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# Purification of recombinant virus-like particles of porcine circovirus type 2 capsid protein using ion-exchange monolith chromatography

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ABSTRACT

Diseases associated with porcine circovirus type 2 (PCV2) infection are having a severe economic impact on swine-producing countries. The PCV2 capsid (Cap) protein expressed in eukaryotic systems self-assemble into virus-like particles (VLPs) which can serve as antigens for diagnostics or/and as vaccine candidates. In this work, conventional adsorbents as well as a monolithic support with large pore sizes were examined for the chromatographic purification of PCV2 Cap VLPs from clarified yeast lysate. Q Sepharose XL was used for the initial separation of VLPs from residual host nucleic acids and some host cell proteins. For the further purification of PCV2 Cap VLPs, SP Sepharose XL, Heparin Sepharose CL-6B and CIMmultus SO3 monolith were tested. VLPs were not retained on SP Sepharose XL. The purity of VLPs after chromatography on Heparin Sepharose CL-6B was only 4–7% and the recovery of VLPs was 5–7%. Using ion-exchange chromatography on the CIMmultus SO3 monolith, PCV2 Cap VLPs with the purity of about 40% were obtained. The recovery of VLPs after chromatography on the CIMmultus SO3 monolith was 15–18%. The self-assembly of purified PCV2 Cap protein into VLPs was confirmed by electron microscopy. Two-step chromatographic purification procedure of PCV2 Cap VLPs from yeast lysate was developed using Q Sepharose XL and cation-exchange CIMmultus SO3 monolith.

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## 1. Introduction

Circoviruses are classified as members of *Circoviridae* family, consisting of a circular single-stranded DNA genome. These viruses infect various animal and bird species. Porcine circovirus 2 (PCV2) has been isolated from diseased pigs in North America, Europe and Eastern Asia [1]. PCV2 is mainly associated with a disease known as postweaning multisystemic wasting syndrome (PMWS) [2–4]. PMWS most commonly affect pigs of 2 to 3.5 months of age [4]. Morbidity and lethality are variable depending on the management within affected units and on the batches of animals. The usual rates are 4–30% and 70–80%, respectively. PCV2-associated diseases are having a huge economic impact on swine-producing countries [5].

The virion of PCV2 is nonenveloped, icosahedral, with a diameter of about 20 nm [6]. Open reading frame 2 (ORF2) of PCV2 encodes a protein of about 30 kDa that is involved in viral capsid formation [7]. Virus-like particles (VLPs) formed by the expression product of ORF2 were similar to intact PCV2 particles. PCV2 capsid (Cap) protein can be used as an antigen for specific serological detection of PCV2 infection [8]. The Cap protein was found to be a major immunogen of PCV2, inducing protection of swine against PMWS in a prime-boost protocol [9].

Bacterial as well as eukaryotic systems are used for the expression of PCV2 Cap protein. The production of full-length PCV2 Cap protein in *E. coli* expression system is laborious due to the high incidence of arginine residues in a nuclear localization signal (NLS) domain [10]. The high-level expression of full-length PCV2 Cap protein in standard *E. coli* expression system was achieved by the replacement of rare arginine codons located at the 5′ end of the *cap* reading frame with codons optimal for *E. coli* [11]. However, the expressed Cap protein failed to-self assemble into VLPs. It has been recently reported that the full-length PCV2 Cap protein (Cap1-233), expressed using a modified *E. coli* expression system, is able to self-assemble into VLPs that resembled the authentic PCV2 capsid [12].

The characteristic feature of the recombinant PCV2 Cap protein expressed in eukaryotic systems is its self-assembly into VLPs [13–15]. The Cap protein of PCV2 was expressed by using a recombinant baculovirus in insect Tn5 cells [13]. The

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28 kDa protein was released into the culture medium and self-assembled into PCV2-like particles with a diameter of 20 nm. *Saccharomyces cereviciae*-derived PCV2 Cap protein self-assembles into VLPs that are morphologically and antigenically similar to insect cell-derived VLPs [14]. It was demonstrated that *S. cerevisiae* is a safe and simple system to produce PCV2 Cap VLPs [14,15].

The common procedure for recovery and purification of PCV2 Cap VLPs is ultracentrifugation in CsCl or sucrose gradients [12–15]. In large-scale purifications, the use of ultracentrifugation has some restrictions due to the lack of scalability and the enhancement of labor intensity [16,17]. Chromatography procedures may be regarded as an attractive alternative to ultracentrifugation. Chromatographic methods are used for the isolation and purification of fused PCV2 Cap protein. Fusions of PCV2 Cap with ubiquitin [18] or 6xHis-SUMO tag [19] were purified using size exclusion or immobilized metal affinity chromatography for ubiquitin-Cap and 6xHis-SUMO-Cap protein, respectively.

Currently, there is no reliable chromatographic purification process for PCV2 Cap VLPs. For the purification of viruses and VLPs, various chromatographic adsorbents are used including ion exchange [20–22], hydrophobic interaction [21,23] and hydroxyapatite [22]. The use of conventional chromatography media allowed obtaining highly purified VLPs [20,22]. However, the pore size of conventional resins used in packed-bed chromatography is generally less than 30–40 nm [24,25]. VLPs, ranging from 20 nm to over 200 nm, are hindered from binding onto the internal surface area of the resin, resulting in sub-optimal usage of such matrices and low recovery [16,26].

For the purification of VLPs, chromatographic supports with large pores might provide a better alternative to the conventional chromatography media. Monoliths are continuous stationary phases with large pores of 1000–5000 nm in diameter [27]. The convective mass transfer through the macro-pores of monoliths explains the high dynamic binding capacity for large molecules. The channels with a diameter above 1000 nm provide enough area for the adsorption of large biomolecules. Polymethacrylate monolithic columns, also called convective interaction media (CIM) monoliths are formed from a continuous porous material with mean pore diameter of 2100 nm. Recently, the use of CIM monolithic columns for the purification of VLPs has been reported [28,29]. The ion exchange CIMac QA monolithic column was demonstrated to be efficient in purifying adenovirus type 3 dodecahedric virus-like particles (Ad3 VLPs) from pre-purified samples as well as directly from crude cell lysate [28]. With the use of CIMac QA column the ultracentrifugation step could be omitted and the purification procedure became significantly shorter. The VLPs of the hepatitis B surface antigen (HBsAg) were successfully purified from clarified yeast homogenate by using a hydroxyl derivatized hydrophobic interaction monolith [29]. The monolith was then compared to a conventional beaded resin method, where the dynamic binding capacity was shown to be three-fold superior for monolith with equivalent 90% recovery of the VLPs.

In this work, conventional chromatographic adsorbents as well as CIMmultus SO3 ion-exchange monolith were examined for the purification of PCV2 Cap VLPs from clarified yeast lysate. Q Sepharose XL was used for the initial separation of VLPs from residual host nucleic acids and some host cell proteins. For the next separation step, SP Sepharose XL, Heparin Sepharose CL-6B and CIMmultus SO3 monolith were tested. Structural properties of the media used for the purification of PCV2 Cap VLPs are presented in Table 1. Two-step chromatographic purification method for PCV2 Cap VLPs was developed. This method consists of chromatography on Q Sepharose XL followed by chromatography on CIMmultus SO3 ion-exchange monolith.

## 2. Experimental

### 2.1. Materials

Adsorbents Q Sepharose XL, SP Sepharose XL, Heparin Sepharose CL-6B and empty columns XK16/20 for liquid chromatography were purchased from GE Healthcare Bio-Sciences AB (Uppsala, Sweden). CIMmultus SO3 Advanced Composite Column (bed volume 1.0 ml) was purchased from BIA Separations (Ajdovščina, Slovenia). Chemicals were obtained from Merck (Darmstadt, Germany) or Sigma–Aldrich Chemie GmbH (Steinheim, Germany).

### 2.2. Chromatographic equipment

Chromatographic experiments were carried out using AKTApurifier 100 chromatography system with fraction collector Frac-920 (GE Healthcare Bio-Sciences AB, Uppsala, Sweden).

### 2.3. PCV2 Cap VLPs production

VLPs were produced in yeast *S. cerevisiae* strain AH22-214 (*a, leu2-3,112, his4-519*). Yeast transformants harboring plasmid pFX7-PCV2Cap-622S with PCV2-cap encoding gene (GenBank KJ128275) were grown in YEPD medium (yeast extract 1%, peptone 2%, and glucose 2%) supplemented with 5 mM formaldehyde overnight at 30 °C and recombinant protein expression was induced after transferring yeast cells into induction medium YEPG (yeast extract 1%, peptone 2%, and galactose 3%) supplemented with 5 mM formaldehyde and culturing for additional 18 h as described previously [15]. Yeast biomass harboring recombinant proteins was harvested by centrifugation and was stored at −20 °C until use.

### 2.4. Cell lysis

An equal volume of glass beads (particle size 425–600 μm, Sigma–Aldrich) was added to the plastic tube containing harvested yeast cells (5 g). 10 ml of breaking buffer (50 mM Tris-HCl, pH 8.0, 20% (v/v) glycerol, 0.15 M NaCl, 1 mM $CaCl_2$, 2 mM $MgCl_2$, 0.01% Triton X-100), containing 100 μl Halt Protease Inhibitor Cocktail (Thermo Scientific, Vilnius, Lithuania) was added to the tube and a total of ten cycles of vortexing and incubation on ice (1 min each) were performed. Sample was centrifuged at 805 × *g* for 10 min. The supernatant was transferred to a new tube and Ribonuclease A from bovine pancreas (Calbiochem, Merck Biosciences Ltd, Nottingham, UK) or Benzonase Nuclease (Merck Millipore, Darmstadt, Germany) was added. The final concentration of Ribonuclease A was 0.1 mg/ml, Benzonase Nuclease – 100 U/ml. Afterwards, the supernatant was kept on ice for 30 min and then cleared by centrifugation at 12,000 × *g* for 25 min. In some cases, nuclease treatment step was omitted and supernatant was simply centrifuged at 12,000 × g for 25 min.

### 2.5. Chromatographic purification of PCV2 Cap VLPs

The purification was performed at +4 °C temperature. 10 ml of Q Sepharose XL was packed into XK 16/20 column and equilibrated with 10 column volumes (CV) of 50 mM Tris-HCl, pH 8.0, containing 20% (v/v) glycerol and 0.15 M NaCl. The supernatant (prepared as described in Section 2.4) was diluted with equilibration buffer by a ratio 1:3, filtered with a 0.45 μm Durapore low binding membrane filter (Merck Millipore, Hertfordshire, UK) and applied to Q Sepharose XL column. The flow rate was 2.0 ml/min. After loading, column was washed with 5-10 CV of equilibration buffer. Next, a linear 0.15 M–1 M NaCl gradient was applied. The flow-through protein fractions were pooled and further purified by using SP Sepharose XL, Heparin Sepharose CL-6B or CIMmultus SO3 column.

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