# Eukaryotic Expression of Porcine Interleukin-17 and Its Biological Activity: Postprint

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## Abstract

Objective: To achieve eukaryotic expression of porcine interleukin-17 (IL-17) and investigate the immunobiological activity of the expressed product in cell culture.

Methods: The porcine IL-17 gene was amplified by PCR and inserted into the eukaryotic expression vector pVAV1, followed by transfection into IPEC-J2 cells, HaCaT cells, and L02 cells. Cells were harvested at 24, 48, and 72 hours post-transfection, while supernatant was collected at 48 hours. Harvested cells were subjected to real-time quantitative PCR to detect expression levels of related immune genes, and the collected supernatant was used in antibacterial assays to assess the biological activity of related antimicrobial peptides.

Results: A recombinant plasmid expressing porcine IL-17 was constructed using the pVAX1 vector and transfected into cells. The IL-17 gene was confirmed to induce expression of antimicrobial peptide genes (RegIII, S100A8, and BD2), and significantly upregulate JAK-STAT signaling pathway genes (JAK1, STAT1, and STAT3) as well as cytokine genes (IL-6, IL-12, and TNF- ). Furthermore, the cell culture supernatant inhibited proliferation of Escherichia coli and Staphylococcus aureus to varying degrees.

Conclusion: Porcine IL-17 was successfully expressed in eukaryotic cells, and its product could induce effector cells to express multiple cytokines, produce various antimicrobial peptides, and exhibit antibacterial activity; this establishes a preliminary foundation for further development of porcine IL-17 as an antibacterial immune molecular agent.

## Full Text

# Eukaryotic Expression of Porcine Interleukin-17 and Its Biological Activity

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## Abstract

**Objective:** To achieve eukaryotic expression of porcine interleukin-17 (IL-17) and investigate the immunobiological activity of the expressed product in cell culture. **Methods:** The porcine IL-17 gene was amplified by PCR and inserted into the eukaryotic expression vector pVAX1. The recombinant plasmid was then transfected into IPEC-J2 cells, HaCaT cells, and L02 cells. Cells were harvested at 24, 48, and 72 hours post-transfection, while supernatants were collected at 48 hours. Gene expression levels were quantified by real-time fluorescence quantitative PCR, and the biological activity of antimicrobial peptides in the supernatants was assessed through antibacterial assays. **Results:** The recombinant plasmid expressing porcine IL-17 was successfully constructed using the pVAX1 vector and transfected into target cells. The IL-17 gene significantly induced expression of antimicrobial peptide genes (Reg , S100A8, and BD2), markedly upregulated JAK-STAT signaling pathway genes (JAK1, STAT1, and STAT3), and elevated cytokine genes (IL-6, IL-12, and TNF- ). Additionally, the cell supernatants inhibited proliferation of *Escherichia coli* and *Staphylococcus aureus* to varying degrees. **Conclusion:** Porcine IL-17 was successfully expressed in eukaryotic cells, and its product induced effector cells to express multiple cytokines and produce various antimicrobial peptides with demonstrable antibacterial capacity. These findings establish a preliminary foundation for further development of porcine IL-17 as an antimicrobial immunomolecular agent.

**Keywords:** Porcine; Interleukin-17; Cell transfection; Immunity; Antibacterial effect

## Introduction

Interleukin-17 (IL-17) is a multifunctional pro-inflammatory cytokine primarily secreted by Th17 cells, monocytes, and neutrophils [1]. In 1993, Rouvier et al. first cloned the CTLA8 cDNA sequence from activated T-cell hybridomas, and its associated protein was designated IL-17 [2]. The IL-17 receptor (IL-17R) is widely distributed throughout the body and expressed at varying levels in diverse cell types including intestinal epithelial cells, fibroblasts, muscle cells,

and hepatocytes [3]. Upon binding to its receptor, IL-17 activates signaling pathways mediated by JAK-STAT, NF- B, and MAP kinases [4-5].

IL-17 plays crucial roles in inflammatory diseases, autoimmune disorders, tumor immunity, innate immunity, and host defense [6-7]. It induces various cells to express pro-inflammatory factors such as IL-6, IL-8, and TNF- , as well as intercellular adhesion molecules (ICAM-1), thereby exerting its biological effects and promoting cell proliferation and angiogenesis [8-9]. IL-17 participates in neutrophil-mediated inflammatory responses by recruiting neutrophils to inflammatory sites, serving as an important bridge connecting innate and adaptive immunity [10]. It also acts directly on tissue epithelial cells to induce immune responses against pathogens and promote tissue repair [11]. Furthermore, IL-17 is essential for intestinal homeostasis by maintaining the integrity of the intestinal mucosal barrier [12].

Research has demonstrated that IL-17 acts on various epithelial or keratinocyte cells to induce production of multiple cytokines, chemokines, and cell adhesion molecules, subsequently activating inflammatory responses and generating diverse antimicrobial peptides to control and eliminate pathogenic invasion, thereby maintaining organismal homeostasis [13]. However, most IL-17 gene studies have focused on humans and laboratory mice [14-16], with limited research on the biological activity of porcine IL-17 [17-18]. Therefore, this study aimed to clone the porcine IL-17 gene, construct its pVAX1 eukaryotic expression vector, and transfect appropriate target cells—porcine intestinal epithelial cells (IPEC-J2), human immortalized epidermal cells (HaCaT), and human normal liver cells (L02)—to investigate its immunobiological activity and the antibacterial activity of induced antimicrobial peptides, providing a foundation for developing novel molecular biological agents for animal disease prevention and anti-infection.

## Materials and Methods

### 1.1.1 Plasmids, Strains, and Cells

The eukaryotic expression vector pVAX1-porcine fusion interleukin-17/22 (PVIL17/22) engineering bacteria and pVAX1 vector engineering bacteria were preserved in our laboratory. Trans-T1 Phage Resistant chemically competent *E. coli* was purchased from Beijing TransGen Biotech. Standard *E. coli* strain (ATCC 15306), drug-resistant *E. coli*, standard *Staphylococcus aureus* strain (ATCC 25923), and drug-resistant *S. aureus* were preserved in our laboratory. L02 cells were kindly provided by Researcher Wang Gang from the National Biomedical Materials Engineering Research Center at Sichuan University. IPEC-J2 cells were kindly provided by Professor Yu Bing from the Animal Nutrition Institute of Sichuan Agricultural University. HaCaT cells were preserved in our laboratory.

### 1.1.2 Reagents

Plasmid extraction kits and gel recovery kits were purchased from OMEGA Bio-Tek. Phanta high-fidelity DNA polymerase was obtained from Vazyme Biotech. 100 bp DNA Ladder, 2K Plus DNA Marker, and TransScript One-Step gDNA Removal and cDNA Synthesis SuperMix were purchased from TransGen Biotech. Restriction endonucleases Kpn and Xho , T4 DNA ligase, Lipo3000 DNA transfection reagent, and Opti-MEM I reduced-serum medium were from Thermo Fisher Scientific. DMEM, DMEM/F12, RPMI1640, penicillin-streptomycin solution, trypsin, and fetal bovine serum were from HyClone. TRIzol reagent was from Invitrogen. SsoAdvance Universal SYBR Green Supermix was from Bio-Rad. Polymyxin B and kanamycin were from Aoke Biotechnology.

### 1.2.1 Amplification and Identification of Porcine IL-17 Gene

Using the existing PVIL17/22 engineering bacteria as template, the target gene was amplified with specific primers IL-17F/R: IL-17F: 5' -CGGGGTACCGCCACCATGGATGC-3', IL-17R: 5'-CCGCTCGAGCTAAGAAATATGGCGGACG-3' . The amplified sequence contained the TPA signal peptide and IL-17 mature peptide. PCR reaction conditions followed the manufacturer' s instructions for Phanta high-fidelity DNA polymerase. Amplified fragments were detected by 1% agarose gel electrophoresis and purified using the OMEGA gel recovery kit according to the manufacturer' s protocol.

### 1.2.2 Construction and Identification of IL-17 Recombinant Eukaryotic Expression Vector

The IL-17 PCR product was double-digested with Kpn /Xho and ligated into similarly digested pVAX1 vector. The ligation product was transformed into chemically competent *E. coli*, and positive transformants were identified by bacterial PCR and double restriction enzyme digestion. Positive plasmids were sequenced by Chengdu Tsingke Biotechnology. The recombinant IL-17 vector was designated PV17.

### 1.2.3 Transfection of PV17 into Effector Cells

*E. coli* containing recombinant plasmid PV17 and pVAX1 empty vector were cultured in LB medium, and plasmids were prepared using the OMEGA endotoxin-free plasmid maxiprep kit for transfection experiments. IPEC-J2, HaCaT, and L02 cells were revived and seeded into 6-well plates. When cells reached 80% confluence, transfection was performed following the Lipo3000 DNA transfection reagent protocol. The experimental group was transfected with PV17, the negative control with pVAX1 empty vector, and an untransfected group served as the control. Cells were harvested at 24, 48, and 72 hours post-transfection, and supernatants were collected at 48 hours.

### 1.2.4 Quantitative PCR Detection of Related Gene Expression

Total RNA was extracted from harvested cells using TRIzol reagent, and cDNA was synthesized using the TransScript One-Step gDNA Removal and cDNA Synthesis SuperMix. Based on gene sequences reported in GenBank, specific primers for quantitative PCR were designed and synthesized, targeting 22 related proteins. PPIA or GAPDH served as reference genes, IL-6, IL-12, and TNF- as cytokines, Reg , S100A8, and BD2 as antimicrobial peptides, and JAK1, STAT1, and STAT3 as JAK-STAT signaling pathway genes (Table 1 ).

Using diluted cDNA as template, gene expression levels were detected using primers from Table 2 and a Bio-Rad CFX Connect real-time PCR system. The reaction volume was 15 L, with the following program: pre-denaturation at 95°C for 30 s, followed by 40 cycles of denaturation at 95°C for 5 s and annealing at optimal temperature for 30 s, with a melting curve from 65°C to 95°C increasing by 0.5°C every 5 s. Relative gene expression was calculated using the 2-ΔΔCT method with PPIA or GAPDH as reference genes.

### 1.2.5 Antibacterial Assay

Four standard and drug-resistant bacterial strains were inoculated into MH broth and cultured to logarithmic phase, then diluted to approximately 1×10 CFU/mL (OD 0.005). The bacterial suspensions were seeded into 96-well plates, and 100 L (undiluted), 50 L (2-fold dilution), and 25 L (4-fold dilution) of the three collected supernatants were added, with cell culture medium added to bring each well to 200 L. Antibiotic treatment served as positive control, bacteria-only as negative control, and no bacteria as blank control. Plates were incubated at 37°C for 16 hours, and OD values were measured using a Thermo Fisher microplate reader.

### 1.2.6 Statistical Analysis

The IL-17 group was designated as the experimental group and the Cell group as the control. Statistical analysis of differences between groups at the same time points was performed using GraphPad Prism 6 software with two-way ANOVA followed by Sidak' s multiple comparisons test. Differences were considered significant at $P<0.05$.

## Results

### 2.1 Amplification of Porcine IL-17 Gene

Bacterial PCR amplification yielded a fragment that, when analyzed by 1% agarose gel electrophoresis, showed a band of approximately 500 bp corresponding to IL-17, consistent with the expected size (Figure 1 [Figure 1: see original paper]).

### 2.2 Construction of Recombinant Eukaryotic Expression Vector PV17

PCR products from PV17 monoclonal colonies showed bands matching expected sizes when analyzed by 1% agarose gel electrophoresis (figure omitted). Plasmid extraction followed by double restriction enzyme digestion yielded two bands of approximately 3000 bp and 500 bp in the PV17 lane, consistent with expected sizes (Figure 2 [Figure 2: see original paper]). Positive plasmids were sequenced by Chengdu Tsingke Biotechnology, and analysis using DNAStar software revealed 100% homology between the IL-17 fragment in the recombinant plasmid and the porcine IL-17 gene sequence published in GenBank (NM_001005729.1), confirming successful construction of the recombinant eukaryotic expression vector PV17.

#### 2.3.1 Quantitative Analysis of IL-17 Gene Expression

As shown in Figure 3 [Figure 3: see original paper], IL-17 gene expression levels in all three effector cell types transfected with PV17 were significantly higher than those in control groups throughout the entire time course ($P<0.05$).

#### 2.3.2 Quantitative Analysis of Antimicrobial Peptide Gene Expression

In IPEC-J2 cells, expression levels of Reg , S100A8, and BD2 genes in the experimental group were significantly higher than controls at 48 and 72 hours ($P<0.05$). In HaCaT and L02 cells, all three antimicrobial peptide genes showed significant upregulation in the experimental group compared to controls at 24 and 48 hours; at 72 hours, only S100A8 expression remained significantly elevated ($P<0.05$) (Figure 4 [Figure 4: see original paper]).

#### 2.3.3 Quantitative Analysis of JAK-STAT Signaling Pathway Gene Expression

Figure 5 [Figure 5: see original paper] demonstrates that expression levels of JAK1, STAT1, and STAT3 genes in the experimental group were significantly elevated compared to controls at various time points in all three cell types ($P<0.05$). Notably, in HaCaT and L02 cells, all three genes showed significant upregulation at 24 hours post-transfection.

#### 2.3.4 Quantitative Analysis of Cytokine Gene Expression

As shown in Figure 6 [Figure 6: see original paper], expression levels of IL-6, IL-12, and TNF- genes in the experimental group were significantly increased compared to controls at different time points in all three cell types ($P<0.05$). Specifically, IL-6 expression was significantly higher at 48 hours, IL-12 expression in HaCaT cells remained elevated from 24-72 hours, and TNF- expression in L02 cells was significantly upregulated throughout the 24-72 hour period ($P<0.05$).

#### 2.4.1 Antibacterial Effects Against *E. coli*

The antibacterial effects of supernatants against *E. coli* are shown in Figure 7 [Figure 7: see original paper]. Compared to controls, all three concentrations of cell supernatants significantly inhibited growth of both standard and drug-resistant *E. coli* strains ($P<0.05$). For standard *E. coli*, significant inhibition was observed with 2-fold diluted supernatants from all three experimental groups, while for drug-resistant *E. coli*, undiluted, 2-fold, and 4-fold diluted supernatants all showed marked inhibitory effects, with similar trends across different supernatant treatments ($P<0.05$).

#### 2.4.2 Antibacterial Effects Against *S. aureus*

As shown in Figure 8 [Figure 8: see original paper], bacterial proliferation of both standard and drug-resistant *S. aureus* strains was significantly inhibited by experimental group supernatants at various dilutions ($P<0.05$). For standard *S. aureus*, significant inhibition occurred only with undiluted supernatant, with IPEC-J2 supernatant showing the strongest effect across all three dilutions ($P<0.05$). For drug-resistant *S. aureus*, only L02 cell supernatant at undiluted or 2-fold dilution demonstrated significant inhibitory effects ($P<0.05$).

## Discussion

Although IL-17 participates in immune regulation at both innate and adaptive immunity levels, its role in infectious diseases primarily involves resistance to pathogen infection by epithelial cells and mucosal tissues in contact with the external environment, which guided our experimental design for activity analysis [19-21]. Among the limited studies on porcine IL-17, Katoh et al. first cloned and prokaryotically expressed porcine IL-17 in 2004, investigating its partial biological activity and tissue expression abundance [22]. Domestic research on porcine IL-17 has also been limited, focusing primarily on gene cloning and identification without further investigation of immunobiological activity [17-18].

In this study, we successfully constructed the eukaryotic expression plasmid PV17 containing a TPA signal peptide sequence at the gene fragment' s N-terminus for secretory expression and supernatant collection. Transfection into IPEC-J2, HaCaT, and L02 cells followed by qPCR and antibacterial assays confirmed high IL-17 expression in all three target cell types, indicating successful transfection. Importantly, expression of three antimicrobial peptide genes—Reg , S100A8, and BD2—was significantly upregulated in experimental groups ($P<0.05$). Antimicrobial peptides possess unique anti-infective and antibacterial properties [23], and previous studies have shown that human IL-17 can induce various epithelial cells to produce multiple antimicrobial peptides such as BD2 and S100A9 in a concentration-dependent manner [24].

Antibacterial activity testing of transfected cell supernatants revealed that all three eukaryotic expression supernatants significantly inhibited growth of four

bacterial strains at various volumes ($P<0.05$). Although bacteria were in stationary overnight phase, growth inhibition was evident, with the most pronounced effects against drug-resistant *E. coli*. These results demonstrate that secreted IL-17 protein induces antimicrobial peptides with significant antibacterial function, including notable effects against drug-resistant bacteria.

Quantitative analysis of JAK-STAT signaling pathway gene expression showed that JAK1, STAT1, and STAT3 expression levels in experimental groups were significantly higher than controls at various time points ($P<0.05$). The JAK-STAT pathway participates in numerous critical biological processes including cell proliferation, differentiation, apoptosis, and immune regulation [25]. JAK1 is involved in many cytokine signal transduction pathways and associated with tumor development; STAT1 mediates interferon responses and promotes Th1 cell differentiation; STAT3 participates in cell growth and inflammatory cytokine gene transcription [26-28]. The significantly elevated expression of IL-6, IL-12, and TNF- genes in experimental groups ($P<0.05$) indicates that IL-17 can induce both Th1- and Th2-type cytokines, further expanding and enhancing immune defense mobilization. These upregulated gene expression levels demonstrate that recombinant vector PV17 transfection enhances target cell immune responses and participates in antibacterial processes.

In summary, we successfully constructed a secretory eukaryotic expression vector for porcine IL-17 (PV17) and transfected it into three cell types, confirming its ability to induce production of various antimicrobial peptides, upregulate JAK-STAT signaling pathway genes and partial cytokine genes, and generate supernatants with antibacterial activity. These findings establish a foundation for further development of IL-17 as a novel molecular biological agent for disease prevention and anti-infection applications.

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