Quantitative Profiling of Prokaryotic Post Translational Modifications

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INTRODUCTION

Post-translational modification (PTM's) of proteins serves to regulate many aspects of their activity and thus cellular biology and physiology. Here we show the qualitative and quantitative changes in site-specific modifications in *E. coli* for a number of PTM's including phosphorylation of serine, threonine, and tyrosine, lysine acylation (acetylation, succinylation, propionylation), and arginine methylation. Immunoaffinity purification (IAP) using highly specific rabbit monoclonal Motif and PTM antibodies to enrich for these modifications allowed identification of thousands of unique modified peptides present in prokaryotes. Western blot screening was used to determine the PTMs sensitive to various treatments to select antibodies for quantitative profiling of changes.

METHODS

E. coli strain K12 DH5α were grown at 30°C and treated by heat shift to 42°C for 10 minutes, or transformation with an ampicillin resistance plasmid. The cells were harvested by centrifugation, proteins extracted, reduced with DTT, and run on SDS-PAGE for modification and motif specific antibody based Western blotting. Extracts were alkylated and digested with trypsin overnight, desalted over Sep-PK C18 columns and lyophilized. The PTM of interest was immunoprecipitated from 10mg of peptide using the PTMScan method (Cell Signaling Technology), LC-MS/MS was performed using an LTQ-Orbitrap Elite, and peptides were identified using SEQUEST.

CONCLUSIONS

Antibodies designed to enrich for specific post-translational modifications or PTM's can be used in multiple applications such as Western blotting and immunoaffinity based peptide enrichment prior to LC-MS/MS. Moreover these PTM's are present in species from different biological kingdoms. Here we show that the PTM's phosphotyrosine, the serine/threonine 14-3-3 substrate binding motif; as well as the lysine acylations: acetyl, succinyl and propionyl are present in *E. coli*.

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PTMScan Method

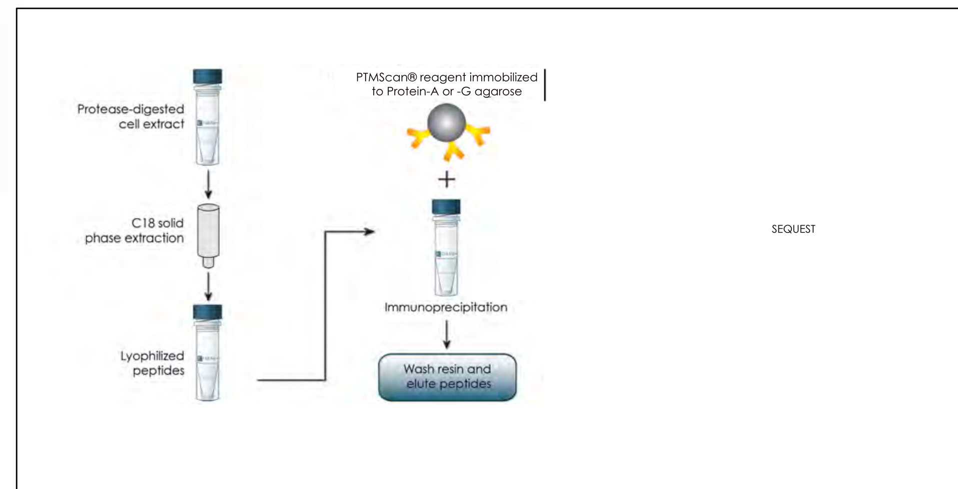

Figure 1: PTMScan Method. The above method was used for immunoafinity LC-MS/MS analysis. Following modification specific peptide enrichment peptides were desalted and run on an Orbi Trap Elite.

PTM and Motif Antibodies

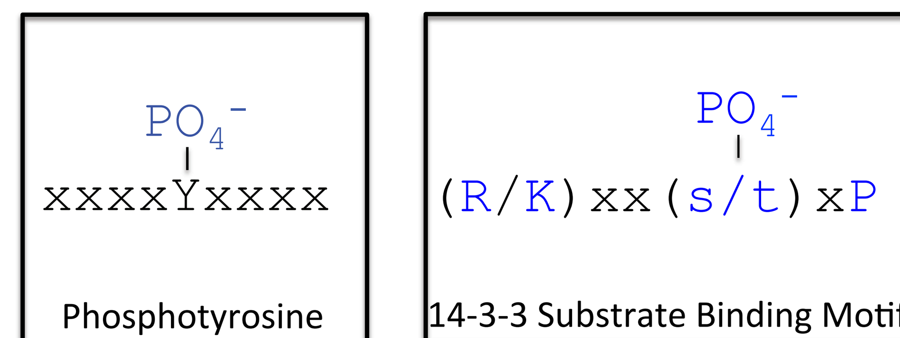

Figure 2: Phosphospecific motifs : Modification and motif specific antibodies were raised against the above phospho-peptides.

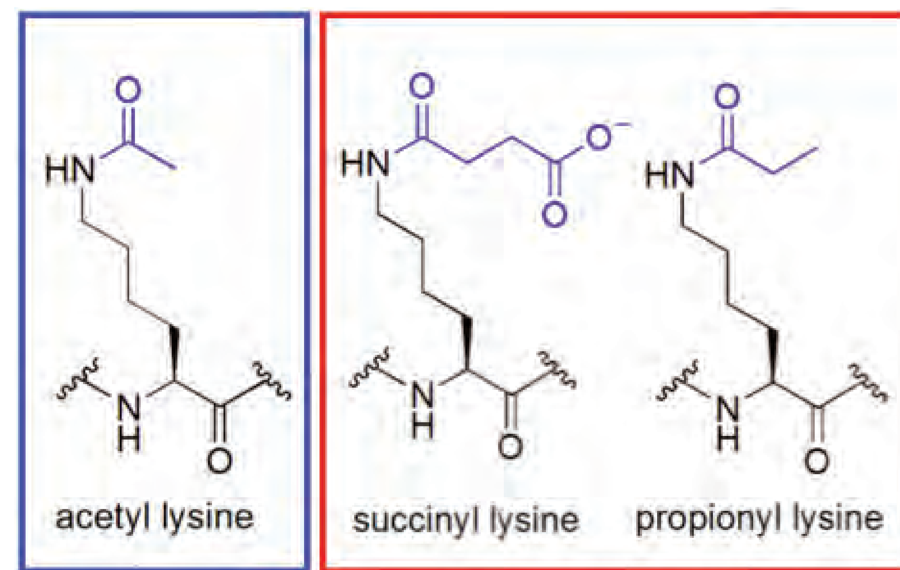

Figure 3. Acyl Lysine Modifications: Antibodies designed to enrich for peptides with the above acyl-lysine modifications, acetylated lysine, succinyl lysine and propionyl lysine were used.

Western Blotting Results

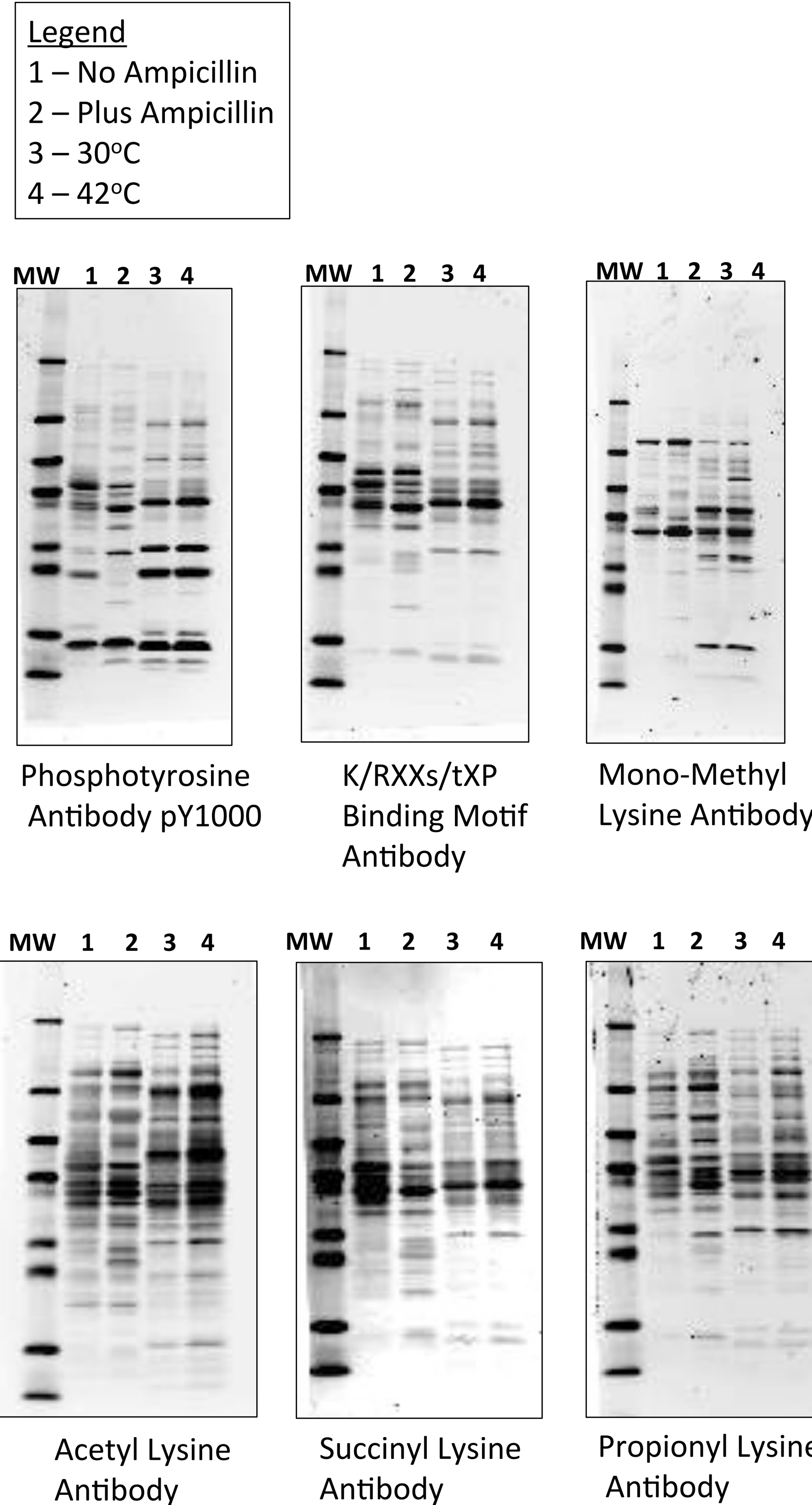

Figure 4. Western blotting results: The same antibodies used for peptide capture were used for western blotting. 25ug of E. coli derived extract was loaded per lane run on 5-20% gradient gels (Invitrogen) before transfer to nitrocellulose. Each 1° antibody was used 1:1,000 overnight. Blots were scanned using a LICOR Odyssey infrared imager. Lane 1, cells grown in the absence of ampicillin; lane 2, cells transformed with pBlueScript plasmid and grown in the presence of ampicillin; lane 3, cells grown at 30°C; lane 4, cells grown after heat shift to 42°C for 15 minutes.

PTMScan Results

Antibody	Unique Peptides	Proteins
Acetyl Lysine	7,362	1,424
Propionyl Lysine	1,476	510
Succinyl Lysine	4,168	903
Phosphotyrosine pY1000	356	249
K/RXX9s/t)XP Binding Motif	167	132
Mono-Methyl Lysine	73	63

Succinyl Lysine Antibody (Number of Peptides or Proteins)

	Unique Peptides No AMP	Unique Peptides Plus AMP	Proteins No AMP	Proteins Plus AMP
Count	3,414	2,872	772	678

Acetyl Lysine Antibody (Number of Peptides or Proteins)

	Unique Peptides No AMP	Unique Peptides Plus AMP	Proteins No AMP	Proteins Plus AMP	Unique Peptides 30	Unique Peptides 40 C	Proteins 30	Proteins 40 C
Count	4,616	6,016	1,059	1,216	2,390	3,755	702	929

Figure 5. Summary of Qualitative Results: Combined number of unique modified peptides and parent proteins per enrichment.

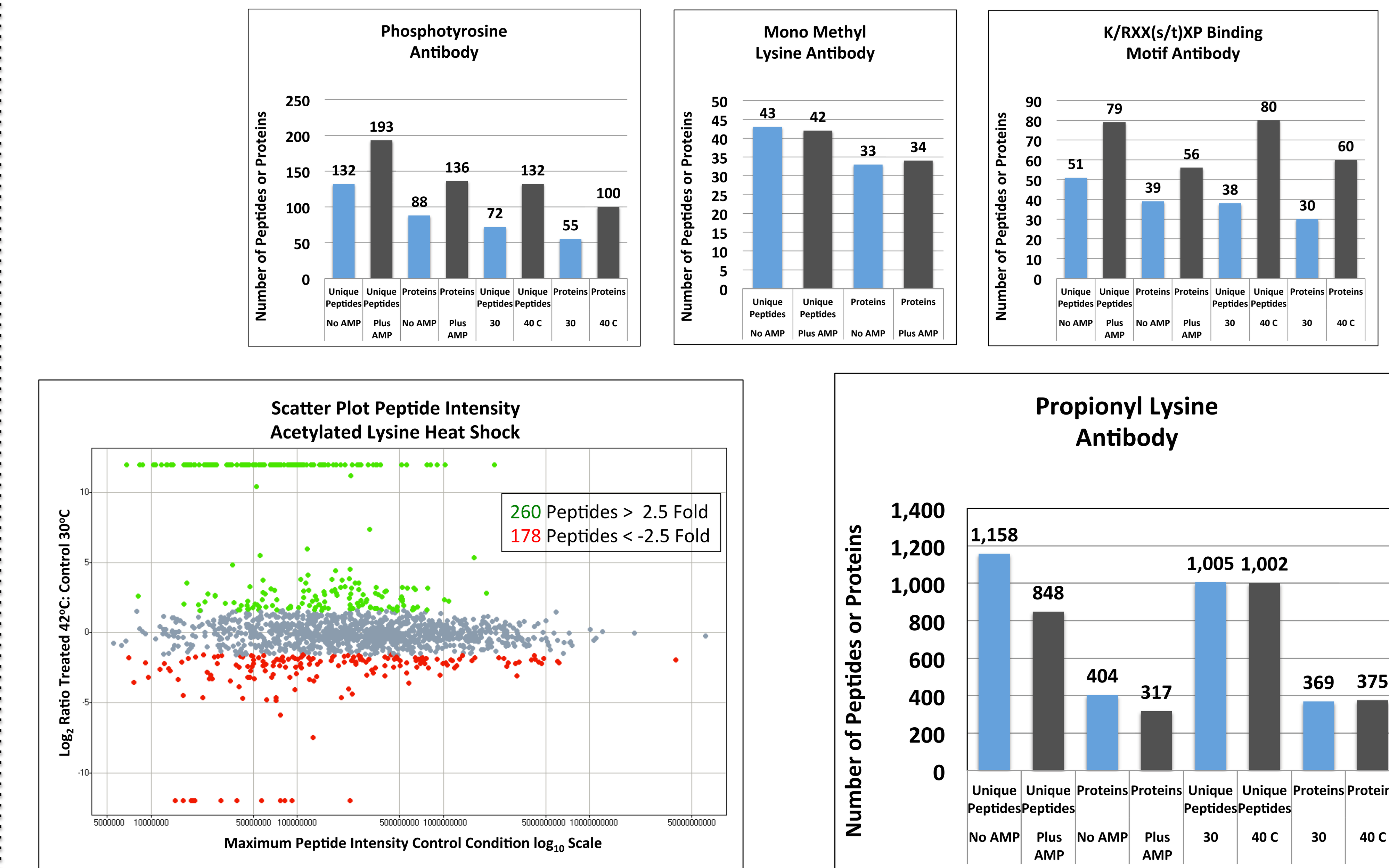

Figure 6. Scatter Plot. Log2ratios were calculated from peptide intensity measurements (Progenesis), treated 42°C: control 30°C. Peptides with ratios showing a fold change greater than 3-fold are in green, between 3 and -.3 are grey, ratios lower than -3 are red. Intensity measurements for peptides found in the treated condition alone were assigned log2 value of 12, and peptides in the control condition alone were assigned -12.

Figure 7. Qualitative Results: Tryptic peptides derived from 5mg of soluble *E. coli* extracts were enriched for the desired PTM using the PTMScan method. Results are total unique peptide numbers and corresponding number of parent proteins. MS/MS spectra were evaluated using SEQUEST 3G.

Acylation Motif Logos

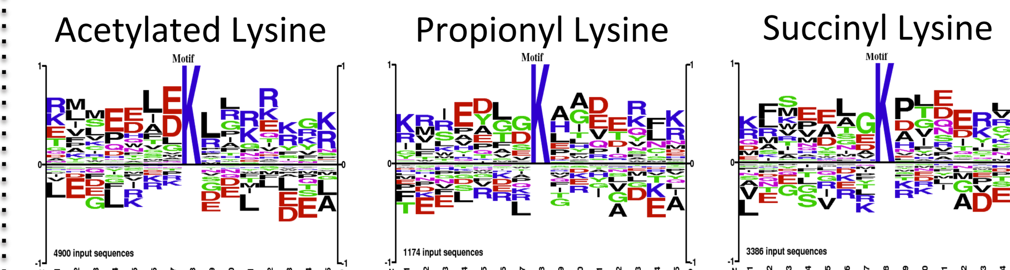

Figure 8, Motif Analysis for Acyelated peptides: Non-redundant peptide sequences were searched for motifs surrounding the modified lysine residue using Motif Generator from PhosphoSitePlus.

Functional Mapping

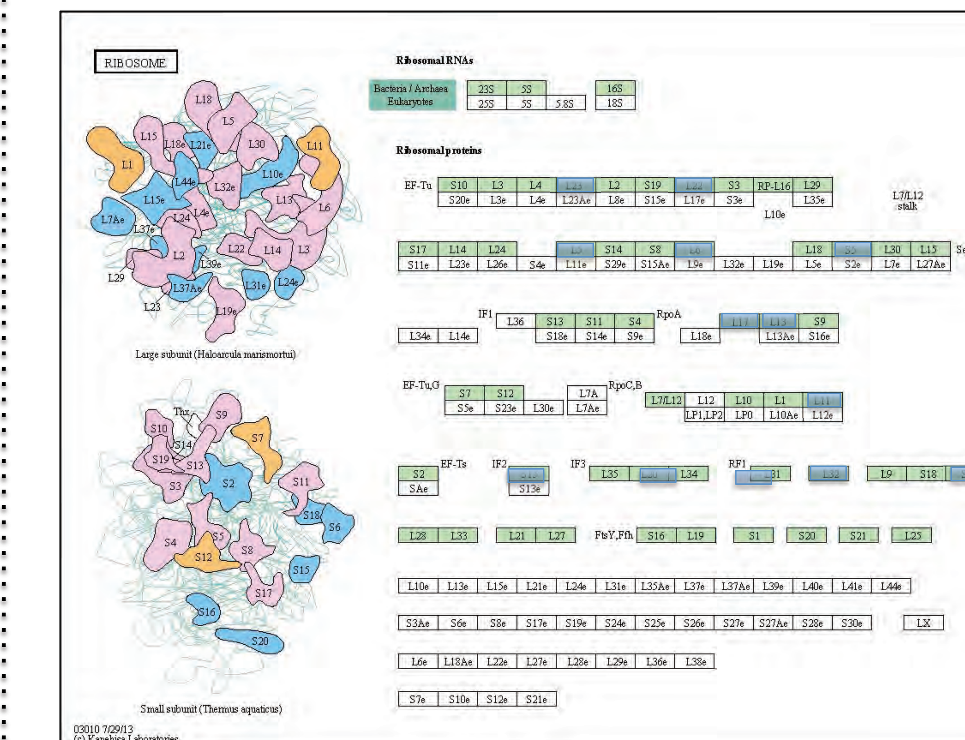

Figure 9. Qualitative Results: Phosphotyrosine enriched peptide identifications were mapped the parent proteins (BLUE BOXES) to functional pathways using KEGG and DAVID informatic resources.

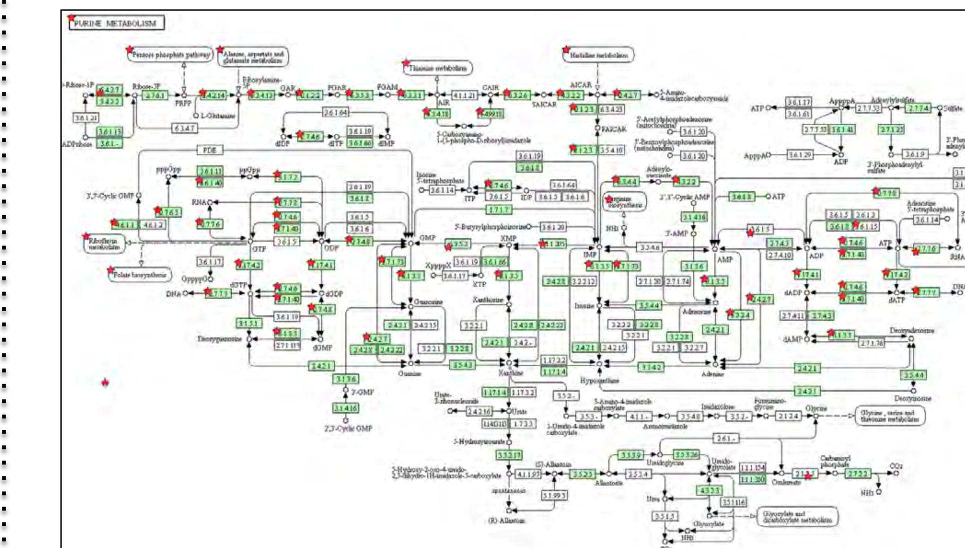

Figure 10. Functional Pathway Annotation: Following PTMScan based enrichment for peptides with acetylated lysine, the parent proteins were mapped to metabolic pathways using KEGG. Here peptides from the cells grown in the absence of ampicillin were used, and the acetylated parent proteins are denoted with a red star.

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