105 年度生醫系專題研究競賽報名表

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(中文):*Nepenthes* 萃取物可抑制 DnaD 所誘增的 PriA ATP 水解活性：找尋新型可阻斷 DNA 複製重啟的抗生素

(英文)：Stimulation of PriA ATPase activity via physical contact with DnaD can be inhibited by the nepenthes extract: New antibiotic for blocking the DNA replication restart

Abstract：

Background and motivation

Staphylococcus aureus is an important etiologicalagent responsible for healthcare-associated infections.*Staphylococcus aureus*has a remarkable ability to developantibiotic resistance, and few therapies are effective against this pathogen. PriA-directed primosome is essential for DNA replication restart in bacteria,and this system is not found in humans. Thus, inhibiting theactivity of PriA and its loaderssuch as DnaD and other proteins will be detrimental to block bacterialgrowth and survival.

Methods

PriA and DnaD were purified by Ni^{2+}-affinity chromatography. Protein-protein interactions were analyzed by surface plasmon resonance technology (SPR) and gold nanoparticle assay. PriA ATPase activity was assayed by thin-layer chromatography (TLC). *Nepenthes* extract was prepared within liquid nitrogen and alcohol. The active compounds targeting the PriA activity were identified using high performance liquid chromatography (HPLC) plus MS/MS.

Results

We found that PriA is a poor helicase but the activity can be significantly stimulated by DnaD, a PriA-specific loader protein. Experimental evidences showed that DnaD is assDNA-binding protein with a binding-site size of approximately 32 nt. Based on results from SPR, gold nanoparticle assay, and mutational analyses, we found that the highly conserved C-terminal region of DnaD is crucial for stimulatingPriA activity via direct physical contact. The PriA activity was

inhibited by the plant extract, *Nepenthes*. Several compounds from the extract were further isolated and identified by HPLC and LC-MS/MS, and some of which were thought to be potential antibiotic leads to block bacterial replication restart. Study on structure-inhibition relationship among PriA, DnaD, and one of these compounds, as well as drug optimization is still ongoing. Part of these results has been published in *PLoS One*, 2016.

Conclusion and future works

In this study, we have found that DnaD is assDNA and PriA binding protein via the C-terminal region. In addition, DnaD can significantly stimulate the PriA activity. Several compounds extracted from *Nepenthes*were identified and selected to be drug leads. Because PriA and DnaD are not found in mammals, inhibitors based on these proteins are potentially safe for human use. We hope that the new antibiotic based on this study can develop in the early future.