Studying the Function of Heterogeneous Ribosomes Using Antibiotics

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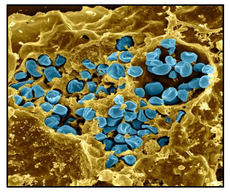
INTRODUCTION

Francisella tularensis

- Gram negative bacterium
- Causes tularemia, a potentially lethal illness
- Potential bioweapon due to its highly infectious nature



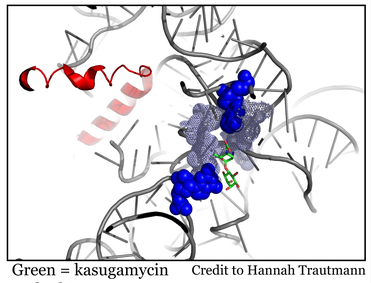
Ribosomal protein bS21

- Involved in translation initiation
- Three homologs encoded in the *F. tularensis* genome: *rpsU1*, *rpsU2*, and *rpsU3*
- Strains were created to focus on each homolog individually

Antibiotics and Kasugamycin

- Antibiotics effective against bacteria often inhibit ribosome function
- Kasugamycin is an inhibitor of translation initiation
- Antibiotics that bind to the 30S ribosome were studied with *F. tularensis*.
- A previous student identified kasugamycin as the only drug that caused altered susceptibility within these strains among the tested 30S inhibitors.

Kasugamycin Binds on the Ribosome



Green = kasugamycin
Red = bS21
Grey = 16S rRNA
Blue = kasugamycin-binding pocket on 16S rRNA

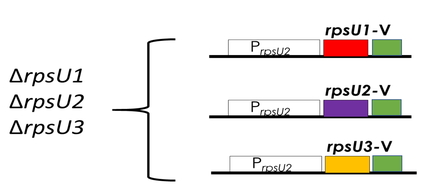
Credit to Hannah Trautmann

Antibiotic Susceptibility of Strains with Single bS21 Homologs is Generally Unaffected

Drug	LVS	LVS Tn7::bS21-1	LVS Tn7::bS21-2	LVS Tn7::bS21-3
Kanamycin	53.5 +/- 0.6	55.3 +/- 0.2	53.6 +/- 0.4	53.2 +/- 0.5
Tetracycline	46.0 +/- 0.9	45.8 +/- 1.4	49.0 +/- 1.9	47.2 +/- 0.3
Streptomycin	55.1 +/- 3.4	54.9 +/- 0.7	56.1 +/- 0.1	58.0 +/- 1.8
Ciprofloxacin	56.5 +/- 1.3	56.0 +/- 1.2	55.1 +/- 0.1	56.0 +/- 0.1

METHODS AND MATERIALS

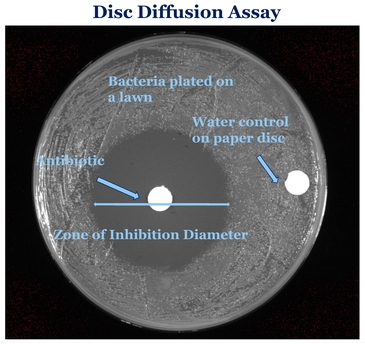
Strains Used



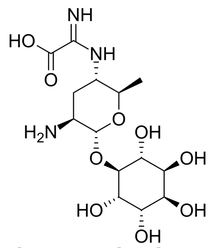
Strain Name
Tn7::*rpsU1*
Tn7::*rpsU2*
Tn7::*rpsU3*

Credit to Hannah Trautmann

Disc Diffusion Assay



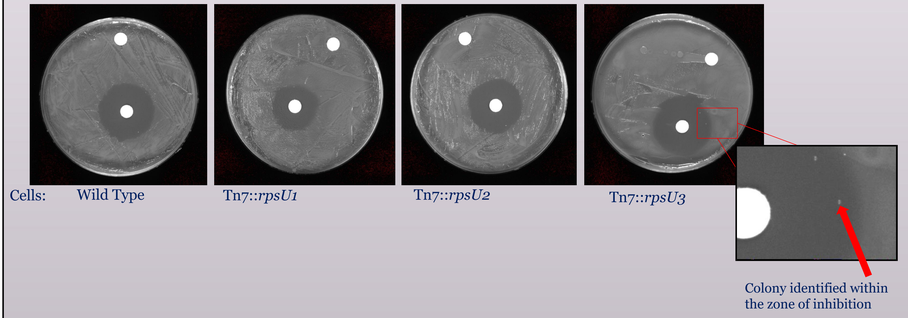
Kasugamycin

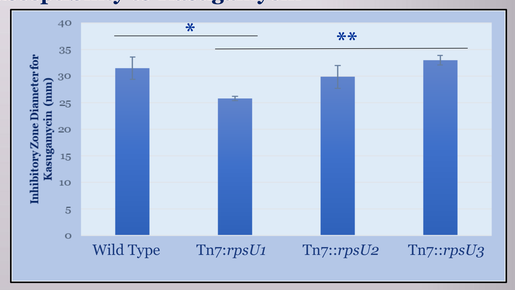


Dissolved in water and used at 50 mg/ml and 150mg/ml as indicated

RESULTS

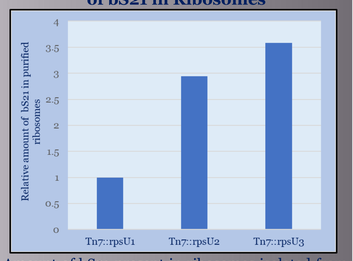
Cells with Different bS21 Content Have Altered Susceptibility to Kasugamycin





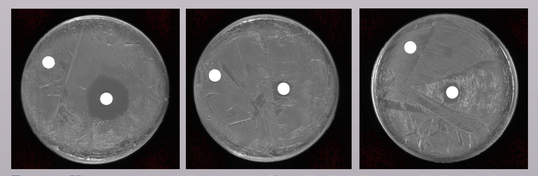
Disc diffusion assays were performed the indicated strains in biological triplicate using discs impregnated with 50 mg/ml kasugamycin. Plates were incubated for 48 hours, and zones of inhibition were measured in mm. Error bars represent standard deviation. *p<.002, **p<.0002

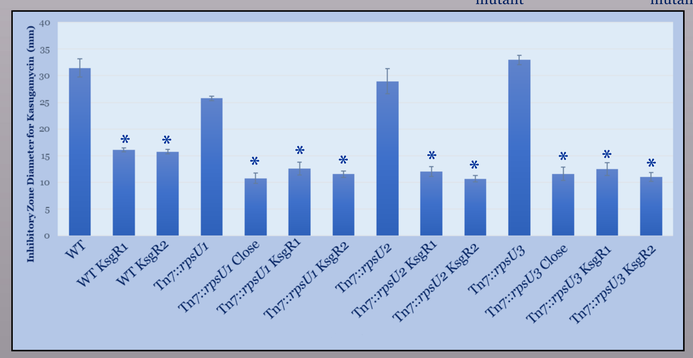
Isogenic Strains Have Different Amounts of bS21 in Ribosomes



Amount of bS21 present in ribosomes isolated from indicated strains. VSV-G tagged bS21 homologs were detected by immunoblotting. Results were normalized to total protein in ribosome samples. Results from Hannah Trautmann.

Identification of Kasugamycin Resistant Mutants





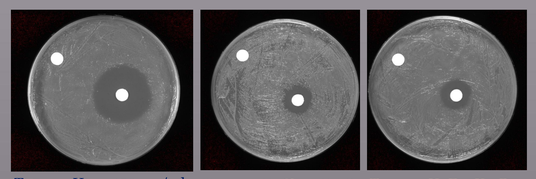
Disc diffusion assays were performed the indicated strains in biological triplicate using discs impregnated with 50 mg/ml kasugamycin. Plates were incubated for 48 hours, and zones of inhibition were measured in mm. Error bars represent standard deviation. *p<.00005 compared to parental strain.

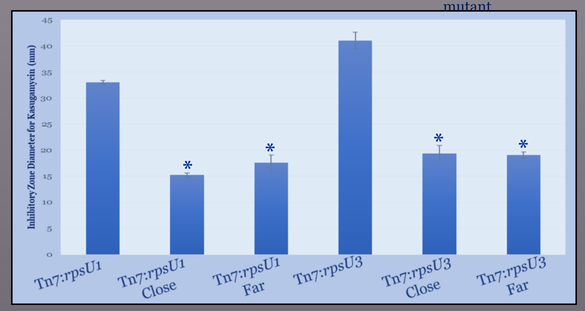
Mutations Identified that Correlate with Kasugamycin Resistance

Strain	Type of Mutation	Original Amino Acid	Mutation
LVS	-	-	-
LVS KsgR1	Missense	Arg217 (AGA)	Ile217 (ATA)
LVS KsgR2	Frameshift Deletion	His82 (CAT)	Deletion (--C)
Tn7::rpsU1	-	-	-
Tn7::rpsU1 Close	Nonsense	Glu206 (GAA)	Stop Codon (TAA)
Tn7::rpsU1 KsgR1	Missense	Thr48 (ACA)	Ile48 (ATA)
Tn7::rpsU1 KsgR2	Frameshift Deletion	His82 (CAT)	Deletion (--C)
Tn7::rpsU2	-	-	-
Tn7::rpsU2 KsgR1	Nonsense	Glu207 (GAA)	Stop Codon (TAA)
Tn7::rpsU2 KsgR2	Nonsense	Ser99 (TCA)	Stop Codon (TAA)
Tn7::rpsU3	-	-	-
Tn7::rpsU3 Close	Nonsense	Tyr202 (TAC)	Stop Codon (TAA)
Tn7::rpsU3 KsgR1	Frameshift Deletion	His82(CAT)	Deletion (--C)
Tn7::rpsU3 KsgR2	Nonsense	Ser10 (TCG)	Stop Codon (TAG)

In all kasugamycin-resistant strains, we identified mutations in the *ksgA* gene by Sanger sequencing; no mutations were identified in the parental strains. The wild-type *KsgA* protein is 262 amino acids long.

Further Testing of Kasugamycin Resistant Mutants

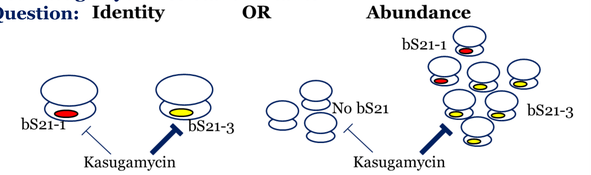




Disc diffusion assays were performed the indicated strains in biological triplicate using discs impregnated with 150 mg/ml kasugamycin. Plates were incubated for 48 hours, and zones of inhibition were measured in mm. Error bars represent standard deviation. *p<.0001 compared to parental strain.

CONCLUSIONS AND FURTHER STEPS

Identified kasugamycin resistant mutants
Open Question: Identity OR Abundance



Exciting future direction: ribosome specific antibiotics?

References

Kasugamycin:
Schuwirth, B. S. *et al.* Structural analysis of kasugamycin inhibition of translation. *Nat Struct Mol Biol* 13, 879–886 (2006).

Translation initiation:
Duval, M., Simonetti, A., Caldelari, I. & Marzi, S. Multiple ways to regulate translation initiation in bacteria: Mechanisms, regulatory circuits, dynamics. *Biochimie* 114, 18–29 (2015).

Acknowledgments

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