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The Bacillus subtilis Quorum-Sensing Molecule CSF Contributes to Intestinal Homeostasis via OCTN2, a Host Cell Membrane Transporter.

Fujiya M, Musch MW, Nakagawa Y, Hu S, Alverdy J, Kohgo Y, Schneewind O, Jabri B, Chang EB.

Quorum sensing CSF of *B. subtilis*, through OCTN2 transport, contributes to intestinal homeostasis

Mikihiro Fujiya[1], Mark W. Musch[1], Yasushi Nakagawa[1], Shien Hu[1], John Alverdy[2], Yutaka Kohgo[3], Olaf Schneewind[4], Bana Jabri[5], Eugene B. Chang[1]

[1]Martin Boyer Laboratories, University of Chicago

[2]Department of Surgery, University of Chicago

[3]Third Department of Internal Medicine, Asahikawa Medical College, Asahikawa, Japan

[4]Department of Microbiology, University of Chicago

[5]Department of Pathology, University of Chicago

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Corresponding author:

Eugene B. Chang, M.D.

Department of Medicine, MC 6084, University of Chicago

5841 S. Maryland Ave.

Chicago, IL 60637

echang@medicine.bsd.uchicago.edu

Tel: 773-702-6458

Fax: 773-702-2281

Summary

Bacteria use quorum sensing molecules (QSMs) to communicate within as well as across species, however, the effects of QSM on eukaryotic host cells are less understood. We report that the quorum-sensing pentapeptide, competence and sporulation factor (CSF), of a Gram-positive bacterium *Bacillus subtilis,* activates key survival pathways including p38 MAP kinase and protein kinase B (Akt) in intestinal epithelial cells and also induces cytoprotective heat shock proteins (Hsps), the latter which prevent oxidant-induced intestinal epithelial cell injury and loss of barrier function. These effects of CSF depend on its uptake by an apical membrane organic cation transporter-2 (OCTN2). OCTN2-transported CSF therefore serves as an example of a unique mechanism of host-bacterial interactions allowing the host to monitor changes in colonic flora behavior or composition.

Introduction

The enteric microbiota is a unique ecological niche where microorganisms live normally in the digestive tract in a balanced relationship with other species and the host. The relationship is complex and incompletely understood, often involving bidirectional signals and interactions that not only influence the behavior of microflora, but also host responses essential to the maintenance of intestinal homeostasis (Rakoff-Nahoum et al., 2006). Prime examples of the latter are the host toll-like receptors (TLR) that continuously monitor luminal microbial pattern molecules that are essential for regulation of innate immune responses and epithelial cytoprotection (Pasare and Medzhitov, 2005). Among bacteria, population dynamics are influenced by the secretion of numerous metabolites and effecter molecules that promote species stability, adaptation, and survival within this environment. Quorum sensing is perhaps the most elegant of these processes, providing bacteria with the ability to communicate and change behavior of the same or other species in response to conditions and perturbations of the environment (Bassler and Losick, 2006 and Camilli and Bassler, 2006). Both Gram-positive and Gram-negative organisms utilize quorum sensing molecules (QSMs) which, in the former, are usually bioactive peptides, whereas in the latter, include non-peptide molecules such as acyl-homoserine lactone (Bassler and Losick, 2006). Because they play a role in determining the diversity and composition of the enteric microbiome, the profile of QSMs at any given time reflects the status or impending changes in the microbiota. Whether eukaryote cells have the ability to detect the complex array of QSMs is unexplored, but such an ability would allow the host to appropriately respond to physiological or

pathophysiological perturbations in the microbiota. The present studies demonstrate that the pentapeptide, QSM, ERGMT, also known as CSF (competence and sporulation factor) from *Bacillus subtilis*, is transported into mammalian intestinal epithelia through a novel cell membrane transporter, organic cation transporter isotype 2 (OCTN2). Once taken up by intestinal epithelial cells, activates key survival pathways including p38 MAP kinase and protein kinase B (Akt) and also induces cytoprotective heat shock proteins (Hsps), the latter preventing oxidant-induced intestinal epithelial cell injury and loss of barrier function**.**

Results

Bacillus subtilis produced-CSF induces Hsps and activates survival pathway (Akt) and stress signal (p38 MAPK) in Caco2$_{bbe}$ cells

Heat shock proteins (Hsp) are essential for maintenance of intestinal homeostasis (Rakoff-Nahoum et al., 2004, Pasare and Medzhitov, 2005 and Rakoff-Nahoum et al., 2006), rendering colonic epithelial cells less susceptible to injury and stress (Arvans et al., 2004). Intestinal epithelia express many heat shock proteins, including inducible Hsp27(human)/Hsp25(murine) and Hsp70 and constitutively expressed heat shock cognate Hsc70. The nomenclature, Hsp27 and Hsp25, defines the related small heat shock proteins of approximate molecular weight of 27 kDa and 25 kDa of human and murine cells, respectively (Morimoto, 1993 and Morimoto, 2002). Physiological expression of inducible heat shock proteins like Hsp27/25 and Hsp70 is maintained by microbial-derived molecules, including pattern recognition ligands (Rakoff-Nahoum et

al., 2004), accounting for their predominant expression in surface colonocytes (see Figure S1 in the Supplemental Data available with this article online). Therefore, we assessed the effects of conditioned media (CM) from several representative strains of enteric bacteria in inducing heat shock protein 27 (Hsp27) expression in human colonic epithelial $Caco2_{bbe}$ cells. As shown in Figure 1A, of the strains that were tested, most Gram-positive, but not Gram-negative, bacteria significantly induced Hsp27 expression in $Caco2_{bbe}$ cells (results expressed as a percent of control Hsp27 response to thermal stress, 41.5^{o}C x 23 mins). In light of this dichotomous response of Gram-positive bacteria, we considered the possibility that secreted agents such as quorum sensing peptides might be mediating the actions of this group of microbes.

B. subtilis (strain JH 642, wild type) was selected for further study because it is a well characterized obligate, Gram-positive aerobe that is not only a common soil and water saprophyte, but frequently part of human enteric flora with known probiotic activity (Solomon et al., 1996, Kunst et al., 1997, Lazazzera et al., 1997, Levin et al., 1998 and Tam et al., 2006). Conditioned medium from the *B. subtilus* strain JH 642 increased Hsp27 expression to nearly the same extent as heat shock (Figure 1B), whereas neither experimental condition altered expression of the constitutively expressed heat shock cognate Hsc70. The latter was anticipated because Hsc70 in most cells is quite stable and less influenced by exogenous stimuli or cell stress. This insures that Hsc70 continues to function in critical processes such as protein folding, chaperone function, and in formation and stabilization of protein complexes (Morimoto, 1993 and Morimoto, 2002). To further characterize the factor(s) that induced Hsp27, CM from JH 642 was size-separated by a 3 kDa molecular mass cutoff filter, with bioactivity largely remaining

in the filtrate indicating a small molecular mass (Figure 1C). Additionally, the Hsp27-inducing bioactivity was heat-stable and pepsin-sensitive.

B. subtilis produces and secretes many bioactive agents, but its competence and sporulation factor (CSF), a QSM, fits the parameters of the above physiochemical characteristics. CSF is a cationic pentapeptide corresponding to the C-terminal 5 amino acids of the 40 amino acid polypeptide encoded by the *phrC gene* (Kunst et al., 1997) and functions in quorum-sensing (Lazazzera et al., 1997) with a physiological concentration range between 10-100 nM (Solomon et al., 1996) to alter Bacillus population behavior. To assess CSF's potential biological role in colonic epithelial cells, CM from wild type (JH 642) and the CSF-deficient JH 642-derived *B. subtilis* strain, RSM 121 were added to $Caco2_{bbe}$ cells. CM derived from RSM121 (delta CSF) failed to induce Hsp27 in $Caco2_{bbe}$ cells, implicating CSF in this effect (Figure 1D). To further evaluate this possibility, CSF (ERGMT) was chemically synthesized and purified. CSF induced Hsp27 in $Caco2_{bbe}$ cells (Top Western blot of Figure 1E) and this induction is a concentration-dependent fashion which are physiologically relevant (Figure S2). Additionally, we tested the activation of other signaling pathways involved in cell survival and found that CSF activated the Akt and p38 MAPK pathways (Lower two sets of western blots in Figure 1E). In intestinal epithelial cells, the former has been shown to be important in promoting Hsp25 expression (Tao et al., 2006) and the latter in blocking apoptosis by inhibiting caspase-3 after polyamine depletion (Zhang et al., 2004). In contrast, other two pathways, JNK and ERK, were not influenced by CSF (data not shown). As a control, a scrambled pentapeptide, EMTRG, did not induce Hsp27 or activate the Akt and p38 MAPK pathways (data not shown).

OCTN2 transports CSF and mediates CSF effect on Hsps induction in Caco2$_{bbe}$ cells

CSF-mediated activation of an early competence promoter (*srfA*) (Lazazzera et al., 1997 and Levin et al., 1998) in *B. subtilis* cells is dependent on the uptake by a Bacillus oligopeptide transporter. Could a convergent mechanism develop in eukaryotic host that would mediate a specific uptake of bacterial QSM peptides? In fact, other peptides, such as bacterial chemotactic peptides, can be transported by eukaryote apical membrane oligopeptidyl transporters (Charrier et al., 2006). We specifically focused on the apical membrane organic cation transporter, OCTN2, as a candidate for CSF-uptake because of its transport preference for substrates having physiochemical properties close to CSF (e.g. cationic oligopeptide) (Tamai et al., 2000 and Peltekova et al., 2004). OCTN2 is believed to be the main transporter for dietary carnitine, but its abundant expression in the colon is unexplained, as most carnitine is absorbed in the small intestine (Tamai et al., 2000 and Ohashi et al., 2001). OCTN2 is primarily expressed by surface epithelial cells of the colon that are in direct contact with the luminal contents and microbes and which exhibit sustained expression of microbial-induced heat shock proteins (Rakoff-Nahoum et al., 2004) (Figure S1). OCTN2, in contrast to OCTN1, is also expressed in Caco2$_{bbe}$ cells (Figure S3A).

As shown in Fig. 2A, ^{14}C-labeled CSF was readily taken up by Caco2$_{bbe}$ cells, an effect that was increased in OCTN2-transfected cells and inhibited in cells with siRNA silenced OCTN2 expression (Figure S3B and C). Similar, but more pronounced, effects were observed in OCTN2-transfected human fibroblast HSWP cells that normally exhibit minimal endogenous expression of OCTN2 (Figure S4A and B). FITC-labeled CSF was

also rapidly taken up by $Caco2_{bbe}$ cells (Figure 2B) and distributed throughout the cytosol within 30 min, an effect competed by L-carnitine (10 mM) implicating OCTN2 transport. In addition, while CSF competed with L-carnitine uptake, a scrambled pentapeptide for CSF (EMTRG) did not, suggesting the specificity of OCTN-mediated transport of the peptide (Figure S4C). Taken together, these studies provide compelling evidence for uptake of CSF by OCTN2. CSF induction of Hsp27 was also blocked by inhibiting OCTN2 expression with siRNA, whereas Hsp27 induction by heat shock (41.5^{o}C x 23 min) was not affected (Figure 2C), showing that OCTN2 is required for CSF to mediate Hsp27 induction. As shown in Figure 2D, conditioned media of other Gram-positive, in contrast to Gram-negative, bacteria also appear to compete with Na-dependent, L-carnitine uptake of $Caco2_{bbe}$ cells, suggesting OCTN2 uptake of soluble molecules derived from these organisms.

OCTN2 mediated-CSF uptake protects epithelial cells from oxidant stress

To assess the functional role of CSF and OCTN2 in epithelial homeostasis, we examined whether CSF protects epithelial cells against oxidant (NH_2Cl, monochloramine)-induced injury using ^{51}Cr release. Pretreatment of $Caco2_{bbe}$ monolayers with wild type JH642 CM protected cells against oxidant-induced injury, whereas pretreatment with CM from Rsm121 (delta - ΔCSF) did not (Figure 3A, left panel). When cells were treated with CSF, cPD1, an unrelated quorum-sensing molecule from Enterococcus (Figure 3A, middle panel), or scrambled pentapeptide of CSF (EMTRG) (Figure 3A, right panel), protection against oxidant stress was only seen with

CSF. Silencing of OCTN2 with siRNA inhibited the protective effects of CSF against oxidant-induced stress in $Caco2_{bbe}$ cells (Figure 3B).

Because intestinal barrier function and viability are highly sensitive to inflammation-associated injury, the above agents were tested to determine their ability to limit oxidant (monochloramine)-induced increases in barrier function. Only CSF and CM of *B. subtilis* mitigated induced increases in ^{3}H-mannitol flux, a measure of barrier function (Figure 3C). Silencing of Hsp27 resulted in nearly complete reversal of the CSF- and CM-induced protection of cell viability (Figure 3D) and epithelial barrier function (Figure 3E) against oxidant-induced stress. In contrast, treatment of human $Caco2_{bbe}$ cells with siRNA to murine Hsp25 (mHsp25) had no effects, indicating the specificity of Hsp27 silencing.

CSF protects intestinal tissues from oxidant stress through OCTN2 transport in *ex vivo* preparation of mice

We next examined the effects of CSF (ERGMT) and a scrambled pentapeptide molecule (EMTRG) on induction of Hsp25 and Hsp70 in *ex vivo* preparations of murine proximal small intestine and colon. Surgically removed segments of bowel were ligated at both ends, filled with buffer containing CSF or scrambled peptide. After 2 hrs, CSF treatment of both small and large intestinal mucosa stimulated a significant increase in Hsp25 and Hsp70 (Figure 4A), best appreciated in small intestine, as basal levels of these proteins were minimal. In contrast, no changes in mucosal Hsc70 (constitutively expressed heat shock cognate) were noted. Despite the higher basal expression in colonic mucosa due to the presence of enteric flora (Arvans et al., 2004), significant increases in

Hsp25 and Hsp70 expression were still observed. In contrast, the scrambled peptide EMTRG slightly induced Hsp25, but not Hsp70, in small intestine. Thus, we cannot rule out the possibility that pentapeptides with similar amino-acid sequence have biological activity in eukaryotic cells. No induction of Hsp25 and Hsp70 was noted in large bowel mucosa exposed to the scrambled peptide. This effect was mediated by OCTN2, as L-carnitine (10 mM) inhibited CSF-induced Hsp25 and Hsp70 induction (Figure 4B). To determine the physiological consequences of the CSF effect, transmural ^{3}H-mannitol fluxes were performed in intact small bowel loops to assess intestinal barrier function. As shown in Fig. 4C, increased mucosal permeability in small intestinal loops caused by exposure to oxidant (NH_2Cl 0.3 mM) was significantly inhibited by luminal CSF (100nM), but not scrambled peptide. This protective action was inhibited when studies were performed with L-carnitine (10 mM), used to competitively inhibit CSF uptake through OCTN2 (Figure 4D). No changes in the basal mannitol permeability were observed with either CSF or the scrambled peptide (data not shown). Additionally, pretreatment of intestinal loops or $Caco2_{bbe}$ monolayers with inhibitors of the Akt and p38 MAPK pathways (LY294002 and SB203580, respectively) had no significant effects on CSF protection (Figure S5), suggesting the induction of heat shock proteins plays a major role in conferring protection in this form of stress. While a contributory role of the Akt and p38 MAPK pathways cannot be categorically ruled out, several other reports have also demonstrated induced heat shock proteins are particularly effective in protecting cells against oxidant-induced stress (Ropeleski et al., 2003 and Arrigo et al., 2005).

Discussion

This study establishes a physiological role for OCTN2-transport of CSF as a mediator of host-microbial interaction. It is notable that polymorphisms of the OCTN1 andOCTN2 encoding genes, SLC22A4 and SLC22A5, are within the IBD5 susceptibility locus of Crohn's disease (Peltekova et al., 2004, Noble et al., 2005, Vermeire et al., 2005, Walter et al., 2006 and Leung et al., 2006), although a disease-causing role for these genes has not been established (Trinth et al., 2005). OCTN2 and other similar pathways for engaging or uptake of QSMs may be essential for the regulation of host responses important for maintenance of intestinal homeostasis. Bacteria use quorum-sensing to communicate and coordinate population behavior in response to environmental changes, nutrient availability, and resisting other competing or pathogenic microorganisms (Bassler and Losick, 2006 and Camilli and Bassler, 2006). Similarly, pathogenic bacteria use quorum-sensing to co-ordinate their virulence, allowing them to evade immune detection and successfully establish infection. The finding that many Gram-positive bacteria, in contrast to Gram-negative organisms, produce compounds that compete L-carnitine uptake (Figure 2D) is interesting because their quorum sensing molecules are typically small peptides, whereas the latter utilize non-peptides (Bassler and Losick, 2006 and Camilli and Bassler, 2006). At any point in time, the profile of quorum-signaling molecules potentially serves as a composite measure of the status of the colonic microbiota. Thus, the uptake or sampling of QSMs by OCTN2 and potentially other transporters like it may provide the host with the ability respond or adapt to changes in the microbiome in order to maintain intestinal homeostasis. Furthermore, our *in vitro* study with OCTN2 siRNA strongly suggested the relevance of OCTN2 transport in

inducing cytoprotective protein Hsps and protecting intestinal epithelial cells by CSF. In this regard, OCTN2 could potentially mediate some the actions of probiotic microorganisms. From our studies, it is difficult to determine the relative contribution of OCTN2-mediated host-microbial interaction relative to other forms of host-microbe interaction, including pattern recognition receptors or cytoplasmic nucleotide-binding-oligomerization domain (NOD) molecules (Mueller et al. 2005). This issue will only be resolved by analysis in animals bearing intestinal epithelial-specific gene targeted deletion of OCTN2. However, differences in the OCTN2 pathway from TLR and NOD signaling bear further discussion. The latter receptors are critically important for recognition of microbial-derived cellular or cell wall-derived ligands that are indicative of potential or impending threats by pathogens. As a consequence, innate immune cells can respond rapidly and appropriately to many types of pathogens. In contrast, OCTN2 is primarily expressed by intestinal epithelial cells and to a far lesser extent by innate immune cells. Thus, OCTN2 is less likely to be involved in meeting pathogen threats head on. While being a fairly promiscuous transporter capable of taking up many molecules, OCTN2 still requires certain structural features (e.g. small organic cations) that is likely to restrict substrates to particular types or classes of bacterial-derived molecules. Our studies would suggest that small peptide quorum sensing molecules secreted primarily by Gram-positive bacteria are among these molecules. It is also notable that many of these bacteria are not pathogenic in the normal host and, in some cases, have been used as probiotic agents. Instead, we propose that OCTN2 is an example of a host mechanism that continuously samples the luminal content for certain microbial constituents within the enteric microbiome, allowing the host to adjust to

perturbations or changes that might otherwise affect intestinal homeostasis. Since OCTN2 function is restricted to particular substrates, we predict that other, similar pathways exist (e.g. OCTN1, MDR-1), allowing the host to survey many constituents of the microbiome. At the moment, the intracellular mammalian receptors or targets for bacterial QSM have not been identified. However, regardless of the nature of these intracellular QSM-receptors, we have demonstrated that the ability of epithelial cells to sense bacterial QSM and deliver them using highly specialized transporter molecule OCTN2. Once taken up by intestinal epithelial cells, CSF activates key survival pathways including p38 MAP kinase and protein kinase B (Akt) and induces cytoprotective heat shock proteins, the latter preventing oxidant-induced intestinal epithelial cell injury and loss of barrier function**.**

Experimental Procedures

Materials. Peptides were purchased from EZ Biolab (Westfield, IN) or Elim Biopharmaceuticals (Hayward, CA) and [^{3}H]-carnitine, [^{14}C]-acetic anhydride, [^{3}H]-mannitol, [^{35}S]-EXPRESS and [^{51}Cr] Cl from Perkin Elmer (Boston, MA). Radiolabeled CSF was made by an exchange method using [1-^{14}C] acetic anhydride (Moravek Biochemicals, Brea CA). Briefly, 1.9 mg of peptide (CSF (ERGMT)) was dissolved in 100µl water and mixed with 1 ml of sodium acetate saturated water. Two ul of acetic anhydride were added 5 times at 15 min intervals at 0°C, and the reaction stopped by addition of 50µl ammonium hydroxide. The acetylated peptide was purified by HPLC using a 5µ 25cm length 4.6mm diameter Lichrosphere-100 RP-18 using a solvent

gradient: starting at 98% A/2% B (A== 0.1% TFA in water and B= 0.1% TFA in acetonitrile) for 5 min and then a linear gradient to 75% A 25%B over 20 min at flow rate of 1 ml/min. Detection was at 220 nm. The fraction containing acetylated ERGMT was analyzed by nuclear magnetic resonance (NMR) spectrometry to confirm structure and identify the acetylated residues (determined to be on amino terminal E).

Cell culture. Human colonic epithelial $Caco2_{bbe}$ cells, a generous gift of Dr. Mark Mooseker (Yale University, New Haven, CT), were grown in high-glucose Dulbecco's Modified Eagle's Medium (DMEM) supplemented with 10% (vol/vol) fetal bovine serum (FBS), 2mM L-glutamine, 50U/ml penicillin, 50µg/ml streptomycin and 10µg/ml transferrin (all from Invitrogen/GIBCO, Grand Island, NY) in a humidified atmosphere of 5% CO_2. Cells were used between passages 55-70. Cells were plated on 6 or 12 well plates at a density of 10^5 cells/ cm^2 and were allowed to differentiate for 10-14 days before experiments.

Mice. These studies were approved by the Institutional Animal Care and Use Committee of the University of Chicago. C57Bl/6 mice were purchased from Jackson Laboratories (Bar Harbor, ME) or Taconic Labs (Germantown, NY). Small and large intestines with or without treatments were removed, rinsed with ice-cold saline, and epithelium was gently sheared off with glass slides for protein or mRNA determination.

Preparation of *B. subtilis* conditioned media (CM). *B. subtilis* JH642 (wt) and RSM121 (delta CSF) were a generous gift of Alan D. Grossman (MIT, Cambridge, MA), the latter strain derived from the former[13]. Single colonies of both strains were picked from LB agar allowed to grow in LB until OD_{600nm} reached 1.0 (about 3 hours), then pelleted (2000 x *g* for 15 min) and washed three times in minimum medium (S7 minimal

salts, 1% wt/vol glucose, 1% wt/vol glutamate and required amino acids (50μg/ml). Bacteria were then resuspended in 10ml of minimal medium and grown for an additional 3 hours. Cells were then pelleted and the supernatant (containing secreted products) was filter-sterilized through a 0.1μ filter and stored at –80°C until use. For radioactively labeled media, [^{35}S]-methionine and cysteine (^{35}S-EXPRESS labeling reagent) were added to the initial LB growth medium.

OCTN2 cDNA transfection. Human OCTN2 (SLC22A5) cDNA (a generous gift from Dr. Vadivel Ganapathy, Medical College of Georgia, Augusta, Georgia) was sublconed into pDsRED2-C1 (Takara/Clontech, Palo Alto, CA), and transfected Caco2$_{bbe}$ and HSWP cells using the polyamine-derived reagent LT-1 (Mirus, Madison, WI). Clones were selected on the basis of G418 (600μg/ml) resistance and individually propagated for flux studies. The degree of OCTN2 transfection of cells was assessed by measuring Na^+-dependent L-carnitine uptake and OCTN2 immunoblotting.

siRNA. To specifically inhibit expression of OCTN2, the Invitrogen BLOCK-iT RNAi designer (Invitrogen, Carlsbad, CA) was used to select the region of the coding sequence of human OCTN2 (1331-1355) for silencing and non-sense sequence (5'-CCATCTAAGTTGCCCGTGAATCGTT-3') as a negative control. dsRNA Stealth oligo was mixed with siLentfect reagent (Bio-Rad, Hercules, CA; 0.6μl of reagent per cm^2 growing surface) in Optimem medium (Invitrogen) and allow to form complexes for 15 minutes. Sufficient dsRNA was used for a final concentration of 100 nM. Complexes were applied when cells were 60% confluent and added for a second time after 2 days. Uptake studies were performed 24-48 hours after second application.

Western blotting. Proteins of $Caco2_{bbe}$ cells or mouse intestinal epithelia were analyzed by Western blotting. Twenty to forty μg of each sample was resolved by SDS-PAGE (10-12%) and immediately transferred to a polyvinylidene difluoride (PVDF) membrane using 1x Towbin buffer (25mM Tris pH 8.8, 192mM glycine with 15% (vol/vol) methanol). PVDF membranes were incubated in TBS with 0.05% (vol/vol) Tween 20 (T-TBS) containing 3% (wt/vol) BSA for 1 hour at room temperature to block nonspecific binding. Blots were incubated overnight at 4°C with the following primary antibodies: anti-mouse Hsp25 antibody (Stressgen, Victoria, British Columbia, Canada), anti-human Hsp27 (Stressgen) or anti-mouse Hsp70 antibodies (Stressgen), anti-total and phosphorylated antibodies to each of the following Akt, p38 MAP kinase, ERK 1/2 (p44/42), SAPK/JNK (Cell Signaling, Beverly, MA), rabbit polyclonal OCTN2 antiserum (Alpha Diagnostic International, San Antonio, TX), and rabbit polyclonal anti-human PepT1 (a gift of Didier Merlin, Emory University) PepT1(Merlin D, et al. Blots were washed five times for 10 min each in T-TBS at room temperature, incubated for 60 min in species-appropriate horseradish peroxidase-conjugated secondary antibody (Jackson Immunoresearch, West Grove, PA) T-TBS, washed four times in T-TBS, once in TBS and developed using the Super-Signal West Pico enhanced chemiluminescence system (Pierce Chemical, Rockford, IL).

Cell viability assay. $Caco2_{bbe}$ cells were grown in 24-well plates until differentiated and then treated with 10% vol/vol of *B. subtilis* conditioned medium, 100 nM CSF or other peptides for 24 hours. Cells were loaded with ^{51}Cr (50 μCi/ml) for 60 minutes, washed, and incubated in media with 0.6 mM monochloramine to induce cell injury. Medium was harvested from the cells after 60 minutes, and the ^{51}Cr remaining in the cells was

extracted with 0.1 wt/vol% SDS. The amounts of ^{51}Cr in the released and cellular fractions were counted by liquid scintillation spectrometer. The amount of ^{51}Cr released was calculated as the amount released divided by the total (cellular and released) ^{51}Cr.

***Ex vivo* intestinal loop studies.** C57Bl/6 mice (18-25 gm) were sacrificed and the small intestine removed beginning at the ligament of Treitz. The first 18 cm were divided into three 6 cm lengths, each end ligated with silk suture and the loops filled with RPMI 1640 medium with 10% vol/vol heat inactivated FBS, with or without peptides (ERGMT (CSF) or EMTRG (scrambled)) at 100nM. Loops were filled to moderate distention, about 1ml per loop. Loops were placed in the outer loop of organ culture dishes (Falcon, Becton Dickinson Labware, Franklin Lakes, NJ) which were filled with 5 mls media as above. Loops were incubated for 2 hours at 37°C in a 5% CO_2 incubator. A 1 cm segment was removed from the middle and mucosa scraped off with glass slides and processed for protein analysis as described in detail in the supplement. To measure permeability effects, the two remaining segments were filled with RPMI 1640 medium containing serum with 1 mM mannitol and 1 μCi/ml [^{3}H]-mannitol, and with or without 0.3 mM freshly prepared monochloramine. Loops were placed into the middle section of the organ culture dish in 2 ml of RPMI 1640 with serum without NH_2Cl. Samples were taken at 5, 20, and 35 minutes to determine flux of mannitol from lumen to medium outside bathing loops.

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References:

Arrigo, A.P., Virot, S., Chaufour, S., Firdaus, W., Kretz-Remy, C., and Diaz-Latoud, C. (2005). Hsp27 consolidates intracellular redox homeostasis by upholding glutathione in its reduced form and by decreasing iron intracellular levels. Antioxid Redox Signal. *7*, 414-22.

Arvans, D.L., Vavricka, S.R., Ren, H., Musch, M.W., Kang, L., Rocha, F.G., Lucioni, A., Turner, J.R., Alverdy, J., and Chang, E.B. (2004). Luminal bacterial flora determines physiological expression of intestinal epithelial cytoprotective heat shock proteins 25 and 72. Am. J. Physiol. Gastrointest. Liver Physiol. *288*, G696-704.

Bassler, B.L., and Losick, R. Bacterially speaking. (2006). Cell *121*, 125(2):237-46.

Camilli, A., and Bassler, B.L. (2006). Bacterial small-molecule signaling pathways. Science *311*, 1113-1116.

Charrier, L., Driss, A., Yan, Y., Nduati, V., Klapproth, J.M., Sitaraman, S.V., Merlin, D. (2006). hPepT1 mediates bacterial tripeptide fMLP uptake in human monocytes. Lab. Invest. *86*, 490-503.

Kunst, F., Ogasawara, N., Moszer, I., Albertini, A.M., Alloni, G., Azevedo, V., Bertero, M.G., Bessieres, P., Bolotin, A., Borchert, S., et al. (1997). The complete genome sequence of the Gram-positive bacterium Bacillus subtilis. Nature *390*, 249–256.

Lazazzera, B.A., Solomon, J.M., and Grossman, A.D. (1997). An exported peptide functions intracellularly to contribute to cell density signaling in B. subtilis. Cell *89*, 917-925.

Leung, E., Hong, J., Fraser, A.G., Merriman, T.R., Vishnu, P., and Krissansen, G.W. (2006). Polymorphisms in the organic cation transporter genes SLC22A4 and SLC22A5 and Crohn's disease in a New Zealand Caucasian cohort. Immunol Cell Biol. *84*, 233-6.

Levin, P.A., and Grossman, A.D. (1998). Cell cycle and sporulation in Bacillus subtilis. Curr. Opin. Microbiol. *1*, 630-635.

Morimoto, R.I. Cells in stress: transcriptional activation of heat shock genes. (1993). Science. *259*, 1409-1410.

Morimoto, R.I. Dynamic remodeling of transcription complexes by molecular chaperones. (2002). Cell. *110*, 281-284.

Mueller, T., and Podolsky, D.K. Nucleotide-binding-oligomerization domain proteins and toll-like receptors: sensors of the inflammatory bowel diseases' microbial environment. (2005). Curr. Opin. Gastroenterol. *21*, 419-425.

Noble, C.L., Nimmo, E.R., Drummond, H., Ho, G.T., Tenesa, A., Smith, L., Anderson, N., Arnott, I.D., and Satsangi, J. (2005). The contribution of OCTN1/2 variants within the IBD5 locus to disease susceptibility and severity in Crohn's disease. Gastroenterology *129*, 1854-1864.

Ohashi, R., Tamai, I., Nezu, Ji. J., Nikaido, H., Hashimoto, N., Oku, A., Sai, Y., Shimane, M., and Tsuji, A. (2001). Molecular and physiological evidence for multifunctionality of carnitine/organic cation transporter OCTN2. Mol. Pharmacol. *59*, 358-366.

Pasare, C., and Medzhitov, R. (2005). Toll-like receptors: linking innate and adaptive immunity. Adv Exp Med Biol. *560*, 11-8.

Peltekova, V.D., Wintle, R.F., Rubin, L.A., Amos, C.I., Huang, Q., Gu, X., Newman, B., Van Oene, M., Cescon, D., Greenberg, G., Griffiths, A.M., St George-Hyslop, P.H., and Siminovitch, K.A. (2004). Functional variants of OCTN cation transporter genes are associated with Crohn disease. Nat. Genet. *36*, 471-475.

Rakoff-Nahoum, S., Paglino, J., Eslami-Varzaneh, F., Edberg, S., and Medzhitov, R. (2004). Recognition of commensal microflora by toll-like receptors is required for intestinal homeostasis. Cell *118*, 229-241.

Rakoff-Nahoum, S., and Medzhitov, R. (2006). Role of the innate immune system and host-commensal mutualism. Curr. Top. Microbiol. Immunol. *308*, 1-18.

Ropeleski, M.J., Tang, J., Walsh-Reitz, M.M., Musch, M.W., Larson, R.A., and Chang, E.B. (2003). Interleukin-11-induced heat shock protein 25 confers intestinal epithelial-specific cytoprotection from oxidant stress. Gastroenterology *124*, 1358- 1368.

Solomon, J.M., Lazazzera, B.A., and Grossman, A.D. (1996). Purification and characterization of an extracellular peptide factor that affects two different developmental pathways in Bacillus subtilis. Genes. Dev. *10*, 2014-2024.

Tam, N.K., Uyen, N.Q., Hong, H.A., Duc le, H., Hoa, T.T., Serra, C.R., Henriques, A.O., and Cutting, S.M. (2006). The intestinal life cycle of Bacillus subtilis and close relatives. J. Bacteriol. *188*, 2692-2700.

Tamai, I., Ohashi, R., Nezu, J.I., Sai, Y., Kobayashi, D., Oku, A., Shimane, M., and Tsuji, A. (2000). Molecular and functional characterization of organic cation/carnitine transporter family in mice. J. Biol. Chem. *275*, 40064-40072.

Tao, Y., Drabik, K.A., Waypa, T.S., Musch, M.W., Alverdy, J.C., Schneewind, O., Chang, E.B., and Petrof, E.O. (2006). Soluble factors from Lactobacillus GG activate MAPKs and induce cytoprotective heat shock proteins in intestinal epithelial cells.Am. J. Physiol. *290*, C1018-1030.

Trinh, T.T., and Rioux, J.D. (2005). Understanding association and causality in the genetic studies of inflammatory bowel disease. Gastroenterology. *129*, 2106-10.

Vermeire, S., Pierik, M., Hlavaty, T., Claessens, G., van Schuerbeeck, N., Joossens, S., Ferrante, M., Henckaerts, L., Bueno de Mesquita, M., Vlietinck, R., and Rutgeerts, P. (2005). Association of organic cation transporter risk haplotype with perianal penetrating Crohn's disease but not with susceptibility to IBD. Gastroenterology *129*, 1845-1853.

Waller, S., Tremelling, M., Bredin, F., Godfrey, L., Howson, J., and Parkes, M. Evidence for association of OCTN genes and IBD5 with ulcerative colitis. (2006). Gut *55*, 809-14.

Zhang, H.M., Rao, J.N., Guo, X., Liu, L., Zou, T., Turner, D.J., and Wang, J.Y. (2004). Akt kinase activation blocks apoptosis in intestinal epithelial cells by inhibiting caspase-3 after polyamine depletion. J. Biol. Chem. *279*, 22539-22547.

Figure legends

Figure 1. Bioactive agents in conditioned media from representative Gram-positive bacteria, including the quorum-sensing molecule CSF of *Bacillus subtilis*, induce heat shock protein 27 (Hsp27) in colonic Caco2$_{bbe}$ cells. A) Conditioned media from most Gram-positive (left 5 bars), but not Gram-negative (right 3 bars) bacterial species significantly induced Hsp27 protein expression in Caco2$_{bbe}$ cells. Densitometry results are expressed as a percent of the Hsp27 response induced by heat shock in paired control cells. B) Secreted factors in *B. subtilis* (JH 642, wild type) conditioned media induce Hsp27. Densitometry mean $\pm$ SEM of Western blots are normalized to unstimulated control values (arbitrarily set at 100 units). No changes in the protein expression of the heat shock cognate, Hsc70, were observed in any of the experimental conditions, including heat shock (HS). C) Bioactive factors from *B. subtilis* (JH 642, wild type) that induce Hsp27 (shown by Western blot) are less than 3kD (top set of panels), heat-stable, and pepsin-sensitive (lower set of panels). Filtrate and retentate were prepared by passing CM through a 3 kD Centricon filter. D) CM from wild type *B. subtilis* JH642, but not CSF-deficient Rsm121 (delta CSF), induces Hsp27 (Western blot). E) CSF (100 nM) also stimulates phosphorylation of Akt and p38 MAPK (shown in Western blots), two additional survival pathways of intestinal epithelial cells. Responses to heat shock (HS) and TNF-alpha (100 ng/ml) stimulation are shown as positive controls. Heat shock cognate, Hsc70, was used as a loading and experimental control. "Con." indicates control cells that were not treated with peptides. Heat shock (HS) control samples are shown, obtained from cells two hrs after transient exposure to 41.5^{o}C x 23 mins. * $p < 0.05$ compared with control at the same time point by analysis of variance.

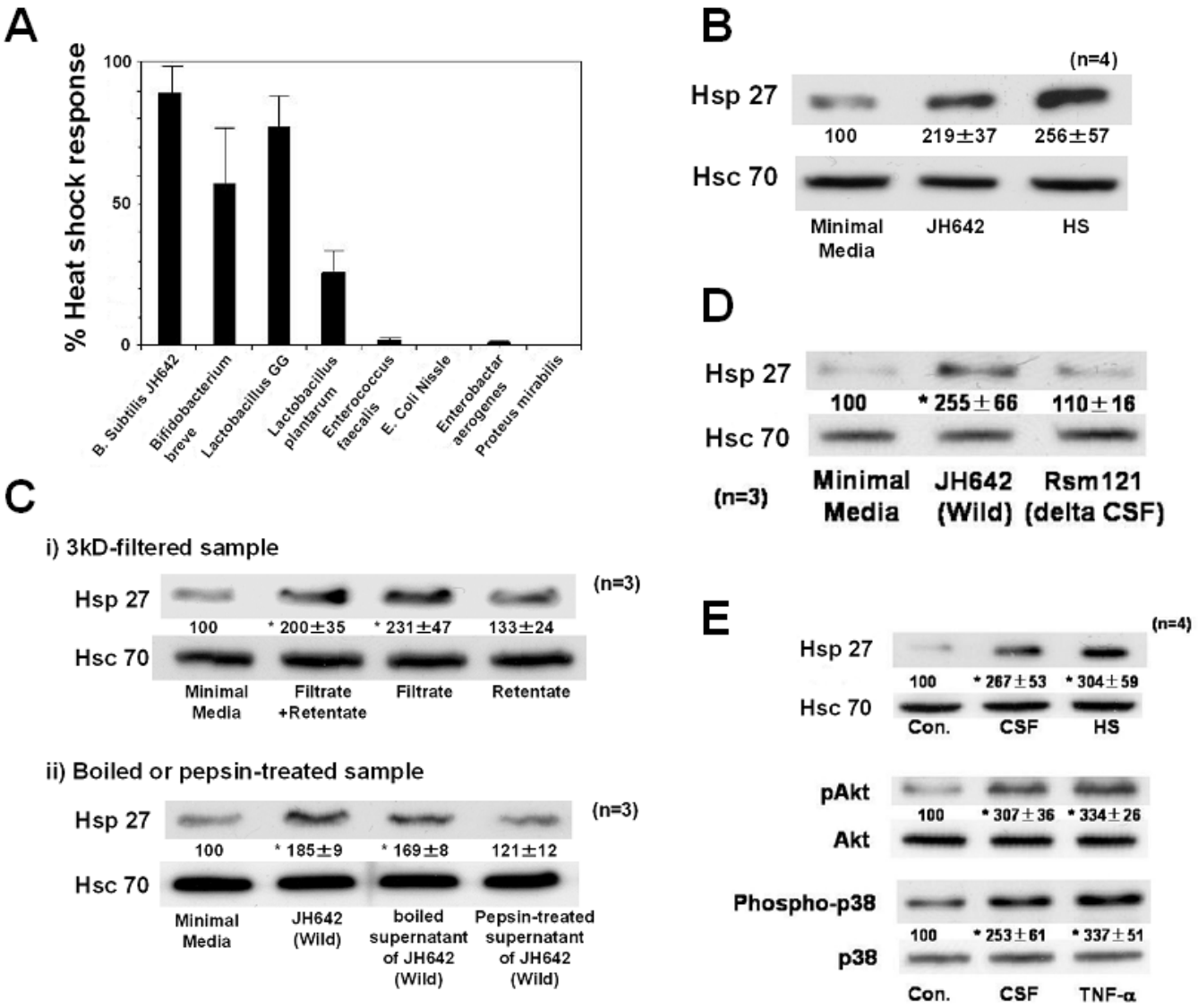
Figure 2. A) Caco2$_{bbe}$ uptake of ^{14}C-labeled CSF (competence and sporulation factor) is enhanced in OCTN2-overexpressed cells and less in OCTN2-siRNA treated cells (left panel). *$p<0.05$ compared to mock transfected control cells. B) Caco2$_{bbe}$ cells take up FITC-labeled CSF which distributes in the cytoplasm (30 min incubation), an effect competed by 10 mM L-carnitine (lower panels). C) CSF-induced Hsp27 (shown by Western blot) is blocked in cells treated with OCTN2-siRNA (without effect on Hsc70 or heat shock (HS) response). Control (Con) cells were not stimulated with CSF. HS, samples obtained from cells two hrs

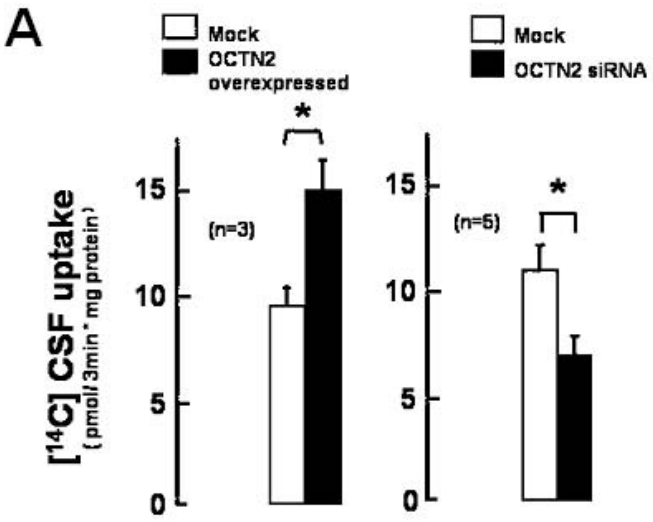
after transient exposure to 41.5°C x 23 mins. D) Effects of CM of Gram-positive (left-most 5 bars) and Gram-negative bacteria (3 right bars) on OCTN2 transport, assessed by competition of [^{3}H]-L-carnitine uptake by confluent human colonic epithelial $Caco2_{bbe}$ monolayers. L-carnitine uptake is used to functionally characterize OCTN2 transport activity (Tamai et al., 2000 and Ohashi et al., 2001). Values represent the amount of [^{3}H]-L-carnitine uptake reduced by bacterial CM. * $p < 0.05$ compared with control at the same time point by analysis of variance.

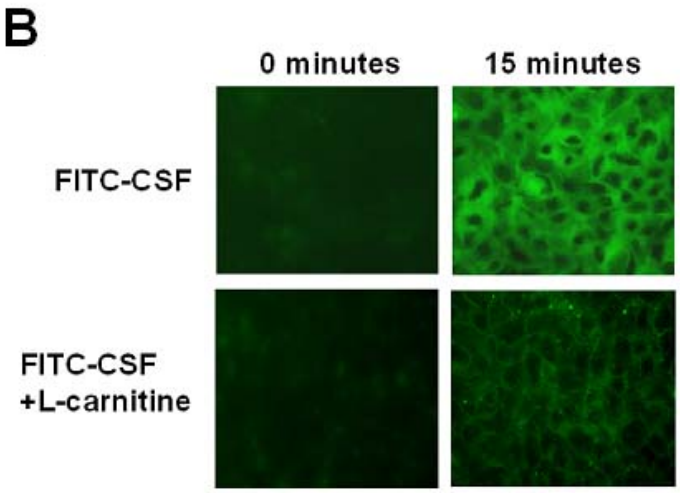
Figure 3. Condition media (CM) of *B. subtilis* and quorum-sensing CSF protect intestinal epithelial cells against oxidant-induced cell death and loss of barrier function. A) Pretreatment of $Caco2_{bbe}$ monolayers with wild type JH642 CM, but not with Rsm121 CM, protected cells against oxidant-induced injury (monochloramine, 0.3 mM). An unrelated quorum-sensing molecule from Enterococcus (cPD1) and scrambled pentapeptide of CSF (EMTRG) had no effects. HBS: HEPES buffered saline. B) Silencing of OCTN2 with siRNA in Caco2 cells inhibits the protective effects of CSF (third set from left) against oxidant-induced stress, as measured by ^{51}Cr release. Clear bars indicate control cells (no CSF treatment), whereas solid bars indicates CSF-treated cells. Cells treated with a non-sense siRNA still showed protection with CSF (second set from left), indicating specificity of siRNA treatment. In the right panel, inhibitors of the Akt (Ly294002) and p38 MAPK (SB203580) pathways, either alone or together, did not significantly inhibit the CSF protective action against oxidant stress. C) When these agents were tested to determine their ability to limit oxidant (monochloramine)-induced increases in barrier function, only CSF and CM of *B. subtilis* mitigated induced increases in ^{3}H-mannitol flux, a measure of barrier function. $^{++}p<0.05$ by ANOVA compared with untreated HBS^+ control (n=5 for all). D, E) Silencing of Hsp27 resulted in nearly complete reversal of the CSF- and BS-CM-induced protection of cell viability (Figure 3C) and epithelial barrier function (Figure 3D) against oxidant-induced stress. siRNA to murine Hsp25 (mHsp25) having nucleotide sequence unrelated to hHsp27 had no effects, indicating the specificity of Hsp27 silencing. * $p \leq 0.01$ compared with corresponding responses in absence of CSF or BS-CM, n=4.

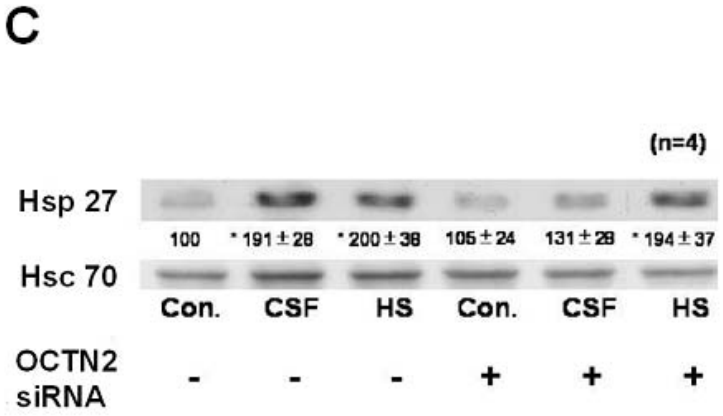
Figure 4. CSF induces Hsp25 and Hsp70 expression and inhibits oxidant-induced alterations in *ex vivo* intestinal preparations, effects that are blocked by the presence of L-carnitine. A) By Western

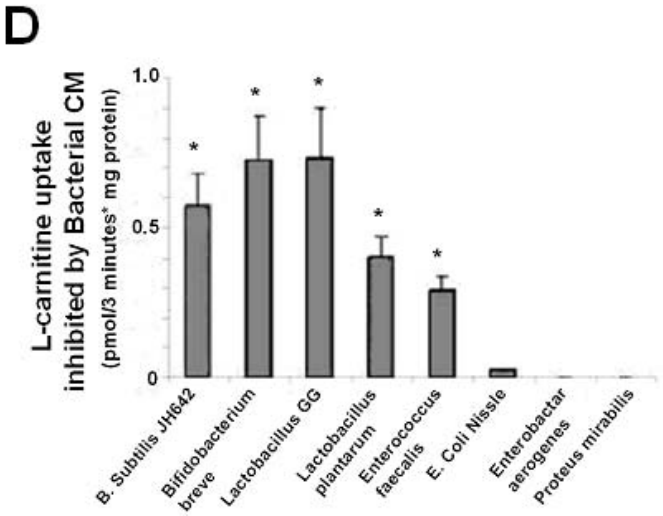
blot, CSF (100nM), but not the scrambled peptide (EMTRG), induces both Hsp25 and Hsp70 in mucosa of *ex vivo* ligated loops of murine small and large intestine (colon, left), B) By Western blot, the induction of Hsp25 and Hsp70 by CSF is inhibited by L-carnitine in the small intestine (10mM, right). "Con." and "Scr" indicate tissues that were not treated and were treated with the scrambled pentapeptide EMTRG, respectively. C) CSF, but not the scrambled peptide protects intestinal barrier function against oxidant-induced stress (NH_2Cl, 0.3 mM). D) CSF-protection of oxidant-induced loss of barrier function (indicated by high mannitol flux) is reversed in the presence of L-carnitine, indicating an effect requiring OCTN2 transport of CSF. No treatment (clear bars) indicates oxidant effects in absence of either CSF or scrambled peptide. Permeability was assessed by passive ^{3}H-mannitol flux, calculated by subtracting the value of NH_2Cl–free- from that of NH_2Cl-treated samples. * $p \leq 0.01$ compared with no treatment, n=3.

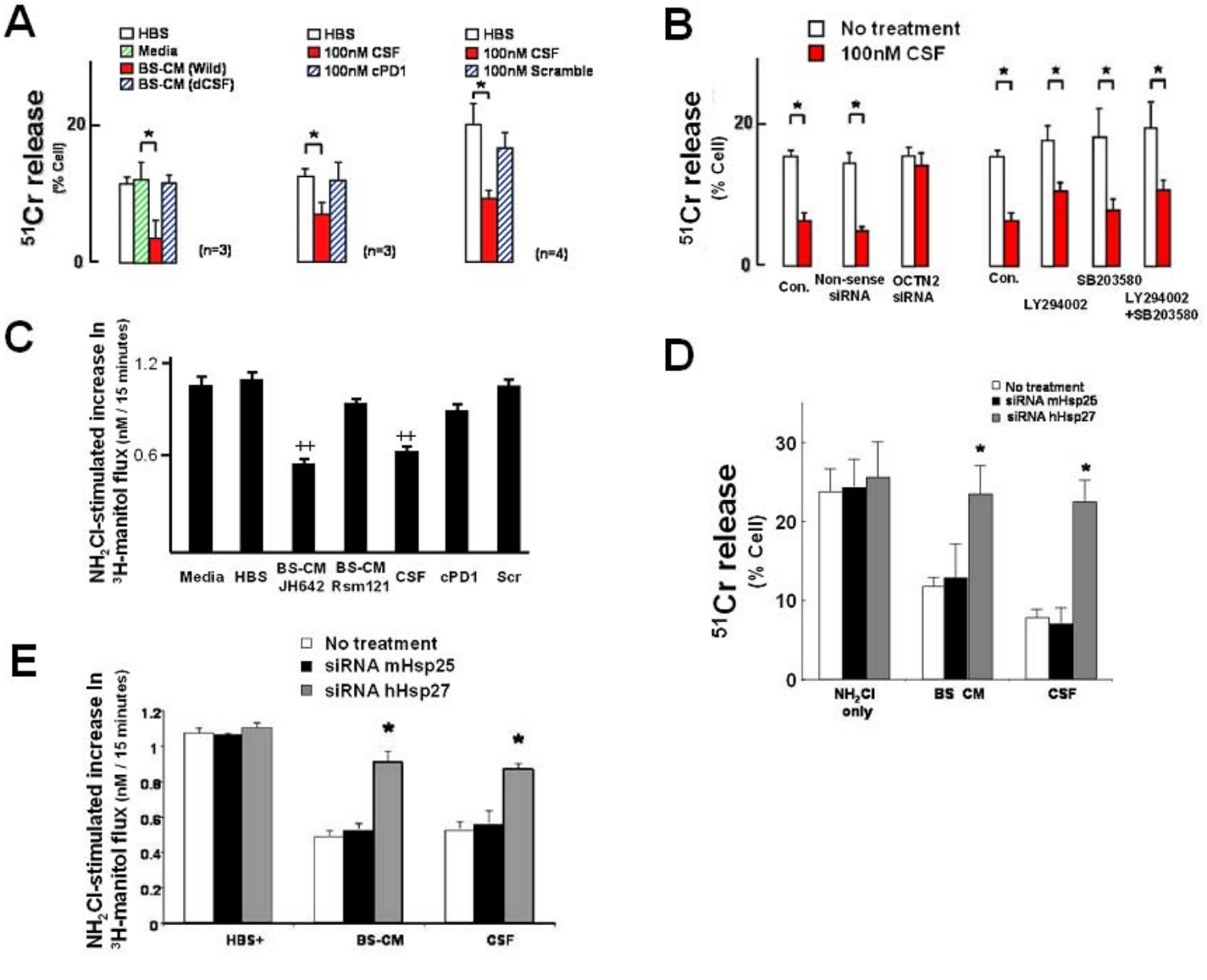

A
% Heat shock response
100
50
0
B. Subtilis JH642
Bifidobacterium breve
Lactobacillus GG
Lactobacillus plantarum
Enterococcus faecalis
E. Coli Nissle
Enterobactar aerogenes
Proteus mirabilis
B
(n=4)
Hsp 27
100
219±37
256±57
Hsc 70
Minimal Media
JH642
HS
D
Hsp 27
100
* 255±66
110±16
Hsc 70
(n=3)
Minimal Media
JH642 (Wild)
Rsm121 (delta CSF)
C
i) 3kD-filtered sample
Hsp 27
(n=3)
100
* 200±35
* 231±47
133±24
Hsc 70
Minimal Media
Filtrate +Retentate
Filtrate
Retentate
ii) Boiled or pepsin-treated sample
Hsp 27
(n=3)
100
* 185±9
* 169±8
121±12
Hsc 70
Minimal Media
JH642 (Wild)
boiled supernatant of JH642 (Wild)
Pepsin-treated supernatant of JH642 (Wild)
E
Hsp 27
(n=4)
100
* 267±53
* 304±59
Hsc 70
Con.
CSF
HS
pAkt
100
* 307±36
* 334±26
Akt
Phospho-p38
100
* 253±61
* 337±51
p38
Con.
CSF
TNF-α

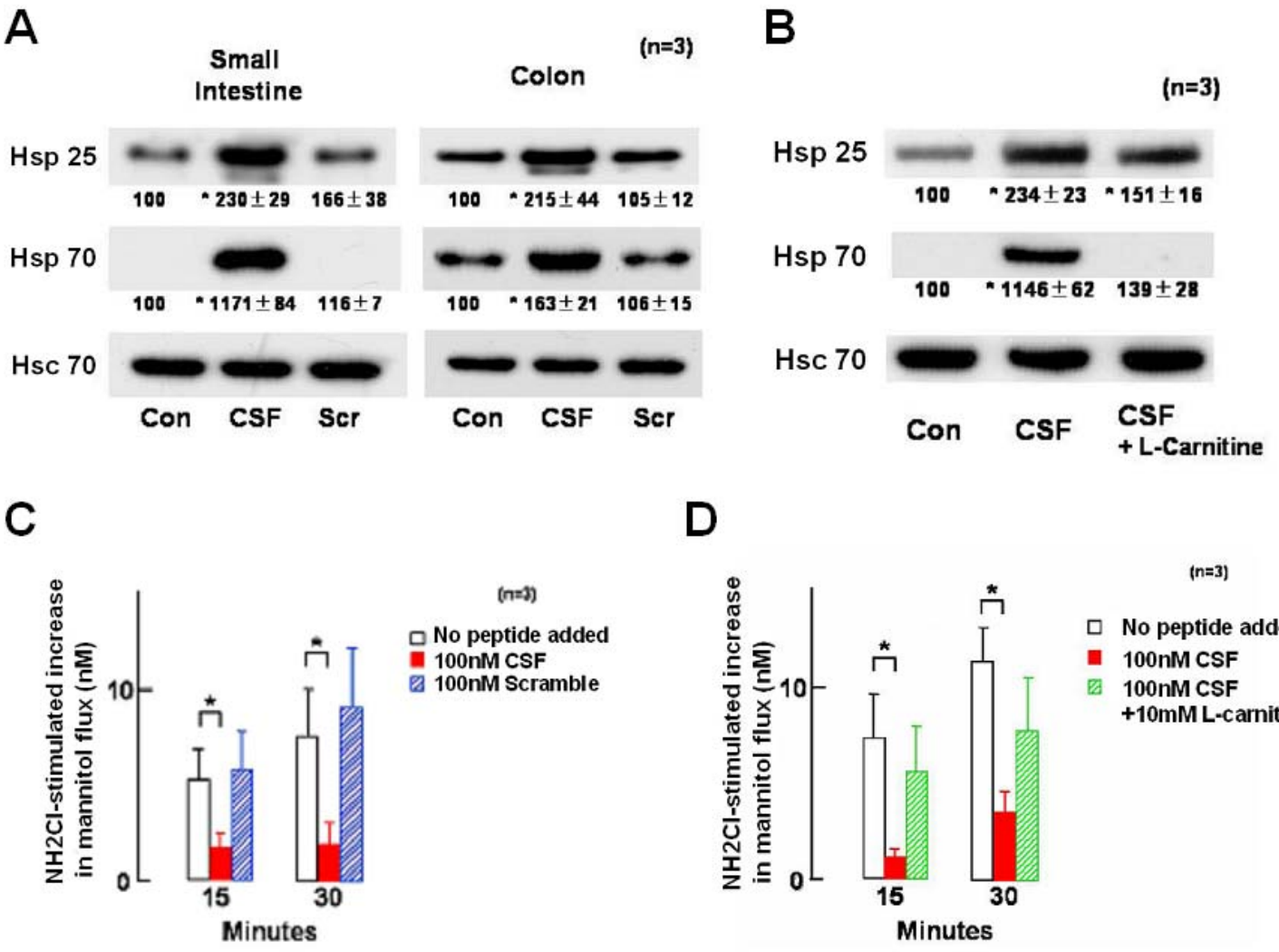

A
Mock
OCTN2 overexpressed
Mock
OCTN2 siRNA
[14C] CSF uptake
(pmol/ 3min * mg protein)
15
10
5
0
(n=3)
(n=5)
*
*


B
0 minutes
15 minutes
FITC-CSF
FITC-CSF
+L-carnitine


C
(n=4)
Hsp 27
Hsc 70
100
* 191 ± 28
* 200 ± 38
105 ± 24
131 ± 28
* 194 ± 37
Con.
CSF
HS
Con.
CSF
HS
OCTN2 siRNA
-
-
-
+
+
+


D
L-carnitine uptake inhibited by Bacterial CM
(pmol/3 minutes* mg protein)
1.0
0.5
0
*
*
*
*
*
B. Subtilis JH642
Bifidobacterium breve
Lactobacillus GG
Lactobacillus plantarum
Enterococcus faecalis
E. Coli Nissle
Enterobactar aerogenes
Proteus mirabilis


A
HBS
Media
BS-CM (Wild)
BS-CM (dCSF)
HBS
100nM CSF
100nM cPD1
HBS
100nM CSF
100nM Scramble
51Cr release
(% Cell)
20
0
*
(n=3)
(n=3)
(n=4)
B
No treatment
100nM CSF
Con.
Non-sense siRNA
OCTN2 siRNA
Con.
SB203580
LY294002
LY294002 +SB203580
C
NH2Cl-stimulated increase in 3H-manitol flux (nM / 15 minutes)
1.2
0.6
++
Media
HBS
BS-CM JH642
BS-CM Rsm121
CSF
cPD1
Scr
D
No treatment
siRNA mHsp25
siRNA hHsp27
30
20
10
0
NH2Cl only
BS CM
CSF
E
No treatment
siRNA mHsp25
siRNA hHsp27
1.2
1
0.8
0.6
0.4
0.2
0
HBS+
BS-CM
CSF


A
Small Intestine
Colon
(n=3)
Hsp 25
100
* 230±29
166±38
100
* 215±44
105±12
Hsp 70
100
* 1171±84
116±7
100
* 163±21
106±15
Hsc 70
Con
CSF
Scr
Con
CSF
Scr
B
(n=3)
Hsp 25
100
* 234±23
* 151±16
Hsp 70
100
* 1146±62
139±28
Hsc 70
Con
CSF
CSF + L-Carnitine
C
NH2Cl-stimulated increase in mannitol flux (nM)
10
0
*
*
15
30
Minutes
(n=3)
No peptide added
100nM CSF
100nM Scramble
D
NH2Cl-stimulated increase in mannitol flux (nM)
10
0
*
*
15
30
Minutes
(n=3)
No peptide added
100nM CSF
100nM CSF +10mM L-carnitine

Supplemental Methods

Human colon tissues. Tissue harvests were approved by the University of Chicago Institutional Review Board and informed consent was obtained from all subjects. Colonic biopsy specimens provided by 3 healthy volunteers were used for immunohistochemistry. For localization of OCTN2 and Hsp27 expression, formalin-fixed specimens of intact small and large intestine were analyzed by immunohistochemistry using the Vector ABC kit per manufacturer's directions.

Real-time PCR. RNA was isolated from differentiated $Caco2_{bbe}$ cells using Trizol reagent (Invitrogen, Carlsbad, CA) according to the manufacturer's instructions. Two µg of RNA were reverse-transcribed using random primers and Superscript II RT (Invitrogen). Three µl of 1:10 reverse transcription reaction were analyzed using ™ iCycler iQ Multicolor Real-Time PCR Detection System (Bio-Rad., Hercules, CA) in triplicate. The averaged OCTN1, 2 or hPepT1 mRNA expression levels were normalized to GAPDH expression and calculated using the comparative threshold cycle method.

Supplemental data

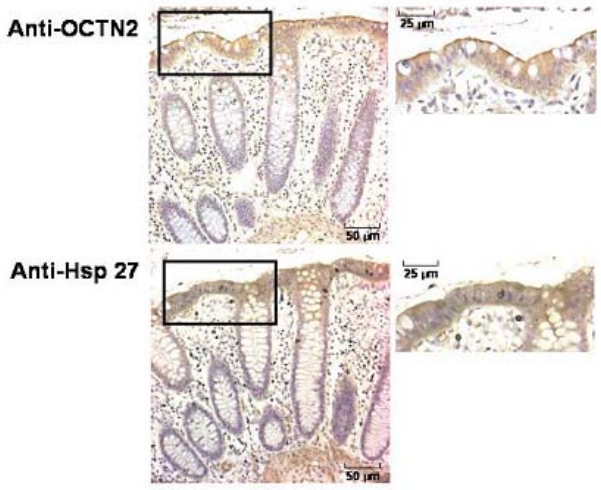


Figure S1: OCTN2 and Hsp27 are co-expressed in colonic epithelia. OCTN2 is primarily expressed by surface epithelial cells of the colon (top panel, brown staining) that are in direct contact with the luminal contents and microbes and which exhibit sustained expression of microbial-induced heat shock proteins, Hsp27 (shown, bottom panel) and Hsp70. Hsp27 is the human homolog of murine Hsp25. Higher power magnifications are shown in the insets.

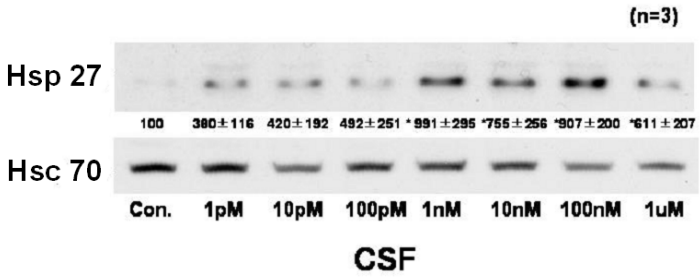


Figure S2: Competence and sporulation factor (CSF) of B. subtilis induces a dose-related increase in Hsp27 expression. CSF induces Hsp 27 in a concentration-dependent manner within the physiological range for CSF (10-100 nM). The bands shown are from Western blots.

Figure S3

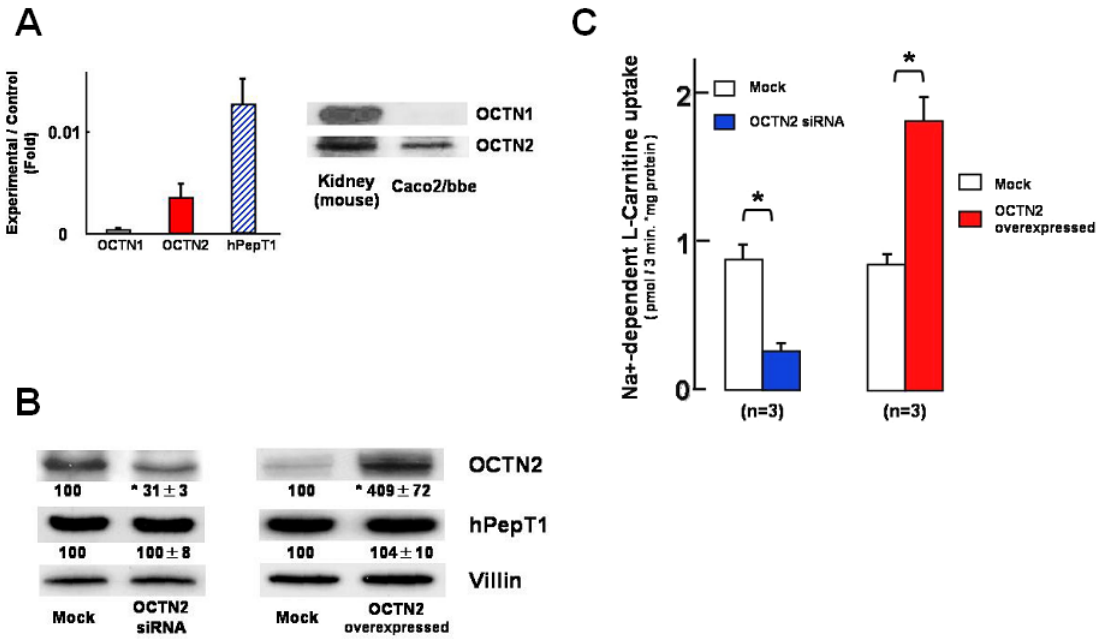


Figure S3: Substances secreted by *B. subtilis* are selectively taken up by OCTN2-expressing human colonic Caco2$_{bbe}$ cells. Validation of OCTN2 expression and activity in OCTN2-siRNA and –transfected cells. A) Differentiated Caco2$_{bbe}$ cells express OCTN2 protein and mRNA, but minimal OCTN1 protein or mRNA. The averaged mRNA abundance of OCTN1, OCTN2 and hPepT1 were determined by real time PCR using the comparative threshold cycle method normalized to GAPDH expression. Rabbit polyclonal anti-mouse OCTN1 or OCTN2 antisera (Alpha Diagnostic International, San Antonio, TX) were used as primary antibodies for Western blotting (right panel). B) Western bllots showing specificity and effectiveness of OCTN2 silencing and overexpression in Caco2$_{bbe}$ colon cells. Villin and hPepT1 protein expression were not affected in either case. C) Silencing and overexpression of OCTN2 in Caco$_{2bbe}$ cells results in inhibition and augmentation of endogenous OCTN2 activity (Na-dependent L-carnitine uptake), respectively. *$p<0.05$ compared to mock transfected cells (n=3).

Figure S4

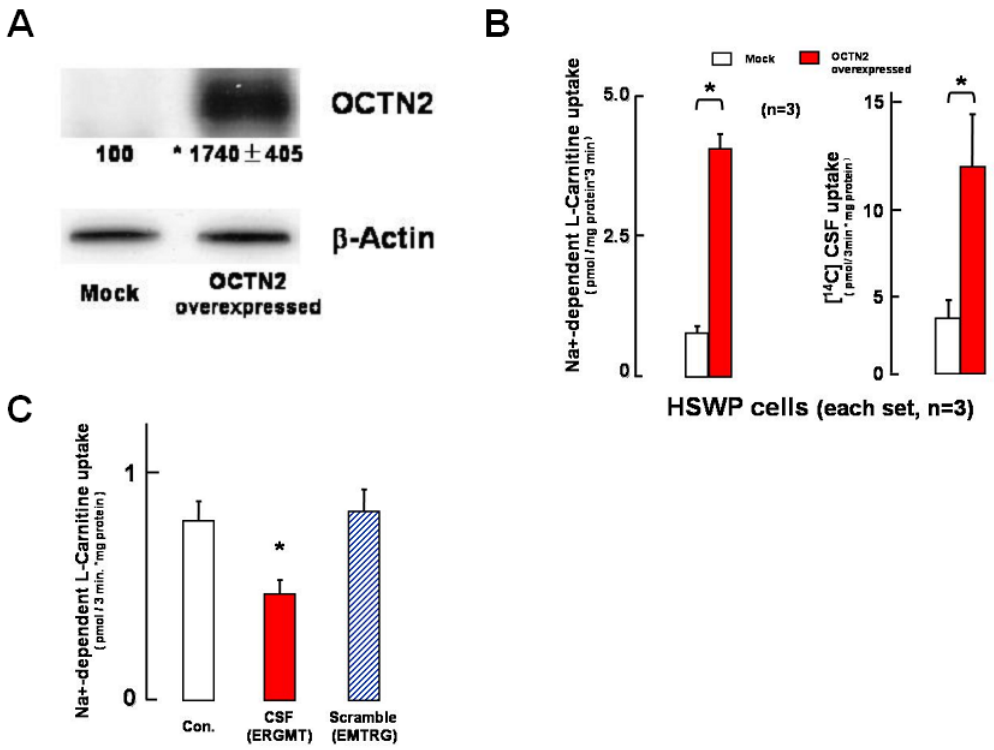


Figure S4: Quorum-sensing CSF competes with L-carnitine uptake by OCTN2 overexpressing Caco2$_{bbe}$ cells. A) OCTN2 protein expression is increased in HSWP cells transfected with CMV-driven OCTN2 cDNA. Note the minimal basal expression of OCTN2 in these cells (mock). The bands shown are from Western blots. B) Na-dependent L-carnitine uptake is significantly increased in OCTN2-transfected (overexpressing) human HSWP cells. In addition, ^{14}C-CSF uptake by OCTN2-overexpressing HSWP cells is also observed. *$p<0.05$ compared to mock transfected cells (n=3). C) The scrambled pentapeptide (EMTRG) does not affect Na^+-dependent L-carnitine uptake in Caco2$_{bbe}$ cells, while CSF inhibits the uptake. [^{3}H] L-carnitine uptake was measured over 3 min in 10-14 day post-confluent monolayers in flux buffer containing CSF or scrambled pentapeptide.

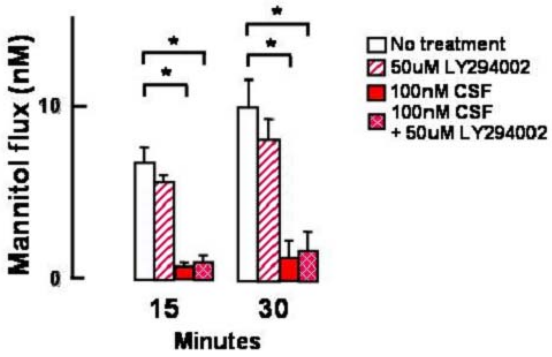


Figure S5: OCTN2 is required for CSF-induced protection of Caco2 cells against oxidant stress. Although p38 MAPK and Akt pathways are activated by CSF, they appear to play only a minor role in protection against oxidant stress. LY294002 did not effect CSF protection against oxidant-induced increases in mucosal permeability (assessed by ^{3}H-mannitol flux) in *ex vivo* small intestinal loops. Additionally, no inhibition of CSF action by SB203580 was observed (data not shown). *$p<0.05$ by ANOVA compared to no treatment control (n=5).

Summary

Bacteria use quorum sensing molecules (QSMs) to communicate within as well as across species, however, the effects of QSM on eukaryotic host cells are less understood. We report that the quorum-sensing pentapeptide, competence and sporulation factor (CSF), of a Gram-positive bacterium *Bacillus subtilis,* activates key survival pathways including p38 MAP kinase and protein kinase B (Akt) in intestinal epithelial cells and also induces cytoprotective heat shock proteins (Hsps), the latter which prevent oxidant-induced intestinal epithelial cell injury and loss of barrier function. These effects of CSF depend on its uptake by an apical membrane organic cation transporter-2 (OCTN2). OCTN2-transported CSF therefore serves as an example of a unique mechanism of host-bacterial interactions allowing the host to monitor changes in colonic flora behavior or composition.