**Lactobacillus** S-layer protein inhibition of *Salmonella*-induced reorganization of the cytoskeleton and activation of MAPK signalling pathways in Caco-2 cells

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Surface layer (S-layer) proteins are crystalline arrays of proteinaceous subunits that are present as the outermost component of the cell wall in several *Lactobacillus* species. The S-layer proteins have been shown to play a role in the antimicrobial activity of certain lactobacilli. However, it is not fully understood how the S-layer proteins exert this biological function. The aim of this study was to test the hypothesis that *Lactobacillus acidophilus* S-layer proteins antagonize *Salmonella Typhimurium* (S. Typhimurium) infection by protecting against F-actin cytoskeleton rearrangements and the activation of mitogen-activated protein kinase (MAPK) signalling pathways. Monolayer transepithelial electrical resistance (TER) was measured after *S. Typhimurium* infection in Caco-2 cultured human intestinal cells with *L. acidophilus* S-layer proteins. F-actin rearrangement and MAPK activation were also assessed by immunofluorescence staining or Western blotting. The results showed that when *S. Typhimurium* was co-incubated with S-layer proteins, the *S. Typhimurium*-induced Caco-2 cell F-actin rearrangement was reduced, and the *S. Typhimurium*-induced TER decrease and interleukin 8 (IL-8) secretion were attenuated. Additionally, *L. acidophilus* S-layer proteins could inhibit *S. Typhimurium*-induced phosphorylation of extracellular signal-regulated kinases 1 and 2 (ERK1/2), c-Jun amino-terminal kinase (JNK) and p38. This study indicates that *L. acidophilus* S-layer proteins are able to inhibit *S. Typhimurium* infection through blocking *S. Typhimurium*-induced F-actin rearrangements and *S. Typhimurium*-induced ERK1/2, JNK and p38 activation in Caco-2 cells. These data provide a rationale for the use of lactobacillus S-layer proteins as therapeutic and preventative agents, at least in infectious diarrhoea.

**INTRODUCTION**

*Salmonella Typhimurium* (S. Typhimurium) is a facultative intracellular pathogen that causes enterocolitis in animals and humans (Gordon *et al.* 2008; Helms *et al.*, 2005; Rugbjerg *et al.*, 2004). *S. Typhimurium* intimately adheres to, invades and interacts with host epithelial cells to cause cytoskeletal rearrangements (mostly of actin filaments), a rapid decrease in transepithelial electrical resistance (TER) and the production of several proinflammatory mediators, including the chemokine interleukin 8 (IL-8), by activating host cell signal transduction pathways (Galañ & Bliska, 1996). A key element in the signalling pathways involved in transducing *S. Typhimurium*-initiated signals to cellular responses is the mitogen-activated protein kinase (MAPK) signalling pathway (Hobbie *et al.*, 1997). The MAPK signal transduction pathway contains the extracellular signal-regulated kinases (ERKs) 1 and 2 (ERK1/2), c-Jun amino-terminal kinase (JNK) and p38 kinase, which are the most extensively studied group and are closely related to threats from pathogenic bacterial (Chen *et al.*, 2001; Kyriakis & Avruch, 2001; Roux & Blenis, 2004; Weston & Davis, 2007). MAPK signalling pathways are important in defence against pathogens (Jones *et al.*, 2008), and may convey ‘alarm’ signals to the host cells (Roux & Blenis, 2004).

Lactobacilli have important antibacterial effects that prevent the adherence, establishment and invasion of numerous enteropathogens; however, to our knowledge the precise mechanisms involved remain unclear. Surface layer (S-layer) proteins are crystalline arrays of proteinaceous subunits present as the outermost component of the cell wall in several *Lactobacillus* species, although not all lactobacillus strains express S-layer proteins (Ávall-Jääskeläinen & Palva, 2001).
2005; Jakava-Viljanen & Palva, 2007). Recently, the S-layer proteins of some lactobacillus strains have been shown to be a key adhesion factor of the cell, and could play a role in probiotic activity (Wang et al., 2008). The S-layer proteins of species such as Lactobacillus helveticus, Lactobacillus crispatus and Lactobacillus kefir are involved in tissue adherence and are able to inhibit bacteria-mediated pathogenesis, for example that of enterohaemorrhagic Escherichia coli (Johnson-Henry et al., 2007) and Salmonella Enteritidis (Golowczyc et al., 2007).

During Salmonella infection, changes in the distribution of the cytoskeletal component and activation of MAPK signalling pathways have been reported (Bhavsar et al., 2007). It is not known whether an increase in mucosal integrity that inhibits Salmonella infection is induced in the presence of S-layer proteins by the stabilized actin filaments and the subsequent increased host barrier defence, or whether the S-layer proteins inhibit S. Typhimurium-induced MAPK activation. Therefore, the aim of this study was to examine changes in the cytoskeleton and MAPK signalling pathways in the Caco-2 cultured human intestinal cell line and to investigate the possible antimicrobial mechanisms of Lactobacillus acidophilus S-layer proteins.

**METHODS**

**Reagents and antibodies.** RIPA buffer [0.1 % (w/v) SDS, 1 % (v/v) Triton X-100, 1 % (w/v) sodium deoxycholate in Tris-buffered saline (TBS; 25 mM Tris/HCl, pH 7.5, 150 mM NaCl)], phosphatase and protease inhibitors (1 mM PMSF, 1 mM Na2VO4, 25 mM NaF) were purchased from the Beyotime Institute of Biotechnology (Jiangsu, China). Horseradish peroxidase (HRP)-conjugated goat anti-rabbit IgG and HRP-conjugated goat anti-mouse IgG were purchased from Bioworld Technology. Antibodies specific to phospho-p38 and p38, phospho-JNK1/2/3 (T183 + Y185) and JNK1/2/3 (T178) were purchased from Cell Signaling Technology. Monoclonal mouse anti-glyceraldehyde-3-phosphate dehydrogenase (GAPDH) antibody was purchased from Kangchen Biotechnology (Shanghai, China). Rabbit antiserum specific to S-layer proteins was obtained in-house. Phalloidin–FITC and all other reagents were from Sigma, unless indicated otherwise.

**Bacterial strains.** S. Typhimurium strain SL1344 was a generous gift from Professor Shulin Liu (Peking University, Beijing, China). L. acidophilus strain ATCC4356 was purchased from the China Committee for Culture Collection of Microorganisms (Beijing, China). For Caco-2 cell infection, S. Typhimurium was routinely grown in Luria–Bertani (LB) medium overnight. The bacteria were collected by centrifugation (4000 g for 5 min) and resuspended in tissue culture medium without antibiotics. L. acidophilus ATCC4356 was grown in De Man–Rogosa–Sharpe (MRS) static cultures with minimal aeration at 37 °C for 24 h and harvested by centrifugation (4000 g for 5 min at room temperature). After washing twice with PBS, cell pellets were resuspended in antibiotic-free culture medium for storage.

**Cell culture.** Caco-2 human colon cancer epithelial cells were obtained from the American Type Culture Collection (ATCC; Rockville, MD, USA). Caco-2 cells (passages 29–45) were cultured in Dulbecco's modified Eagle’s medium (DMEM) supplemented with 100 mg penicillin G, 100 mg phytofmcytin (v/v), 10 % (v/v) heat-inactivated fetal calf serum (FCS), 4.5 g d-glucose g⁻¹, 25 mM HEPES, 1 % (v/v) non-essential amino acids and 2 mM l-glutamine (Gibco).

**S-layer protein.** S-layer proteins were obtained from L. acidophilus ATCC4356, as reported previously (Li et al., 2010). Briefly, S-layer proteins were extracted from L. acidophilus ATCC4356 using 4.0 M guanidine hydrochloride (Boot et al., 1993) and then purified by anion-exchange column chromatography (DE52, Whatman).

**Infection of Caco-2 cells by S. Typhimurium.** Our previous study demonstrated that the S-layer protein-induced inhibitory effects against S. Typhimurium invasion are dose-dependent. In the present study, 100 μg S-layer proteins ml⁻¹ were added in the following assays (Li et al., 2010). Caco-2 cells were seeded into 24- or six-well plates and grown to confluence for 2 weeks prior to the assay. Tissue culture medium was replaced with antibiotic-free medium 1 day before bacterial infection. L. acidophilus S-layer proteins (100 μg ml⁻¹) and S. Typhimurium SL1344 (bacteria : epithelial cells m.o.i. of 20 : 1) were added to the Caco-2 cell monolayers and co-incubated for 2 h at 37 °C. All experiments were performed in triplicate on three consecutive cell passages.

**Scanning electron microscopy (SEM).** To confirm the inhibitory effect of S-layer protein on Salmonella association, infected Caco-2 monolayers were examined by SEM. Caco-2 cells were differentiated on glass coverslips, which were placed in 24-well tissue culture plates for 2 weeks with changes of medium every 3 days. The differentiated Caco-2 cells were then incubated in DMEM/10 % FCS, infected with S. Typhimurium strains (m.o.i. 20 : 1, opsonized for 2 h at 37 °C, 5 % CO₂), and washed three times with PBS. The cells were then fixed in glutaraldehyde, post-fixed in OsO₄ for 1 h, dehydrated with a graded series of ethanol, and freeze-dried by t-butyl alcohol substitution. The dried samples were examined under a Hitachi S-3000N scanning electron microscope.

**Fluorescently-labelled actin staining.** Phalloidin–FITC staining was performed as described elsewhere (Cocconier et al., 2000). Caco-2 cells were differentiated on glass coverslips that were placed in 24-well tissue culture plates until each well was 50–75 % confluent. S-layer proteins (100 μg ml⁻¹) and S. Typhimurium (m.o.i. 20 : 1) were added to Caco-2 cells and co-incubated for 2 h (37 °C, 5 % CO₂). Next, cell preparations were fixed for 10 min at room temperature in PBS/3.7 % paraformaldehyde. The fixed cells were permeabilized with 0.1 % (v/v) Triton X-100 in PBS for 5 min before incubation with phalloidin–FITC (50 μg ml⁻¹) in PBS for 40 min, and then the coverslips were washed several times with PBS to remove any trace of non-specific fluorescence. Finally, the cells were examined under a fluorescence microscope (Axiovert, Zeiss).

**Measurement of TER and IL-8 assay.** The assay was performed as described by Huang et al. (2004). Caco-2 cells were seeded on collagen-coated 12 mm, 0.4 μm pore-size Millicell culture plate inserts (Millipore). The cells were grown until a TER value of at least 600 Ω cm⁻² was achieved. Prior to bacterial infection, the cells were washed and placed in antibiotic-free medium. Infection was started by the addition of Salmonella suspension (m.o.i. 20 : 1) to the upper chamber of the Millicell and was allowed to proceed for 1 h (37 °C, 5 % CO₂); cells were co-incubated simultaneously with L. acidophilus S-layer proteins. The medium was aspirated from both the upper and the lower chamber at the end of the infection period. The cell monolayer was washed twice with sterile PBS, and medium containing gentamicin (40 μg ml⁻¹) was added to both chambers. Gentamicin is very poorly internalized in mammalian cells, killing only the extracellular bacteria and leaving the intracellular bacteria protected from the gentamicin (Veiga et al., 2007). After incubation for 3 h (37 °C, 5 % CO₂), the basolateral medium was collected and used for IL-8 assay. The IL-8 concentration in the basolateral medium
was determined using a human IL-8 ELISA kit (Boster, Wuhan, China). Meanwhile, Caco-2 cell monolayers were used in the TER assay. The changes in the integrity of the cell monolayer were verified at 0, 1, 2, 3, 4, 6 and 10 h by measuring the TER using a Millipore ERS-2 Volt-Ohm meter (Millipore). All infections were carried out in triplicate.

**Extraction of host cell proteins.** In this assay, *L. acidophilus* ATCC4356 (m.o.i. 200:1) was added to Caco-2 cells as another control. Caco-2 cells were washed twice with ice-cold PBS (pH 7.2–7.4) and then lysed on ice for 30 min with RIPA buffer (with protease and phosphatase inhibitors added immediately before use to prevent proteolysis and maintain the phosphorylation status of proteins). Cell lysates were centrifuged (14,000 g for 5 min at 4 °C), and the supernatants were aliquoted and stored at −70 °C until use. Protein concentrations were determined using a bicinchoninic acid protein determination kit (Multisciences Biotech, Hangzhou, China).

**Western blot analysis.** Protein preparations from Caco-2 cells were separated by 4–12 % SDS-PAGE and transferred onto nitrocellulose (NC) membranes. Loaded NC membranes were blocked with 5 % BSA in TBST (10 mM Tris/HCl, pH 7.5, 150 mM NaCl, 0.1 % Tween 20). The membranes were incubated first with antibodies specific for phosphorylated forms of JNK1/2/3 and p38 overnight at 4 °C. After washing, the membranes were incubated with goat anti-rabbit (or anti-mouse) IgG–HRP (Bioworld) at room temperature for 1 h. Antibodies specific to total JNK1/2/3 and p38 were used to detect the expression of the respective proteins. Monoclonal mouse anti-GAPDH was used to monitor sample loading. The washing procedure was repeated, and specific signals were visualized with SuperSignal West Pico chemiluminescent substrate (Pierce Biotechnology) according to the manufacturer’s instructions, and recorded on film or with a Kodak Image Station 2000MM Multi-Modal Imager (Eastman Kodak). The membranes were then stripped and probed again with antibodies to total JNK1/2/3, or total p38 kinase, as appropriate. Autoradiograms were scanned and analysed using Quantity One software (Bio-Rad) to quantify the band densities. The mean density of a phosphorylated MAPK band was divided by the mean density of the corresponding total MAPK band to yield a normalized band density value.

**Data analysis.** Results are expressed as mean ± SEM. Analysis of variance (ANOVA) and unpaired Student’s *t* tests were employed to determine statistical differences among multiple groups.

**RESULTS**

**Inhibition by S-layer proteins of *S. Typhimurium* adherence to Caco-2 cells**

Our previous study demonstrated that when *S. Typhimurium*-infected Caco-2 monolayers are treated with *L. acidophilus* S-layer proteins, the adhesion and invasion of *S. Typhimurium* decrease significantly as compared with untreated cells (Li et al., 2010). This reduction in bacterial adherence and invasion is not due to bactericidal effects of S-layer proteins on *S. Typhimurium* (Supplementary Fig. S1, Supplementary Methods). In the current study, S-layer proteins were examined by SEM for their ability to inhibit the adhesion of *S. Typhimurium* to Caco-2 cells. At 2 h post-infection, many *S. Typhimurium* cells had adhered to the Caco-2 cells (Fig. 1b). Although complete protection was not achieved when Caco-2 cells were treated with *S. Typhimurium* and S-layer proteins simultaneously, visually, the number of adhered *S. Typhimurium* appeared to be diminished (Fig. 1d). No adhered *S. Typhimurium* was observed in the Caco-2 cells treated with S-layer proteins alone (Fig. 1c) or in the control cells (Fig. 1a).

**Activity of S-layer proteins against *S. Typhimurium*-induced F-actin rearrangements and disruption of barrier integrity in Caco-2 cells**

It is well known that in epithelial cells, invading *S. Typhimurium* is surrounded by a large extension of the host cell membrane, correlated with the size and extent of an F-actin-dense region (Ly & Casanova, 2007; Patel & Galán, 2005). In this experiment, the alterations in F-actin were examined when Caco-2 cells were treated with S-layer proteins and *S. Typhimurium* either alone or simultaneously (Fig. 2). In the uninfected cells, the fine, long and uniform distribution of thick fibres located centrally in the cells represented F-actin (Fig. 2a). When treated with *S. Typhimurium* alone, F-actin was redistributed and characterized by the disappearance of the fine, thick fibres located centrally in the cells and the appearance of intense localized accumulations (Fig. 2c). In contrast, S-layer proteins did not modify the distribution of F-actin in Caco-2 cells (Fig. 2b). Furthermore, when the cells were treated with *S. Typhimurium* and S-layer proteins simultaneously, the *S. Typhimurium*-induced F-actin accumulation was weakened markedly at 2 h post-infection as compared with the cells treated with *S. Typhimurium* alone (Fig. 2d).

We also measured the TER, a sensitive measure of barrier function, in Caco-2 cells. Consistent with earlier findings (Tafazoli et al., 2003), we detected a significant decrease in TER at 1 h post-infection (Fig. 3). However, the S-layer proteins did not affect the TER in Caco-2 cells. Interestingly, when cells were co-cultured with S-layer proteins simultaneously, the *S. Typhimurium*-induced F-actin accumulation was weakened markedly at 2 h post-infection as compared with untreated cells (Fig. 3).
S-layer proteins inhibit the activation of MAPK signalling pathways in the host cell response to S. Typhimurium

Based on an earlier report (Huang et al., 2004) and our observations (Supplementary Fig. S2), the phosphorylation levels of ERK1/2, JNK and p38 in Caco-2 cells peaked at 1 h post S. Typhimurium infection, indicating that S. Typhimurium was able to induce the activation of the ERK1/2, JNK and p38 MAPK pathways. We have previously demonstrated that L. acidophilus S-layer proteins can inhibit S. Typhimurium-induced ERK1/2 activation (Li et al., 2011), and we found that L. acidophilus could mediate ERK1/2 activation (data not shown). Here we focused on the involvement of JNK and p38 signalling pathways in the antagonistic activity of S-layer proteins against S. Typhimurium in Caco-2 cells. S-layer proteins and L. acidophilus did not affect JNK and p38 activation. Interestingly, the S-layer proteins were able to significantly suppress S. Typhimurium-induced JNK activation (phosphorylated JNK/total JNK value of 0.64 ± 0.05 for cells treated with S-layer proteins versus 1.77 ± 0.19 for untreated cells; P<0.001; Fig. 5a, b) and p38 phosphorylation (phosphorylated JNK/total JNK value of 0.61 ± 0.04 for cells treated with S-layer proteins versus 0.93 ± 0.07 for untreated cells; P<0.05; Fig. 5c, d).

DISCUSSION

Previously, we have reported that L. acidophilus S-layer proteins can inhibit the adhesion to and invasion of Caco-2 cells by S. Typhimurium (Li et al., 2010). In the present study, the antagonistic activity of L. acidophilus S-layer proteins was examined by SEM to obtain a more detailed understanding of our previous findings. Caco-2 cells infected with S. Typhimurium showed high frequencies of bacterial adherence, whereas the number of adhered S. Typhimurium cells was much lower when S. Typhimurium was co-incubated with the S-layer proteins. In recent years, the antagonistic activity of lactobacillus S-layer proteins against pathogen infection has been studied extensively (Chen et al., 2007; Golowczyc et al., 2007; Johnson-Henry et al., 2007). However, the antimicrobial mechanisms of S-layer proteins are poorly understood. Some researchers think that there may be competitive binding of the S-layer proteins and pathogens to the mucin or cell receptor of the intestinal epithelium, which may block bacterial adherence and invasion (Vadillo-Rodrı´guez et al., 2005). The general mechanisms of the antagonistic activity therefore remain unclear.

One report indicates that the spent culture supernatant of L. acidophilus LB (LB-SCS) can decrease the number of apical S. Typhimurium-induced F-actin rearrangements in infected Caco-2 cells (Coconnier et al., 2000). S-layer proteins can be present in spent culture supernatants of Lactobacillus strains (Golowczyc et al., 2007). This suggests that S-layer proteins may play a key role in LB-SCS antimicrobial activity. In the present study, we demonstrated that S-layer proteins from L. acidophilus ATCC4356 are able to modify S. Typhimurium-induced F-actin rearrangements in infected Caco-2

Fig. 1. SEM analysis of Caco-2 cells infected with S. Typhimurium. (a) Intact monolayer of uninfected Caco-2 cells. (b) At 2 h post-infection, many bacterial cells (arrows) attached to the monolayer were detected. (c) Treatment of Caco-2 cells with S-layer protein alone. (d) Caco-2 cells infected with S. Typhimurium were co-incubated with S-layer proteins. Although complete protection was not achieved, the number of adhered S. Typhimurium appeared to be markedly diminished. Bars, 2 μm.
cells. This may result from the blocking by the S-layer proteins of the initial crosstalk between *Salmonella* effectors and Caco-2 cell F-actin. The F-actin cytoskeleton is essential for the formation and maintenance of intestinal cell barrier integrity (Banan *et al.*, 2000, 2007; Galán, 1996; Patel *et al.*, 2005). Therefore, these results correspond with the findings of the cell barrier integrity assay that S-layer proteins can modify the *Salmonella*-induced reduction in TER.

IL-8 has been found to contribute to *Salmonella* pathogenesis and to decrease epithelial barrier function (Eckmann *et al.*, 1993; Kucharzik & Williams, 2002). IL-8 released from the basolateral aspect of infected epithelial cells plays an important role in the initial movement of neutrophils from the circulation into the subepithelial region. However, several studies have demonstrated that persistent secretion of IL-8 leading to neutrophil infiltration often causes

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**Fig. 2.** Evolution of the *S. Typhimurium*-induced F-actin alteration in Caco-2 cells upon S-layer protein treatment. High-magnification micrographs reveal the localization of F-actin labelled by phalloidin–FITC. Magnification ×400. (a) Uninfected control cells. The fine fibriform actin located centrally in the cells represents F-actin (arrow). (b) Uninfected cells treated with S-layer proteins for 2 h showed no change in F-actin distribution (arrow). (c) Cells infected for 2 h with *S. Typhimurium*. The disappearance of F-actin and the localized dense spots of fluorescence represent F-actin accumulation (arrows) resulting from a *Salmonella*-induced lesion. (d) *S. Typhimurium*-induced localized dense F-actin accumulation in cells with S-layer protein treatment at 2 h post-infection. The dispersed localized *Salmonella*-induced F-actin accumulation changed in intensity (arrow). Bars, 20 μm.

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**Fig. 3.** Effect of co-incubation with S-layer proteins on the *S. Typhimurium*-induced reduction in the TER in Caco-2 cells. Caco-2 monolayers were infected with *S. Typhimurium* (■), co-incubated with S-layer proteins and the pathogen (▲), or treated with S-layer proteins (○) or medium alone (×), and TER values were determined at different time points. The results represent the mean and SEM of three experiments, each performed in triplicate.
chronic inflammation and may subsequently culminate in epithelial cell damage (Huang et al., 2004; Mumy & McCormick, 2009). In the present study, we found that infection of polarized Caco-2 cells by S. Typhimurium was followed by the release of IL-8. However, S-layer protein treatment reduced S. Typhimurium-induced IL-8 secretion. The results also correspond with the results of TER determination in Caco-2 cells. The reduced IL-8 levels in S. Typhimurium-infected Caco-2 cells in the presence of S-layer proteins could promote the recovery of cell barrier integrity. Previously, we have reported that under these conditions of infection, the S-layer protein treatment inhibits the adherence to and invasion of Caco-2 cells by S. Typhimurium (Li et al., 2010). The inhibition of bacterial adhesion may block adhesion-dependent, S. Typhimurium-induced IL-8 release (Coconnier et al., 2000). Consequently, our results suggested that the S-layer protein treatment did not directly interfere with IL-8 synthesis, but was able to block adhesion-dependent cytokine production induced by the presence of the pathogen.

MAPK signalling pathways play a critical role in widespread mechanisms of eukaryotic cell regulation (Chen et al., 2001). The ERK1/2 pathway mainly mediates cell proliferation and differentiation (Roux & Blenis, 2004). L. acidophilus S-layer proteins are able to mediate ERK1/2 activation (Li et al., 2011). The results indicate that S-layer proteins can promote cell development via the ERK1/2 pathway. We have also demonstrated that L. acidophilus S-layer proteins protect against Salmonella-induced apoptosis through reduced caspase-3 activation. In addition,
Salmonella-induced apoptotic cell damage is modified by S-layer proteins through the ERK1/2 signalling pathway (Li et al., 2011). This process offers an explanation of why in the presence of S-layer proteins the TER of infected cells increased and also returned to the normal level at 10 h post-infection with S. Typhimurium. This result suggests that the recovery of the Caco-2 cell TER may benefit from the antagonistic activity of S-layer proteins.

In contrast, L. acidophilus and its S-layer proteins do not mediate JNK and p38 activation. Both the JNK and p38 signalling pathways can mediate inflammatory responses (Roux & Blenis, 2004; Weston & Davis, 2007). This is consistent with the present observation that L. acidophilus S-layer proteins did not induce IL-8 secretion. Toll-like receptors (TLRs) are pattern-recognition receptors for micro-organisms. We speculated that L. acidophilus and its S-layer proteins might bind to ERK1/2 signal receptors, such as TLR2 (Rakoff-Nahoum et al., 2004; Vizoso Pinto et al., 2009) or other receptors, to induce ERK1/2 activation. p38 signal transduction can be mediated by TLR2 and TLR4 (Cario et al., 2000). L. acidophilus and S-layer protein-induced cellular biology may occur via the pathway TLRs–MyD88 (an adaptor molecule essential for TLR-mediated induction)–NF-κB (the transcription factor) (Ishii et al., 2008; Rakoff-Nahoum et al., 2004; Thomas & Versalovic, 2010), but not via the p38 or JNK signalling pathway.

An important feature of Salmonella pathogenesis is its ability to activate the host cell signal transduction pathways. S. Typhimurium can induce the activation of ERK1/2, JNK and p38 MAPK pathways (Bhavsar et al., 2007). MAPK signal transduction is conducive to S. Typhimurium diffuse infection (Jones et al., 2008; Roux & Blenis, 2004). In this study, our results were consistent with those of previous studies. Strikingly, when S. Typhimurium-infected Caco-2 monolayers were co-incubated with L. acidophilus S-layer proteins, S. Typhimurium-induced phosphorylation of ERK1/2, JNK and p38 was suppressed. S-layer proteins have the ability to autoassemble on bacterial surfaces (Antikainen et al., 2002; Golowczyc et al., 2007). We also demonstrated that S-layer proteins could bind directly to both S. Typhimurium and Caco-2 cells (Supplementary Fig. S3, Supplementary Methods). It has been reported that S-layer proteins are secreted by probiotic bacteria as mediators that affect mucosa–bacteria interactions (Sánchez et al., 2010). S-layer proteins may be associated with the S. Typhimurium surface and could interact with specific sites on S. Typhimurium or the epithelial cell surface involved in the first step of mucosal infection. Alternatively, S-layer proteins may either modify or mask the S. Typhimurium structures necessary for secreting the effectors of MAPK signalling pathways to inhibit ERK1/2, JNK and p38 activation. The suppressed MAPK signal transduction may be related to non-specific competition between the S-layer protein and S. Typhimurium for MAPK receptors, as well as the initial interaction of S-layer protein with specific sites of the pathogen before Caco-2 cells are challenged.

Taken together, these results demonstrate that L. acidophilus S-layer proteins are able to reduce S. Typhimurium-induced F-actin rearrangements, modify S. Typhimurium-induced TER reduction and IL-8 secretion, and inhibit S. Typhimurium-induced ERK1/2, JNK and p38 activation. The proposed specific mechanisms of action of lactobacillus S-layer proteins include competitive exclusion, enhancement of host cytoskeleton and host barrier defences, and modification of host signalling during Salmonella infections. Future studies will address the ability of lactobacillus S-layer proteins to inhibit infection by other enteropathogens, as well as their role in evoking beneficial effects in animal models.

**ACKNOWLEDGEMENTS**

We thank all other staff at the College of Veterinary Medicine of Nanjing Agricultural University for their assistance. We appreciate the support of Professor Shulin Liu in providing access to S. Typhimurium strain SL1344. This work was supported by grants from the National Natural Science Foundation of China (no. 30871858) and the Program of Jiangsu Province in China (no. BE200830155).

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Edited by: P. W. O’Toole