Use of fluorescence quantitative polymerase chain reaction (PCR) for the detection of *Escherichia coli* adhesion to pig intestinal epithelial cells

C.H. Dai¹, L.N. Gan¹, W.U. Qin¹, C. Zi¹, G.Q. Zhu², S.L. Wu¹, W.B. Bao¹

¹ College of Animal Science and Technology, Key Laboratory for Animal Genetics, Breeding, Reproduction and Molecular Design of Jiangsu Province, Yangzhou University, Yangzhou, China
² College of Veterinary Medicine, Yangzhou University, Yangzhou, Jiangsu, China

Abstract

An efficient and accurate method to test *Escherichia coli* (E. coli) adhesion to intestinal epithelial cells will contribute to the study of bacterial pathogenesis and the function of genes that encode receptors related to adhesion. This study used the quantitative real-time polymerase chain reaction (qPCR) method. qPCR primers were designed from the *PILIN* gene of *E. coli* F18ab, F18ac, and K88ac, and the pig *β-ACTIN* gene. Total deoxyribonucleic acid (DNA) from *E. coli* and intestinal epithelial cells (IPEC-J2 cells) were used as templates for qPCR. The $2^{-\Delta\Delta C_T}$ formula was used to calculate the relative number of bacteria in cultures of different areas. We found that the relative numbers of F18ab, F18ac, and K88ac that adhered to IPEC-J2 cells did not differ significantly in 6-, 12-, and 24-well culture plates. This finding indicated that there was no relationship between the relative adhesion number of *E. coli* and the area of cells, so the method of qPCR could accurately test the relative number of *E. coli*. This study provided a convenient and reliable testing method for experiments involving *E. coli* adhesion, and also provided innovative ideas for similar detection methods.

Key words: Adhesion, *Escherichia coli*, intestinal epithelial cells, pig, quantitative real-time PCR

Introduction

Enterotoxigenic *Escherichia coli* (ETEC), one of the main pathogens that cause diarrhea in piglets, which seriously threatens the economic benefits of scale pig farms with high mortality (Turner et al. 2006, Zhang et al. 2007). The pathogenic *Escherichia coli* (*E. coli*) can adhere to pig intestinal epithelial cells (IPEC-J2 cells) in a process mediated by fimbriae, then secrete enterotoxin and ultimately lead to edema and diarrhea in piglets (Bertschinger et al. 1972, Hohmann et al. 1975, Imberechtset al. 1994). Common enterotoxigenic *E. coli* bacillosis in scale pig farms are mainly caused by F18 and K88. Studies showed that whether they could cause disease in piglets depended upon the existence of specific receptors for adhesion.