CHARACTERIZATION OF RECOMBINANT VEROTOXIN 2e DURING THE PRODUCTION PROCESS

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BACKGROUND AND OBJECTIVES

VEPURED® is a vaccine for the protection of piglets against edema disease. The antigen of VEPURED® is a recombinant verotoxin 2e (rVt2e). The rVt2e is purified during the production process in order to guarantee the safety and immunogenicity of the vaccine.

In this study, we present the characterization of the production process for the rVt2e.

MATERIAL AND METHODS

The rVt2e production starts with a fermentation culture, the culture is induced to express rVt2e. The downstream processing of the fermentation culture involves a separation of the biomass from the supernatant, a cellular fragmentation to obtain the active ingredient, a purification of the active ingredient by chromatography, a diafiltration and a sterilizing filtration.

Samples were obtained after cellular fragmentation, purification and sterilization. The rVt2e purity and the endotoxin levels in each sample were evaluated by SDS-PAGE analysis and Limulus Amoebocyte Lysate (LAL) assay, respectively.

RESULTS

The cellular fragmentation fraction contains a rVt2e together with other *E. coli* proteins. The percentage of rVt2e is less than 1% of the total proteins, and the endotoxin levels are higher than $1x10^6$ EU/mL (Table 1).

Sample	Purity (%)	Endotoxins (EU/mL)
Fragmentation	0.5	>$1x10^6$
Purified rVt2e	93.3	500
Final rVt2e	95.9	<10

Table 1. rVt2e purity, and endotoxin levels obtained in the different fractions of the production process.

After the purification step, there was a 90-fold increase in purity and the majority of endotoxins were removed.

So the purification step is essential in order to obtain a pure active ingredient (Figure 1).

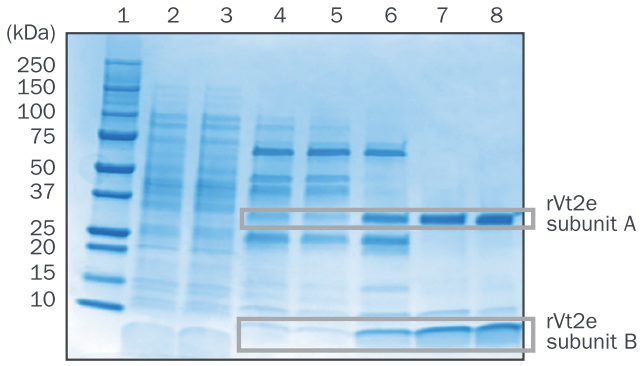

Figure 1. SDS-PAGE of samples obtained during the rVt2e production process. 1) Molecular weight marker; 2) Cellular fragmentation; 3) Purification: flow through; 4-6) Purification: wash; 7) Purification: purified rVt2e; 8) Final rVt2e.

At the end of the production process (final rVt2e), the rVt2e had greater than 95% purity and the endotoxin levels were below 10 EU/mL (Table 1).

All the final rVt2e batches that were analysed had greater than 90% purity, indicating that the production process is consistent (Figure 2).

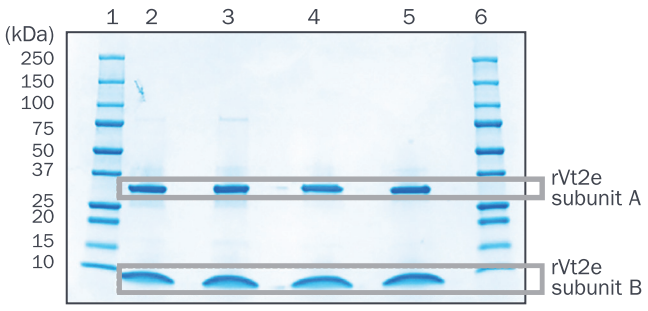

Figure 2. SDS-PAGE of different final rVt2e batches. 1,6) Molecular weight marker, 2) rVt2e batch 15/001, 3) rVt2e batch 15/002, 4) rVt2e batch 15/003, 5) rVt2e batch 15/004.

CONCLUSION

The purification step used in the rVt2e production process is essential in order to obtain a final rVt2e protein with a purity higher than 90% and with low endotoxin levels.

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