Assess if other sulfur anions are reduced

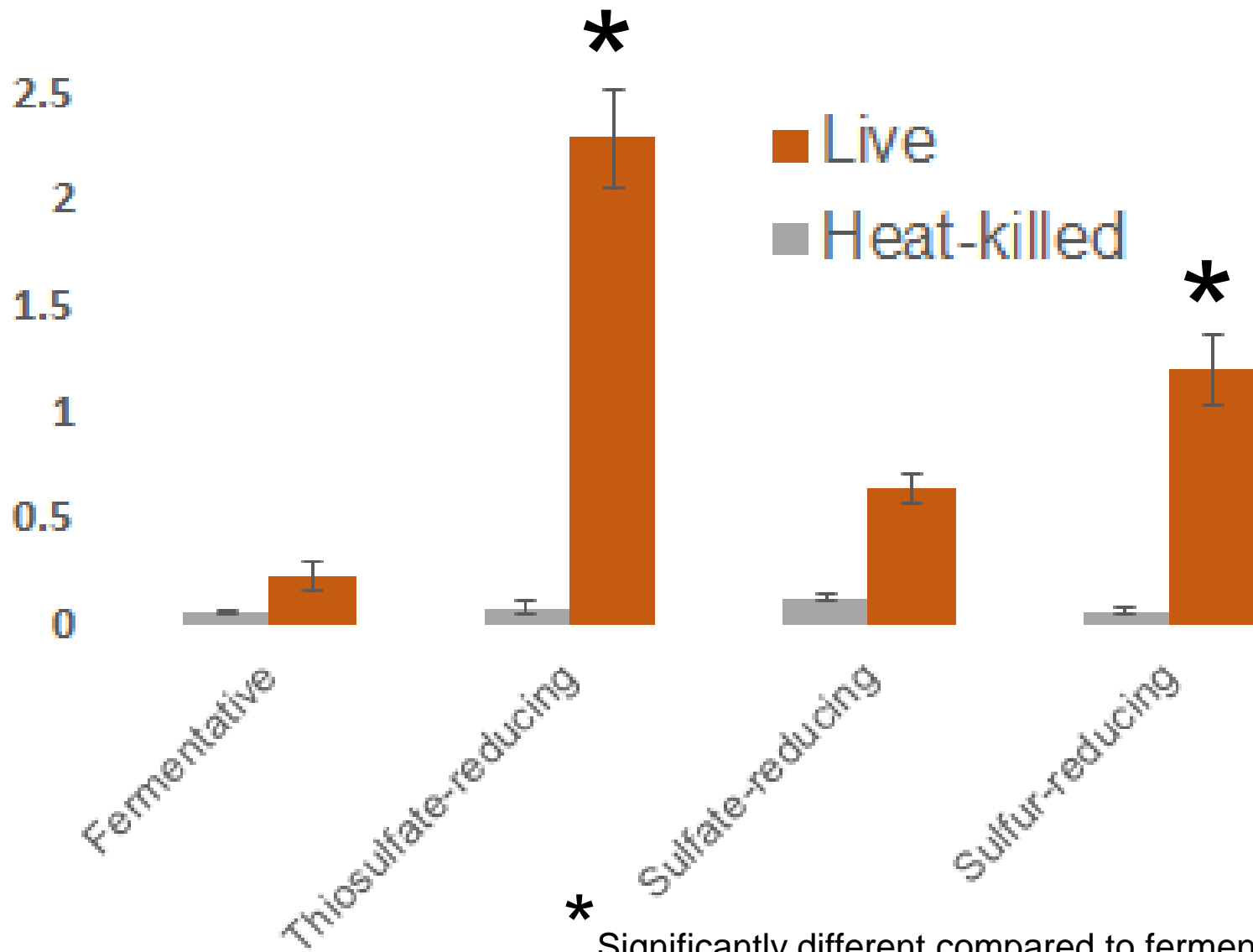
Evaluate rhodanese activity

Identify end products of thiosulfate reduction

Determine sulfide/thiosulfate ratio

Genomic analysis

Sulfidogenesis in *H. vreelandii*

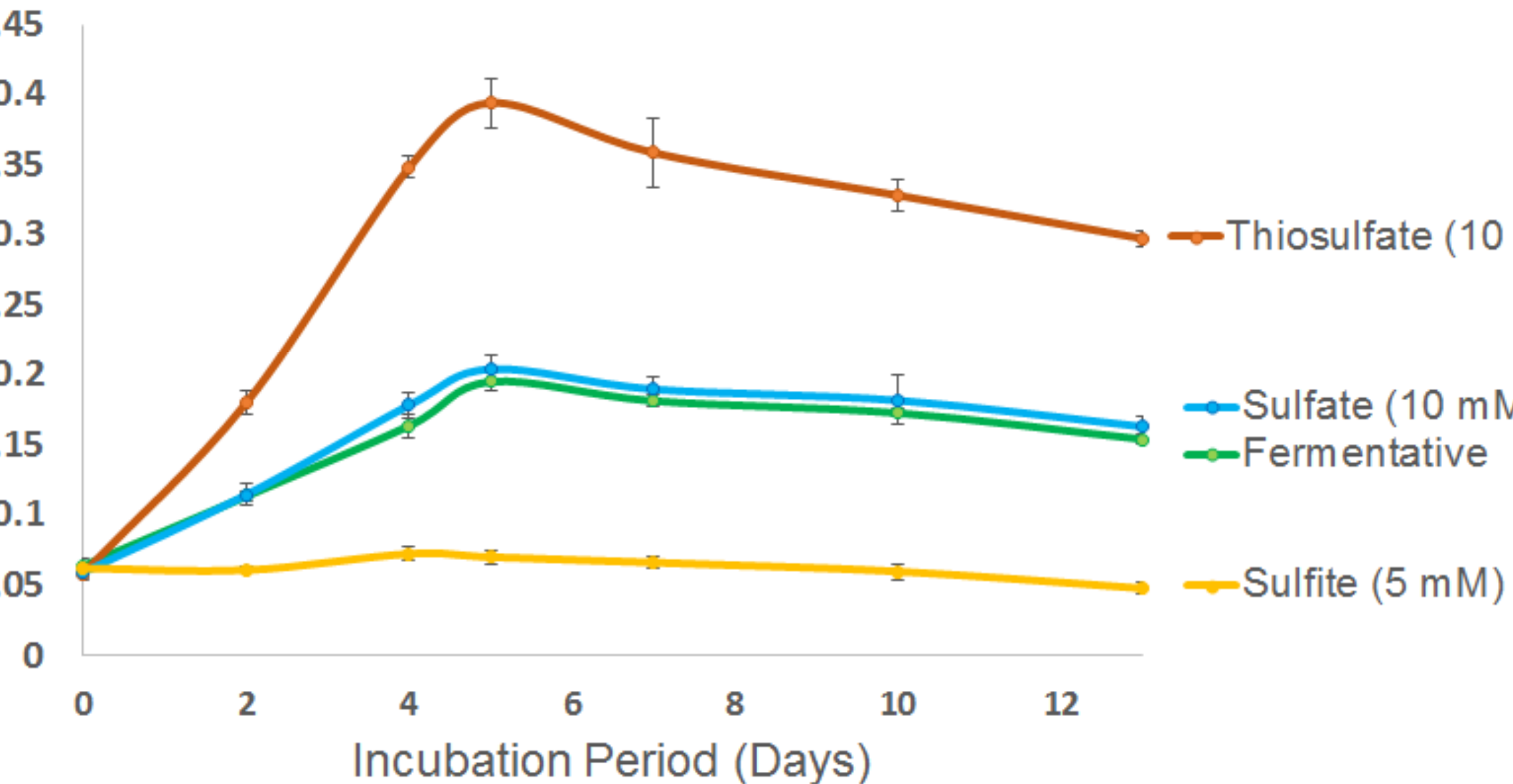


Sulfide produced from reduction of $S_2O_3^{2-}$ and S_8

Thiosulfate reduction also confirmed in *H. congolense* and OKU7

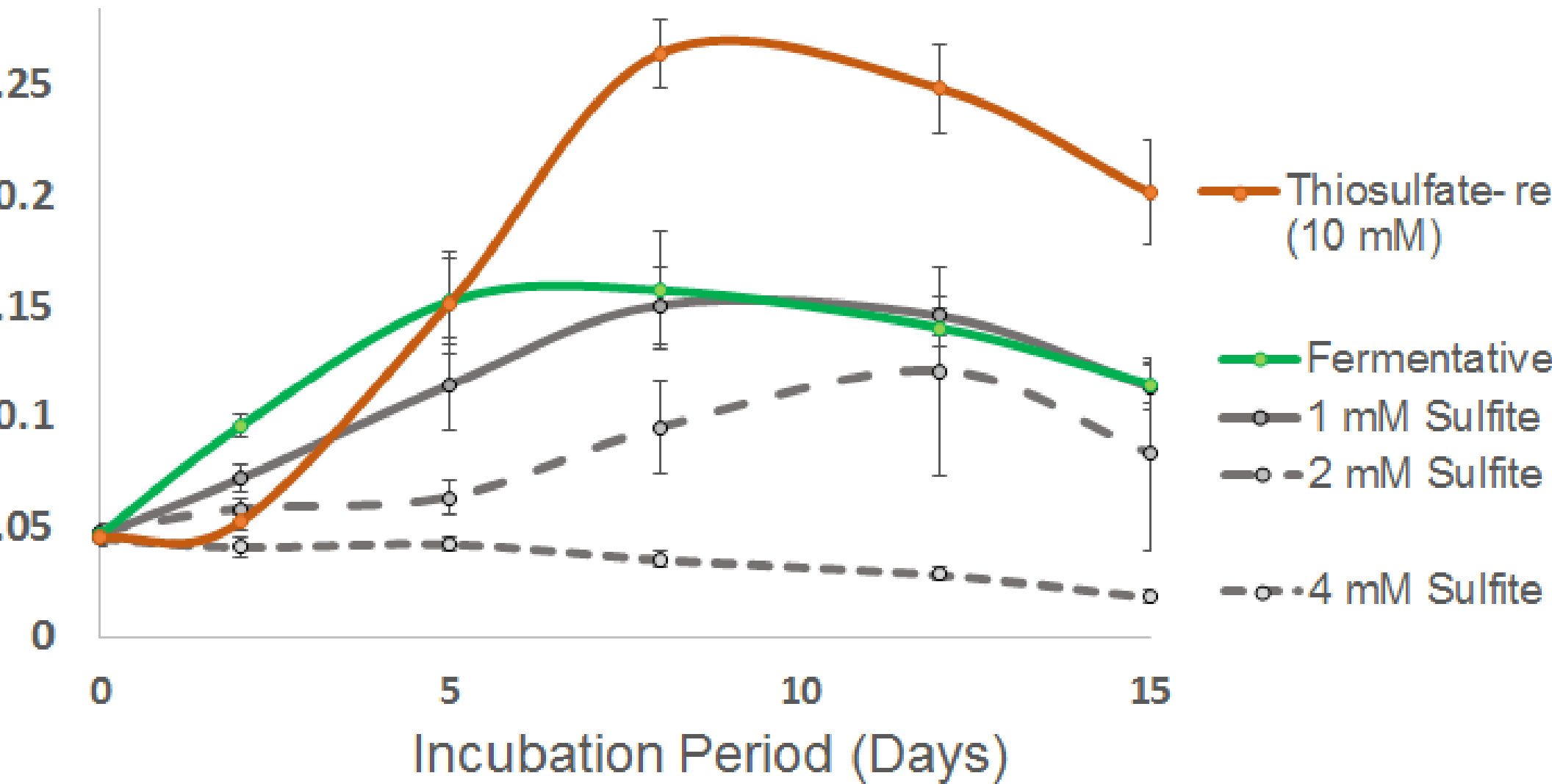
* Significantly different compared to fermentative, Student's t-test, p-value

Growth of *H. vreelandii* in the Presence of Sulfur Anions



Similar results observed for *H. congolense* and *C.*

The Effect of Sulfite on Growth of *H. vreelandii*



with is improved as sulfite concentration decre

Investigating Enzymatic Pathways

Assess if other sulfur anions are reduced

- Elemental sulfur and thiosulfate are reduced to sulfide.
- Sulfite inhibits growth

2. Evaluate rhodanese activity

Rhodanese Activity: Quantification of Thiocyanate



thiocyanate



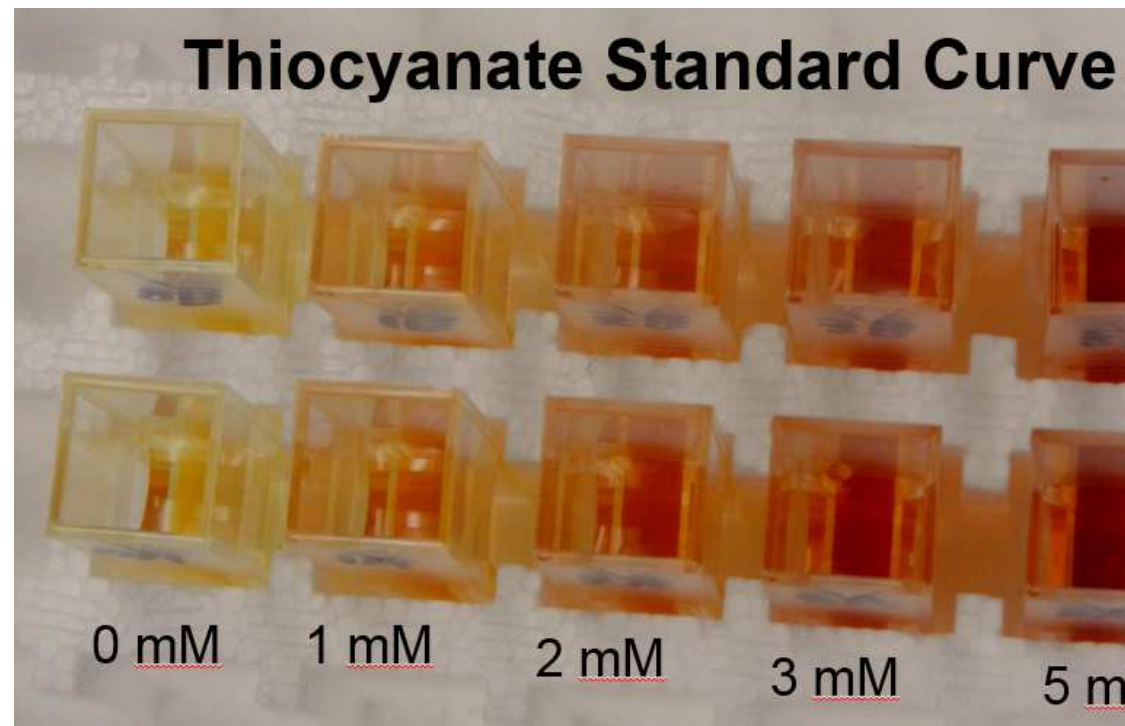
Ferric nitrate
reagent

Iron-thiocyanate complex

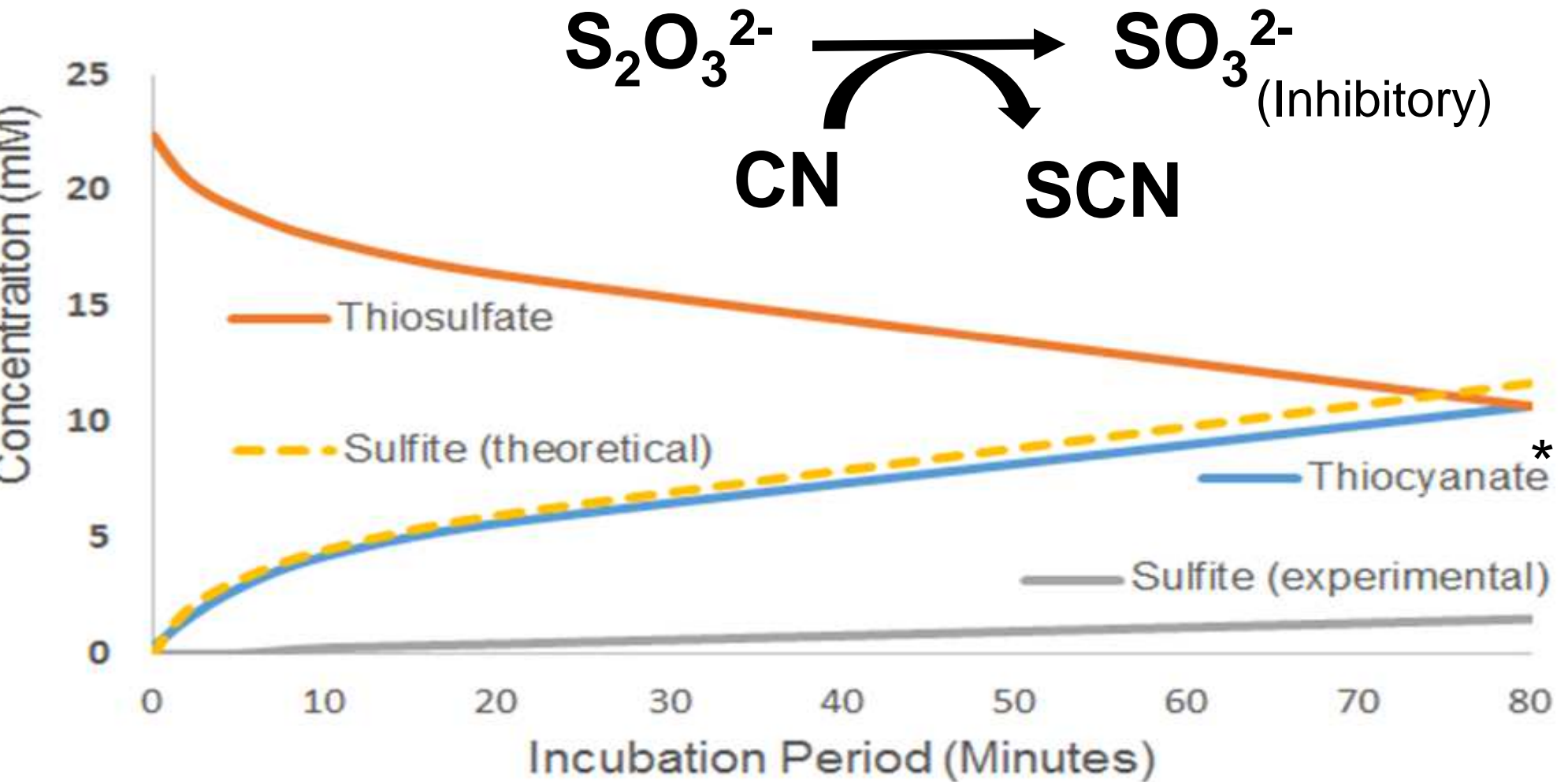
Measure absorbance of
supernatant at 470 nm

thiocyanate can only be
produced from rhodanese!

**Rhodanese activity observed
in all *Halanaerobium* strains
tested!**



Thiosulfate Activity in *H. vreelandii*



Thiocyanate is only detected when culture is amended with cyanide.

Investigating Enzymatic Pathways

Evaluate rhodanese activity

- Both sulfidogenic and non-sulfidogenic species of *Halanaerobium* possess rhodanese activity.
- Only a fraction of the predicted sulfite is detected in the rhodanese assay.
 - Where does the sulfite go?

Identify end products of thiosulfate reduction

State of Sulfur Anions

sulfate was not produced.

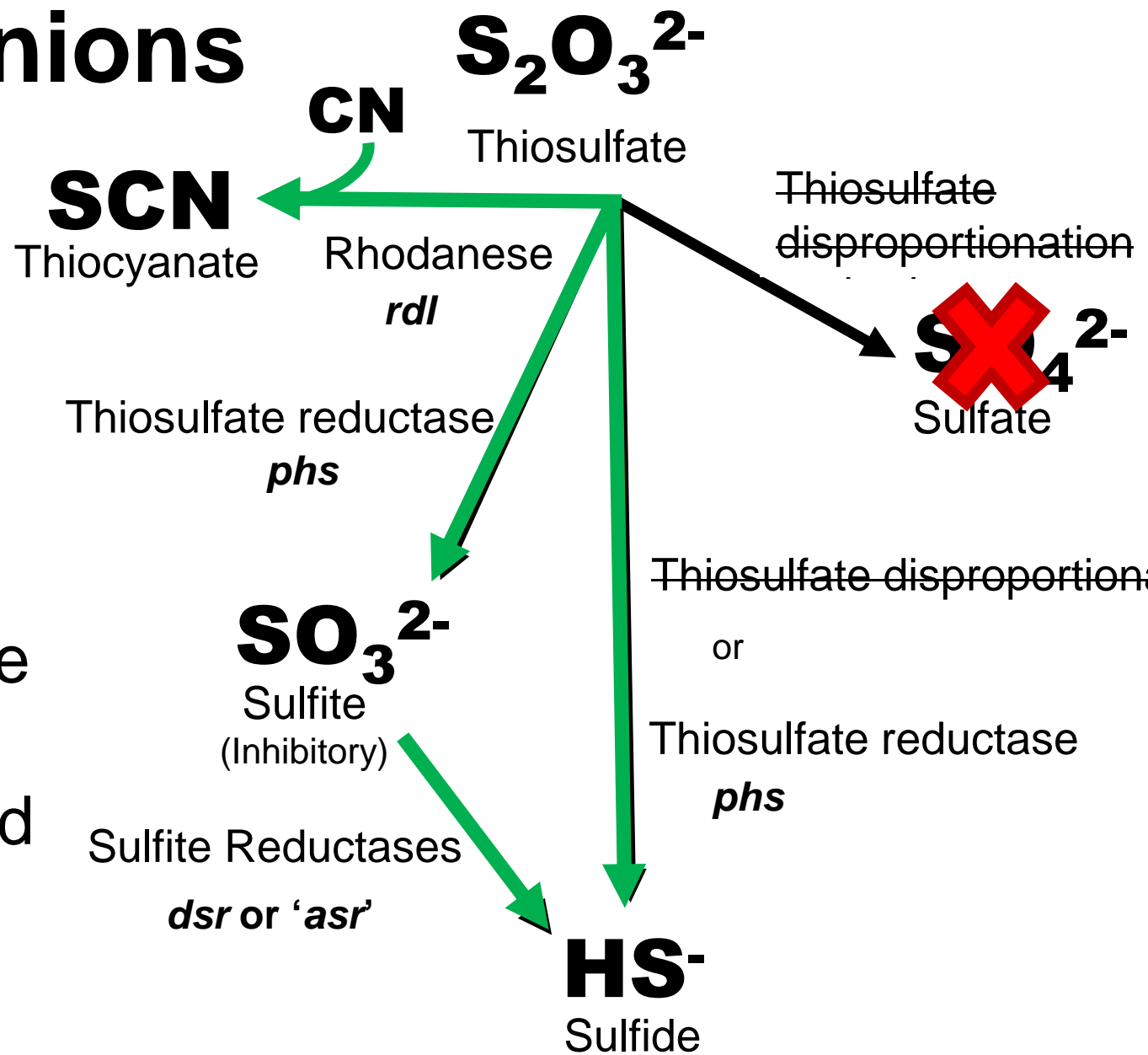
sulfide was generated as an end product of thiosulfate reduction

thiocyanate was produced when cyanide

was present.

sulfite was not detected *in vivo*, but detected in

low quantities in the rhodanese assay.



Investigating Enzymatic Pathways

Identify end products of thiosulfate reduction

- Thiosulfate disproportionation can be ruled out as an operable pathway.
- Rhodanese and thiosulfate reductase cannot not be excluded.
- Sulfite must be metabolized to prevent growth inhibition

Determine sulfide/thiosulfate ratio

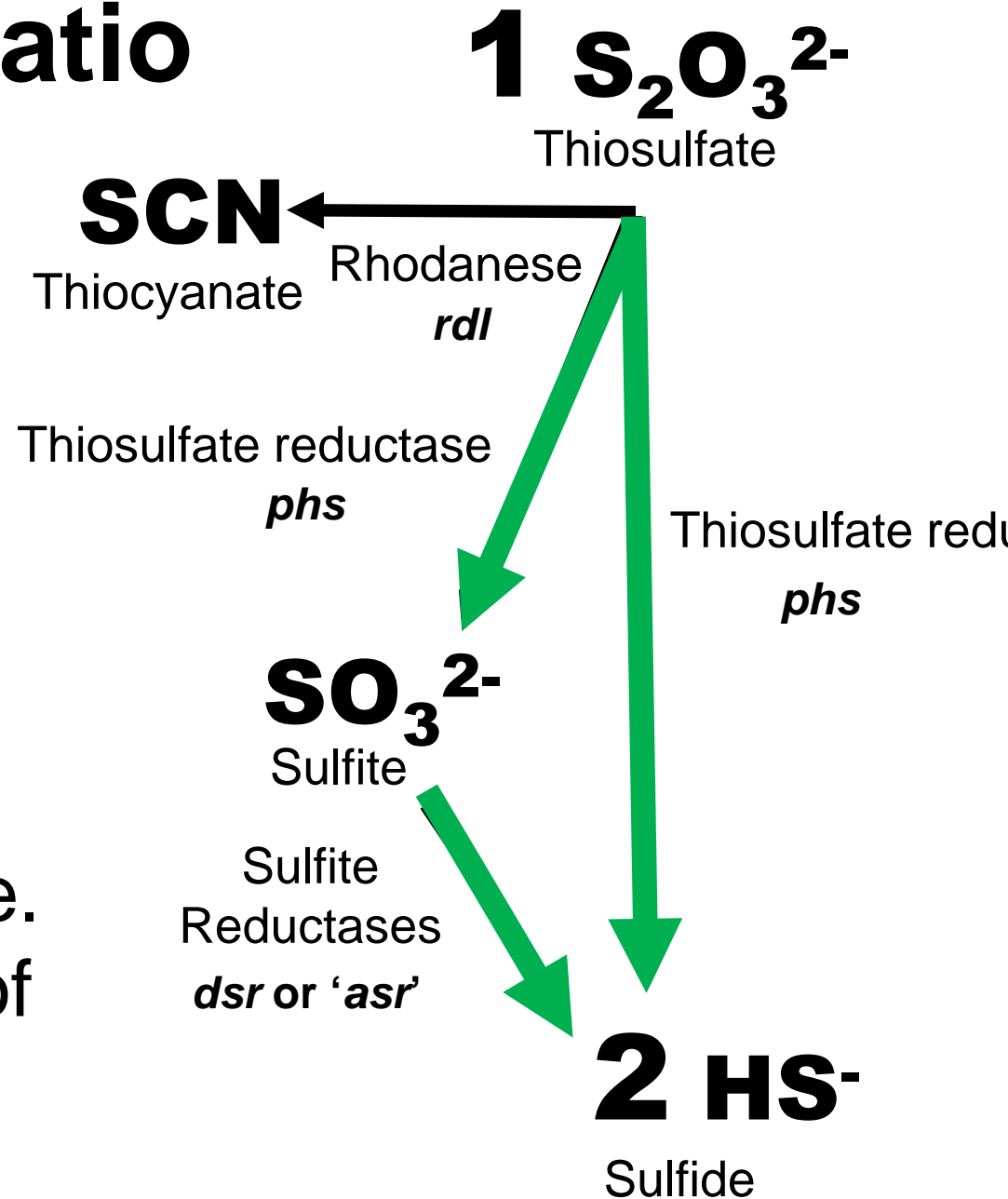
Thiosulfate: Sulfide Ratio

For every molecule of thiosulfate reduced, two molecules of sulfide are generated (2:1).

Suggests that both thiosulfate and sulfite reductases are operable.

➤ Sulfite as an intermediate.

Questions the applicability of the rhodanese reaction.



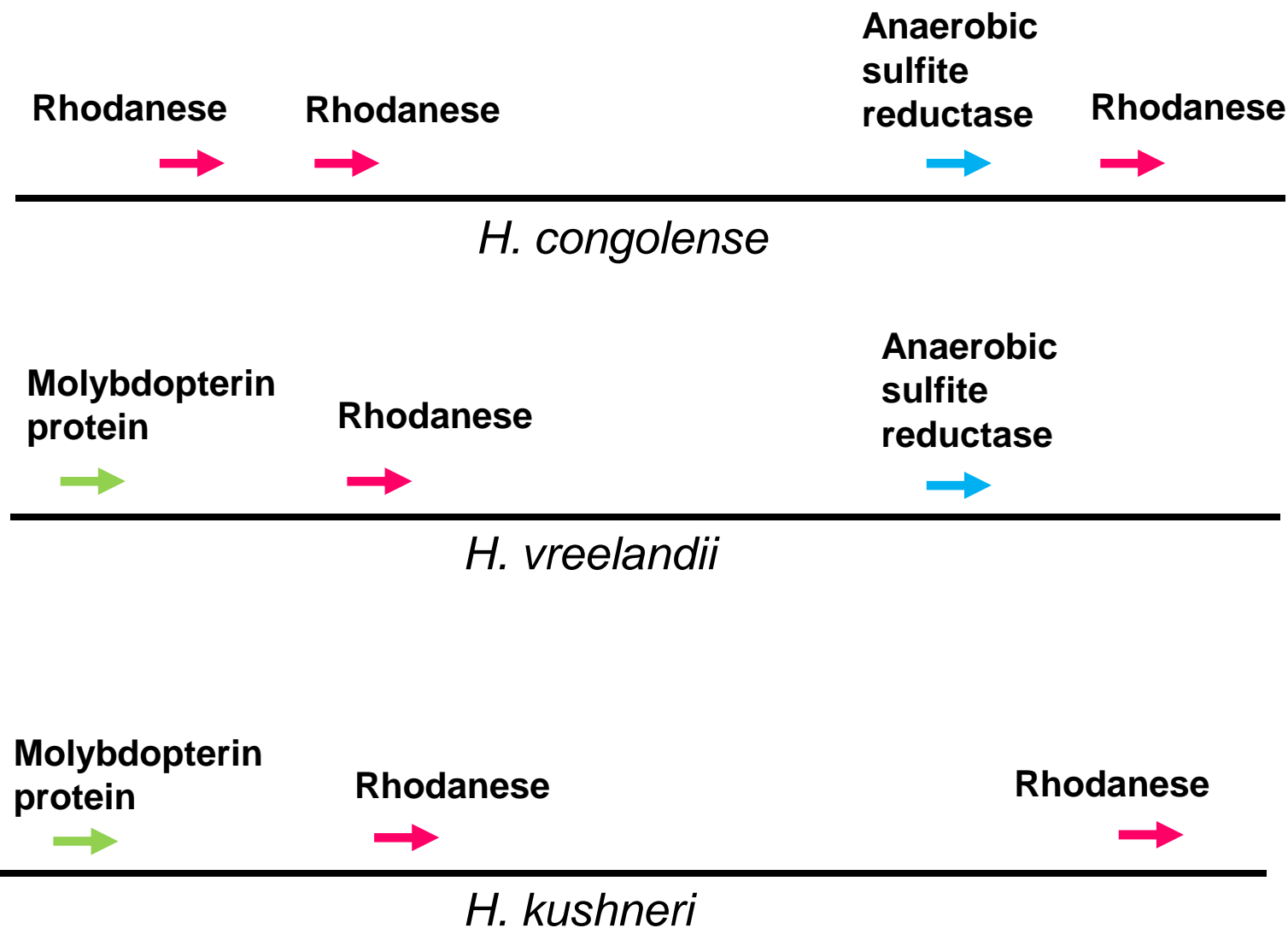
Investigating Enzymatic Pathways

Determine sulfide/thiosulfate ratio

- 2:1 Both sulfur atoms of thiosulfate are reduced to sulfide
- Strongly suggests the activity of a sulfite reductase

Genomic analysis

Genome Results



Genes for dissimilatory sulfite reductase were not retrieved.

Molybdopterin protein could potentially function as thiosulfate reductase.

Compiled Results

	<i>H. congolense</i>	<i>H. vreelandii</i>	<i>H. kushneri</i>	<i>H. salsug</i>
Thiosulfate-reduction	Yes	Yes	Yes?	No
Rhodanese Activity	Yes	Yes	Yes	Yes
Thiosulfate: Thiosulfate Ratio	2 to 1	2 to 1	1 to 1	NA
Predicted Genes	Rhodanese, sulfite reductase	Rhodanese, thiosulfate reductase, sulfite reductase	Rhodanese, thiosulfate reductase	NT

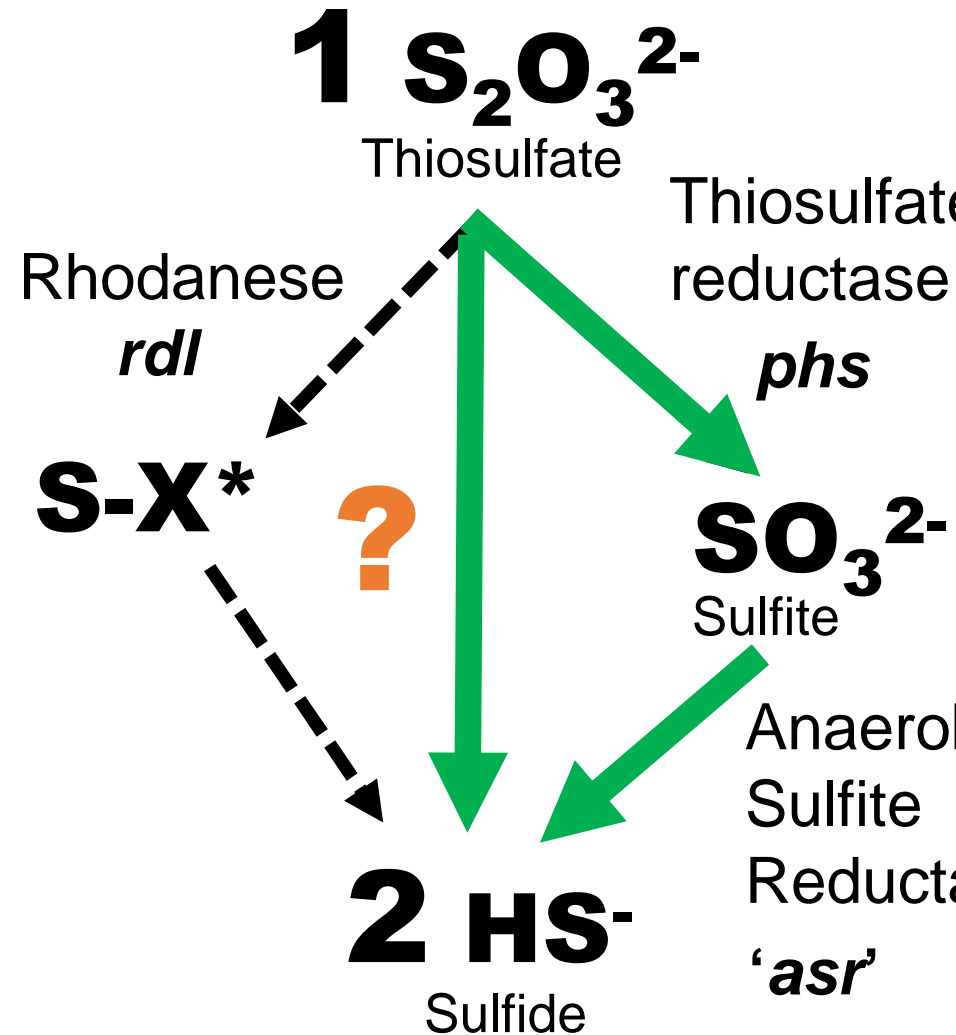
Investigating Enzymatic Pathways

Genome Analysis

Initial thiosulfate cleavage remains unclear.

- Can a rhodanese cleave thiosulfate into sulfite and sulfide?

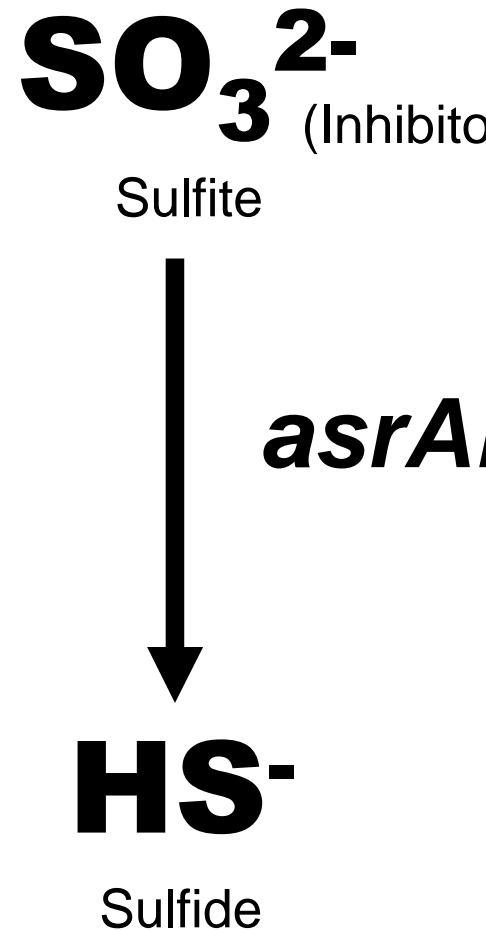
Suggests that an 'anaerobic sulfite reductase' is involved in the metabolism of sulfite.



* Sulfur accepting molecule
(traditionally CN in rhodanese)

anaerobic sulfite reductase'

- Also observed in *Thermoanaerobacter*, *Clostridia* and *Salmonella* species
- May be involved in reducing sulfite to prevent growth inhibition.
- Is not amplifiable using gene targets for dissimilatory sulfite reductase from SRB.



Rethinking TRB Gene Targets

Prodanese-like proteins are not practical
targets for sulfidogenic
Halanaerobium species.

Aerobic sulfite reductase is prime target
Directly responsible for the production
of sulfide.

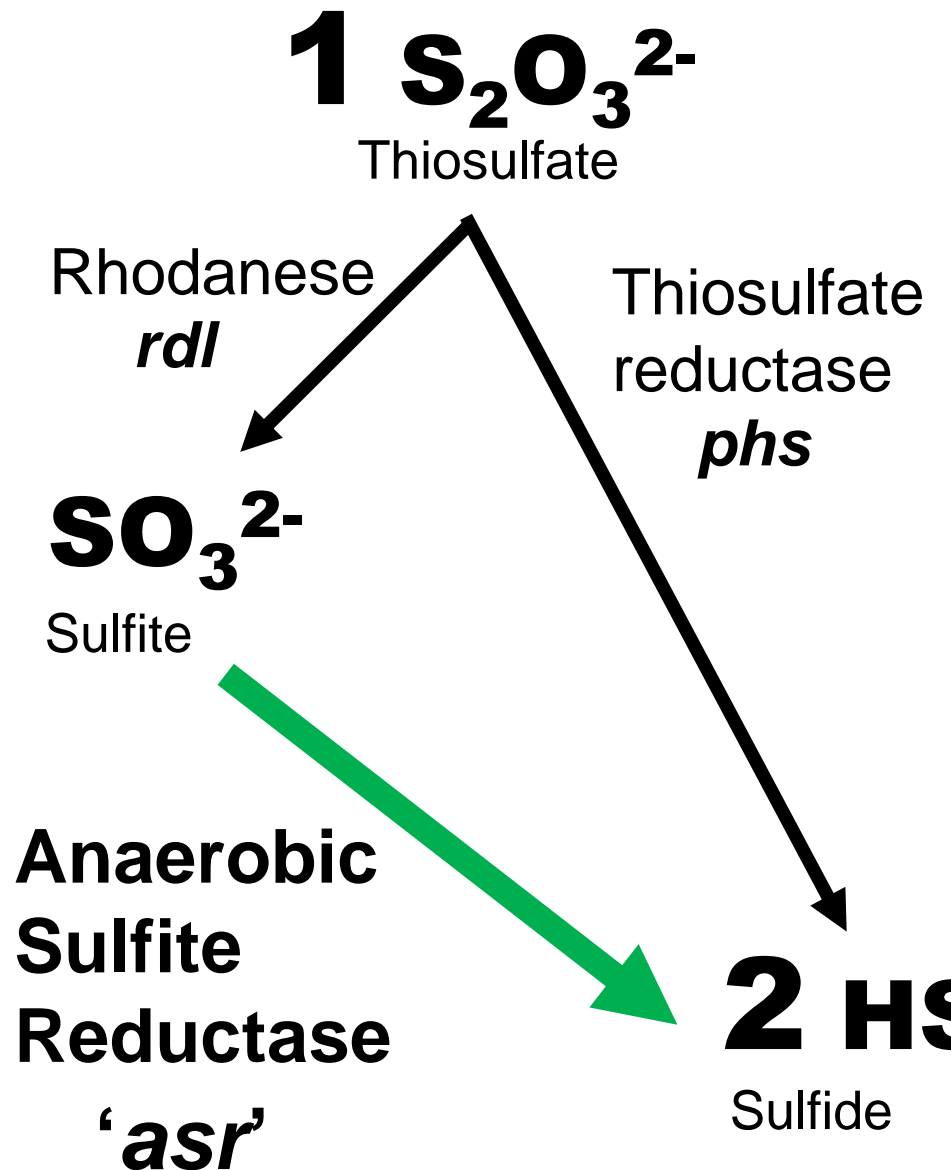
Inhibition of this enzyme be an effective
means of control for sulfidogenic
Halanaerobium species.



ke Home Messages

Sulfidogenesis from various sulfur anions and alternative enzymatic pathways must be considered.

Anaerobic sulfite reductase may be a more appropriate gene target for sulfidogenic *Halanaerobium* species.



Acknowledgements

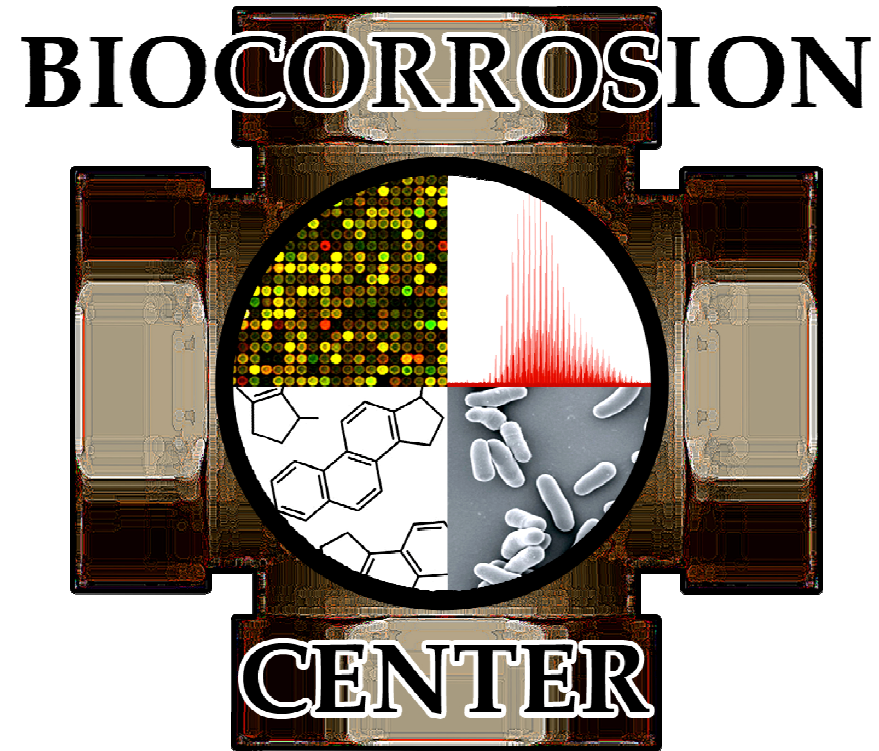
Thesis Committee:

r. Kathleen Duncan

r. Ralph Tanner

r. Joseph Suflita

r. Michael McInerney



Thank you for your time.

I welcome your questions!