WP_Mining metagenomes for traits and species

Date: 2020-02-17
Created by: Maarten Verhoeven

Summary: Prior to construction and design of the expression vectors that will be used to transform and screen for bio-augmentation candidates, several aspects considering selection markers, replication origins and transformation methods need to be research. The experiments in this workplace aim to gain more insight into what is required on these expression vector to be able to target the most abundance (and most appropriate) species in activated sludge. For this, 16s rRNA data as well as MAGs will be used, which will be cross referenced with literature data on selection markers, plasmid replication mechanisms and transformation methods.

Background: Wastewater treatment currently relies on complex microbial communities that are formed from microorganism originated from the environment. Through application of selection pressures (e.g. sludge retention time, aeration rate, temperature etc..). While this method is excellent for removal of most of the organic compounds that are present at high(er) quantities in wastewater, micro-pollutant (small molecules present in low quantities) are often not removed sufficiently. As a result these pollutants are often released into the environment, which in some cases can have severe detrimental effects. A cost-effective option to remove micro-pollutants could be through bio-augmentation, the introduction of microbial species or strains that have capacity to degrade specific pollutants into the community. The greatest challenge for successful bio-augmentation is in obtaining microorganisms that can degrade the pollutants while concurrently also sustaining growth within the activated sludge community. Introduction of exogenous microorganisms have so far had limited success due to their low survival after prolonged cultivation. An alternative approach is to generate recombinant strains from species that already have a dominant presence in the activated sludge environment. As part of the Rubicon project I will be exploring whether we can apply recombinant technology to construct strains that can be used to augment wastewater treatment plants. Successful recombinant bio-augmentation requires that the host microorganism is genetically accessible.Therefore, a novel screening method will be developed that can be used to select the species capable of expressing transfected DNA. In order to design expression vectors as well as setup transformation protocols the metagenome data that is available at the PHN lab is very helpful to gain more insight into which components are required and what transformation methods to use.

Aim of the workplan: The aim of the experiments related with this workplan is to investigate the meta-genomic data gathered on WWTPs sample for the presence of antibiotic resistance genes (ARGs) and estrogenic genes. Additionally, 16s rRNA sequencing data from MIDAS will be used in conjunction with the abundance data from meta-genome sequencing to assess the microbial community. This data will provide valuable input for the plasmid designs and the transformation

methods to express these vectors in (preferable the most abundant) members of the microbial community in WWTPs.

Approach and activities:

- **Assess the microbial species abundance**: Successful recombinant bio-augmentation requires that a significant population of cells will need to contain the heterologous pathway in the eventual WWT culture. To ensure this, we prefer to target high abundant species during transformation and expression. Using 16s rRNA data that has been gathered for MIDAS and the meta-genome data gathered for WWTPs in Denmark a list of most abundant species can be made. Cross referencing with literature will allow us to select the most appropriate replication origin sequences for the expression vectors as well as aid the setup of the transformation methods.
- **Investigate the presence of ARGs**: The presence of antibiotic resistance in the WWTP community will be assessed in order to determine which antibiotic selection markers are most suitable for expression in the Interspecific expression system plasmids. Using the toolbox provided by Abricate databases such as the Comprehensive Antibiotic Resistance Database (CARD) or ResFinder. will be used to identify antibiotic resistance features in the MAGs of WWTP samples. the final selection will be based on both this data as well as literature research on previously used selection markers for engineering of (closely) related species. The table below shows the currently most used antibiotic selection marker that are used for bacteria:

Name	Class	Mode of Action*	
Kanamycin	aminoglycoside	Binds 30S ribosomal subunit; causes mis-translation	Bactericidal
Spectinomycin	aminoglycoside	Binds 30S ribosomal subunit; interrupts protein synthesis	Bactericidal
Streptomycin	aminoglycoside	Inhibits initiation of protein synthesis	Bactericidal
Ampicillin	beta-lactam	Inhibits cell wall synthesis	Bactericidal
Carbenicillin	beta-lactam	Inhibits cell wall synthesis	Bactericidal

Bleomycin	glycopeptide	Induces DNA breaks	Bactericidal
Erythromycin	macrolide	Blocks 50S ribosomal subunit; inhibits aminoacyl translocation	Bacteriostatic
Polymyxin B	polypeptide	Alters outer membrane permeability	Bactericidal
Tetracycline	tetracyclin	Binds 30S ribosomal subunit; inhibits protein synthesis (elongation step)	Bacteriostatic
Chloramphenicol		Binds 50S ribosomal subunit; inhibits peptidyl translocation	Bacteriostatic
Blasticidin S		Inhibiting termination step of translation and peptide bond formation (to lesser extent) by the ribosome.	Bacteriostatic

- **Search for Estrogen degradation genes in the MAGs:** Chen et al 2018 (see attached pdf) shows an overview of the estrogen degradation products and the corresponding species for which the conversion were identified (see figure 1). Only for one of the pathways the associated genes are known. To see if these genes are present in the WWTP microbial communities the metagenome data present at the AAU will be used to search for homologs in species.
    - oecA: https://www.genome.jp/dbget-bin/www_bget?spkc:KC8_09390
    - oecB: https://www.genome.jp/dbget-bin/www_bget?spkc:KC8_16650
    - oecC: https://www.genome.jp/dbget-bin/www_bget?spkc:KC8_05325

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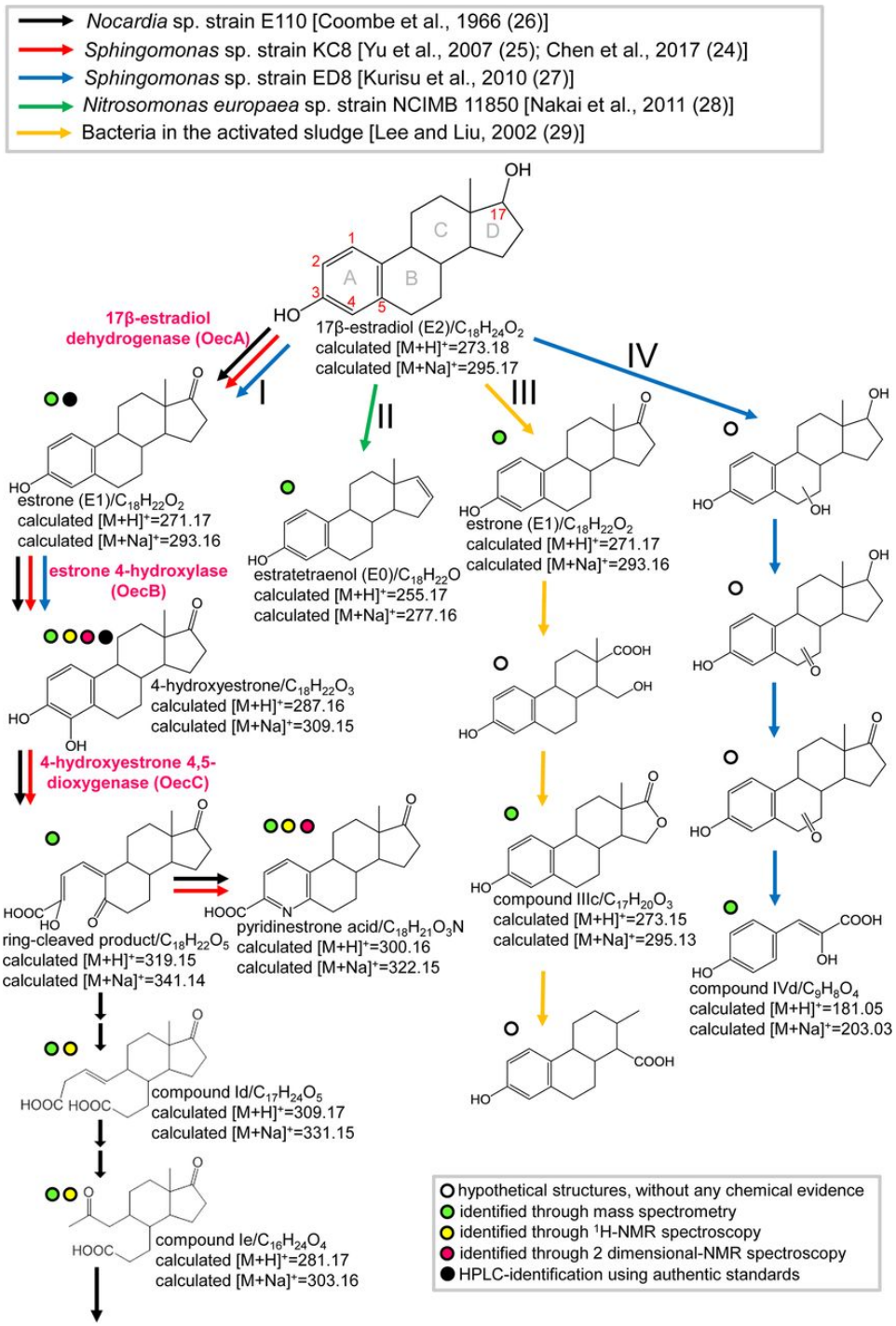

Figure 1: Overview of identified estrogen degradation in bacteria from activated sludge.

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- **Combine the information from previous three activities:** To fully assess which of the species in activated sludge are suitable for recombinant bio-augmentation the table below will need to filled in. Most of the information can be extracted from the 16s rRNA and MAGs gathered at AAU. Additionally, the predicted replication origin and suitable transformation methods will be curated from previous studies.

Species	Kingdom	Phylum	Class	Order	Family	**Abundance**	**Antibiotic Resistance**	**Predicted Replication Origin**	**Predicted Transformation Method**

Planning:

Activity:	week 1	week 2	week 3	week 4	week 5
Assess the microbial species abundance					
Investigate the presence of ARGs					
Search for estrogen degradation genes in the MAGs					
Combine the information from previous three activities					

Data management:

Data will be stored in CSV files in elabFTW.

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Required materials (and non-standard equipment):

- Software: Abricate, PANDAS, Fasttree,

Steps

Retrieve MAG data (2020-03-16 12:42:27)

Run Abricate (2020-03-16 12:42:29)

Make list with species abundance

Cross reference replication origin usage

Literature research on transformation methods

Combine information in table

Attached files

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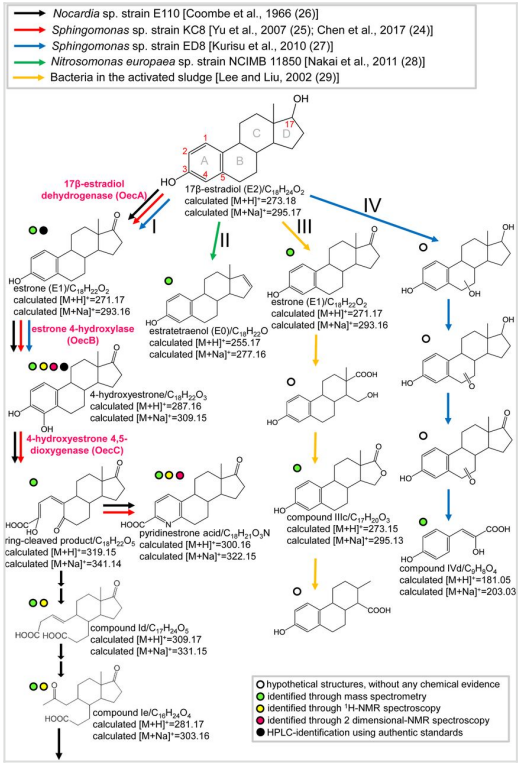

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