DEVELOPING BIOSENSORS TO IDENTIFY ANTIMYCIN-PRODUCING ACTINOMYCETES

Antibiotic Resistance is one of the biggest challenges for 21st Century healthcare and is being exacerbated by the failure to discover new antibiotics. Team NRP-UEA_Norwich 2013 has developed a Biosensor Reporter System to screen soil bacteria for the recently sequenced antibiotic antimycin. Investigating the biosynthetic pathways of the novel antimycin structures will aid future engineering of antimycins to create potential new antibiotics for healthcare.

BACKGROUND

Streptomyces, a sub species of the actinomycete order, are bacteria renowned for producing two thirds of naturally-occuring antibiotics. They produce the antibiotics antimycins, active against various fungi by inhibiting the final stage of electron transport chain.

Homologues of the AntA sigma factor, the key regulatory protein activating antimycin biosynthesis have been found in all 14 known gene clusters. Biosensors have been developed consisting of a promoter regulated by AntA, the *antG* promoter, the most highly transcribed of the biosynthetic genes, controlling expression of our variable reporters.

BIOSENSOR

Utilising the specificity of the AntA sigma factor we have produced a cheap, high-throughput method of screening for antimycins (Fig. 1).

Without the biosensor, the process either relies on costly sequencing/mining endeavours or blind strain processing.

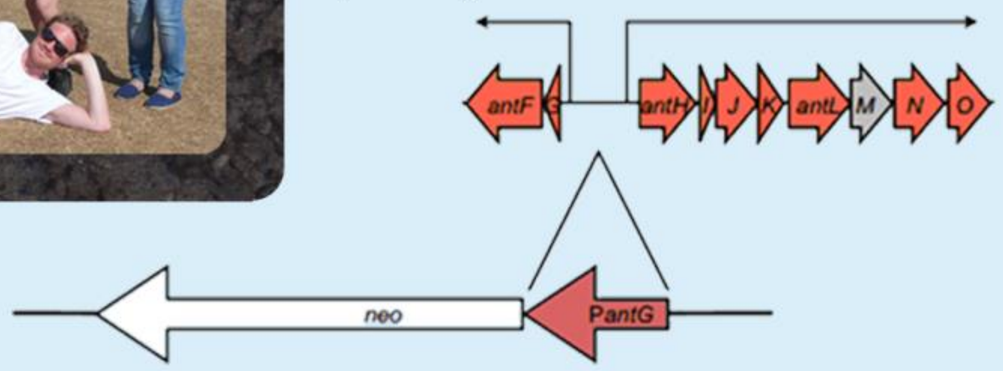


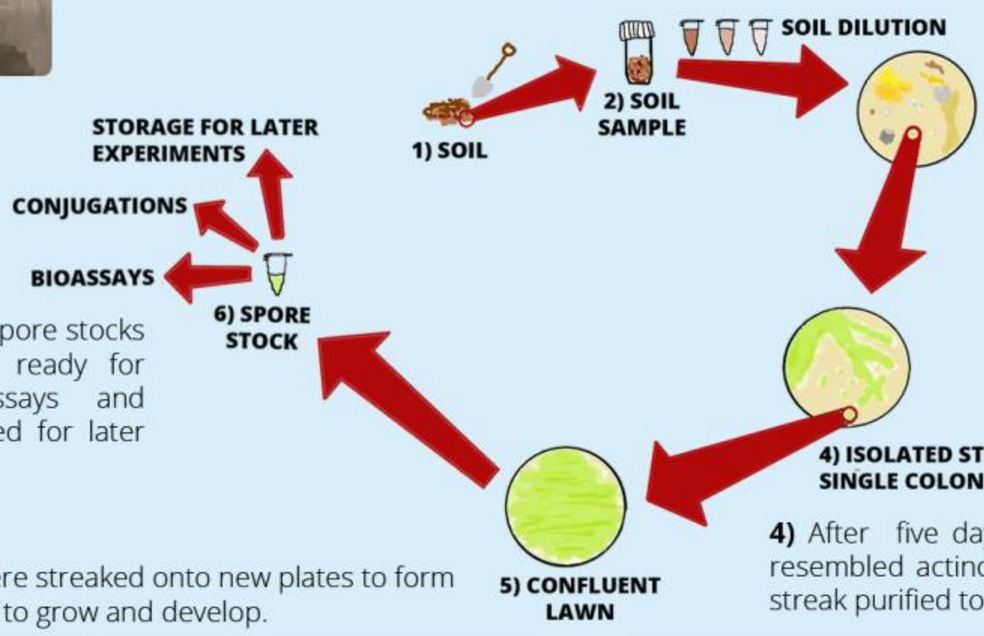
Figure 1. Design of a biosensor reporter construct including promoter *antGP*, regulated by antA sigma factor, which controls expression of our variable reporters (neo gene shown).

PROCEDURE

One focus of our project was to produce a library of filamentous actinomycetes and screen them, using our biosensor, for antimycin production.

Figure 3. Eight spore stocks showing the variety in strain colour and texture that exists in the strain library created. All are suspended in 20% glycerol and frozen.

1) In collabration with other iGEM teams we received soil samples from all over the world to increase the likelihood of finding new and interesting antimycins.



2) Serial soil dilutions were cultured on SFM (Soya Flour Mannitol) agar medium to isolate the filamentous bacteria (Fig. 2).

Figure 2. An example of soil dilution sample plated on SFM agar.

4) After five days of growth, the colonies that resembled actinomycetes (the furry ones!) were streak purified to single colonies for analysis.

5) Once grown these were streaked onto new plates to form confluent lawns and left to grow and develop.

6) Over 300 individual Spore stocks (Fig. 3) were created ready for analysis by bioassays and conjugations, and stored for later experiments.

RESULTS

Over 100 strains were challenged in *C. albicans* bioassays (Fig. 4). Of these 37 produced zones of clearing, 10 of which are representitive of antimycin clearings.

These 10 were 16s sequenced, those that were successful, following BLAST analysis, returned predominantly *streptomyces* sp.

Five spore stocks closely matched five different *Streptomyces* strains.

During our project we trialled and optimised three reporters, neomycin (Kanamycin resistance), RFP (red fluorescent protein) and GUS (B-glucoronidase breaks down a glucoride substrate producing a blue halo around the colonies).

We successfully cloned two Biosensor Reporter constructs (BBa_K1041001 BBa_K1041002) (Fig. 5) , submitted these to the Parts Registry and designed a third biosensor (BBa_K1041004).

We improved an exsisting biobrick Bba_J04450 and experimentally validated the part (BBa_K1041000).

FURTHER WORK

Following the competition our host lab will utilise HPLC and LC-MS to identify if novel antimycins are being produced. If indeed a novel antimycin is detected, identification of the biosynthetic cluster and characterisation of the pathway and associated tailoring enzymes is essential.

It is hoped that in the future these pathways can be engineered to enhance antimycin properties or create new structures and functions. The biosensor design can also be adapted to identify bacterial strains that produce antibiotics or useful secondary metabolites by varying the promoter component.

Figure 4. Plates showing 6 of the bioassays of *Candida albicans* overlays on colonies of unknown actinomycetes, with a noticeable zone of clearance surrounding the spots of bacteria.

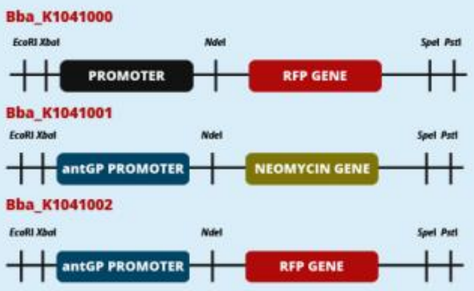


Figure 5. Design of our three characterised biobricks.

HUMAN PRACTICES

Our team carried out a wide range of collaborations and outreach activities to publicise iGEM, Synthetic Biology and our project to a wide range of audiences. In addition we developed a resource library for future teams to access.

COLLABORATIONS

- Copenhagen's Bricks of Knowledge
- Purdue's standardisation of the parts registry
- Soil samples received from worldwide
- Attended the Young Synthetic Biologists Conference in London

OUTREACH ACTIVITIES

- Stall at the Norwich Forum
- UEA 50 Anniversary Festival
- SGM Podcast
- Eastern Daily Press (EDP) newspaper article
- Summer Student Afternoon
- Talk to secondary school students
- Students as Partners Summit at the HEA

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