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CpmA and 80α scaffolding proteins interaction in staphylococcus aureus

SaPI are super antigen carrying pathogenicity islands. This is a mobile genetic element that is discretely integrated in the DNA of staphylococcus aureus. A mobile genetic element is a type of DNA that can move around within the genome (Dearborn et. al). One example is staphylococcus aureus SaPI1. This particular SaPI is responsible for the spread of toxins. These toxins released by SaPI1 can cause shock syndrome, rare life threating disease (Damle et. al).The SaPI are lured out of the host genome by helper phages to replicate. Helper phages are a virus which helps a separate and unrelated defective virus reproduce by infecting the same host cell that is already occupied by the defective virus and providing the proteins which the defective virus is missing and needs to complete its life cycle (Damle et. Al).

Each SaPI has a certain helper page or pages that will lure it out. For SaPI1 in staphylococcus aureus it is phage 80α.The way it uses the page 80α the SaPI1 highjacks it replication process. It does this to redirect the formation of capsid size. This causes the formation of the capsid to be smaller than the 80α capsid size. The smaller capsid will only fit the DNA from the SaPI1 because the DNA from the phage 80α is too large to fit into the smaller capsid (Christie, Dokland).

During the formation of the smaller capsid there are two major proteins that responsible for the smaller capsid size they are CpmA and CpmB (Poliakov et. al). In a previous experiment it was shown that for efficient capsid size reduction both of these proteins needed to be present. With the deletion of CpmA there was no change in capsid size. With the deletion of CpmB there was approximately 3% of the capsids that formed were small (Damle et. al). In another experiment results showed that the CpmB interacted with the scaffolding proteins that form the capsid. In this experiment it will determine if there is any interaction between the protein CpmA and scaffolding proteins in the absence of CpmB (Spilman et.al).

The way this will be determined is by adding a histone tag to the CpmA protein and running it down a nickel column. The histone tag will stick to the nickel column and any proteins that are interacting with CpmA will be attached to the CpmA (Liu et.al). The following will give a brief description of the procedure on how this will be accomplished. First a plasmids will be purchased that has the histone tag and antibiotic resistance incorporated into it. Then the plasmid will be modified so it carries deletion for CpmB made by SOEPCR. After modification the plasmid will be inserted into E.Coli by electroporation. After the insertion of the plasmid the E. Coli is grown. After the E. Coli is grown it is put on agar plate with ampicillin and allowed to grow. Only the bacteria that have the plasmids modification that were made will grow. Next samples off the agar plate will be grown. The next step is to get the purified plasmids out of the E.Coli. This will be done by the use of a PureYield™ Plasmid Miniprep System, which uses minicolumns and microcentrifuge to separate the plasmids. After plasmids are purified they will be inserted into staphylococcus aureus by electroporation. Then the temperature is raised to 42 degrees to allow for integration of the plasmids. Then it is cooled to 30 degrees to solve the issue of cointegration. Then the temperature is raised to 42 degrees and the plasmid is lost leaving wild type and mutant with the deletion of CpmB (Bose et al). After resolution of the cointegrate the wild type will carry the plasmid with it that has the antibiotic resistance. The mutant will not have the plasmid. Then grow bacteria. Plate the bacteria on agar and grow. Then use replica plating to determine mutant by growing on plate with ampicillin ("Isolation of Spontaneous Mutations."). Then collect the mutant bacteria and grow it. Next introduce 80α to the mutants and stress the cell with ultraviolet light to induce replication. Then lyse the cell with a mild detergent forming a lysate, a fluid containing the contents of the lysed cell. Then run it down a nickel column and wash with imidazole. After collecting the assay from the column run a gel electrophoresis to determine size of the protein or proteins. Then cut the individual protein out of the gel and preform electro elution which will extract the protein form the gel into a buffer solution and it will ready for mass spec. which will determine the weight of the protein. Then using bioinformatics to cross check data bases to determine what proteins are present.

The results of this experiment will determine if there is protein interaction between CpmA and scaffolding proteins or any other proteins. This also will lead to a better understanding of how helper bacteriophage help the SaPI1 replicate. This better understanding could lead to better treatment of this type of bacterial infection.

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