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Small P particles formed by Taiwan native norovirus P domain overexpressed in *Pichia pastoris* and purified by tag-free purification schemes

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Statement of the Problem: The Protrusion (P) domain of the major structural protein VP1 of Norovirus (NoV) is critical for host immune response and receptor binding. Most heterologous P domain expressed in *Escherichia coli* or *Pichia pastoris* forms P particles consisted of 24 P monomers through intermolecular contact in the P regions and end-linked cysteine tag. Particularly, the small P particle consisted of 12 P monomers is only found in the P domain with terminal modification. *P. pastoris* expression system exist advantages over bacteria including no endotoxin risk, low costs and easy scale-up. Currently, the purification of the recombinant P protein from the crude extract mainly relied on purification tags. However, the purification tags increase the cost and labor in downstream processes and the risk on authentic structure alternation.

Method: The NoV P domain of the most predominant NoV strain GII.4 isolated from Taiwan was expressed in the *P. pastoris*. A high-yield fermentation process of the recombinant NoV P complexes and the tag-free purification schemes were developed. The recombinant NoV P complexes were also verified by LC-MS/MS, ELISA and saliva binding assay and its polymer formation was analyzed by gel filtration, dynamic light scattering and transmitted electronic microscopy.

Findings: The majority of NoV P proteins expressed in *P. pastoris* formed small P particles, composed by 12 copies of the P domain with a diameter of 14 nm and in square and ring shapes.

Conclusion: The high cell density fermentation processes and tag-free purification schemes of NoV P protein were successfully developed. The small P particles formed by Taiwan native norovirus P domain might provide further morphogenesis study and vaccine development.

Biography

Yu-Ling Chen is currently a doctoral student at Department of Biochemical Science and Technology, National Taiwan University in Taiwan. Her research interests include heterologous gene expression systems of the methylotrophic yeast *Pichia pastoris*, the edible mushroom *Flammulina velutipes* and antigen presentation system of norovirus P particle.

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