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Expression and Purification of E. coli YoaA, a Putative Helicase

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ABSTRACT: All cells must maintain their genomic integrity to survive, which they achieve through several repair mechanisms that necessitate unwinding the damaged DNA by helicases. In *Escherichia coli* (*E. coli*), YoaA has been genetically shown to be involved in DNA repair and shares conserved sequences with helicase DinG. The goal of our study was to purify YoaA for further biochemical characterization. For expression, YoaA was fused to a His tag and overexpressed in MG1655 E.coli under the lacZ or T7 promoters for 2 hours, 4 hours, or overnight at 24ºC, 30ºC or 37ºC. For purification, crude lysate was applied to a Nickel-NTA column, then single-stranded-DNA (ssDNA) or MonoQ columns. Expression did not appreciably change between the lacZ and T7 promoters but exclusively produced full-length YoaA. Expression increased with temperature and decreased with time. YoaA best eluted from the Ni-NTA column at 100mM imidazole, then from the MonoQ column at 1.5M NaCl, but not the ssDNA column. In this study, we found that expressing YoaA under the lacZ promoter in 5L MG1655 *E. coli* cells for 2 hours at 37ºC, Ni-NTA then MonoQ column purification yielded 11µg YoaA. Future studies using a linear elution gradient could be performed to fully isolate YoaA. Biochemical characterization of YoaA will expand our understanding of the molecular mechanisms of DNA repair in prokaryotes which can be extended to the study of the same function in humans, allowing for the development of more targeted therapeutics for human diseases rooted in defects in DNA repair.