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Edwardsiella induces microtubule-severing in host epithelial cells

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ABSTRACT

Edwardsiella bacteria cause economic losses to a variety of commercially important fish globally. Human infections are rare and result in a gastroenteritis-like illness. Because these bacteria are evolutionarily related to other Enterobacteriaceae and the host cytoskeleton is a common target of enterics, we hypothesized that *Edwardsiella* may cause similar phenotypes. Here we use HeLa and Caco-2 infection models to show that microtubules are severed during the late infections. This microtubule alteration phenotype was not dependant on the type III or type VI secretion system (T3SS and T6SS) of the bacteria as ΔT3SS and ΔT6SS mutants of *E. piscicida* EIB202 and *E. tarda* ATCC15947 that lacks both also caused microtubule disassembly. Immunolocalization experiments showed the host katanin catalytic subunits A1 and A like 1 proteins at regions of microtubule severing, suggesting their involvement in the microtubule disassembly events. To identify bacterial components involved in this phenotype, we screened a 2,758 transposon library of *E. piscicida* EIB202 and found that 4 single mutations in the *atpFHAGDC* operon disrupted microtubule disassembly in HeLa cells. We then constructed three *atp* deletion mutants; they all could not disassemble host microtubules. This work provides the first clear evidence of host cytoskeletal alterations during *Edwardsiella* infections.

1. Introduction

Enteric bacteria from the genus *Edwardsiella* cause hemorrhagic septicaemia, petechial hemorrhage and holes in the bodies of an array of commercially important fish such as the Japanese eel, red sea bream, yellowtail, channel catfish, and turbot (Mohanty and Sahoo, 2007; Xu and Zhang, 2014; Buján et al., 2018B). Of the five known species within the *Edwardsiella* genus (*E. tarda*, *E. piscicida*, *E. anguillarum*, *E. ictaluri*, and *E. hoshinae*), only *E. tarda* is pathogenic to humans; causing various gastrointestinal or extraintestinal diseases in immunocompromised people (Janda et al., 1991a; Abayneh et al., 2013; Shao et al., 2015; Leung et al., 2019). *E. tarda* does not have a type III secretion system (T3SS) or type VI secretion system (T6SS) (Shao et al., 2015). However, the related *Edwardsiella* species, *E. piscicida* and *E. ictaluri* contain 1 T3SS and 1 T6SS, and *E. anguillarum* has 2 T3SSs and 3 T6SSs (Leung et al., 2019). It has been shown that *E. piscicida* is able to invade and replicate in epithelial cells such as human epithelial type-2 cells (HEp-2) (Janda et al., 1991b), and fish epithelioma papillosum of carp cells (Ling et al., 2000), as well as phagocytic murine macrophages (J774A.1 and RAW264.7) (Qin et al., 2017; Okuda et al., 2006), and fish primary macrophages (Ishibe et al., 2008). Using a blue gourami fish model, T3SS and T6SS deletion mutants of *E. piscicida* were attenuated by 1 and 2 logs, respectively in LD_{50} values; these two secretion systems are believed to be the key virulence mechanisms in *Edwardsiella* pathogenesis (Leung et al., 2019).

Cytoskeletal systems are crucial for maintaining tissue integrity, but are also common targets of bacterial pathogens (Yoshida and Sasakawa, 2003; Stevens et al., 2006; Kumar and Valdivia, 2008). Consequently, intracellular (e.g. *Shigella flexneri*, *Listeria monocytogenes*, and *Salmonella enterica* serovar Typhimurium) as well as extracellular pathogens (enteropathogenic *Escherichia coli*) hijack the actin filaments, microtubules and/or intermediate filaments networks during their infectious process (Yoshida and Sasakawa, 2003; Loisel et al., 1999; Rottner et al., 2004; Ashida et al., 2012). The close evolutionary relationship amongst

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many of those enterics to *Edwardsiella* (Tan et al., 2005; Nakamura et al., 2013) suggested that *Edwardsiella* may target similar cytoskeletal components during their infection of host cells. Supporting this notion is the evidence that *E. piscicida* (old name as *E. tarda*) PPD130/91 induced host actin condensation underneath the bacteria upon adhesion to internalize invading *Edwardsiella* (Ling et al., 2000). Thus, we hypothesized that other host cytoskeletal structures might be altered by these microbes.

Here we used two epithelial cell culture models to study the host cytoskeletal organization during *Edwardsiella* infections. We showed that host microtubules are severed six hours post-infection. This severing coordinates with the localization of the katanin catalytic subunits A1 and AL1 microtubule-severing proteins at the microtubule cutsites. The bacterial T3SS and T6SS are dispensable for this event to occur. To decipher which bacterial genes were required for the microtubule break-down phenotype, we screened a defined transposon insertion library in *E. piscicida* EIB202 (Yang et al., 2017) and found 4 *atp* transposon mutants that lost microtubule-severing abilities. This was further confirmed by three *atp* deletion mutants suggesting that ATP synthesis is involved in the microtubule disassembly process caused by *Edwardsiella* infected host epithelial cells.

2. Materials and methods

2.1. Bacterial strains and growth conditions

Bacterial strains used in this study included 4 wild type *Edwardsiella* strains: *E. piscicida* EIB202 and PPD130/91, *E. tarda* ATCC15947, and *E. anguillarum* ET080813, and 11 transposon and deletion mutants of EIB202 (Table 1). All strains were grown using tryptic soy broth (TSB, BD Biosciences), supplemented with antibiotics where appropriate. The non-redundant EIB202 defined transposon library was constructed and described previously (Yang et al., 2017). For the growth studies of *Edwardsiella* strains, they were first grown in TSB statically at 30 °C for overnight, and then subcultured in Dulbecco's Modified Eagle's medium (DMEM) next day at 35 °C without shaking. Aliquots were taken hourly from the well-mixed cultures for optical density (OD) reading at 600 nm.

2.2. Cell culture and infections

Human colon adenocarcinoma and human cervical epithelial cell lines were cultured in DMEM containing high-glucose (HyClone) supplemented with 10% fetal bovine serum (FBS, Sigma) and 1% non-essential amino acids (Gibco, Life Technologies), and DMEM containing high-glucose supplemented with 10% FBS, respectively. Both cell lines were grown at 37 °C with 5% CO_2 before *Edwardsiella* infection. Cells were trypsinized with 0.05% Trypsin-EDTA (Gibco) and seeded onto glass coverslips (Fisher Scientific) that were placed into 24-well plates (Corning).

For standard immunofluorescence and infection experiments, 6×10^4 HeLa or Caco-2 cells were seeded on 12 mm diameter glass coverslips in 24-well plates containing 1 ml of culture medium one day prior to the infections. Before infecting, tissue culture cells were checked for 90% confluence and the culture medium was replaced with 1 mL of culture medium with containing the bacterial inoculum. For all the *Edwardsiella* strains, both HeLa and Caco-2 cells were infected with the overnight culture for 6 h, at a multiplicity of infection (MOI) of about 360; while for the *E. tarda* ATCC 15947, overnight inoculum was used to infect for a period of 3 h, with MOI about 36. As for the *atp* transposon and deletion mutants, MOI of 720 was used to infect for 8 h to compensate their slow growth rates and to reach the same bacterial density as the wild type *E. piscicida* EIB202.

2.3. Immunofluorescence staining

At the completion of the infections, the samples were washed three times with phosphate-buffered saline (PBS) without magnesium and calcium (PBS -/-) (Hyclone) warmed to 37 °C and then fixed with warm 3% paraformaldehyde for 15 min. To permeabilize the cells, we used PBS −/− with 0.2% Triton-X-100 for 5 min at room temperature (RT), then washed three times with PBS −/−. The samples were then blocked with 5% normal goat serum (NGS) (Life Technologies) for 20 min and stained with primary antibodies overnight at 4 °C. Primary antibodies consisted of mouse anti-α tubulin (1:50) (Developmental studies hybridoma bank), rabbit anti-katanin A1 and rabbit anti-katanin AL1 (Sigma). After multiple washes with TPBS-0.1% BSA, Alexa-488 and Alexa-594 conjugated goat antibodies (1:1000) (Life Technologies) against mouse and rabbit IgG were added to the appropriate samples

Table 1
Strains and plasmids used in this study.

Strain or plasmid	Characteristics	Reference or source
Wild type		
E. anguillarum ET080813	Type strain; from Japanese eel; Col^r	Shao et al. (2015)
E. piscicida EIB202	CCTCC No. M208068; Col^r Cm^r	Wang et al. (2009)
E. piscicida PPD130/91	From serpae tetra; Km^s Col^r Amp^s	Ling et al. (2000)
E. tarda ATCC15947	Type strain; from human feces	Ewing et al. (1965)
Mutants of EIB202		
Δ*eseBCD* (ΔT3SS)	Deletion of *eseB-eseD*	Yang et al. (2015)
Δ*esrB*	In-frame deletion of *esrB*	Lv et al. (2012)
Δ*evpAB* (ΔT6SS)	Deletion of *evpA-evpB*	Yang et al. (2015)
Δ*eseBCD*, Δ*evpAB* (ΔT3/ΔT6SS)	Deletion of *eseB-eseD* and *evpA-evpB*	Yang et al. (2015)
Tn mutants of EIB202		
atpA::Tn	Transposon insertion mutant at locus inside *atpA*	This study
atpF::Tn	Transposon insertion mutant at locus inside *atpF*	This study
atpG::Tn	Transposon insertion mutant at locus inside *atpG*	This study
atpH::Tn	Transposon insertion mutant at locus inside *atpH*	This study
Deletion mutants of EIB202		
Δ*atpC*	In-frame deletion of atpC	This study
Δ*atpD*	In-frame deletion of atpD	This study
Δ*atpG*	In-frame deletion of atpG	This study
Escherichia coli		
DH5α λpir	Host for π requiring plasmids	Lv et al. (2012)
SM10 λpir	Host for π requiring plasmids, conjugation donor	Simon et al. (1983)
Plasmid		
pDMK	*pir* dependent, R6K, SacBR, Cm^r	Xiao et al. (2009)

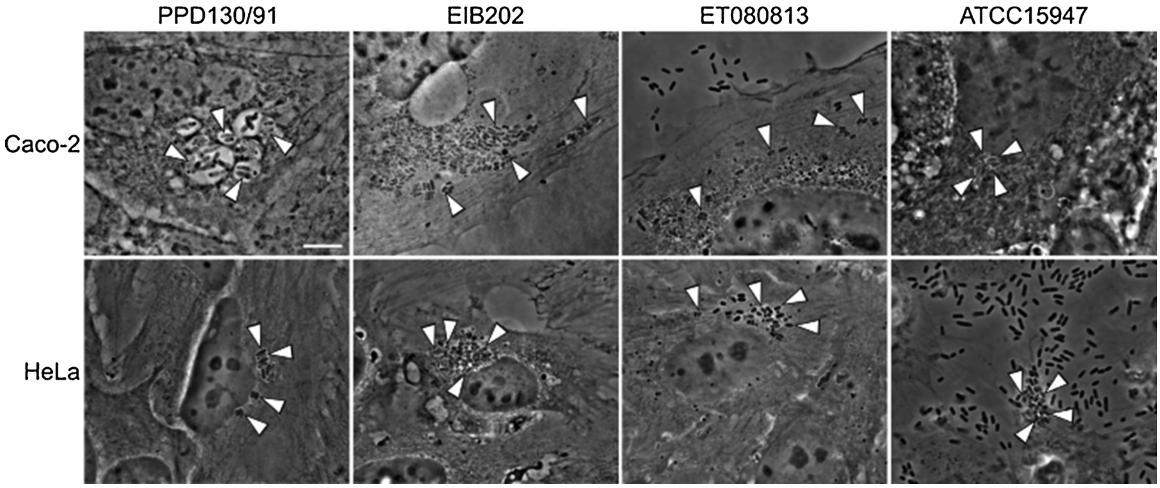


Fig. 1. HeLa and Caco-2 cell models showing successful internalization of different *Edwardsiella* isolates (*E. anguillarum* ET080813, *E. piscicida* EIB202, *E. piscicida* PPD130/91, and *E. tarda* ATCC15947). Both HeLa and Caco-2 cells were infected with wild type *E. piscicida* and *E. anguillarum* strains for 6 h at MOI 360, while the infection time for *E. tarda* strain was 3 h at MOI 36. Arrowheads point towards internalized bacteria. Scale bar = 10 μm.

and incubated for 2 h at RT. After the secondary antibody incubation, the samples were washed and mounted with Prolong Gold containing DAPI (Invitrogen).

2.4. Fluorescence microscopy imaging

A Leica (Wetzlar, Germany) DMI4000b inverted fluorescent microscope with a Hamamatsu Orca R2 CCD camera (Hamamatsu, Japan), connected to a PC with Metamorph Imaging System software (Molecular Devices, USA) was used for visualization and analyze of all samples. Images were processed without compromising the integrity of the data using ImageJ (Bethesda, USA) and Inkscape.

2.5. Cell culture transfection and live cell imaging

HeLa cells were transfected with a mKate-2-EB3 (end-binding 3) construct to label microtubules (Evrogen). Transfection was done using the jetPRIME transfection reagent according to manufacturer's protocol (Polypus Transfection). Cells were incubated at 37 °C for 24 h to allow expression of the EB3 protein. For live cell imaging, a Chamlide IC top stage incubator system was used to maintain a constant temperature of 35 °C and 5% humidified CO_2 gas in air. Images were captured every 2 min, after the infected cell culture dish was incubated at 35 °C for 1 h and 30 min.

2.6. Screening of the defined-transposon library of EIB202

30,000 HeLa cells were seeded into 96-well glass bottom plates containing 75 μL DMEM and 10% FBS media. The next day, each of the mutant library strains (Yang et al., 2017) were inoculated into 96-well dilution blocks and grown statically overnight for 16 h at 30 °C under gentamicin and colistin (Sigma) selection. Inoculations were done using a 48 pin metal multi-blot replicator. The following day, 11 μl of the overnight inoculum was used to infect HeLa cells in 96-well glass bottom microtitre plates for 3.5 h at 35 °C at a MOI of about 360. Cells were fixed and stained as described in Section 2.3. This protocol was repeated twice for all 2,758 mutants. Mutants were checked for microtubule disassembly phenotype in the 96-well dishes and their abilities to grow in DMEM. Mutants that did not disassemble host cell microtubules were repeated for two additional screens in 24-well plates as described in Section 2.2.

2.7. Construction of deletion mutants and characterization of mutants using growth studies

In-frame deletion mutants were generated by *sacB*-based allelic exchange as described previously (Xiao et al., 2009). Upstream and downstream fragments were produced by PCR using the primer pairs P1/P2 and P3/P4, respectively; the primer sequences are given in Supplementary Table S1. Overlap PCR with primer pair P1/P4 was used to generate fragments with in-frame deletions. The resulting fragments were cloned into the *sacB* suicide vector pDMK after linearization of the vector with XbaI and were transformed into *Escherichia coli* DH5α λpir; clones were validated using the primer pair pDMK-F/R. After sequencing, the plasmids were transformed into *E. coli* sm10 λpir and then mated into EIB202 by conjugation. Double crossover processes were selected sequentially on tryptic soy agar (TSA) medium containing colistin and chloramphenicol (Sigma) and then on TSA with 12% (v/v) sucrose to complete homologous recombination. The targeted mutants were confirmed by PCR using the primer pairs of out-F/R and in-F/R, and by sequencing of the region surrounding the deletion.

3. Results

3.1. Microtubules are severed during Edwardsiellainfections

Key to study host proteins during bacterial infections is the ability to view and observe the alteration of these proteins. Although the use of fish-based cell lines would be ideal to study the infections, antibodies that are commercially available rarely cross-react with fish proteins. Because *E. tarda* also infects humans (Michael and Abbott, 1993), we opted to develop and use human cell culture infection models. HeLa and Caco-2 cells both showed high bacterial loads, with bacteria clearly present within the monolayers (Fig. 1). This was evident with four different *Edwardsiella* strains [EIB202 (*E. piscicida* strain, 1 T3SS and 1 T6SS), ATCC15947 (*E. tarda* type strain, no T3SS or T6SS), PPD130/91 (*E. piscicida* strain, 1 T3SS and 1 T6SS), and ET080831 (*E. anguillarum* type strain, 2 T3SS and 3 T6SS)] at MOIs ranging from about 36 for *E. tarda* ATCC15947 to 360 for the remaining three *Edwardsiella* wild types in both HeLa and Caco-2 cells (Fig. 1).

Using these infection models, we immunolocalized tubulin and found that alterations to microtubules were observed by six hours post-infection. The microtubules were dramatically changed from the normal continuous radiating filaments throughout the HeLa and Caco-2 cells (Fig. 2A & B) to fragments within the cultured cells. These cut microtubule phenotypes were evident throughout the entire dish

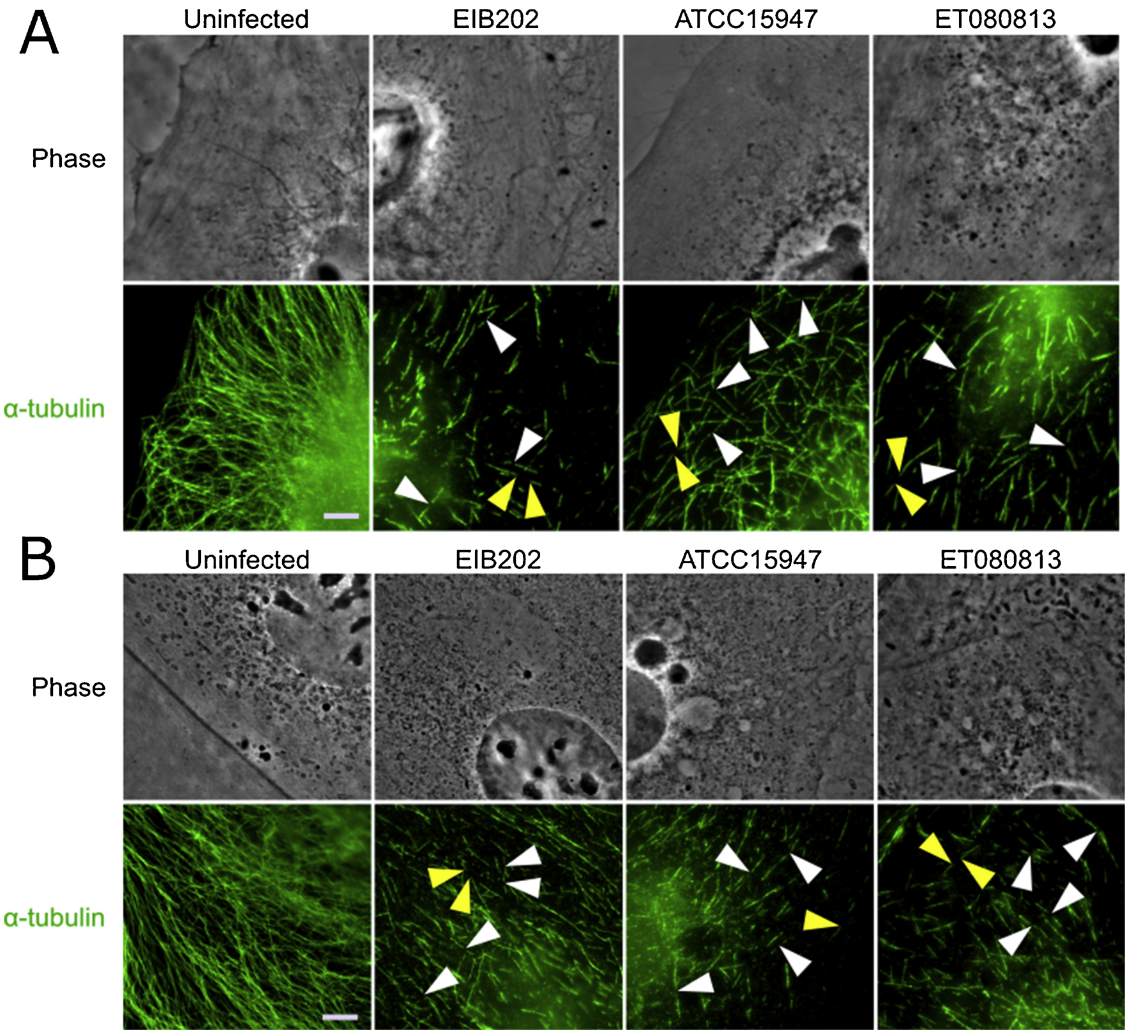


Fig. 2. Host microtubule severing phenotype observed during *Edwardsiella* infections. (A) HeLa cells showing microtubule disassembly during infection with *E. anguillarum* ET080813, *E. piscicida* EIB202, and *E. tarda* ATCC15947. (B) Caco-2 cells showing microtubule disassembly during infection with *E. anguillarum* ET080813, *E. piscicida* EIB202, and *E. tarda* ATCC15947. Both HeLa and Caco-2 cells were infected with wild type *E. piscicida* and *E. anguillarum* strains for 6 h at MOI 360, while the infection time for *E. tarda* strain was 3 h at MOI 36. After the infections, both HeLa and Caco-2 cells were fixed, stained for microtubules with a mouse monoclonal anti-α-tubulin antibody (green), and visualized by fluorescence microscopy. White arrowheads point to cuts in microtubules in both host cell types, and yellow arrowheads point to larger breaks in host microtubules. Scale bar = 10 μm.

whether bacteria were observed within the cells or not and held true for all strains tested (Fig. 2A & B). More than 88% of infected HeLa cells with the four wild types of *Edwardsiella* showed the microtubule disassembly phenotype; whereas this phenotype was absent in uninfected control cells (Table 2). This phenotype was validated by live cell imaging with *E. piscicida* EIB202, as microtubules were simultaneously cut at various locations within the same host cell, which was followed by microtubule network disintegration (Video S1). Most of these microtubule broken HeLa and Caco-2 cells had no *Edwardsiella* attached or inside the host cells.

Our evidence using the *E. tarda* ATCC15947 strain that naturally lacks both T3SSs and T6SSs strongly indicated that effectors released by these secretion systems are not needed for the observed microtubule disassembly events (Fig. 2, Table 2). However, there is a T3SS effector (EseG) that has been shown to disassemble microtubules when overexpressed in transfected host cells (Xie et al., 2010). To further confirm that T3SS or T6SS bacterial effectors were not responsible for the observed microtubule severing, we infected HeLa cells with mutated *E. piscicida* EIB202 strains (ΔT3SS, ΔT6SS, ΔT3SS/ ΔT6SS, and Δ*esrB*), which all continued to show identical microtubule severing phenotypes (Fig. 3).

3.2. *Host microtubule severing enzymes are present at microtubule cut-sites in host cells*

Katanins are common microtubule-severing enzymes (Sharp and Ross, 2012). Using antibodies against two katanin catalytic subunit proteins - A1 and AL1, we identified these two enzymes at the microtubule cut-sites during *E. piscicida* EIB202 infections in HeLa cells (Fig. 4A & B). Being able to observe this event was difficult as katanins rapidly release from the microtubules once severing is occurring (McNally and Vale, 1993; Sonbuchner et al., 2010; Johjima et al., 2015). The localization of these 2 proteins on the microtubules suggests that redundant mechanisms could be involved to ensure microtubule severing during *Edwardsiella* infections and katanin is playing a major role (Fig. 4A & B).

3.3. *Bacterial genes involved in the microtubule severing phenotype*

To determine bacterial factor(s) needed for the microtubule-severing phonotype that we observed during the epithelial cell infections, we screened an *E. piscicida* EIB202 defined transposon insertion library that covered ~70% of the genome (Yang et al., 2017). This negative screening approach was used to find *E. piscicida* mutants that did not

Table 2
Percentage of microtubule disassembly in HeLa cells infected with different *Edwardsiella* strains.

Edwardsiella strain	% of microtubule disassembly out of 50 cells [%, (disassembled/total)]		
	Exp. #1	Exp. #2	mean±SD
Wild type			
E. anguillarum ET080813	90.7 (49/54)	90.9 (50/55)	90.4 ± 0.1
E. piscicida EIB202	84.6 (44/52)	92.3 (48/52)	88.5 ± 5.4
E. piscicida PPD130/91	83.6 (46/55)	96.2 (50/52)	90.0 ± 8.9
E. tarda ATCC15947	100 (58/58)	96.4 (54/56)	98.2 ± 2.5
Tn mutants of *E. piscicida* EIB202			
atpA::Tn	1.9 (1/53)	5.4 (3/55)	3.7 ± 2.5
atpF::Tn	0 (0/55)	3.4 (2/58)	1.7 ± 2.4
atpG::Tn	0 (0/53)	0 (0/56)	0
atpH::Tn	1.9 (1/54)	3.8 (2/52)	2.9 ± 1.3
Deletion mutants of *E. piscicida* EIB202			
Δ*atpC*	2.0 (1/51)	5.4 (3/55)	3.7 ± 2.4
Δ*atpD*	0 (0/55)	3.6 (2/55)	1.8 ± 2.5
Δ*atpG*	0 (0/57)	3.4 (2/58)	1.7 ± 2.4
Uninfected control	0 (0/54)	0 (0/55)	0

About fifty HeLa cells, randomly chosen from at least six different fields of the monolayers, were counted in duplicate experiments to show microtubule disassembly after 6 h of infection with the wild types at MOI 360 (except for ATCC 15947 was 3 h at MOI 36), or 8 h at MOI 720 with various *Edwardsiella* mutants. Disassemble means microtubule filaments are severed into smaller fragments within a single HeLa cell; whereas, no disassemble means continuous and intact microtubule filaments are found to form a network throughout a single HeLa cell. Percentage of microtubule disassembly was calculated as the number of microtubule disassembled cells over 50 HeLa cells in percentage, mean±SD.

cause microtubule disassembly in the HeLa cells. The candidates were scored based on the amount of disassembly observed at a specific timepoint: high disassembly (>80% disassembly), low disassembly (<10%), and no disassembly (0%). We marked the wells with no disassembly as those with bacteria harbouring mutations in potential gene (s) responsible for the host cell microtubule-severing event. During the screen, we also eliminated transposon mutants that could not grow in DMEM. Ultimately, we identified 4 bacterial gene candidates (*atpA*, *atpF*, *atpG*, and *atpH*) in the transposon screen that were encoded for components of a F-type H+-transporting ATPase (the protein tag numbers are ETAE_3531, ETAE_3529, ETAE_3532, and ETAE_3530, respectively) that could not disassemble host microtubules.

Because the four mutations all fell in the same *atpFHAGDC* operon, we constructed a deletion mutant Δ*atpG* as well as in the last two genes of the *atp* operon, Δ*atpD* and Δ*atpC*. The hypothesis was that any of these six genes in the *atp* operon could disrupt ATP biosynthesis and these last two genes would be affected in all the four *atp* transposon mutants due to the polar effect. The growth rates of these 4 *atp* transposon and 3 deletion mutants were reduced; they all could grow in DMEM but grow rates of these *atp* mutants were slower as compared to the *E. pisicicda* wild type EIB202 in DMEM (Fig. S1). We then repeated the HeLa cells infection experiment by doubling the inoculum size of these *atp* mutants at a MOI of 720 and incubated for extra two hours to make sure the mutants reached the same or higher extracellular bacterial concentration as compared to the wild type at the end of the infection assay. None of the *atp* transposon and deletion mutants showed significant microtubule disassembly phenotype (less than 4% as in Table 2) and the infected HeLa cell monolayers were intact even when we prolonged the infection time to a total of eight hours.

4. Discussion

Pathogens often target the host cytoskeleton as part of their disease processes (Ashida et al., 2012; Ribet and Cossart, 2015). By coupling an assortment of *Edwardsiella* species with host cell infections, we demonstrated that microtubules were disassembled by these microbes. This microtubule alteration appears to have occurred through the activation of katanin and katanin-like proteins, which are known host microtubule severing proteins (Sonbuchner et al., 2010; McNally et al., 1996), as these proteins localized precisely at microtubule cut-sites. This disassembly event did not require bacterial T3SS effectors as host cells infected with non-functional T3SS and/or T6SS mutants as well as with *E. tarda* ATCC15947 (possessing no T3SS or T6SS) also showed the same microtubule severing phenotype.

Using our negative selection approach, we were able to identify four genes in ATP synthesis within *E. piscicida* that were needed for microtubule severing. Katnin and katanin-like proteins are closely related microtubule-severing enzymes that process AAA + domain for ATPase

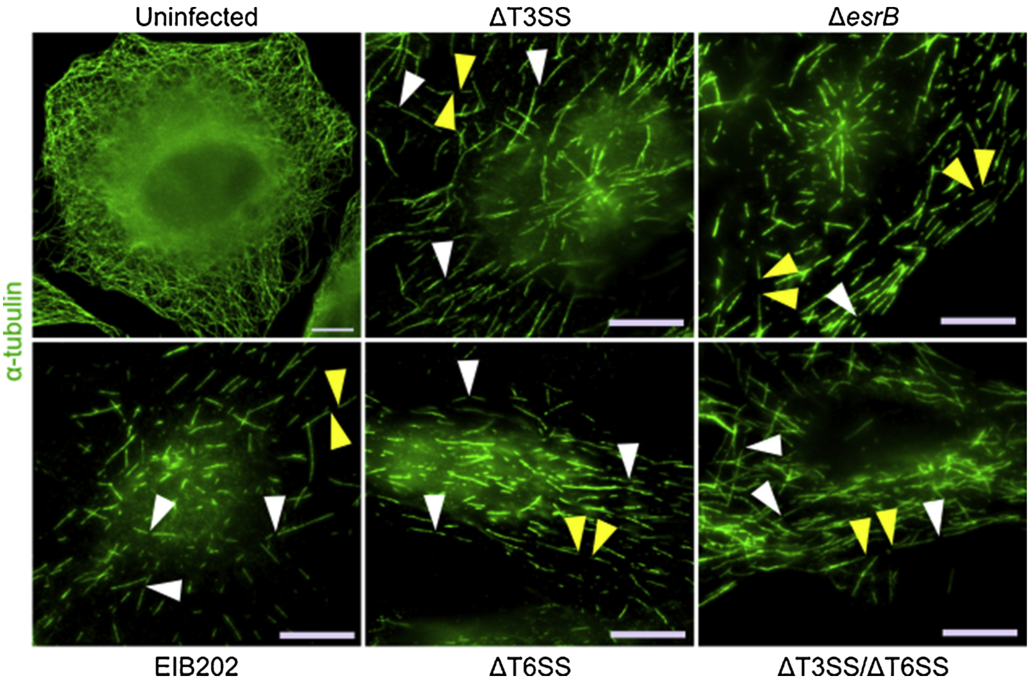
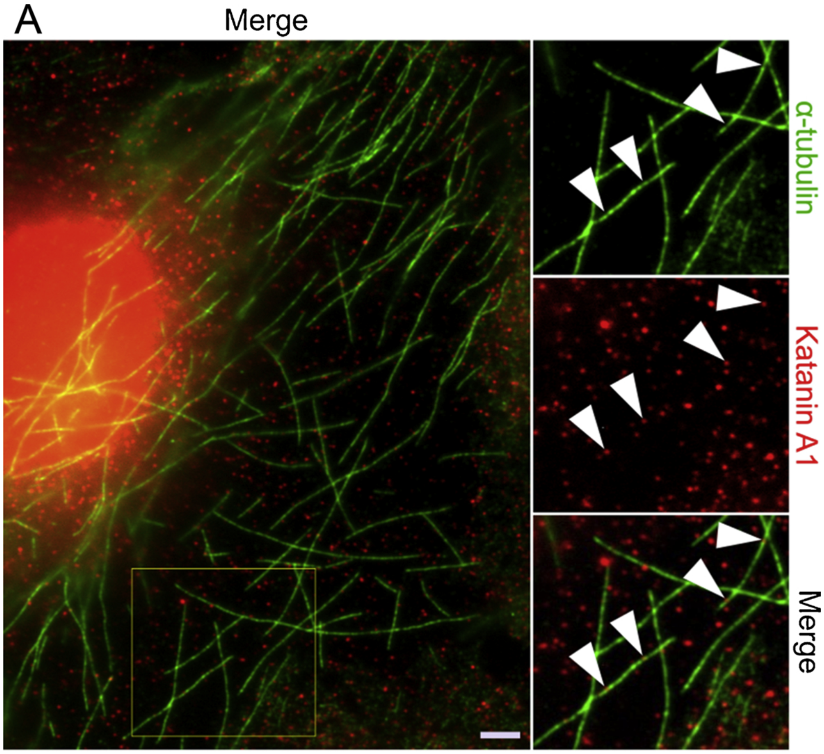


Fig. 3. *E. piscicida* EIB202 T3SS and T6SS mutants sever microtubules in HeLa cells. HeLa cells were infected with wild type *E. piscicida* EIB202 as well as *E. piscicida* EIB202 T3SS and T6SS mutants for 6 h at 35 °C at MOI 360. After the infections the cells were fixed and stained for microtubules with a mouse monoclonal anti-α-tubulin antibody (green). White arrowheads point to cuts in microtubules in host HeLa cells, and yellow arrowheads point to larger breaks in host microtubules. Scale bar = 10 μm.



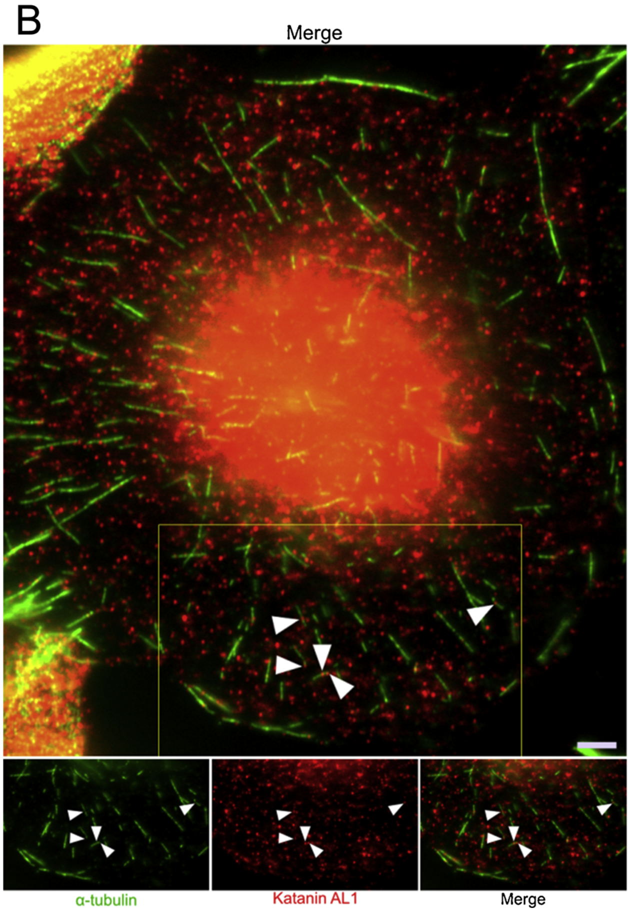


Fig. 4. Katanin catalytic subunits (A) A1 and (B) AL1 are involved in microtubule severing during *E. piscicida* infections. Katanin catalytic subunit A1 or AL1 localizes at microtubule cut-sites in HeLa cells post 6-h infection at MOI 360 with *E. piscicida* EIB202. Arrowheads point to cellular localization of katanin A or A1 at the cut-sites. Scale bar, = 10 μm.

activity. The resulting reaction is to bind to the microtubule and cut them into uneven fragments. Katanin has two catalytic subunits, p60 (subunit A1) and p80 (Rezabkova et al., 2017). The C-terminal of p60 consists of an AAA + domain, the catalytic site to hydrolyse ATP to energize the microtubule severing. ADP or ATP can bind to katanin. Formation of katanin hexamers is ATP-dependent and increase the enzyme's microtubule-severing activity (Roll-Mecak and McNally, 2010). On the other hand, the monomeric form is ADP-dependent and has low affinity for microtubules. We therefore speculate that during the infection process, *Edwardsiella* bacteria can provide extra ATP molecules through its ATP biosynthesis pathway. This exogenous pool of ATP may be possible to directly interact with katanin and katanin-like proteins, or indirectly through the activation of an unknown released *Edwardsiella* proteins to accelerate the host microtubule-severing enzymes. It is unclear how the bacterial synthesized ATP can target into the host cells and interacts with katanin and katanin-like proteins. To our knowledge, there are no other reports to implicate or suggest that the production of ATP from invading bacteria could influence the cellular functions in the host cells. This microtubule-severing phenotype did not require bacterial attachment or intracellular bacteria inside the host cells. The molecular basis of this mechanism is unknown and will be further explored and studied in the future.

Why would *Edwardsiella* want to disassemble the host microtubules of all cells in the sample? Potential reasons may lie with the functions of microtubules as tracts for vesicle movement, maintenance of cell structure, and cell motility; shutting down those processes run by microtubules leave the cells more vulnerable to *Edwardsiella* attack. The stopping of microtubule-based transport processes inside the host cells may also induce program cell death and influence the integrity of the epithelia itself so that *Edwardsiella* can invade further for systemic infection. Although this microtubule-severing phenotype was examined in human cells and not fish cells, the observed alteration to the cytoskeleton could conceivably influence the gross phenotypes of infected fish with holes in their tissues and liquefaction of internal tissues and organs.

Microtubule severing is a novel strategy used by microbes to dismantle the host cytoskeletal structures. By identifying the key bacterial proteins involved and crucial steps in the katanin-regulatory pathway, our work will contribute not only to the bacterial pathogenesis field but also the general field of cell biology. This work sets the foundation for our ultimate goal of blocking the key pathways used by *Edwardsiella* and to halt these infections.

Declaration of Competing Interest

The authors declare no conflicts of interest.

Acknowledgments

JAG, QW, and KYL conceived and designed the study. PA, LW, and YC performed the experiments. QL, JAG, QW, and KYL provided strains or reagents. PA, JAG, and KYL drafted the paper. All authors contributed to and approved the manuscripts in its final format. This research was supported by the National Natural Science Foundation of China (grant number #31873048) and the National Science and Engineering Research Council of Canada (grant number #06385-2014) to Dr. Ka Yin Leung.

Appendix A. Supplementary data

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