The Synthesis of Colicin M using Cell-Free Protein Synthesis

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Bacteriocins are proteins that are toxic to bacteria, yet are made by them. These proteins may provide an alternative to traditional antibiotics but synthesizing them using recombinant methods is problematic. A bacteria transformed with a plasmid containing a bacteriocin gene can potentially kill itself by expressing the gene; thus, making it impossible to sustain a growth of living cells and to produce the desired bacteriocin protein. The use of cell-free protein synthesis (CFPS) to create bacteriocins, such as colicin M, avoids this problem. Since the synthesis process does not require live cells, viability is not a concern. This allows for a high yield of the bacteriocins. To conduct CFPS , two major components, a plasmid containing the target gene and bacterial extract containing ribosomes and enzymes, were mixed with amino acids, energy sources, and other reagents to create an artificial cell. The plasmid was created through PCR and Gibson Assembly, and the extract was created by lysing bacteria and isolating relevant cellular components. Both the plasmid and the bacterial extract were successfully made and were mixed with the reagents to synthesize colicin M. After a day of incubation, the protein was purified and the concentration of the protein was found using a NanoDrop. The total concentration of the protein synthesized was 736.67ug/mL. This supports the idea that bacteriocins, can be created using cell-free protein synthesis. Further investigation would involve synthesizing different types of bacteriocins and testing their efficacy at killing bacteria.

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