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Analysis of protein phosphorylation in prokaryotes
–Identification of phosphorylated protein by mass spectrometry–

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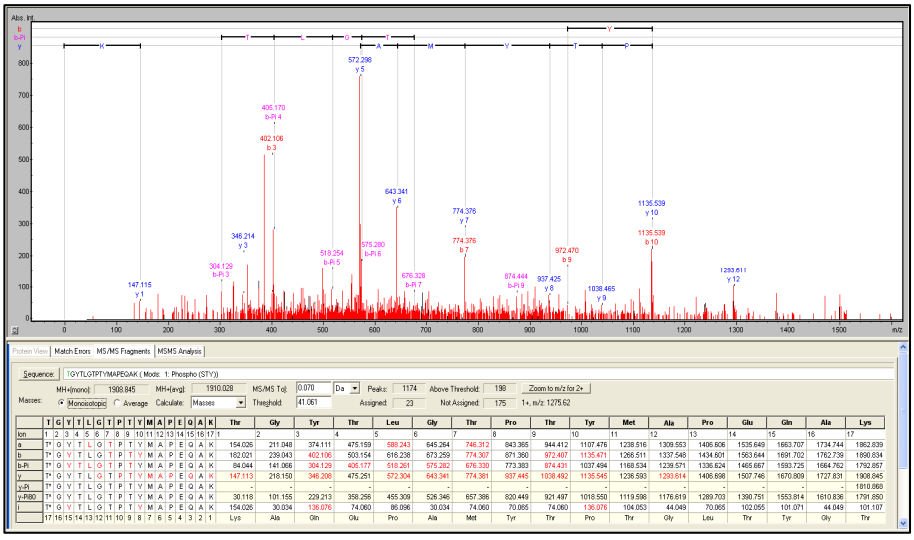
Protein phosphorylation/dephosphorylation on serine, threonine, tyrosine (Ser/Thr/Tyr) by the protein kinase and phosphatase controls many cellular processes such as intercellular communication, cell growth, proliferation, differentiation, and apoptosis in eukaryotes. Many researchers have studied protein kinases and phosphatases as a target of drug development. In bacteria, protein phosphorylation on histidine and aspartate residues, associated with two-component signal transduction systems is well established. In contrast, few studies of phosphorylation cascades involving Ser/Thr/Tyr had been reported for bacteria. However, as a result of advances in mass analysis and phosphopeptide enrichment methods, recent experimental evidences indicated that a large number of proteins in bacteria are also phosphorylated on Ser/Thr/Tyr. *Thermus thermophilus* HB8 is often recognized as a model organism that can grow at an extremely high temperature due to its small genome size and high stability of its proteins. These proteins are suitable for functional and structural analysis that can give an imformation to understand fundamental biological systems. We selected *T. thermophilus* HB8 to characterize the signalling pathway by Ser/Thr/Tyr phospholylation/dephospholation mechanism in bacteria.

First, we tried to identify phosphorylated proteins in *T. thermophilus* HB8 by mass spectrometry. Whole-cell proteins were prepared from *T. thermophilus* HB8 cells cultivated in complex media and the lysates were digested with trypsin. The phosphopeptide enrichment was performed by titanium oxide chromatography. The enriched phosphopeptides were separated by nano-LC prior to mass spectrometry. We identified 40 phosphoproteins and their phosphorylation sites, with 54%, 34%, 16% distribution of Ser, Thr, and Tyr residue, respectively, of the proteins involved in carbohydrate metabolism, protein synthesis, and nucleotide metabolism.



Representative MS/MS result of phosphopeptide (protein kinase, TTHA0138) by Q-TOF.