Reprogramming Microbes to Silence or Cater to their Hosts:
Beta-Carotene Production and RNAi Delivery

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Beta-carotene Production

Roughly 400 million people worldwide are at risk of vitamin A deficiency, of whom 300 million are children (1). Deficiency can lead to progressive blindness and death and is preventable with a healthy diet containing significant plant derived beta-carotene pigments which are readily converted in the body to vitamin A. For many socio-economic and environmental reasons which are further explored by the IGEAM 2008 Calgary Ethics team on their website (1), improved diet is not a likely treatment for everyone. Knowing this, we propose a synthetic biology answer to this world health problem: to create a plug and play beta-carotene production system in a broad host range plasmid to reprogram human and corn associated bacteria to produce this vitamin when it had not been synthesized before. For transgenic intestinal vitamin production, we targeted the probiotic E. coli strain Nissle 1917 and several lactobacilli which might be ingested in locally made yoghurts. A corn endosymbiotic bacteria, Klebsiella pneumoniae 342 was also picked for testing vitamin production inside a different biological system.

Results: Beta-carotene

We obtained Erwinia uredovora genes for beta carotene synthesis from Ethan Johnson of iGEM Minnesota and these were modified by PCR mutagenesis to conform to Biobrick standards before submission to the registry. These Biobricks were then employed in the construction of a synthetic operon by serial suffix addition cutting the host plasmid with SpeI and PstI and ligating in the next XbaI and PstI cut fragment (see figure 1). To provide a transcriptional terminator and a visual marker for complete operon transcription, we included the GFP Biobrick Bba_E0030 as the fifth gene in the composite device. Two different promoters were inserted to drive operon expression: the chloroplast 16S ribosomal promoter (PSBA) from a herbicide resistant Amaranthus plant (2) and the arabinoide inducible promoter Bba_R0800 as the fifth gene in the composite device. Different promoters were inserted to drive operon expression: the chloroplast 16S ribosomal promoter (PSBA) from a herbicide resistant Amaranthus plant (2) and the arabinoide inducible promoter Bba_R0800 as the fifth gene in the composite device. Two different promoters were inserted to drive operon expression: the chloroplast 16S ribosomal promoter (PSBA) from a herbicide resistant Amaranthus plant (2) and the arabinoide inducible promoter Bba_R0800 as the fifth gene in the composite device. Two different promoters were inserted to drive operon expression: the chloroplast 16S ribosomal promoter (PSBA) from a herbicide resistant Amaranthus plant (2) and the arabinoide inducible promoter Bba_R0800 as the fifth gene in the composite device. Two different promoters were inserted to drive operon expression: the chloroplast 16S ribosomal promoter (PSBA) from a herbicide resistant Amaranthus plant (2) and the arabinoide inducible promoter Bba_R0800 as the fifth gene in the composite device. Two different promoters were inserted to drive operon expression: the chloroplast 16S ribosomal promoter (PSBA) from a herbicide resistant Amaranthus plant (2) and the arabinoide inducible promoter Bba_R0800 as the fifth gene in the composite device. Two different promoters were inserted to drive operon expression: the chloroplast 16S ribosomal promoter (PSBA) from a herbicide resistant Amaranthus plant (2) and the arabinoide inducible promoter Bba_R0800 as the fifth gene in the composite device. Two different promoters were inserted to drive operon expression: the chloroplast 16S ribosomal promoter (PSBA) from a herbicide resistant Amaranthus plant (2) and the arabinoide inducible promoter Bba_R0800 as the fifth gene in the composite device. Two different promoters were inserted to drive operon expression: the chloroplast 16S ribosomal promoter (PSBA) from a herbicide resistant Amaranthus plant (2) and the arabinoide inducible promoter Bba_R0800 as the fifth gene in the composite device. Two different promoters were inserted to drive operon expression: the chloroplast 16S ribosomal promoter (PSBA) from a herbicide resistant Amaranthus plant (2) and the arabinoide inducible promoter Bba_R0800 as the fifth gene in the composite device. Two different promoters were inserted to drive operon expression: the chloroplast 16S ribosomal promoter (PSBA) from a herbicide resistant Amaranthus plant (2) and the arabinoide inducible promoter Bba_R0800 as the fifth gene in the composite device. Two different promoters were inserted to drive operon expression: the chloroplast 16S ribosomal promoter (PSBA) from a herbicide resistant Amaranthus plant (2) and the arabinoide inducible promoter Bba_R0800 as the fifth gene in the composite device.

Discussion

We attempted to build plug and play plasmids which could be easily transferred to different bacterial species to co-opt their growth habits for human benefit. We were able to boot up a beta carotene production function in the lab strains of E. coli DH5α when expressed off the high copy plasmid pSB1A2 and supplemented with exogenous arabinose (figure 2), but this effect was not observed using the low copy plasmid pDSK-GFPuv. When put into intestinal model however these lab strains of E. coli were not able to survive or produce beta carotene after only two days, while pDSK-GFPuv in the probiotic strain Nissle 1917 was observed to survive and express GFP. Clearly further work will need to be done to adapt the synthetic operon for beta-carotene production from low copy plasmids and in biologically relevant microbial species. The TB1 RNAi construct expressed in pDSK-GFPuv was successfully created, electroporated into kp342, and injected into corn plants, but as the silencing phenotype manifests itself at later stages of plant maturity, it is not possible to comment on effectiveness or necessary additions to the strategy yet. BIGS will hopefully be promoted as a cheap, quick, and stable for studies in plant functional genomics worldwide.

References

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Thank You!

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Bacterial Induced Gene Silencing (BIGS) by RNAi

Double stranded RNA is recognized by eukaryotes as a signal for post transcriptional gene silencing and is so significant a discovery that it received the 2006 Nobel prize for Physiology or Medicine. It has gained importance in plant science as a tool for functional genomics to tally gene function by mutant phenotype analysis, however in plants like corn, transgenic plant production takes up to a year. Viral induced gene silencing (VIGS) is a quick and transient alternative in plants where it has been developed, however it has transient effects and does not result in stable phenotypes. As a quick and stable alternative, we propose to deliver RNAi signals into plants using plasmid, or endophytes. A strategy similar to transkingdom RNAi developed for mice using E. coli (4). Since bacteria like Klebsiella pneumoniae 342 can colonize corn plants in large numbers (5), we believe that constitutive RNAi expression in these stages may be carried by the plasmid within the host plant, will result in DNA sequestration into the plant resulting in systemic silencing of the targeted gene. We propose to call this technique BIGS (Bacterial Induced Gene Silencing). We designed a BIGS construction tool, which will accept any existing Biobrick and allow its orientation in an RNAi orientation (figure 5 in the sense orientation followed by a copy in the antisense orientation). To test our device, we targeted the corn TB1 gene for silencing which should result in a distinctive branching phenotype (3).

Results: BIGS

To test our bacterial vector and delivery plasmid, we injected corn plants with pDSK-GFPuv tagged kp342 and evaluated plasmid maintenance and bacterial survival over a period of one month (figure 4). Based on colony forming units counts (CFU) in 1.07 million microbes were counted 200 bp primer and 200 bp primers from IDT. The TB1 RNAi construct was made and electroporated into kp342 (figure 6). V2 stage corn was injected with 20 µl of this endophyte at OD600 = 0.8 and evaluated for phenotype two weeks later relative to tb1 mutants injected with pDSK-GFPuv tagged kp342 (figure 5).