系统生物学

Bacterial evolution of antibiotic hypersensitivity.

汇报人：李新颖
导师：何璟
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BACKGROUND

- 抗生素（antibiotics）
  - 高等动植物
  - 微生物
    - 真菌
    - 细菌
    - 放线菌

抗病原菌 → **次级代谢产物**

阿莫西林、青霉素、“沙星”

抗生素：

- 抑制细胞壁的合成
- 与细胞膜相互作用
- 干扰蛋白质的合成
- 抑制核酸的转录和复制

抗生素滥用 → 耐药性 → ? → 敏感

Antibiotic name	Abbreviation	action	Bacteriostatic
Ampicillin	AMP*	Cell wall	Bactericidal
Pipericallin	PIP	Cell wall	Bactericidal
Cefoxitin	FOX*	Cell wall	Bactericidal
Fosfomycin	FOS	Cell wall	Bactericidal
Lomefloxacin	LOM	Gyrase	Bactericidal
Ciprofloxacin	CPR*	Gyrase	Bactericidal
Nalidixic acid	NAL*	Gyrase	Bactericidal
Fosmidomycin	FSM	Lipid	Bactericidal
Nitrofurantoin	NIT*	Multiple mechanisms	Bactericidal
Amikacin	AMK	Aminoglycoside	Bactericidal
Gentamicin	GEN	Aminoglycoside	Bactericidal
Kanamycin	KAN*	Aminoglycoside	Bactericidal
Tobramycin	TOB*	Aminoglycoside	Bactericidal
Streptomycin	STR	Aminoglycoside	Bactericidal
Tetracycline	TET*	Protein synthesis, 30S	Bacteriostatic
Doxycycline	DOX*	Protein synthesis, 30S	Bacteriostatic
Chloramphenicol	CHL*	Protein synthesis, 50S	Bacteriostatic
Erythromycin	ERY*	Protein synthesis, 50S	Bacteriostatic
Fusidic acid	FUS	Protein synthesis, 50S	Bacteriostatic
Sulfamonomethoxine	SLF	Folic acid biosynthesis	Bacteriostatic
Trimethoprim	TRM*	Folic acid biosynthesis	Bacteriostatic
Muporicin	MUP	Gram positive	NA
Cycloserine	CYC	Gram positive	NA
Vancomycin	VAN	Gram positive	NA

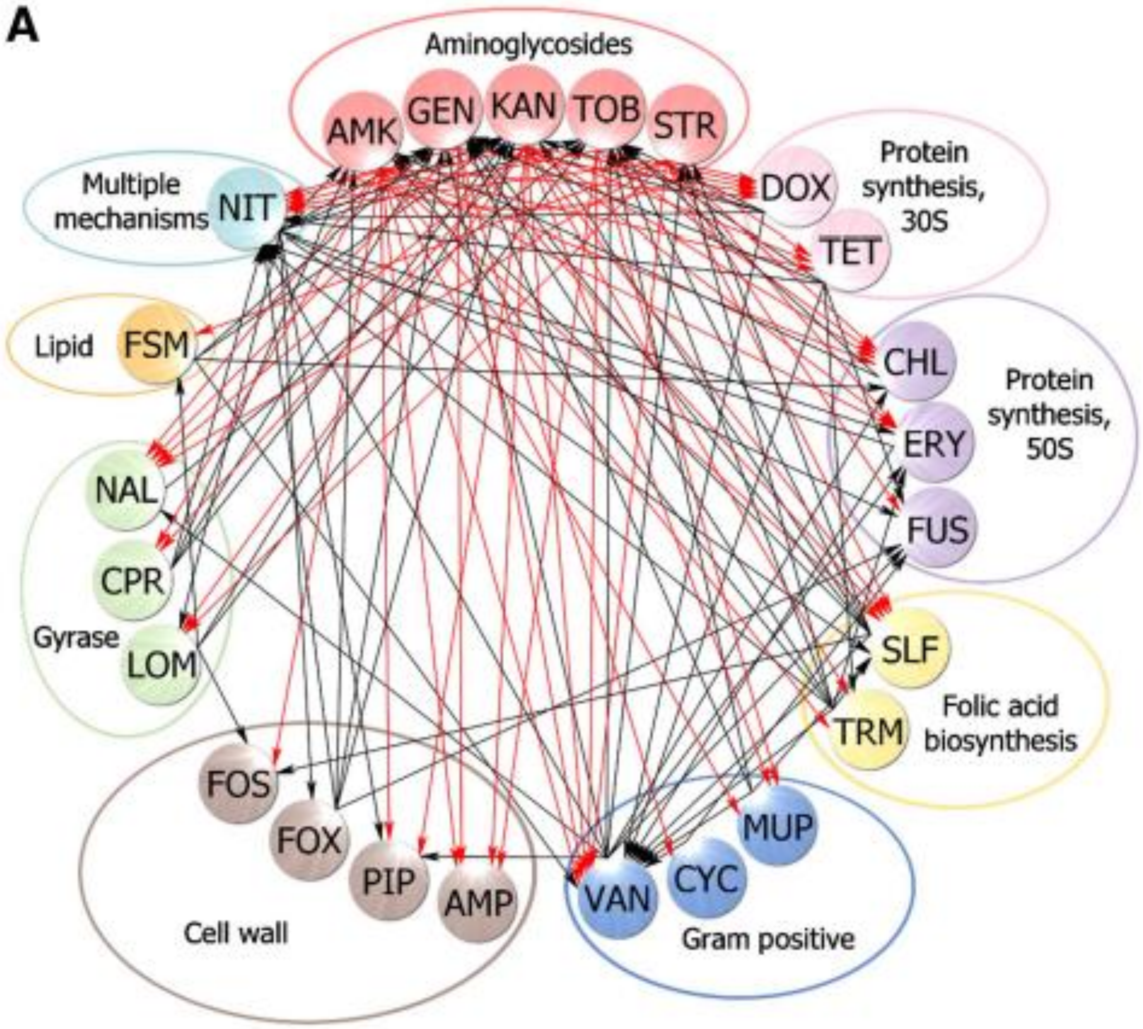
抗生素敏感性的进化：

1.每种抗生素24×10个平行
2.12种抗生素进行互补实验(96)
3.10个对照（无抗性）

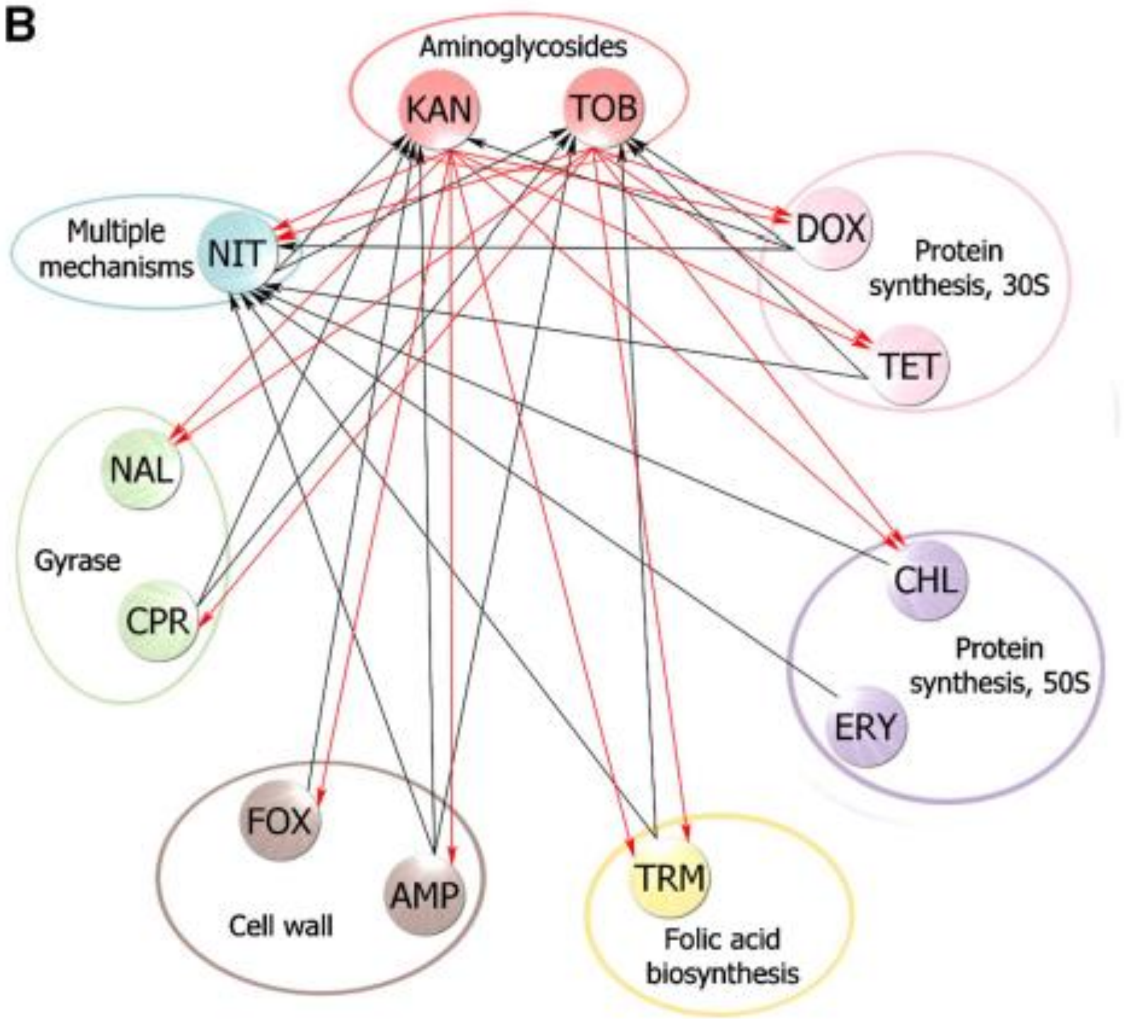
↓ MIC为原来的20～328倍

测试对其他抗生素的耐药性

低浓度

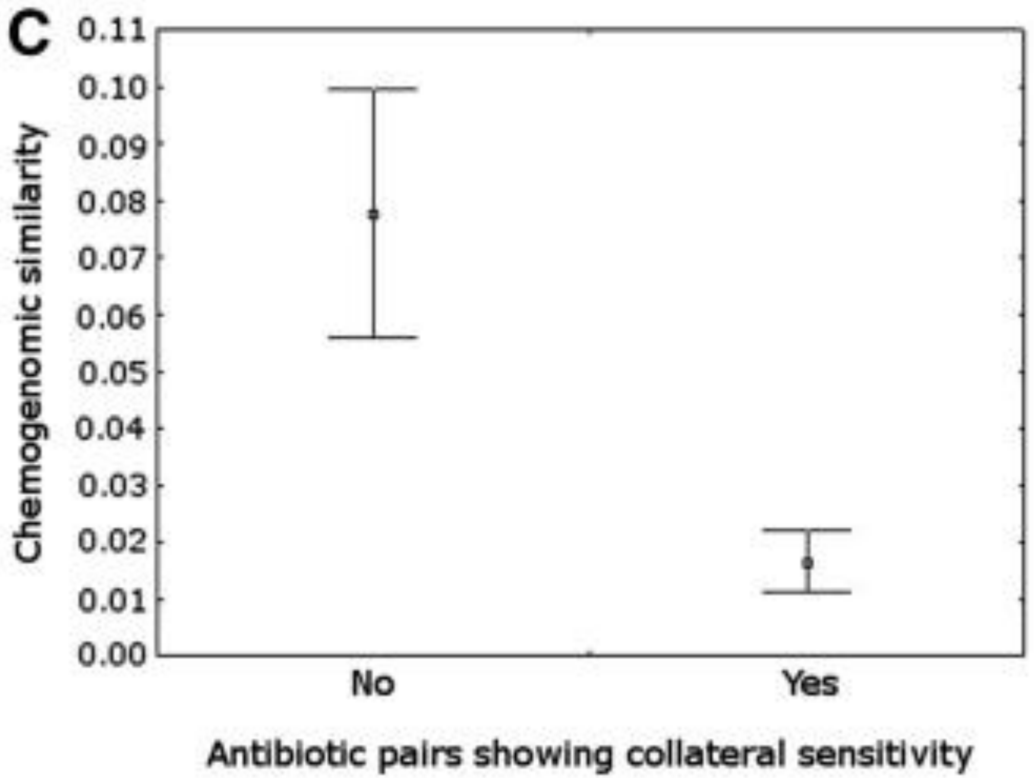

A
Aminoglycosides
AMK
GEN
KAN
TOB
STR
Protein synthesis, 30S
DOX
TET
Multiple mechanisms
NIT
Lipid
FSM
NAL
CPR
Gyrase
LOM
CHL
ERY
FUS
Protein synthesis, 50S
SLF
TRM
Folic acid biosynthesis
FOS
FOX
PIP
AMP
Cell wall
VAN
CYC
MUP
Gram positive

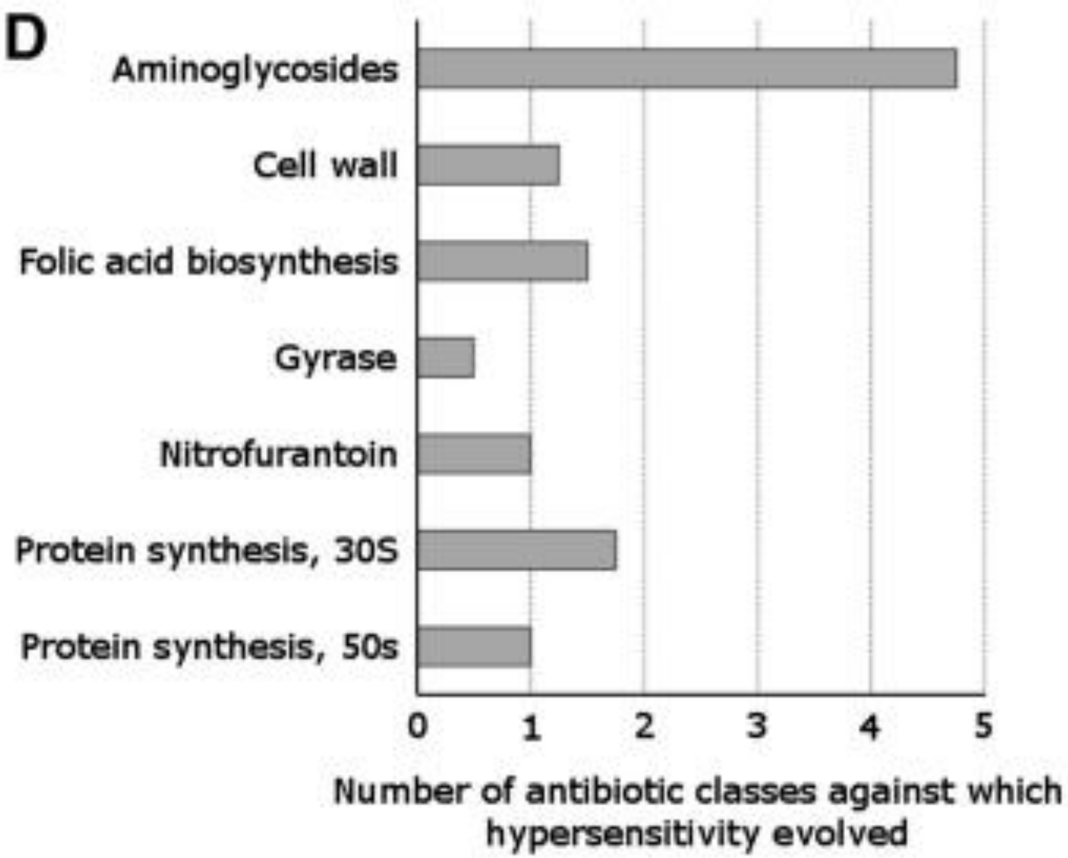
高浓度

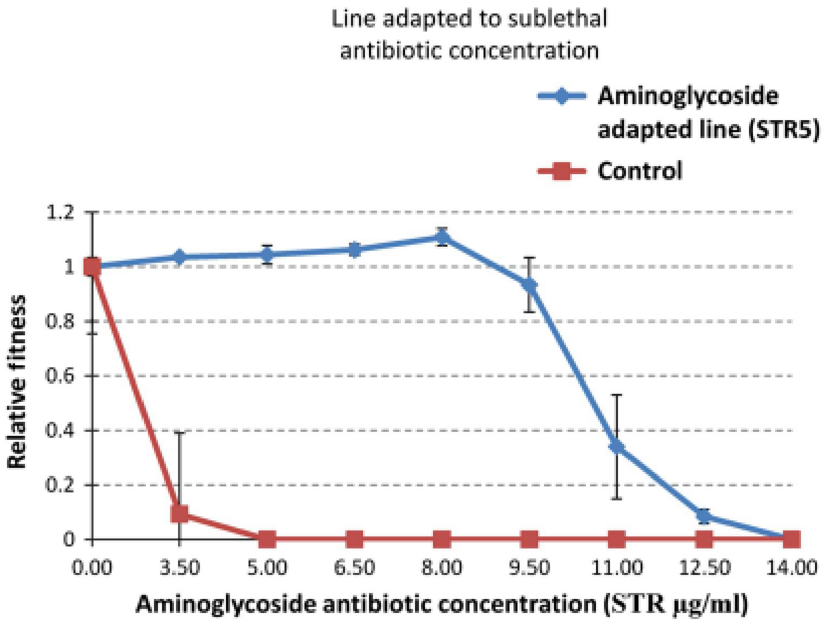

B
Aminoglycosides
KAN
TOB
Multiple mechanisms
NIT
DOX
Protein synthesis, 30S
TET
NAL
Gyrase
CPR
CHL
Protein synthesis, 50S
ERY
FOX
AMP
Cell wall
TRM
Folic acid biosynthesis

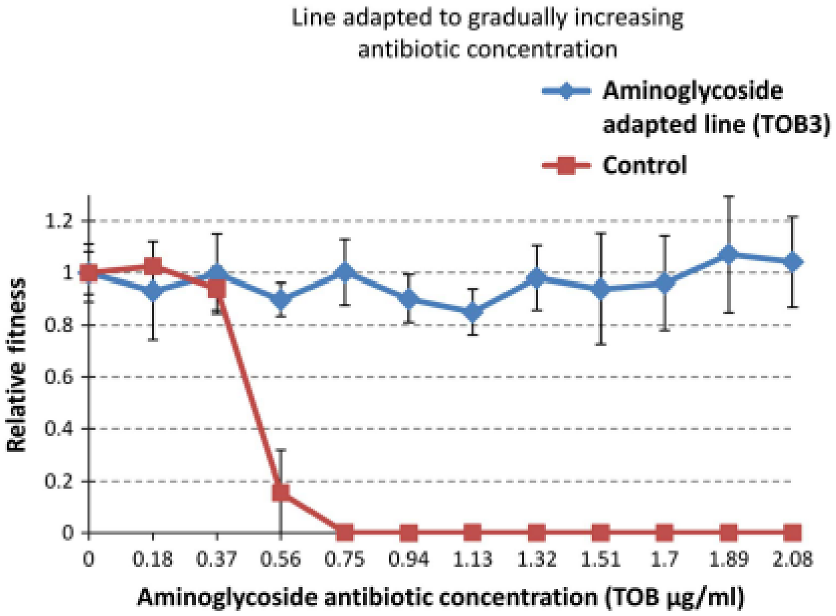
基因低重叠率

多不具有相互作用

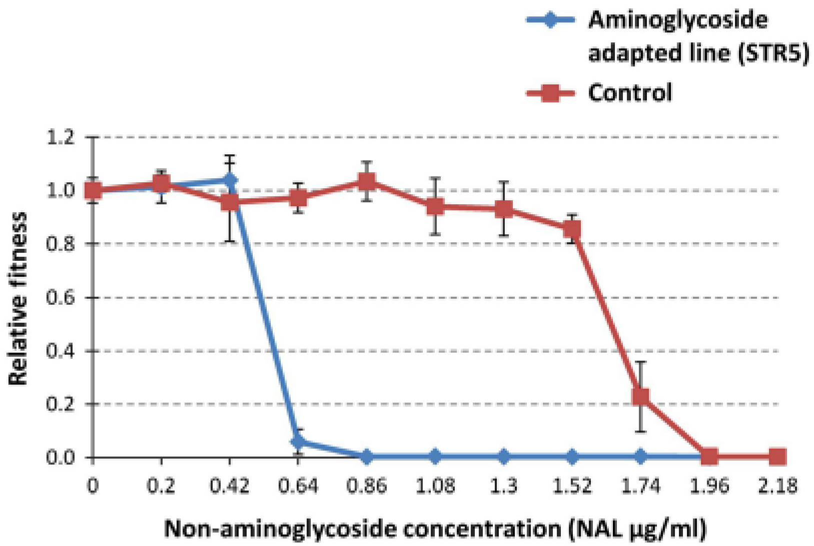


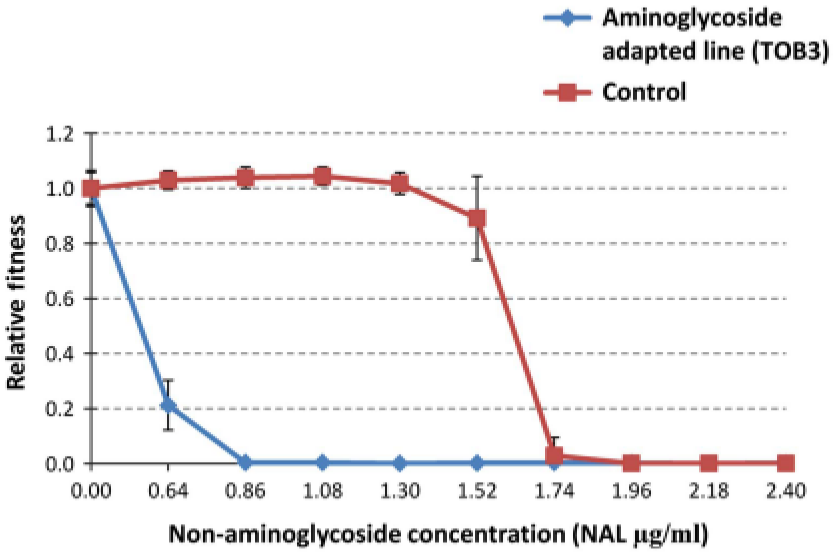



Line adapted to sublethal antibiotic concentration
Aminoglycoside adapted line (STR5)
Control
Relative fitness
0 0.2 0.4 0.6 0.8 1 1.2
0.00 3.50 5.00 6.50 8.00 9.50 11.00 12.50 14.00
Aminoglycoside antibiotic concentration (STR µg/ml)


Line adapted to gradually increasing antibiotic concentration
Aminoglycoside adapted line (TOB3)
Control
Relative fitness
0 0.2 0.4 0.6 0.8 1 1.2
0 0.18 0.37 0.56 0.75 0.94 1.13 1.32 1.51 1.7 1.89 2.08
Aminoglycoside antibiotic concentration (TOB µg/ml)

AG耐药


Aminoglycoside adapted line (STR5)
Control
Relative fitness
0.0 0.2 0.4 0.6 0.8 1.0 1.2
0 0.2 0.42 0.64 0.86 1.08 1.3 1.52 1.74 1.96 2.18
Non-aminoglycoside concentration (NAL µg/ml)


Aminoglycoside adapted line (TOB3)
Control
Relative fitness
0.0 0.2 0.4 0.6 0.8 1.0 1.2
0.00 0.64 0.86 1.08 1.30 1.52 1.74 1.96 2.18 2.40
Non-aminoglycoside concentration (NAL µg/ml)

N-AG敏感

氨基糖苷类抗性的三个主要机制：

- 通过氨基糖苷修饰酶灭活药物；
- 修饰核糖体；
- 降低膜通透性（部分通过膜电位的变化）；

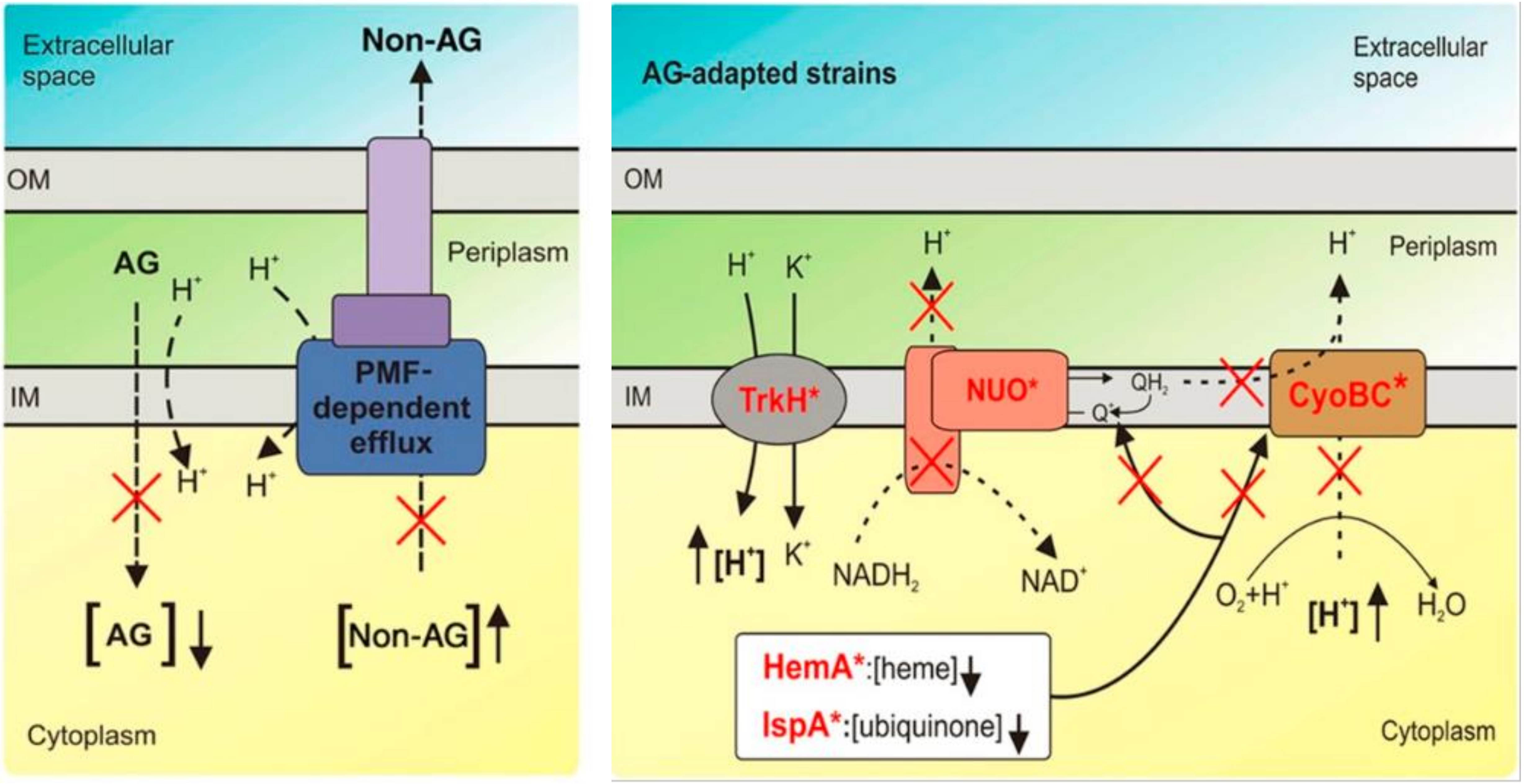
14种菌株（卡那霉素，妥布霉素或链霉素）

↓ SNP

44个蛋白质编码基因的100个突变

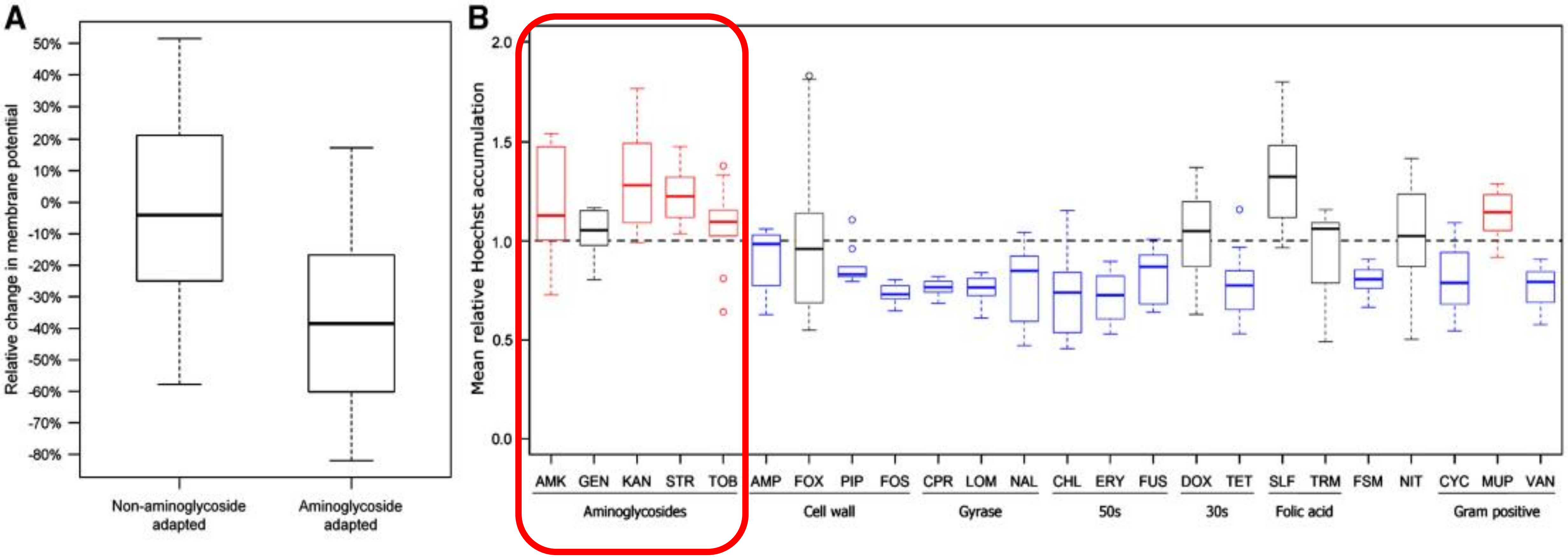
- 影响翻译
- 膜转运
- 影响膜电化学电位 ？

氧化磷酸化：trkH、hemA、cyoB、cyoC



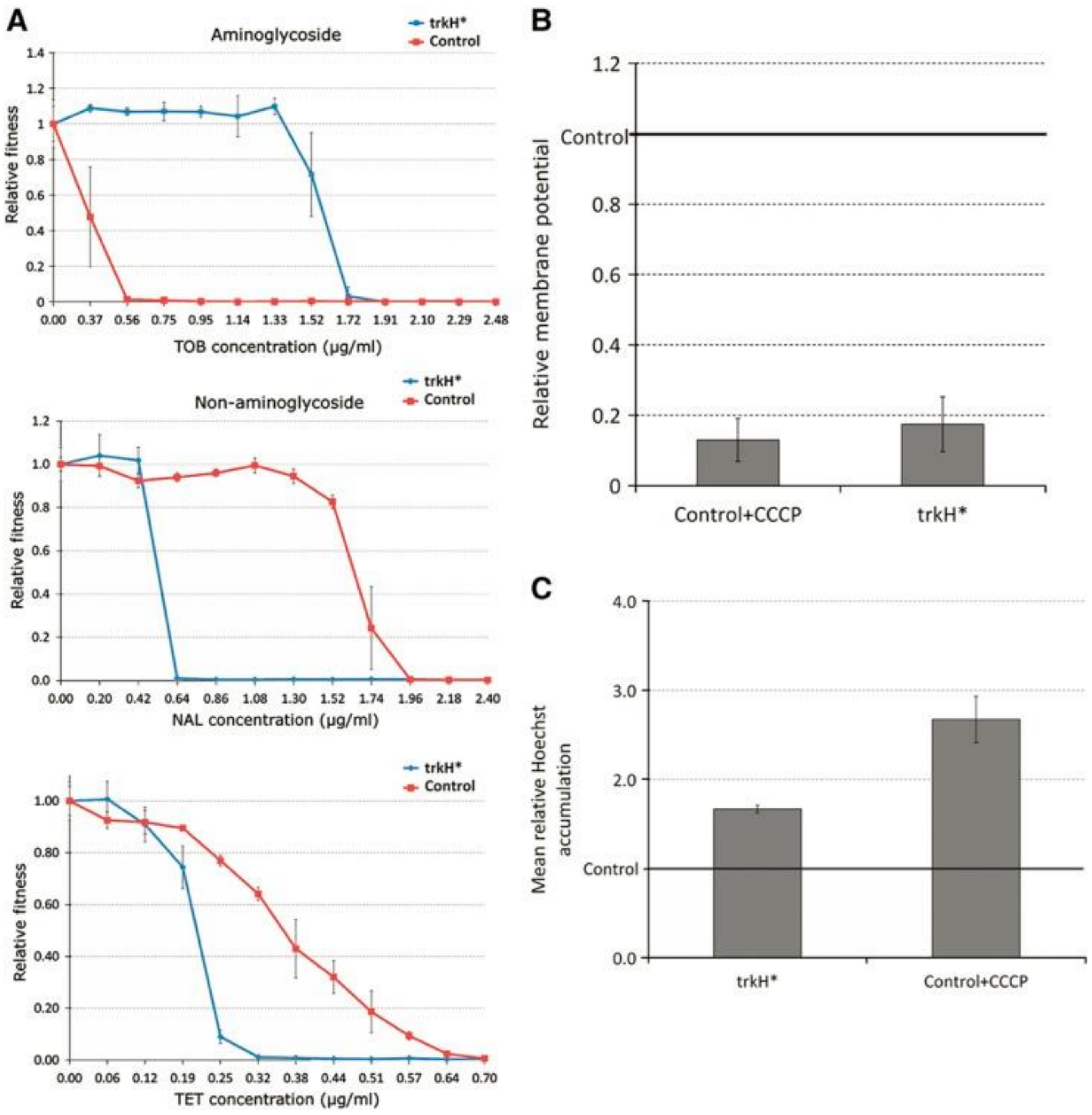
Extracellular space
Non-AG
OM
AG
H+
H+
Periplasm
IM
PMF-dependent efflux
H+
H+
[AG]
[Non-AG]
Cytoplasm
AG-adapted strains
Extracellular space
OM
H+
H+
K+
H+
Periplasm
IM
TrkH*
NUO*
QH2
Q+
CyoBC*
[H+]
K+
NADH2
NAD+
O2+H+
[H+]
H2O
HemA*:[heme]
IspA*:[ubiquinone]
Cytoplasm

碳菁染料DiOC2



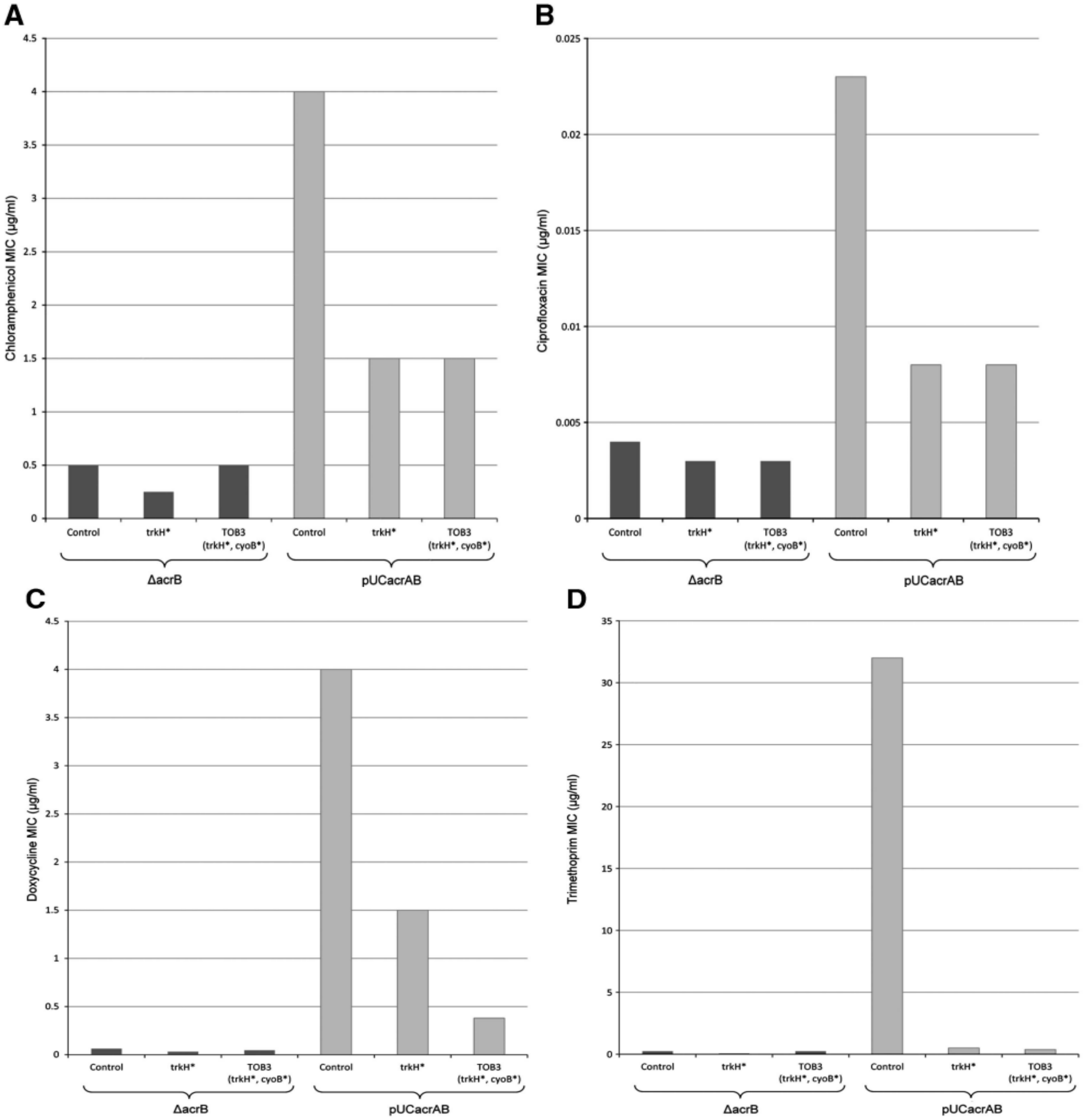
A
Relative change in membrane potential
50%
40%
30%
20%
10%
0%
-10%
-20%
-30%
-40%
-50%
-60%
-70%
-80%
Non-aminoglycoside adapted
Aminoglycoside adapted
B
Mean relative Hoechst accumulation
2.0
1.5
1.0
0.5
0.0
AMK GEN KAN STR TOB AMP FOX PIP FOS CPR LOM NAL CHL ERY FUS DOX TET SLF TRM FSM NIT CYC MUP VAN
Aminoglycosides
Cell wall
Gyrase
50s
30s
Folic acid
Gram positive

*trkH*突变（K^+）



A
Aminoglycoside
trkH*
Control
Relative fitness
1.4
1.2
1
0.8
0.6
0.4
0.2
0
0.00 0.37 0.56 0.75 0.95 1.14 1.33 1.52 1.72 1.91 2.10 2.29 2.48
TOB concentration (μg/ml)
Non-aminoglycoside
trkH*
Control
Relative fitness
1.2
1.0
0.8
0.6
0.4
0.2
0.0
0.00 0.20 0.42 0.64 0.86 1.08 1.30 1.52 1.74 1.96 2.18 2.40
NAL concentration (μg/ml)
trkH*
Control
Relative fitness
1.00
0.80
0.60
0.40
0.20
0.00
0.00 0.06 0.12 0.19 0.25 0.32 0.38 0.44 0.51 0.57 0.64 0.70
TET concentration (μg/ml)
B
Relative membrane potential
1.2
Control
0.8
0.6
0.4
0.2
0
Control+CCCP
trkH*
C
Mean relative Hoechst accumulation
4.0
3.0
2.0
Control
0.0
trkH*
Control+CCCP

AcrAB外排泵系统


A
Chloramphenicol MIC (µg/ml)
4.5
4
3.5
3
2.5
2
1.5
1
0.5
0
Control
trkH*
TOB3 (trkH*, cyoB*)
Control
trkH*
TOB3 (trkH*, cyoB*)
ΔacrB
pUCacrAB
B
Ciprofloxacin MIC (µg/ml)
0.025
0.02
0.015
0.01
0.005
0
Control
trkH*
TOB3 (trkH*, cyoB*)
Control
trkH*
TOB3 (trkH*, cyoB*)
ΔacrB
pUCacrAB
C
Doxycycline MIC (µg/ml)
4.5
4
3.5
3
2.5
2
1.5
1
0.5
0
Control
trkH*
TOB3 (trkH*, cyoB*)
Control
trkH*
TOB3 (trkH*, cyoB*)
ΔacrB
pUCacrAB
D
Trimethoprim MIC (µg/ml)
35
30
25
20
15
10
5
0
Control
trkH*
TOB3 (trkH*, cyoB*)
Control
trkH*
TOB3 (trkH*, cyoB*)
ΔacrB
pUCacrAB

CONCLUTIONS

- 耐氨基糖苷类菌株对几种其他抗生素敏感；
- 全基因组测序揭示了氨基糖苷类耐药性的多种机制，包括翻译，膜转运，磷脂合成、内膜的质子 - 动力（PMF）的减少，通过降低依赖PMF的主要外排泵（AcrAB转运蛋白）的活性，可以导致对其他几种抗生素的过敏反应，从而达到一个平衡。

局限：

- 首先，只能解释氨基糖苷类物质耐药性的机理，其他类型抗生素需要相应的机理解释；
- 其次，我们通进行基因突变却忽视了环境的影响；
- 第三，缺乏系统的研究，PMF突变的频率难以控制。

启发：

利用本实验的研究，可以用来治愈抗生素引起的耐药性；

在科研中，要多思考，不断完善实验方案，做到尽善尽美。

THANKS