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Short communication

Sensitivity of *Chlamydia suis* to cathelicidin peptides

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Abstract

Nine *Chlamydia suis* isolates, obtained from pigs with conjunctivitis, were molecularly characterized by *ompA* sequencing and their *in vitro* susceptibility to six cathelicidin peptides (SMAP-29, BAC-7, BMAP-27, BMAP-27, BMAP-28, PG-1, LL-37) determined in cell culture. SMAP-29 was the most active peptide, reducing the intracellular inclusion number by $\geq$50% at a concentration of 10 μg/ml (3 μM) in six of the nine isolates tested. Three molecularly identical isolates were insensitive at a concentration as high as 80 μg/ml (25 μM). Of the remaining cathelicidin peptides tested, BAC-7 and BMAP-27 were active against six *C. suis* isolates at a concentration of 80 μg/ml (25 and 26 μM, respectively). Cathelicidins LL-37 and PG-1 did not show any anti-chlamydial activity at 80 μg/ml.

Keywords: *Chlamydia suis*; Cathelicidin peptides

1. Introduction

Chlamydia suis cause conjunctivitis, pneumonia, enteritis, genital tract diseases and a high incidence of apparently asymptomatic infections in swine; the high incidence of *C. suis* in enteric porcine specimens indicates that it may be endemic in pig herds (Longbottom, 2004).

Several studies have reported that granular protein extracts from mammalian polymorphonuclear leukocytes inactivate *Chlamydia* spp. (Register et al., 1987): these antimicrobial peptides include defensins and

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cathelicidins. The cathelicidin peptides are heterogeneous in size and sequence and exhibit marked structural diversity (Gennaro and Zanetti, 2000). Previous studies have investigated the antimicrobial activity (Frank et al., 1990; Agerberth et al., 1995; Skerlavaj et al., 1999) of cathelicidin peptides against bacteria, fungi and protozoa. Recently, cathelicidin peptides have been tested against *Chlamydia* isolated from humans (Yasin et al., 1996a,b; Turner et al., 1998; Donati et al., 2005) as well as from various animal species (Donati et al., 2005), but not from pigs. The susceptibility of *Chlamydia* to cathelicidin peptides differs with *C. trachomatis* showing far more sensitive to antimicrobial peptides than chlamydiae isolated from animals (Donati et al., 2005). In the present study, we investigated the *in vitro* activity of six cathelicidin peptides against nine *C. suis* isolates.

2. Materials and methods

2.1. Origin of samples

Nine *C. suis* isolates (MS04 and MS06 1–8) were collected from conjunctival swab specimens obtained from pigs with conjunctivitis from different herds in Northern Italy.

2.2. C. suis DNA molecular analysis

DNA of *C. suis* isolates grown in LLC-MK2 cells was extracted for molecular analysis employing a commercially available kit (Tissue Kit, Qiagen, Düsserldorf, Germany) and used as a template for an 1050-bp *ompA* gene fragment amplification (Sayada et al., 1995).

The amplicons were purified using a commercially available kit (QIAquick PCR purification kit; Qiagen) and sequenced. Nucleotide sequences were compared with the same regions of the *C. suis* type strain S45 available in the GenBank database using BLAST software.

2.3. In vitro testing of cathelicidin peptides

To determine the activity of the cathelicidin peptides, *C. suis* isolates were grown in LLC-MK2 cells (Donati et al., 2005) on 24-well plates with a glass coverslip at the bottom. Chlamydial elementary bodies were purified using sucrose gradients (Moroni et al., 1996), resuspended in 0.2 M sucrose/phosphate/glutamic acid (SPG) and frozen in 0.5 ml aliquots at −70 °C.

The six cathelicidin peptides – SMAP-29 from sheep, BAC-7, BMAP-27 and BMAP-28 from cattle, PG-1 from pigs and LL-37 from humans – were chemically synthesized, purified, characterized and provided as lyophilized peptides, as previously reported (Donati et al., 2005). To determine the lowest peptide concentration required to achieve ≥50% reduction in chlamydial inclusions with respect to untreated controls, individual peptides were diluted two-fold with SPG, from 80 to 2.5 μg/ml in a volume of 150 μl in polypropylene tubes and added to an equal volume of 10^6 IFU/ml of elementary bodies in SPG medium. After incubation at room temperature for 2 h, a 100 μl aliquot from each sample was inoculated in triplicate into LLC-MK2 cells grown on 24-well plates. After centrifugation at 800 × *g* for 1 h at 33 °C and incubation at 35 °C for 48 h, the cultures were fixed and stained for the presence of chlamydial inclusions by immunofluorescence with a fluorescein-conjugated monoclonal antibody specific for the chlamydial LPS genus-specific antigen, as previously described (Donati et al., 2002).

3. Results and discussion

The *ompA* amplification showed the expected products. Alignment of the deduced amino acid sequences of the MOMP protein of the isolates with the same sequence of the reference *C. suis* S45 strain revealed an amino acid homology of 83–88%. Most of the differences were clustered in the region of the variable segments (VS) I–IV of the *ompA* gene locus (Fig. 1), in agreement with previous data (Kaltenboeck et al., 1997; Hoelzle et al., 2000). In particular, the amino acid sequences of the MS06 2, 4 and 6 isolates were identical; the sequences of MS06 3, 5 and 8 also showed 100% homology. The amino acid sequences of MS06 1, 7 and MS04 differed individually.

The activities of the six cathelicidin peptides tested against *C. suis* isolates are shown in Table 1. Of the six cathelicidin peptides tested, SMAP-29 was the most

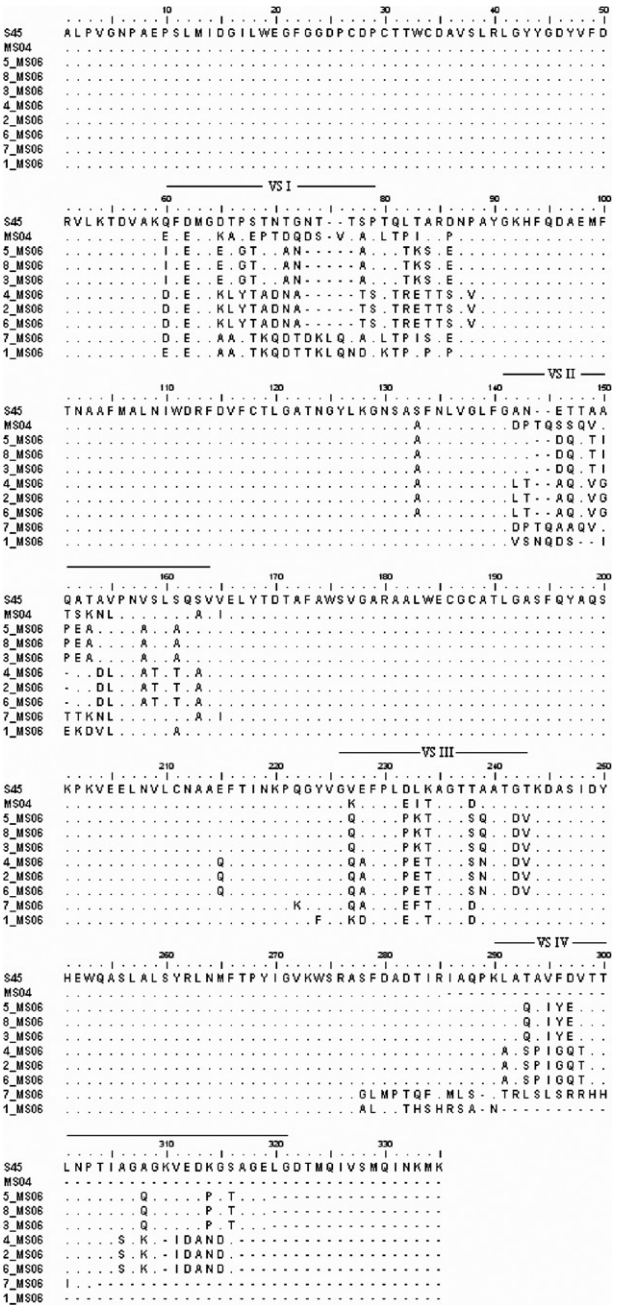

Fig. 1. Alignment of deduced amino acid sequence of *ompA* PCR products from nine *C. suis* isolates with reference strain *C. suis* S45.

active compound against *C. suis* strains, reducing the inclusion numbers by $\geq$50% in six of nine isolates at a concentration of 10 μg/ml, whereas BAC-7 and BMAP-27 inhibited replication of the same *C. suis* isolates at a concentration of 80 μg/ml. BMAP-28, PG-1 and LL-37 had no inhibitory effect on these isolates even at a concentration of 80 μg/ml. Three isolates, MS06 3, 5 and 8, molecularly identical, were

Table 1
Activity of cathelicidin peptides against nine *Chlamydia suis* isolates

Strains	Peptide concentration (μg/ml (μM)) reducing chlamydial inclusion by ≥50%					
	SMAP-29	BAC-7	BMAP-27	BMAP-28	PG-1	LL-37
MS04	10 (3)	80 (19)	80 (>25)	>80 (>26)	>80 (>37)	>80 (>18)
5MS06	>80 (>25)	>80 (>19)	>80 (>25)	>80 (>26)	>80 (>37)	>80 (>18)
8MS06	>80 (>25)	>80 (19)	>80 (25)	>80 (>26)	>80 (>37)	>80 (>18)
3MS06	>80 (>25)	>80 (>19)	>80 (>25)	>80 (>26)	>80 (>37)	>80 (>18)
4MS06	10 (3)	80 (19)	80 (25)	>80 (>26)	>80 (>37)	>80 (>18)
2MS06	10 (3)	80 (19)	80 (25)	>80 (>26)	>80 (>37)	>80 (>18)
6MS06	10 (3)	80 (19)	80 (25)	>80 (>26)	>80 (>37)	>80 (>18)
7MS06	10 (3)	80 (19)	80 (25)	>80 (>26)	>80 (>37)	>80 (>18)
1MS06	10 (3)	80 (19)	80 (25)	>80 (>26)	>80 (>37)	>80 (>18)

not sensitive to cathelicidins at a concentration as high as 80 μg/ml.

In a previous study (Donati et al., 2005), we comparatively analyzed the action of cathelicidin peptides against several *Chlamydia* spp., including chlamydiae from men (*C. trachomatis* and *C. pneumoniae*) and chlamydiae (*C. psittaci*, *C. pecorum*, *C. abortus, C. felis*) isolated from animals other than pigs. *Chlamydia* showed different susceptibilities, with *C. trachomatis* showing far greater sensitive to antimicrobial peptides (SMAP-29, BAC-7, BMAP-27, BMAP-28) than *C. pneumoniae*; SMAP-29 was the most active peptide, being active against all *C. trachomatis* strains tested at a concentration of 10 μg/ml. Animal chlamydiae were not sensitive; the only exception being four *C. felis* isolates that were partially susceptible to BAC-7 and SMAP-29 at a concentration of 80 μg/ml. In this study, six *C. suis* isolates were sensitive to SMAP-29 at a concentration of 10 μg/ml, and to BAC-7 and BMAP-27 at 80 μg/ml, whereas the remaining three isolates were insensitive to all cathelicidins tested. Therefore, the majority of *C. suis* isolates were sensitive to cathelicidins, in particular to SMAP-29, showing a sensitivity approaching that of human *C. trachomatis* isolates (Donati et al., 2005). This observation is not unexpected, since *C. suis* strains were previously referred to as *C. trachomatis*. Even if porcine strains are genetically different to human strains, a close relationship between *C. suis* and *C. trachomatis* is indicated by the *ompA* DNA sequence homology (Kaltenboeck et al., 1997), together with morphology and other characteristics, such as the production of glycogen by *C. suis* in cell culture (Rogers et al., 1996). It has been also reported that several *C. suis* strains possess a plasmid (Everett, 2000). This close biological relationship is further confirmed by the results of this study in relation to sensitivity against cathelicidin antimicrobial peptides. Finally, it is of interest to note that the three *C. suis* isolates insensitive to cathelicidins were molecularly identical compared to the sensitive isolates, which showed nucleotide differences, mostly clustered in the region of the VS I to IV of the *ompA* gene locus. This observation deserve further study to evaluate whether these genetic differences could be correlated with insensitivity to cathelicidin peptides.

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