Don't let waste get wasted!

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1. Abstract

The Edinburgh iGEM 2013 team, WastED, is focusing on remediation and valorization of industrial waste streams, with a particular focus on Scottish leather and whisky industry waste waters, containing toxic heavy metal ions as well as fermentable organic components. Using Bacillus subtilis as chassis, we are engineering organisms to capture ions using chelators and metal binding proteins, and to ferment organic components to produce biofuels. We are also testing a new assembly procedure, GenBrick, based on the Genabler assembly system. GenBrick allows assembly of multiple RFC10-compatible BioBricks in a single reaction, and is also well suited to the preparation of fusion proteins and addition of terminal tags. Enzyme fusions may enhance metabolic pathways through substrate channeling. We are testing the effect of protein fusions on fermentation efficiency for biofuel production. In addition, we are examining the implications of possible Scottish independence, following the 2014 referendum, for Synthetic Biology in Scotland.

2. Introduction

Our aim was to create a self-contained system for bioremediation and valorization of toxic waste waters produced by the key Scottish industries. We have identified two major components of the industrial waste streams, which are believed to have the most detrimental effects on the environment: heavy metals and fermentable organic compounds.

Heavy metals can be dangerous to both health and the environment and, unlike other pollutants, they do not decay. They can lay dormant and have the potential for bioaccumulation and biomagnification. This leads to heavier exposure for some organisms, such as coastal fish and seabirds, than is present in the environment alone. Fermentable organic waste on the other hand is deleterious in a less direct way. When released to the water bodies, it can lead to occurrence of harmful algal blooms, which are of increasing concern in Scotland and worldwide through their negative effects on the biodiversity, human health and economy.

We have decided to use Bacillus subtilis as our chassis and the first step in our project was to characterize it by looking at its responses to varying ethanol and heavy metal concentrations. We then went on to create a sensory system for metal detection, followed by metal binding. We have decided to convert the fermentable organic compounds into bioethanol, which can have many potential application. As co-localization of enzymes has been previously shown to speed up metabolic pathways (see Team Slovenia 2010), we wanted to exploit this principle to increase ethanol production in bacteria by generating pET fusion protein(Whitaker et al., 2012,Conrado et al., 2012). To achieve this we have employed and tested a new assembly method, called GenBrick. Finally, we wanted to see how our manipulations might affect cell metabolism, by combining a modular model of the whole cell, to which different pathways can be slotted in.

a. Fur transcription factor and the fur box
b. Ferric binding protein A
c. Production of bioethanol
d. Aggregation by biofilms

3. Results

a. Characterization of Bacillus subtilis 168 as a chassis

Names
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Aggregation – metal induced biofilm formation in B. subtilis

4. Conclusions

5. Future Work

6. Reference List

7. Appendix 1 – List of Parts Submitted

	Type	Description	Designer	Length
BBa_K1122001	Composite	GFP (BBa_E0040) under the control of lac promoter	Lina Gasiūnaitė	1151
BBa_K1122003	Composite	mTagBFP (BBa_K592100) under the control of lac promoter	Lina Gasiūnaitė	1137
BBa_K1122004	Coding	GFP (BBa_E0040) compatible with Genabler assembly	Lina Gasiūnaitė	736
BBa_K1122007	Coding	AmilCP (BBa_K592009) compatible with Genabler assembly	Lina Gasiūnaitė	685
BBa_K1122008	Coding	mTagBFP (BBa_K592100) compatible with Genabler assembly	Lina Gasiūnaitė	721
BBa_K1122009	Plasmid	pDonor2	Lina Gasiūnaitė	2462
BBa_K1122010	Reporter	Genabler acceptor RFP cassette	Lina Gasiūnaitė	1034
BBa_K1122011	Reporter	Genabler acceptor lacZα cassette	Lina Gasiūnaitė	376
BBa_K1122674	Translational_Unit	Plac+fused PDC-ADH	Aleksandra Lewicka, Jan Lyczakowski	3459
BBa_K1122000	Coding	SinR transcription factor	Dainius Tautvaisas	333
BBa_K1122005	Coding	dsRED (BBa_E1010) compatible with Genabler assembly	Lina Gasiūnaitė	697
BBa_K1122006	Coding	mCherry (BBa_J06504) compatible with Genabler assembly	Lina Gasiūnaitė	730
BBa_K1122069	Regulatory	Ferric uptake repressor box	Hugo Villanueva	15
BBa_K1122222	Regulatory	pSpac - LacZ	Aleksandra Lewicka	390
BBa_K1122666	Coding	Ferric uptake repressor	Hugo Villanueva	450
BBa_K1122667	Coding	Enterobactin synthase component F	Kyle Rothschild-Mancinelli	4020
BBa_K1122668	Coding	Enterobactin synthase component D	Kyle Rothschild-Mancinelli	630
BBa_K1122669	Coding	Enterobactin synthase component E	Kyle Rothschild-Mancinelli	1611
BBa_K1122670	Coding	Enterobactin synthase component B	Kyle Rothschild-Mancinelli	858
BBa_K1122671	Coding	Enterobactin synthase component S	Kyle Rothschild-Mancinelli	1251
BBa_K1122672	Coding	Alcohol dehydrogenase E (AdhE)	Aleksandra Lewicka, Jan Lyczakowski	2589
BBa_K1122673	Coding	Ethanol production module	Aleksandra Lewicka, Jan Lyczakowski	3014
BBa_K1122675	Other	SinI Upstream Flank	Kyle Rothschild-Mancinelli	999
BBa_K1122676	Translational_Unit	IPTG inducible pdc and adhB	Aleksandra Lewicka, Jan Lyczakowski	3660
BBa_K1122678	Translational_Unit	pSpac - LacZ - pdc- adhB	Aleksandra Lewicka, Jan Lyczakowski	3450
BBa_K1122679	Translational_Unit	pSpac - LacZ - fused pdc and adhB	Aleksandra Lewicka, Jan Lyczakowski	3412

	Type	Description	Designer	Length
BBa_K1122680	Translational_Unit	pLac - LacZ - Fur	Aleksandra Lewicka, Jan Lyczakowski	1056
BBa_K1122681	Translational_Unit	pSpac - LacZ - Fur	Aleksandra Lewicka, Jan Lyczakowski	846
BBa_K1122682	Other	SinR Downstream Flank	Kyle Rothschild-Mancinelli	999
BBa_K1122683	Regulatory	Plac_LacZ_RBS (B0030)	Aleksandra Lewicka	623
BBa_K1122684	Regulatory	Plac_LacZ_RBS (B0032)	Aleksandra Lewicka	621
BBa_K1122685	Regulatory	Plac_LacZ_RBS (B0033)	Aleksandra Lewicka	619
BBa_K1122700	Coding	L lactate dehydrogenase (L-LDH)	Jan Lyczakowski	993
BBa_K1122701	Coding	D lactate dehydrogenase (D-LDH)	Jan Lyczakowski	1002
BBa_K1122702	Coding	Ferric ion-binding protein (FbpA)	Harry Thornton	999
BBa_K1122703	Coding	Ferric ion-binding protein (FbpA) without a signal peptide	Harry Thornton	936

8. Appendix 2 – Methods

9. Appendix 3 – RFC for GenBrick