

Diversity of Estrogen Degrading Microorganisms in Las Vegas Wash and Lake Mead, Nevada, USA

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Introduction

Endocrine disrupting chemicals (EDCs) are a subject of intense research as more studies reveal their persistence in the environment and detrimental effects on wildlife. Steroid hormones, including the natural and synthetic estrogens estrone (E1), 17-beta-estradiol (E2) and 17-alpha-ethinyl estradiol (EE2), are among the most bioactive and have been detected at low concentrations in waterways downstream from wastewater treatment plants. Las Vegas Wash, a stream flowing into Lake Mead and fed primarily by treated wastewater, provides a unique experimental system in which to study the role microorganisms play in the fate and dispersal of these compounds in surface waters.

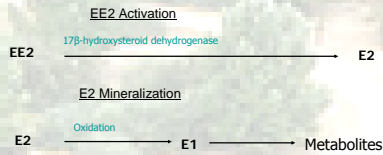
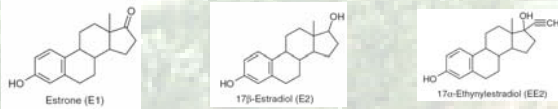


Fig 1. Chemical structures of E1, E2, and EE2 and microbially mediated degradation pathways

Objectives

- Isolate and identify estrogen-degrading bacteria
- Analyze degradation kinetics in the presence and absence of co-substrates
- Measure estrogen degradation by natural bacterial populations
- Determine abundance of estrogen degraders in Las Vegas Wash and Lake Mead

Materials and Methods

Cultivation

Aquatic samples were inoculated into minimal and nutrient-rich media containing the steroid estrogens E1, E2 and EE2. Microbial density was estimated by dilution. Bacterial growth (absorbance) was measured spectrophotometrically. Isolates were individually tested (in progress) for estrogen degradation. Degradation analysis was conducted using an Agilent HPLC and an Applied Biosystems 4000 Q Trap LC/MS/MS.

Molecular

Isolates were identified via PCR amplification (16S rRNA gene). DNA sequencing was performed at Functional Biosciences, Inc. Madison, WI.



Fig 2. Sampling Sites

Individual reads were assembled using MEGA and identified using the BLAST algorithm. Phylogenetic analyses were performed using the neighbor joining functions of MEGA (Tamura et al. 2007).

Results

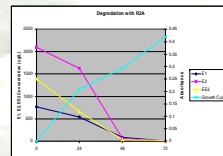


Fig 3. Las Vegas Wash water inoculated into R2A (nutrient rich) media

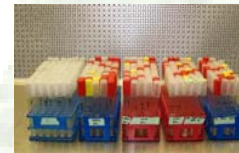


Fig 4. Dilution cultivation revealed 10¹ estrogen degrading cells/mL out of a total of 10⁶ culturable cells

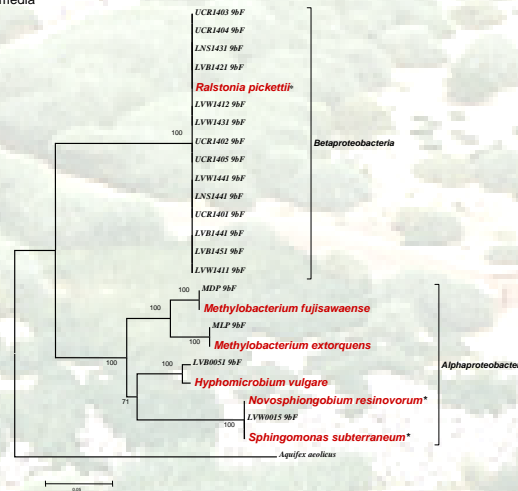


Fig 5. 16S tree based on forward read only. Neighbors are 99-100% matches using BLAST, except for LVB0051 and *H. vulgare*, which was 98%
 * Genus previously shown to degrade estrogen

Results: *M. fujisawaense* degradation

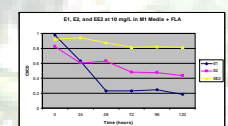
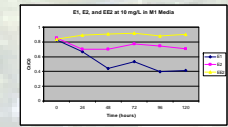
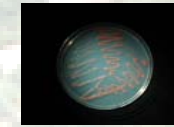


Fig 6. *Methylobacterium fujisawaense* on minimal media with estrogen as sole carbon source and microscopically

Fig 7. *M. fujisawaense* inoculated into M1 minimal media and M1 with additional organic acids

In Progress

Spiked Mesocosm Experiment (collaboration with SNWA)

- Water collected from 4 sites in the Lake Mead System spiked with pharmaceuticals and estrogens at ~500 ng/L
- Total cell counts and T-RFLP community fingerprinting
- Time points at 0, 1, 2, 4, 7, 14, 28, 56, 112 days
- Solid phase extraction, LC/MS analysis
- Will enable measurement of degradation kinetics under more environmentally relevant concentrations



Fig 8. Setup for current mesocosm experiment

Conclusions

- Six estrogen-degrading bacteria have been isolated from the Lake Mead system to date (in progress)
- M. fujisawaense* shows degradation capability for E1 and E2 but not EE2
- Native microorganisms show ability to degrade E1, E2, and EE2 to levels below detection within 72 hours (from ~2 mg/L starting conc.)
- Out of 10⁶ culturable cells/mL in Las Vegas Wash, only 10¹ are capable of growing on estrogen as sole carbon source

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