ABSTRACT

Cancer of uterine cervix is the second most common cancer in women. Approximately 520,000 new cases and 274,000 deaths have been reported each year worldwide. The incident rate of cervical cancer in India is approximately 134,000 per year; which is nearly 52% of the recorded incidence of the disease in the Asia-Pacific region. It is estimated that by the year 2025, the cervical cancer incidence rates in the developing world would account for nearly 86% of the global rates. Prof. Harold zur Hausen began to postulating and established the etiological link between Human papillomavirus (HPV) and cervical cancer.

Frequent monitoring of cervical exfoliates for an abnormality requires a large number of trained professionals and persistent public funding to support the requisite infrastructure. Prophylactic vaccination has the potential to augment cervical screening programme, promoting the reduction in disease burden by prevention of HPV infection. Since authentic HPV can only be cultured in a tedious oragnotype raft cultures, production of inactivated vaccine, at present, is neither feasible at production scales nor acceptable, since the vaccine would contain the viral oncogenes. The VLP based bivalent HPV16/18 or quadrivalent HPV6/11/16/18 vaccines have been successfully introduced into the market. Despite the introduction of these prophylactic vaccines into the
market, the prohibitive cost of the vaccines is likely to affect their availability to women in developing countries.

In the present study, recombinant clones of *Pichia pastoris* that express HPV 16 or 18 L1 major capsid proteins and another expressing HPV 16 L2 minor capsid protein were generated. Stability of HPV 16 or 18 L1 and 16 L2 clones were confirmed.

The major capsid protein (L1) of human papillomaviruses (HPV) expressed in *Pichia pastoris* assembled into virus-like particles (VLP). The VLPs produced in *Pichia pastoris* were purified either using size exclusion or heparin sepharose chromatography. The purified VLPs were characterized using conformation-specific monoclonal antibodies in ELISA and by transmission electron microscopy.

Mice immunized with monovalent and bivalent formulation of HPV vaccine produced in *Pichia pastoris* developed high serum antibody titers to both HPV 16 & 18 types, which persisted for 190 days post vaccination. Serum of mice immunized with the HPV-VLP preparations could neutralize homologous pseudoviruses in an *in-vitro* assays.

In this study we have demonstrated conclusively, that *Pichia pastoris* can express HPV 16 & 18 VLPs. The VLPs are capable of eliciting neutralizing antibodies in immunized mice.

Efficiency of vaccine was assessed in an *in-vitro* pseudovirion neutralization assay, which is designated as the gold standard by WHO advisory group.
To our knowledge, a detailed characterizations of the *Pichia pastoris* expressed HPV VLPs and their ability to induce neutralizing antibodies have not been reported thus far. Also, this study is first to describe cloning and expression of HPV 18 VLPs & HPV 16 L2 in *Pichia pastoris*. The data generated from this work has formed the basis for developing prophylactic vaccine against cervical cancer for human use, where more HPV types have been added to expand the type specificity of the vaccine.