Sanford Burnham Prebys Metabolomics Core: Data Report [SECIM SUBMISSION]

Date: 07/07/2016	**SBP Project #**: 16-016	**Researcher:** Jessica Kajfasz	**PI:** Jose Lemos

Biological Samples Submitted

Species: *Enterococcus faecalis*
Sample Type: Cell Pellet
Experimental Variables: 1. DMSO (N = 3)
2. Mupirocin (N = 2)
3. Decoyinine (N = 3)

N: 3 for DMSO, 2 for Mupirocin*, 3 for Decoyinine; **Total N = 8 samples**

* A microcentrifuge tube shattered while removing from storage container

Assay Modules Performed

Nucleotides (1-methyl NAM, NAM, NMN, NAD, NADP, AMP, ADP, ATP, GDP, GTP, CDP, CTP, UDP, UTP)

Sample Preparation and LC-MS: AZ
Data Analysis and Reporting: AZ and SJG
Instrumentation: Thermo Quantiva LC/MS/MS

LC/MS/MS quantitation utilized authentic, heavy isotope-labeled internal standards (IS) for NAM, NMN, NAD, ATP, GTP, CTP, and UTP. AMP and ADP quantitation utilized the ATP IS, GDP quantitation utilized GTP IS, CDP quantitation utilized CTP IS, UDP quantitation utilized UTP IS, NADP quantitation utilized NAD IS.

Background

Enterococcus faecalis is a gram-positive bacterium frequently found in the mouth and has a naturally high level of antibiotic resistance. It was formerly classified as *Streptococcus faecalis* until it was placed into a separate genus classification with further genomic DNA analysis in 1984. *Enterococcus faecalis*, while normally abundant within the body, is the cause of many serious human infections.

Mupirocin is an antibiotic used to treat gram-positive bacteria. The drug inhibits protein synthesis by binding to isoleucyl t-RNA synthetase. In addition, DNA and cell wall formation are also negatively impacted.

Decoyinine has certain effects on Bacteria including inhibiting guanine nucleotide synthesis, RNA synthesis, cell wall synthesis, and initiating sporulation.

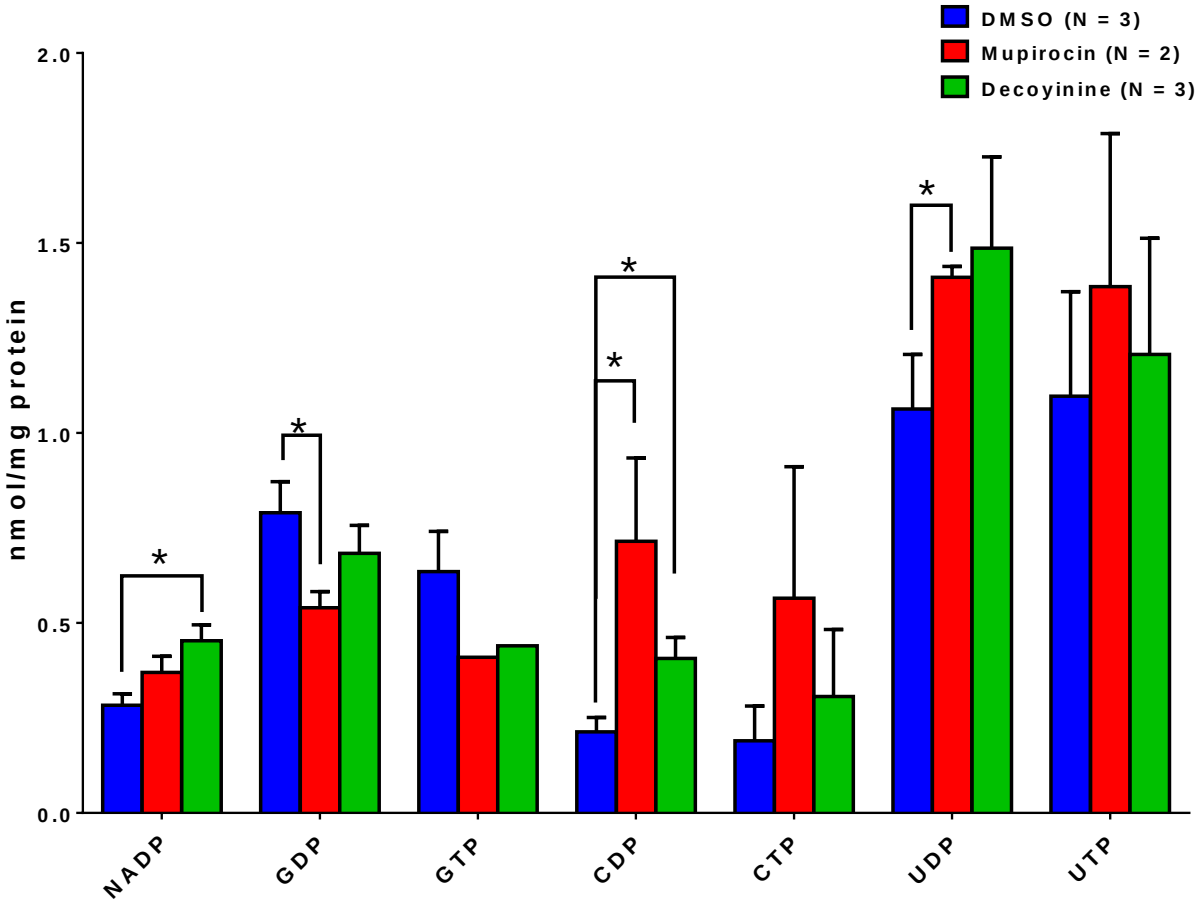
Study Overview

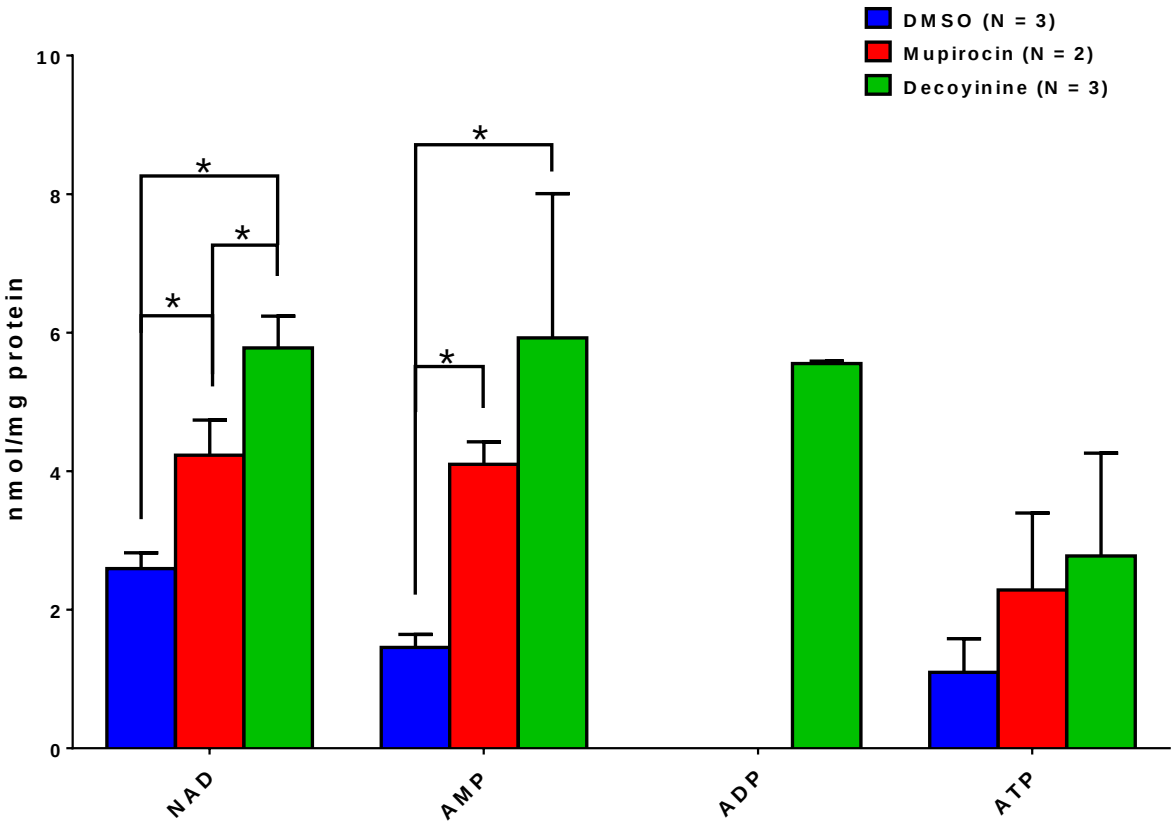
Frozen cells pellets were received on dry ice and stored at -80° C. Before the assay was performed, the samples were lyophilized to dryness. The lyophilized pellet was homogenized in 200 uL of 0.5 M PCA using a Precellys (bead beating) system. A 100 µL aliquot of the homogenate was used to perform the nucleotides assay.

The data which follow are normalized to protein measurements (mg) provided by the researcher.

Nucleotides

Determination of metabolite profile of E. faecalis OG1RF control as compared to exposure to mupirocin or decoyinine





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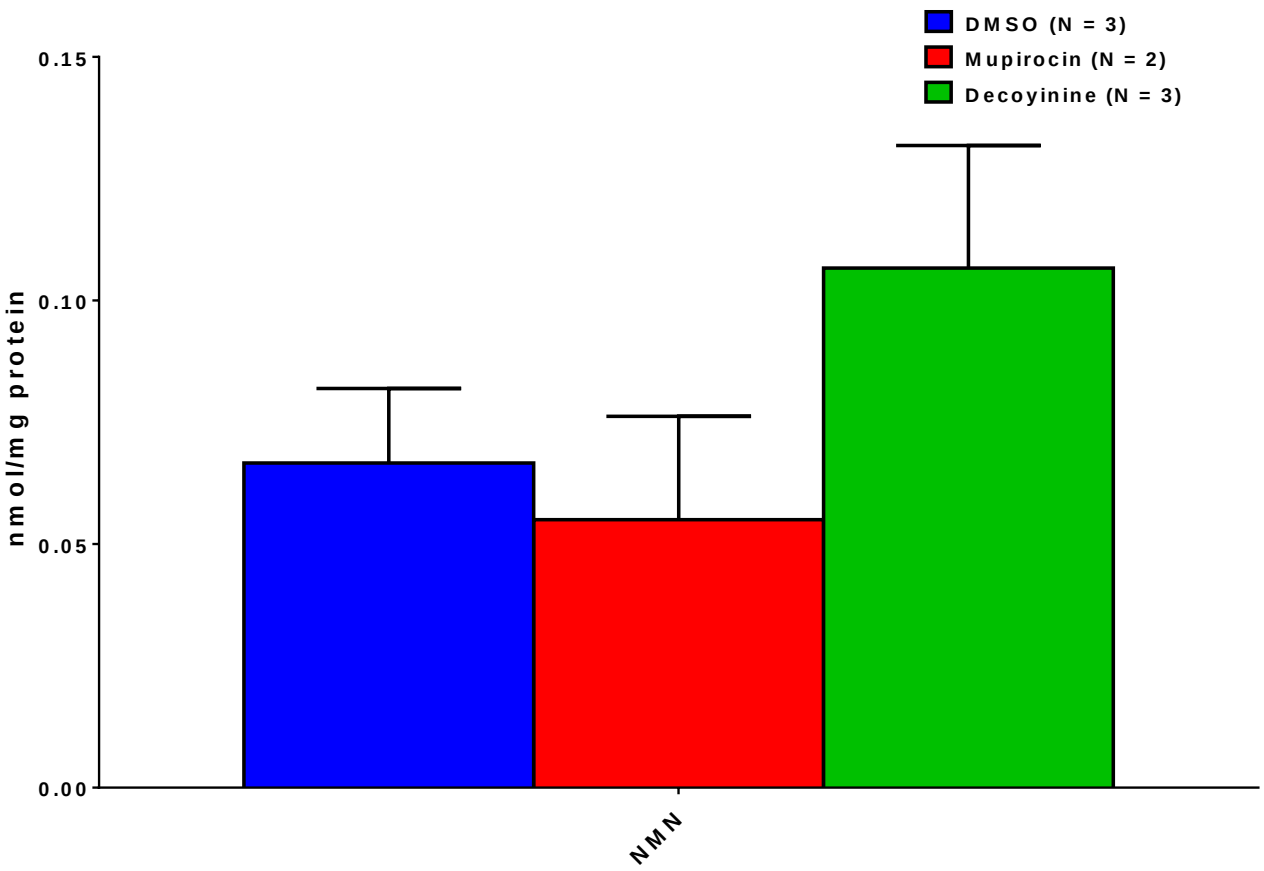

SIGNIFICANT CHANGES

The following tables depict the analytes that display statistically significant changes (p < 0.05). All P values were determined by a Student's t-test.

	Mupirocin vs. DMSO		Decoyinine vs. DMSO		Decoyinine vs. Mupirocin	
	% change	P value	% change	P value	% change	P value
NAD	63.2	0.014	123.0	< 0.001	36.6	0.038
NADP			59.5	0.004		
AMP	181.9	0.001	307.1	0.021		
GDP	-31.6	0.034				
CDP	237.3	0.022	92.1	0.007		
UDP	32.4	0.049				

COMMENTS

- Statistically significant differences between Mupirocin treatment and DMSO are observed for NAD (63.2%, p = 0.014), AMP (181.9%, p = 0.001), GDP (-31.6%, p = 0.034), CDP (237.3%, p = 0.022), and UDP (32.4%, p = 0.049).
- Statistically significant differences between Decoyinine treatment and DMSO are observed for NAD (123.0%, p = <0.001), NADP (59.5%, p =0.004), AMP (307.1%, p = 0.021), and CDP (92.1%, p = 0.007).
- ADP concentrations for DMSO and Mupirocin treated cells were below the limit of quantitation and thus have been omitted from the above graphical representation of the data. Nevertheless, we provide the data in the table shown below. Interestingly, the Decoyinine treated cells had higher levels of ADP that were quantifiable in two of the cell extracts.

	ADP nmol/mg protein
OG1RF_1 DMSO	1.38
OG1RF_2 DMSO	1.81
OG1RF_3 DMSO	1.98
OG1RF_1 Mup	3.74
OG1RF_3 Mup	4.64
OG1RF_1 Decoy	4.95
OG1RF_2 Decoy	5.58
OG1RF_3 Decoy	5.53

Below the limit of quantitation