The clinical *Pseudomonas fluorescens* MFN1032 strain exerts a cytotoxic effect on epithelial intestinal cells and induces Interleukin-8 via the AP-1 signaling pathway

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**Abstract**

**Background:** *Pseudomonas fluorescens* is present in low number in the intestinal lumen and has been proposed to play a role in Crohn’s disease (CD). Indeed, a highly specific antigen, I2, has been detected in CD patients and correlated to the severity of the disease. We aimed to determine whether *P. fluorescens* was able to adhere to human intestinal epithelial cells (IECs), induce cytotoxicity and activate a proinflammatory response.

**Results:** Behaviour of the clinical strain *P. fluorescens* MFN1032 was compared to that of the psychrotrophic strain *P. fluorescens* MF37 and the opportunistic pathogen *P. aeruginosa* PAO1. Both strains of *P. fluorescens* were found to adhere on Caco-2/TC7 and HT-29 cells. Their cytotoxicity towards these two cell lines determined by LDH release assays was dose-dependent and higher for the clinical strain MFN1032 than for MF37 but lower than *P. aeruginosa* PAO1. The two strains of *P. fluorescens* also induced IL-8 secretion by Caco-2/TC7 and HT-29 cells via the AP-1 signaling pathway whereas *P. aeruginosa* PAO1 potentially used the NF-κB pathway.

**Conclusions:** The present work shows, for the first time, that *P. fluorescens* MFN1032 is able to adhere to IECs, exert cytotoxic effects and induce a proinflammatory reaction. Our results are consistent with a possible contribution of *P. fluorescens* in CD and could explain the presence of specific antibodies against this bacterium in the blood of patients.
latter has a chemotactic role and can recruit polymorphonuclear cells into the infected site and promote their infiltration of the epithelial layer infected by invasive or noninvasive bacteria [10,11]. IL-8 gene expression is regulated by two major transcriptional factors: nuclear factor kappa B (NF-κB) and activator protein (AP)-1 [12]. NF-κB has a pivotal role in the immune and inflammatory response, but also controls cell survival, proliferation and differentiation [13,14]. Recent works demonstrated that NF-κB signaling is a critical element of the homeostatic immuno-inflammatory function in the gut. Indeed, epithelial NF-κB preserves the integrity of the gut epithelial barrier and coordinates the antimicrobial actions of the innate and adaptive immune systems [15]. Nevertheless, hyperactivation of this transcription factor results in chronic inflammatory bowel diseases [16]. Activation of AP-1 is dependent on mitogen-activated protein kinases (MAPK) that are central in many physiological processes, including regulation of cytokine and stress responses and cytoskeletal reorganization [17,18].

P. fluorescens MFN1032 is a clinical strain recently isolated in our laboratory [19]. It displays hemolytic activity toward sheep erythrocytes [20,21], however, its infectious potential on human IECs is still unknown.

In the present study, we investigated adhesion and cytotoxic properties of P. fluorescens MFN1032 on Caco-2/TC7 and HT-29 cell lines in comparison to the psychrotrophic strain, P. fluorescens MF37 and the well-known opportunistic pathogen P. aeruginosa PAO1. The proinflammatory potential of P. fluorescens MFN1032 was also evaluated by the measurement of IL-8 secretion on both Caco-2/TC7 and HT-29 cells, and analysis of NF-κB and AP-1 activation using the reporter gene strategy.

Results
Adhesion to intestinal epithelial cells
The binding index of the clinical strain P. fluorescens MFN1032 on Caco-2/TC7 and HT-29 cells was determined after 5 h of incubation and compared to P. fluorescens MF37 and P. aeruginosa PAO1. The data presented in Figure 1 show that these bacterial strains adhere to both cell lines but the binding index was higher for Caco-2/TC7 (Figure 1A) than for HT-29 (Figure 1B).

P. aeruginosa PAO1 showed the highest adhesion potential on Caco-2/TC7 cells compared to P. fluorescens MF37 and P. fluorescens MFN1032. When the cells were infected with a 10^6 CFU or 10^8 CFU ml^-1 bacterial solution, the mean adhesion index of P. aeruginosa PAO1 reached 12.6 ± 2.6 or 32.1 ± 1.9 bacteria cell^-1, respectively, whereas the adhesion of P. fluorescens was quite similar for the two strains with 10.6 ± 0.5 or 18.1 ± 1.9 bacteria cell^-1 and 8.2 ± 0.6 or 19.8 ± 2 bacteria cell^-1 for MF37 and MFN1032, respectively.

The same experiment using HT-29 cells showed that the binding index of P. aeruginosa PAO1 remained the highest (7.1 ± 0.8 or 10.1 ± 1.0 bacteria cell^-1) but the index of P. fluorescens MFN1032 (4.3 ± 0.6 or 8.3 ± 1.6 bacteria cell^-1) was significantly higher than that of MF37 (1.4 ± 0.2 or 2.3 ± 0.5 bacteria cell^-1).

Cytotoxicity assay
The cytotoxic effect of Pseudomonas strains on Caco-2/TC7 and HT-29 cells was determined by quantification of lactate dehydrogenase (LDH) released in culture medium (Figure 2).

P. fluorescens MF37 exhibited the lowest cytotoxic activity (expressed as % of maximal LDH release) with only 7.8 ± 1.9% (at 10^6 CFU ml^-1) or 30 ± 16.4% (at 10^8 CFU ml^-1) of cell lysis after 24 h of infection on Caco-2/TC7 (Figure 2A) and 17.5 ± 1.1% (at 10^6 CFU ml^-1) or 22 ± 2.0% (at 10^8 CFU ml^-1) of cell lysis for HT-29 cells (Figure 2B). The cytotoxicity of MFN1032 was higher with 34 ± 15.2% or 74.7 ± 4.6% lysis for infection respectively with 10^6 or 10^8 CFU ml^-1 on Caco-2/TC7 and 33.2 ± 1.5 or 60.3 ± 5.5% lysis after infection with 10^6 or 10^8 CFU ml^-1 respectively on HT-29. P. aeruginosa PAO1 led to a total lysis of Caco-2/TC7 at the two bacterial concentrations tested and on HT-29, with infection rates of 10^6 or 10^8 CFU ml^-1, LDH release was 67.9 ± 7.2% or 85.6 ± 3.4% respectively. At the end of infection, Caco-2/TC7 and HT-29 cells were observed by light microscopy. Figure 3 shows the cell monolayers after infection with MOI of 100 (10^8 CFU ml^-1). When Caco-2/TC7 cells where infected with P. fluorescens MF37, a slight cell detachment was detectable while more cells were detaching after infection with MFN1032. Infection with P. aeruginosa PAO1 led to a complete disappearance of the organized Caco-2/TC7 and HT-29 monolayers.

Induction of IL-8 secretion
The bacterial proinflammatory effect was assessed by measuring IL-8 secretion. Compared to untreated cells, the three Pseudomonas strains induced significant stimulation of IL-8 secretion in both Caco-2/TC7 (Figure 4A) and HT-29 cells (Figure 4B). Mean values of IL-8 on HT-29 and Caco-2 in response to P. fluorescens MF37 and MFN1032 were similar for these two strains and it is noteworthy that IL-8 secretion was significantly increased in HT-29 compared to Caco-2 cells.

NF-κB and AP-1 activation in Caco-2 and HT-29 reporter cell lines
To further explore the immuno-modulatory properties of P. fluorescens MFN1032, we tested the effects of this
bacterium on NF-κB or AP-1 activation using Caco-2 and HT-29 reporter cell lines. We observed that *P. aeruginosa* PAO1 stimulated NF-κB activity by 2.5-fold over control in both Caco-2/NF-κB-seap-7 and HT-29/NF-κB-seap-25 reporter clones (Figure 5) while it had no effect on the AP-1 pathway (Figure 6). Interestingly, *P. fluorescens* MF37 and MFN1032 had an opposite effect. Indeed, none of these strains induced NF-κB activation (Figure 5) whereas they both activated the AP-1 pathway by 2.2-fold over control in Caco-2/AP-1-luc-1 and HT-29/AP-1-luc-6 reporter clones (Figure 6).

**Discussion**

*P. fluorescens* is present at low level in the human gut and has been linked to Crohn’s disease (CD) [7,8], however little is known about the potential interaction of this bacterium with the intestinal mucosa. In the present paper, we aimed at determining its potential to adhere to IEC, to induce cell cytotoxicity and trigger a proinflammatory response. We selected two strains, a classical psychrotrophic strain (MF37) and a recently characterized clinical strain adapted to grow at 37°C (MFN1032). The behaviour of these bacteria was compared to that of the opportunistic pathogen *P. aeruginosa*.

Since adhesion and cytotoxicity to IECs are crucial events in the infection process, the three strains were tested on two epithelial cell lines. Except for adhesion, the two IECs models used in this study gave similar responses to the three strains of *Pseudomonas*. Indeed, a dose dependent adhesion of bacteria to Caco-2/TC7 and HT-29 cells was observed with the greatest effect obtained with the opportunistic pathogen...
P. aeruginosa. It is noteworthy that, compared to the psychrotrophic strain MF37, the clinical strain P. fluorescens MFN1032, which is adapted to develop at 37°C displayed statistically significant higher adhesion potential to HT-29 but not to Caco-2/TC7 cells. This observation suggests that the clinical strain may express a greater diversity of adhesion factors than MF37 and could explain, at least in part, the higher cytotoxicity effect of MFN1032.

Although differences exist between surface proteins expressed by Caco-2/TC7 and HT-29 cell lines in comparison to normal human IECs, our results support the hypothesis that P. fluorescens should be able to colonize the intestinal mucosa. Pseudomonad are rarely searched for and detected as fecal bacteria, and are usually considered as a sub-dominant population [22]. In addition, there is now ample evidence that the circulating bacterial population in the intestinal lumen is very different from the resident microbiota that comes in contact with the apical surface of the enterocytes and is tightly associated to the mucus/glycocalyx layer [23,24]. For an aerobic bacterium such as P. fluorescens, the best ecological niche should be at the vicinity of the epithelium, where oxygen concentration is the highest in the

Figure 3 Effects of P. fluorescens MF37 (A), P. fluorescens MFN1032 (B) and P. aeruginosa PAO1 (C) on the morphological aspect of Caco-2/TC7 and HT-29 monolayers compared to a non-infected monolayer (D). The figure only shows the results obtained after 24 h of infection with a concentration of 10^8 CFU.ml⁻¹. Scale bar = 100 μm.

Figure 4 Induction of IL-8 release by P. fluorescens MF37, P. fluorescens MFN1032 and P. aeruginosa PAO1 in Caco-2/TC7 (A) and HT-29 (B) cells. IL-8 content was estimated in the cells supernatant after 24 h of infection. * P < 0.05, *** P < 0.001.
intestinal environment [25]. This is supported by the evidence showing that the P. fluorescens-specific I2 antigen sequence is systematically detected in ileal mucosa samples [7]. Moreover, in CD patients, there is a positive correlation between blood level of circulating anti-I2 antibodies and the severity of the disease [8] suggesting that the I2-producing bacteria, i.e. P. fluorescens, are in close contact with enterocytes and could contribute to CD pathogenesis.

The LDH release assay showed that the cytotoxicity of P. fluorescens on Caco-2/TC7 and HT-29 cells is lower than that of P. aeruginosa. This limited virulence of the P. fluorescens strains seems to be normal for a species that should be a resident in the intestine whereas P. aeruginosa is typically an opportunistic pathogen only detected in case of declared infection [26]. This hypothesis is also in agreement with the hierarchy of the cytotoxic activity of the two tested strains of P. fluorescens, the clinical strain MFN1032 being more virulent than the environmental and psychrotrophic strain MF37. Bacterial cytotoxicity is a highly complex phenomenon combining the virulence of the prokaryote and the intrinsic sensitivity of the eukaryotic cell. In opposition to the present results, Chapalain et al found that the cytotoxic activity on glial cells was higher for P. fluorescens MF37 than MFN1032 [4]. These observations are in agreement with the work of Picot et al showing that in the case of P. fluorescens, the necrotic and apoptotic activities are not simply correlated to the adhesion potential of the strain [27].

In contrast to P. aeruginosa, the proinflammatory effect of P. fluorescens strains has not been elucidated.
In this study, we demonstrated that similarly to *P. aeruginosa*, *P. fluorescens* MFN1032 and MF37 exerted a direct proinflammatory effect on IECs as demonstrated by induction of IL-8 secretion. The homogenous proinflammatory response of IECs induced by the two *P. fluorescens* strains studied suggests a link between the proinflammatory properties and a common pathogenic factor of these strains. IL-8 gene expression is regulated by several signaling pathways including mainly NF-κB and AP-1 transcription factors. Previous studies have shown that *P. aeruginosa* activates NF-κB in mouse monocyte/macrophage cell line [28] and MAPK signaling pathways in lung epithelial cells [29], which in turn leads to the production of proinflammatory cytokines, such as IL-6, IL-8, and TNF-α (tumor necrosis factor alpha). In our study, the two *P. fluorescens* strains failed to activate the NF-κB pathway in contrast to *P. aeruginosa*, however the two strains were able to activate AP-1 signaling, suggesting that the proinflammatory effect of these bacteria in IECs is linked to the activation of MAPK signaling pathways. The MAPK form a group of three pathways, including extracellular signal-regulated protein kinases (ERK1/2) and two stress-activated protein kinases p38 and JNK (c-jun N-terminal kinase) [30]. The activation of MAPK has been reported to be involved in response to infection by invasive bacteria, such as *Salmonella enterica* serovar typhimurium or *Listeria monocytogenes*, in IECs [31,32] or in macrophages [33]. Moreover, it has been shown that enterodherent *Escherichia coli* activate this pathway and both bacterial attachment and secreted proteins might be implicated in cytokine responses [34]. *P. aeruginosa* as well as *P. fluorescens* contain multiple cell surface factors, including flagellin, pili, LPS, type III-mediated toxin secretion and quorum-sensing molecules which could interact with distinct epithelial membrane proteins, such as asialylated glycolipid receptors, toll like receptors (TLRs), or combinations of these proteins [28,35,36]. We have shown that purified flagellin strongly activated NF-κB pathway in HT-29 and to a lower extent in Caco-2, whereas both cell lines poorly responded to LPS (Lakhdari et al, submitted manuscript). In contrast, purified flagellin and LPS do not activated the AP-1 pathway in the two cell lines (data not shown). Thus, we can conclude that *P. fluorescens* activated AP-1 pathway in Caco-2 and HT-29 independently of flagellin and LPS expression. Further investigations will be needed to identify the exact nature and function of *P. fluorescens* compounds responsible for MAPK activation in IECs.

Conclusions

*P. fluorescens* MFN1032, *P. fluorescens* MF37 and *P. aeruginosa* PAO1 were found to adhere to Caco-2/TC7 and HT-29 cells and the cytotoxicity towards these cell lines was higher for the clinical strain MFN1032 than for MF37. We showed that the two strains of *P. fluorescens* induced IL-8 secretion by Caco-2/TC7 and HT-29 cells via the AP-1 signaling pathway whereas *P. aeruginosa* PAO1 potentially used the NF-κB pathway. To our knowledge, this work is the first to demonstrate the interaction and the proinflammatory potential of *P. fluorescens* on IECs.

Methods

Cell culture

The human colon adenocarcinoma cell lines Caco-2/TC7 [37] and HT-29 were used between passages 10 and 35. Caco-2/TC7 cells were grown in Dulbecco’s modified Eagle Minimal Essential Medium (Sigma) containing 20% foetal calf serum (FCS) supplemented with 2 mM of L-glutamine, 100 U ml⁻¹ each of penicillin and streptomycin and 1% non-essential amino acids at 37°C with 5% CO₂. HT-29 cells were grown in Dulbecco’s modified Eagle Minimal Essential Medium (Sigma) containing 10% FCS supplemented with 2 mM of L-glutamine, 100 U ml⁻¹ each of penicillin and streptomycin at 37°C with 5% CO₂.

Bacterial strains and culture conditions

*P. fluorescens* MF37 is a rifampicin-resistant natural mutant of the strain MF0 (Biovar V), originally identified in crude milk [38]. *P. fluorescens* MFN1032, is a clinical biovar I strain collected in a hospital of Haut-Normandie (France) [4]. *P. aeruginosa* PAO1 was obtained from an international collection. Bacteria were grown overnight in ordinary nutrient broth (Merck) at 28°C for the two strains of *P. fluorescens* and at 37°C for *P. aeruginosa* PAO1. For adhesion and cytotoxicity assays, bacteria in stationary phase were harvested by centrifugation (5000 x g, 5 min, 20°C) and resuspended in antibiotic-free and serum-free cell culture media at densities of 10⁶ and 10⁸ CFU ml⁻¹, corresponding to a multiplicity of infection (MOI) of 1 and 100 respectively.

Adhesion assay

For adhesion assays, Caco-2/TC7 and HT-29 cells were seeded at a concentration of 1 x 10⁵ cells ml⁻¹ on coverslips coated with 50 μg ml⁻¹ poly-L-lysine and used at 80% confluence as recommended by Li et al [39]. Cells were incubated for 5 h with 1 ml of the bacterial suspensions. After incubation, the medium and non-adherent bacteria were removed by washing. Then, the coverslips were fixed with methanol (10 min), stained with Giemsa solution (20 min) and observed using an Axiovert S100TM light microscope (Zeiss). The adhesion index (mean number of bacteria adherent per cell) was determined by direct counting on a minimum of 100 cells following the technique of Darfeuille-Michaud et al [40].
Cytotoxicity assay
Confluent Caco-2/TC7 and HT-29 cells cultivated in 24-well culture plates were infected for 24 h with 1 ml of the bacterial suspensions. At the end of incubation, lactate dehydrogenase (LDH) present in the supernatant was measured in each well using the Cytox 96® enzymatic assay (Promega). LDH is a stable cytosolic enzyme released by eukaryotic cells and an overall indicator of necrosis. Caco-2/TC7 and HT-29 cells exposed to Triton X100 (0.9%) were used as a control of total release (100% LDH release). The background level (0% LDH release) was determined with serum free culture medium. The percentage of cytotoxicity was calculated following the manufacturer’s instructions.

IL-8 ELISA
IL-8 assays were performed on confluent Caco-2/TC7 and HT-29 cells monolayers grown in 24-well culture plates. After 24 h of infection with the bacterial suspensions (MOI of 100), immunoreactive IL-8 protein levels in cell culture supernatant were quantified using an ELISA Quantikine kit (R&D systems) according to the manufacturer’s instructions.

Construction of stable NF-κB and AP-1 reporter cells
The NF-κB reporter clones Caco-2/kb-seap-7 and HT-29/kb-seap-25 were obtained after a stable transfection of parental cells with the reporter plasmid pNiFty2-SEAP (Invivogen), which contains SEAP (secreted alkaline phosphatase) as reporter gene downstream of five repeats of the NF-κB binding consensus.

The AP-1 reporter clones Caco-2/ap1-luc-1 and HT-29/ap1-luc-6 were obtained after a stable co-transfection of the reporter plasmid pAP-1-luc (Stratagen), which contains luciferase as reporter gene downstream of seven repeats of the AP-1 binding consensus, together with pTK-Hyg (Clontech) a hygromycine-based selection vector. Transfection of HT-29 was performed by lipofection using TFX-50 (Promega) according to the manufacturer’s instructions and quantified using an ELISA Quantikine kit (R&D systems) according to the manufacturer’s protocol.

Analysis of NF-κB and AP-1 activation
For each experiment, reporter cells were seeded at 50,000 cells per well, into 96-well plates and pre-incubated 24 hours before adding live bacteria at an MOI of 100.

For NF-κB activation assays, Caco-2/kb-seap-7 and HT-29/kb-seap-25 cells were incubated with live bacteria for 8 hours and IL-1β (10 ng/ml) was used as a positive control. SEAP activity in the supernatant was measured using the Quanti-Blue reagent (Invivogen) using the manufacturer’s instructions while Caco-2 cells were transfected using the Amaxa Nucleofector system (Lonza).

For AP-1 activation assays, cells were incubated for 12 h with live bacteria. Phorbol-myristate acetate (PMA, 1 μM) or butyric acid (2 mM) was used as a positive control for Caco-2/ap1-luc-1 or HT-29/ap1-luc-6 reporter cells respectively. Luciferase activity was measured using the ONE-Glo™. Luciferase Assay System (Promega) according to the manufacturer’s instructions and quantified as relative luminescence units (RLU). All measurements were performed using a microplate reader (Infinite 200, Tecan).

Statistical analysis
Data are expressed as a mean ± standard error (SEM) calculated over three independent experiments performed in triplicate. Analysis of statistical significance were performed by ANOVA with Bonferroni post hoc test (adhesion and cytotoxicity assays) or Student’s t-test (IL-8 secretion, NF-κB and AP-1 activation assays)

Acknowledgements
This work was supported by a BRI grant (Bourse Régionale Industrielle) from the Région Haute-Normandie and BIOGALENYS. OL is supported by the European Community’s Seventh Framework Programme (FP7/2007-2013) MetaHiT, grant agreement HEALTH-F4-2007-201052. We thank Mihai Covasa and Christine Farmer for revising the English manuscript.

Authors’ contributions
AM carried out most experiments and analyzed most of the data. NC wrote the manuscript, participated in the design of the study and analyzed most of the data. MG carried out the IL-8 ELISA assay. OL carried out the construction of NF-κB reporter cells. KR carried out the construction of AP-1 reporter cells. JD and HB participated in the design of the construction of NF-κB and AP-1 reporter cells and help to draft the manuscript. PS and NO were involved in the design of the study and writing of the manuscript. AG performed the statistical analysis. All authors read and approved the final manuscript.

Received: 21 May 2010 Accepted: 10 August 2010
Published: 10 August 2010

References
1. Hirakata Y, Iizumikawa K, Yamaguchi T, Igimi S, Futuya N, Maesaki S, Tomono K, Yamada Y, Kohno S, Yamaguchi K, et al. Adherence to and penetration of human intestinal Caco-2 epithelial cell monolayers by Pseudomonas aeruginosa. Infect Immun 1998, 66(4):1748-1751.
2. Plotkowski MC, de Bentzmann S, Pereira SH, Zahm JM, Bajolet-Laudinat O, Roger P, Puchelle E. Pseudomonas aeruginosa internalization by human epithelial respiratory cells depends on cell differentiation, polarity, and junctional complex integrity. Am J Respir Cell Mol Biol 1999, 20(5):880-890.
3. Zaborina O, Kohler JE, Wang Y, Bethel C, Shevchenko O, Wu L, Turner JR, Alverdy JC. Identification of multi-drug resistant Pseudomonas aeruginosa clinical isolates that are highly disruptive to the intestinal epithelial barrier. Ann Clin Microbiol Antimicrob 2006, 5(14).
4. Chapalain A, Rossignol G, Lesouhaitier O, Meneau A, Guffaz C, Guerillon J, Meyer JM, Orange N, Feuilloley MG. Comparative study of 7 fluorescent pseudomonad clinical isolates. Can J Microbiol 2008, 54(1):19-27.
Enzymes from isolates of *Pseudomonas fluorescens* involved in food spoilage. J Appl Microbiol 2002, 93(2):205-213.

Wei B, Huang T, Dalwadi H, Sutton CL, Bruckner D, Braun J. *Pseudomonas fluorescens* encodes the Crohn’s disease-associated I2 sequence and T-cell superantigen. Infect Immun 2002, 70(12):6567-6575.

Sutton CL, Kim J, Yamane A, Dalwadi H, Wei B, Landers C, Targan SR, Braun J. Identification of a novel bacterial sequence associated with Crohn’s disease. Gastroenterology 2000, 119(1):23-31.

Dalwadi H, Wei B, Kronenberg M, Sutton CL, Braun J. The Crohn’s disease-associated bacterial protein I2 is a novel enteric T cell superantigen. Immunity 2001, 15(1):149-158.

Eckmann L, Kagnoff MF, Ferter J. Epithelial cells secrete the chemokine interleukin-8 in response to bacterial entry. Infect Immun 1993, 61(11):4569-4574.

McCormick BA, Miller SJ, Carnes D, Madara JL. Transepithelial signaling to neutrophils by *salmonella*: a novel virulence mechanism for *gastrenterocteritis*. Infect Immun 1995, 63(6):3202-3209.

Sakovski SD, Koutsouris A, Hecht G. Attachment of a noninvasive enteric pathogen, *enteropathogenic Escherichia coli*, to cultured human intestinal epithelial monolayers induces transmigration of neutrophils. Infect Immun 1996, 64(11):4480-4487.

Mukaida N, Okamoto S, Iwakawa Y, Matsushima K. Molecular mechanism of interleukin-8 gene expression. J Leuk Biol 1994, 56(5):554-558.

Karim M, Lin A. NF-kappaB at the crossroads of life and death. Nat Immunol 2002, 3(3):221-227.

Hayden MS, Ghosh S. Shared principles in NF-kappaB signaling. Cell 2008, 132(3):344-362.

Hayden MS, West AP, Ghosh S. NF-kappaB and the immune response. Oncogene 2006, 25(51):6758-6780.

Schreiber S, Nikolaus S, Hampe J. Activation of nuclear factor kappa B inflammatory bowel disease. Gut 1998, 42(4):477-484.

Davis RJ. The mitogen-activated protein kinase signal transduction pathway. J Biol Chem 1993, 268(20):14553-14556.

Davis RJ. Signal transduction by the JNK group of MAP kinases. Cell 2000, 103(2):239-252.

Chapalain A, Chevalier S, Orange N, Murillo L, Papadopoulos V, Feuillie MG. Bacterial ortholog of mammalian translocase protein (TSP0) with virulence regulating activity. PLoS One 2009, 4(6):e6096.

Rossignol G, Merieau A, Guerillon J, Veron W, Lesouhaitier O, Feuillie MG, Orange N. Involvement of a phospholipase C in the hemolytic activity of a clinical strain of *Pseudomonas fluorescens*. BMC Microbiol 2008, 8:189.

Sperandio D, Rossignol G, Guerillon J, Connin N, Orange N, Feuillie MG, Merieau A. Cell-associated hemolysis activity in the clinical strain of *Pseudomonas fluorescens* MNF1032. BMC Microbiol 2010; 10:124.

Matsuda T, Tsuji H, Asahara T, Kado Y, Nomoto K: Identification of a novel bacterial sequence associated with *Pseudomonas aeruginosa* infection. Infect Immun 2003, 71(10):6035-6044.

Robinson MJ, Cobb MH: Mitogen-activated protein kinase pathways. Curr Opin Cell Biol 1997, 9(2):180-186.

Hobbie S, Chen LM, Davis RJ, Galan JE: Involvement of mitogen-activated protein kinase pathways in the nuclear responses and cytokine production induced by *Salmonella typhimurium* in cultured intestinal epithelial cells. J Immunol 1997, 159(11):5550-5559.

Tang P, Sutherland CL, Gold MR, Finlay BB: *Listeria monocytogenes* invasion of epithelial cells requires the MEK-1/ERK-2 mitogen-activated protein kinase pathway. Infect Immun 1998, 66(3):1106-1112.

Schwan WR, Kugler S, Schaller S, Kopecko DJ, Goebel W: Detection and characterization by differential PCR of host eukaryotic cell genes differentially transcribed following uptake of intracellular bacteria. Infect Immun 1996, 64(1):91-99.

Dahan S, Busuttil V, Imbert V, Peyton JF, Rampal P, Czerucka D: Enterohemorrhagic *Escherichia coli* infection induces interleukin-8 production via activation of mitogen-activated protein kinases and the transcription factors NF-kappaB and AP-1 in T84 cells. Infect Immun 2002, 70(5):2304-2310.

Ratner AJ, Bryan R, Weber A, Nguyen S, Banes D, Pitt A, Gelber S, Cheung A, Prince A: Cystic fibrosis pathogen acquires Ca2+-dependent mitogen-activated protein kinase signaling pathways in airway epithelial cells. J Biol Chem 2001, 276(22):19267-19275.

Zhang Z, Reenstra W, Weiner DJ, Louboutin JP, Wilson JM. The p38 mitogen-activated protein kinase signaling pathway is coupled to Toll-like receptor 5 to mediate gene regulation in response to *Pseudomonas aeruginosa* infection in human airway epithelial cells. Infect Immun 2007, 75(12):5985-5992.

Chantret I, Rodolosie A, Barbat A, Dussaulx E, Brot-Laroche E, Zewiba A, Rousset M: Differential expression of sucrose-isoamylase in clones isolated from early and late passages of the cell line Caco-2: evidence for glucose-dependent negative regulation. J Cell Sci 1994, 107( Pt 1):213-225.

Burri JF, Gugi B, Merieau A, Guerin-Michel JF: Uspase and acidic phosphatase from the psychrotrophic bacterium *Pseudomonas fluorescens*: two enzymes whose synthesis is regulated by the growth temperature. FEMS Microbiol Lett 1994, 122(1-2):13-18.

Li XJ, Yue LY, Guan XF, Qiao SY: The adhesion of putative probiotic *lactobacilli* to cultured epithelial cells and porcine intestinal mucus. J Appl Microbiol 2008, 104(4):1082-1091.

Darfeuille-Michaud A, Aubel D, Chauviere G, Rich C, Bourges M, Servin A, Joly B: Adhesion of enterotoxigenic *Escherichia coli* to the human colon carcinoma cell line Caco-2 in culture. Infect Immun 1990, 58(4):893-902.

Cite this article as: Madi et al.: The clinical *Pseudomonas fluorescens* MNF1032 strain exerts a cytotoxic effect on epithelial intestinal cells and induces Interleukin-8 via the AP-1 signaling pathway. *BMC Microbiology* 2010 10:124.