Prokaryotic Protein-Protein Interactions

Using biotin labeling to find the proteins that interact with LapD in *Pseudomonas fluorescens*

Amaya Okogeri
Biomedical Importance of Pseudomonas Species

- *Pseudomonas aeruginosa* causes infections in hospitals such as cystic fibrosis, gastrointestinal infections, and skin and soft tissue infections.
- The World Health Organization ranked *Pseudomonas aeruginosa* as the #2 pathogen to watch because it is highly resistant to antibiotics.
- *Pseudomonas fluorescens* is used as the model organism for the experiment.
- *Pseudomonas fluorescens* and *aeruginosa* both forms biofilms which uses LapD to regulate its formation.
Biofilm formation is when a single free-floating bacterial cell switches to living in a community while attached to a surface due to changes in the bacteria’s environment.

The bacterial cells communicate through quorum sensing which increases the growth of the biofilm.

The community of cells are embedded in extracellular polymeric substances (EPS) which are composed of polysaccharides and proteins.

It has been accredited with causing 80% of chronic infections, such as burn wounds infections, infections from implants, heart infection and ear infection.
Cyclic di-GMP and LapD

- *Pseudomonas fluorescens* is a gram-negative bacterium which means it has an inner and outer membrane.
- c-di-GMP is a second messenger molecule in the bacteria’s cytoplasm that directly affects biofilm formation.
TurboID Method

- The biotin labeling method has been done in mainly eukaryotic cells, but never in prokaryotic cells.
- It labels proteins with biotin through proximity labeling to show the protein of interest’s interactions with other proteins.
- BirA is a biotin ligase that aims for specific amino acid sequences and then fuses with the protein of interest.
- BirA has three mutant versions which are “BioID1”, “BioID2”, and “TurboID”.
Experiment Process

1. Cloning
   a. Standard cloning procedure
   b. TurboID fusion done through PCR to amplify the inserts and infusion to insert them into the vectors
      i. Step 1: Amplify TurboID-HA and insert into pJN105
      ii. Step 2: Amplify LapD and GcbC and insert separately into the new pJTurbo- HA vector.

2. Expression of *Pseudomonas fluorescens*
   a. Introduce the Turbo- fusion plasmids into *P. fluorescens* by electroporation
   b. Induce expression with 0.2% arabinose for three hours

3. Western Blot for streptavidin
   a. Streptavidin blot to show biotinylated proteins

4. Western Blot for anti-HA
   a. Use antibody against HA tag to confirm fusion protein expressions in cells
Cloning Reactions

pJT-HA and pJmT-HA were cut with HindIII to check for the presence of Turbo and MiniTurbo inserts.

pJTurbo and pJMiniTurbo were cut with NdeI and EcoRI to check for the presence of LapD and GcbC.

*DNA sequencing confirmed the Turbo and MiniTurbo insert.

1. Turbo-LapD
2. MiniTurbo- LapD
3. Turbo- GcbC
4. MiniTurbo- GcbC

*DNA sequencing confirmed the LapD or GcbC inserts into the Turbo-HA or MiniTurbo-HA vector.
Biotin Labeling Results

Western Blot: biotinylation in LapD- TurboHA and GcbC- TurboHA and protein expression in the HA blot is shown.
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