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Short communication

Comparative analysis of different methods to enhance porcine circovirus 2 replication

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A B S T R A C T

Porcine circovirus 2 (PCV2) is an extremely slow-growing virus, and PCV2 infection and replication in cell culture yield very low viral titers. The effects of different methods of PCV2 cultivation in vitro were compared with the purpose of increasing viral yield. The results showed that treatment with IL-2, ConA, and D-glucosamine increased PCV2 yield more effectively than other treatments. Additionally, treatment with IL-2, ConA, D-glucosamine and MβCD consistently increased PCV2 infection in PK-15 cells during consecutive viral passages. A combinatorial treatment with ConA, MβCD and D-glucosamine increased PCV2 yield significantly in PK-15 cells, to 1.81×10^{10} genome copy numbers per mL of cell lysate at 72 hpi, and the viral titer (−lg TCID50/100 μL) was 8.6. The results of this study may be helpful for the investigation of PCV2 replication and the production of a PCV2 vaccine.

Porcine circovirus 2 (PCV2) has been identified as the etiological agent of postweaning multisystemic wasting syndrome (PMWS) (Chae, 2005; Finsterbusch and Mankertz, 2009). It is an extremely slow-growing virus, and PCV2 infection and replication in cell culture yield very low viral titers (Allan et al., 1998). During productive infection, it has been shown that viral antigens, RNA transcripts and progeny viruses all increase in a time-dependent manner (Cheung and Bolin, 2002). The PK-15 cell line, which is widely used for PCV2 propagation, does not undergo efficient viral infection (Zhu et al., 2007). The results of previous studies have shown that PCV2 propagation in cell culture was not efficient, and the virus isolated from the samples cannot be passaged more than 3 times (data not shown). Different methods have been described to increase the viral yield (Meerts et al., 2005; Tischer et al., 1987; Lefebvre et al., 2008; Misinzo et al., 2008a,b, 2009; Ramamoorthy et al., 2009; Beach et al., 2010). PCV multiplication was found to be inducible by treating infected cell cultures with 300 mM glucosamine (Tischer et al., 1987). Misinzo et al. (2009) reported that PCV2 infection of epithelial cells is actin- and Rho-GTPase-mediated and enhanced by cholesterol depletion. Stimulation of the porcine immune system or PBMC was shown to increase replication of PCV2 (Meerts et al., 2005; Lefebvre et al., 2008). It was also demonstrated that IFN-alpha and IFN-γ influenced PCV2 infection in PK-15 cells (Meerts et al., 2005), and combined IFN-γ and endosomal–lysosomal system acidification inhibitors increased PCV2 yields by up to 50 times compared to untreated PK-15 (Misinzo et al., 2008a). To find a better way to enhance PCV2 yield, the effects of different methods of PCV2 cultivation were compared in the present study. The results of this study may be helpful for the investigation of PCV2 replication and the production of PCV2 vaccines.

PCV-free PK-15 cells were plated onto 25 cm^2 cell culture flasks and cultured in Dulbecco's modified Eagle's medium (DMEM, GIBCO) supplemented with 2% fetal bovine serum (PAA) and incubated at 37 °C in an atmosphere with 5% CO_2. A total of 40–50% confluent PK-15 cells were infected with PCV2 strain CC1 (GenBank accession number: JQ955679) (Yang et al., 2012).

PCV2 DNA from infected PK-15 cells was extracted and purified with the TIANamp Virus DNA/RNA Kit (Tiangen Biotech, China) according to the manufacturer's protocol. Genomic DNA was dissolved in 50 μL of DNase-free ddH_2O and stored at −20 °C. Quantitative real-time PCR was performed in a BIO-RAD IQ™5 Multicolor Real-Time PCR Detection System using a BioEasy SYBR Green I Real-Time PCR Kit (BIOER, China). The amplification reaction was performed as follows: 1 cycle at 95 °C 3 min, then 40 cycles at 95 °C 10 s, 55 °C 15 s and 72 °C 30 s. The primers used in this study were described by Shen et al. (2008). Samples were amplified at least three times. In all cases, cells and supernatants were frozen and thawed, and the cell debris was removed by low-speed centrifugation at 24, 36, 48, 60 and 72 h post treatment. All experiments were performed three times for each condition.

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To evaluate PCV2 virus titrations in PK-15 cells, virus was collected and serially diluted 10-fold from 10^{-9} to 10^{-1}. At 50% confluency, PCV-free PK-15 cells in 96-well plates were infected and cultured at 37 °C in an atmosphere with 5% CO_2. Each dilution was replicated eight times and was examined for viral proliferation by immunofluorescence assay (IFA) at different time points. IFAs were performed according to McNeilly et al. (2002). Briefly, the cells in 96-well plates were infected with PCV2 for 24, 36, 48, 60 and 72 h and fixed with 80% ice-cold acetone for 30 min. The cells were then washed three times with PBS and incubated for 1 h with Porcine Circovirus Type 2 Direct FA Conjugate (VMRD, Inc.) at 37 °C. After another three PBS washes and the addition of 10% glycerol, the cells were examined using an Eclipse TE2000-V (Nikon) microscope. The TCID50 values were then calculated according to the method of Reed–Muench.

To compare the effects of different methods for PCV2 replication, PK-15 cells were treated with D-glucosamine (glucosamine, Sigma), methyl-beta-cyclodextrin (MβCD, Sigma), concanavalin A (ConA,

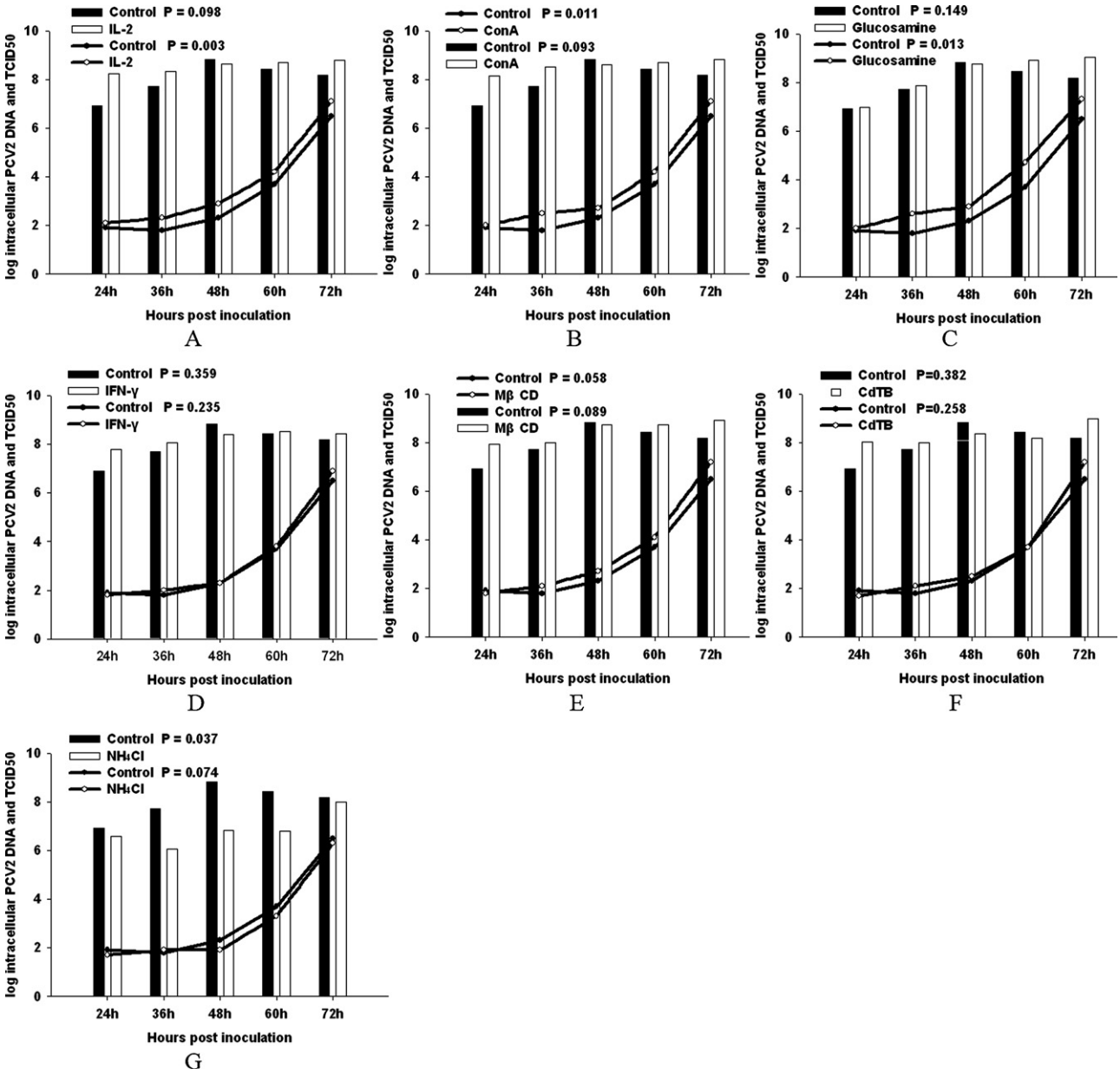

Fig. 1. Kinetics of PCV2 replication in untreated PK-15 cells and PK-15 cells treated with IL-2 (A), ConA (B), D-glucosamine (C), IFN-γ (D), MβCD (E), CdTB (F), or NH_4Cl (G). For D-glucosamine, cells were incubated with 300 mM D-glucosamine at 21 h post-inoculation (hpi) for 30 min at 37 °C. The cells were then washed and cultured in DMEM supplemented with 10% fetal bovine serum (FBS) until they reached 100% confluence. Then, cells were cultured in fresh DMEM supplemented with 2% fetal bovine serum. For treatment with NH_4Cl, the medium was aspirated at 1 hpi, and cells were incubated in DMEM with or without 25 mM NH_4Cl in a 5% CO_2 incubator at 37 °C. Cell culture medium was then replaced with fresh DMEM containing 2% FBS at 24 hpi. For MβCD, cells were incubated in fresh medium for 2.5 h at 1 hpi. Then, cells were treated with or without 2.5 mM MβCD for 2 h, washed and cultured in fresh DMEM containing 2% FBS. For IL-2, ConA and IFN-γ, cell culture medium with or without IL-2 (0.5 ng/mL), ConA (5 μg/mL), and IFN-γ (500 U/mL) was added to cells at 6 h post-seeding. Eighteen hours later, cells were washed and inoculated with PCV2 strain CC1 for 1 h. Cells were again washed and cultured in fresh DMEM (with IL-2, ConA or IFN-γ, respectively) containing 2% FBS. For CdTB, PK-15 cells were washed, incubated for 4 h in DMEM with CdTB (12.5 ng/mL) in a 5% CO_2 incubator at 37 °C, washed again and inoculated with PCV2 strain CC1 for 1 h. Following another wash step, cells were then cultured in fresh DMEM containing 2% FBS. At 24, 36, 48, 60 and 72 h post-infection, cells were harvested. Viral titers were determined by IFA in PK-15 cells. The results are represented as the mean values from eight experimental replicates. Intracellular PCV2 DNA was also detected by real-time PCR. The line plot indicates TCID50 (−lg TCID50), and the bar chart indicates PCV2 DNA (log 10).