

PROTEIN PHOSPHORYLATION PLAYS A MAJOR ROLE IN STREPTOMYCES DIFFERENTIATION

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Streptomyces species undergo a complex developmental cycle that includes programmed cell death (PCD) events and sporulation. Streptomycetes are widely used in biotechnology as they produce approximately two thirds of clinically relevant secondary metabolites. Although *S. coelicolor* is one of the bacterium encoding the largest number eukaryotic type kinases, the role of protein phosphorylation in *Streptomyces* remains to be established. We characterized the *Streptomyces* phosphoproteome by using calcium phosphate precipitation (CPP) for phosphopeptide recovery and tandem mass spectrometry for accurate phosphopeptide sequencing and annotation. CPP proved highly efficient for phosphopeptide enrichment, enabling the mapping of a total of 229 phosphorylation sites in 129 *S. coelicolor* phosphoproteins across three developmental stages. Most distinct phosphorylation events were detected during the presporulation and sporulation stages (81%), but phosphorylation events were also detected in the vegetative phase (19%). A fraction of the phosphoproteins (11%) were found to be phosphorylated in both the vegetative state and in the sporulation phases. Several proteins that are known to regulate sporulation processes, such as FtsZ, DivIVA, and FtsH2, transcriptional regulators and kinases, were found to be phosphorylated. This work establishes the widespread occurrence and functional role of Ser/Thr/Tyr protein phosphorylation in *Streptomyces* development. Our database of *S. coelicolor* phosphorylation sites provides a valuable resource for future experiments aimed at more mechanistic understanding of the molecular processes that govern *Streptomyces* differentiation.