Investigation of a *Streptomyces nodosus* glycosyltransferase

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Introduction

- Amphotericin B (AmB) is an antibiotic used to treat systemic fungal infections[1]
- Naturally synthesized by *Streptomyces nosodus*[2]
- Potency is recognized but its uses are limited due to the toxic side effects for patients[3]
- One of the enzymes involved in the synthesis of AmB is AmphDI, a glycosyltransferase[4]
- Many glycosyltranferases can accept a variety of sugars are their substrate[5]
- Mycosamine sugar plays an important role in AmB's antifungal activity[6]

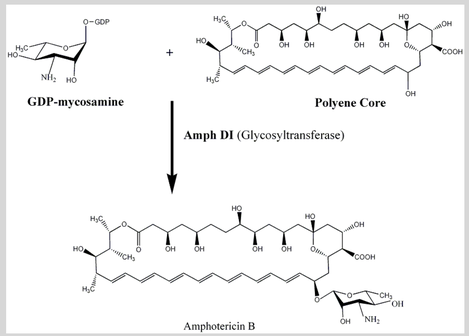

Figure 1. AmphDI function

Central Question

- Can the substitution of sugars attached to the polyene core of AmB alter its toxicity to humans and provide more potent antibiotics?

Research Goals

- Ascertain the expression of AmphDI in the *Streptomyces venezuelae* strain NJB122
- Isolate the plasmid AmphDI/pSE34 from *S. venezuelae* strains

Materials and Methods

- *S. venezuelae* (DHS7038) containing the pSE34 plasmid was the control strain (NJB115)
- *S. venezuelae* (DHS7038) containing the *amph*DI/pSE34 vector is the production strain (NJB122)
- Kanamycin and thiostrepton were used to select for the specific strains (50 mg/ml stock concentration)
- 50 uL spores cultured in 5ml TSB broth (50uL/ml Kan/Tsr) for 48 hours (seed cultures) followed by 48 hours in 50 ml SCM broth, both at 29°C

Materials and Methods

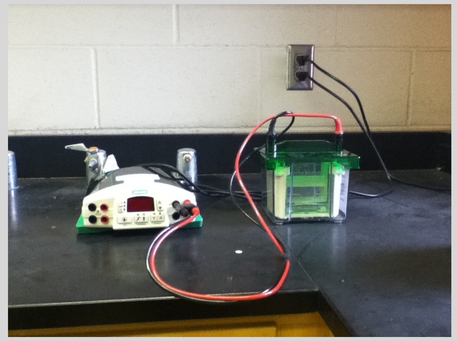

Figure 2. BioRad SDS-PAGE electrophoresis apparatus

BioRad SDS-PAGE electrophoresis

- Bacteria harvested by centrifugation
- Analyzed samples for *S. venezuelae* only, not *E. coli*
- Protein isolated using nickel resin from Promega
- Samples consisted of increasing concentrations of binding buffer + imidazole
- SDS-PAGE run on 10% Precise gel (Thermo Scientific) with Tris-HEPES running buffer
- Used constant current of 18 mA per gel

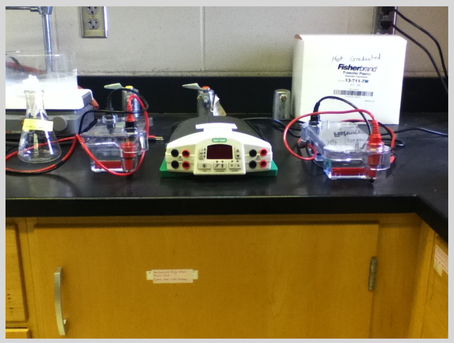

Figure 3. BioRad DNA gel electrophoresis apparatus

DNA purification method

- Plasmid DNA extraction with alkaline lysis preparation
- DNA digestion with HindIII
- Digestion samples incubated for 1 hour at 37°C
- Analyzed on 0.7% agarose gel in 1x TAE buffer
- Gel run using constant voltage 80V
- Product expected size

7.5 (vector) + 1.4 (gene) = ~9 kb[2]

Results – SDS-PAGE analysis

- Marker used was prestained protein ladder
- Expected size of AmphDI protein is 53.8 kDa[2]
- Observed bands in both control strain (115) and production strain (122) around expected size of AmphDI
- Protein bands in control strain much lighter than in production strain – may not be AmphDI protein

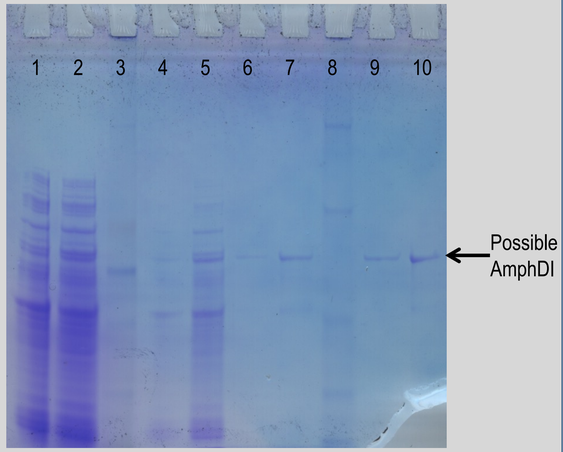

Figure 4. SDS-PAGE gel for *S. venezuelae* 115/122: lane 1 10mM 115, lane 2 10mM 122, lane 3 marker, lane 4 30 mM 115, lane 5 30 mM 122 lane 6 50 mM 115, lane 7 30mM 122, lane 8 marker, lane 9 250mM 115, lane 10 250 mM 122

Results – DNA gel electrophoresis

- Marker used was New England Biolab 1kb ladder
- Visualized plasmid DNA isolated from 115 or 122 after digestion with HindIII
- Observed a difference between 115 and 122 lanes

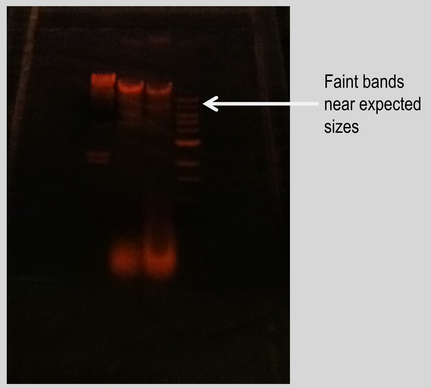

Figure 5. DNA gel for *S. venezuelae* 115/122 plasmid isolation: lane 1 marker ,lane 2 115, lane 3 122, lane 4 marker.

Conclusions

- *amph*DI gene appears to be present in pSE34 construct in production strain of *S. venezuelae* (NJB122) but not in control strain (115)
- Protein of size similar to AmphDI appears to be present in both control strain and production strain of *S. venezuelae*
- Need to determine identity of protein observed by SDS-PAGE in production strain NJB122
  - Gene is present in plasmid construct, but protein expression is not certain

Ongoing Research

- Obtain protein sample from NJB122 for amino acid sequence analysis
- Once identity of protein confirmed, grow large-scale cultures of NJB122 to mass produce AmphDI protein
- Study AmphDI catalyzed reaction with natural and alternative sugar substrates for novel compounds[7]

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