IN VITRO CHARACTERIZATION OF THE ABILITY OF *YERSINIA ENTEROCOLITICA* BT4 TO COLONIZE PIGS AND STAINLESS STEEL SURFACES

Pierre Raymond[1], Bastien Frémaux[2], Martine Denis[1], Carole Feurer[3], Sabine Jeuge[2], Emilie Esnault[1*]

[1]Anses, Laboratory of Ploufragan/Plouzané, Hygiene and Quality of Poultry and Pig Products Unit, University of Bretagne-Loire, Ploufragan, France
[2]IFIP-Institut du Porc, 7 avenue du Général de Gaulle, 94 700, Maisons-Alfort, France.
[3]IFIP-Institut du Porc, La Motte au Vicomte, BP35104, 35650 Le Rheu, France.
** Corresponding author: emilie.esnault@anses.fr*

Abstract

Yersiniosis is, after campylobacteriosis and salmonellosis, the third most frequently reported zoonosis in Europe. Humans become infected with *Y. enterocolitica* through the consumption of undercooked pork and raw food having been in contact with contaminated surfaces. Pigs, the main reservoir for human pathogenic strains, do not develop clinical signs. In France and worldwide, biotype 4 (BT4) is the biotype the most frequently isolated from both pigs and clinical yersiniosis. In this study, a collection of 26 pathogenic BT4 strains isolated from pig tonsils was used to investigate their ability to adhere and invade intestinal pig cells (IPEC-J2) and to adhere to abiotic surfaces (stainless steel coupons) using two *in vitro* tests. Regression analysis was performed between data sets obtained from IPECJ2 cells assays *versus* stainless steel assays.

All BT4 strains were able to adhere and invade IPEC-J2 cells. However, the results showed heterogeneity between strains with respect to their ability to adhere to IPECJ2 cells, with a percentage of adhesion varying from 9% to more than 90%. The BT4 population displayed a more homogeneous ability to invade IPECJ2 cells with percentages varying from 10% to 26%. The BT4 strains displayed a great ability to adhere to the stainless steel surface, percentage of adhesion varying from 0.3% to 4.2%. No correlation was observed between IPEC-J2 cell adhesion, cell invasion and adhesion to the stainless steel surface ($R^2 < 0.02$). In conclusion, these results reflect the ability of the different BT4 strains to colonize the intestinal tract of pigs and to contaminate the stainless steel surfaces of the food processing environment.

Introduction

Yersiniosis is, after campylobacteriosis and salmonellosis, the third most frequently reported zoonosis in Europe (EFSA and ECDC 2016). Humans become infected with *Y. enterocolitica* through the consumption of undercooked pork meat and raw food having been in contact with contaminated surfaces (Bottone 1999). The ability of *Y. enterocolitica* to infect humans may depend on their capability to colonize pigs and to develop biofilm on conventional materials used in food industries. In France and worldwide, biotype 4 (BT4) is the biotype the most frequently isolated from both pigs and clinical yersiniosis (Fondrevez et al. 2014; Le Guern et al. 2016). In this study, a collection of 26 pathogenic BT4 strains isolated from pig tonsils were investigated for their ability to adhere and invade intestinal pig cells (IPEC-J2) and to adhere to abiotic surfaces (stainless steel coupons) using two *in vitro* tests.

Material and methods

IPEC-J2 cells assay

A suspension of 2.10^7 CFU *Y. enterocolitica* bacteria were added to the 2.10^5 Porcine IPEC-J2 cells, then the plates were incubated at 37°C in the presence of 5% CO_2. Three hours following infection, the cells were washed extensively with PBS (Phosphate Buffered Saline). The total number of adherent bacteria was determined by cell lysis using 0.1% Triton X-100 and plating on a bacterial agar medium. Bacterial uptake was assessed by adding 100µg of gentamicin per well. After two hours of incubation. The percentage of bacteria that survived killing was determined after plating on a bacterial agar medium. For each strain, the relative level of bacterial adhesion and uptake was determined by calculating the number of CFU relative to the total number of bacteria introduced into cells.

Stainless steel assay

The INOX coupons were contaminated by immersing them in a saline solution (0.9%) containing 10^6 CFU of *Y. enterocolitica* bacteria during 6h at 12°C. Non-adherent cells were removed by successive soaking in sterile saline solution (0.9%). Adherent bacteria were detached from the coupon by a 40s-vortex and a 2 min-sonication steps. The adherent bacteria were then enumerated after plating on a bacterial agar medium. For each strain, the relative level of bacterial adhesion was determined by calculating the ratio of adherent cells to the total number of bacteria in the immersion suspension.

Statistical analysis

Regression analysis was performed between data sets obtained from IPEC-J2 cells assays *versus* stainless steel assays by using logiciel R (R Development Core Team, 2015).

Results

All BT4 strains were able to adhere and invade IPEC-J2 cells. However, the results showed heterogeneity between strains with respect to their ability to adhere to IPEC-J2 cells, with a percentage of adhesion varying from 9% to more than 90%. The BT4 population displayed a more homogeneous ability to invade IPEC-J2 cells with percentages varying from 10% to 26%. The BT4 strains displayed a great ability to adhere to the stainless steel surface, with a percentage of adhesion varying from 0.3% to 4.2%.

No correlation was observed between IPEC-J2 cell adhesion, cell invasion and adhesion to the stainless steel surface ($R^2 < 0.02$).

Discussion

Y. enterocolitica has been reported to colonize pigs. The ability of *Y. enterocolitica* BT4 strains to adhere and invade epithelial cells is crucial for host colonization. The 26 strains studied were able to adhere and enter the intestinal porcine cells *in vitro*. This observation is in accordance with the fact that pigs are considered a reservoir for human pathogenic BT4 strains (Fondrevez et al. 2014; Le Guern et al. 2016). In IPEC-J2 cells assay, the adhesion profile was different from the invasion profile. The percentage of adhesion reached about 90% *versus* a maximal percentage of invasion of only 26%, regardless of the strain tested. These results may support the fact that *Y. enterorocilita* BT4 is able to colonize pork asymptomatically. *In vivo*, pigs do not develop clinical signs, but they do carry pathogenic *Y. enterocolitica* in their oral cavity and excrete the bacterium in their feces (Thibodeau et al. 1999).

Various bacteria including food spoilage bacteria and pathogens can form biofilms on different food processing surfaces, leading to potential food contamination or spoilage. All the *Y. enterocolitica* BT4 strains exhibited the ability to adhere to the stainless steel surface. This observation is in concordance with the study of Allan *and al*. which reported the fact that like *L. monocytogenes* and *Salmonella*, *Y. enterocolitica* strains are able to form biofilm on stainless steel surface (Allan, Yan, and Kornacki 2004).

Some cellular components, like the protein OmpR, seem to be involved in adhesion-invasion capacities and biofilm formation (Raczkowska et al. 2011). Pleiotropic effect of a component can be highlighted by demonstrating a correlation between IPEC-J2 cell adhesion, cell invasion and adhesion to the stainless steel surface at the cell level. In the present study, no correlation between IPEC-J2 cell adhesion, cell invasion and adhesion to the stainless steel surface was observed, for the 26 strains studied.

Conclusion

These results reflect the ability of the different BT4 strains to colonize the intestinal tract of pigs and to contaminate the stainless steel surfaces of the food processing environment.

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