Abstract:
The Ndc80 protein in budding yeast is a subunit of the outer kinetochore responsible for triggering the spindle assembly checkpoint, and ensures proper chromosome attachment and alignment with the spindle during division. Previous research has identified specific phosphorylation sites in the Ndc80 protein via mass spectrometry; however, it is still unknown which of the identified phosphorylation sites, if any, are critically important for the function of the Ndc80 protein. The goal of our research is to determine which of these identified phosphorylation sites are important for the functioning of the Ndc80 protein, which allows us to better the understanding of this protein’s mechanistic role in preserving genome integrity. In order to identify critical phosphorylation sites, we used bioinformatics methodologies, homology searching and multiple sequence alignments, to identify areas of certain stretches of amino acids within budding yeast Ndc80 that are well conserved in orthologous sequences in closely and distantly related species. Specifically, we employed protein BLAST and PSI-BLAST methods in combination with sequence and pairwise alignment. We found that the serine 205 residue is the most conserved among yeasts as well as in more distantly related species using these approaches. We identified several other well conserved positions. Sequences surrounding Serine 205 were well conserved even in distantly related species implying this phosphorylation site likely holds a critically important role in the function of Ndc80. Given this we can follow up in vivo via site-directed mutagenesis to identify the function of Ndc80 phosphorylation in further research.