Available online at www.sciencedirect.com
ScienceDirect
Veterinary Microbiology 127 (2008) 106–115
veterinary microbiology
www.elsevier.com/locate/vetmic

An *Actinobacillus pleuropneumoniae arcA* deletion mutant is attenuated and deficient in biofilm formation

Falk F.R. Buettner, Alexander Maas, Gerald-F. Gerlach *

Institute for Microbiology, Department of Infectious Diseases, University of Veterinary Medicine Hannover, 30173 Hannover, Germany

Received 3 May 2007; received in revised form 6 August 2007; accepted 7 August 2007

Abstract

Actinobacillus pleuropneumoniae is a facultative anaerobic pathogen of the porcine respiratory tract requiring anaerobic metabolic activity for persistence on lung epithelium. The ArcAB two-component system facilitating metabolic adaptation to anaerobicity was investigated with regard to its impact on virulence and colonization of the porcine respiratory tract. Using pig infection experiments we demonstrate that deletion of *arcA* renders *A. pleuropneumoniae* significantly attenuated in acute infection and reduced long-term survival on unaltered lung epithelium as well as in sequesters. Contrary to its role in enterobacteria, the deletion of *arcA* in *A. pleuropneumoniae* does not affect growth and survival under anaerobic conditions. Instead, other than the parent strain *A. pleuropneumoniae* Δ*arcA* does not show autoaggregation under anaerobic conditions and is deficient in biofilm formation. It is hypothesized that the lack of these functions is, at least in part, responsible for the reduction of virulence.

Keywords: *Actinobacillus pleuropneumoniae*; Virulence; *arcA*; Biofilm

1. Introduction

Actinobacillus pleuropneumoniae is the causative agent of a porcine pleuropneumonia causing high economic losses worldwide (Fenwick and Henry, 1994). After inhalation of *A. pleuropneumoniae* containing aerosols the pathogen colonizes the respiratory epithelium of its host and persists on tonsils, on healthy lung epithelium, and in sequestered lesions (Sidibe et al., 1993). Our previous studies implied that the ability to adapt to anaerobic conditions is crucial for the development of persistent infections; thus, the deletion of *hlyX*, the homologue to the global regulator for anaerobic metabolism FNR in *Escherichia coli* (Shaw and Guest, 1982), caused a clear attenuation of *A. pleuropneumoniae* (Baltes et al., 2005).

The ArcAB two-component system, like FNR, is a global regulatory mechanism facilitating the adaptation to a changing redox potential and anaerobic

* Corresponding author at: Institut fuer Mikrobiologie, Zentrum fuer Infektionsmedizin, Stiftung Tieraerztliche Hochschule Hannover, Bischofsholer Damm 15, 30173 Hannover, Germany. Tel.: +49 511 856 7598; fax: +49 511 856 7697.
E-mail address: gfgerlach@gmx.de (G.-F. Gerlach).

0378-1135/$ – see front matter © 2007 Elsevier B.V. All rights reserved.
doi:10.1016/j.vetmic.2007.08.005

condition in enterobacteria (Iuchi and Lin, 1988). The ArcAB system is composed of the transmembrane sensor kinase ArcB and the cytosolic response regulator ArcA (Iuchi and Lin, 1995; Lynch and Lin, 1996). *Salmonella arcA* mutants have been shown to grow more slowly than the wild-type strain in both aerobic and microaerobic conditions (Sevcik et al., 2001), and *E. coli arcA* mutants are unable to survive prolonged starvation (Nystrom et al., 1996). For *Haemophilus influenzae* deletion of *arcA* is accompanied with attenuation in septic infection (Souza-Hart et al., 2003), and in the intestinal pathogen *Vibrio cholerae arcA* was found to modulate the expression of virulence factors (Sengupta et al., 2003).

The role of *arcA* in an extracellular respiratory tract pathogen has not been investigated to date. Therefore, we constructed an isogenic *A. pleuropneumoniae arcA* deletion mutant, investigated its growth under aerobic and anaerobic conditions, and determined its virulence in a pig aerosol infection model.

2. Materials and methods

2.1. Bacterial strains, plasmids, and primers

Bacterial strains, plasmids, and primers used in this work are listed in Table 1.

2.2. Media and growth conditions

E. coli strains were cultured in Luria–Bertani medium supplemented with the appropriate antibiotics (ampicillin, 100 μg/ml; chloramphenicol, 25 μg/ml) at 37 °C; for cultivation of *E. coli* 2155 (Δ*dapA*), diaminopimelic acid (1 mM; Sigma Chemical Company, Deisenhofen, Germany) was added. *A. pleuropneumoniae* parent and mutant strain were cultured at 37 °C and 5% CO_2 in PPLO medium or on PPLO agar (Difco GmbH, Augsburg, Germany) supplemented with NAD (10 μg/ml; Merck AG, Darmstadt, Germany), L-cysteine hydrochloride (260 μg/ml; Sigma), L-cystine dihydrochloride (10 μg/ml; Sigma), and dextrose (1 mg/ml). The aerobic culture grown for experimental aerosol infection in addition contained Tween®80 (0.1%). For selection of *A. pleuropneumoniae* transconjugants (single crossing-overs) chloramphenicol (5 μg/ml) was added, and the medium for counter selection was prepared as described previously (Tonpitak et al., 2002). For cultivation of the complemented mutant kanamycin (25 μg/ml) was added.

For anaerobic growth supplemented medium was preincubated in an anaerobic chamber (DonWhitley Scientific, Shipley, England) in an atmosphere containing 5% CO_2, 10% H_2, and 85% N_2 at 37 °C, and then inoculated with 1% of log phase aerobic culture with an optical density at 600 nm (OD_{600}) of 0.3. Anaerobicity of the medium was confirmed using a dissolved oxygen sensor (CellOx® 325, WTW, Weilheim, Germany) linked to an inoLab® instrument (WTW, Weilheim, Germany). For recording of growth curves bacteria were harvested at 2, 4, 6, 8, 10 and 24 h post-inoculation by centrifugation. As *A. pleuropneumoniae* showed heavy clumping upon growth under anaerobic conditions the protein content of whole-cell lysates was determined. Statistical analyses were performed using the Student's *t*-test.

2.3. Preparation of whole-cell lysates

Cells were harvested by centrifugation (13,000 × *g*, 10 min, 4 °C), washed once with a buffer containing 10 mM Tris–HCl (pH 8.0) and 5 mM magnesium acetate. Pellets were resuspended in 50 mM Tris–HCl (pH 7.3) and stored at −70 °C overnight. Cells were thawed; ruptured using the FastPrep® Instrument (Qbiogene, Heidelberg, Germany) three times for 40 s at intensity setting 5.0, and the cell debris was removed by centrifugation (15,000 × *g*, 30 min). Protein concentration was determined with the MicroBC® assay (Uptima Interchim, Montlucon Cedex, France).

2.4. Manipulation of DNA

DNA modifying enzymes were purchased from New England Biolabs (Bad Schwalbach, Germany) and used according to the manufacturer's instructions. Taq polymerase was purchased from Gibco-BR Life Technologies (Karlsruhe, Germany). Chromosomal and plasmid DNA preparations, PCR, Southern blotting, transformation, and gel electrophoresis were done by standard procedures (Sambrook et al., 1989), and pulsed field gel electrophoresis (PFGE) was performed as described previously (Oswald et al., 1999a).