

## **SENSITIZATION OF *E. COLI* O157:H7 ON FRUITS AND VEGETABLES TO HALOGENATED SANITIZERS**

**ANN LARSON AND ERIC JOHNSON**

Outbreaks of foodborne illness caused by *E. coli* O157:H7 and other bacterial foodborne pathogens in produce have raised concerns about the safety of fresh fruits and vegetables that are consumed raw or have been only minimally processed to reduce or eliminate pathogens. Currently used and proposed methods for reducing the numbers of such organisms on produce, including washing with water and the use of sanitizers, are often ineffective, inconsistent, or cost-prohibitive under certain conditions. The object of this study was to examine methods to enhance the effect of sanitizers on produce and thus reduce the risk of illness caused by *E. coli* O157:H7 and other pathogens.

The first objective of this study was to determine whether acid adaptation increased the sensitivity of *E. coli* O157:H7 on the surface of fruits (apples, oranges, melons), vegetables (iceberg lettuce, alfalfa sprouts, cabbage), and sprout seeds to chlorinated and iodine-based sanitizers. In some instances, acid-adapted *E. coli* O157:H7 on certain types of produce was somewhat more sensitive than non-adapted cells to sodium hypochlorite, calcium hypochlorite, or iodine-based sanitizer. The second objective of this study was to develop economically viable combinations of pre-treatment/sanitizer method(s) to reduce *E. coli* O157:H7 populations on produce. Pre-treatment with steam, although somewhat bactericidal, was found to negatively affect the quality of all types of produce tested. In this study, chlorinated sanitizers were more bactericidal to *E. coli* O157:H7 than an iodine-based sanitizer. Although none of the sanitizer/pre-treatment combinations this study resulted in a 5-log CFU/g decrease in *E. coli* O157:H7, numbers of the pathogen decreased approximately 1–3 log CFU/g after exposure to many of the treatments tested. In general, however, pre-treatment with organic acid did not significantly enhance the bactericidal effect of sanitizers on produce. This study should provide a basis for improved sanitation procedures for gram-negative pathogens on produce.

## PHENOTYPIC CHARACTERIZATION OF AN *ESCHERICHIA COLI* O157:H7 *HNS* MUTANT

IRFAN EROL, C. W. KASPAR, AND S.-H. CHOI

H-NS is a nucleoid protein with central roles in the regulation of approximately 30 proteins in *Escherichia coli*. A number of the genes regulated by H-NS are involved in growth and responses to environmental conditions, such as *rpoS* which encodes for the alternative stationary-phase sigma factor ( $\sigma^{38}$ ). Because *rpoS* is important to acid tolerance and animal passage in *E. coli* O157:H7, an *hns* mutant of *E. coli* O157:H7 was generated (*hns::nptI*, FRIK47001) and compared to the parent strain (ATCC 43895) and strain FRIK47001 carrying a plasmid (pSC0061) with a functional *hns* and the *hns* promoter (ca. 90 bp). FRIK47001 exhibited reduced cell size, coccoid morphology when examined using electron microscopy and was non-motile in motility agar and tested negative for the H7 antigen by latex agglutination.

Biochemical characterization determined that FRIK47001 was negative for sucrose and rhamnose metabolism as well as 40 other carbohydrates in comparison to the parent strain. In addition, FRIK47001 metabolized 20 of the 95 nitrogen sources tested while the parent was capable of using 39 of these nitrogen sources. The parent and complemented strains were able to grow in LB containing 14 to 15% bile salts, bile, or oxgall while FRIK47001 grew in 6.5, 9, and 14% of these compounds, respectively. There was no significant difference in heat tolerance. Survival of log- and stationary-phase cells in acid was evaluated in pH 2.0 simulated gastric fluid. Log phase cells of hte FRIK47001 were the most tolerant to acid with a  $D_{pH2.0} = 103.5$  min. The average  $D_{pH2.0}$  values of log phase cells of hte parent strain and FRIK47001 containing pSC006 were 41.8 and 60.5 min, respectively. In contrast, stationary-phase cells had significantly greater acid tolerance nad FRIK47001 was most sensitive to acid. Stx1 and Stx2 was not significantly influenced by H-NS. These results demonstrate that H-NS is responsible for the regulation key metabolic and survival properties in *E. coli* O157:H7 and is a potential target for control of this human pathogen.

## **TETRACYCLINE CAUSES ACID-SENSITIVITY IN STATIONARY-PHASE *E. COLI* CONTAINING *TET A* OR *TET O* BY REDUCING PROTEIN SYNTHESIS**

**J. L. BOSE, C. W. KASPAR, AND C.-M. CHENG**

The mechanism by which tetracycline causes acid sensitivity in stationary-phase cells of *E. coli* DH1 was studied using tetracycline (Tc) -resistant and -sensitive strains. Two resistance genes, *tetA* and *tetO*, were used to elucidate the role of tetracycline in this phenotype. These genes were introduced into DH1 in low-copy-number plasmids (pJB3TA and pJB3TO). Previous studies demonstrated that growth of DH1 with pJB3TA in the presence of tetracycline or with chloramphenicol for 3.5 hours preceding acid challenge resulted in an acid-sensitive phenotype. Growth in the presence of both tetracycline and chloramphenicol (last 3.5 h) did not increase acid sensitivity. These data suggested that protein synthesis was influenced by tetracycline despite the presence of *tetA*. Protein synthesis in stationary-phase (16-hr) cells was monitored using b-galactosidase (b-gal) as a reporter. Non-induced, stationary-phase DH1 cells express 38 units of b-gal while IPTG induced DH1 cells produced 330 units. Production of b-gal was examined with tetracycline-resistant strains (*tetA* or *tetO*) grown in the absence or presence of 12 and 36  $\mu\text{g}$  tetracycline/ml. DH1 cells containing pJB3TA produced 256 units of b-gal when grown in the absence of tetracycline. But, these cells produced 47 and 27 units of b-gal when grown in the presence of 12 and 36  $\mu\text{g}$  tetracycline/ml, respectively. This represents an 80 to 90% reduction in b-gal production. DH1 containing pJB3TO expressed 326 units of b-gal when grown in the absence of tetracycline, but only 173 and 133 units of b-gal when grown in the presence of 12 and 36  $\mu\text{g}$  tetracycline/ml, respectively. This represents a reduction of 47% and 59%. Interestingly, there did not appear to be a difference in b-gal production in log-phase cells of the parent and plasmid harboring DH1 strains grown with or without tetracycline. These data suggest that a reduction in protein synthesis in stationary-phase cells caused by tetracycline is responsible for acid-sensitivity.

## TRANSCRIPTIONAL AND TRANSLATIONAL REGULATION OF *DPS* EXPRESSION IN *ESCHERICHIA COLI* O157:H7

K. JEONG<sup>1</sup>, C. W. KASPAR<sup>1</sup>, AND S. CHOI<sup>2</sup>

<sup>1</sup>Food Research Institute, UW–Madison

<sup>2</sup>Chonnam National University, Kwang-Ju, Republic of Korea

Dps, a non-specific DNA-binding protein in *Escherichia coli*, is important in protection of DNA from oxidative and acidic stress. Northern blot and primer extension analysis of RNA from *E. coli* O157:H7 (ATCC43895) and an *rpoS* mutant (FRIK47004) revealed that the transcription of *dps* was regulated by sigma 70 and sigma 38. *dps* RNA was produced continuously from log phase to late stationary phase and reached a maximum level at early stationary phase. In log phase cells, transcription of *dps* was driven by sigma 70 which was negatively regulated by *crp*. In stationary phase cells, transcription of *dps* was mediated by sigma 70 or sigma 38; however, transcription was initiated at the same site. Using a *dps*-E5 fusion, we found that translational regulation is involved in expression of *dps*. Synthesis of Dps was significantly increased at 16 hr, but transcription levels of *dps* reached a maximum level after 8 hr of incubation. This suggests that a specific factor enhances translation of *dps* mRNA. Studies of translational fusions with *crp*, *cspE*, *hns*, *lrp*, and *oxyR* mutants found that there was little Dps produced in an *hns* mutant even though mRNA of *dps* was present in late stationary phase cells. These results indicate that Dps expression is directed by different sigma factors depending on the physiological state of the cell and positively impacted by H-NS.

**STcE, A METALLOPROTEASE SECRETED  
BY *ESCHERICHIA COLI* O157:H7, SPECIFICALLY  
CLEAVES C1 ESTERASE INHIBITOR**

**RODNEY A. WELCH**

*Department of Medical Microbiology & Immunology, UW–Madison*

A goal of our laboratory's research is to examine how StcE, a metalloprotease secreted by *Escherichia coli* O157:H7, contributes to the pathogenesis of this foodborne pathogen. We have recently completed an extensive epidemiological survey of diarrheagenic *E. coli* clinical isolates, correlating the presence of *stcE* with other potential virulence factors acquired during the evolution of enterohemorrhagic *E. coli* O157:H7. It appears that the acquisition of *stcE* was among the first steps in the evolution of *E. coli* O55:H7, an enteropathogenic strain of *E. coli*, into the current O157:H7 serotype. The presence of *stcE* appears to be linked with the *etp* type II secretion operon, a general secretory cluster required for the secretion of StcE. Additional research suggests that StcE, which cleaves the critical serpin C1 esterase inhibitor (C1-IHN), may potentiate C1-INH activity as a possible mechanism of serum resistance.

*PUBLICATION:*

Lathem, W. W., T. E. Grys, S. E. Witowski, A. G. Torres, J. B. Kaper, P. I. Tarr, and R. A. Welch. StcE, a metalloprotease secreted by *Escherichia coli* O157:H7, specifically cleaves C1 esterase inhibitor. *Molec. Microbiol.* (in press) 2002.