

Interaction between Viral Envelope Protein VP28 and *Pm*Rab7 Variants through Yeast Surface Display Platform

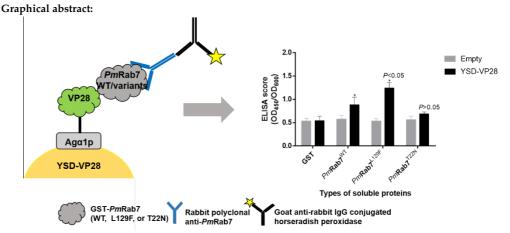
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Abstract: Currently, yeast surface display (YSD) is being employed for protein engineering and characterization. By fusion of interested protein with anchoring domain of yeast cell wall protein, the display protein could efficiently function with a specific purpose. White Spot Syndrome Virus (WSSV) causes White Spot Disease (WSD) in *Penaeus monodon* shrimp in which the shrimp receptor protein PmRab7 interacts with the viral envelope protein VP28. PmRab7 was identified in the same family as human Ras which some mutations affected the increase or decrease interaction with effector proteins. We hypothesized that the same mutations in PmRab7 affected binding with VP28. In this study, we demonstrated the interaction of both proteins in the methylotrophic yeast $Pichia\ pastoris$. The $PmRab7^{VI}$, $PmRab7^{L129F}$ (dominant active) and $PmRab7^{T22N}$ (dominant negative) genes were fused with GST for ease of purification and expressed in pET28a plasmid in $Escherichia\ coli\ C41$ (DE3). The interaction was performed using $P.\ pastoris$ GS115 cells displaying VP28 induced by 0.5% methanol for 48 h incubating with purified different PmRab7 form. Our results showed that YSD-VP28 increased binding with $PmRab7^{L129F}$ (1.24±0.12) of about 1.5 folds as compared to $PmRab7^{WT}$ (P < 0.05) whereas the binding with $PmRab7^{T22N}$ (0.68±0.05) was not different from the wild type (P > 0.05). Fusion of GST to PmRab7 did not affect binding as the signal was similar to the negative control harboring expression empty cassette. In conclusion, YSD platform is robustness to characterize PmRab7-VP28 interaction.



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Keywords: Yeast Surface Display, Pichia pastoris, Penaeus monodon Rab7, White Spot Syndrome Virus, VP28

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