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Characterization of the omptin protease, OmpT, in Escherichia coli

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Determine whether Ompt expressed from new construct cleaves ICSA when expressed in shigella MBG34 (7csP-). I will be testing whether Ompt can protect shigella (and salmonella) from LL-37.

Omptins are outer membrane proteases found in gram negative bacteria that cause diseases in humans, such as pathogenic *Escherichia coli*, *Shigella flexneri***,** *Salmonella typhimurium*, and *Yersinia pestis.* Bacterial species that express omptins cause diseases such as highly fatal plague and severe diarrhea and dysentery. The genes that encode these proteases are *ompT*, *icsP*, *pgtE*, and *pla*, respectively. These proteases are highly related in structure and share approximately 50% sequence identity. In *S. flexneri,* IcsP has been shown to cleave a key virulence determinant, IcsA (Egile *et al.,* 1997). IcsA recruits host actin and allows for intracellular movement within host cells (Steinhauer *et al.,* 1999). In *S. typhimurium*, PgtE has been shown to cleave a human a-helical cationic antimicrobial peptide (CAMP), LL-37 (Guina *et al.,* 2000). LL-37 is a major component of the innate immune defense system (Zasloff, 1992). It functions by permeabilizing the bacterial membrane, which ultimately results in bacterial cell lysis. PgtE cleaves LL-37, thereby protecting *Salmonella* against the bactericidal effects of CAMPs. In *E. coli*, OmpT has been shown to cleave protamine, an antimicrobial peptide that acts on the bacterial membrane and causes problems in cellular energy transduction and nutrient accumulation (Aspedon and Groisman, 1996) (Figure 2). Like PgtE, OmpT circumvents the immune defense by cleaving protamine into smaller fragments.

Characterization of the Omptin Protease, OmpT, in *Escherichia coli*

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INTRODUCTION

Omptins are outer membrane proteases found in gram negative bacteria that cause diseases in humans, such as pathogenic *Escherichia coli*, *Shigella flexneri*, *Salmonella typhimurium*, and *Yersinia pestis*. Bacterial species that express omptins cause diseases such as highly fatal plague and severe diarrhea and dysentery. The genes that encode these proteases are *ompT*, *icsP*, *pgtE*, and *pla*, respectively. These proteases are highly related in structure and share approximately 50% sequence identity (Figure 1).

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- In *E. coli*, OmpT has been shown to cleave protamine, an antimicrobial peptide that acts on the bacterial membrane and causes problems in cellular energy transduction and nutrient accumulation (Aspedon and Groisman, 1996) (Figure 2). Like PgtE, OmpT circumvents the immune defense by cleaving protamine into smaller fragments.

OBJECTIVES

Due to the high sequence identity at the amino acid level of the omptin proteases, we hypothesized that these proteins may share similar proteolytic activity and function.

- To characterize the proteolytic activity of OmpT,
  - Experiments were conducted to determine whether inducible OmpT recognized and cleaved one substrate, IcsA , in similar manner to natively expressed IcsP.
- To determine whether the LPS environment surrounding the omptin influences its activity or site specificity
  - Omptin switching experiments were conducted by introducing inducible *ompT* into a *Shigella* background.
- To determine whether OmpT could promote resistance to the cationic antimicrobial peptide, LL-37 in *E. coli*
  - Minimum inhibitory concentration (MIC) assays were conducted.

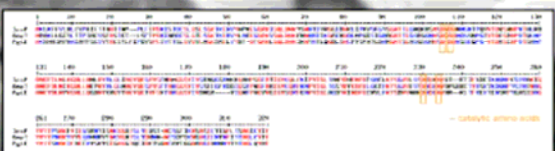

Fig. 1. Alignment of amino acid sequences of IcsP, OmpT, and PgtE

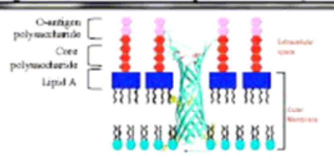

Fig. 2. Cartoon of OmpT embedded in the bacterial cell membrane

MATERIALS AND METHODS

Western Blot Analysis

- Cultures were grown overnight in Tryptic Soy Broth (TSB) or Luria-Bertani (LB) with appropriate antibiotics and 0.2% (w/v) glucose
- Following day, cultures were back-diluted 1:30 in TSB or LB medium to an OD_{600} 0.4-1.0 and induced with 0.2% (w/v) L-arabinose for 1 hour at 37°C
- Whole cell proteins were prepared from these cultures by harvesting equivalent number of cells, hence protein, loaded onto a 10% (w/v) SDS-polyacrylamide gel, separated by electrophoresis at 35 mA, and transferred to polyvinylidene fluoride (PVDF) membrane for 2 hours at 150 mA
- PVDF membrane was probed with:
  1° Antibody: Rabbit IcsA polyclonal antibody (1:10,000) for 2 hours at 37°C
  2° Antibody: Anti-rabbit IgG, horseradish peroxidase linked $F(ab')^2$ fragment from donkey (1:10,000) for 1 hour at 25°C
- IcsA was detected by ECL kit (Amersham) and visualized on a Typhoon scanner using ImageQuant Software

Assay to measure resistance to cationic antimicrobial peptide (CAMP), LL-37

- Cultures were grown overnight in LB medium with appropriate antibiotics and 0.2% (w/v) glucose
- Following day, cultures were back-diluted 1:30 in LB medium to an OD_{600} 0.4-1.0 and induced with 0.2% (w/v) L-arabinose for 1 hour at 37°C
- Cultures were diluted to 2×10^5 cells ml^{-1}
- Test peptide (LL-37) was assayed at final concentrations of 10 μg ml^{-1} to 500 μg ml^{-1} in 96-well microtiter plates
- Minimum inhibitory concentrations (MICs) were determined as the lowest concentration of the peptide that did not allow visible bacterial growth after 18 to 25 hours

RESULTS

-IcsP is known to decrease levels of IcsA in *Shigella* cells (panel 1)

-When IcsA is expressed in *E. coli*, OmpT removes IcsA completely from the bacterial cell (panel 2)

-When IcsA is expressed in *E. coli* carrying an inducible copy of *ompT*, under conditions of induction, IcsA is removed from the bacterial cell (panel 3)

-When IcsA is expressed in a *Shigella icsP-* mutant carrying an inducible copy of *ompT*, under conditions of induction, IcsA levels are decreased (panel 4)

-The MICs of both induced and non-induced *ompT* in *E. coli* were the same, 320 μg ml^{-1}, suggesting that OmpT does not protect *E. coli* from LL-37.

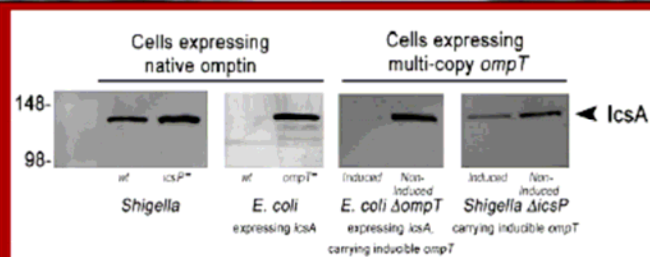

Fig. 3. Western blot analysis

Table 1. MIC's of cationic antimicrobial peptide, LL-37

Strain	Genotype	MIC (μg/ml) LL-37
Salmonella		
CS022	wild-type *Salmonella*	80
TG61	CS022-Δ*pgtE*	10
MBG283 pEHK23	MC1061-Δ*ompT* *Induced*	320
MBG283 pEHK23	MC1061-Δ*ompT* *Non-Induced*	320

CONCLUSIONS

- Although the catalytic amino acids are completely conserved among IcsP and OmpT, it is clear that these proteases cleave IcsA differently, as judged by western blot analysis.
- The LPS background may affect the cleavage specificity of OmpT because the IcsA cleavage pattern in the omptin switching experiment.
- OmpT has a dissimilar proteolytic activity and function to other members of the Omptin family.
- OmpT does not promote resistance to LL-37 in *E. coli*.

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