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Modifying the amino acid sequence in the surface-exposed loops of the omptin family of proteins to determine their effect on function

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Modifying the Amino Acid Sequence in the Surface-Exposed Loops of the Omptin Family of Proteins to Determine Their Effect on Function

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Methods

• Site Directed Mutagenesis of icsP gene:
  - Key nucleotides are changed in the gene to produce a change in the amino acid sequence
  - AAC changed to ATG ⇒ asparagine into methionine
  - IcsP now appears the same as OmpT and PgtE in that region (fig. 5)
• pBAD33 (ΔicsP), pAJH02 (icsP), and pKNM02 (mutated icsP) were introduced into Shigella strain MB341
• SDS-PAGE (PolyAcrylamide Gel Electrophoresis) and Western Blot
  - SDS-PAGE to separate whole cell and supernatant proteins according to their size
  - proteins then transferred to a PVDF membrane
  - IcsA detected by immunoblotting with and anti-IcsA antibody
  - IcsP now appears the same as OmpT and PgtE in that region (fig. 1, L2)

• Imaged with UVP Imaging System

Results and Future Direction

• It is expected for IcsP to produce the same cleavage fragments as OmpT

• Mutation of additional surface-exposed loops to compare function to other members of the omptin family

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Figure 2: IcsP cleavage of IcsA in Shigella. Whole cell and supernatant proteins were separated by SDS-PAGE, and transferred to a PVDF membrane. IcsA was detected by immunoblotting with an anti-IcsA antibody. A: IcsA was detected in the whole cell as well as the supernatant of 2457T (wild-type). However, it was only detected in the whole cell of MBG341 (2457T-ΔicsP). This shows that IcsP cleaves IcsA, removing a fragment of approximately 95kDa from the bacterial surface. B: pMBG270 (icsA) was introduced into the Shigella strain MBG263 (2457T-ΔicsA). IcsA was detected in the supernatant of MBG161 (wild type producing OmpT). This shows that OmpT cleaves IcsA, removing a fragment of approximately 85kDa from the bacterial surface.

Figure 3: OmpT cleavage of IcsA in E. coli. pMBG270 (icsA) was introduced into the E.coli strains MC1061 (wild-type) and MBG263 (MC1061-ΔompT). Whole cell and supernatant proteins were separated by SDS-PAGE, and transferred to a PVDF membrane. IcsA was detected by immunoblotting with an anti-IcsA antibody. IcsA was detected only in the whole cell of MBG263 (lacking OmpT). IcsA was detected in the supernatant of MC1061 (wild type producing OmpT). This shows that OmpT cleaves IcsA, removing a fragment of approximately 95kDa from the bacterial surface.

Figure 4: PgtE cleavage of IcsA in Salmonella. pMBG270 (icsA) was introduced into the Salmonella strains CS202 (wild-type) and TG61 (CS202-Δpge). Whole cell and supernatant proteins were separated by SDS-PAGE, and transferred to a PVDF membrane. IcsA was detected by immunoblotting with an anti-IcsA antibody. IcsA was detected only in the whole cell of TG61 (lacking PgtE). IcsA was detected in the supernatant of CS202 (wild type producing PgtE). This shows that PgtE cleaves IcsA, removing two fragments of approximately 95kDa and 85kDa from the bacterial surface.

Figure 5: Comparison of the amino acid sequence for IcsP, OmpT, and PgtE. Outlined in green is the specific amino acid in IcsP changed to resemble the corresponding amino acid in OmpT and PgtE (fig. 1, L2).