

## On the production of Shiga-like toxins by *E. coli* O157:H7

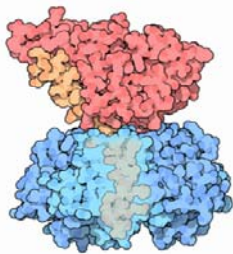
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## Problems

- Shiga-like toxins (SLT)-producing *Escherichia coli* (STEC) pose a significant threat to public health because ingestion of small numbers of the viable organisms (<100 cells) may cause hemorrhagic colitis (HC)
- Need of new methodology to detect SLTs to minimize the possibility of any STEC entering into the food chain.

## SLT facts



- 2 subunits
- 68,000 mw
- A subunit conveys toxic enzymatic activity
- B subunit is a pentamer that binds to glycolipids on host cell (Gb<sub>3</sub>)
- Halt protein synthesis at ribosome by stripping bases from m-RNA

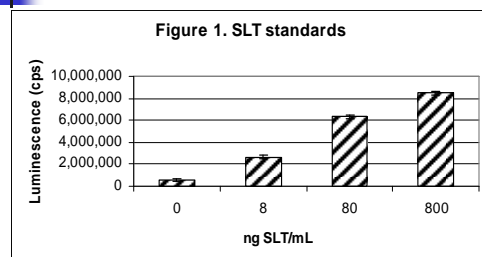
## Production of SLTs by *E. coli* O157:H7

- The bacteria only produce minimal amounts of SLT during log phase growth
- After reaching the steady state of growth (~16 h), the bacteria reach lytic cycle & SLT's are produced.
- Addition of antibiotics or other stresses to cells increase SLT production

## IMB-luminescence methodology

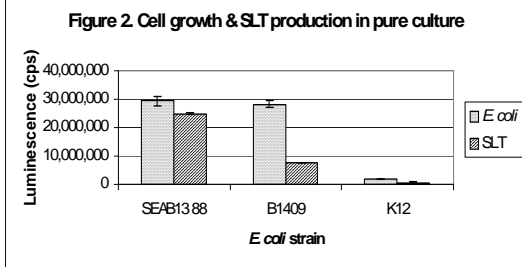
- Magnetic beads coated with anti-SLT antibodies were used to capture the SLTs in bacterial lysate suspensions.
- Beads were collected and then formed sandwiches with with peroxidase conjugated anti-SLT antibodies
- Addition of proper substrates, chemiluminescence was read immediately by a Victor 2 counter.

## Sensitivity of the luminescence method

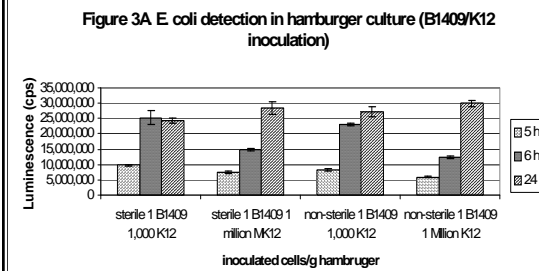




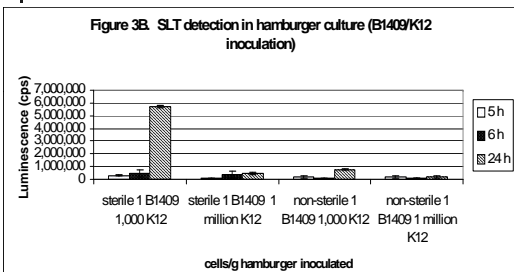
## Production of SLTs by STEC



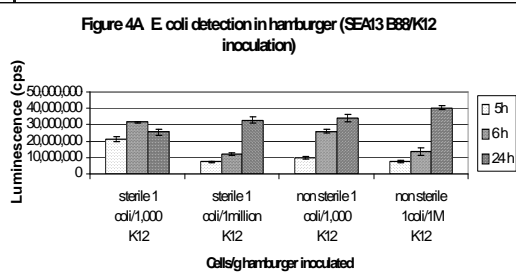
## Growth of B1409 in mixed cultures



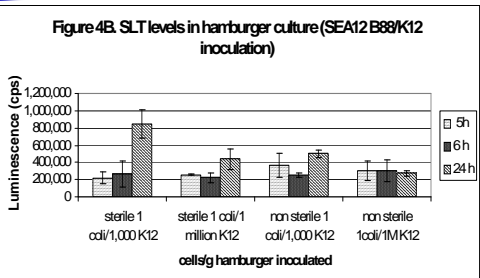
## SLT production by B1409 in mixed cultures



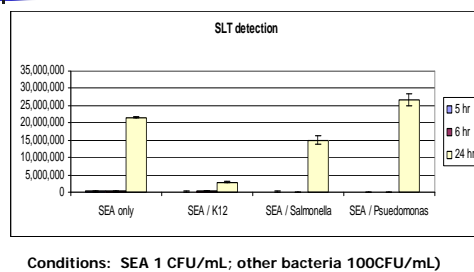
## Growth of SEA13 B88 in mixed cultures



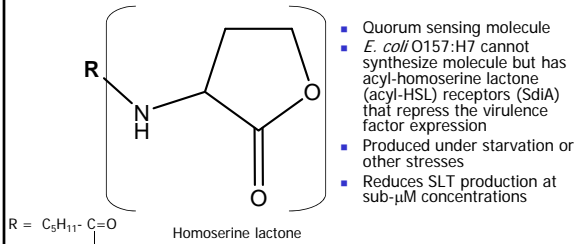
## SLT production by SEA12 B88 in mixed culture



## Effects of other bacteria on SLT production



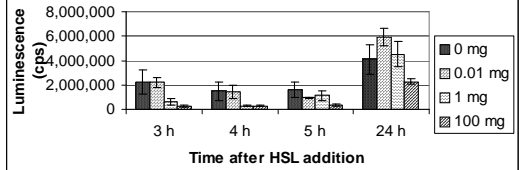
## Acyl-homoserine lactone



- Quorum sensing molecule
- *E. coli* O157:H7 cannot synthesize molecule but has acyl-homoserine lactone (acyl-HSL) receptors (SdiA) that repress the virulence factor expression
- Produced under starvation or other stresses
- Reduces SLT production at sub- $\mu$ M concentrations

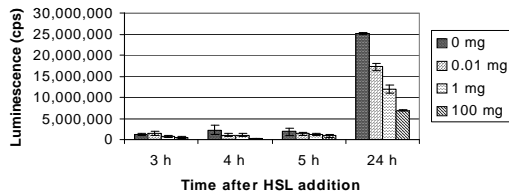
## Effects of HSL on Toxin production

Figure 5A. HSL effects on SLT production in B1409



## Effects of HSL on toxin production

Figure 5B. HSL effects on SLT production in SEA13 B88



## Conclusions

- *E. coli* O157:H7 and SLT could be detected from the same samples by IMB-luminescence or TRF
- Need ~16 h to detect SLT
- The production of SLT is sensitive to the presence of other bacteria.
- Analogs of quorum sensing molecules also suppress SLTs production by *E. coli* O157:H7.