

# Isolation of a *Shewanella* species that grows anaerobically on nitrate and acetate and aerobically on LB

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## Introduction

## Methods and Data

**Isolation media** Isolation media was concocted as follows: First, “brackish base” (BB) was made by combining a number of salts (Table ) per liter final volume and autoclaving to sterilize. Then, sodium bicarbonate and potassium phosphate (5g NaHCO<sub>3</sub> and 0.5g KH<sub>2</sub>PO<sub>4</sub>) were dissolved in 50ml H<sub>2</sub>O, sterile filtered, and added, along with 1ml sterile filtered trace metal solution (JRL 2016 special sauce).

To this base BB media, one or more of sterile-filtered 10 mM thiosulfate (“S”), 20 mM potassium nitrate (“N”), and/or 10 mM sodium acetate (“A”) was added as described below.

To make plates, 1 gram-percent agar was added to the basic solution.

**16s Colony PCR** Each colony was picked and touched to 20ul of alkaline PEG200 (“ALP”). This was then boiled for 5 minutes to lyse the cells. 1 ul of cell lysate was then added to a 25 ul Promega GoTaq G2-based PCR reaction with the bacterial 16s primers 8F and 1391R. 16 rounds of PCR was performed with a 1.5 minute extension time. After PCR, bands were checked on a gel; then, Sanger sequencing was performed directly from the PCR reactions with the primer 515F.

Amount	Chemical
10.5g	NaCl
1.7g	MgCl <sub>2</sub> .6H <sub>2</sub> O
0.125g	CaCl <sub>2</sub> .2H <sub>2</sub> O
0.5g	KCl

Table 1: BB base media salts

**Data availability** All data is available at <https://github.com/ctb/2016-micdiv-report/>.

## Results and Discussion

### An enrichment from Trunk River grew with the addition of acetate

I initially designed media to enrich for sulfur oxidizing denitrifiers microbes (“BB+SN”). I inoculated two Pfennig bottles (approx. 40ml) with approximately 2-3 cc of material. The material for the first enrichment, culture 4, was taken directly from the sediment layer on top of the sand in the main channel of Trunk River, approximately 8m from the start of the narrow outflow channel. The material for the second enrichment, culture 8, was taken from the under-water surface of a sea table enrichment that originated from a microbial mat, also taken from Trunk River. Both enrichments were incubated at 30 deg in a foil-lined box to prevent phototroph growth.

After 18 hours, significant turbidity was observed in both enrichments, along with substantial amounts of supersaturated gas, indicating growth. I therefore transferred 1 ml from each enrichment to another Pfennig bottle containing BB+SN. These transfer enrichments, however, failed to grow.

Based on scent, Dr. Leadbetter suspected that the original transfer of sediment contained acetic acid, indicating the presence of significant amounts of acetate. I therefore added 800ul of 1M sodium acetate to both enrichment cultures, to a final concentration of 20 mM.

After the addition of acetate, both transfers grew to white opacity within about 16 hours at 30 deg. Two subsequent transfers of each culture (1ml into 40 ml “BB+SNA”, BB with thiosulfate, nitrate, and acetate) also exhibited similar growth.

### Enrichments exhibited nitrate and acetate dependent growth

To further analyze growth conditions, I employed a simple “differential diagnosis” approach and transferred each enrichment to four culture conditions: BB, BB with thiosulfate and acetate (BB+SA), BB with nitrate and acetate (BB+NA), and BB with acetate (BB+A). After incubation for 16 hours, only the BB+NA grew, indicating that the enrichments required both nitrate and acetate but not thiosulfate (Figure 1).

### Enrichments yielded colonies when grown on solid media

I plated 1:500 and 1:5000 dilutions of enrichments 4 and 8 on BB+SNA solid media, and incubated the plates both anaerobically and aerobically at 30 deg. I also plated the same dilutions on LB and grew aerobically at 30 deg. All plates showed density-dependent growth, although the colonies on the LB plates grew much faster (2-4 times) than either the aerobic or anaerobic BB plates.



Figure 1: Both enrichments 4 and 8 (first and third row) exhibit nitrate and acetate-dependent growth (third column) in anaerobic BB medium. No growth was observed in base BB media, BB+thiosulfate and acetate, or BB+acetate.

### **Isolate colonies from aerobic LB plates grew successfully in anaerobic culture**

I picked 4 colonies grown aerobically on LB from each enrichment (for a total of 8), and inoculated anaerobic BB+SNA cultures with them. 7/8 of the cultures grew within 48 hours, with three (culture 3 from enrichment 8, and cultures 5 and 8 from enrichment 4) growing overnight to opacity.

I then transferred these three isolates (3, 5, and 8) from BB+SNA anaerobic liquid culture back to LB plates, where they again grew (see Figure 2).

### **Isolate colonies were identified by 16s colony PCR as *Shewanella* spp.**

I picked colonies and performed 16s PCR on the eight LB isolate colonies as described in the Methods. All 8 yielded bands, which were then Sanger sequenced. BLAST of all 8 colonies against NCBI's "nt" database showed strong similarity (99%) to two known species of *Shewanella* (Table 2).

### **Isolate cultures contain Spirilla-shaped microbes**

I examined all three cultures with light microscopy and saw Spirilla-like bacteria (Figure 3). These bacteria appeared to move in a corkscrew-like fashion. This



Figure 2: All three isolate colonies grew after transfer from LB through BB+SNA anaerobic culture followed by streaking on LB.

Enrichment	Colony	Match percentage	BLAST match	Accession
1	8	99%	Shewanella algae strain MAS2736	GQ372874.1
2	8	99%	Shewanella algae strain MAS2736	GQ372874.1
3	8	99%	Shewanella algae strain MAS2736	GQ372874.1
4	8	99%	Shewanella algae strain MAS2736	GQ372874.1
5	4	99%	Shewanella sp. Chr-15	JQ863373.1
6	4	99%	Shewanella sp. Chr-15	JQ863373.1
7	4	99%	Shewanella sp. Chr-15	JQ863373.1
8	4	99%	Shewanella sp. Chr-15	JQ863373.1

Table 2: BLAST-based characterization of Sanger-sequenced 16s regions from colony PCRs. All colonies are clearly identified as *Shewanella* at 99% identity.

morphology is at odds with their molecular identification as *Shewanella* spp, which are typically rod-shaped bacilli.

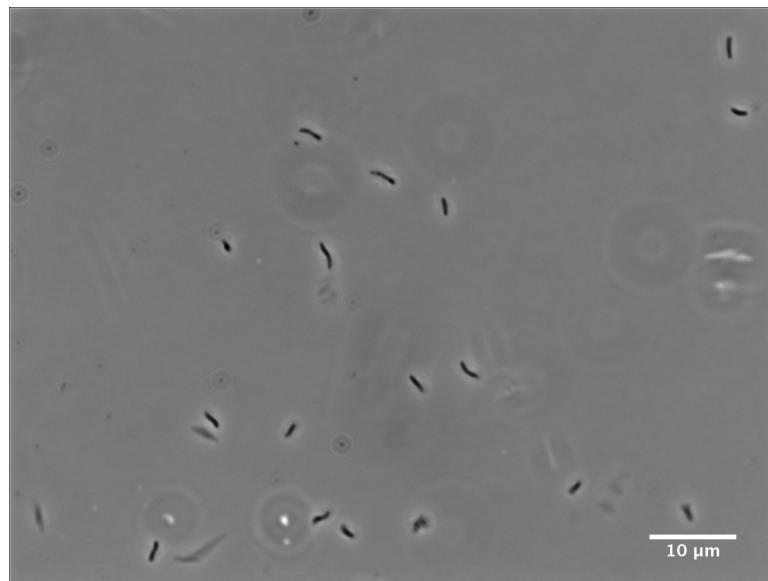


Figure 3: Light microscopy image of isolate colony 8, showing a bent-rod shape characteristic of spirillae; all three isolate colonies (3, 5, and 8) exhibited identical morphology.