Characterization of two *Idiomarina* sp. isolated from electrodes and capable of solid substrate oxidation

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Why *Idiomarina* sp.?

The transfer of electrons to and from solid-substrates and cells, a process termed extracellular electron transport (EET), has remodeled our understanding of microbial physiology as well as the potential for technologies at the microbe-electric interface. The term EET is analogous to "eating" or "breathing" solid-substrates such as inorganic minerals and metals. In a similar manner to the way we eat carbohydrates, proteins, and sugars, microbes are able to "eat" solid rocks and inorganic minerals by taking up their electrons, a process called oxidation. They are also able to "breath" minerals by depositing their own electrons onto these solids, similarly to the way in which we humans deposit electrons onto oxygen. This study seeks to learn more about two species of microbes belonging to the *Idiomarina* genus in hopes that they will be great model organisms to further study these processes.

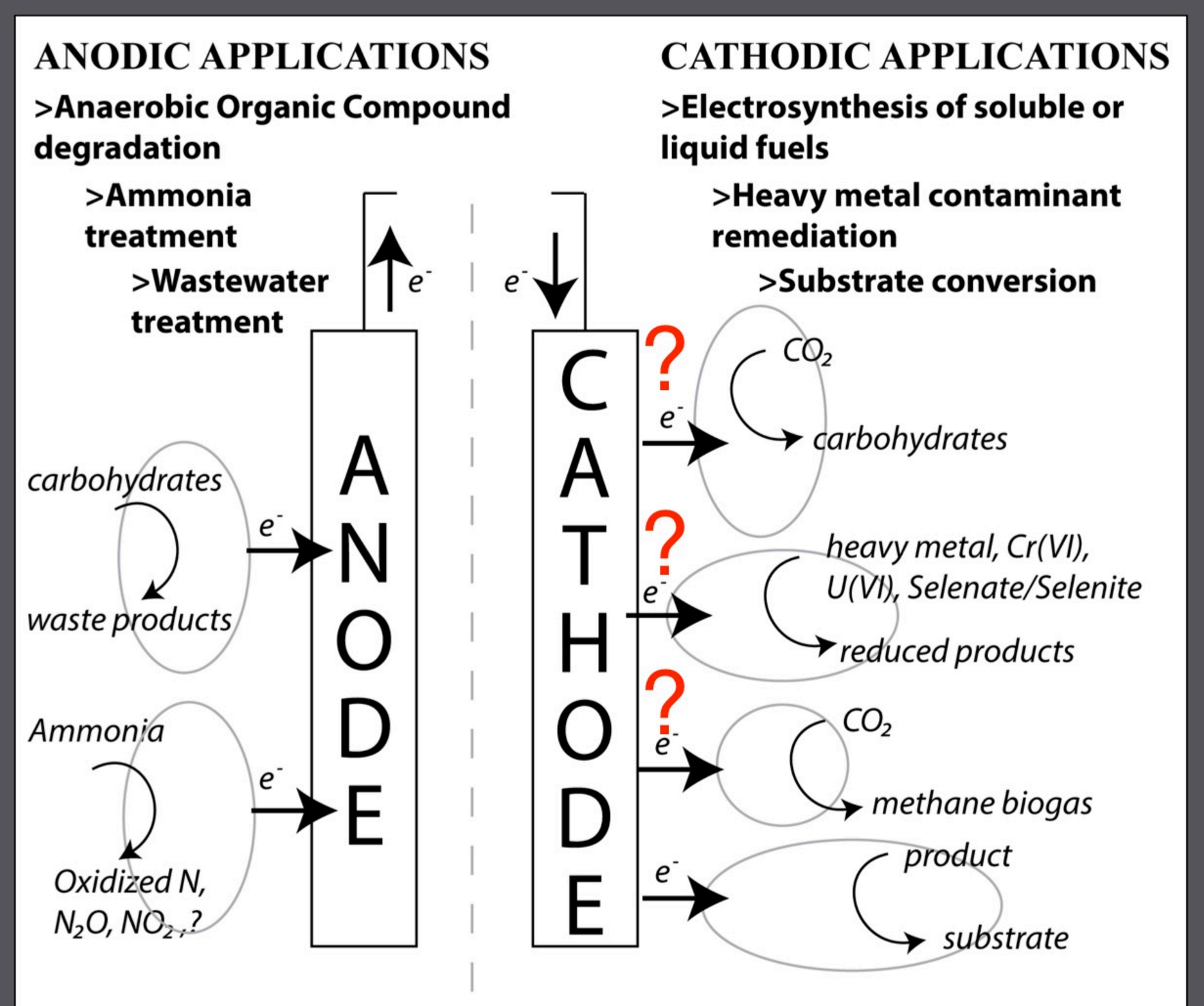

Fig.1. Schematic overview of described bioelectrochemical applications

Goal: Understand basic growth physiology and genomic context of *Idiomarina sp.* in order to pursue understanding its EET capabilities.

Who are *Idiomarina sp.*?

- Isolated from Catalina Island, California
- Motile gram negative rod bacterium (~1x0.2 microns)
- Grows in saltwater media
- Electrochemically active
- Can form biofilms on electrodes

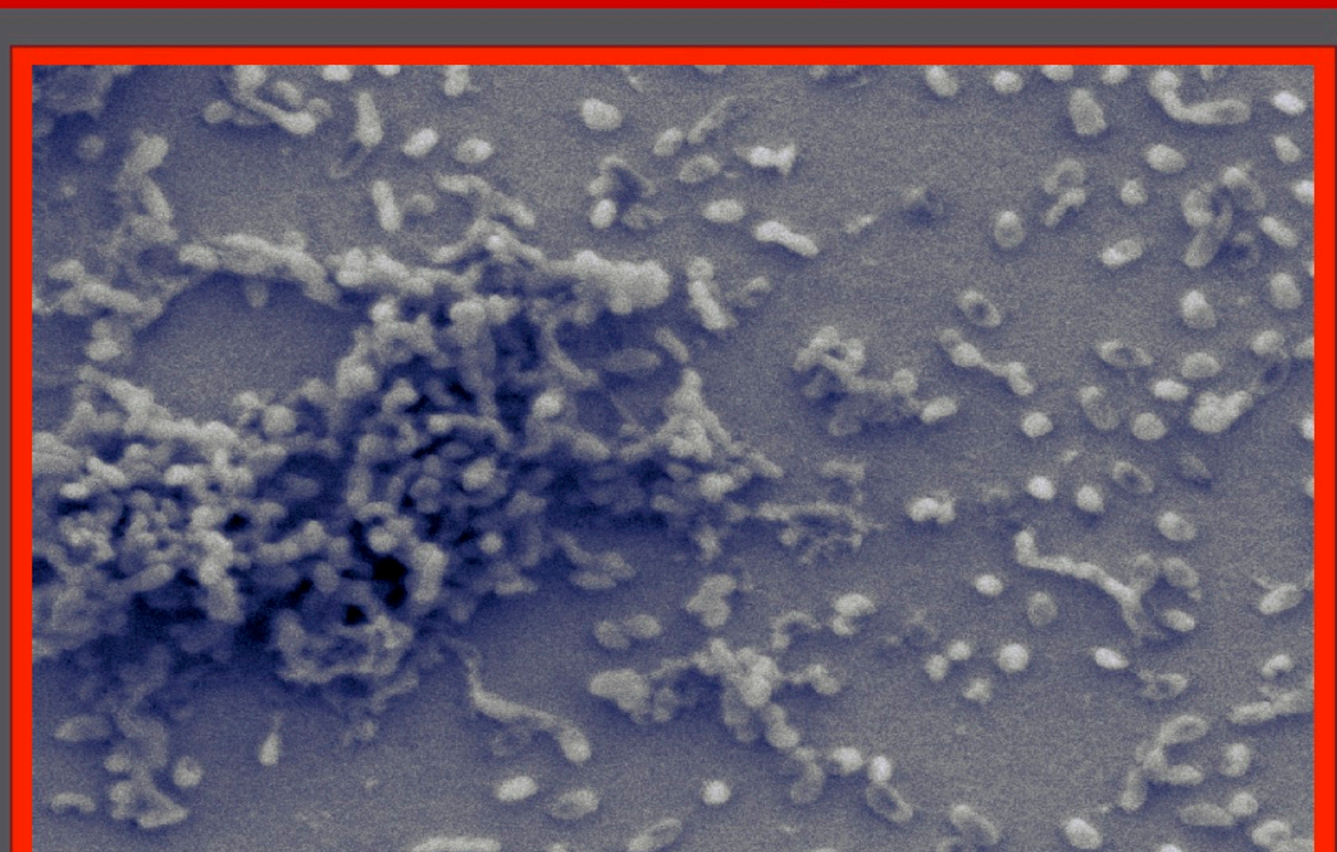
Figure 1. Scanning Electron Microscopy (SEM) image of *Idiomarina sp.*

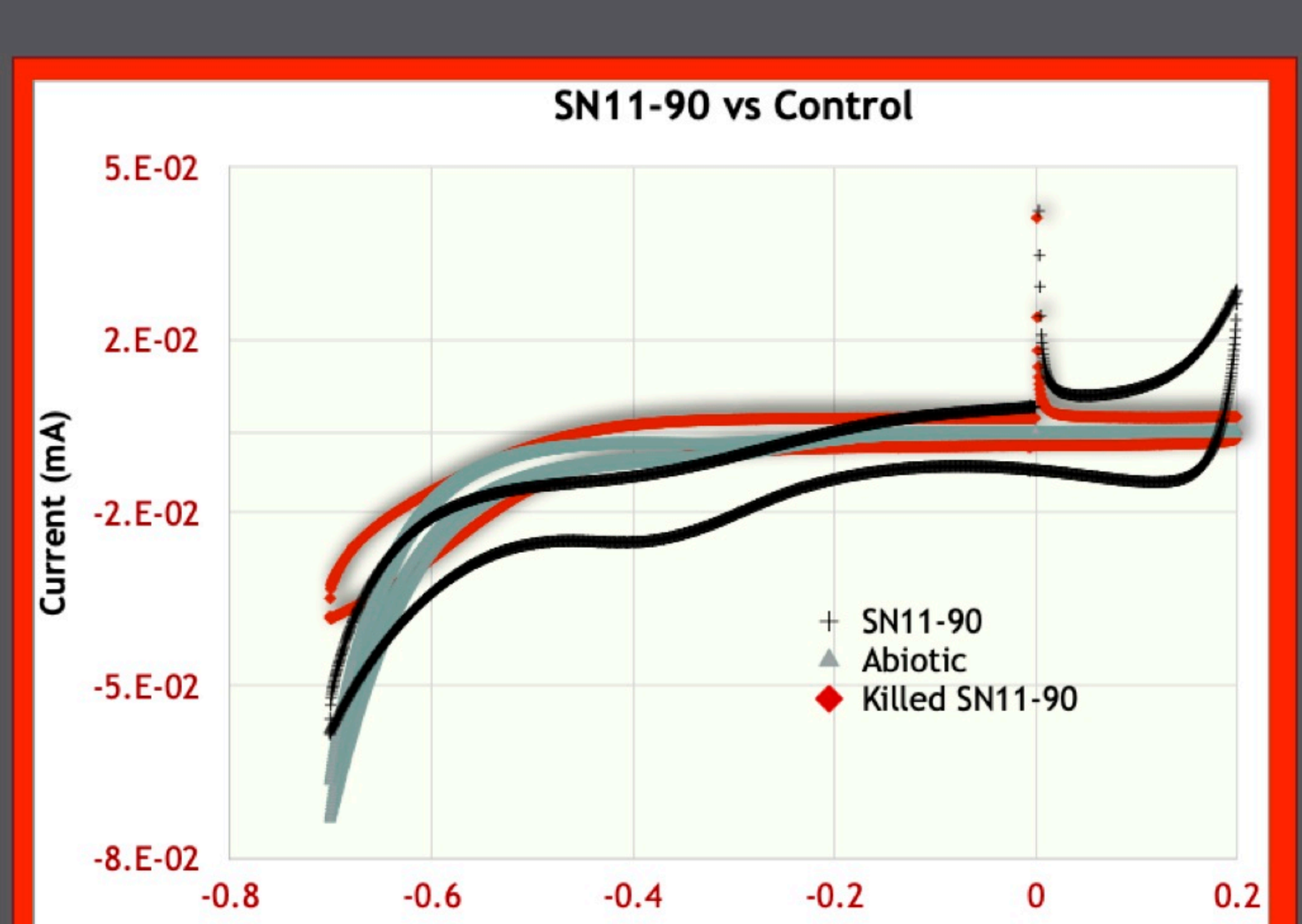

Figure 2. CV of SN11-90 showing biotic, abiotic, and killed samples.

- Cyclic voltammetry (CV) is a technique used to measure current production in response to a change in voltage.
- Current production can be observed when compared to controls beginning at -0.2 V and peaking at -0.4V. This gives us confidence that electron uptake is happening on the electrode.

Phylogeny

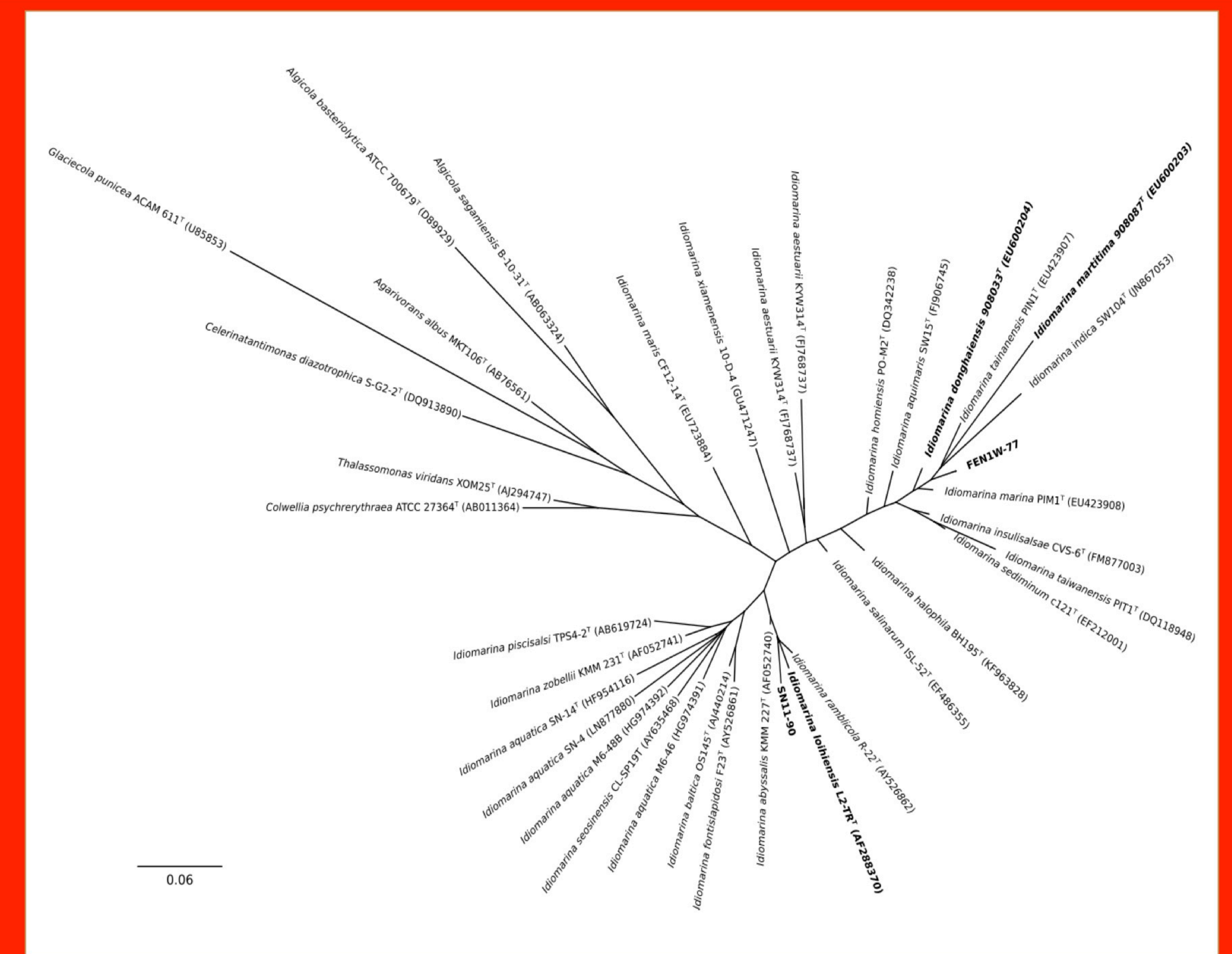
Figure 3. Phylogenetic tree using 16s rRNA gene sequences

Analysis of the genomic features across *Idiomarina sp.* and close relatives was determined using various metrics including genome to genome distance calculation, average nucleotide identity, and Guanine+Cytosine content. SN11-90 and *I. Loihiensis* again displayed very similar data with a GGDC of 0.0286 and an ANI of 97.46% (Table 2.0). FEN1W-77 displayed a genome most similar to *I. Maritima* (73.49%). Guanine and Cytosine contents were are similar, ranging from 46-48%.

Table 1. Genome-to-genome Distance and Average Nucleotide Identity

Query Genome	Reference Genome	Distance	ANI
SN11-90	*I. Loihiensis*	0.0286	97.46%
SN11-90	*I. Donghaiensis*	0.2445	69.80%
SN11-90	*I. Maritima*	0.2014	69.62%
SN11-90	FEN1W-77	0.1899	69.73%
FEN1W-77	*I. Loihiensis*	0.1796	69.63%
FEN1W-77	*I. Donghaiensis*	0.2344	73.36%
FEN1W-77	*I. Maritima*	0.2371	73.49%

Despite the similarities among genomes of Idiomarina spp., SN11-90 and FEN1W-77 display unique physiological differences.

What do *Idiomarina sp.* do?

Table 2. Growth under varying temperature and salinity content

Strain	FEN1W-77	SN11-90	*I. Loihiensis*	*I. Donghaiensis*	*I. Maritima*
Cell Length (μm)	~1x0.2	~1x0.2	0.7-1.8	1.0-1.4	1.4-2.0
Growth with NaCl (w/v,%)	0.1-18	0.1-18	0.25-17.5	0.1-12.5	0-13.0
Growth Range (°C)	4-40	4-40	6-39	7-42.5	7-45
pH Range	6.1-10.1	6.1-9.4	6.5-9.4	6.1-10	6.5-9.4
DNA G+C Content (mol%)	48.12	47.01	47.04	48.36	47.22
Metabolic Tests:					
Hydrolysis of Protease (Gelatin)	+	-	+	+	-

What do *Idiomarina sp.* do? con...

All physiologic tests were incubated at 30°C over 48 hours. Temperature experiments were given extra time given slower growth rates.

Table 3 shows the development of a minimal media for *Idiomarina* sp.. Microorganisms grown on minimal media form colonies using the minimum amount of nutrients possible. These media are often used to control the growth of microorganisms in order to to perform standardized experiments and select for growth of specific microbes. The minimal media developed for *Idiomarina sp.* is founded on salt water base, minerals ,and amino acids. Genomic analysis showed 11 key amino acids for growth, however, 15 total were needed.

Table 3. Growth using various sources of energy

Source	SN11-90	FEN1W-77
Acetate	-	-
Citrate	-	-
Glucose	-	-
Lactate	-	-
Fumarate	-	-
Casamino acids	-	-
+Acetate	-	-
+Glucose	-	-
20 Amino Acids	+	+
-Aspartic Acid	+	+
-Glycine	+	+
-Cysteine	+	-
-Glutamic Acid	+	+
-Alanine	+	+
-Methionine	-	-
-Asparagine	+	-
-Glutamine	+	+
-Proline	-	-
11 Amino Acids	-	-
+Met, Pro	+	-

- Minimal media (g/L):
  - 20g NaCl
  - 0.113g CaCl+2H2O
  - 3g MgCl2+6H2O
  - 0.5g KCL
  - 20mg amino acids (15 total)
- Because growth required at least 2 more amino acids than the genome suggested we hypothesize that methionine might play a role in a quorum sensing mechanism given their inability to grow without it.

FUTURE WORK:

In the future we will continue to grow *Idiomarina spp* on electrodes to better understand electron uptake capabilities and attempt to observe physiology anaerobically and with inorganic materials as the primary electron donor. Further genetic testing will also give insight on what genes are essential to the transport of electrons across membranes. These data will be invaluable for the use of EET for various biotechnology applications.

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