細菌的攻防戰：
農桿菌第六型分泌系統解密

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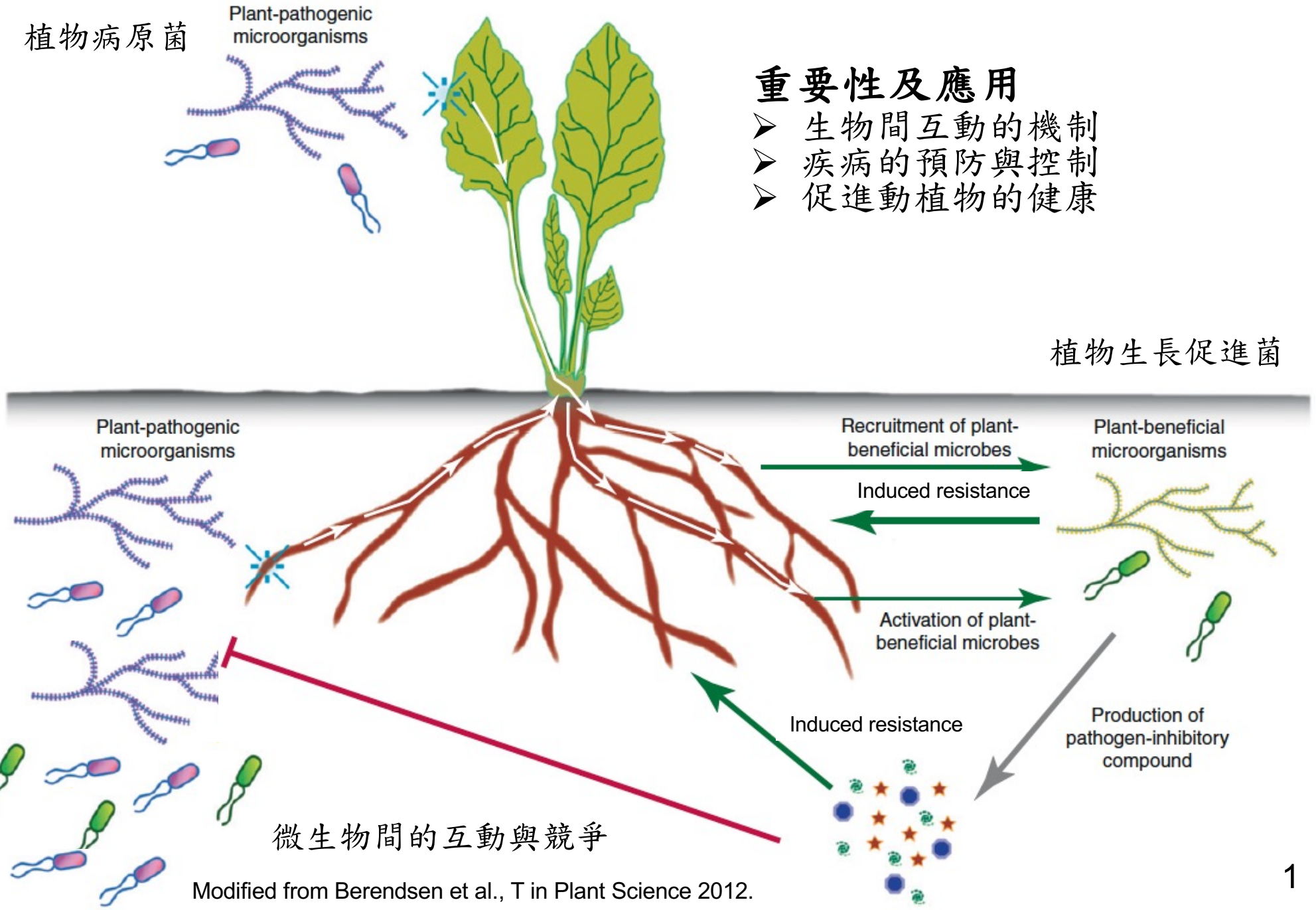
中研院植微所暑期生系列講座

生物間的互動關係：敵或友？

植物病原菌



植物生長促進菌

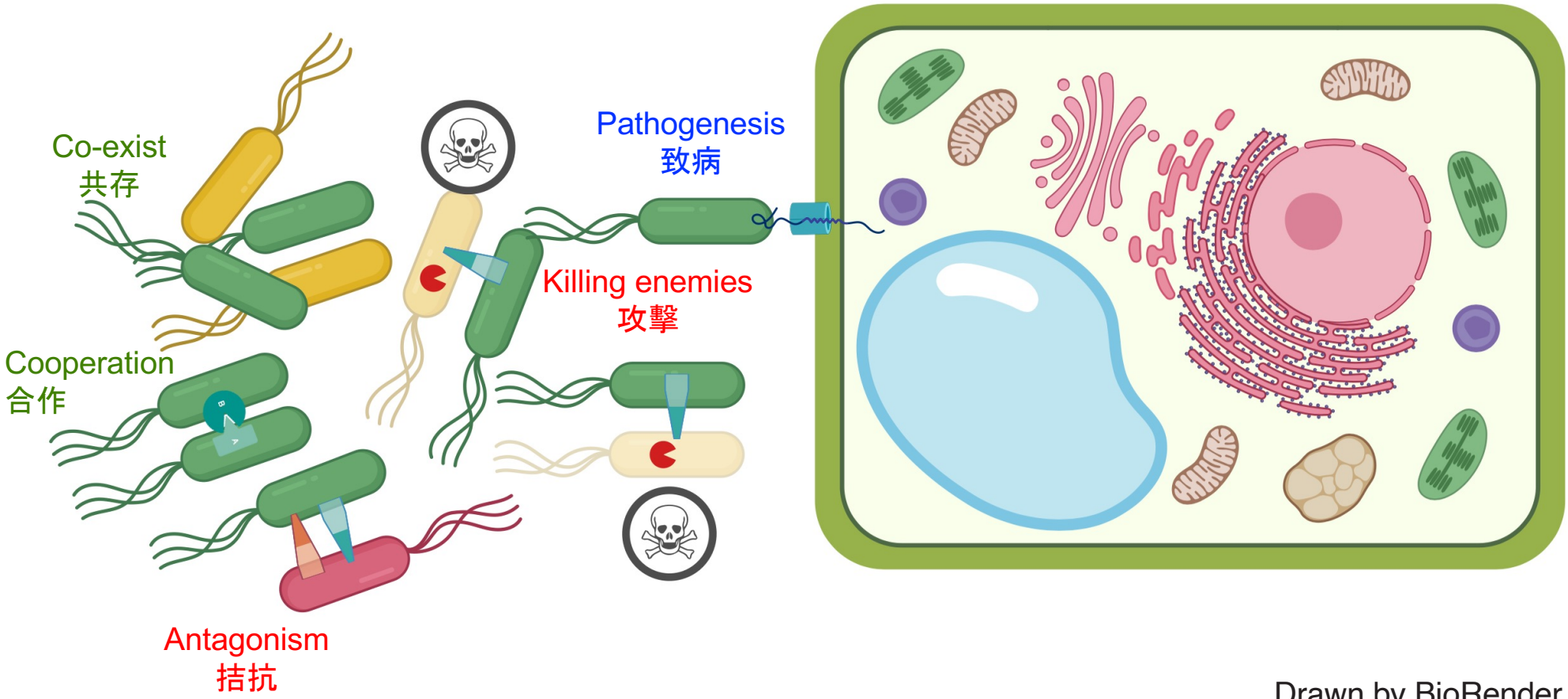
重要性及應用

- 生物間互動的機制
- 疾病的預防與控制
- 促進動植物的健康

微生物間的互動與競爭

Modified from Berendsen et al., T in Plant Science 2012.

細菌與環境中的生物互動

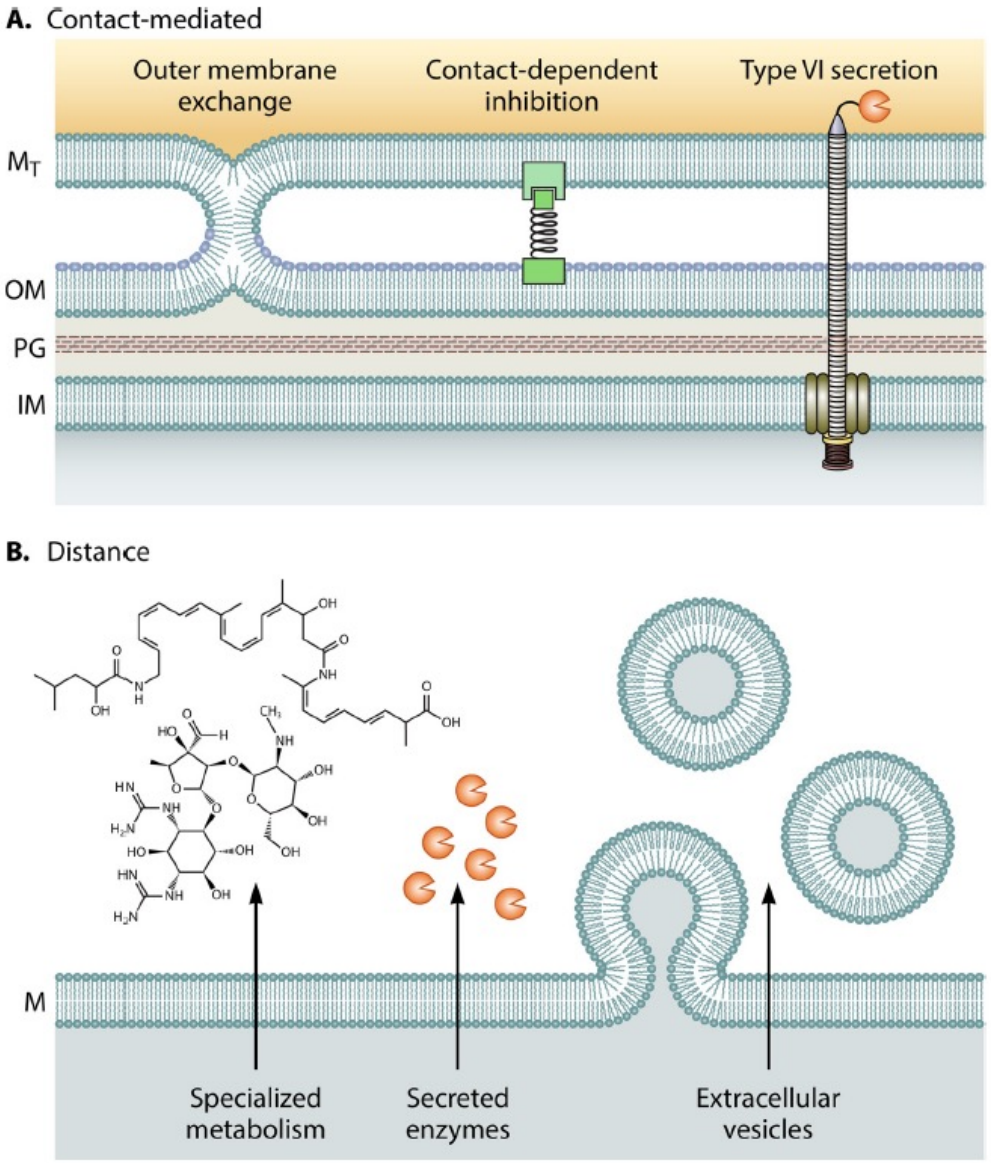


Drawn by BioRender

抗菌機制及毒素(Antibacterial toxins) 種類

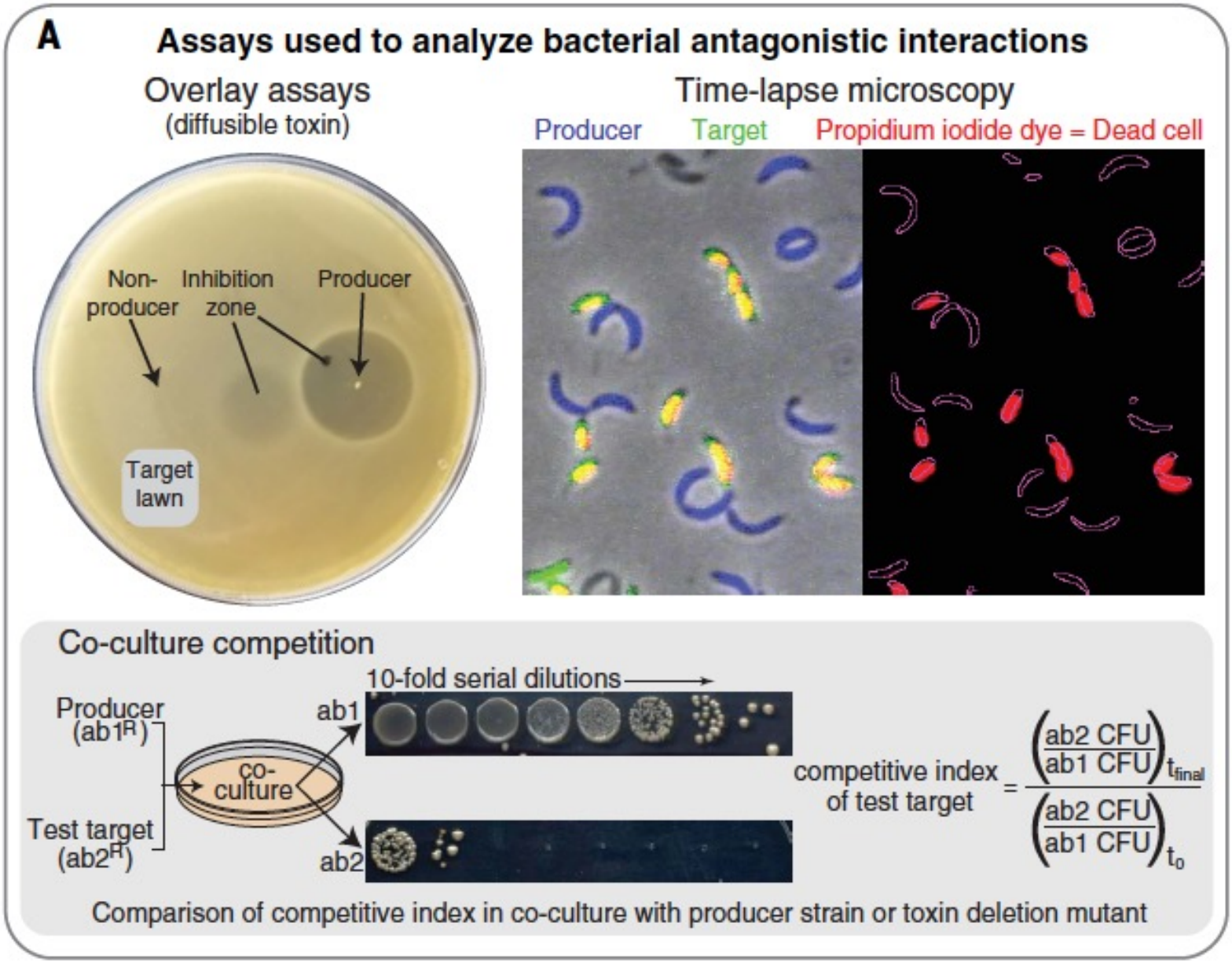
(A)接觸型攻擊毒素：
Toxins that are delivered directly to the target cell via cell-to-cell contact through a variety of secretion systems
-蛋白質毒素 protein toxins
核酸分解酶 nuclease
細胞壁分解酶 cell wall degradation enzyme
磷脂酶 phospholipase

(B) 擴散型毒素：
Diffusible molecules that are released into the extracellular milieu
-抗生素 antibiotics
-抗菌胜肽 antimicrobial peptides
-蛋白質毒素 protein toxins



Stubbendieck RM, Straight PD. 2016. J Bacteriol 198:2145–2155

鑑定及研究抗菌毒素的方法



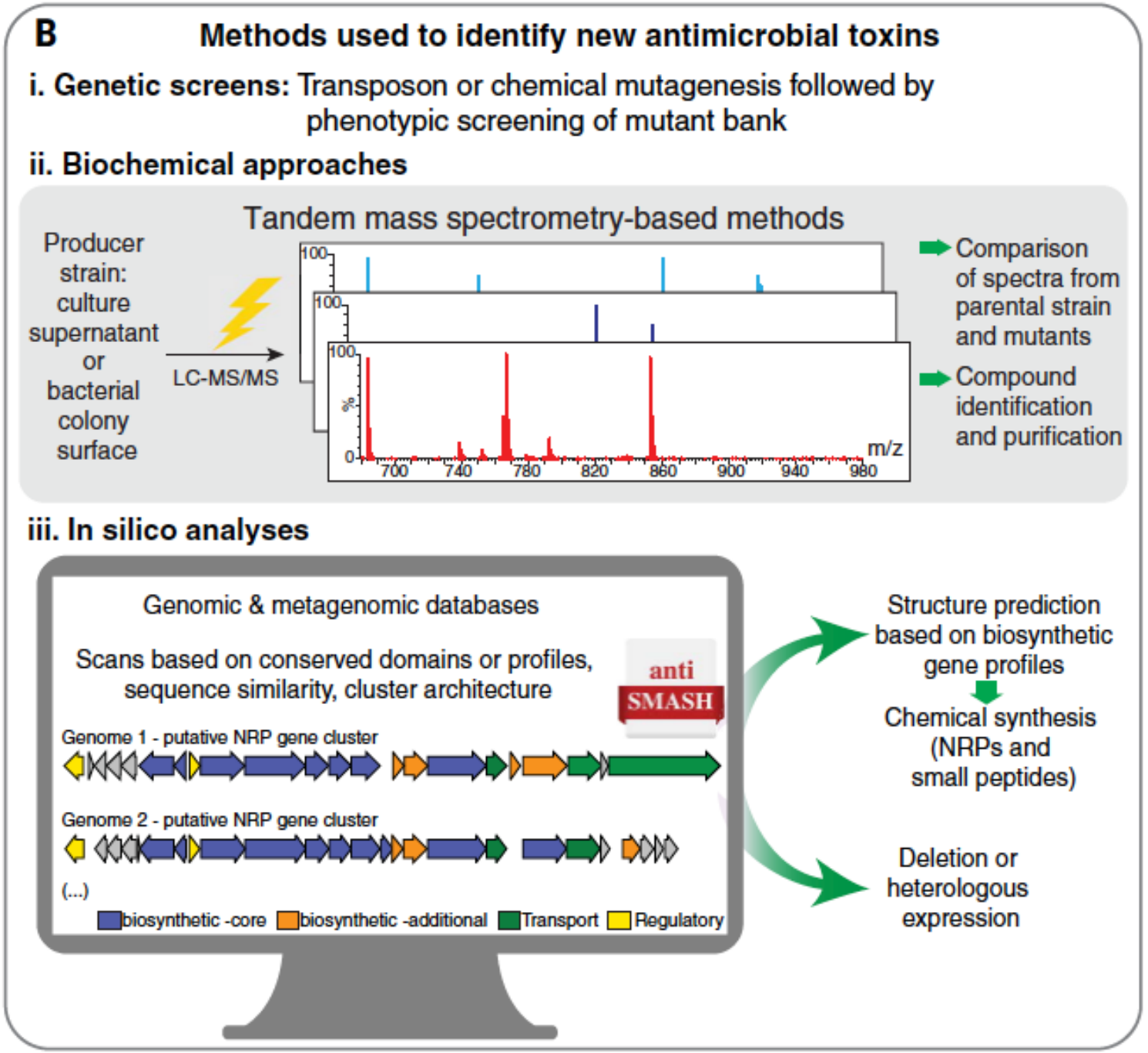
García-Bayona et al., Science 361, eaat2456 (2018)

篩選及鑑定抗菌毒素的方法

遺傳篩選

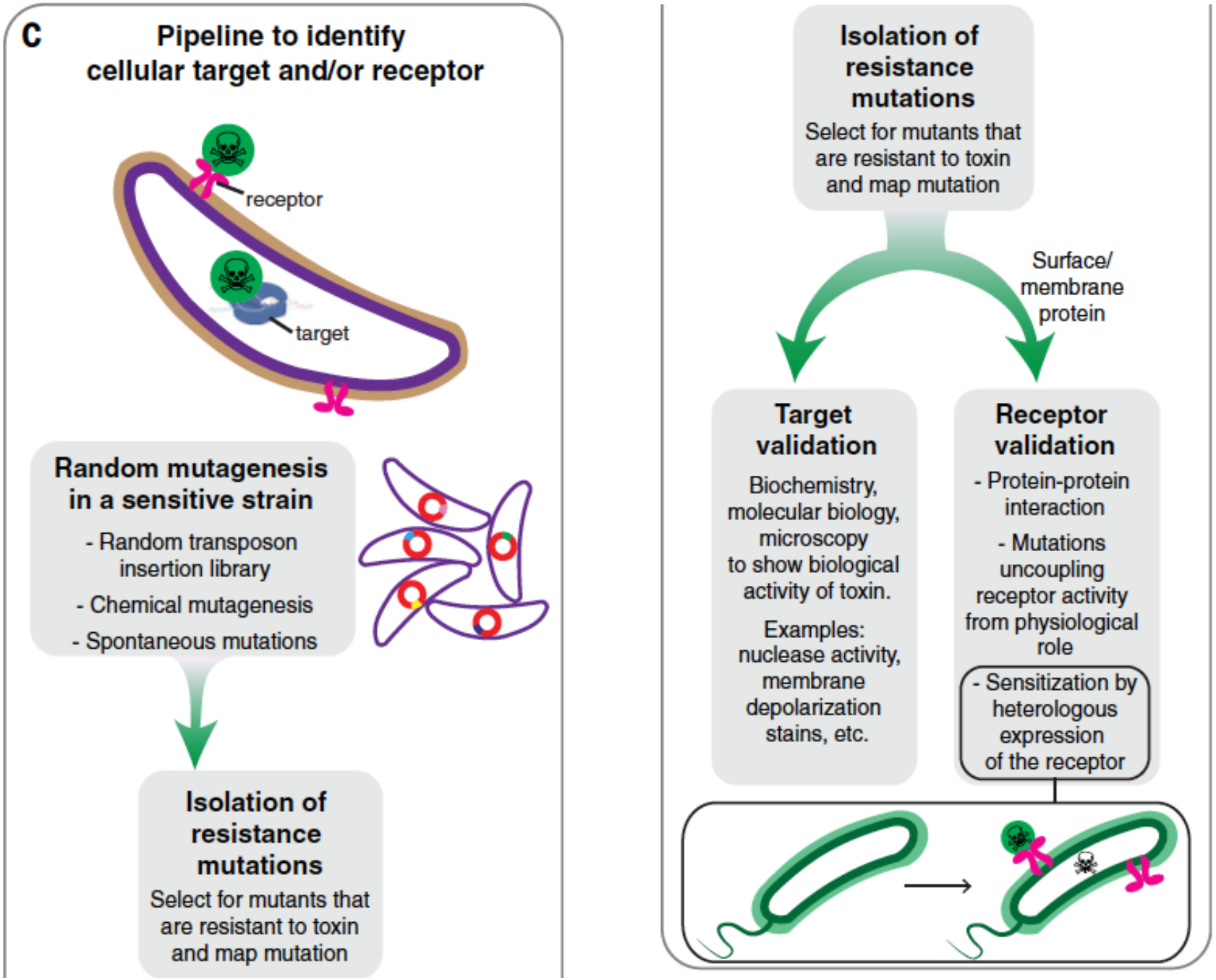
生化篩選

生物資訊分析



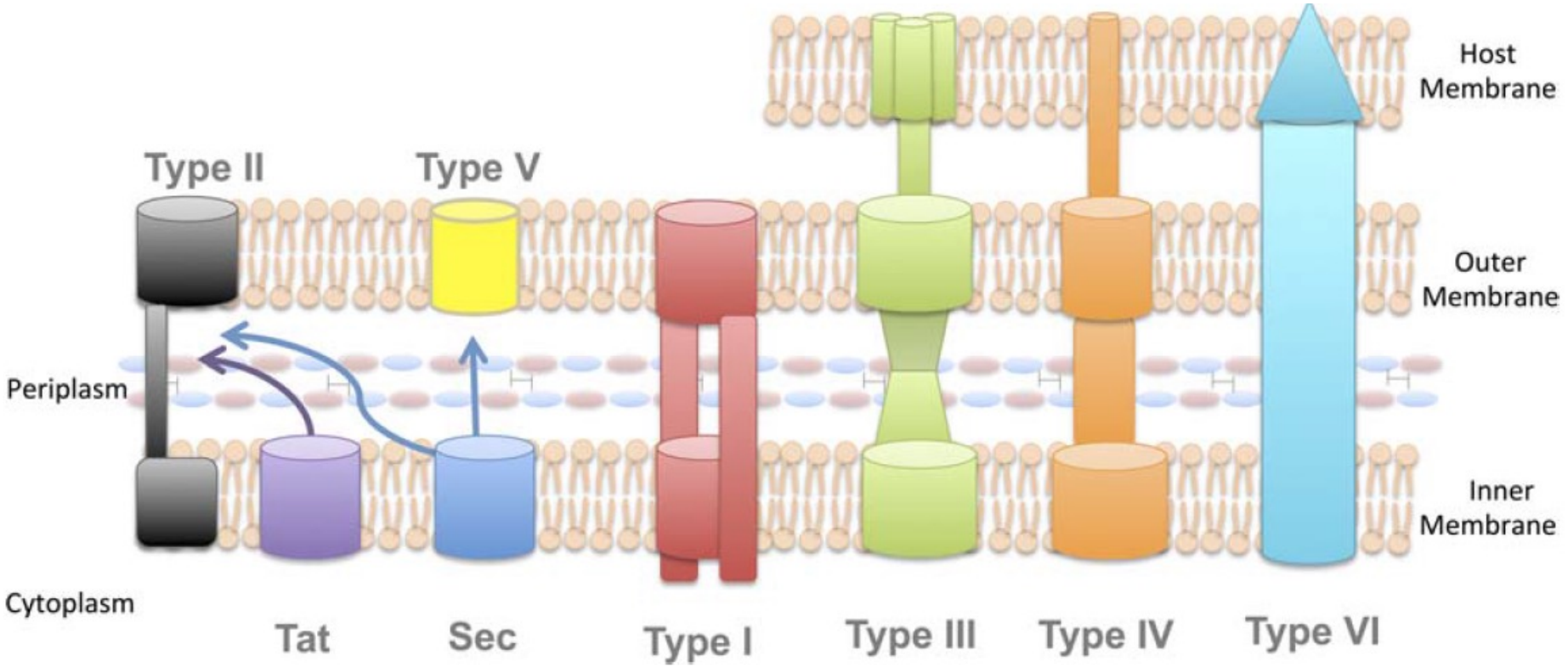
García-Bayona et al., Science 361, eaat2456 (2018)

篩選及鑑定抗菌毒素受體的方法



García-Bayona et al., Science 361, eaat2456 (2018)

細菌蛋白質分泌系統對於病原菌致病力及生存競爭力扮演重要角色

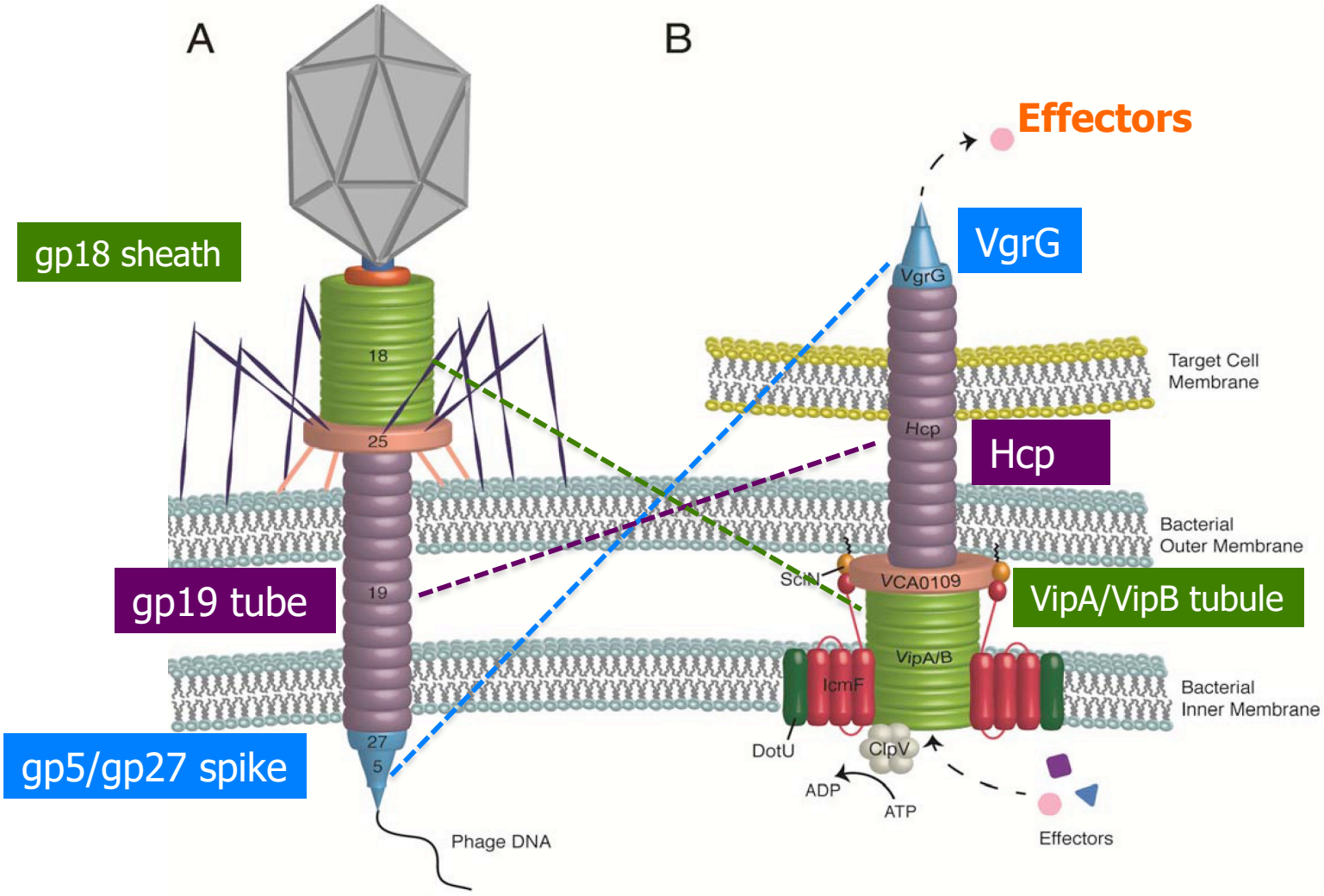


Green ER, Mecsas J. 2016. Microbiol Spectrum

第六型分泌系統(T6SS)普遍存在於約四分之一的格蘭氏陰性細菌

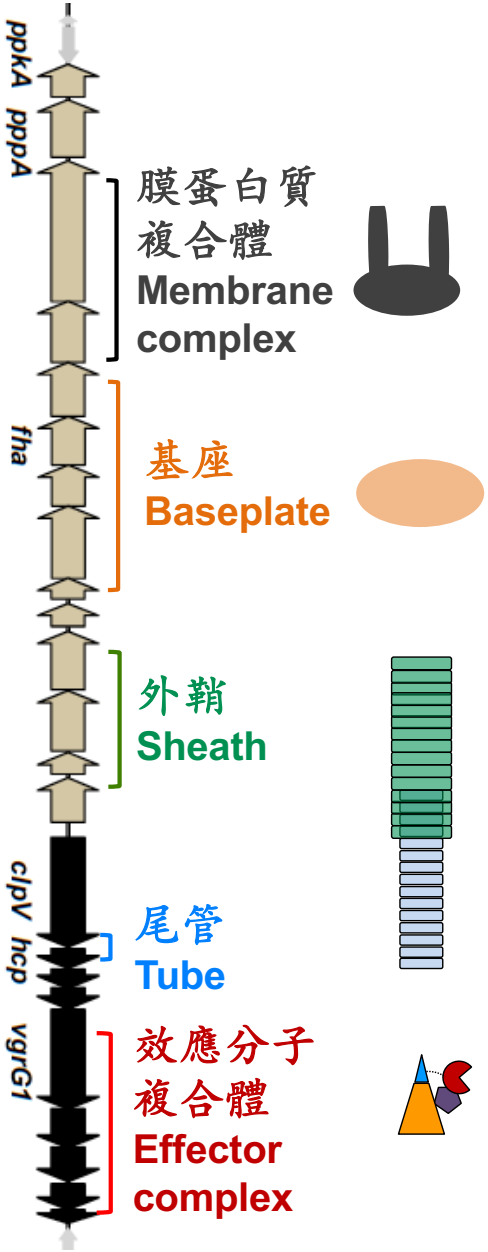
第六型分泌系統：噬菌體倒裝的分泌系統

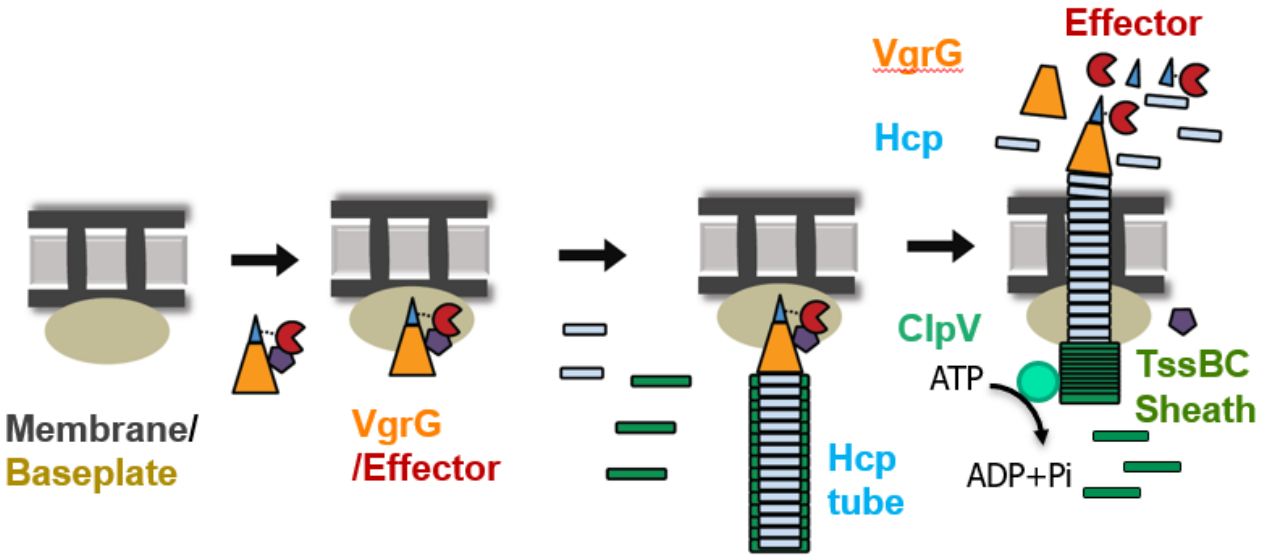
T6SS: an inverted phage-tail structure for effector delivery

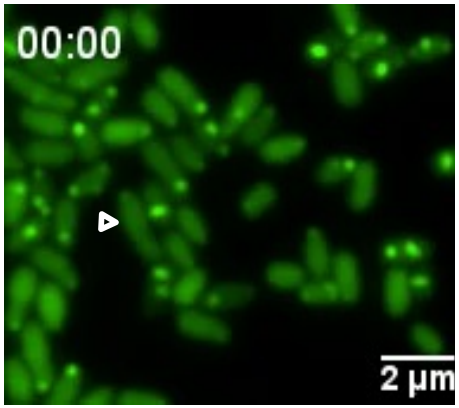


Records, Mol Plant-Microbe Interact, 2011

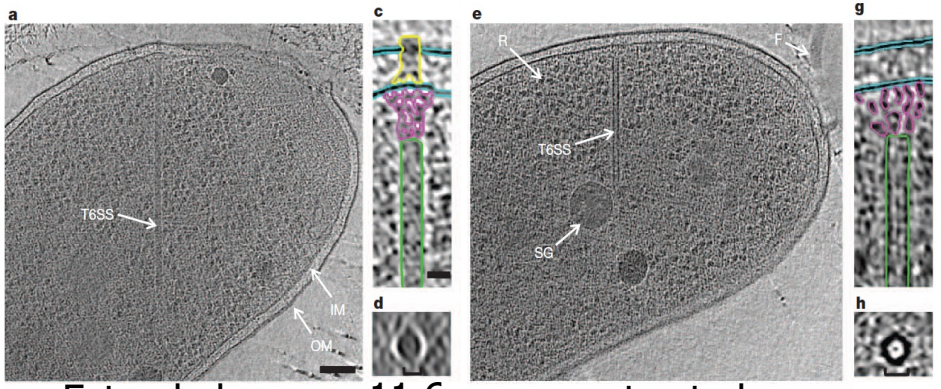
第六型分泌系統：一個伸縮性的注射器
T6SS is a contractile injection machine







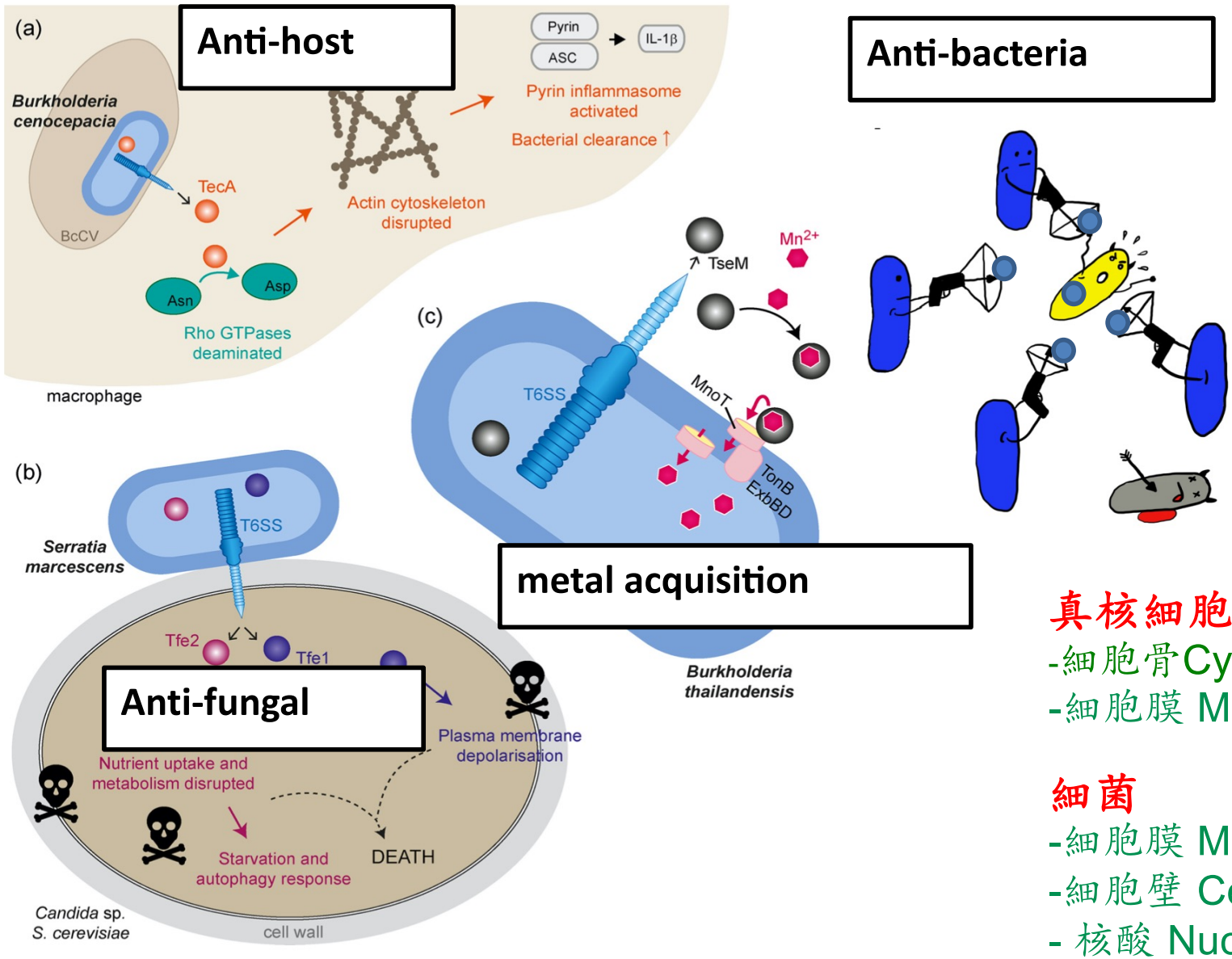
Agrobacterium TssB-GFP



Cryotomography of TssBC sheath *(Basler, Pilhofer et al., 2012)*

Movie website: http://www.nature.com/nature/journal/v483/n7388/extref/nature10846-s12.mov Basler et al., Nature (2012)

第六型分泌系統具效應分子及目標細胞多樣性

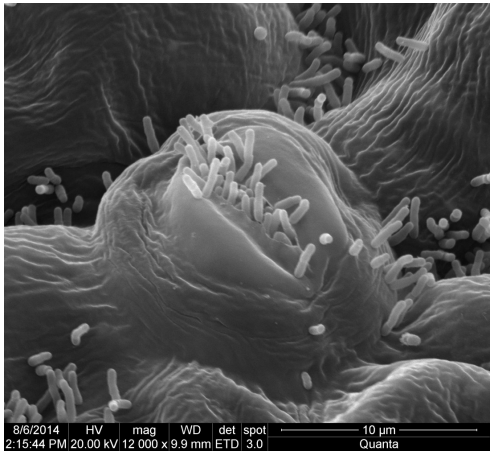


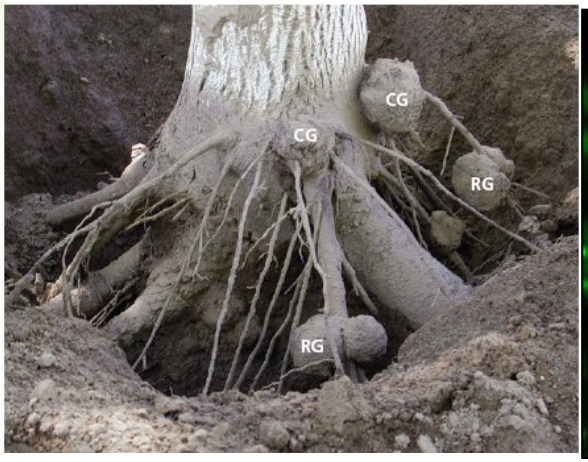
真核細胞
-細胞骨Cytoskeleton
-細胞膜 Membrane

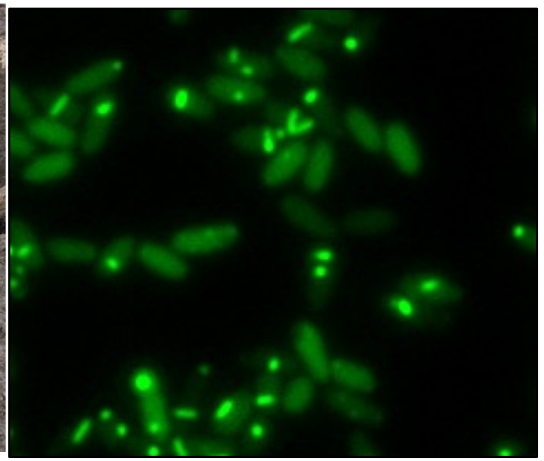
細菌
-細胞膜 Membrane
-細胞壁 Cell Wall
- 核酸 Nucleic acid

Coulthurst. Microbiology, 2019. ***165****: 503-15. Sana, et al, PLOS Pathogens, 2017.* ***13****(6): p. e1006325*

農桿菌: 從植物腫瘤病原菌化身為基因魔術師





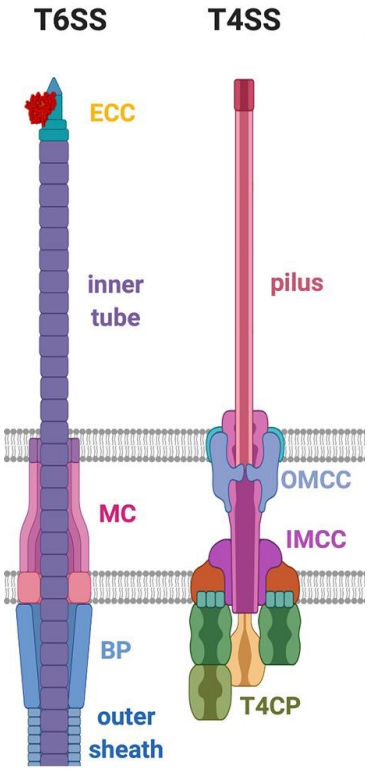


研究主題:

- 研究第四型及第六型蛋白質分泌系統 (T4SS, T6SS)的生化及生理功能
- 探討農桿菌如何運用蛋白質分泌系統來增進其感染力、植物的基因轉殖效率及在環境中的生存競爭力

農業及生技應用:

- 提高植物基因轉殖效率
- 相關藥物開發進而控制動植物細菌病原菌之感染



Photos by Wang, YC (Jane WN, IPMB Plant Cell Biology Core); Santos, MNM, Hsieh, K (IBC, Shih, YL lab), Lin et al., Front. Microbiol., 2020
http://californiaagriculture.ucanr.org/landingpage.cfm?article=ca.v062n03p111&fulltext=yes

農桿菌的生活史 (癌腫病)

Disease cycle of crown gall

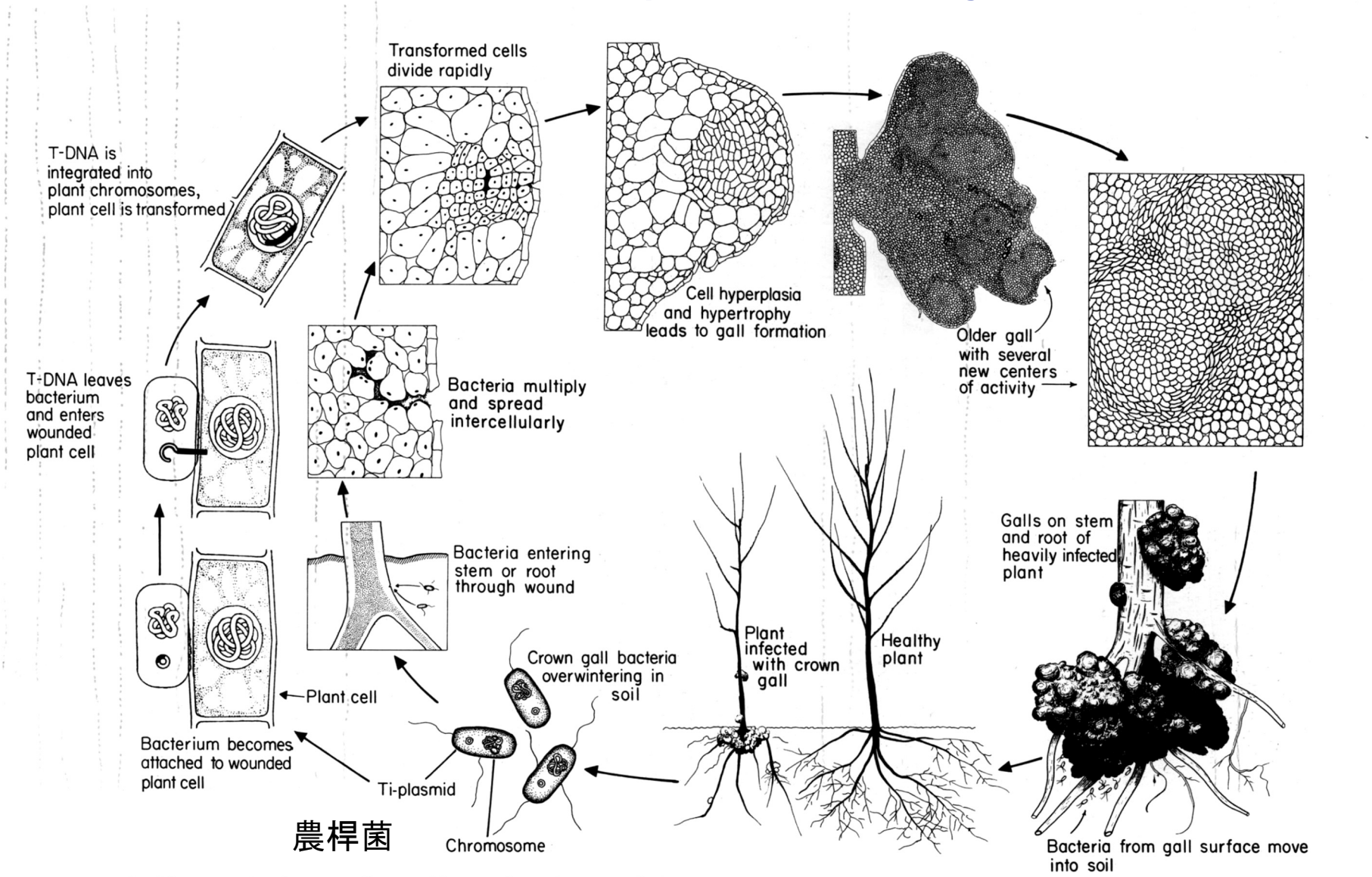
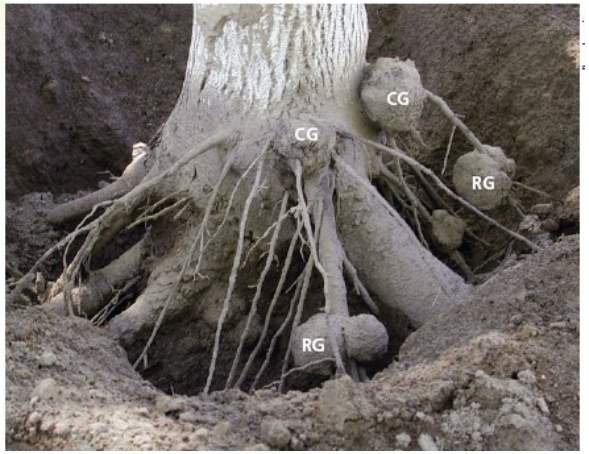


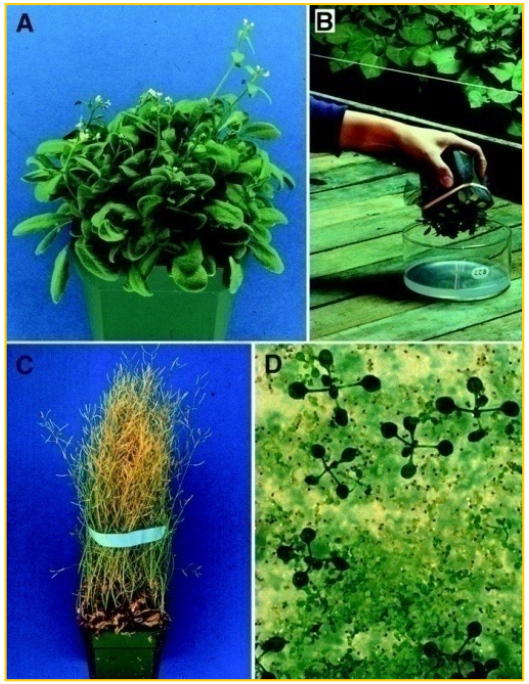
FIGURE 12-26 Disease cycle of crown gall caused by *Agrobacterium tumefaciens*.

農桿菌: 從植物腫瘤病原菌化身為基因魔術師
Agrobacterium tumefaciens: from causal agent of plant tumors transformed to gene engineering magician

- Crown gall disease
- Interkingdom DNA transfer
- Gene transfer tool



Walnut crown and root galls



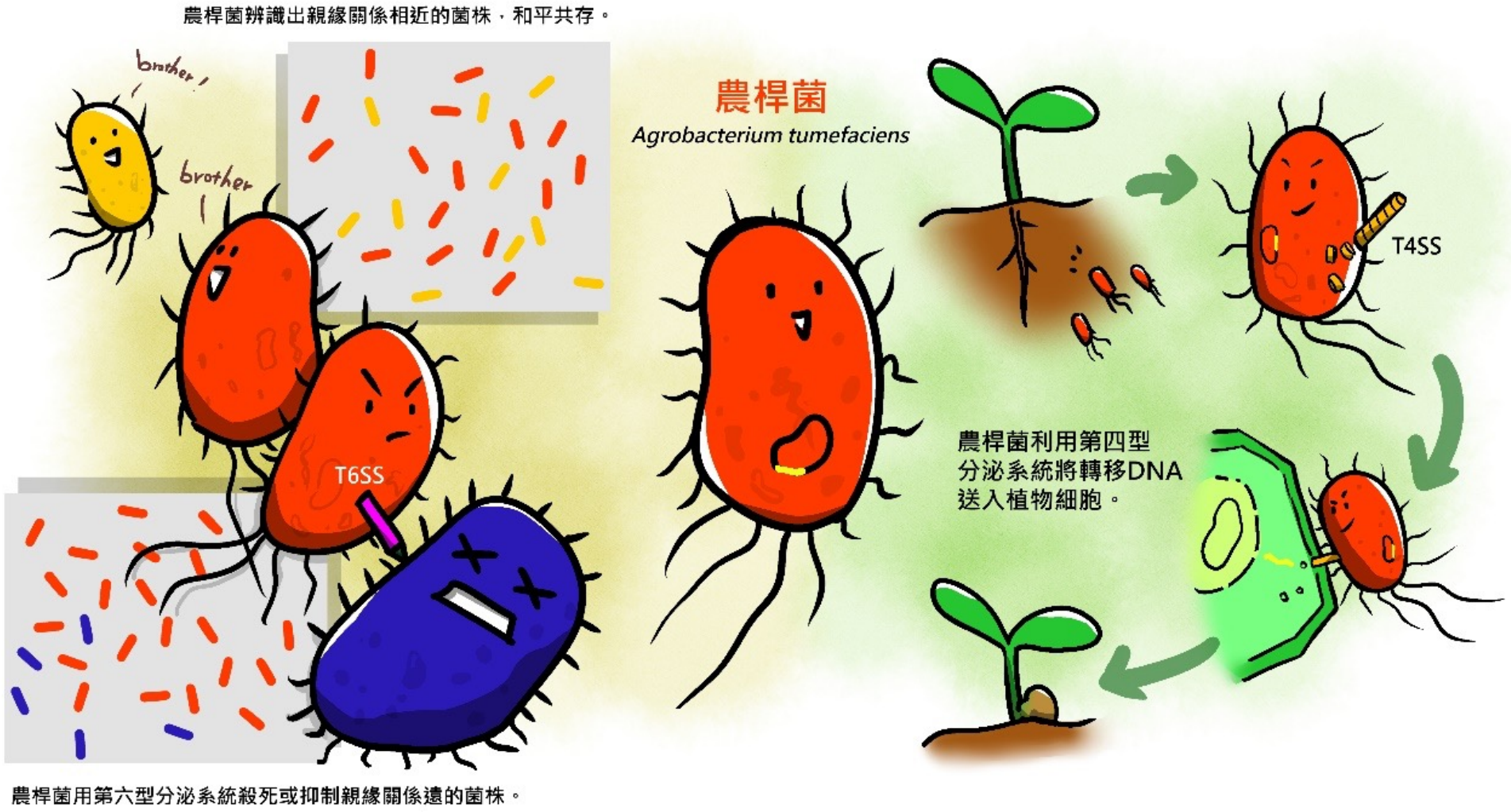
Bent, Plant Physiol (2000)

Apple rootstock

Grape vine

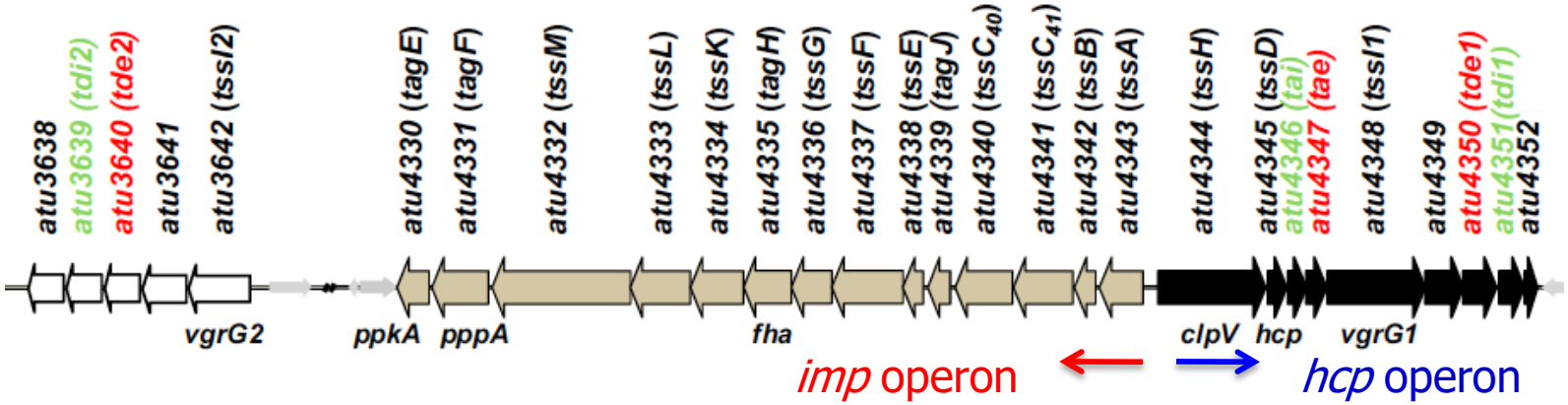
Several economically important crops suffer serious crown gall diseases and many plants resistant to *Agrobacterium*-mediated transformation!!!

農桿菌生存競爭及感染植物宿主的策略



農桿菌C58菌株具有三個T6SS細菌毒素效應分子

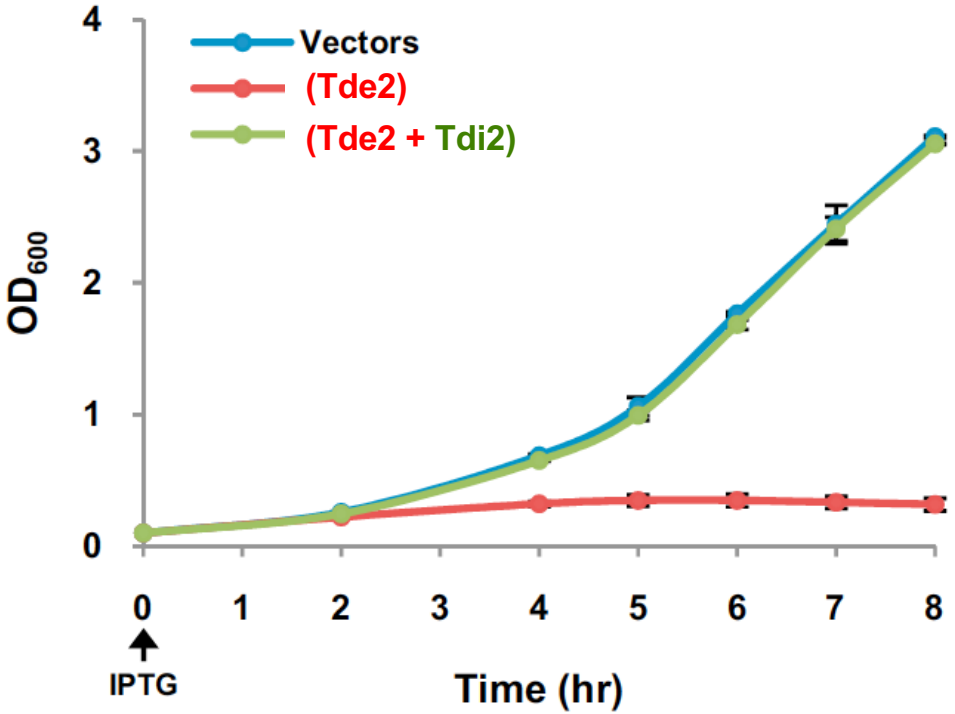
Agrobacterium strain C58 produces three toxins



- **Tae-Tai**: 細胞壁分解酶-免疫蛋白質
  type VI secretion peptidoglycan amidase effector and immunity

Tde1-Tdi1: 核酸分解酶1-免疫蛋白質1
type VI DNase effector and immunity protein 1

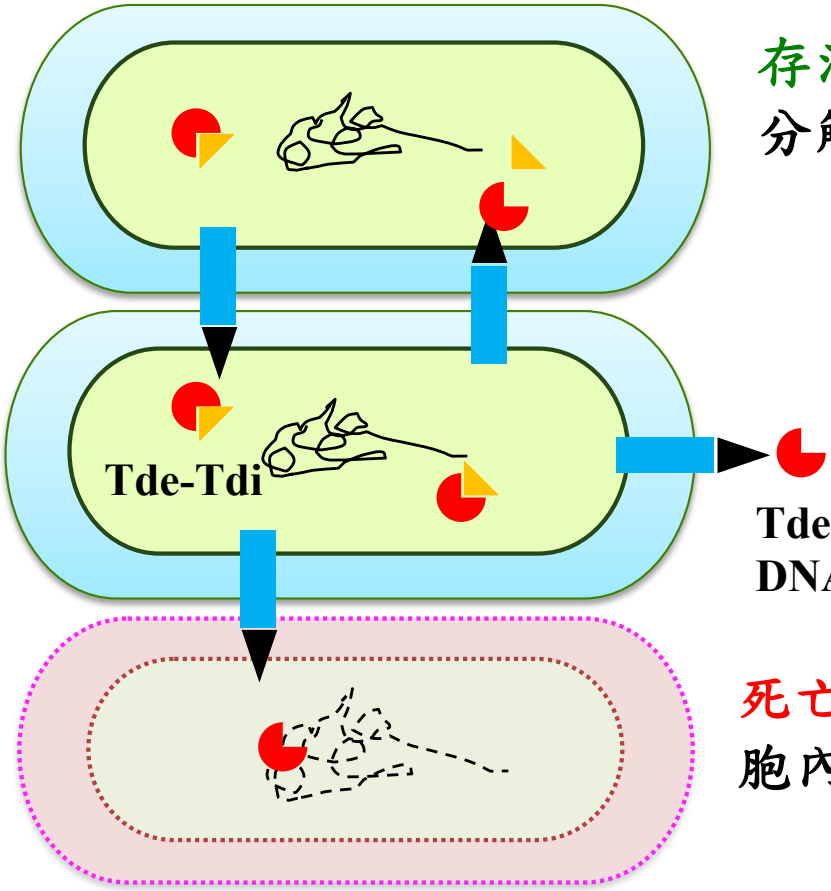
Tde2-Tdi2: 核酸分解酶2-免疫蛋白質2
type VI DNase effector and immunity protein 2



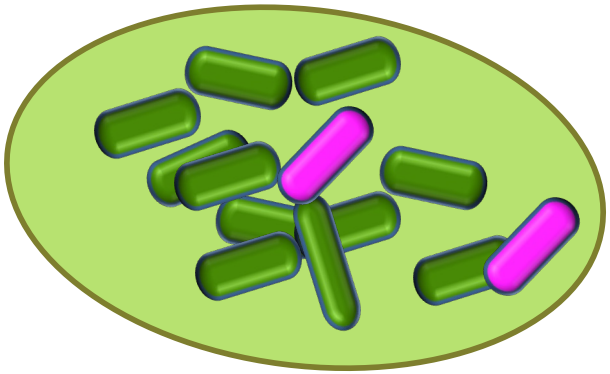
Bacterial growth inhibition assay

Ma et al., Cell Host Microbe, 2014

農桿菌第六型分泌系統為細菌攻防戰的新武器
T6SS as a weapon for interbacterial competition



存活:同源免疫蛋白質Tdi與Tde DNA分解酶結合以保護自身的存活

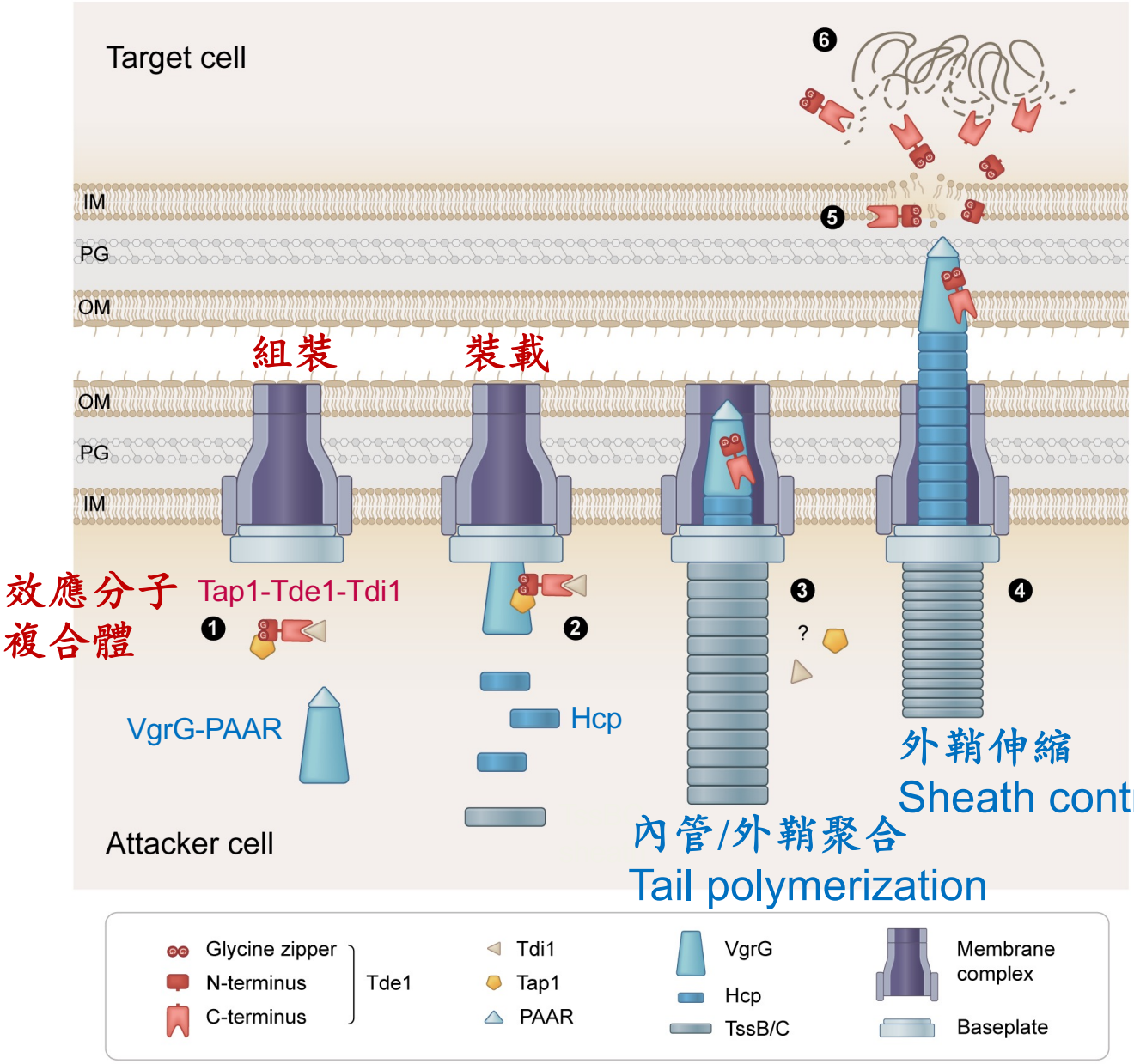


死亡: Tde注射至所接觸的細菌競爭對手胞內並分解競爭對手細胞內的DNA分子

Tde-Tdi: type VI DNase effector and immunity protein

Ma et al., Cell Host Microbe, 2014

第六型分泌系統效應分子的裝載、活化及運輸機制
Tde1 loading, firing, and translocation to target cells

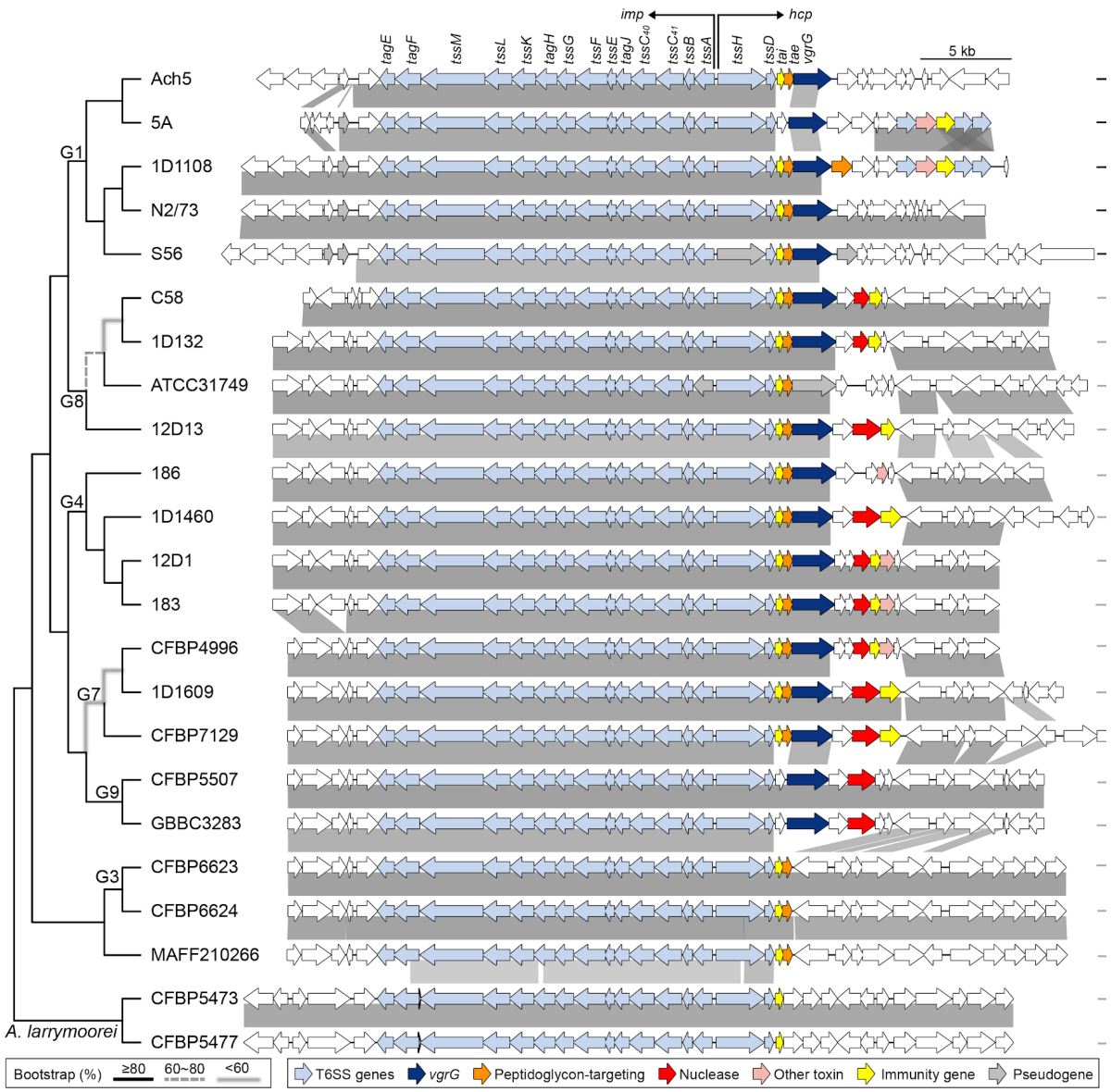


Ali et al., EMBO Rep, 2023

農桿菌物種複合體普遍具有第六型分泌系統殺菌武器

T6SS is widespread across *A. tumefaciens* species complex and functions as an antibacterial weapon

Conserved T6SS gene cluster with diverse effectors



Genomo-species	Predator genotype	Prey: *E. coli* (pRL662)
G8	C58	++
	C58Δ*tssL*	
	1D132	++
	1D132Δ*tssL*	
G7	1D1609	++
	1D1609Δ*tssL*	
G4	1D1460	+
	1D1460Δ*tssL*	
	12D1	+
	12D1Δ*tssL*	
G1	1D1108	+++
	1D1108Δ*tssL*	
	15955	+++
	15955Δ*tssL*	
	A6	+++
	A6Δ*tssL*	

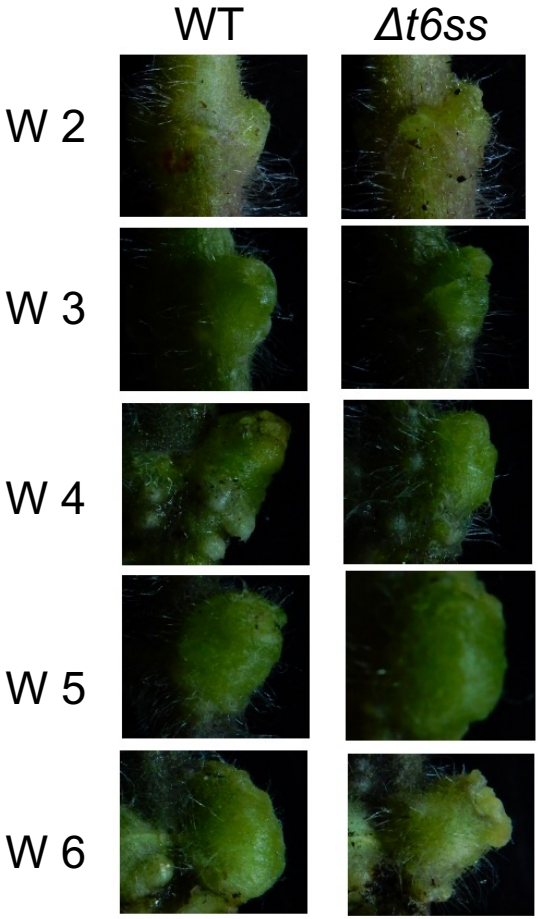
10X dilution →

Chou, Lin et al., 2021; Wu et al., 2018

第六型分泌系統的有無不影響農桿菌的致病能力

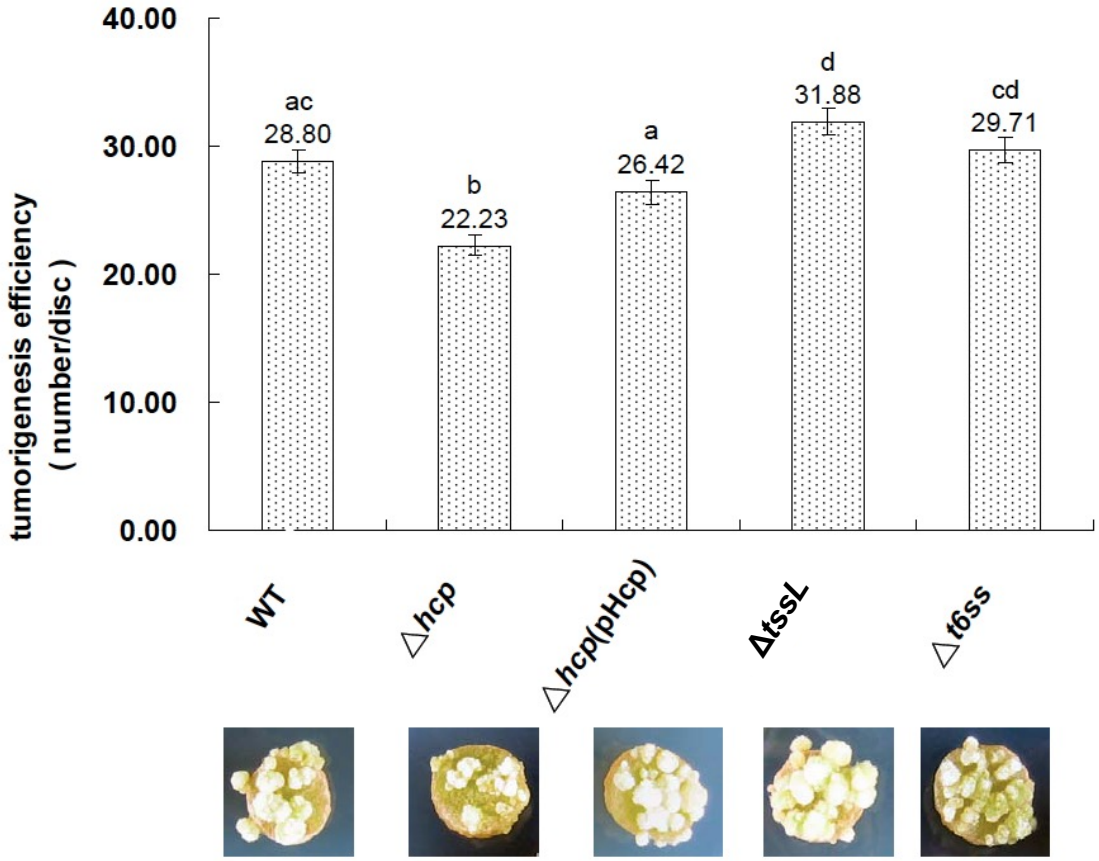
T6SS is not required for gall formation when inoculated directly on plant wounding sites

Tumor assay on tomato stems



(by Chien CF)

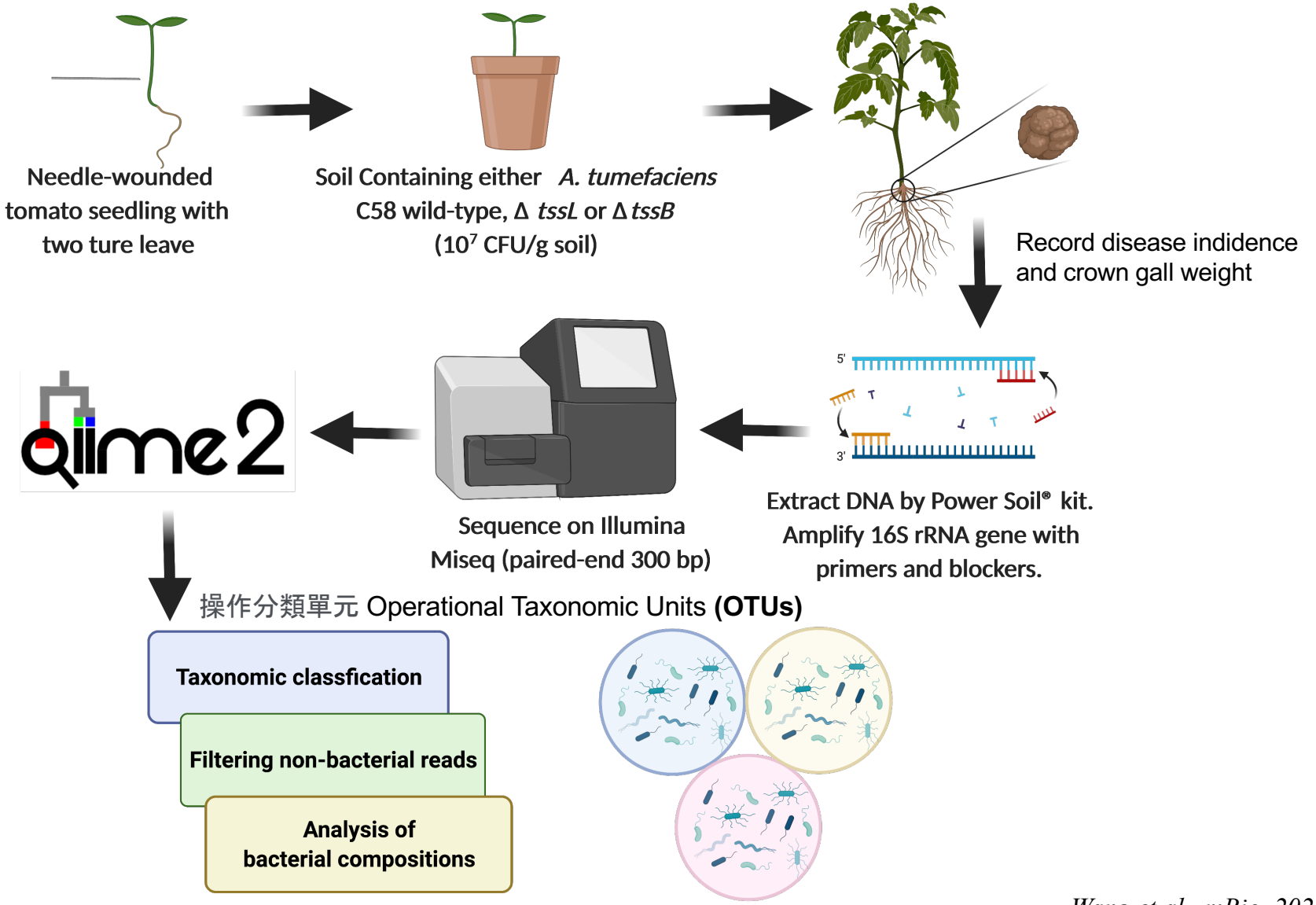
Tumor assay on potato tuber disc



Wu et al., J Bacteriol. (2008)

- We tested the role of T6SS in :
- Tumorigenesis under a more natural setting
- *A. tumefaciens*-associated microbiota in crown gall (termed as gallobiome)

農桿菌土壤接種法及腫瘤微生物相分析流程
Workflow of soil inoculation and gallobiome analysis

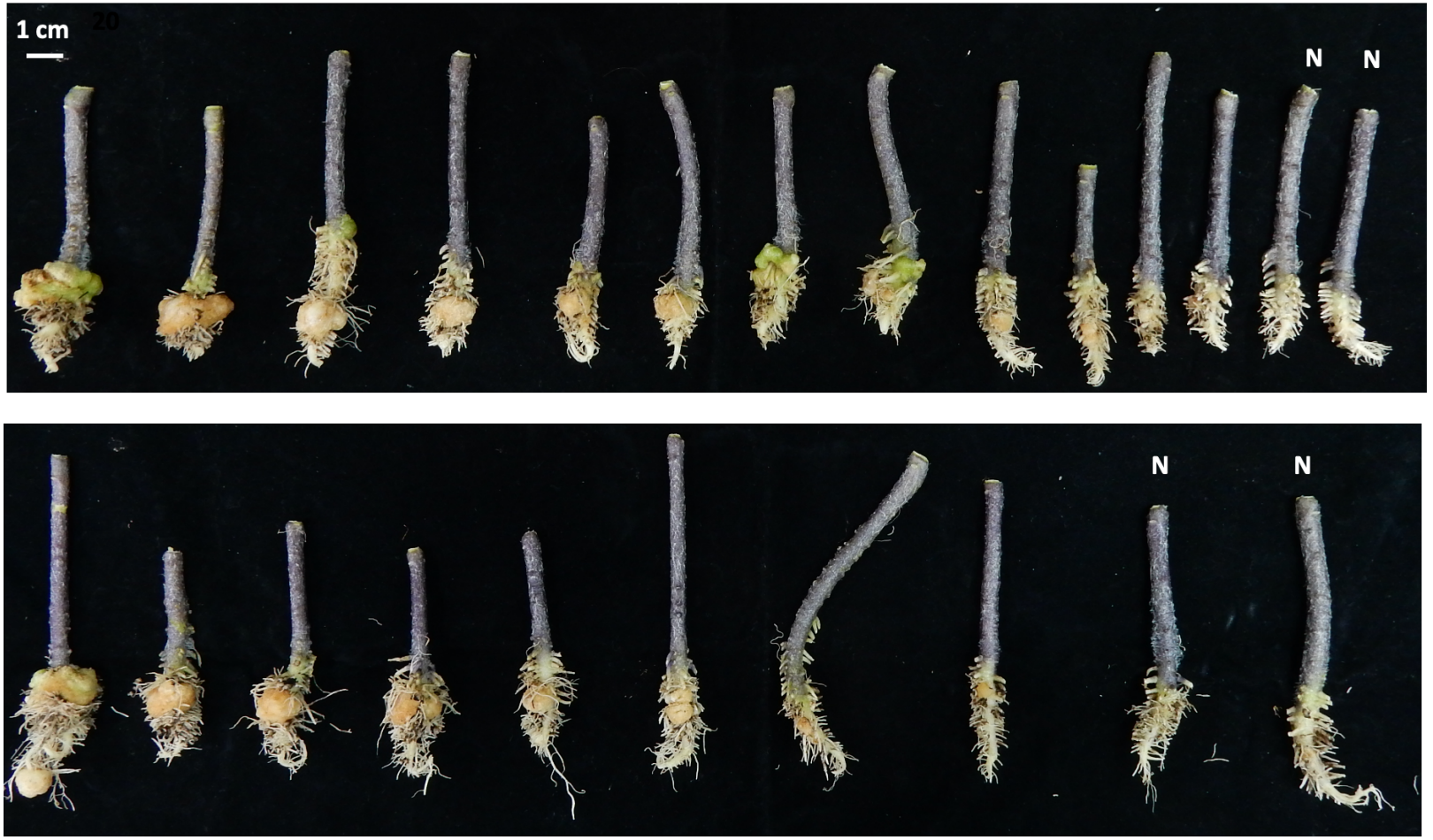


Wang et al., mBio, 2023

模擬自然感染的土壤接種法可以觀察腫瘤發生率及多樣性
Diversity of crown galls generated through soil inoculation

WT

ΔtssL

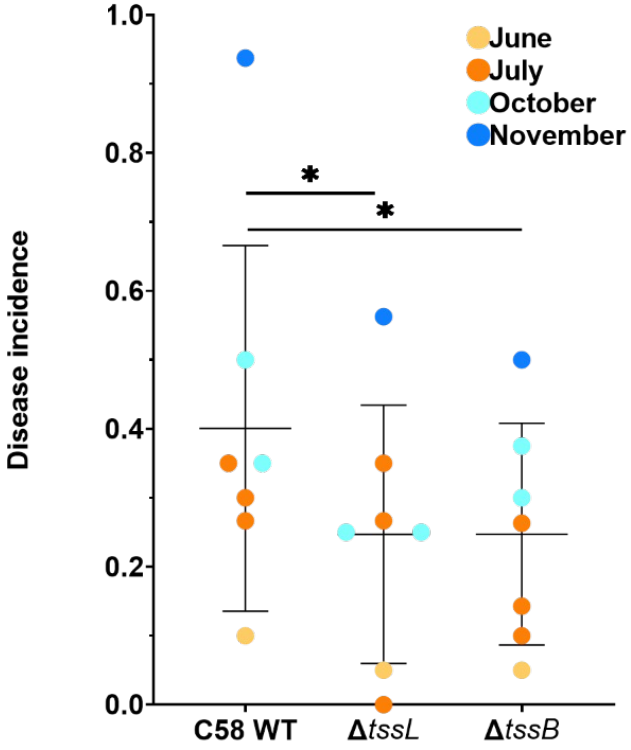


Not all infected plants produce tumors, in contrast to 100% disease incidence when inoculated directly on wounded stems.

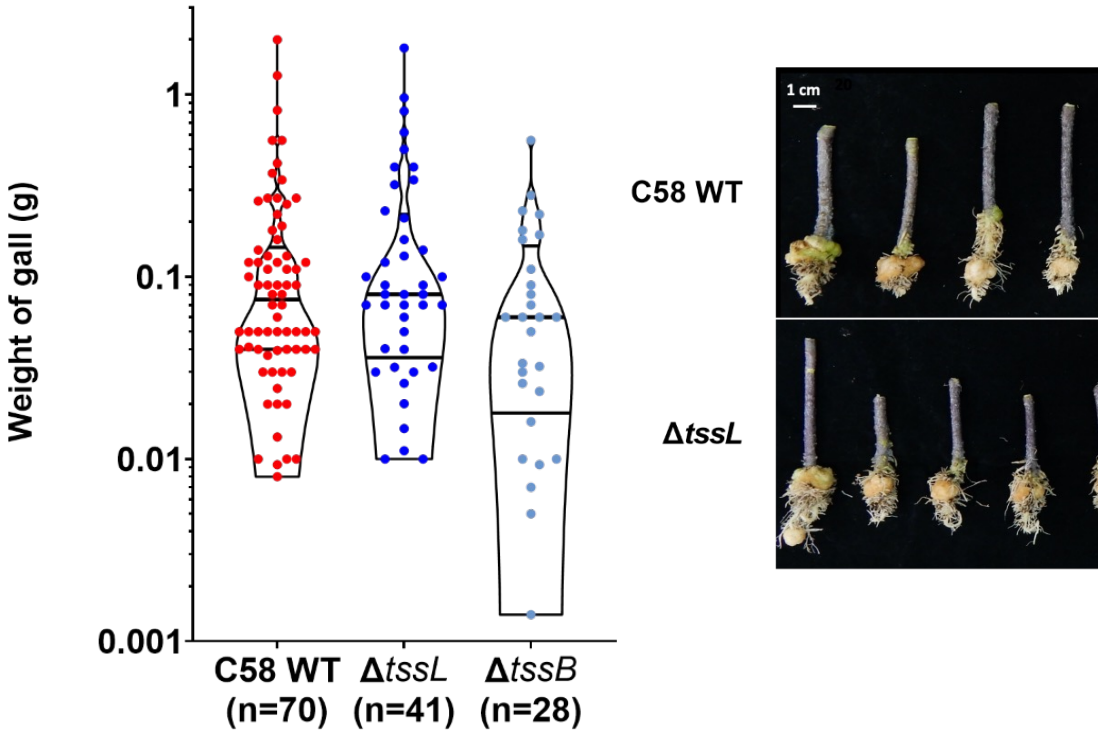
Wang et al., mBio, 2023

第六型分泌系統缺失的突變株誘導較低的腫瘤發生率
T6SS mutants induced lower crown gall disease incidences

Disease incidence

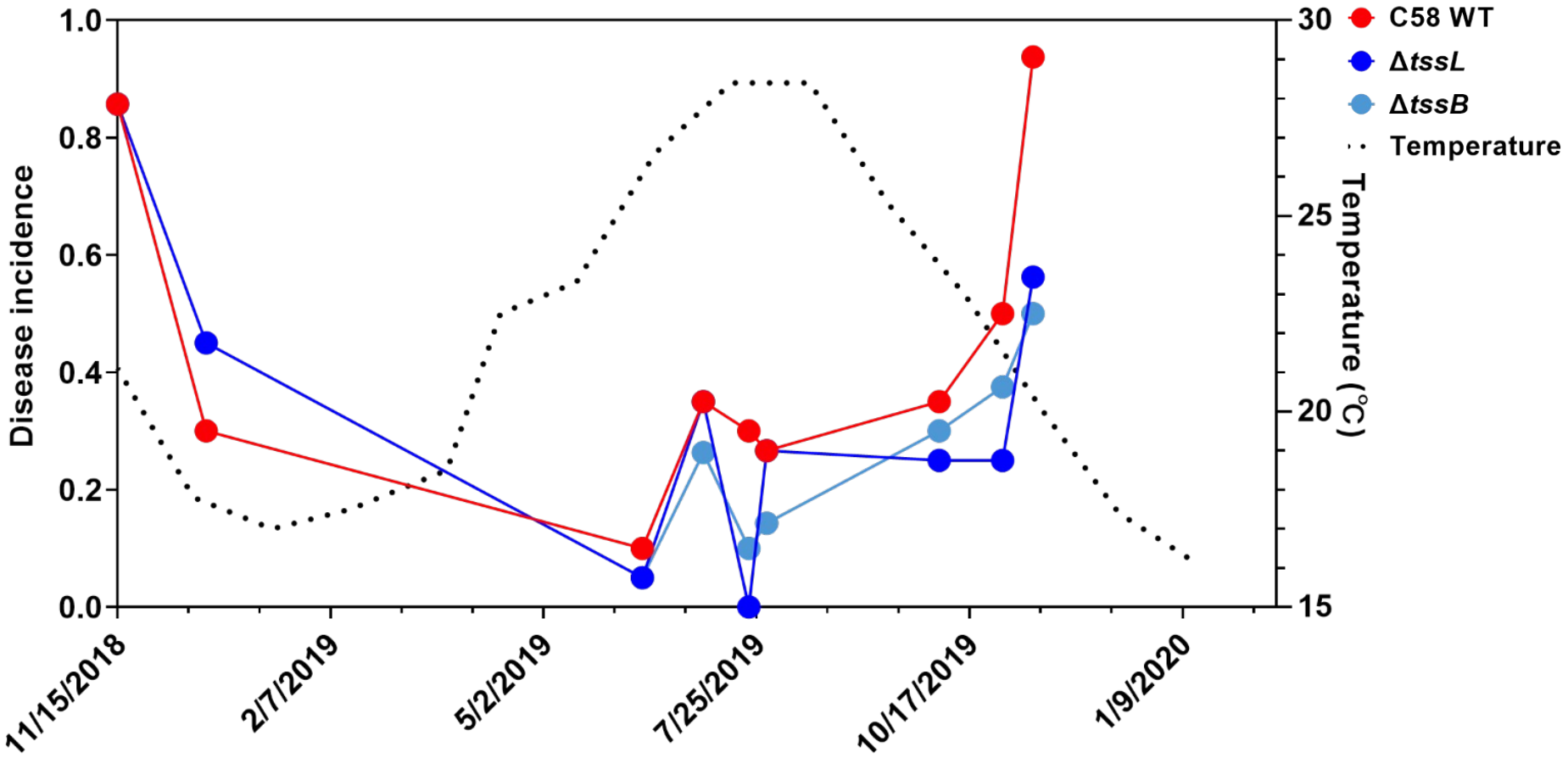


Crown galls weight and morphology



Wang et al., mBio, 2023

腫瘤發生率受環境因子的影響
Tumorigenesis is affected by environmental factors



- An inverse correlation between disease incidence and temperature

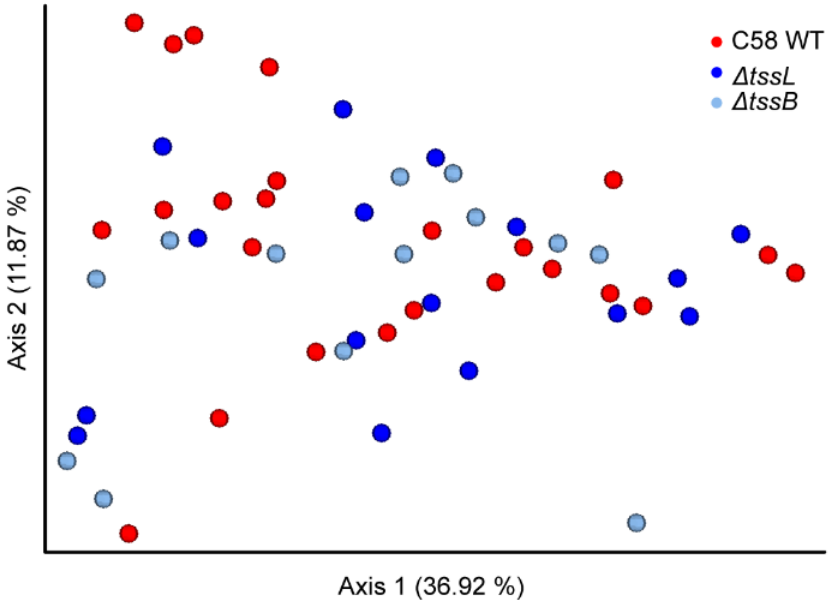
Wang et al., mBio, 2023

第六型分泌系統對腫瘤細菌相的主要組成沒有顯著影響

Gallobiomes induced by T6SS mutants were not significantly different

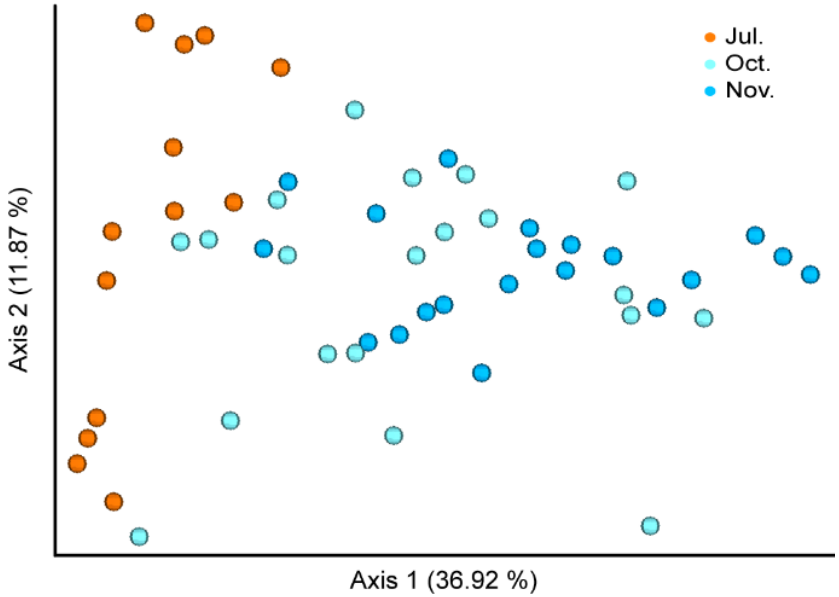
- PCoA analysis of 53 galls microbiota on OTUs (operational taxonomic units)

Strains



p-value = 0.362, R^2 = 0.023

Month of inoculation

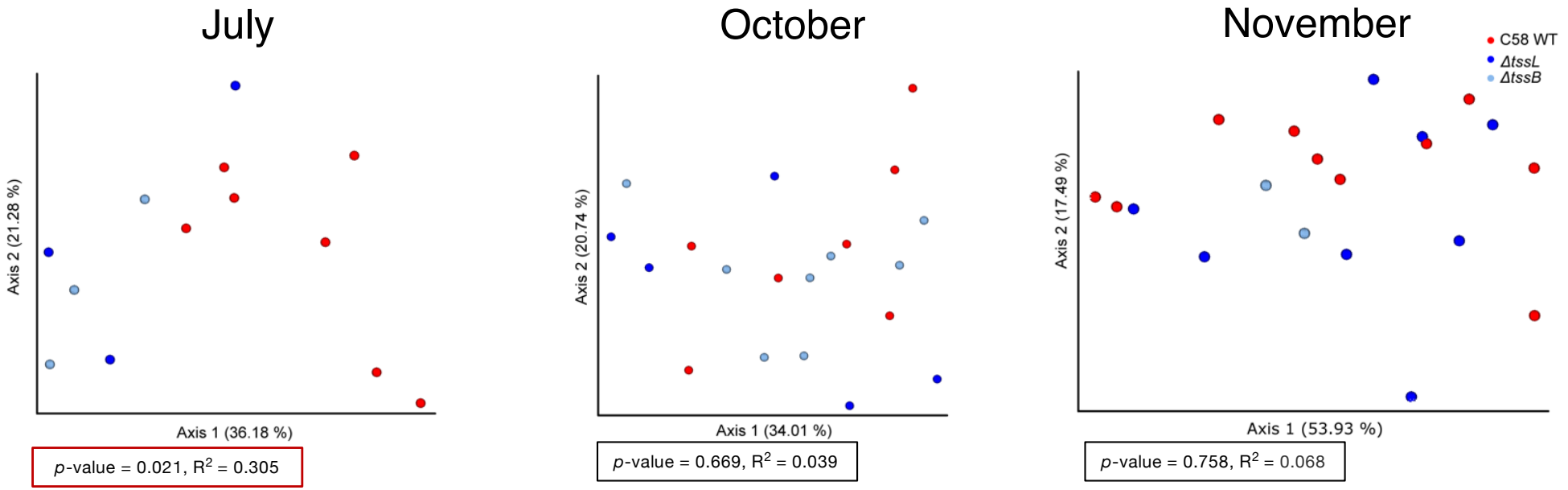


p-value = 0.001, R^2 = 0.260

- 24 induced by WT, 16 by Δ*tssL*, and 13 by Δ*tssB*.
- ➢ Most of the variation between samples were contributed by different seasons (July vs. October/November).

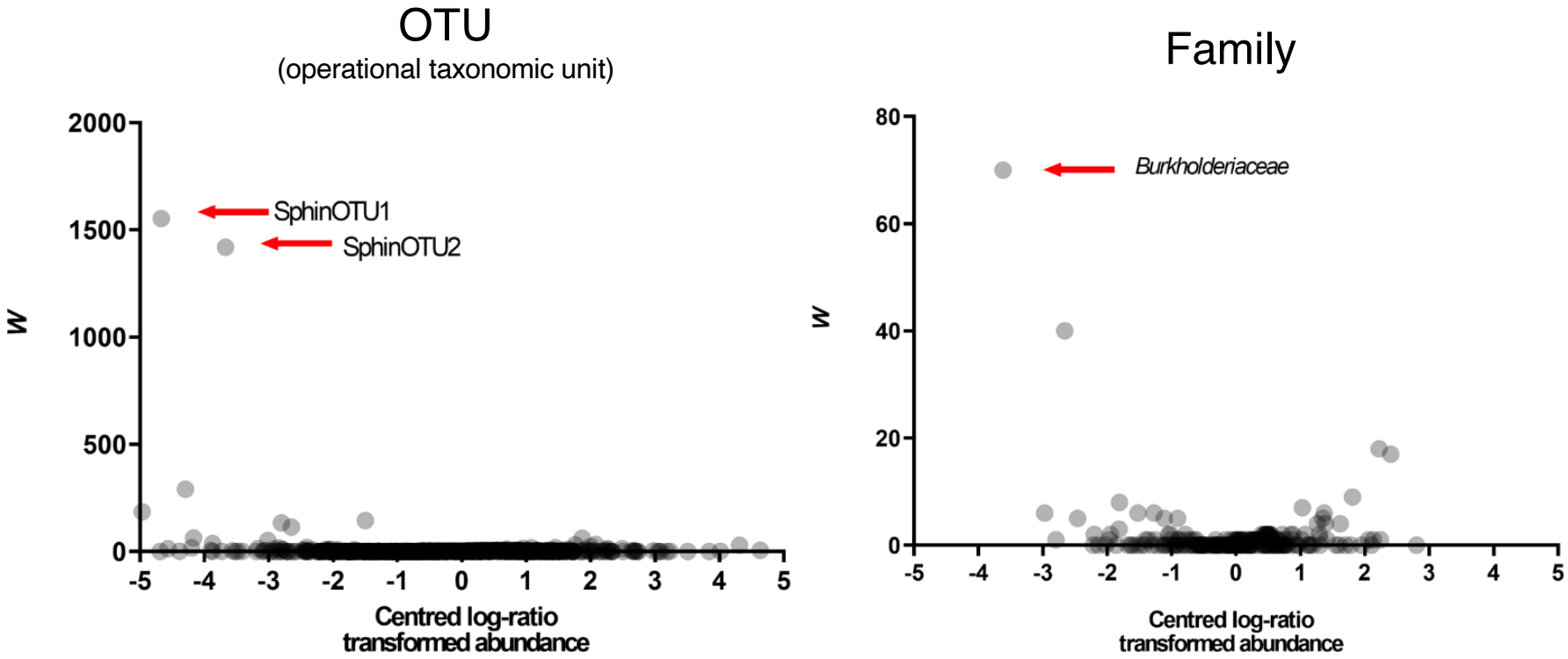
第六型分泌系統對七月接種的腫瘤細菌相有影響

Distinct gallobiome compositions associated WT and T6SS mutants in July



第六型分泌系統的缺失使腫瘤細菌相之特定物種有顯著較高的相對量

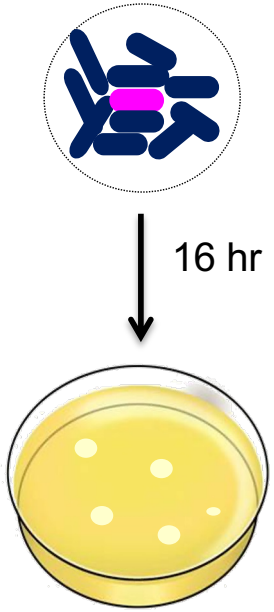
Sphingomonas OTUs and Burkholderiacea were more abundant in the gallobiomes induced by the T6SS mutants in July



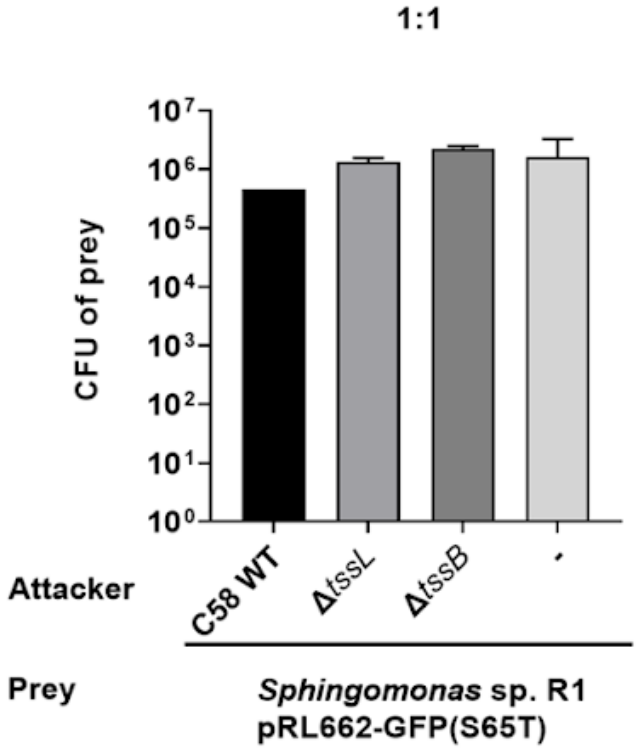
農桿菌第六型分泌系統會抑制蕃茄根圈細菌的生長

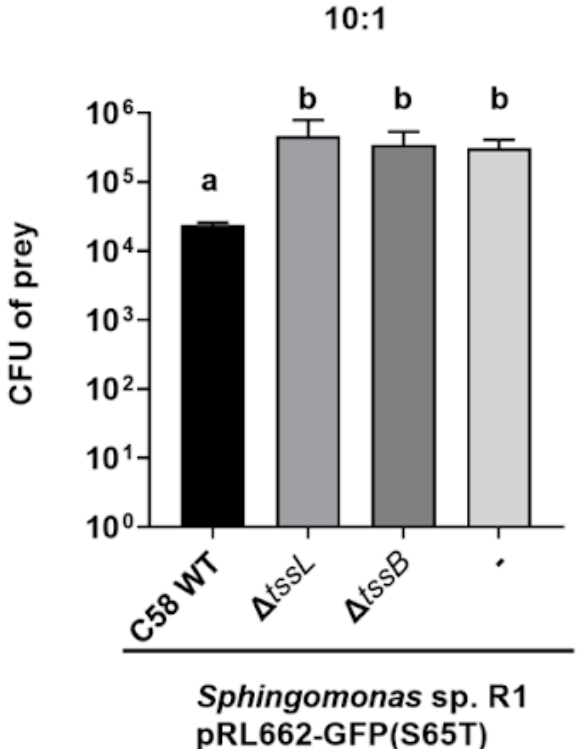
Agrobacterium exhibits T6SS antagonism to a tomato isolate *Sphingomonas* sp. R1

Attacker (10 or 1)
Prey (1)



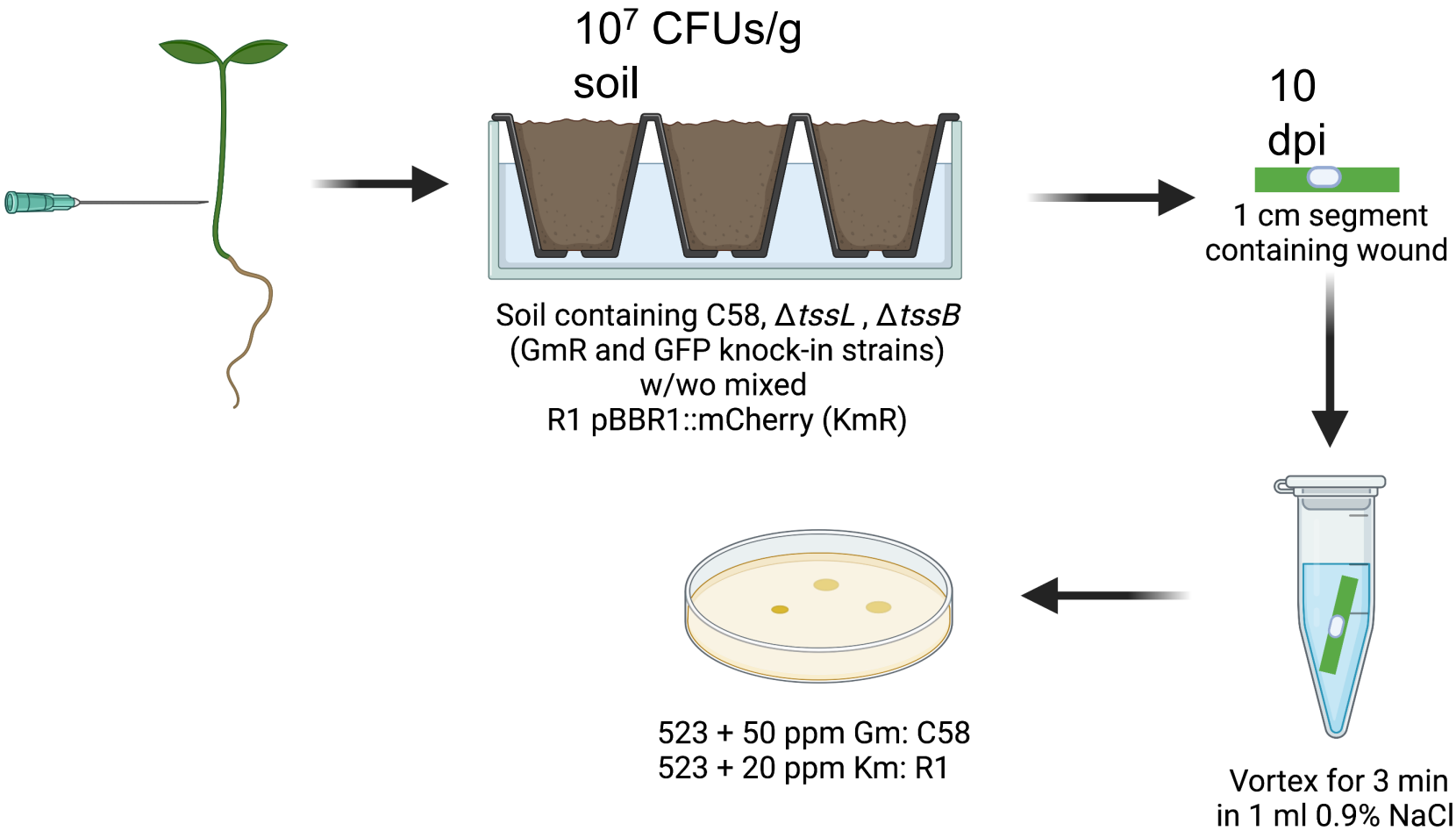
Prey survival by counting CFU (colony forming unit)





-*In vitro* interbacterial competition

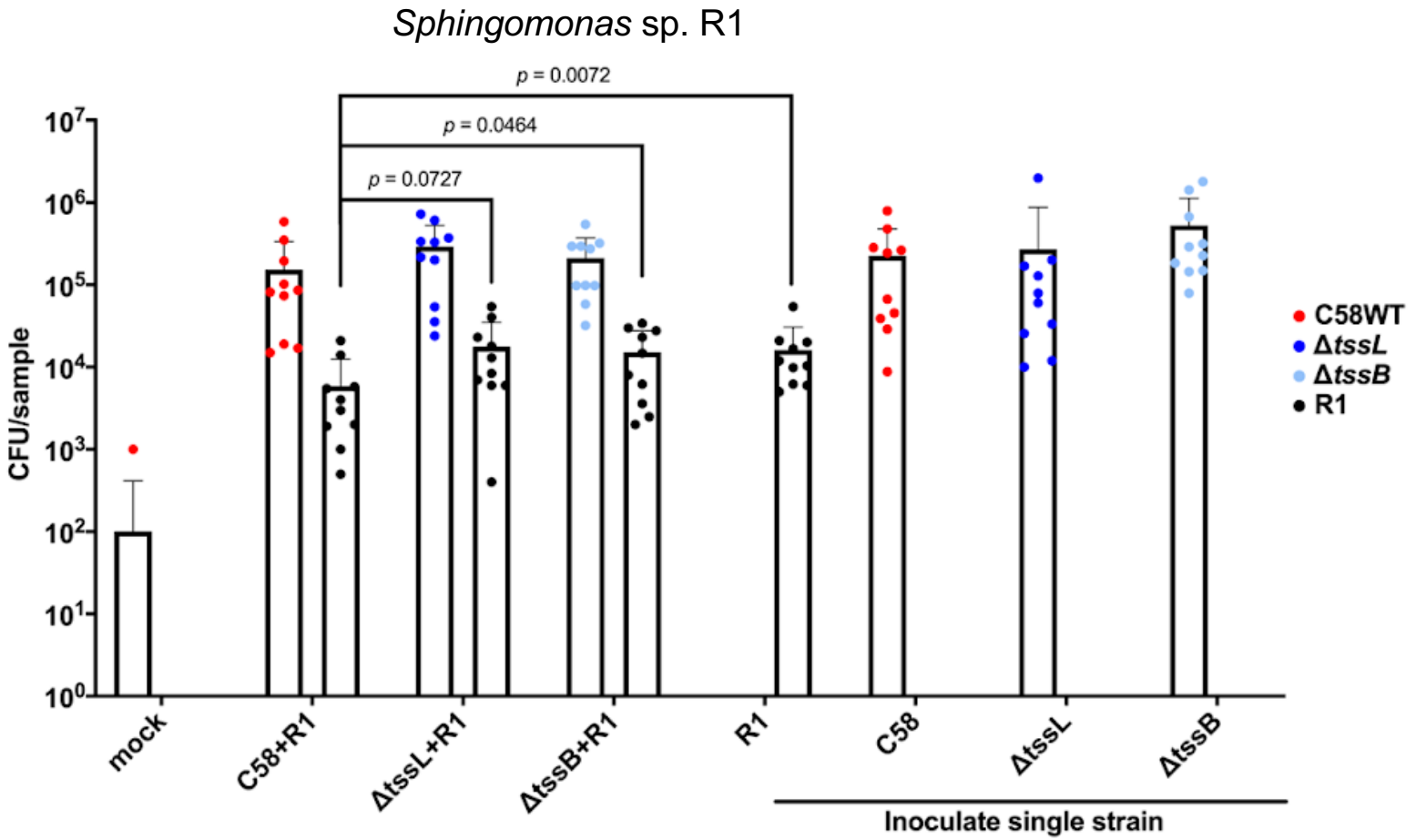
農桿菌土壤接種法在蕃茄小苗植物傷口纏據試驗流程
Colonization on wounded site of tomato seedlings after co-inoculation in soil



Wang et al., mBio, 2023

第六型分泌系統提升農桿菌對蕃茄根圈細菌的生存競爭優勢

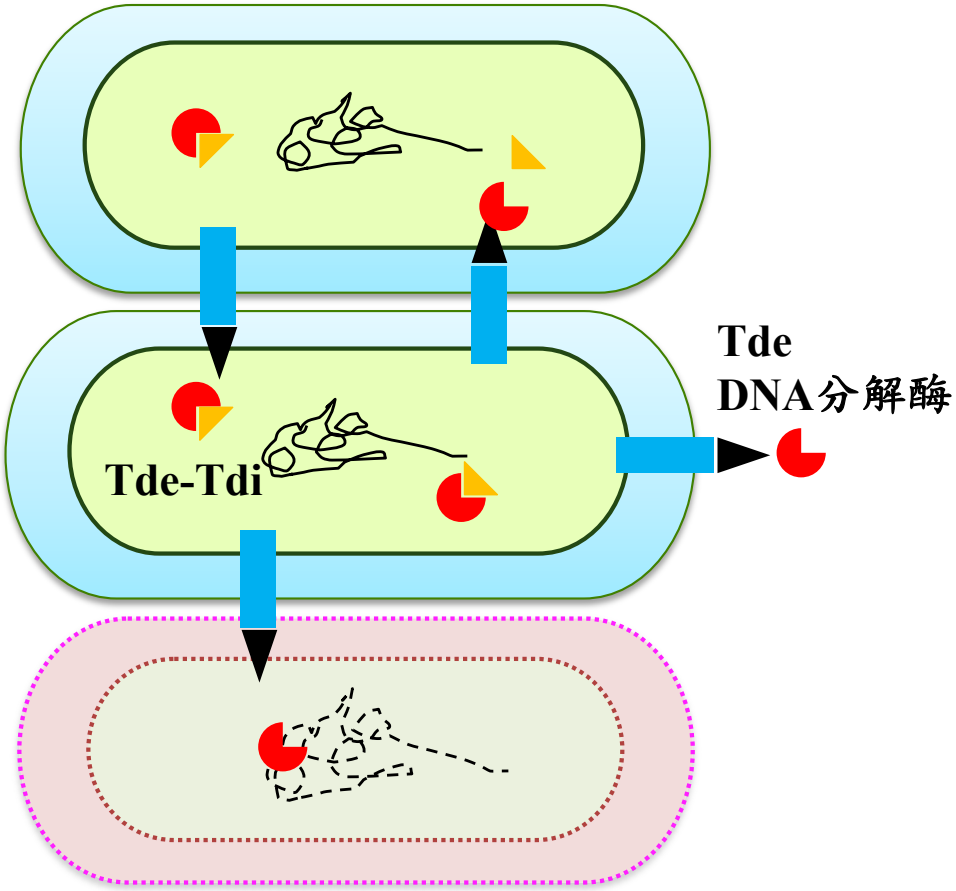
Agrobacterium exhibits T6SS antagonism to *Sphingomonas* sp. R1 during plant colonization



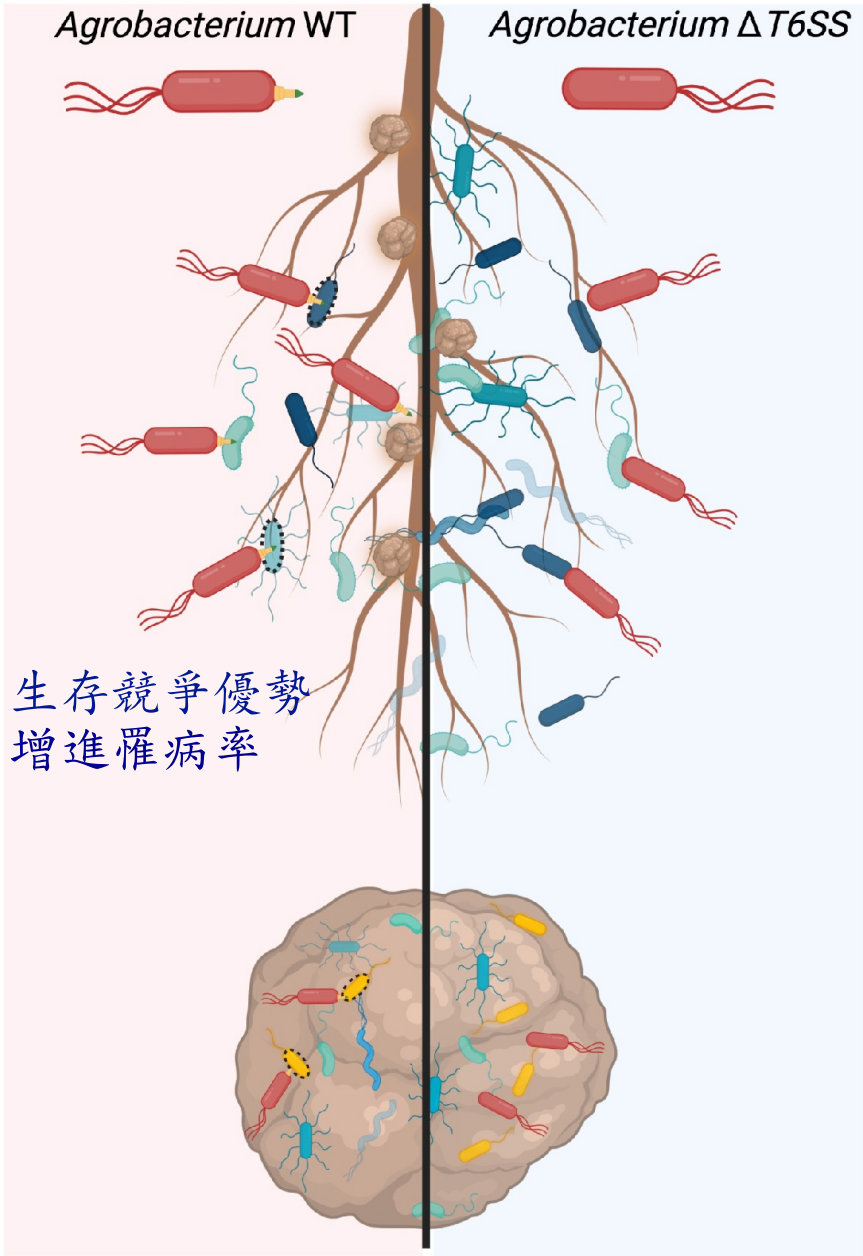
Wang et al., mBio, 2023

農桿菌第六型分泌系統為細菌攻防戰的武器

存活:同源免疫蛋白質Tdi與Tde
DNA分解酶結合以保護自身的存活

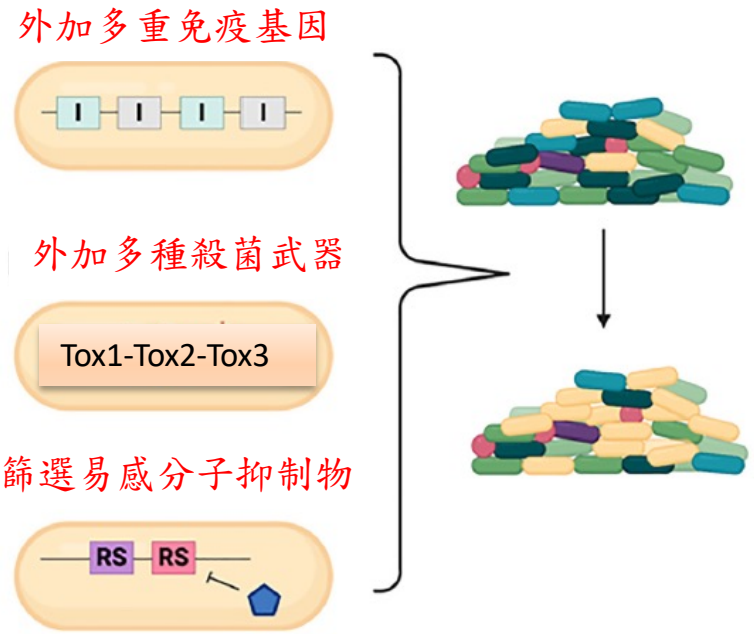


死亡: Tde注射至所接觸的細菌競爭對手胞內並分解競爭對手細胞內的DNA分子

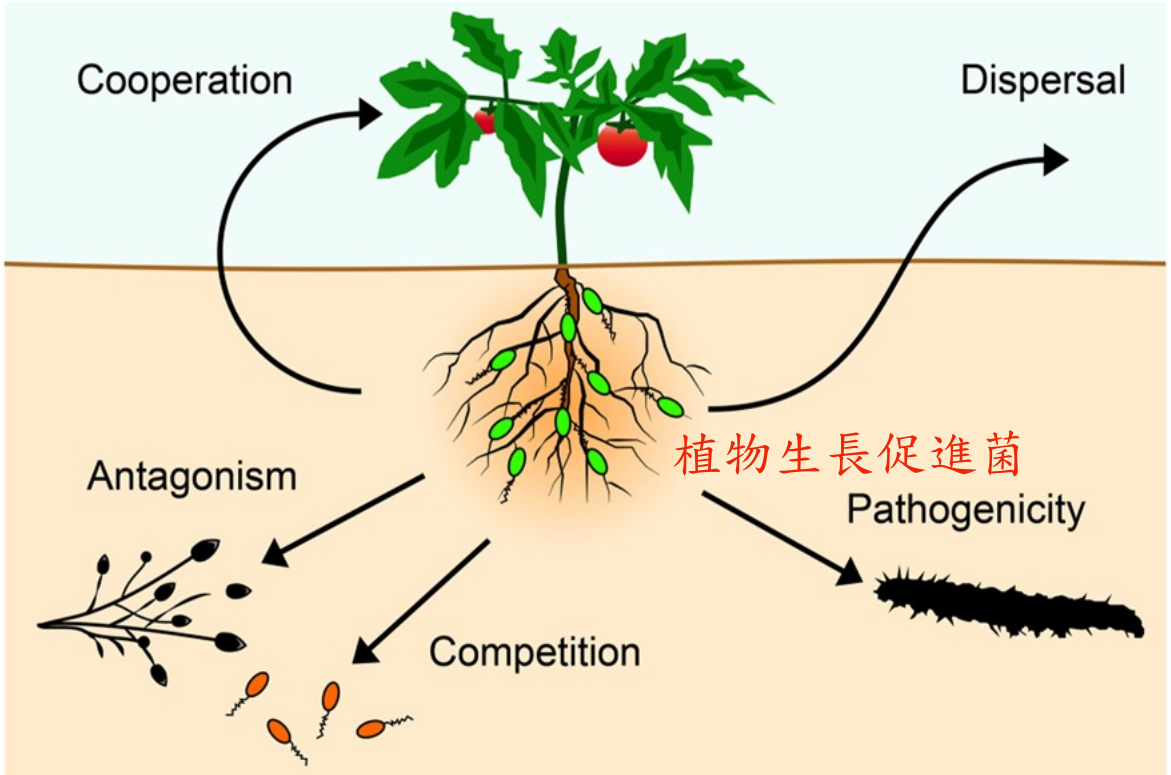


生物防治改良新思考

- 以合成生物學的方式在植物生長促進菌或益生菌的基因組中加入不同的殺菌武器或篩選具多樣性抗菌武器的細菌，或可增進生物防治或益生菌的存活及其效應的強度及廣泛性。



I: Immunity gene (免疫基因)
Tox: Bacterial toxin/effector
RS: Recipient susceptibility gene (受體易感基因)

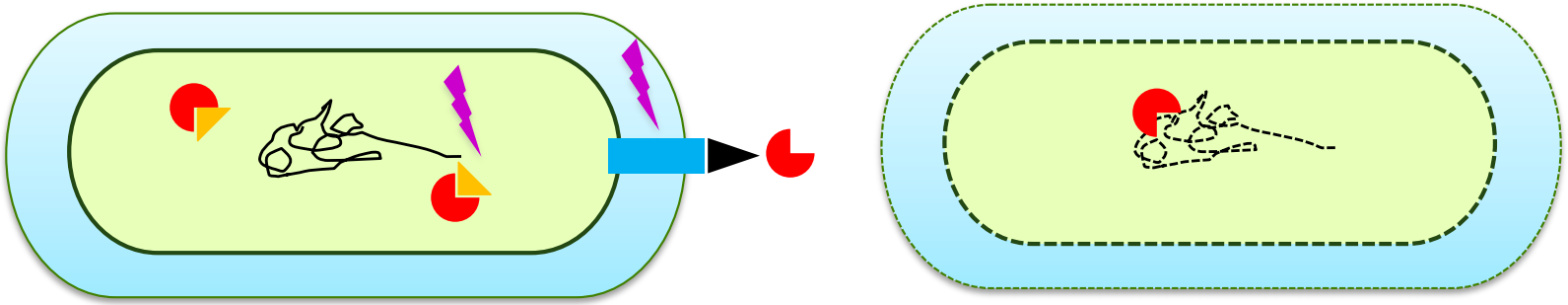


Modified from Lin et al (2020).Front. Microbiol. doi: 10.3389/fmicb.2020.603652

Modified from Front. Plant Sci., 31 July 2013 | doi: 10.3389/fpls.2013.00287

醫藥改良新思考: 篩選新抗生素？

- 分泌系統裝置或同源免疫蛋白質immunity protein或許可作為發展新藥的攻擊對象來抑制特定病原細菌的族群生長。



DNA分解酶 DNase toxin

免疫蛋白質 Immunity protein