Undergraduate Research Symposium
ADVANCING RESEARCH AND STEM FIELD ENGAGEMENT
PROJECT 34

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Drew University, Class of 2021
Major: **Biology**
Minor: **Public Health; Biochemistry; Molecular Biology**

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Research Institute for Scientists Emeriti (RISE)

DNA Sequencing of the Ribosomal Protein Gene, Rpsl, in Strains of Kibdelopsporagium Sp. that Produce Increased Levels of the Medically Important Antibiotic, Kibdelomycin

Kibdelomycin is a natural product antibiotic produced by *Kibdelosporangium sp*. discovered at Merck Research Laboratories. The mechanism of action of kibdelomycin is inhibition of DNA gyrase and topoisomerase IV similar to marketed quinolone antibiotics. Kibdelomycin is of interest because it inhibits the growth of microorganisms resistant to quinolone antibiotics. Unfortunately, kibdelomycin has demonstrated poor activity in animal models due to poor pharmacokinetics. Our goals are to produce sufficient quantities of fermentative kibdelomycin and then modify the chemical structure to improve the pharmacokinetics. In order to increase the production of kibdelomycin two new strains of *Kibdelosporangium sp.* were obtained by selecting strains resistant to streptomycin and gentamicin. These strains produced up to 50% more kibdelomcyin. Increased natural product production via this method has been correlated to mutant ribosomal genes: *rpsL* and *rpoB* (Hu & Ochi 2001). This research project involved Sanger DNA sequencing of the *rpsL* gene in three *Kibdelosporangium sp.* strains: the wild-type strain, a strain resistant to streptomycin, and a strain resistant to both streptomycin and gentamicin. No mutations were found within *rpsL*, showing 100% sequence match between the three strains. Currently, the *rpoB* gene is being sequenced to determine if this gene is responsible for increased kibdelomycin production.

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Acknowledgments: ***Dr. Bayne, Dr. Connors, Dr. Gullo and DSSI***

DNA sequencing of the ribosomal protein gene, rpsL*, in strains of* Kibdelosporangium sp. *that produce increased levels of the medically-important antibiotic, kibdelomycin.*

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Abstract

Kibdelomycin is a natural product antibiotic produced by *Kibdelosporangium sp.* discovered at Merck Research Laboratories. The mechanism of action of kibdelomycin is inhibition of DNA gyrase and topoisomerase IV similar to marketed quinolone antibiotics. Kibdelomycin is of interest because it inhibits the growth of microorganisms resistant to quinolone antibiotics. Unfortunately, kibdelomycin has demonstrated poor activity in animal models due to poor pharmacokinetics. Our goals are to produce sufficient quantities of fermentative kibdelomycin and then modify the chemical structure to improve the pharmacokinetics. In order to increase the production of kibdelomycin two new strains of *Kibdelosporangium sp.* were obtained by selecting strains resistant to streptomycin and gentamicin. These strains produced up to 50% more kibdelomycin. Increased natural product production via this method has been correlated to mutant ribosomal genes: *rpsL* and *rpoB* (Hu & Ochi 2001). This research project involved Sanger DNA sequencing of the *rpsL* gene in three *Kibdelosporangium sp.* strains: the wild-type strain, a strain resistant to streptomycin, and a strain resistant to both streptomycin and gentamicin. No mutations were found within *rpsL*, showing 100% sequence match between the three strains. Currently, the *rpoB* gene is being sequenced to determine if this gene is responsible for increased kibdelomycin production.

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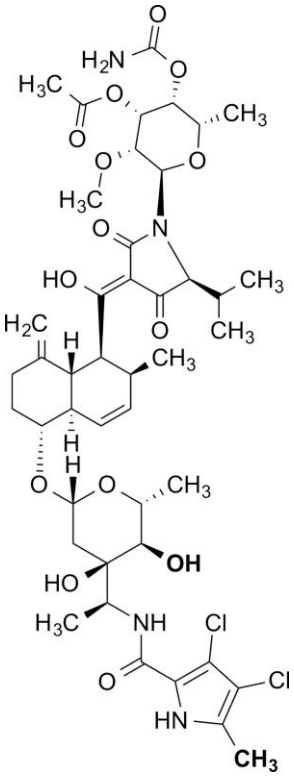
Dr. Marvin Bayne
Dr. Vincent Gullo
Allergan
Drew Summer Science Institute (DSSI)
Dr. Alan Rosan

Kibdelomycin: Natural Product of Kibdelosporangium sp.

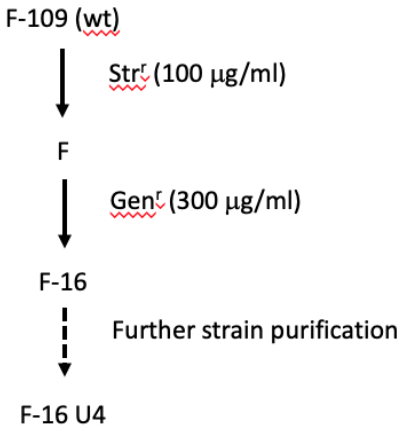
- Discovered by Merck in early 2000's
- Bacterial Type II topoisomerase and DNA gyrase inhibitor
- Antibacterial activity against Gram-positive bacteria
- Genome of *Kibdelosporangium sp.* sequenced
- Poor pharmacokinetics due to binding of serum proteins
- Research Goal: Improve pharmacokinetics through chemical modification of kibdelomycin structure
    - Need to improve kibdelomycin production in fermentation

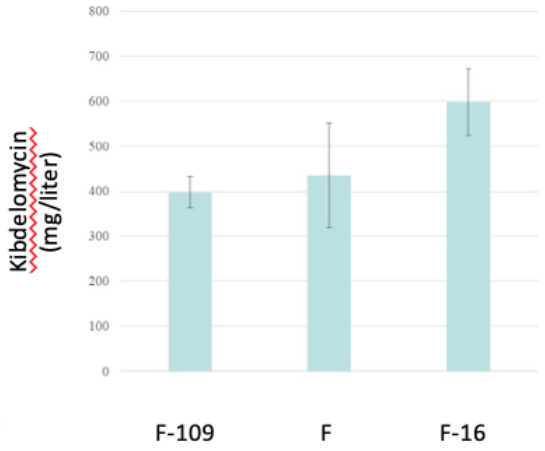


Kibdelomycin
MW:938

Strain Improvement (Classical): Cumulative Antibiotic Resistance Mutations

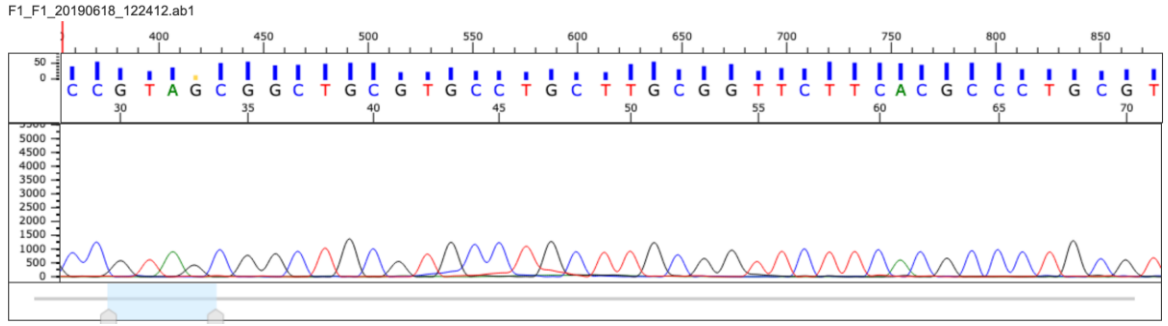
- Production of natural product in actinomycetes can be increased by selecting for resistance to antibiotics that inhibit RNA synthesis
    - Increased NP production correlated to mutants in ribosomal genes - *rpsL* and *rpoB.* (Hu & Ochi 2001)
- Previously sequentially selected for strains resistant against streptomycin, gentamicin, and rifampin (Ryann Callaghan)
    - Resulted in Isolation of Str^r Gen^r mutant strains increases kibdelomycin production
- Objective: Sequence responsible ribosomal genes, to find mutations





rpsL Sequencing Results: No Mutations Present in *rpsL*

- Methods and Analysis
  - Primer Design
  - PCR Nesting for further *rpsL* isolation
  - Sanger Sequencing with ThermoFisher SeqStudio, in house
  - Clustal Matrix for sequence comparisons
- Current and Future Research
  - Currently sequencing *rpoB*
  - Mutagenesis of *Kibdelosporangium* strains to select for rifampin antibiotic resistance to increase kibdelomycin production



Percent Identity Matrix - created by Clustal2.1

1: seq_OriginalSequence	100.00	100.00	100.00	100.00	100.00	100.00	100.00
2: seq_Strain109_Forward	100.00	100.00	100.00	100.00	100.00	100.00	100.00
3: seq_Strain109_Reverse	100.00	100.00	100.00	100.00	100.00	100.00	100.00
4: seq_StrainF_Forward	100.00	100.00	100.00	100.00	100.00	100.00	100.00
5: seq_StrainF_Reverse	100.00	100.00	100.00	100.00	100.00	100.00	100.00
6: seq_StrainF16_Forward	100.00	100.00	100.00	100.00	100.00	100.00	100.00
7: seq_StrainF16_Reverse	100.00	100.00	100.00	100.00	100.00	100.00	100.00