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STABILITY OF L-A1 VIRUS-LIKE PARTICLES PURIFIED FROM SACCHAROMYCES CEREVISIAE

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ScV-L-A1, a member of the virus family *Totiviridae*, is a double-stranded RNA virus often associated with a phenomenon referred to as the killer yeast phenotype. Although this infectious element is known to lack an extracellular phase, it was found to be stably maintained in 15 out of 70 wild *Saccharomyces cerevisiae* strains by transmission through cell-cell mating [1]. The genome of L-A1 virus is shielded and delivered from cell to cell by a capsid composed of 60 asymmetric homodimers of single major capsid protein Gag. The pores in the capsid serve as a molecular sieve, allowing transcripts and metabolites to pass through while retaining dsRNA and blocking entry for degradative enzymes [2]. In this sense, L-A1 virus, as well as any other virus, may be considered a natural nano-delivery system.

Synthesis of virus-like particles is an approach to take advantage of viruses' innate capacity to protect and transport cargo to their intended destination. These are nanoparticles self-assembled from viral capsid proteins, yet incapable of replication due to the lack of genetic material [3]. Therefore, VLPs have been used for the delivery of drugs, nucleic acids, peptides, and proteins [4]. Although a broad range of host cells can be cultivated for the production of VLPs, yeast expression systems – particularly *S. cerevisiae* – are prevalent for this purpose due to inexpensive culturing, rapid cell growth, effective protein expression, scalability and native post-translational protein modifications [3].

The main goal of this study was to examine the stability of L-A1 VLPs purified from *S. cerevisiae*. The synthesis of recombinant major capsid protein of L-A1 in *S. cerevisiae* was induced, and the self-assembled nanoparticles were consequently purified by ultracentrifugation of the yeast lysate through sucrose cushion and cesium chloride density gradient. Transmission electron microscopy (TEM) and dynamic light scattering (DLS) techniques were used to demonstrate the formation of 41.3 ± 1.6 nm L-A1 VLPs, corresponding the size of the native L-A1 virus (40.0 nm) [2]. Since evaluation of the particle stability in different conditions is valuable for the development of nano-delivery systems, the effects of temperature, buffer identity, ionic strength, pH, and Mg^{2+} ions on the VLP size stability were assessed. After the monitoring of the particle size by DLS method for 4 weeks, substantial change in size (aggregation) was observed only at the end of the experiment in Tris-HCl-based buffer at 37°C. Although the purified VLPs can be regarded as fairly stable, additional investigation into their cytotoxicity and cargo encapsulation is necessary prior to applying them for nano-delivery.

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