Impact of Quorum Sensing on Cell Aggregation in Enteropathogenic and Enterohaemorrhagic *Escherichia coli*

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Abstract

Quorum sensing has been shown to regulate phenotypic traits such as motility and biofilm production in pathogenic bacteria. *Escherichia coli* utilise both AI-2 and *N*-acylhomoserine lactones (AHL) quorum sensing systems to initiate phenotypic switches, such as changes in dissemination, in response to overall microbial population density. In addition HosA, a transcriptional regulator present in pathogenic strains of *E. coli*, was shown to be important in bacterial dissemination during this study. Deletion of *hosA* from enteropathogenic *E. coli* strain E2348/69 resulted in a non-motile population at lower temperatures, an effect that was reversed in the presence of exogenous AHL. Furthermore, addition of the same AHLs to wild-type E2348/69 decreased population motility. Bacterial aggregation has been linked to the motility of the population. Deletion of *hosA* was shown to increase aggregation, corresponding to an observed decrease in motility. Furthermore, addition of AHL was shown to decrease the propensity of the HosA mutant population to aggregate. Opposing effects were observed in the non-aggregating wildtype population. A hypothetical hierarchical association between HosA and quorum sensing was modelled to explain the relationship between motility and aggregation in E2348/69. It was observed the EAF plasmid is not essential for the formation of A/E lesions on human cell-line HT-29 by E2348/69. Infection of *Galleria mellonella* revealed increased virulence in highly aggregative populations and further highlighted the effect of HosA and environmental conditions on the pathogenicity of E2348/69. Ruminant animals, in particular cattle, are the main reservoir of enterohaemorrhagic *E. coli* O157:H7 with infection remaining asymptomatic. By comparison infection in humans can result in a range of sequalae from mild to life-threatening. *E. coli* O157:H7 is derived from an EPEC progenitor and as expected, dissemination was affected by specific AHLs in a similar manner to E2348/69. However, no aggregation was observed in the presence or absence of signal. This suggested a difference in the genes affected by quorum sensing between enteropathogenic and enterohaemorrhagic *E. coli*. Development of biocontrol strategies targeting AHL-dependent quorum sensing regulated processes, such as aggregation to reduce *E. coli* O157:H7 contamination of meat products is possible, based on the data presented in this thesis.

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Abbreviations

(v/v)	volume/volume ratio
(w/v)	weight/volume ratio
A/E Lesions	Attaching-effacing lesions
AB	Autoinducing Bioassay medium
AHL	N-acylhomoserine lactone
AI	Aggregation Indices
AI-2	Autoinducer-2
AIP	Autoinducing Peptide
AMC	Activated Methyl Cycle
ANOVA	Analysis of Variance
Bfp	Bundle forming pili
BSA	Bovine serum albumin
DAEC	Diffusely adherent *Escherichia coli*
dDHL	*N*-dodecanoyl-L-HSL
dH_2O	de-ionised water
DMEM	Dulbecco's Modified Eagles Medium
DMSO	Dimethyl Sulphoxide
D-OdDHL	*N*-(3-oxododecanoyl)-D-HSL
D-OHHL	*N*-(3-oxohexanoyl)-D-HSL
DPD	4,5-hydroxy-2,3-pentanedione, the AI-2 precursor
EAEC	Enteroaggregative *Escherichia coli*
EAF	EPEC adherence factor
EHEC	Enterohaemorrhagic *Escherichia coli*
EIEC	Enteroinvasive *Escherichia coli*
EPEC	Enteropathogenic *Escherichia coli*
ETEC	Enterotoxigenic *Escherichia coli*
FAS	Fluorescence Actin Staining
FBS	Foetal Bovine Serum
HosA	Homologue of SlyA

HUS	Haemolytic Uraemic Syndrome
K-S	Kolmogorov-Smirnov test
LA	Luria Agar
LB	Luria Broth
LEE	Locus of Enterocyte Effacement
OD_{600}	Optical Density (600nm)
OHHL	*N*-(3-oxohexanoyl)-L-HSL
PBS	Phosphate Buffer Saline
PFGE	Pulsed Field Gel Electrophoresis
QS	Quorum Sensing
RLU	Relative Light Units
RT	Room Temperature
SAH	S-adenosylhomocysteine
SAM	S-adenosylmethionine
SdiA	Suppressor of division
SRH	S-ribosylhomocysteine
STEC/VTEC	Shiga-toxin containing/ verocytotoxin-producing *Escherichia coli*
Stx	Shiga toxin
sv.	serovar
VFA	volatile fatty acids

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