温度对志贺氏菌毒力的影响

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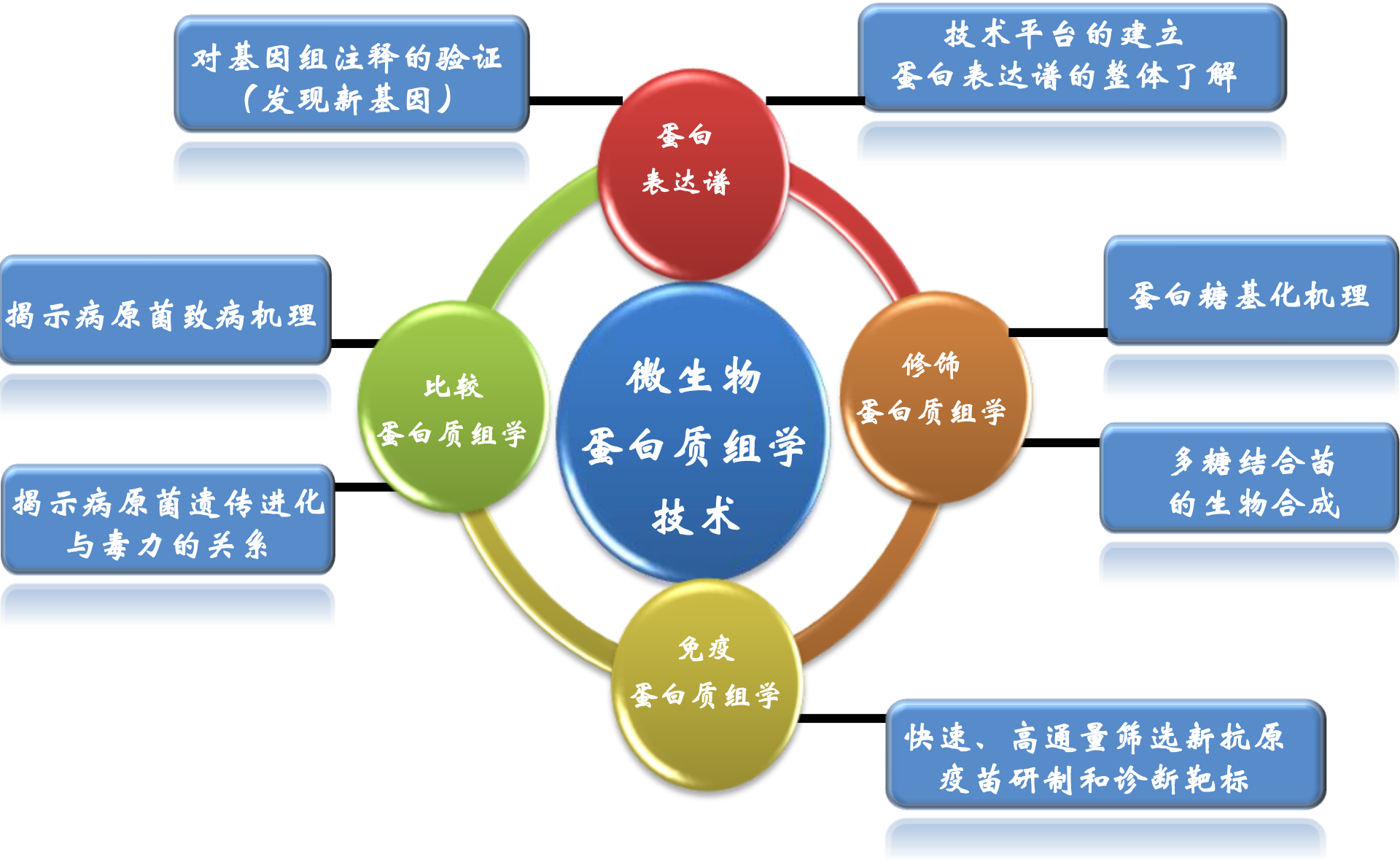
学科研究方向——病原细菌的防控研究

主要研究对象：志贺氏菌和炭疽芽胞杆菌

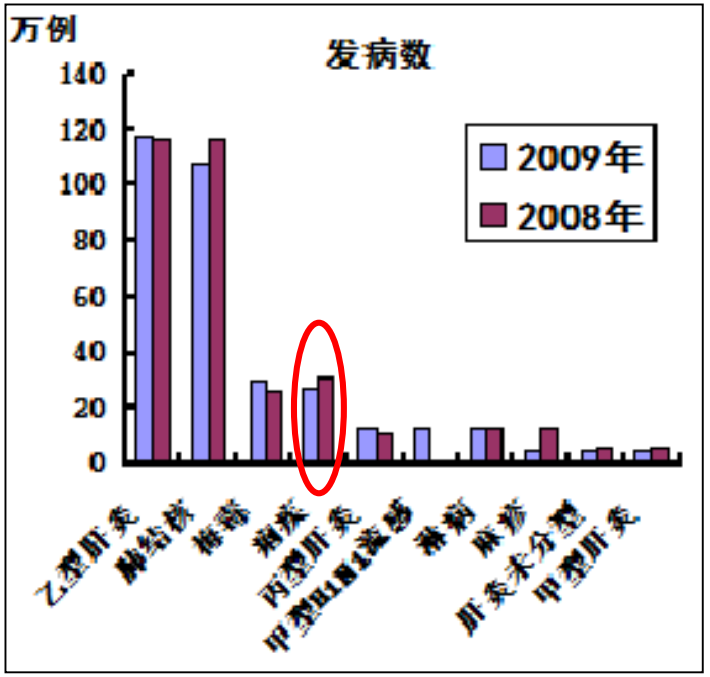
微生物蛋白质组学技术在病原菌防控中的应用



研究志贺氏菌的重要性(一) (志贺氏菌俗称痢疾杆菌)

- 高发病率(极低的感染剂量)

Test strain	Reference	Administered dose*	No. volunteers	No. ill (%)†
S. flexneri 2a,				
2457T	3–5	100	36	14 (39)
		180	36	9 (25)
		5×10^3	49	28 (57)
		10^4	103	58 (56)
		10^5–10^8	59	38 (64)
S. dysenteriae 1				
A-1	6	200	8	3 (38)
		10^4	6	2 (33)
M-131	6	10	10	1 (10)
		200	4	2 (50)
		2×10^3	10	7 (70)
		10^4	6	5 (83)
S. sonnei				
53G	none	500	20	7 (35)
53G	7	500	38	19 (50)

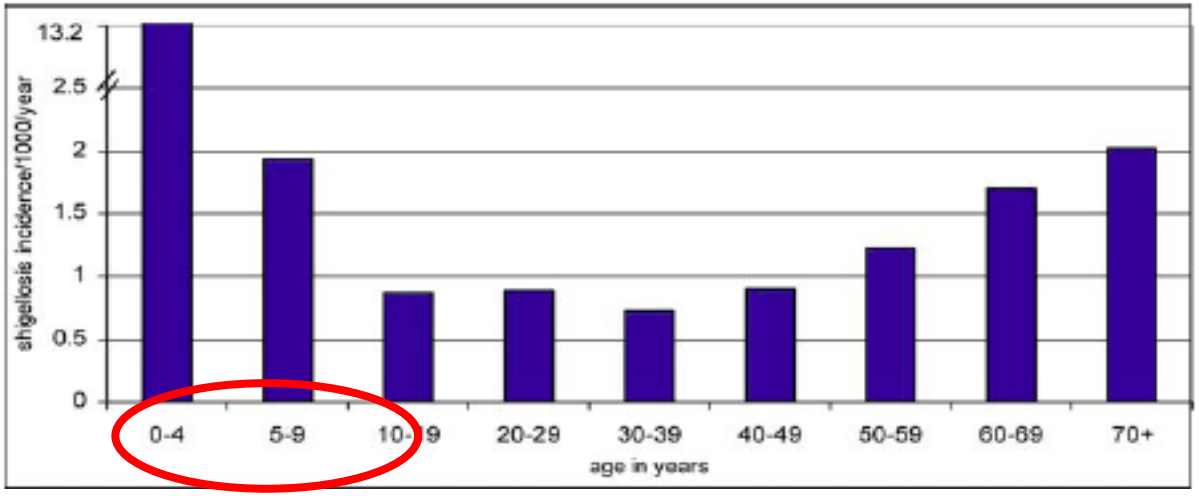


The Enterobacteria（2nd ed）, 2006, ASM press

J Infect Dis, 1989, 159-162

研究志贺氏菌的重要性(二)

- 对儿童危害巨大



Available online at www.sciencedirect.com
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ELSEVIER
Vaccine 24 (2006) 2732–2750
Vaccine
www.elsevier.com/locate/vaccine

Review

A review of vaccine research and development: human enteric infections☆

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[b] *Initiative for Vaccine Research, World Health Organization, 20 Avenue Appia, CH 1211-Geneva, Switzerland*
[c] *Communicable Disease Cluster, World Health Organization, 20 Avenue Appia, CH 1211-Geneva, Switzerland*

***PLoS Medicine*, 2006, 3(9): e353**　　***Vaccine*， 2006，24：2732–2750**

研究志贺氏菌的重要性(三)

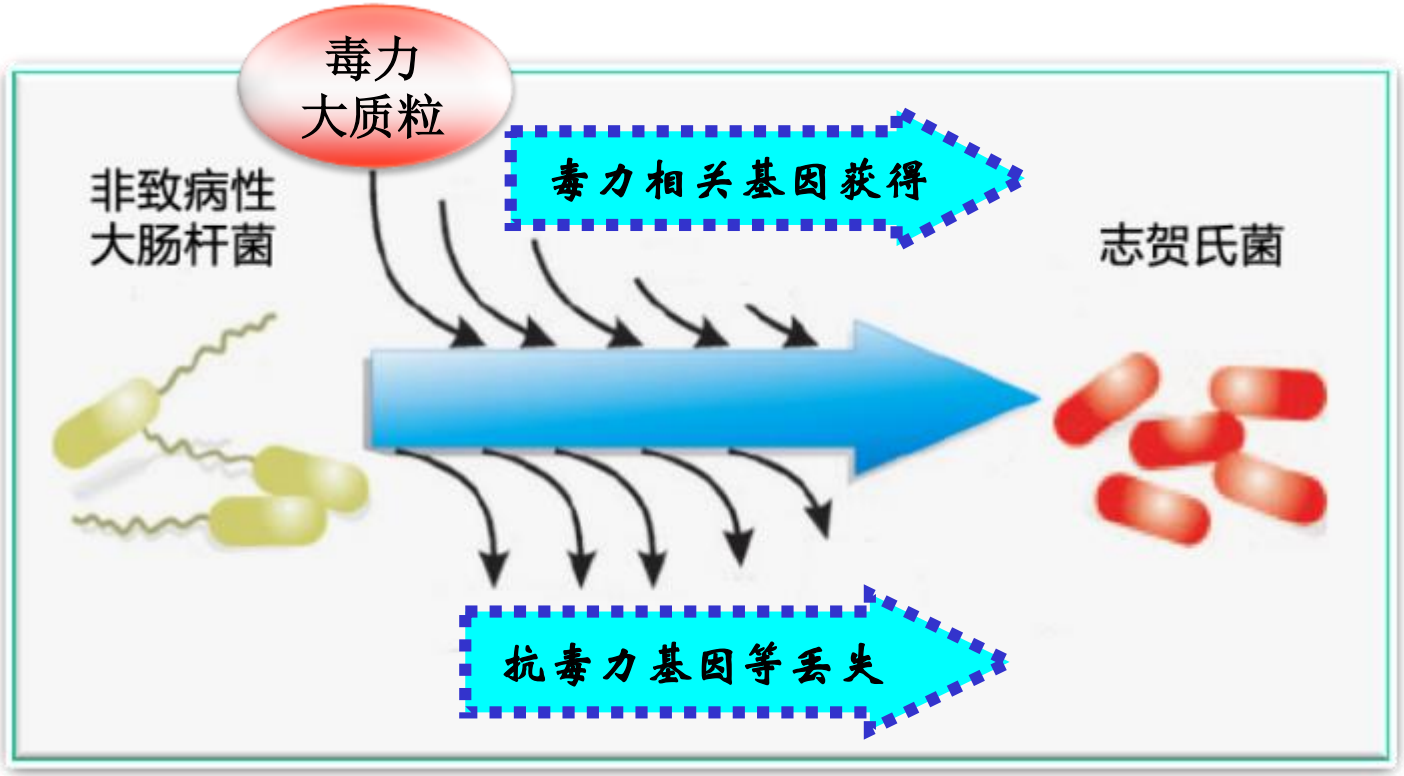
- 高耐药性
- 缺乏理想的疫苗
- 在生物安全方面的潜在危害（Category B）

Antimicrobial agent[a]	% of strains[b]		
	R	I	S
Ampicillin	100	0	0
Amoxicillin-clavulanic acid	0	0	100
Ampicillin-sulbactam	2.6	68.1	24.1
Ticarcillin-clavulanic acid	0	0.9	99
Piperacillin-tazobactam	0	0	100
Piperacillin	12.9	5.2	73.3
Aztreonam	38.8	2.6	58.6
Cefepime	34.5	0.9	95.7
Cefotaxime	6.1	0.9	93.1
Ceftazidime	0	0.9	99
Ceftriaxone	5.2	0	94.8
Ciprofloxacin	13.8	0.9	85.3
Gatifloxacin	0	0.9	99
Levofloxacin	0.9	10.3	88.8
Imipenem	0	0	100
Trimethoprim-sulfamethoxazole	89.7	0	10.3
Nalidixic acid	100		0
Chloramphenicol	96.20		0.8
Tetracycline	99.06		0.94

J Clin Microbiol, 2010, 48: 419-426　　*Vaccine*，2006，24：2732–2750

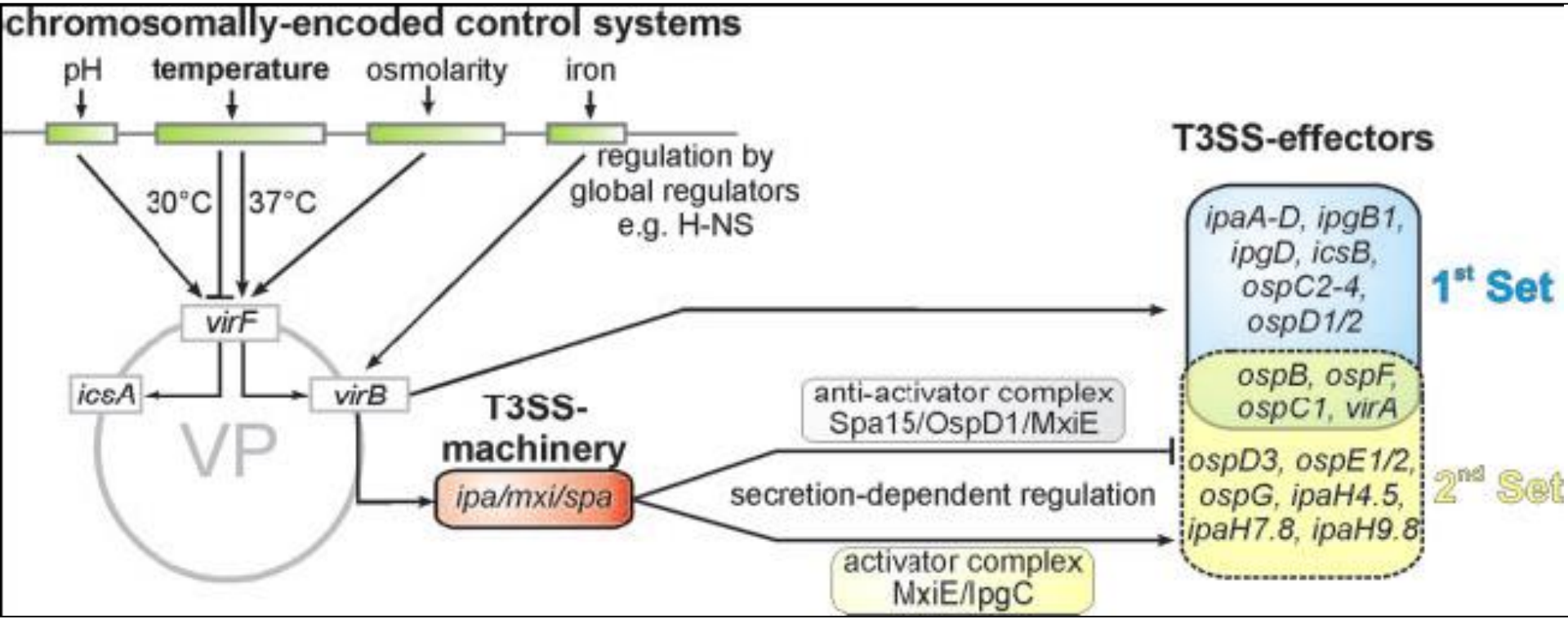
http://www3.niaid.nih.gov/topics/BiodefenseRelated/Biodefense.htm

从非致病性大肠杆菌到志贺氏菌的进化



Clin Microbiol Rev, 2008, 21(1): 134–156

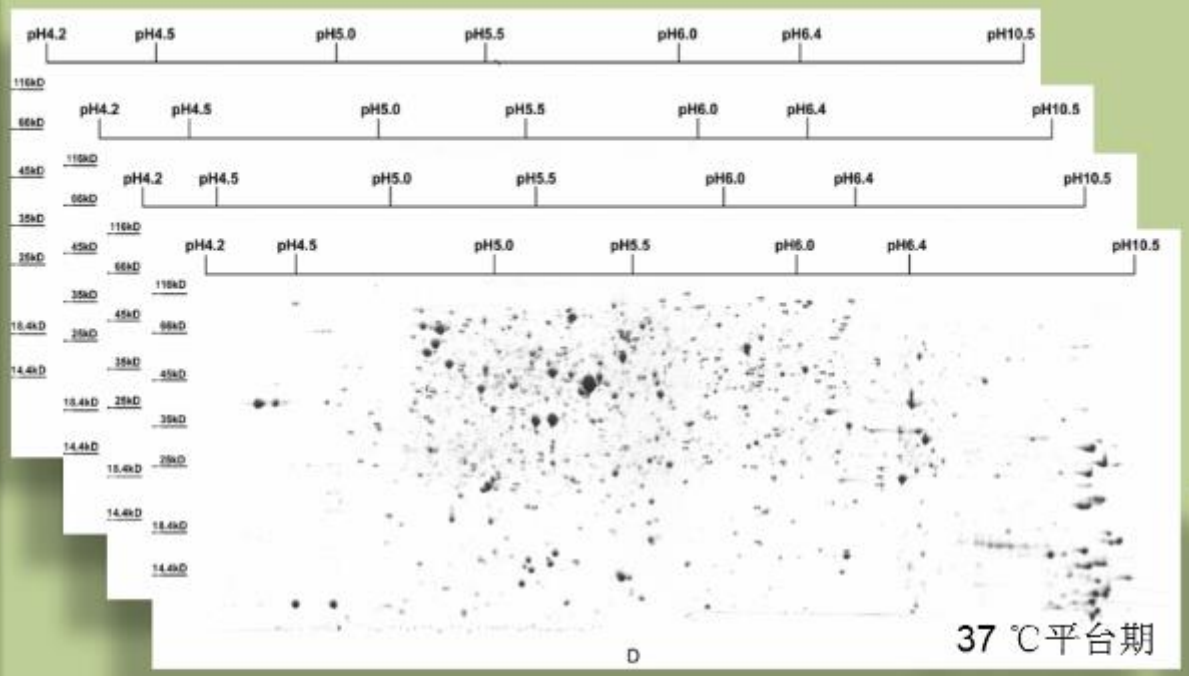
志贺氏菌毒力调控因素

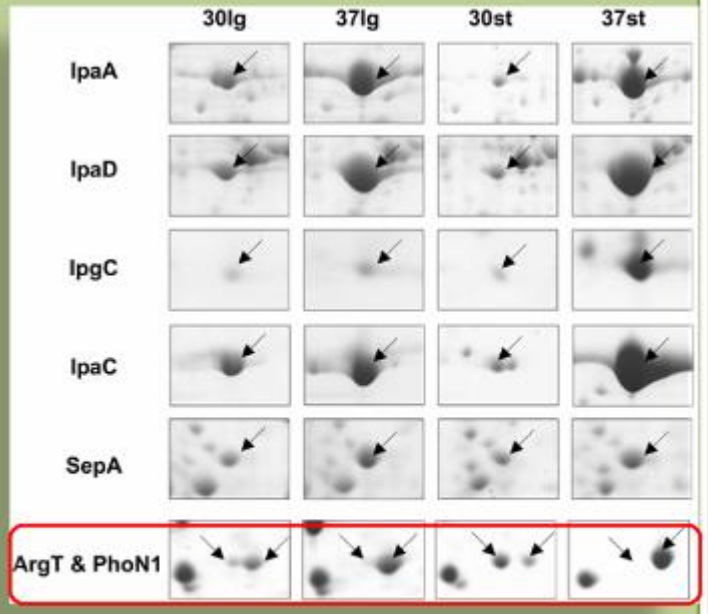


CLINICAL MICROBIOLOGY REVIEWS, Jan. 2008, 21(1), p. 134–156

➢用比较蛋白质组学发现了潜在的抗毒力基因*argT*

(不同温度条件下——30℃和37℃)





ArgT: 赖氨酸、精氨酸等碱性氨基酸转运蛋白

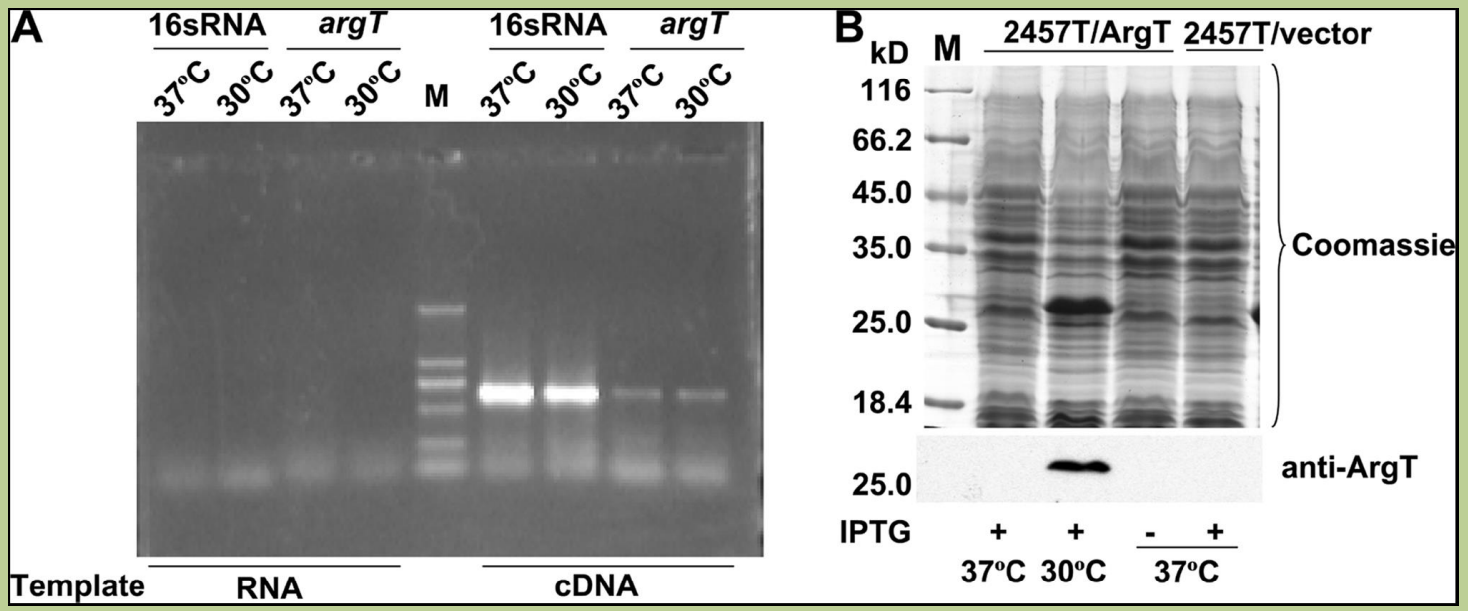
抗毒力基因

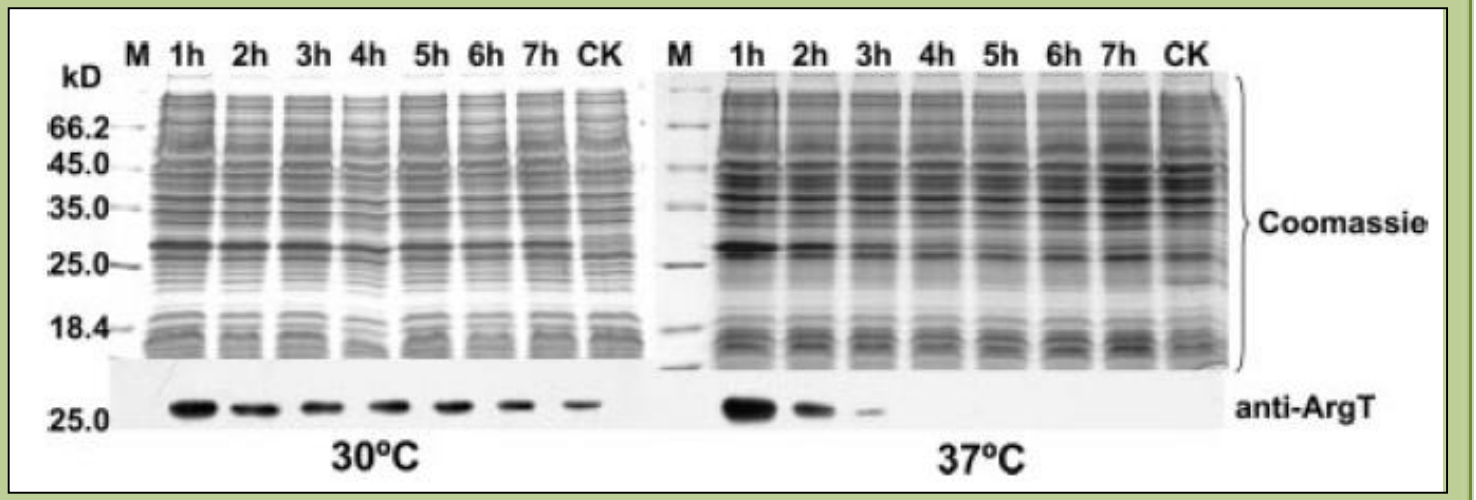
将是今后病原菌研究中的一大热点。

抗毒力基因(anti-virulence genes):

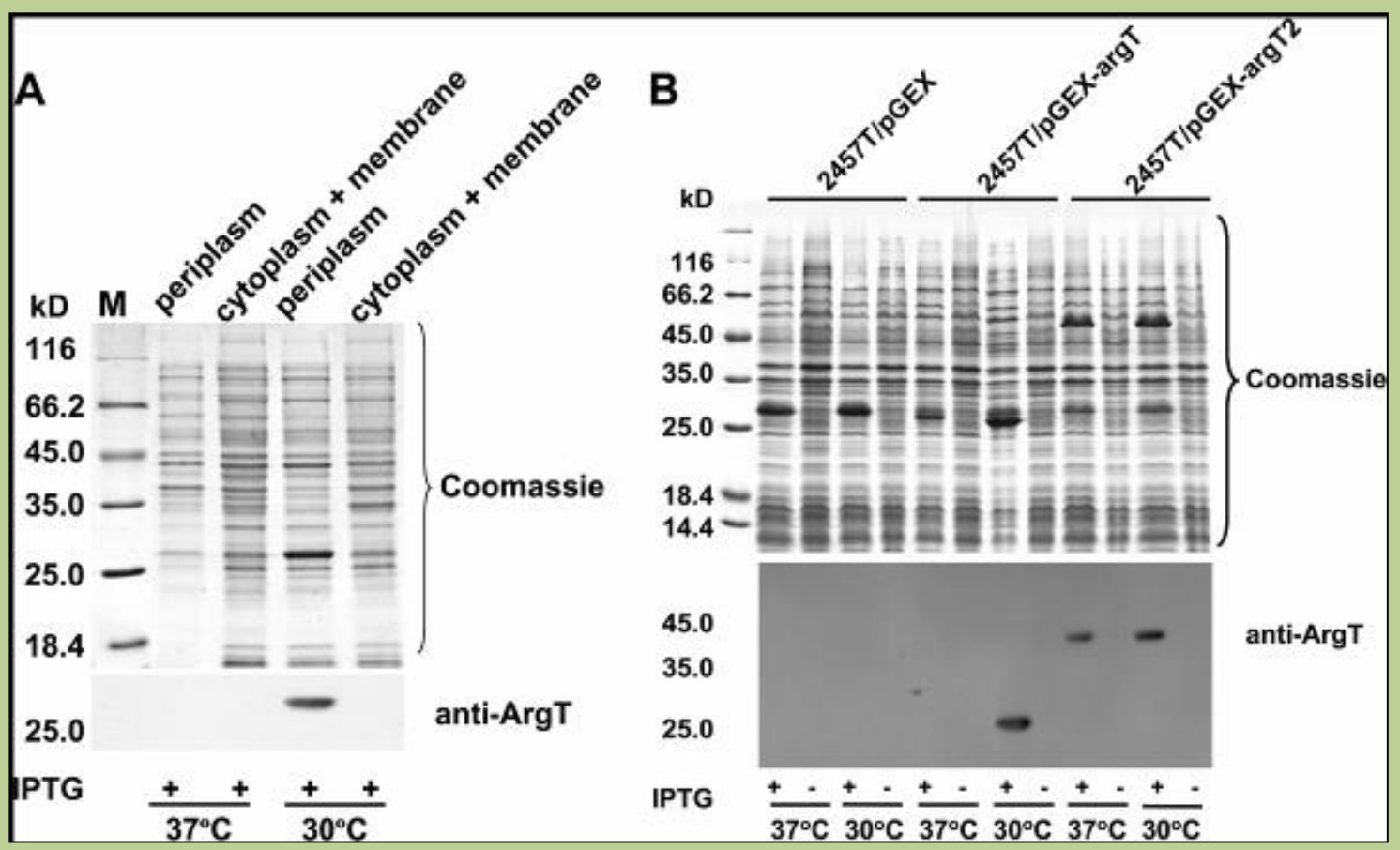
- 在其进化的无毒祖先菌株中稳定存在和表达，而在病原菌中缺失或不表达；
- 在病原菌中强制表达该基因，会降低病原菌的毒力。

➢验证了ArgT 在毒力状态下被降解

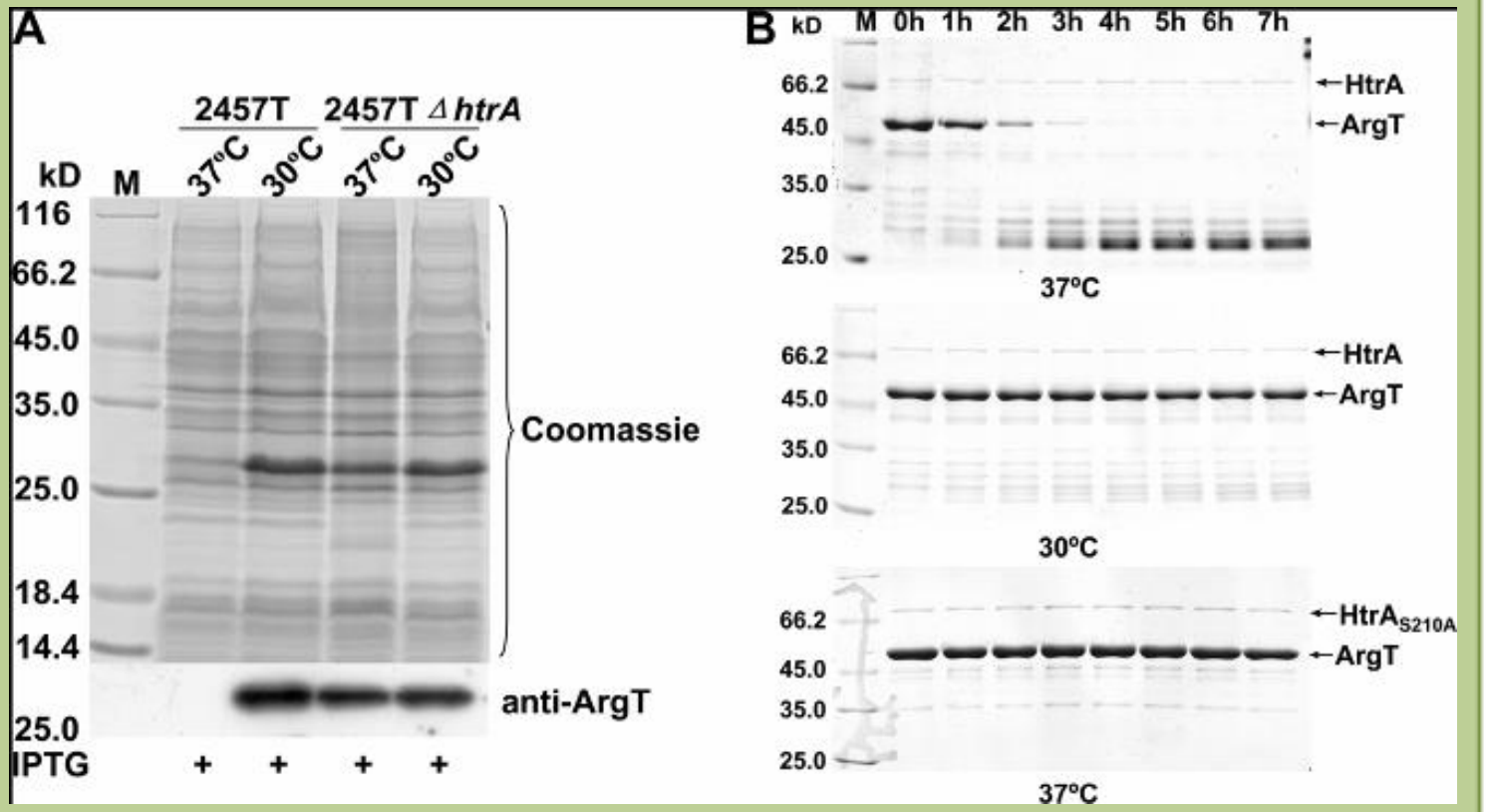




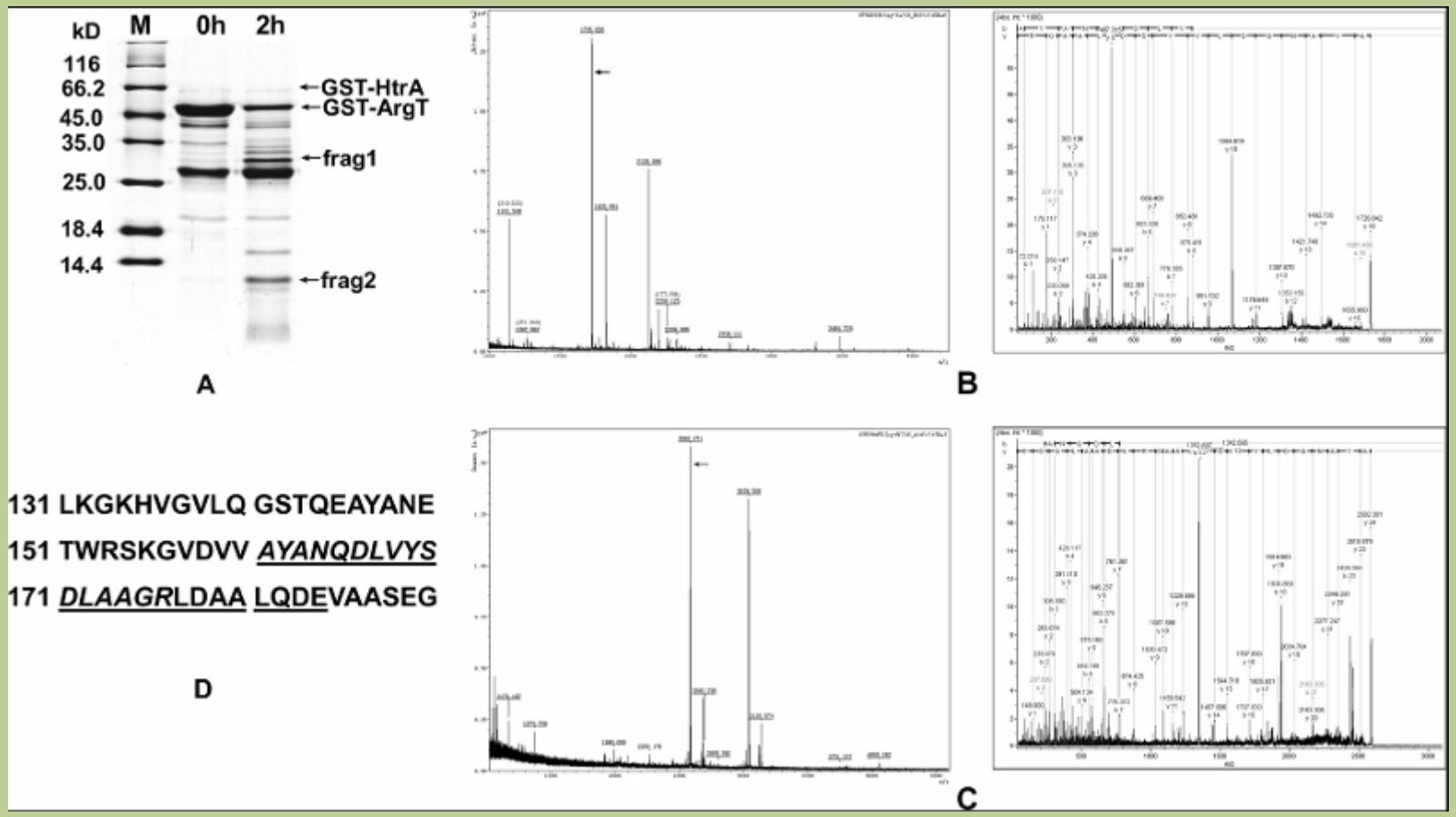
- 发现了**ArgT** 的降解发生在周间质中



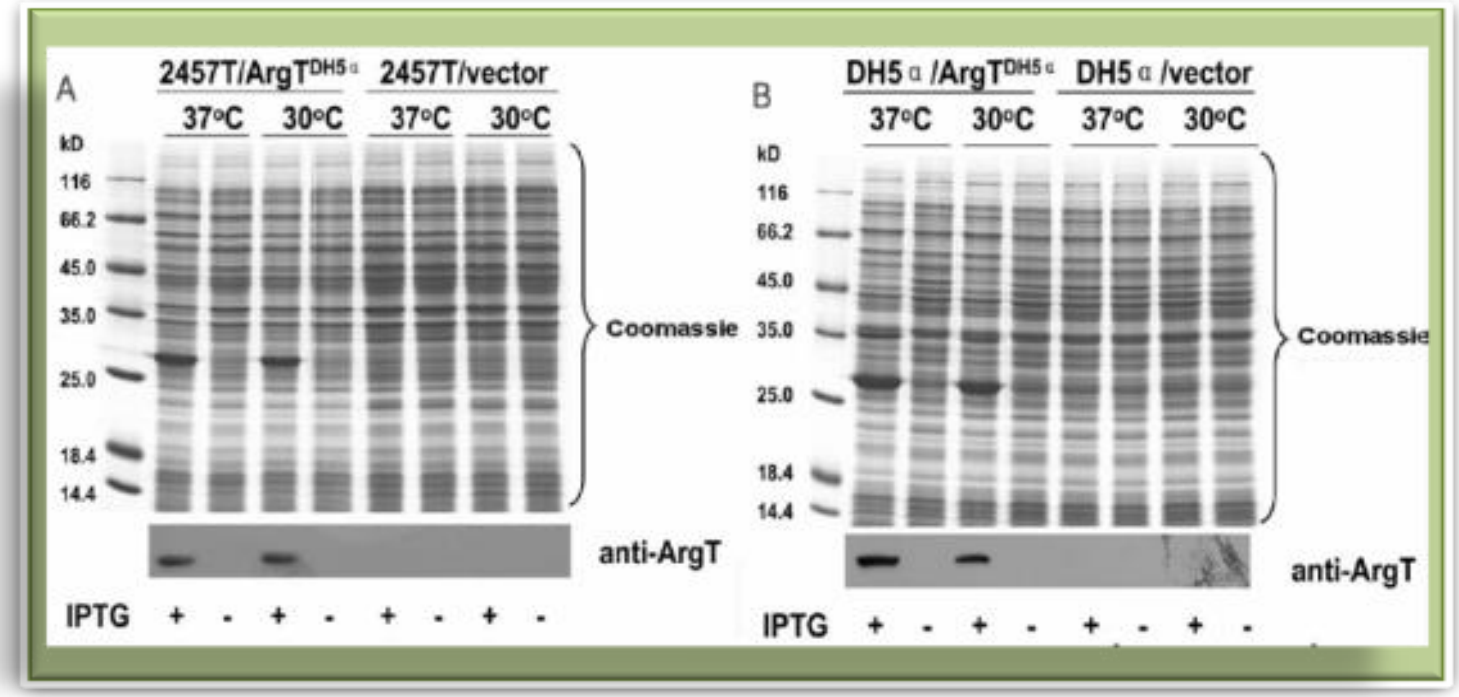
发现了降解ArgT的蛋白酶HtrA

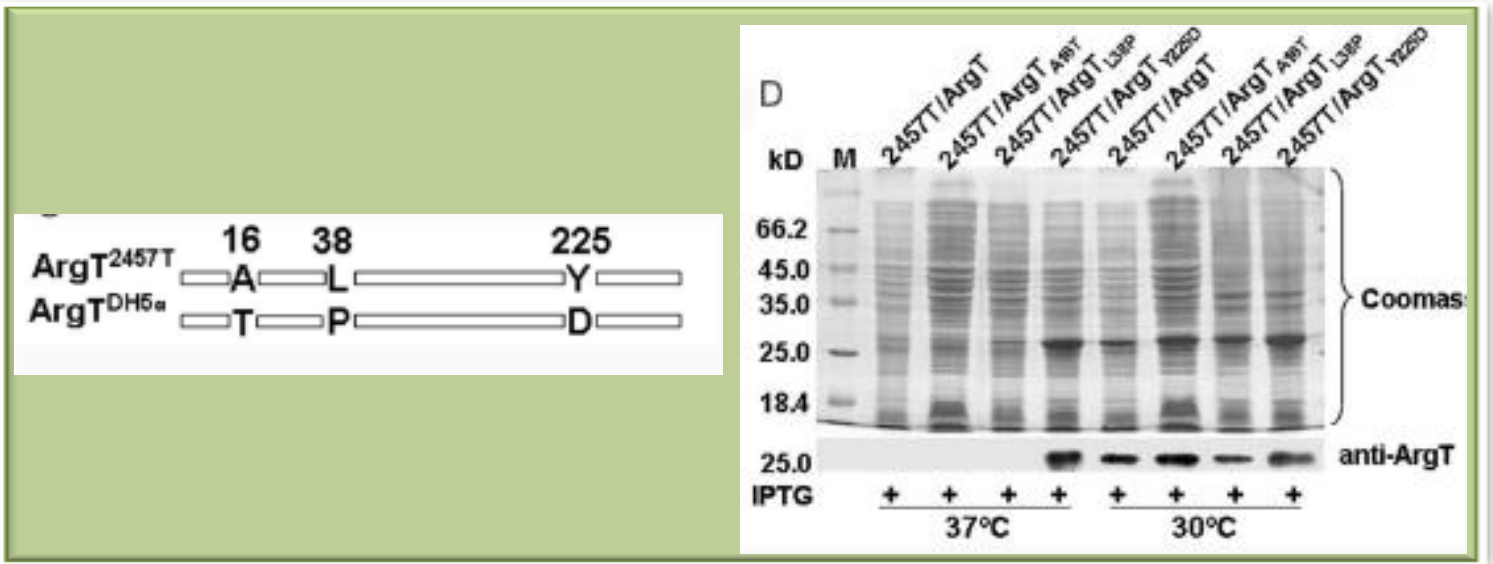


➢ 阐明了ArgT被降解的切割位点

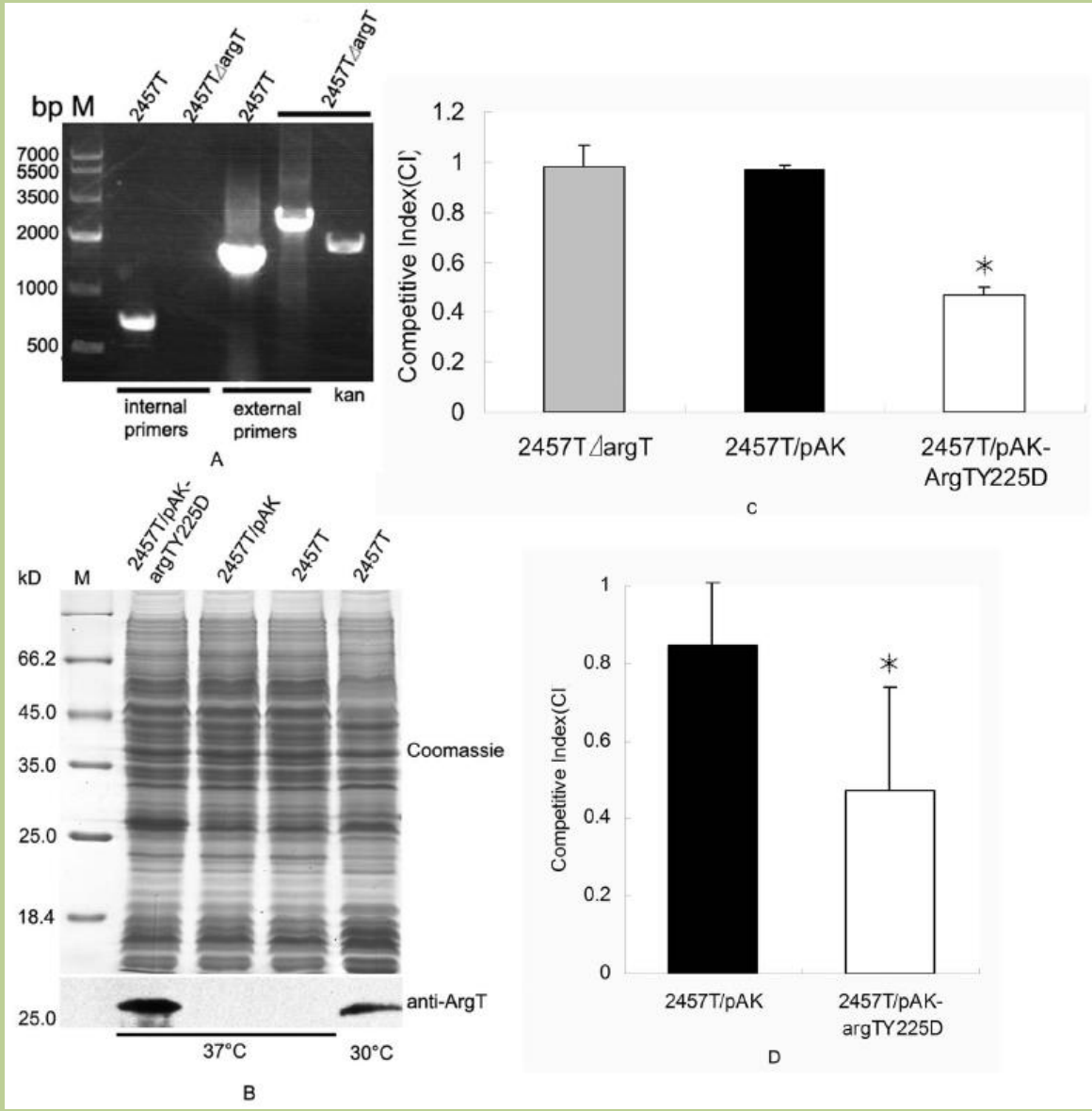


➢ ArgT在志贺氏菌和大肠杆菌中存在差异

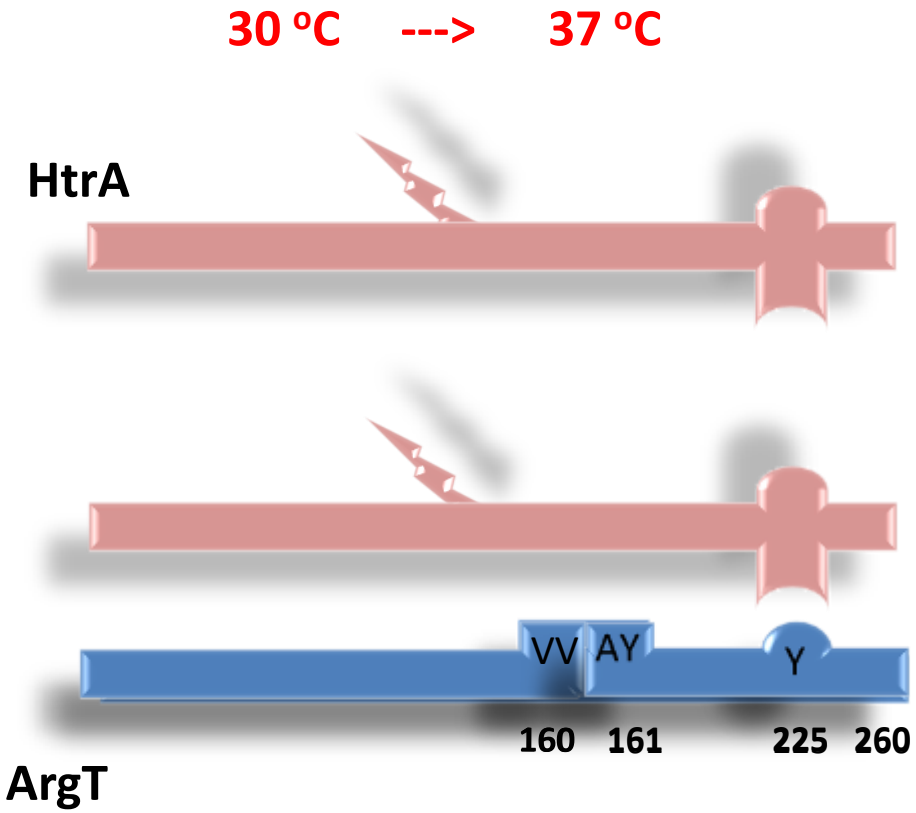




➢验证了ArgT 的抗毒力功能

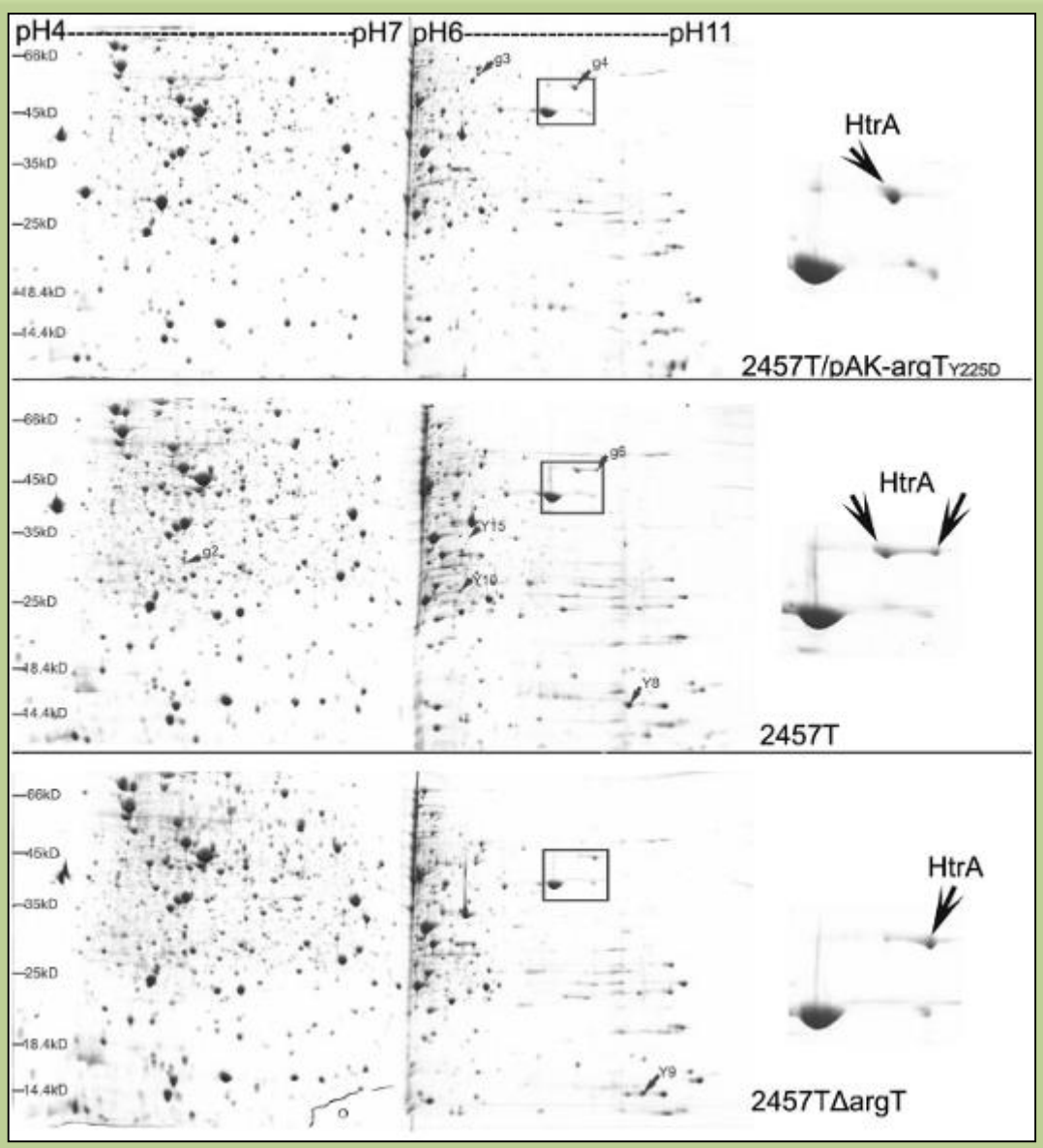


➢ 抗毒力因子ArgT的降解调控示意图

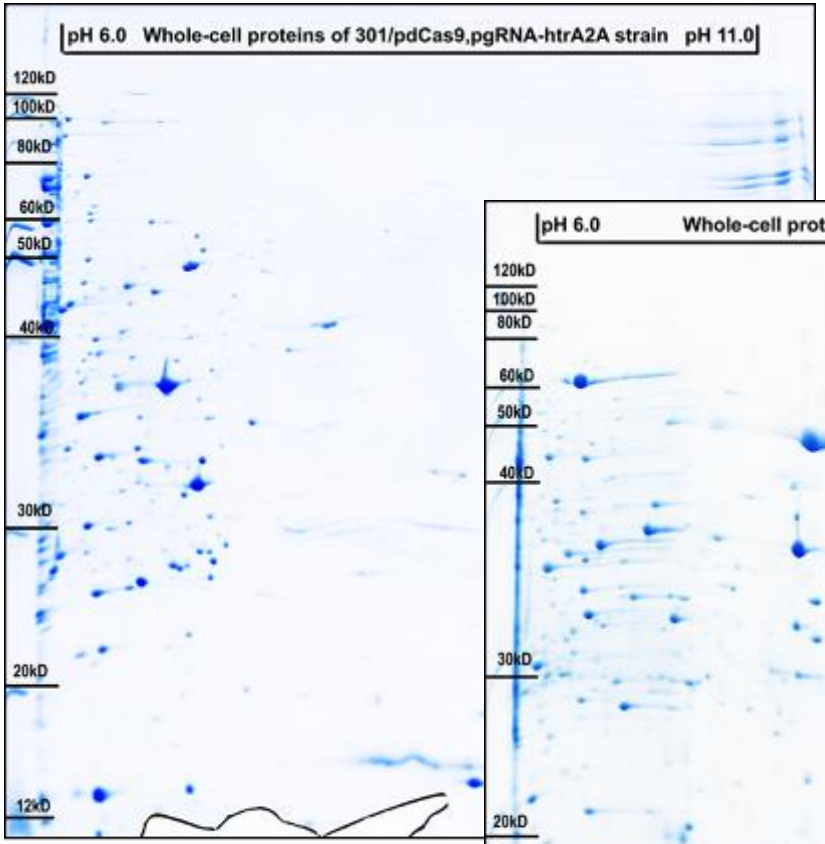


细菌微进化（SNP）与毒力的关系

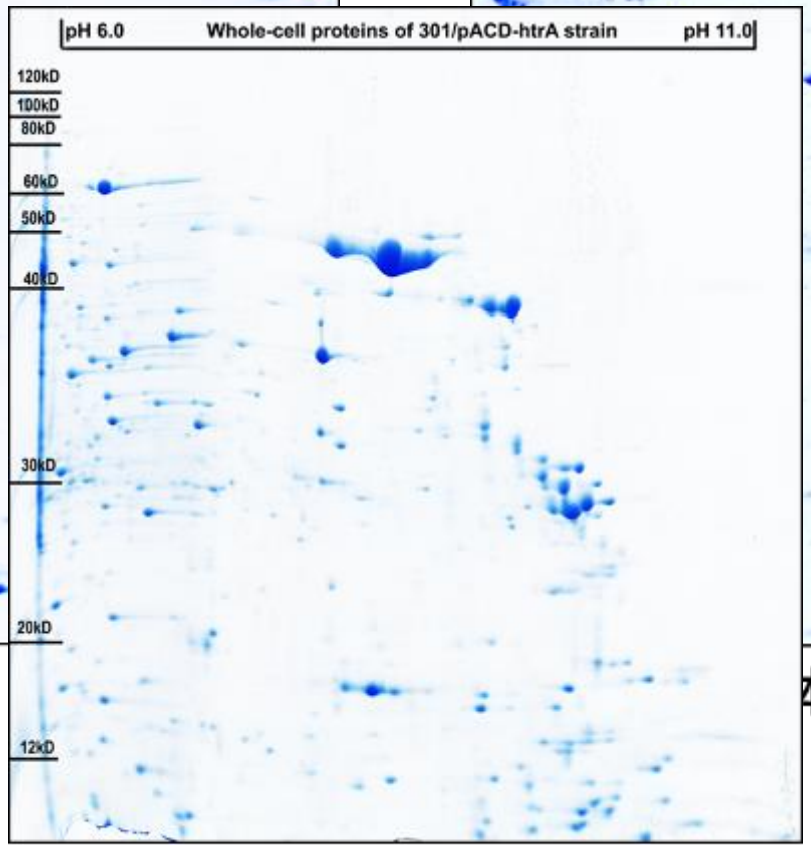
发现ArgT的丰度与HtrA 的修饰有关



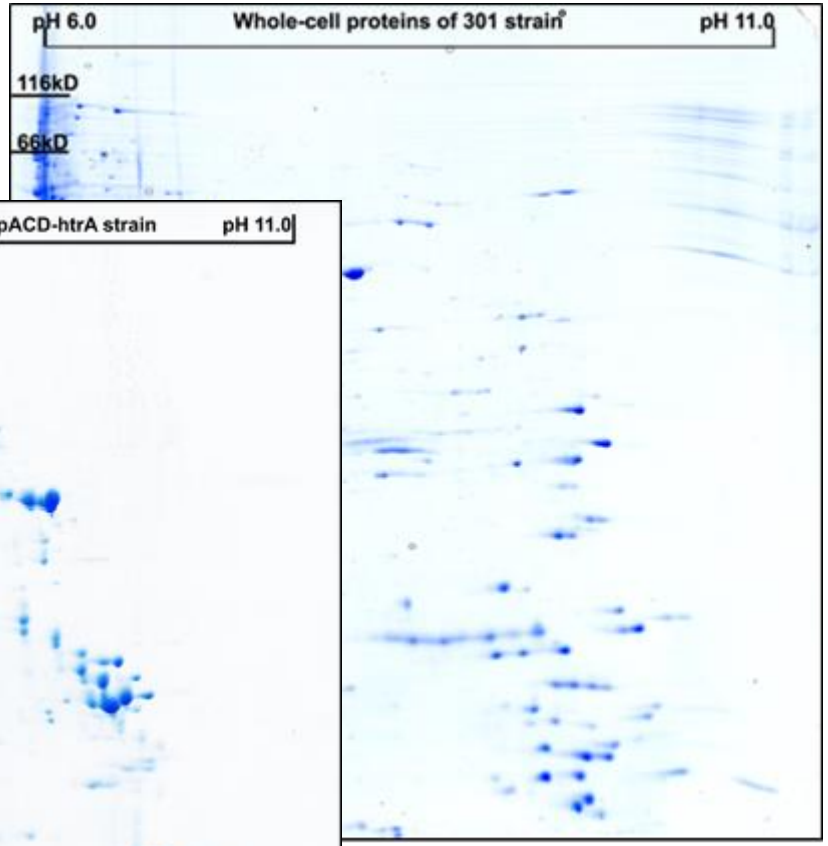
➢ HtrA不同表达模式全菌蛋白2D结果（pH6-11）



下调表达

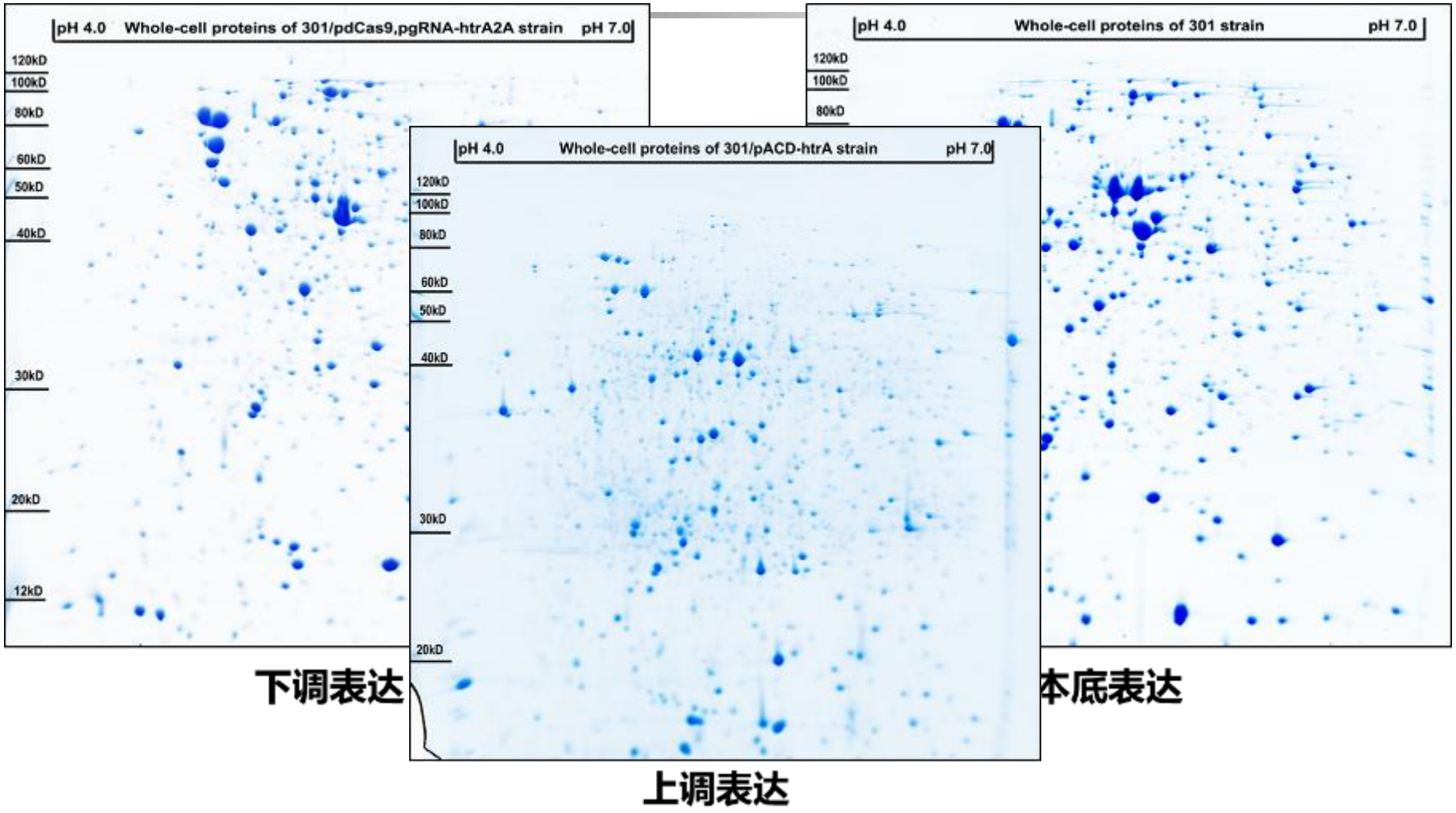


上调表达

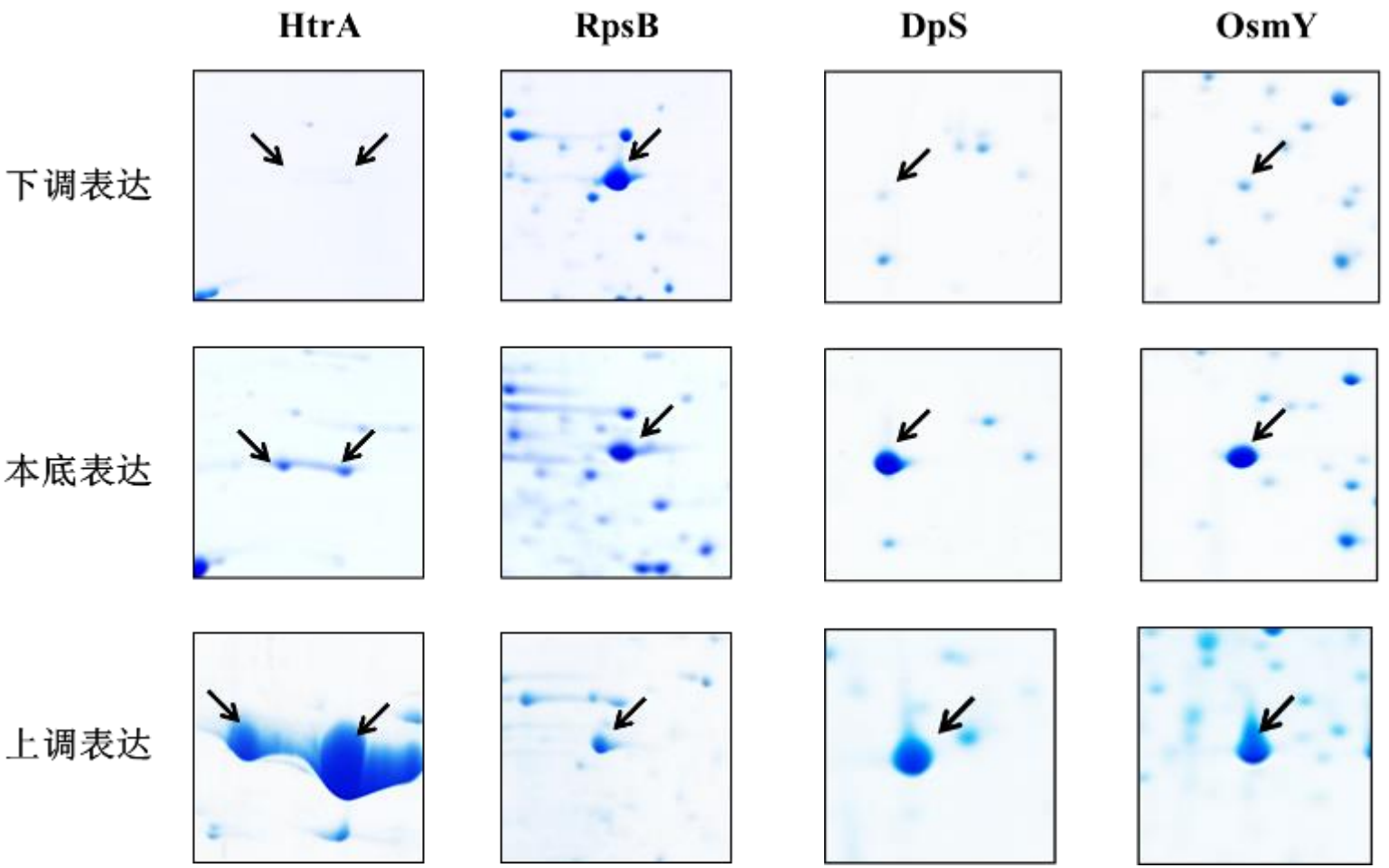


本底表达

➢ HtrA不同表达模式全菌蛋白2D结果（pH4-7）

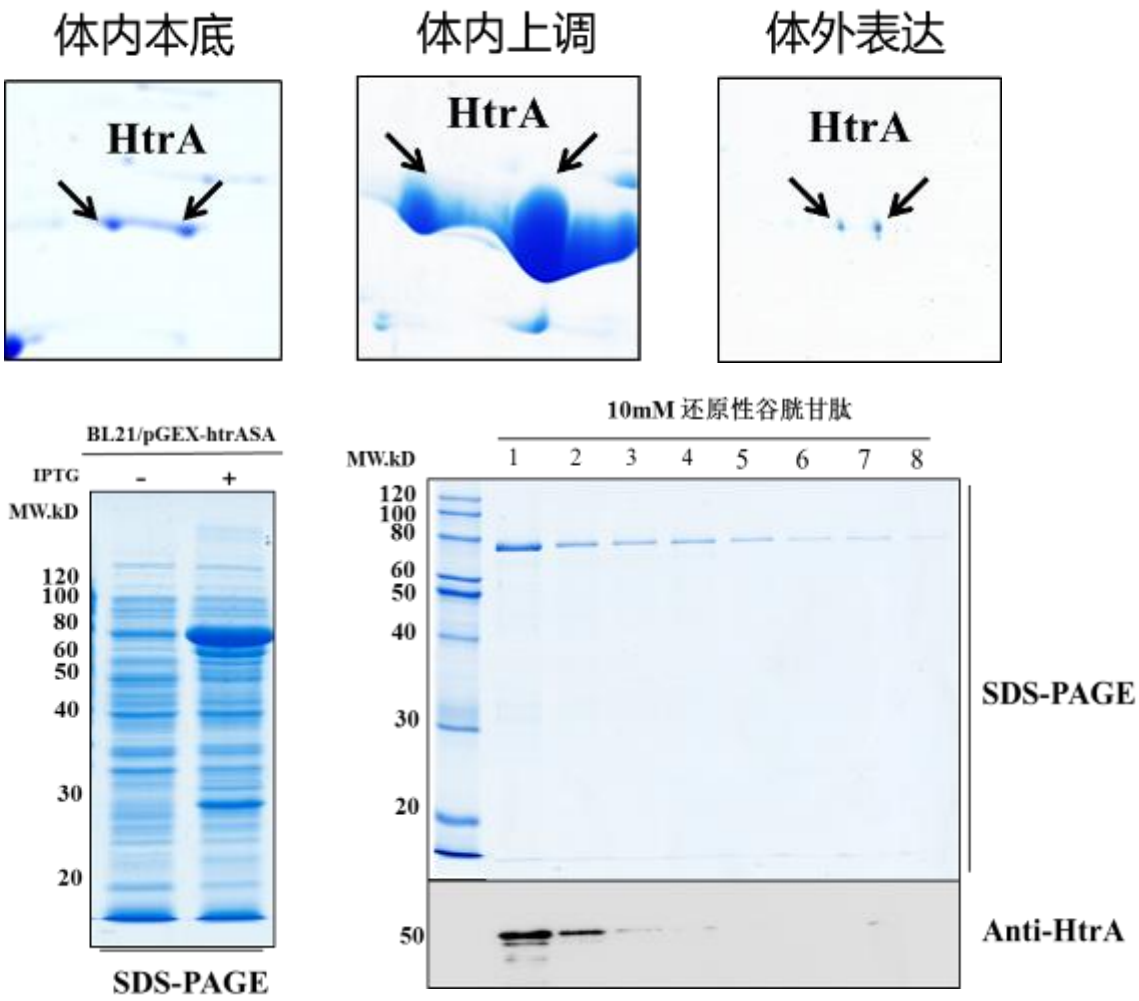


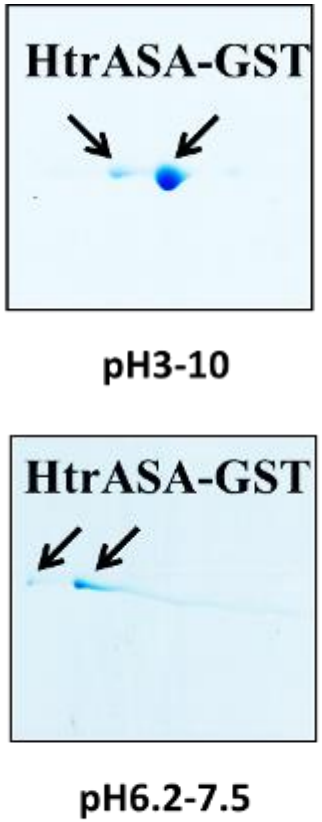
➢ 蛋白差异点质谱分析结果



OsmY是一种周间质蛋白，Dps具有与铁离子结合和抗氧化损伤功能，这种变化表明HtrA与应激系统有关连。

➢ 进一步确认翻译后修饰存在的可能性



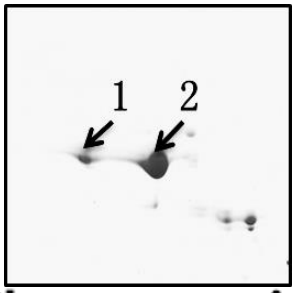


排除酶活位点存在修饰的可能性

➢ 高分辨率质谱分析(LC-MS/MS)

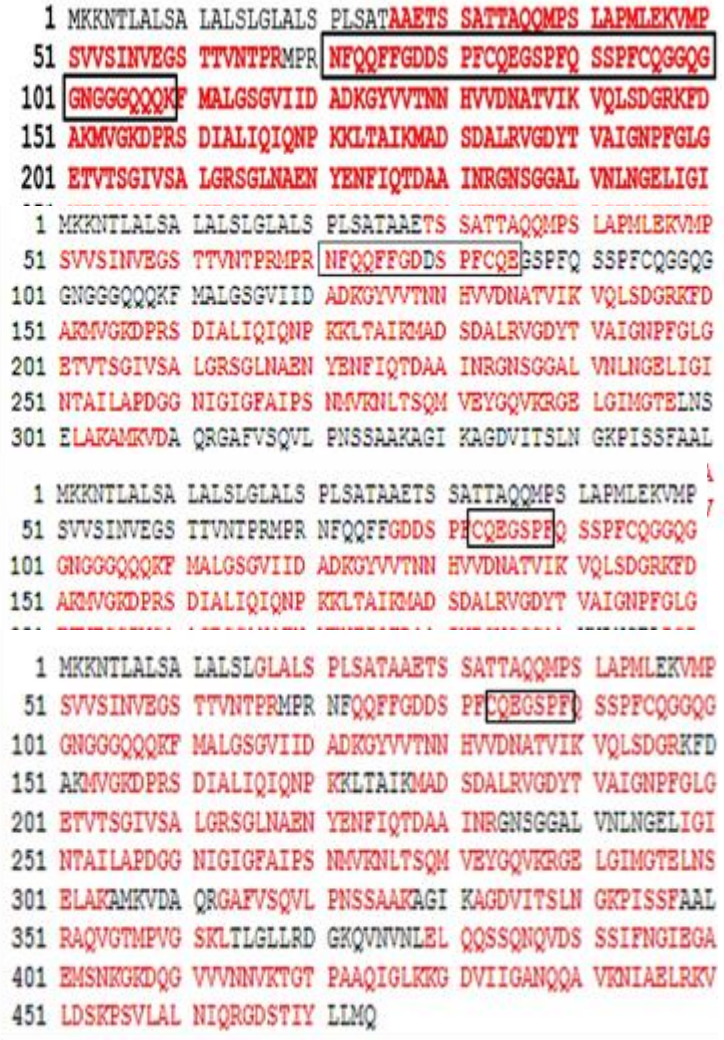
Trypsin

Glu-C

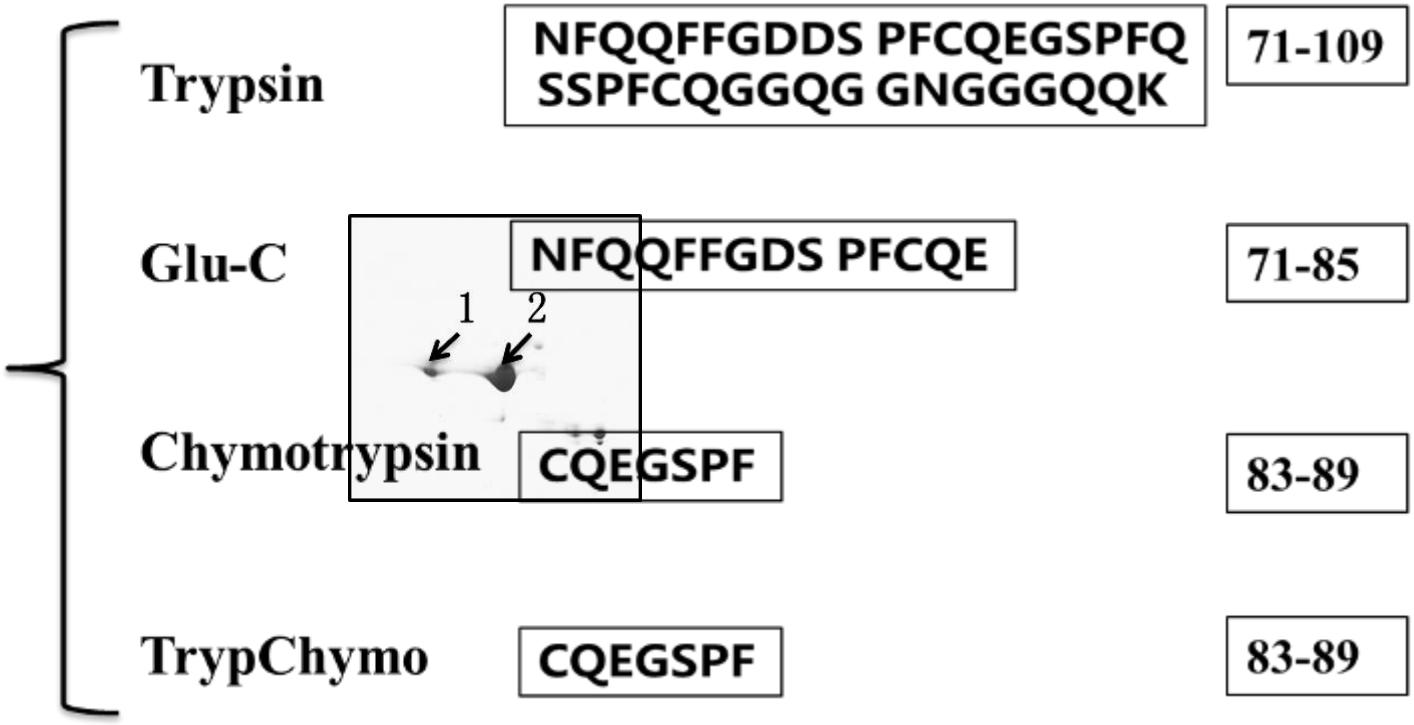


Chymotrypsin

TrypChymo

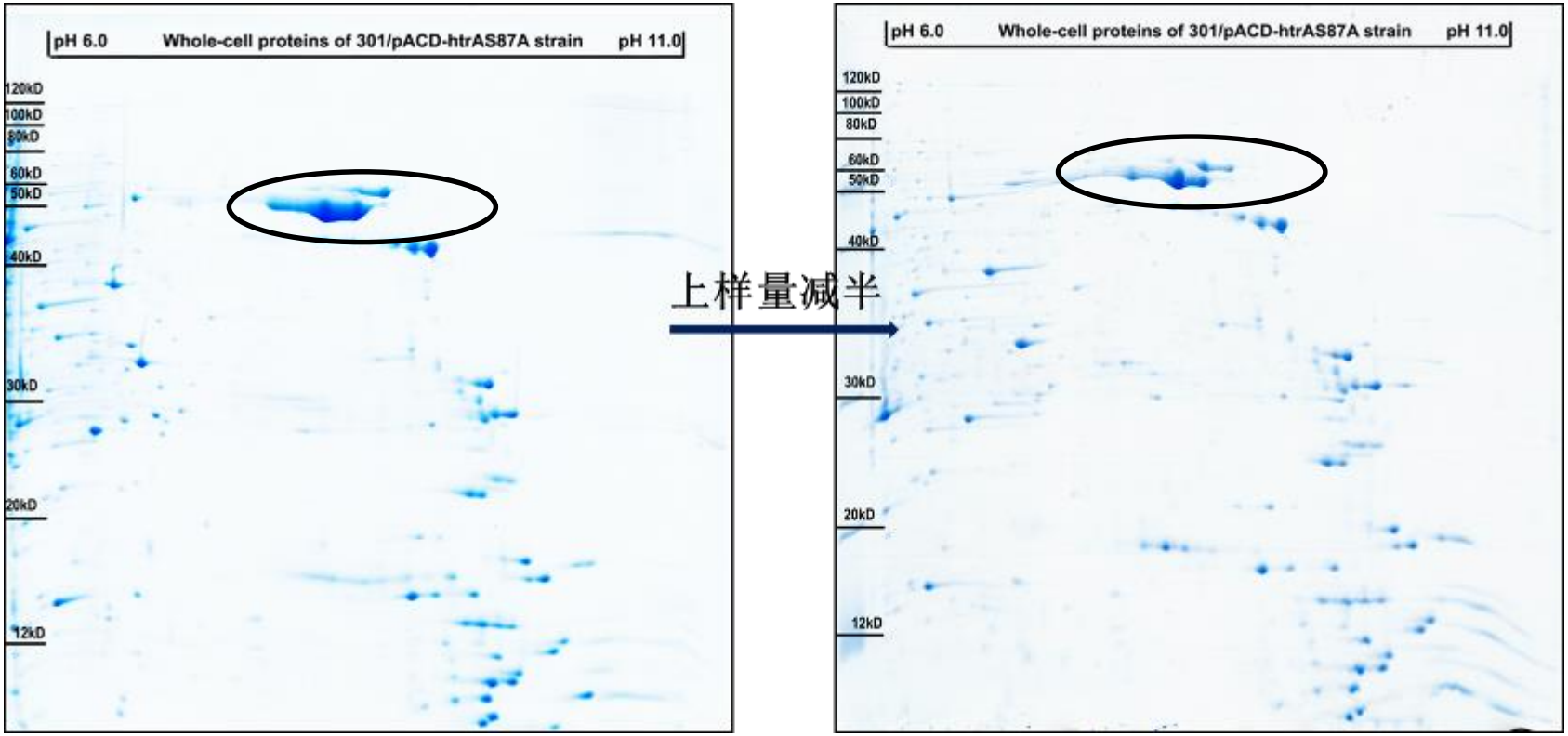


LC-MS/MS结果小结


Trypsin
NFQQFFGDDS PFCQEGSPFQ
SSPFCQGGQG GNGGGQQK
71-109
Glu-C
NFQQFFGDS PFCQE
71-85
1
2
Chymotrypsin
CQEGSPF
83-89
TrypChymo
CQEGSPF
83-89

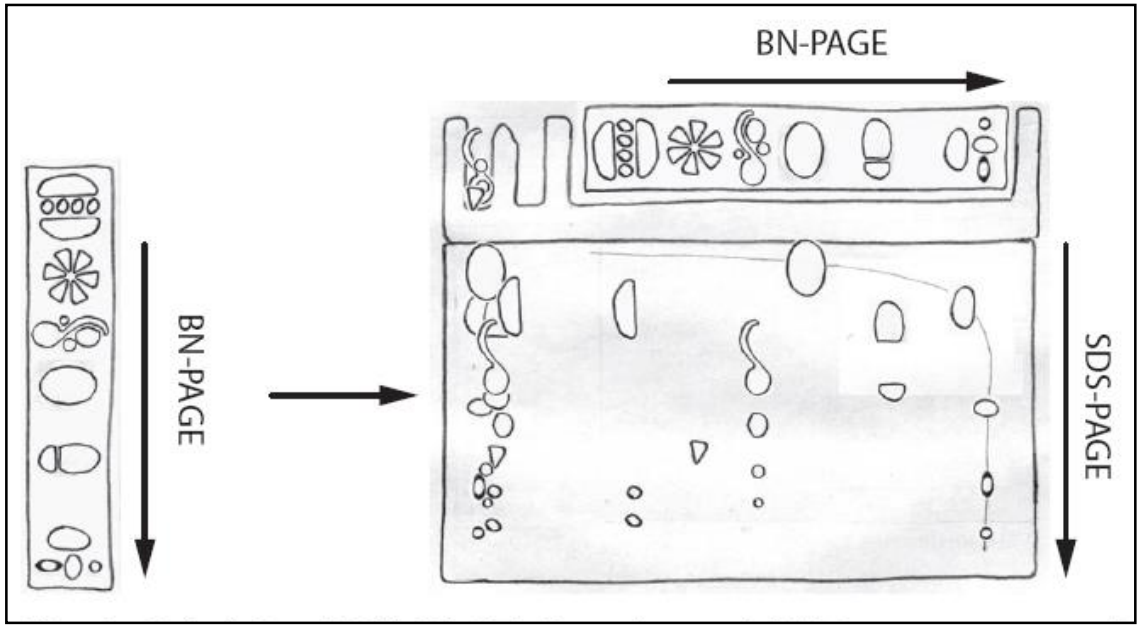
利用点突变寻找修饰位点

C Q E G S P F



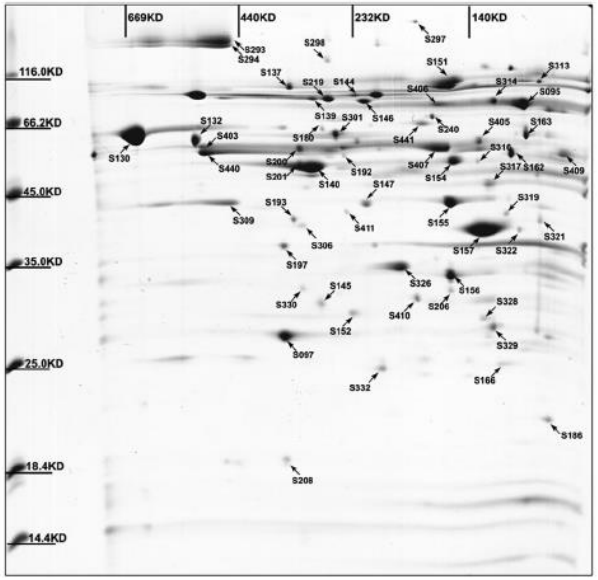
排除87位S存在修饰的可能性

志贺氏菌复合物的分离与鉴定

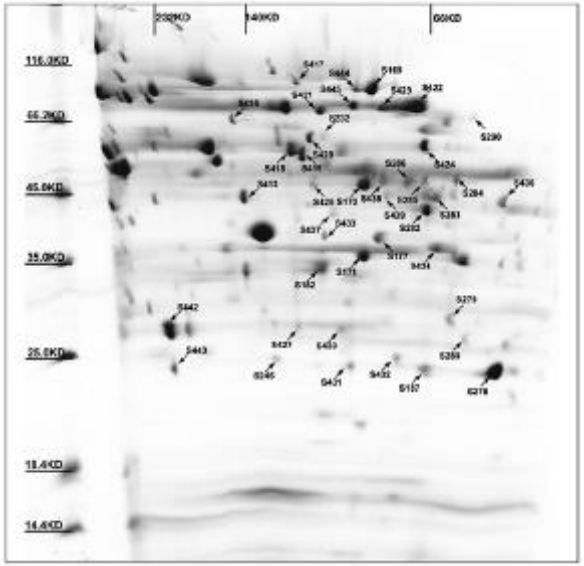


Blue-Native/SDS-PAGE双向电泳

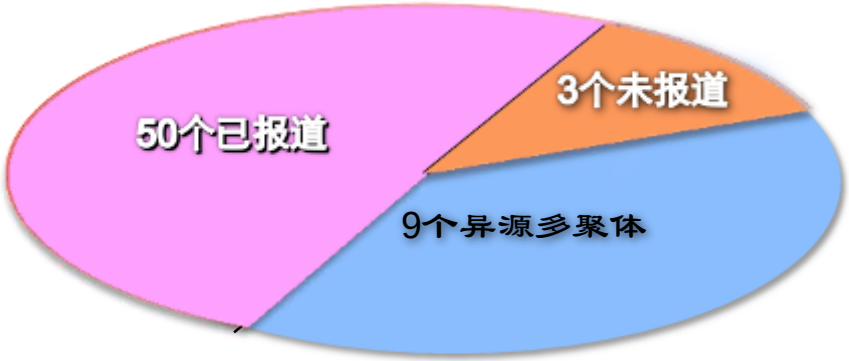
志贺氏菌复合物的分离与鉴定



A. 6%-11% 梯度BN-PAGE及12.5% SDS-PAGE



B. 10%-16% 梯度BN-PAGE及12.5% SDS-PAGE

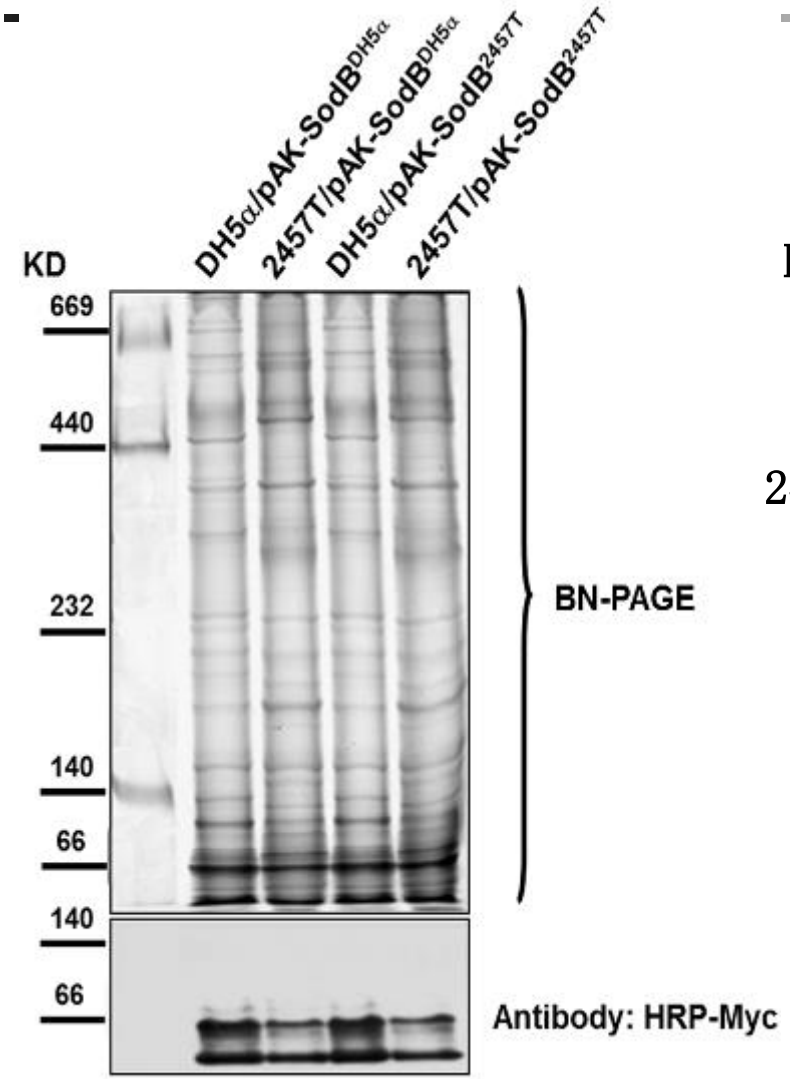


PhoN1-毒力大质粒编码的未知功能蛋白

UshA-双功能酶

YghZ-假想还原酶

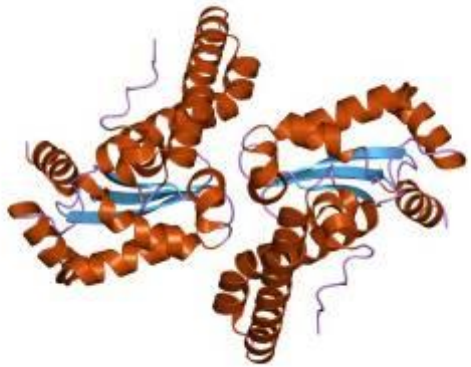
大肠杆菌和志贺氏菌SOD复合物的比较



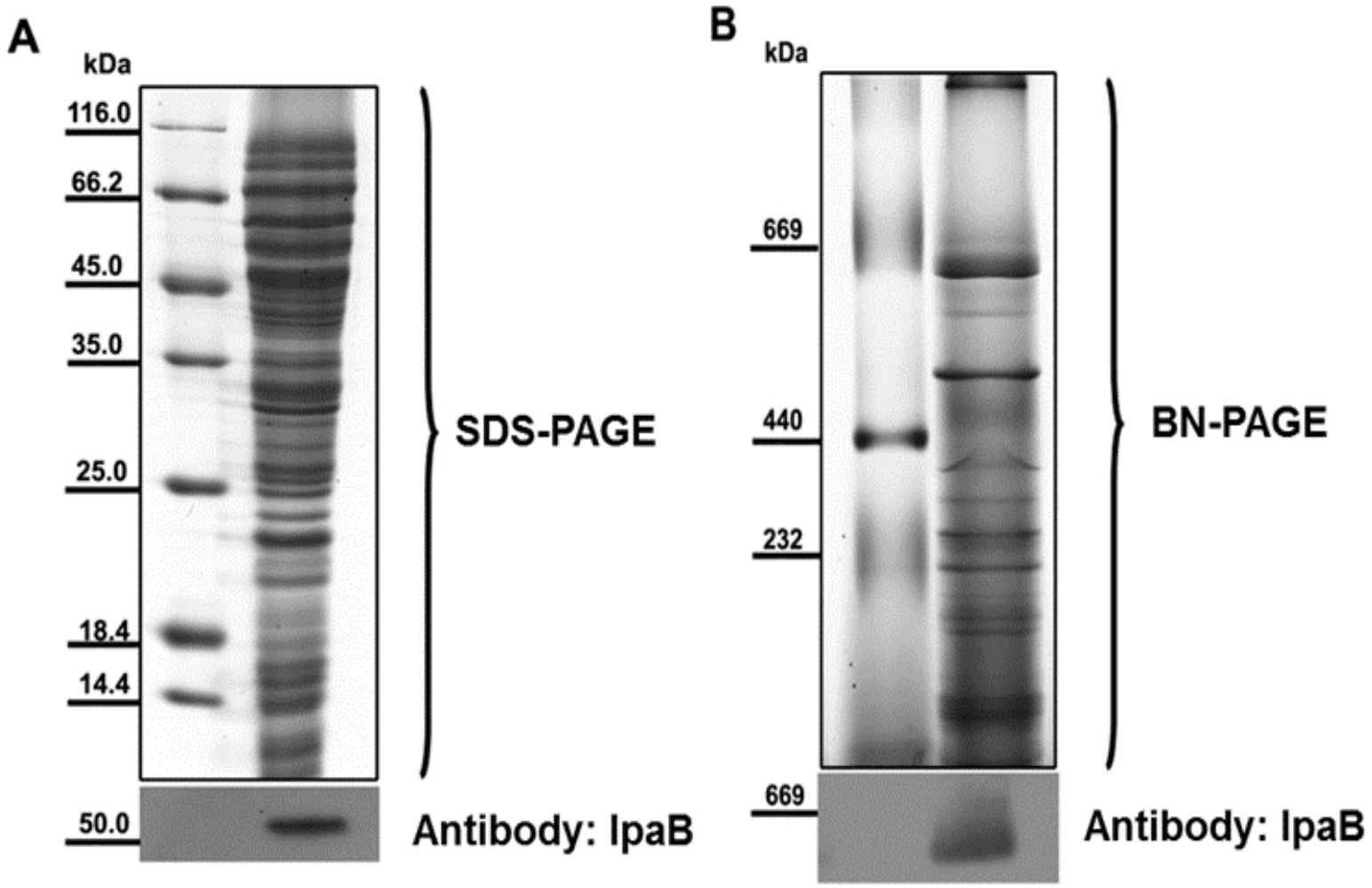
SodB

E.coli —文献报道→ 43KD同源二聚体

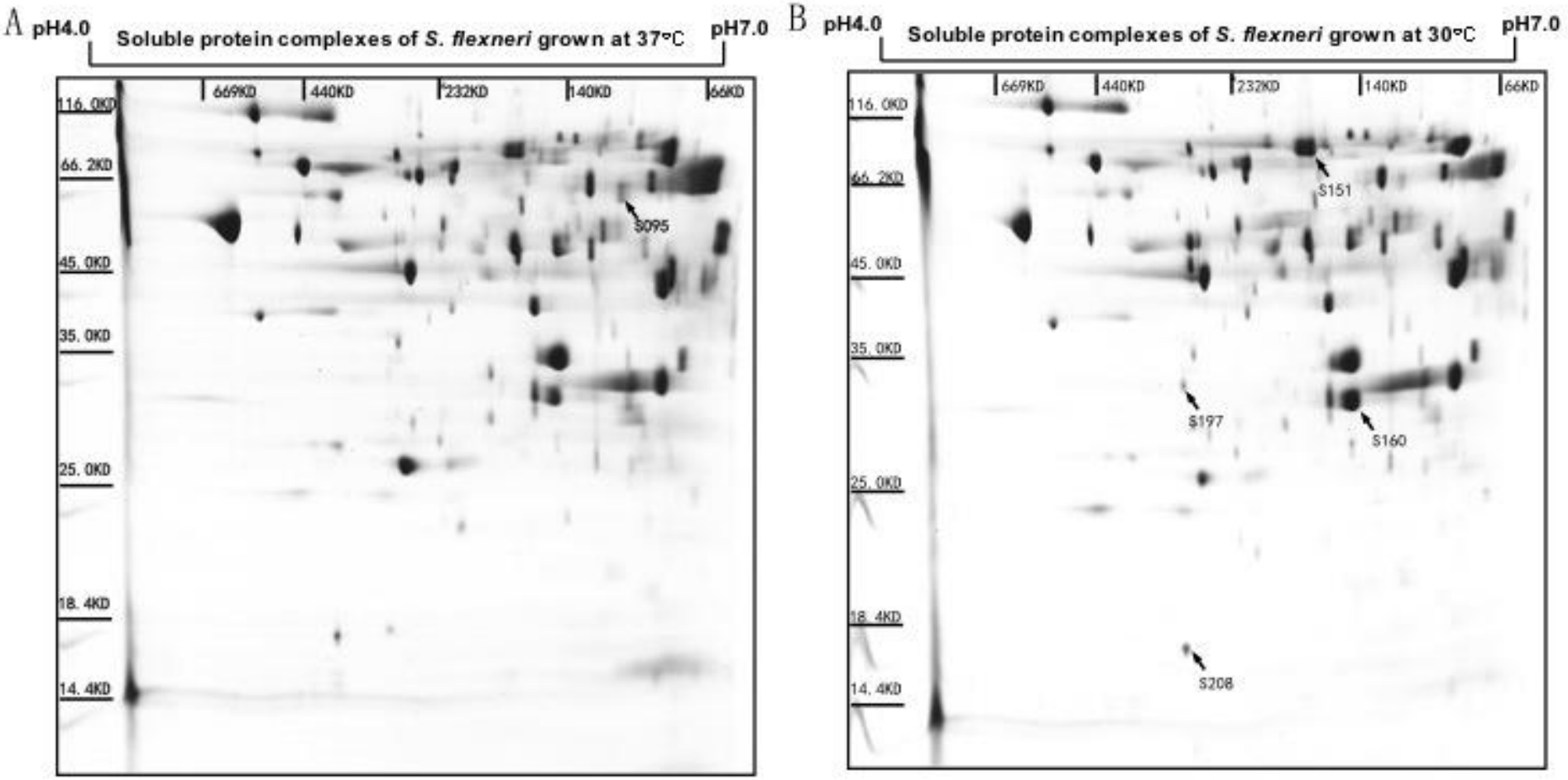
2457T —2-D电泳结果→ 66KD同源多聚体



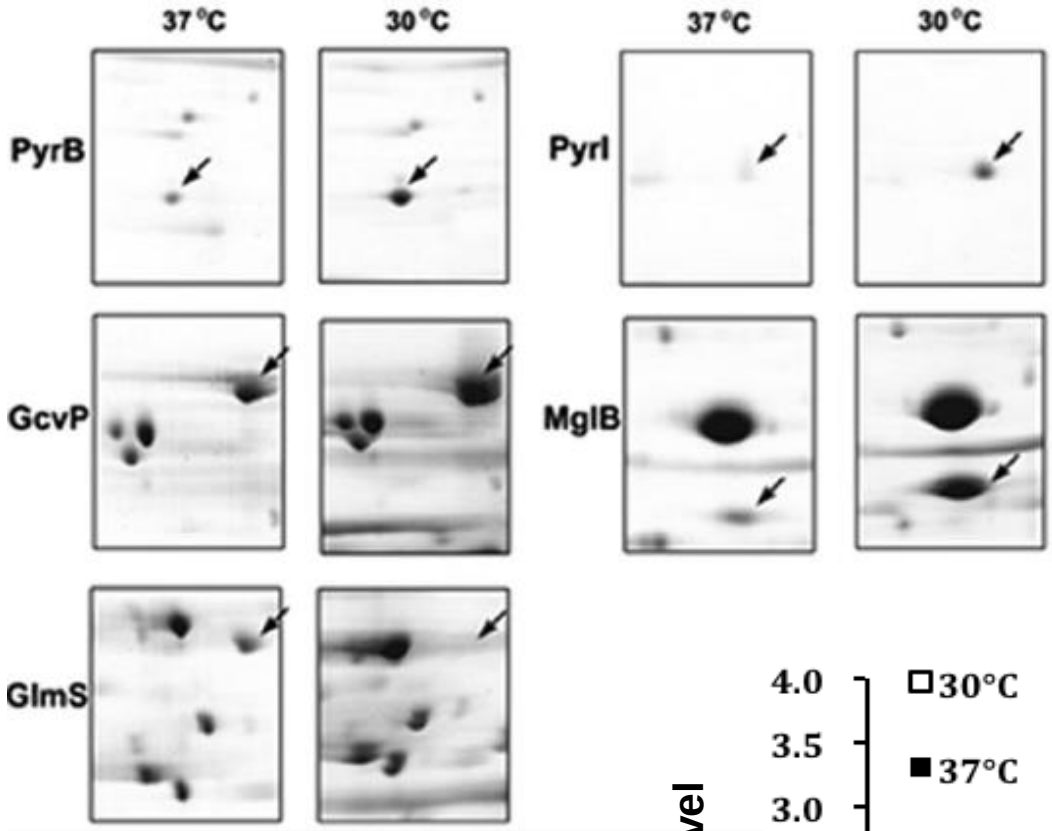
志贺氏菌毒力因子复合物的分析



不同温度下志贺氏菌复合物差异的比较



差异复合物的鉴定与验证



四个蛋白复合物：

PyrB/PyrI　　ATCase

（天冬氨酸转氨甲酰酶复合物）

MglB　　MglABC

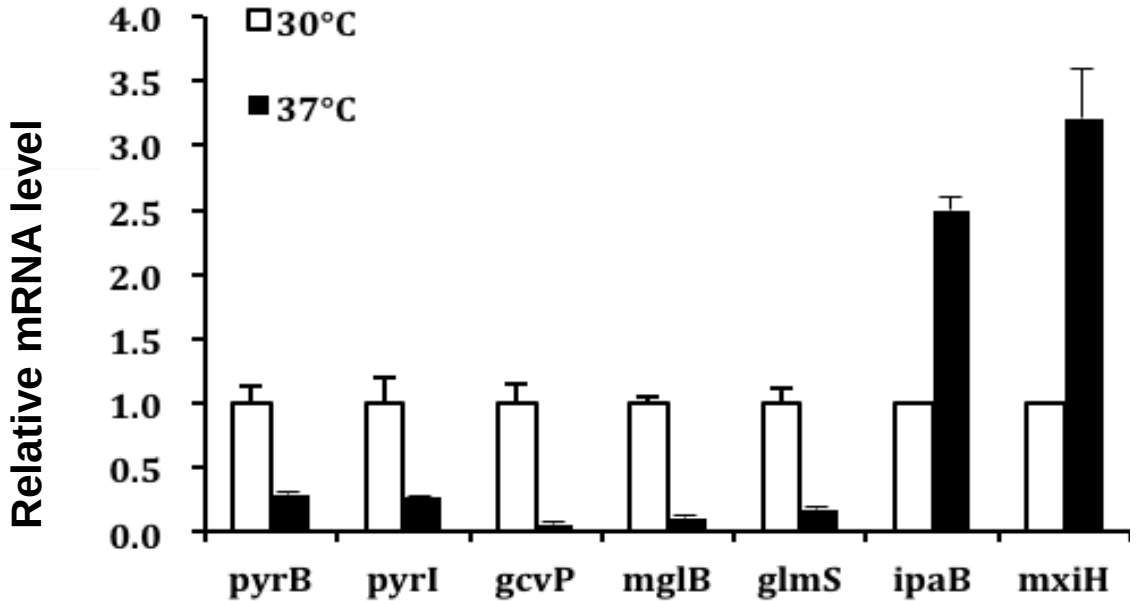
（半乳糖**ABC**转运蛋白复合物）

GlmS　　GFAT

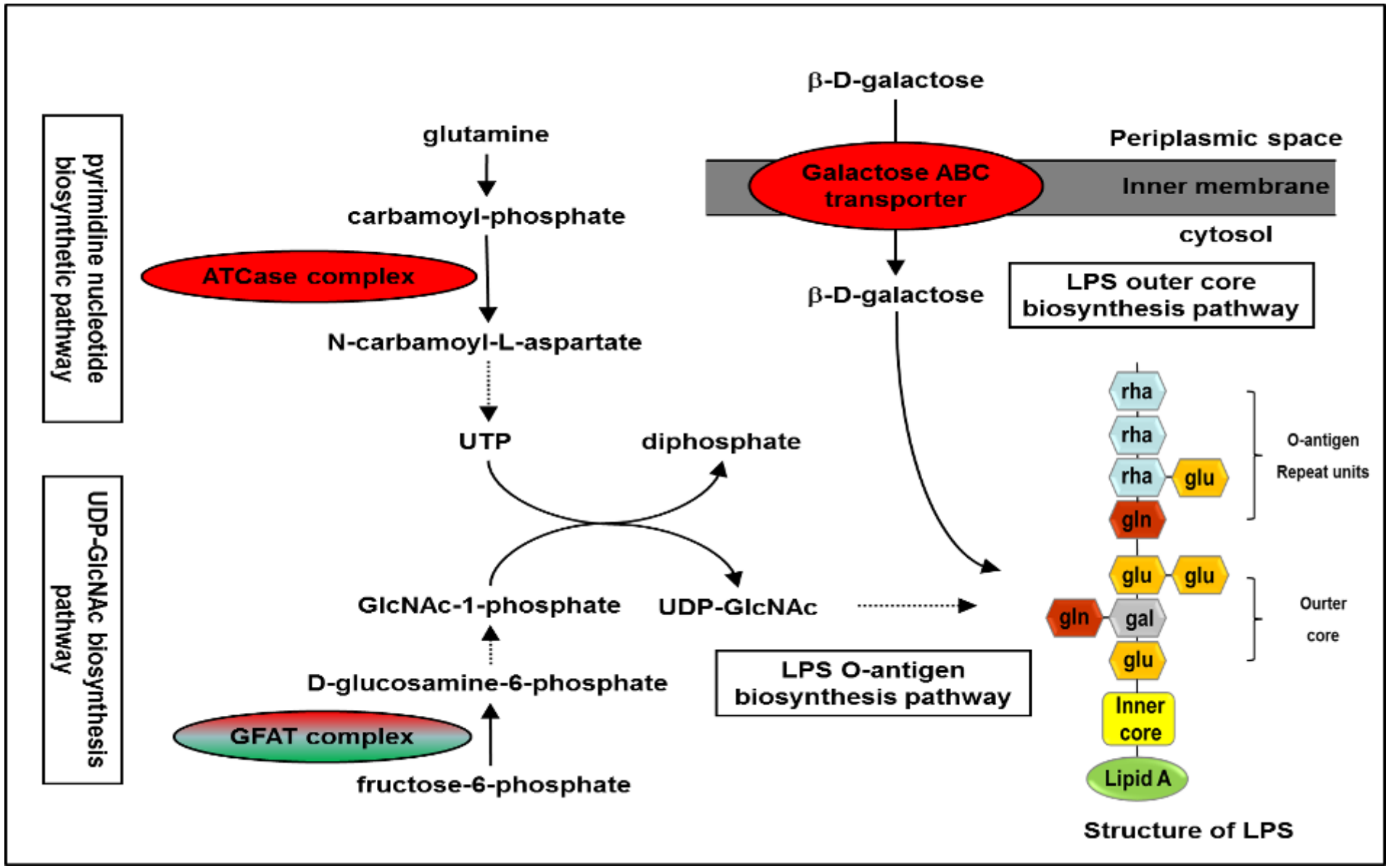
L-谷氨酰胺:D-果糖-6-磷酸氨基转移酶复合物

GcvP　Glycine decarboxylase

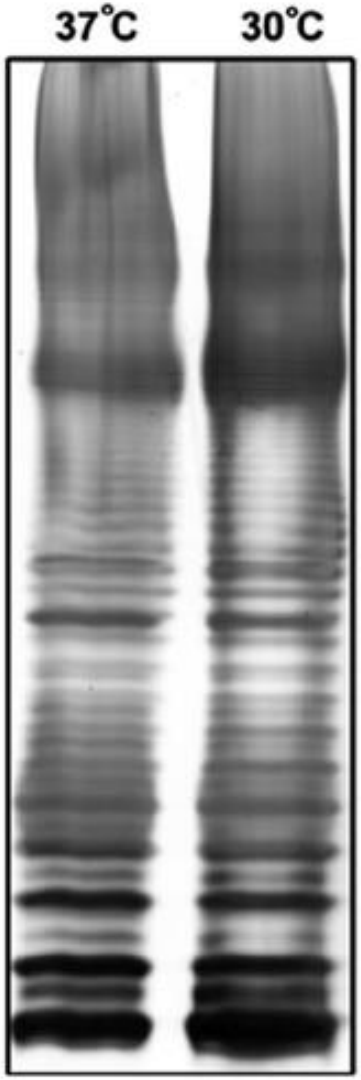
（甘氨酸裂解系统复合物）



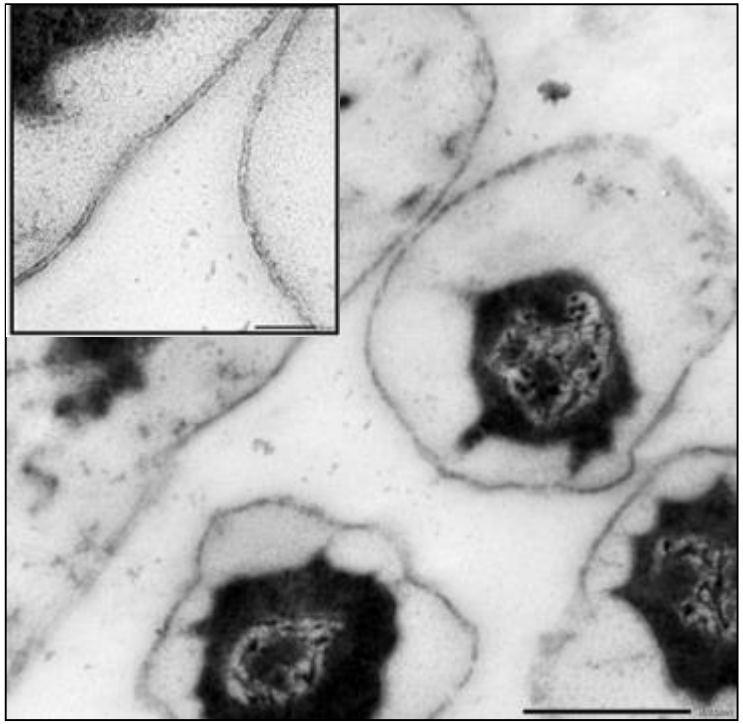
差异表达复合物参与LPS的合成



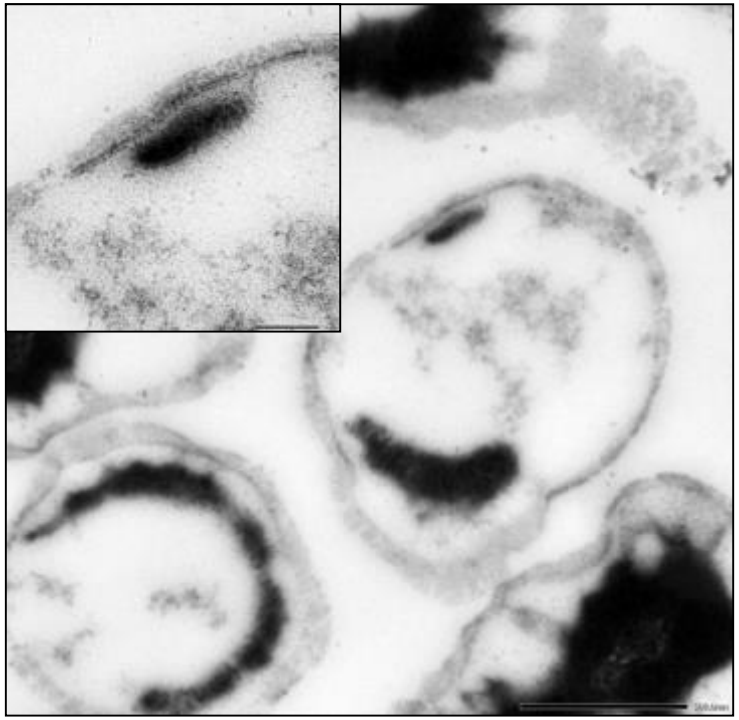
温度影响LPS的合成



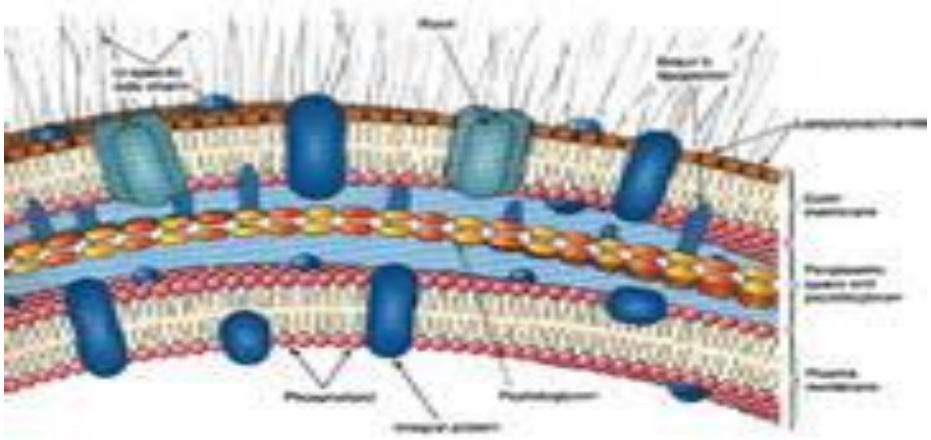
2457T 37℃

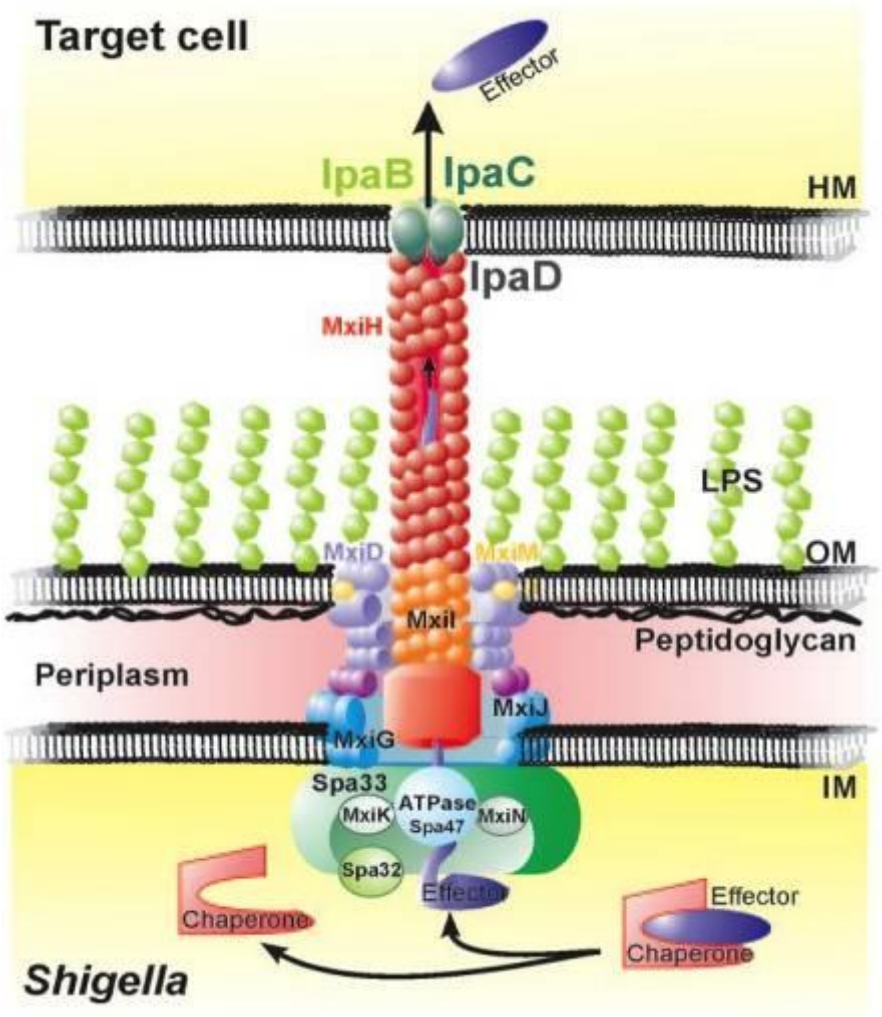


2457T 30℃



志贺氏菌T3SS与LPS间的关系





致　　谢

- 科技部
- 国家自然科学基金委员会
- 总后卫生部

- 课题组成员

刘先凯　朱　力
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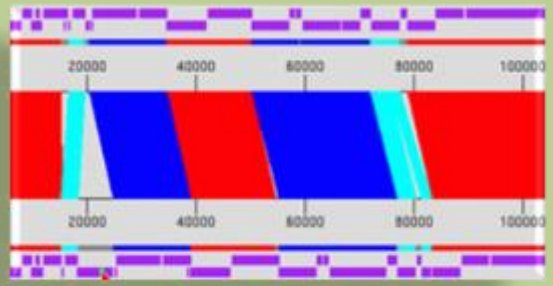
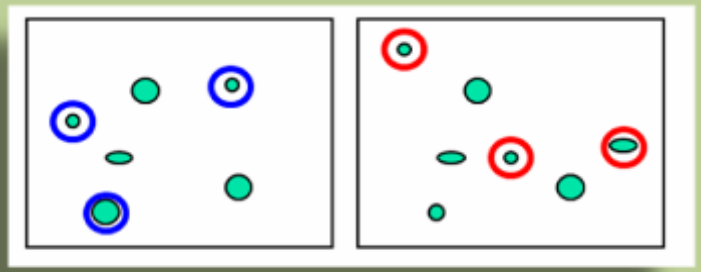
抗毒力基因的研究策略

比较基因组学方法（文献）

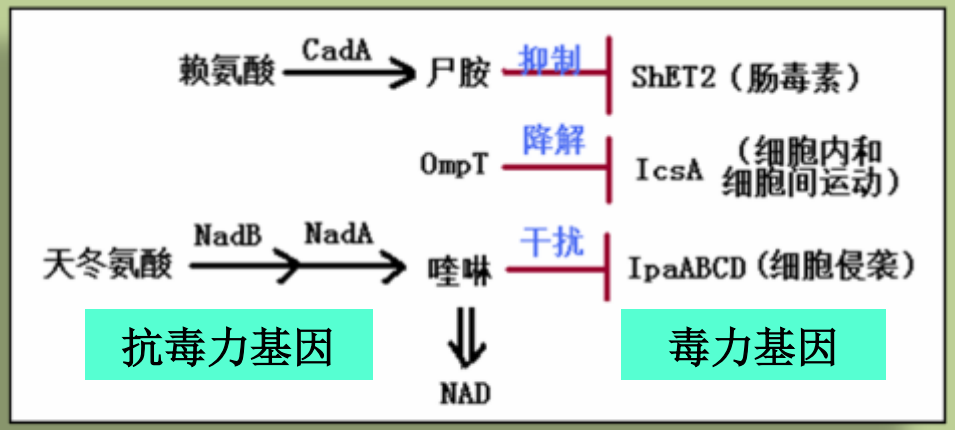


比较蛋白质组学方法（本研究）

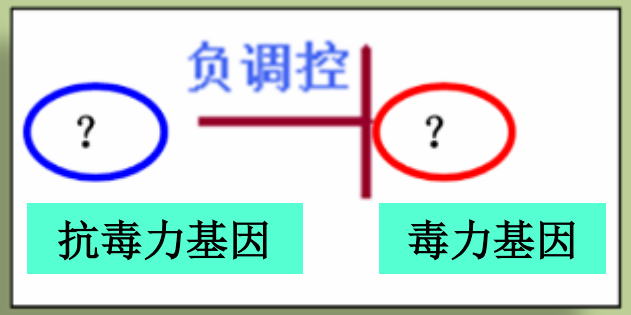


非毒力状态　　毒力状态

已发现的抗毒力基因及其作用机制：



将发现一类新的抗毒力基因：



获得致病菌适应性进化的新证据和新途径。