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Microbial Biodegradation of Steroid Hormones

CEE 697z Organic Contaminants

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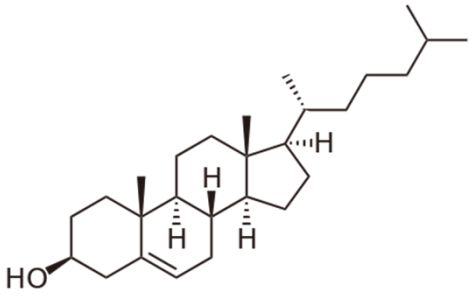
Background

- Estrogens, naturally or synthetically produced, are steroidal hormones
- Regulate a wide range of important biological functions in humans and animals
- All natural steroids are synthesized from Cholesterol
- Steroid hormones interact with intracellular receptors, forming complexes that can increase or decrease transcription of specific genes

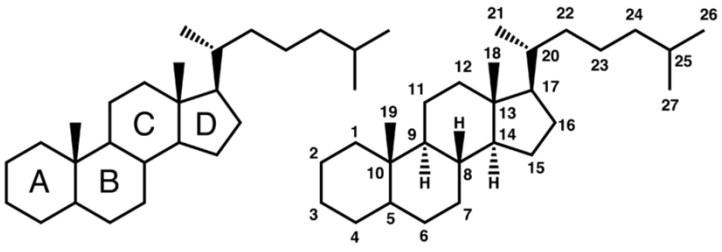
CHOLESTEROL



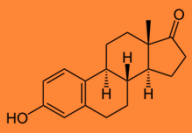
Cholesterol is required to build and maintain membranes; it modulates membrane fluidity over the range of physiological temperatures. The hydroxyl group on cholesterol interacts with the polar head groups of the membrane phospholipids, while the bulky steroid and the hydrocarbon chain are embedded in the membrane, alongside the nonpolar fatty-acid chain of the other lipids. Within the cell membrane, cholesterol also functions in intracellular transport, cell signaling, and nerve conduction. Within the cells, cholesterol is the precursor molecule to several biochemical pathways. Cholesterol is an important precursor molecule for the synthesis of vitamin D and steroid hormones, including cortisol and aldosterone, as well as progesterone, estrogens, and testosterone, and their derivatives.

STEROIDS

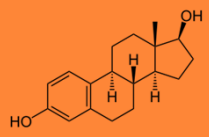
- Consist of 4-cycloalkane rings
  - 3-cyclohexane (A,B,C)
  - 1-cyclopentane (D)
- Methyl groups at C-10, C-13, C-17



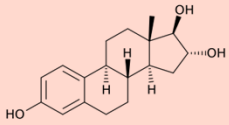
Natural Estrogens



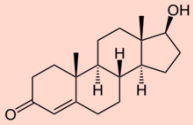
Estrone (E1)



Estradiol (E2)

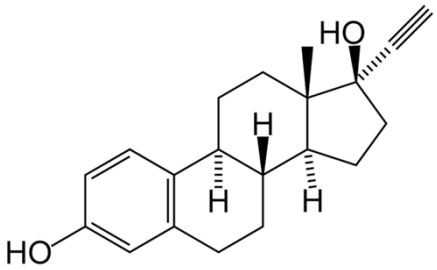


Estriol (E3)



Testosterone

Synthetic Estrogen



Ethinylestradiol (EE2)

Biodegradation

- Process by which microbial organisms transform or alter (through metabolic or enzymatic action) the structure of chemicals introduced into the environment (US EPA, 2010)
- **However,** biodegradation products can be more harmful than the parent substance (International Union of Pure and Applied Chemistry, 1993)

Three Main Categories of Processes that have been Researched

1. Physical Processes
   - Sorption onto Activated sludge
   - Sorption onto adsorbent materials
   - Membrane filtration
2. Advanced Oxidation Processes (AOP)
   - Photolysis
   - Heterogeneous photocatalysis
   - Strong oxidizers
   - Combination of UV and strong oxidizers
   - Sonolysis
3. **Biological Processes**
   - **Bacteria and Archaea (from AS and anaerobic sludge)**
   - **Microalgae**
   - **Enzymes**

Removal of Estrogens during Biological Treatment

- Sorption to biosolids
  - Biosolids may be used for land application which may become a long-term source
- **Biodegradation by microorganisms**
  - Transformed products may still possess estrogenicity

WWTP Estrogen Removal

- E1: 19-94%
- E2: 76-92%
- EE2: 83-87%
- (Ternes et al., 1999a; Johnson and Sumpter, 2001)

For activated sludge plants

- Estradiol Equivalents: 28% (Svenson et al.,2003)

For Trickling Filters

- Higher removal efficiencies for membrane bioreactor and fixed bed reactor systems in comparison to AS (Clara et al., 2005; Joss et al., 2004)

Variation between WWTPs

- Differences in biological (fixed film or suspended) growth and other processes
- Operating conditions
  - **SRT** and HRT
- Geological locations of WWTPs
- Influent concentrations of estrogens
- Adsorption vs. biodegradation

Table 4
Biodegradation processes used for estrogens (E1, E2, E3 and EE2) elimination from water.

Estrogens	Agent and/or Matrix	Experimental Conditions	Conclusions	Reference
E1, E2, EE2	STP	Aerobic batch experiments containing a diluted slurry of AS from a real STP in Germany	- While in contact with AS, E2 was oxidized to E1, which was further eliminated without any E1 degradation products observed - EE2 was predominantly persistent under the selected aerobic conditions - Two glucuronides of E2 were cleaved in contact with the diluted AS solution and, thus, E2 was released	Ternes et al. (1999b)
E1, E2, E3, EE2	AS	Grab samples from influents and effluents, filtered before analysis. Estrogens were extracted by a 6/5-g Carbograph 4 extraction cartridge	- High concentrations of E3 were found in the influent, with a mean of 57 ng L^{-1} - AS process appears to remove 88% of E2 and 74% of E1	Johnson et al. (2000)
^{14}C-labelled estrogenic compounds	Municipal and industrial wastewater	Biosolids from four municipal treatment plants and one industrial plant	- Dramatic differences were observed: in the municipal STP the mineralization was 70–80%, during the 24 h, while for the industrial plant EE2 mineralization was 25–75-fold less than that of E2 and did not reach completion within the 24 h	Layton et al. (2000)
EE2	Nitrifying AS	Ammonium oxidised by the sludge at a rate of 50 mg NH_4^+ g^{-1} DW h^{-1}	- Oxidation of EE2 resulted in the formation of hydrophilic compounds, that were not further identified - Most probably degradation results in a loss of estrogenicity	Vader et al. (2000)
E1, E2, EE2	Waters from English rivers in urban/industrial and rural environment	E2 spikes in the range 20 ng L^{-1}–500 mg L^{-1}	- Microorganisms in river water samples were capable of transforming E2 to E1, with half-lives of 0.2–9 d when incubated at 20 °C. E1 was then further degraded at similar rates - E2 was degraded also when incubated with anaerobic bed sediments - EE2 was much more resistant to biodegradation, but both E2 and EE2 were susceptible to photodegradation, with half-lives in the order of 10 d under ideal conditions	Jürgens et al. (2002)
E1, E2, EE2	STP	Municipal STP in Germany with an AS system for nitrification and denitrification; $C_{sol.}$ = 100 ng g^{-1}	- All estrogen concentrations decreased gradually along the treatment - The elimination efficiency of the natural estrogens (E1 and E2) exceeded 98% and EE2 was reduced by more than 90% - E1 and E2 were found to be degraded in the AS system while EE2 was degraded only in the nitrifying tank	Andersen et al. (2003)
E1, E2, EE2	AS	Batch experiments	- The removal of steroid estrogens with excess sludge was estimated to be only 1.5–1.8%, making sorption not important for the fate of steroid estrogens in STPs compared to biodegradation - In the range low ng L^{-1} to high µg L^{-1}, K_d was independent of water concentration	Andersen et al. (2005)
E2, EE2	Water sediments, groundwater	Aerobic and anaerobic conditions. Sorption test performed at room temperature	- The studied estrogens had modest sorption - There was no degradation of estrogens under anaerobic condition; under aerobic condition, EE2 concentration decreased, within 70 d from 1 to 0.62 mg g^{-1}	Ying et al. (2003)
E2, EE2	Activated and inactivated sludge	$C_{sol.}$ = 2.50 × 10^{-5}–50 mg L^{-1}. Inactivation of the sludge with mercury(II) sulphate.	- It was observed a high adsorption affinity of the compounds to the adsorbent and the adsorption was found to be dependent on pH	Clara et al. (2004)
E1, EE2	Municipal STPs	Various redox conditions were performed in batch experiment	- Both estrogens had an efficiency removal of >90% - The importance of aerobic conditions for the removal of the estrogens was highlighted - The removal efficiency of E1 and EE2 was reported as clearly dependent on redox conditions	Joss et al. (2004)
E1, E2, E3, EE2	*Nitrosomas europaea* from nitrifying AS	Batch experiment $C_{sol.}$ = 0.4 mg L^{-1}	- The addition of allylthiourea reduced the estrogen degrading activity; first order reaction kinetics were: 0.035 h^{-1} for EE2, 0.030 h^{-1} for E3, 0.056 h^{-1} for E1 and 1.3 h^{-1} for E2	Shi et al. (2004)
E1, E2, E3	STPs effluents	Laboratory-scale treatment plants were operated at four different SRTs characteristic for wastewater treatment design - 1, 5, 13 and approx. 26 d; T in the lab-scale reactors varied between 20.3 and 27.4 °C	- This work shows the dependency of the elimination from wastewater of the natural estrogens E2, E1 and E3, on the SRT 10 °C - At SRT 10 °C higher than 10 d nearly complete removal of those compounds was	Clara et al. (2005)

Table 4 (*continued*)

Estrogens	Agent and/or Matrix	Experimental Conditions	Conclusions	Reference
			achieved and effluent concentrations in the limit of detection range were measured	
E1, E2, E3, EE2	STP and AS	Effluents of STPs, included primary chemical treatment only, submerged aerated filter, oxidation ditch, AS and TF combined with AS	- The highest estrogen removal was observed in the effluent from a STP using primary treatment only - EE2 was detected only in two effluents of STPs	Johnson et al. (2005)
E2	AS from a aeration basin of a STP	E2 at 10, 30 and 50 μg L^{-1}; temperatures of 5, 20 and 35 °C; microbial population densities of 1750, 875 and 435 mg L^{-1}	- The removal of E2 was found to be strongly dependent on influent concentrations of E2, microbial population densities and temperatures	Li et al. (2005)
E2, EE2	Cultures established from lake water and sediments	Methanogenic, sulphate, iron and nitrate reducing anaerobic conditions $C_{est.}$ = 5 mg L^{-1}	- E2 was transformed to E1 - No anaerobic degradation of EE2 occurred over a long incubation period (over three years)	Czajka and Londry (2006)
E1, E2	Municipal sewage and AS	HRT: 40–48 h; SRT: 30–40 d; MLSS: 1500–2000 mg L^{-1}	- E2 and E1 adsorbed on the sludge were deconposed in 4 h - Adsorption and decomposition of estrogens in contact with AS were inactivated by sterilizing the sludge	Suzuki and Maruyama (2006)
EE2	Nitrifying AS	Sorption and biodegradation were performed in lab-scale bioreactors	- The relationship between biomass particle size, hydrophobicity and sorption capacity were assessed - Biodegradation of EE2 was more important than biosorption under the condition of high initial ammonia concentration (>48 mg L^{-1})	Yi et al. (2006)
E1, E2, EE2	Anaerobic sludge	Raw sewage sludge used in this work was collected from a STP located in Spain	- Natural estrogens were among the compounds with the higher removal efficiencies - In general, no influence of SRT and temperature on PPCPs removal was observed	Carballa et al. (2007)
EE2	MBR	EE2 in two different labelled radioactive forms MBR operated with a SRT of 25 d	- Radioactivity mainly remained sorbed in the reactor – removal of 80% - The elimination pathway did not involve the removal of the ethinyl group from EE2 molecule	Cirja et al. (2007)
E1, E2, EE2	Nitrifying activated sludge	AS from SBR. Maximum storage of three weeks at 5–8 °C	- The involvement of ammonia monooxygenase in the biotransformation of EE2 into EE2-OH was studied - The EE2 biological stability was compared to that of E1	Ren et al. (2007b)
EE2	Ammonium monooxygenase containing culture extract	Nitrifying bioreactor; HRT 0.75 d and SRT 20 d	- A linear relationship between nitrification and EE2 removal in enriched nitrifying cultures was found	Yi and Harper (2007)
E2, EE2	AS	For each batch experiment, two reactors were set up: aerobic and alternating anoxic/aerobic E2 and EE2 used from methanolic stock solutions	- Potential relationships between availability of oxygen, nitrification rate and estrogen removal were evaluated - EE2 was persistent under anoxic conditions; under aerobic conditions, a removal of 22% was achieved. E2 was readily converted to E1 – faster under aerobic (nitrifying) than anoxic (denitrifying) conditions - Higher removal rates of estrogens were associated with higher nitrification rates	Dytczak et al. (2008)
EE2	*Nitrosomas europora* and *Nitrosopira multiformis* from nitrifying AS	Batch tests with the addition of 200–500 mg L^{-1} of NH_4–N $C_{est.}$ = 300 mg L^{-1}	- EE2 removal was accomplished via nitration - At high NH_4–N concentration, degradation occurred by AOB, and at low concentration degradation is due to heterotrophic bacteria	Gaulke et al. (2008)
E1, E2, E3, EE2	STP	Nitrifying activated sludge plant from England	- The EE2 removal represented only 3%, for 24 h, and 5.6% in the end of 7 d of treatment. However, was observed an excellent removal for the other estrogens (97–99%)	Kanda and Churchley (2008)
E1, E2, EE2	AS	Under strictly anaerobic conditions	- Under anaerobic conditions, E1 was reduced to E2 but the extent of this reduction depends on the type of inoculum - No significant loss of the sum of E1, E2 and EE2 was observed - Adsorption accounted for a 32–35% loss of E1 and E2 from the liquid phase	Mes et al. (2008)
E2, E2	AS	AS reactors operated in a semi continuous flow mode	Under aerobic conditions, E2 and E1 dropped rapidly due firstly to sorption onto AS and then through biodegradation	Li F. et al. 2008
E1, E2, EE2	Nitrite-accumulating sequencing batch reactors		- EE2 removal was observed to be adversely affected by SRT shorter than 5.7 d, and	

(*continued on next page*)

Table 4 (*continued*)

Estrogens	Agent and/or Matrix	Experimental Conditions	Conclusions	Reference
		Two reactors operated with different sludge ages, under aerobic conditions and anoxic/anaerobic/aerobic conditions	significantly lower when SRT was longer than 7.5 d	Phukchan et al. (2008)
E2, EE2	Groundwater, aquifer material and effluent	Both aerobic and anoxic conditions performed in aquifer material and groundwater or effluent mixture, in presence of glucose	- Under anoxic conditions, only the E2 biodegradation occurs, in either type of water - Under aerobic conditions rapid degradation of E2 occurred, but not of EE2: its half-life was 26 d in groundwater and 15 d in effluent	Ying et al. (2008a)
E1, E2, E3, EE2	STPs	Were considered four Australian STPs with different technologies	- E1 and EE2 were the ones more persistent during the treatment - EE2 degradation was faster under aerobic conditions; biodegradation was not significant under anoxic conditions	Ying et al. (2008b)
E2, EE2	Aerated nitrifying submerged fixed bed bioreactors	Lab scale bioreactors with volume 1.4 L and flow velocity 1 m h^{-1}	- In the batch test, was no or little removal of EE2; however, EE2 removal continued after several months of starvation when by AOB - 90% of EE2 removal in aerated nitrifying batch reactor and a complete biological EE2 removal, from the synthetic effluent in a fixed bed reactor were accomplished	Forrez et al. (2009a)
EE2	AS	Sludge from Shanghai Changqiao Municipal STP; Two SBRs	- No biodegradation of EE2 was observed in the absence of nitrate - In the presence of nitrate, the overall removal rate of EE2 was greater than 97% and mostly due to biodegradation (95%)	Zeng et al. (2009)
EE2	AS and membrane bioreactor	SRT: 50 d; aerobic cycle: 2 h; anoxic cycle: 1 h	- AS acclimated in the MBR resulted in the stabilization of EE2 removal (65%) - Conventional activated sludge (CAS) resulted in EE2 accumulation in the permeate - In batch kinetics, CAS removed EE2 only through sorption and AS acclimated to the MBR showed biodegradation abilities	Clouzot et al. (2010)
E1, E2, E3, EE2	Anaerobic sludge	Sludge from three different STPs in France	- Plant-scale anaerobic digestion showed low efficiency (<40%) for removing estrogens	Muller et al. (2010)
EE2	Laccase from *Trametes* sp. and *Pycnoporus coccineus*	Degradation performed in a test tube and a rotating reactor EE2 was adsorbed on sea sand	- Laccase activity of 0.8 U mL^{-1} - Within 48 h, EE2 was removed in the test tube, to the extent of 90%	Tanaka et al. (2001)
E1, E2, E3, EE2	*C. vulgaris*	Batch experiments with incubation for 48 h (light and dark)	- E1 and E2 were interconvertible in both light and dark conditions - In the light, 50% E2 was further metabolized to an unknown product - All the tested estrogens exhibited a degree of partitioning to *C. vulgaris*	Lai et al. (2002)
EE2	Pathogenic ascomycete *Fusarium proliferatum*	$C_{est.}$ = 25 mg L^{-1} Batch experiment	- After 15 d, EE2 was removed from the culture in 97%	Shi et al. (2002)
E2, EE2	Manganese peroxidase and laccase from *P. chrysosporium* and from *T. versicolor* cultures	Mediator system with 1-hydroxybenzotriazole	- MnP activity of 10 nkat mL^{-1} - E2 and EE2 were completely transformed within 1 h. In the same amount of time estrogenic activity disappeared	Suzuki et al. (2003)
EE2	*Cunninghamella elegans*	EE2 from the previous microbial transformation of oral contraceptive norethisterone	- The microbial transformation of EE2 was achieved - Several EE2 metabolites were identified	Choudhary et al. (2004)
E1, E2, E3, EE2	*Rhodococcus zopfii* and *R. equi*	Cultivation under aerobic conditions in test tubes at 25 °C, for 24 h	- 100 mg L^{-1} EE2 were completely removed, within 24 h, by *R. zopfii*	Yoshimoto et al. (2004)
E1, E2, E3, EE2	HRP	Effect of pH and temperature on enzymatickinetics	- HRP activity of 0.017 U mL^{-1} - At pH = 8.0 the removal efficiency was 96–100%, for E2, E3 and EE2, within 1 h	Auriol et al. (2006)
E1	White-rot fungus *Phanerochaete sordida*, *MnP* and *laccase*	Ligninolytic conditions with low-nitrogen and high-carbon culture medium	- E1 decreased by 98% after 5 d of treatment - The activities of ligninolytic enzymes MnP and laccase were detected during treatment, which suggested that the disappearance of E1 is related to their production	Tamagawa et al. (2006)
E1, E2, E3, EE2	HRP and H_2O_2	Initial HRP activity of 0.02 U mL^{-1}	- The initial HRP activity was sufficient to completely remove EE2 - HRP doses up to 0.06 U mL^{-1} were required to remove E1, E2 and E3 - The optimal molar H_2O_2-to-substrate ratio was determined to be ~0.45	Auriol et al. (2007a)
E1, E2, E3, EE2	Laccase	Synthetic water and municipal wastewater	- Laccase (20 U mL^{-1}) was able to produce complete removal; 1-hydroxybenzotriazole mediator improved laccase efficiency	Auriol et al. (2007b)

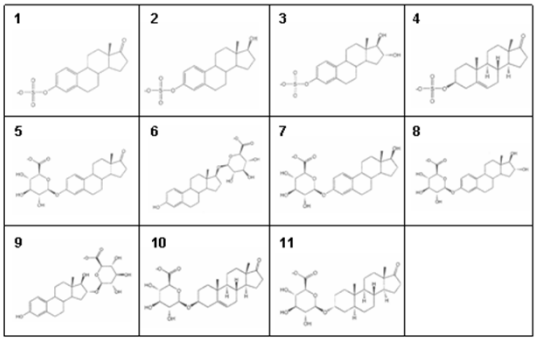
Table 4 (*continued*)

Estrogens	Agent and/or Matrix	Experimental Conditions	Conclusions	Reference
E1, E2, E3, EE2	*Acinetobacter, Agromyces and Sphingomonas*	Aerobic and anoxic conditions were performed with sand and ultra filtrated secondary effluent	- Under aerobic conditions, degradation rates of estrogens increased with the initial concentration (range 50–200 mg L^{-1}) - EE2 remained stable during cultivation with the three isolates	Ke et al. (2007)
E1, E2, E3, EE2	*Sphingobacterium* sp. JCR5	Bacterial strains were isolated from a STP of a producing factory of oral contraceptives, in China; $C_{est.}$ = 30 mg L^{-1}	- Within 10 d, the removal efficiency was 87% - Was suggested a metabolic pathway for EE2 degradation - EE2 was metabolized in 87% within 10 d; the strain can also be cultivated on the other estrogens cited	Haiyan et al. (2007)
E1, E2, E3, EE2	*Trametes versicolor* laccase and HRP	Batch reaction at 25 °C with the buffered (pH = 7.0) reaction mixture or wastewater; $C_{est.}$ = 100 ng L^{-1}	- Laccase activity of 2 U mL^{-1}; HRP activity of 8–10 U mL^{-1} - Estrogens were completely oxidized with both enzymes in the wastewater reaction mixtures after 1 h of treatment	Auriol et al. (2008)
E2, EE2	*T. versicolor*	$C_{est.}$ = 10 mg L^{-1} Experiments in batch and bioreactor (HRT 120 h, operated during 26 d)	- Batch: removal of E2 and EE2 was of more than 97%, in 24 h, corresponding to removal rates of 0.43 and 0.44 mg L^{-1} h^{-1}, respectively - Bioreactor: E2 and EE2 completely removed with removal rates of 0.16 and 0.09 mg L^{-1} h^{-1}, respectively	Blánquez and Guieysse (2008)
EE2	Eleven microalgae strains	10 mg of EE2 to 50 mL of axenic algal cultures with an initial concentration of 160 mg L^{-1}	- Several transformation products were identified - *Selenastrum capricornutum, Scenedesmus quadricauda, S. vacuolatus* and *Ankistrodesmus braunii* were able to biotransform EE2	Della Greca et al. (2008)
E1, E2, E3, EE2	Laccase from five white-rot fungi	Each strain was cultured at 28 °C for 7–10 d	- The removal characteristics among tested strains were almost the same - Complex mixtures of EDCs had an enhanced removal ratio compared to that of single application	Sei et al. (2008)
EE2	*Irpex lacteus, Bjerkandera adusta, Phanerochaete chrysosporium, P. magnoliae, Pleurotus ostreatus, T. versicolor, Pycnoporus cinnabarinus, Dichomitus squalens*	$C_{est.}$ = 10 mg L^{-1} Static cultures were incubated with EE2	- *I. lacteus* and *P. ostreatus* totally degraded EE2, within 3 d - The estrogenic activity determination was assessed by a recombinant yeast assay	Cajthaml et al. (2009)
E1, E2, EE2	Mixture of pure cultures of six different algae genera	Synthetic wastewater was used and the estrogens' concentrations were measured by enzyme-linked immunosorbent assays	- Removal of estrogens is accelerated by the presence of algae, in the 6 d batch tests - Processes like sorption, biodegradation and photolytic degradation were given importance in the removal of estrogens	Shi et al. (2010)

Summary of Table

- Estrogen removal was not always complete due to variations mentioned
- Microorganisms present in treatment plants can convert the excreted conjugates
  - Active conjugates
  - Inactive conjugates

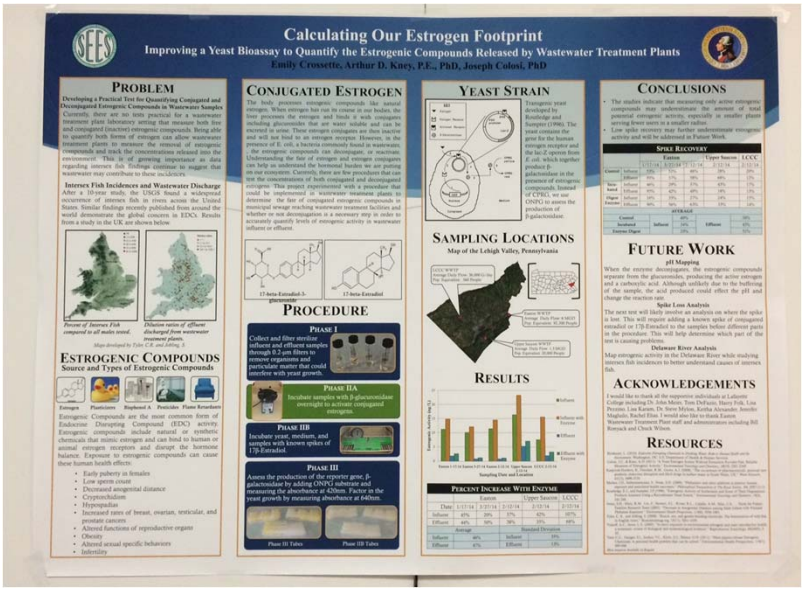
Active vs. Inactive



Inactive= conjugates of suplhuric and glucuronic acids

1. Estrone-3-sulfate
2. Beta-Estradiol-3-sulfate
3. Estriol-3-sulfate
4. Dehydroepiandrosterone-3-sulfate
5. Estrone-3-glucuronide
6. Beta-Estradiol-17-glucuronide
7. Beta-Estradiol-3-glucuronide
8. Estriol-3-glucuronide
9. Estriol-16-glucuronide
10. Dehydroepiandrosterone-3-glucoronide
11. Androsterone-3-glucuronide

NEGSWS



Conclusions from NEGSWS study

- Increased percent of estrogen with enzyme addition to samples to measure inactive estrogens
- Measuring only active estrogenic compounds may underestimate the amount of total potential estrogenic activity, especially in smaller plants serving fewer users in a smaller radius
- Low spike of recovery may further underestimate estrogenic activity

Bacteria in AS

- Variable and mixed community of microorganisms (aerobic, anaerobic, and/or facultative)
- Higher or lower number of bacteria that obtain energy from the conversion of ammonia nitrogen to nitrate nitrogen (nitrification) are also present in AS

Two ways microorganisms can transform (degrade) steroidal hormones

1. Metabolic (growth-linked)- utilization of steroidal hormones as energy and/or carbon source

2. Co-metabolic (non-growth linked)- utilization of bacterial **enzymes** to "degrade" hormones; primary growth substrate is required for sustainable bacterial growth
   - Hydrolase- EC 3: formation of two products from a substrate by hydrolysis
   - Oxidase- EC 1: Catalyzes oxidation reactions; transfer of electrons from on substance to another

Proposed E2 degradation

i. Hydroxylation of ring A at C-4
ii. Hydroxylation of saturated ring (B, C, or D ring)
iii. Dehydration of ring D at C-17
iv. Dehydrogenation of ring D at C-17

E2 Degradation Pathways (Aerobic)

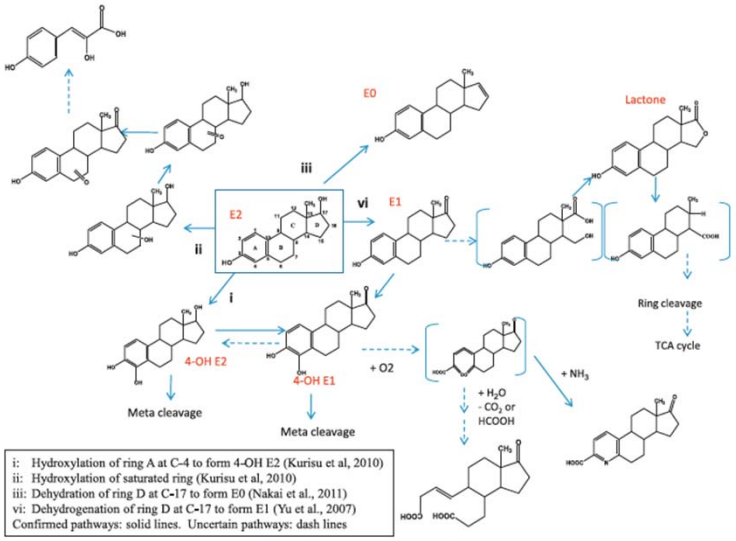
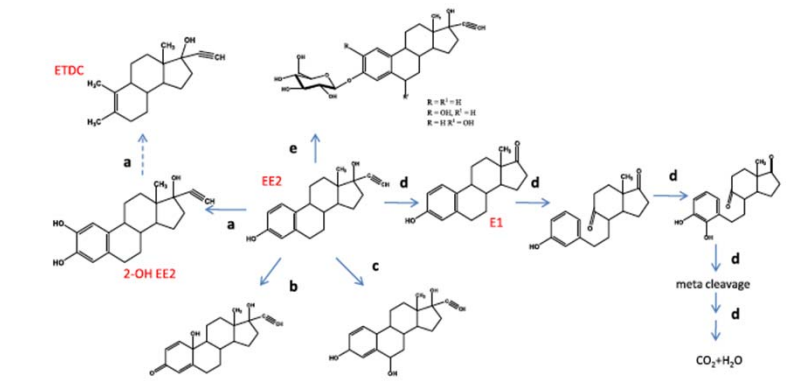


Fig. 2. Degradation pathways of E2 by aerobic bacteria.

Proposed EE2 Degradation

a) A-ring C-2 hydroxylation and ring A cleavage by nitrifying activated sludge
b) Conversion of 3-OH into 3-keto in A ring of EE2 by algal culture
c) B-ring C-6 hydroxylation by an algal culture
d) D-ring C-17 conversion to keto
e) Formation of EE2 conjugation by algal cultures

EE2 Degradation Pathways



(a) A-ring C-2 hydroxylation and ring A cleavage by nitrifying activated sludge (Yi and Harper, 2007)
(b) Conversion of 3-OH into 3-keto in A ring of EE2 by an algal culture, *Scenedesmus quadricauda* (Della Greca et al., 2008).
(c) B-ring C-6 hydroxylation by an algal culture *Ankistrodesmus braunii* (Della Greca et al., 2008).
(d) D-ring C-17 conversion to keto by *Sphingobacterium* sp. JCR5 (Ren et al., 2007)
(e) Formation of EE2 conjugation by an algal cultures, *Scenedesmus capricornutum* (Della Greca et al., 2008).
ETDC = (3-ethynyl-3a, 6, 7-trimethyl -2,3,3a,4,5,5a,8,9,9a,9b-decahydro-1*H*-cyclopenta[a]naphthalen-3-ol)
Confirmed pathways: solid lines. Uncertain pathways: dash lines

Fig. 3. Degradation pathways of EE2 by bacteria and algae.

Degradation Pathways

- Similar to cholesterol degradation- oxidation of A-ring is thought to initiate- catabolic pathway with elimination of alkyl side chain (Wael et al., 2011)
- Under aerobic conditions, the first step of E2 degradation is the oxidation of the C-17 alkyl ketone group to E1. Numerous enzymes can perform this step. The steps following are still controversial and there are two mechanisms currently in suspected (Wael et al., 2011).
- Under anaerobic conditions steps still unclear.
- It is not yet clear what pathways are responsible for the degradation of EE2. Although it is known that E2 is degrading to E1, this may not be the case for EE2 (Yu et al., 2007; Ribeiro et al., 2009)

Half-Lives of Estrogens in WWTPs

Table 3: Half-lives of E2, E1 and EE2 degradation in aerobic and denitrifying tanks of WWTP.

Conditions:	Treatment:	Half-life E2/min	Half-life E1/min	Half-life EE2/min	Initial conc./ ng/L[c]	Reference:
aerobic:	Activated sludge	1.3	2.5	336	500	Kjølholt J (2004) in De Mes et al. (2005)
aerobic:	Activated sludge	2.9	6.2	126	500[d]	Joss et al. (2004)
	MBR[a]	1.1	2.3	168	500[d]	Joss et al. (2004)
aerobic:	Activated sludge	2.1	45	n.d[b]	1000	Ternes et al. (1999a)
denitrifying:	Activated sludge	2	72	5940	500	Kjølholt J (2004) in De Mes et al. (2005)
denitrifying:	Activated sludge	2.2	33.3	834	500[d]	Joss et al. (2004)
denitrifying:	MBR[a]	3.6	8.7	336	500[d]	Joss et al. (2004)

a: membrane bio reactor
b: not degraded
c: if initial concentrations were higher, much higher half-lives were found (De Mes et al. 2005)
d: initial concentration of EE2 was 100 ng/L

Aerobic Microorganisms

Table 1
List of aerobic microorganisms capable of degrading or utilizing steroidal hormones.

Phylogenetic affiliation		Degradation ability and mechanism	Source of isolates	References
Alpha-proteobacteria	*Aminobacter aminovorans* KC7	Degradation of E1, E2	Activated sludge	Yu et al. (2007)
	Aminobacter sp. KC6	Degradation of E1, E2	Activated sludge	Yu et al. (2007)
	Brevundimonas diminuta 1	Conversion of E2 to E1	Activated sludge	Muller et al. (2010)
	Brevundimmonas vesicularies KC12	Conversion of E2 to E1	Activated sludge	Yu et al. (2007)
	Novosphingobium sp. strain JEM-1	Degradation of E1, E2, EE2	Activated sludge	Hashimoto et al. (2010)
	Novosphingobium tardaugens ARI-1	Metabolism of E1, E2, E3	Activated sludge	Fujii et al. (2002)
	Phyllobacterium myrsinacearum BP1	Degradation of E1, E2, E3; cometabolism of EE2 in the presence of E1, E2, E3	Compost	Pauwels et al. (2008)
	Sphingomonas sp. CYH	Degradation of E1, E2 under both aerobic and anoxic conditions	Artificial sandy aquifer	Ke et al. (2007)
	Sphingomonas sp. KC8	Metabolism of E2, E1, testosterone	Activated sludge	Yu et al. (2007) and Roh and Chu (2010)
	Sphingomonas sp. KC9	Conversion of E2 to E1	Activated sludge	Yu et al. (2007)
	Sphingomonas sp. KC10	Conversion of E2 to E1	Activated sludge	Yu et al. (2007)
	Sphingomonas sp. KC11	Conversion of E2 to E1	Activated sludge	Yu et al. (2007)
	Sphingomonas sp. KC14	Conversion of E2 to E1	Activated sludge	Yu et al. (2007)
	Sphingomonas sp. ED8	Metabolism of E2, E1	Soil samples from agricultural fields	Kurisu et al. (2010)
	Sphingomonas sp. ED9	Metabolism of E2, E1	Soil samples from agricultural fields	Kurisu et al. (2010)
Beta-proteobacteria	*Achromobacter xylosoxidans*	Metabolism of E1, E2	Activated sludge	Weber et al. (2005)
	Alcaligenes sp.	Metabolism of E2, testosterone	Soil	Payne and Talalay (1985)
	Alcaligenes faecalis	Conversion of E2 to E1 and *vice versa*	Intestinal microorganisms	Jarvenpaa et al. (1980)
	Leptothrix discophora (LMG 8142)	Production of biogenic Mn oxides to oxidize EE2	Belgian coordinated collections of microorganisms	Sabirova et al. (2008)
	Nitrosomonas europaea ATCC 19718	Cometabolism and Nitration of EE2	ATCC	Gaulke et al. (2008) and Skotnicka-Pitak et al. (2009)
	Ralstonia pickettii BP2	Degradation of E1, E2, E3; cometabolism of EE2 in the presence of E1, E2, E3	Compost	Pauwels et al. (2008)
	Ralstonia sp.	Metabolism of E1, E2	Activated sludge	Weber et al. (2005)

Aerobic Cont.

Gamma-proteobacteria	*Acinetobacter* sp. LHJ1	Conversion of E2 to E1	Artificial sandy aquifer	Ke et al. (2007)
	Acinetobacter sp. BP8	Degradation of E1, E2, E3; cometabolism of EE2 in the presence of E1, E2, E3	Compost	Pauwels et al. (2008)
	Acinetobacter sp. BP10	Degradation of E1, E2, E3; cometabolism of EE2 in the presence of E1, E2, E3	Compost	Pauwels et al. (2008)
	Buttiauxella	Metabolism of E2 and Testosterone	Baltic Sea	Zhang et al. (2011)
	Escherichia coli KC13	Conversion of E2 to E1	Activated sludge	Yu et al. (2007)
	Pseudomonas aeruginosa	Conversion of E2 to E1 and *vice versa*	Intestinal microorganisms	Jarvenpaa et al. (1980)
	Pseudomonas aeruginosa BP3	Degradation of E1, E2, E3; cometabolism of EE2 in the presence of E1, E2, E3	Compost	Pauwels et al. (2008)
	Pseudomonas aeruginosa TJ1	Metabolism of E2	Activated sludge	Zeng et al. (2009b)
	Pseudomonas putida MnB1 (LMG 2321)	Production of biogenic Mn oxides to oxidize EE2	Belgian coordinated collections of microorganisms	Sabirova et al. (2008)
	Pseudomonas putida MnB6 (LMG 2322)	Production of biogenic Mn oxides to oxidize EE2	Belgian coordinated collections of microorganisms	Sabirova et al. (2008)
	Pseudomonas putida MnB29 (LMG 2323)	Production of biogenic Mn oxides to oxidize EE2	Belgian coordinated collections of microorganisms	Sabirova et al. (2008)
	Pseudomonas sp. BP7	Degradation of E1, E2, E3; cometabolism of EE2 in the presence of E1, E2, E3	Compost	Pauwels et al. (2008)
	Vibrio sp. H5	Metbolism of E2 and Testosterone	Baltic Sea	Sang et al. (2012)
Actinobacteria	*Agromyces* sp. LHJ3	Degradation of E2 and E3 with formation of E1 under aerobic condition; degradation of E2 with formation of E1 under anoxic condition	Artificial sandy aquifer	Ke et al. (2007)
	Mycobacterium smegmatis	Conversion of E2 to E1 and *vice versa*; conversion of 16α-hydroxyestrone to E3	Intestinal microorganisms	Jarvenpaa et al. (1980)
	Microbacteria testaceum KC5	Conversion of E2 to E1	Activated sludge	Yu et al. (2007)
	Nocardioides simplex KC3	Conversion of E2 to E1	Activated sludge	Yu et al. (2007)
	Rhodococcus equi Y50155	Metabolism of E1, E2, E3, EE2	Activated sludge	Yoshimoto et al. (2004)
	Rhodococcus equi Y50156	Metabolism of E1, E2, E3, EE2	Activated sludge	Yoshimoto et al. (2004)
	Rhodococcus equi Y50157	Metabolism of E1, E2, E3, EE2	Activated sludge	Yoshimoto et al. (2004)
	Rhodococcus equi ATCC 13557	Partial degradation of EE2 in the presence of a cosubstrate	ATCC	O'Grady et al. (2009)
	Rhodococcus erythropolis ATCC 4277	Partial degradation of EE2 in the presence of a cosubstrate	ATCC	O'Grady et al. (2009)
	Rhodococcus rubber KC4	Conversion of E2 to E1	Activated sludge	Yu et al. (2007)
	Rhodococcus sp. ED6	Metabolism of E2, E1	Soil samples from agricultural fields	Kurisu et al. (2010)

Aerobic Cont.

Table 1 (*continued*)

Phylogenetic affiliation		Degradation ability and mechanism	Source of isolates	References
	Rhodococcus sp. ED7	Metabolism of E2, E1	Soil samples from agricultural fields	Kurisu et al. (2010)
	Rhodococcus sp. ED10	Metabolism of E2, E1	Soil samples from agricultural fields	Kurisu et al. (2010)
	Rhodococcus zopfii Y50158	Metabolism of E1, E2, E3, EE2	Activated sludge	Yoshimoto et al. (2004)
	Rhodococcus zopfii ATCC 51349	Partial degradation of EE2 in the presence of a cosubstrate	ATCC	O'Grady et al. (2009)
Bacteroidetes	*Flavobacterium* sp. KC1	Conversion of E2 to E1	Activated sludge	Yu et al. (2007)
	Flavobacterium sp. KC2	Conversion of E2 to E1	Activated sludge	Yu et al. (2007)
	Sphingobacterium sp. JCR5	Metabolism of E1, E2, E3, EE2.	Oral contraceptives producing factory activated sludge	Ren et al. (2007)
Firmicutes	*Bacillus cereus* Socransky 67	Conversion of E2 to unknown metabolites	Dental plaque	Ojanotkoharri et al. (1991)
	Bacillus sp. E2Y1	Degradation of E1, E2	Activated sludge	Jiang et al. (2010)
	Bacillus sp. E2Y2	Conversion of E2 to E1	Activated sludge	Jiang et al. (2010)
	Bacillus sp. E2Y3	Conversion of E2 to E1	Activated sludge	Jiang et al. (2010)
	Bacillus sp. E2Y4	Degradation of E1, E2	Activated sludge	Jiang et al. (2010)
	Bacillus sp. E2Y5	Conversion of E2 to E1	Activated sludge	Jiang et al. (2010)
	Staphylococcus aureus	Conversion of E2 to E1 and *vice versa*; conversion of 16α-hydroxyestrone to E3	Intestinal microorganisms	Jarvenpaa et al. (1980)
	Streptococcus faecalis	Conversion of E2 to E1; conversion of E1 to 16α-hydroxyestrone	Intestinal microorganisms	Jarvenpaa et al. (1980)
	Streptococcus mutans Ingbritt	Conversion of E2 to E1	Dental plaque	Ojanotkoharri et al. (1991)
	Streptococcus mutans NCTC 10449	Conversion of E2 to E1	Dental plaque	Ojanotkoharri et al. (1991)
	Streptococcus sanguis NCTC 10904	Conversion of E2 to E1	Dental plaque	Ojanotkoharri et al. (1991)

Anaerobic Microorganisms

Table 2
List of anaerobic and anoxic steroid hormone-degrading and -transforming bacteria.

Phylogenetic affiliation		Degradation ability and mechanism	Source of isolates	References
Alpha-proteobacteria	*Sphingomonas* sp. CYH	Degradation of E1, E2 under both aerobic and anoxic conditions	Artificial sandy aquifer	Ke et al. (2007)
Beta-proteobacteria	*Denitratisoma oestradiolicum* AcBE2-1^T	Metabolism of E1, E2 under the denitrifying condition	Activated sludge	Fahrbach et al. (2006)
Gamma-proteobacteria	*Steroidobacter denitrificans* FS^T	Metabolism of E1, E2 testosterone, 4-androstene-3,17-dione under the denitrifying condition	Anoxic digested sludge	Fahrbach et al. (2008)
Actinobacteria	*Actinomyces viscosus* 378.5	Degradation of E2 and progesterone anaerobically	Subgingival plaque samples	Komman and Loesche (1982)
	Agromyces sp. LHJ3	Degradation of E2 and E3 with formation of E1 under aerobic condition; degradation of E2 with formation of E1 under anoxic condition	Artificial sandy aquifer	Ke et al. (2007)
Bacteroidetes	*Bacteroides fragilis*	Conversion of E1 to E2 and E1 to 16α-hydroxyestrone anaerobically	Intestinal microorganisms	Jarvenpaa et al. (1980)
	Bacteroides gingivalis w	Degradation of progesterone anaerobically	Subgingival plaque samples	Komman and Loesche (1982)
	Bacteroides gingivalis 167.5	Degradation of E2 and progesterone anaerobically	Subgingival plaque samples	Komman and Loesche (1982)
	Bacteroides gingivalis 208.1	Degradation of E2 and progesterone anaerobically	Subgingival plaque samples	Komman and Loesche (1982)
	Bacteroides melaninogenicus subsp. Intermedius 155.6	Degradation of E2 and progesterone anaerobically	Subgingival plaque samples	Komman and Loesche (1982)
	Bacteroides melaninogenicus subsp. Intermedius 166.5	Degradation of E2 and progesterone anaerobically	Subgingival plaque samples	Komman and Loesche (1982)
	Bacteroides melaninogenicus subsp. Intermedius 167.4	Degradation of E2 and progesterone anaerobically	Subgingival plaque samples	Komman and Loesche (1982)
	Bacteroides melaninogenicus subsp. Melaninogenicus ATCC 25845	Degradation of E2 and progesterone anaerobically	ATCC	Komman and Loesche (1982)
Unclassified	Iron-reducing bacteria with 16S rRNA gene 84% similar to *Shewanella baltica*	Degradation of E1, E2, E3 under iron-reducing condition	Anaerobic digester	Ivanov et al. (2010)

Bacteria Found That Degrade Estrogens

- 2002- *Novosphingobium tardaugens sp. nov.*, strain ARI-1^T (Fujii et al., 2002; 2003)
  - E2 degrading activity- utilizes E2 as carbon source
  - E1 and E3
- 2004- *Rhodococcus equi, strains* Y50155, Y50156, and Y50157 (Yoshimoto et al., 2004)
  - Thought E2 did not degrade to E1
  - E2 and E1 degraded completely
  - E3 thought to degrade
- 2004- *Rhodococcus zopfii,* strain Y50158 (Yoshimoto et al., 2004)
  - Hypothesized to degrade EE2 but was not sole carbon source
  - Degrades E2 completely

Bacteria Cont.

- 2006- *Denitratisoma oestradiolicum*, strain AcBE2-1 (Fahrbach et al., 2006)
  - Degrades E2 with nitrate as the electron acceptor
- 2007- *Aminobacter* (strains KC6 and KC7), *Brevundimonas* (strain KC12), *Escherichia* (strain KC13), *Flavobacterium* (strain KC1), *Microbacterium* (strain KC5), *Nocardioides* (strain KC3), *Rhodococcus* (strain KC4), and *Sphingomonas* (strains KC8-KC11 and KC14) (Yu et al., 2007).
  - Strains KC6-8 were only three capable of degrading E1
  - All 14 isolates converted E2 to E1, hypothesis of E1 as metabolite of E2 degradation
  - E2 oxidized to E1 under aerobic conditions and slower degradation under anaerobic conditions
  - These isolates were of three Phyla: *Proteobacteria, Actinobacteria*, and *Bacteroidetes*
  - Degradation pathways begin to be explained

Bacteria Cont.

- 2007- Bacteria found in sediment, (Ke et al., 2007)
- Under Aerobic Conditions
  - All isolates, *Acinetobacter* (LHJ1), Agromyces (LHJ3), and CYH, oxidize E2 to E1
  - *Agromyces* (strain LHJ3)- degrades E3
  - Strain CYH had a 95% similarity with *Sphingomonas*- degrades E1
- Under Anaerobic Conditions
  - Strain CYH degrades E1
  - *Agromyces* (strain LHJ3)- degrades E2
  - E3 and EE2 were not degraded by isolates

Bacteria Cont.

- EE2 was found to be metabolized by ammonium-oxidizing bacteria with suspected ammonium monoxygenase involvement (Muller et al., 2009; Vader et al., 2000)
  - In an enriched culture where EE2 was the sole carbon source
  - more evidence of E2 degradation to E1
  - Consortium of *Novosphingobium tardaugens, Denitratisoma oestradiolicum, Rhodococcus zopfii, Rhodococcus equi, Achromobacter xyloxidans, Ralstonia* and *Brevundimonas*
- *Novospingobium sp.* (strain (JEM-1) was isolated (Hashimoto et al., 2011) is closely related to the strain ARI-1^T, first isolated by Fujii et al., 2002, with 96.6% similarity
  - No additional information was provided on the abilities of JEM-1 to degrade EE2 so study seems unjustified

So…

- Complete degradation of EE2 by nitrifying AS(NAS) resulting in formation of hydrophilic compounds
- E1, E2, EE2 all degraded by NAS
- Involvement of ammonia monoxygenase (AMO) in biotransformation of EE2 supporting co-metabolic degradation
- Most likely due to heterotrophic bacteria

Case Study of *Novosphingobium* sp. Strain JEM-1 (Hashimoto et al., 2011)

- Previous studies have no information on the abundance of isolates in AS of the contribution of isolates to estrogen removal in WWT processes
- Isolated using an enrichment culture from WWTP
- Able to degrade E2 and E1 from initial conc. of 10µg/L to below detection limit (0.5 ng/L) in less than 1 hr.
- Strain JEM-1 is able to degrade EE2
- JEM-1 investigated using rt-PCR and estrogen conc. using LC/MS/MS in two full-scale WWTP and a bench-scale bio-augmentation experiment

Case Study of Actinobacteria-*Rhodococcus equi* (Yoshimoto et al., 2004)

- *R. equi* is a facultative, opportunistic pathogen that causes fatal pyogranulomatous bronchopneumonia in foals (as well as HIV patients)
- Essential steps in catabolic pathway (encoded by following genes) are involved in the pathogenicity of *R.equi*
- Genes important for methylhexahydroindanone propionate (HIP, 5OH-HIP) degradation, as part of steroid catabolic pathway, are targets for development of live-attenuated vaccine against *R.equi* infections
- Two genes within cholesterol catabolic gene cluster: *ipdA* and *ipdB* and mutant *ipdAB* encode heterodimeric CoA transferase important for growth on steroids and help with steroid catabolic pathway in degradation
- *ipdAB* thought to remove intermediates by beta-oxidation during steroid degradation
- Inactivation of *ipdAB* induced a substantial protective immunity
- Study also found other gene involved in steroid ring degradation and may help to disrupt the immune homeostasis (*fadE30)*

WHAT' S NEXT? Future Directions…

- Degradation pathways need to be investigated further
- EE2 degradation needs to be researched and more isolates need to be provided
- Better understand metabolites involved in EE2, E2, and E1 degradation
- Degradation under anaerobic and anoxic conditions
- Studies with real world parameters…estrogen concentrations, longer SRTs, longer HRTs

END

- To next lecture