文献汇报

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Article

TRANSPARENT PROCESS

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molecular systems biology

Growth-dependent bacterial susceptibility to ribosome-targeting antibiotics

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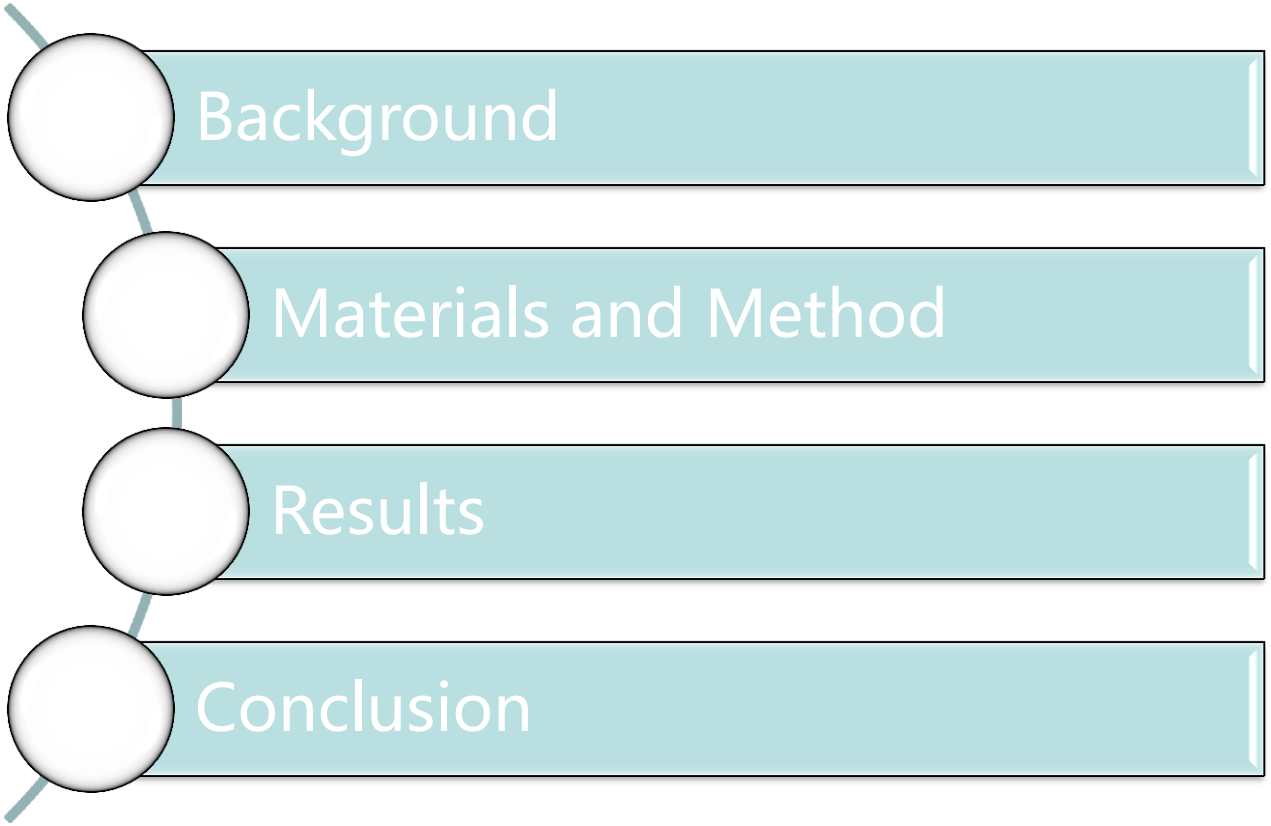
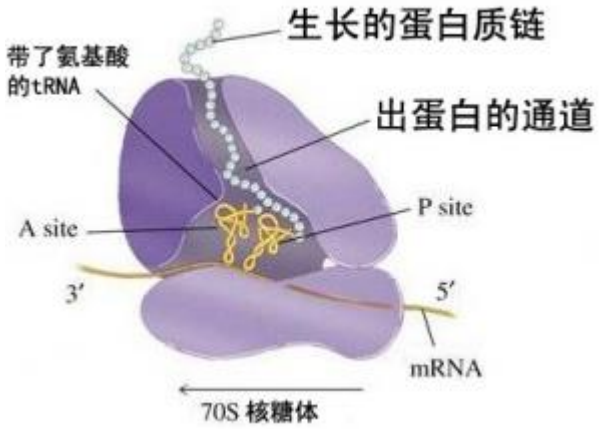
Introduction



- Ribosome

核糖体是联系生命蓝图的重要原件————编码在DNA 中的遗传信息和生命活动的执行者蛋白质之间的桥梁。

2009 年诺贝尔化学奖授予了在核糖体结构和功能研究方面有突出贡献的Ramakrishnan(英国) ，Steitz(美国) 和Yonath(以色列) 。这3 位科学家各自领导的科研小组用X-ray 晶体学技术测定了核糖体的高分辨率分子结构并研究其结构-功能关系，独立地解析了核糖体30S 和50S 亚基的精细结构，阐释了其作为蛋白质翻译机器的工作机制，并发现核糖体作为核糖酶的新功能。



Background

- Antibiotics

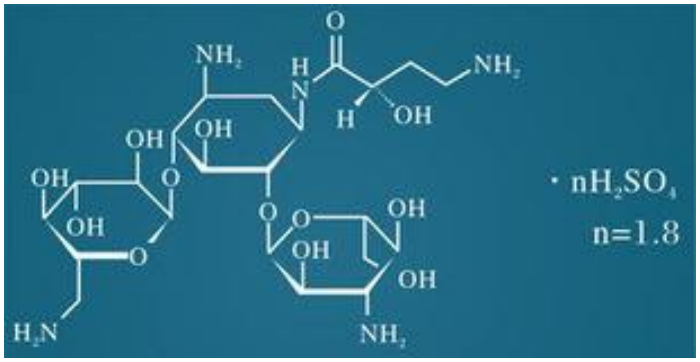
抗生素是由微生物（包括细菌、真菌、放线菌属）或高等动植物在生活过程中所产生的具有抗病原体或其它活性的一类次级代谢产物，能干扰其他生活细胞发育功能的化学物质。现临床常用的抗生素有转基因工程菌培养液液中提取物以及用化学方法合成或半合成的化合物

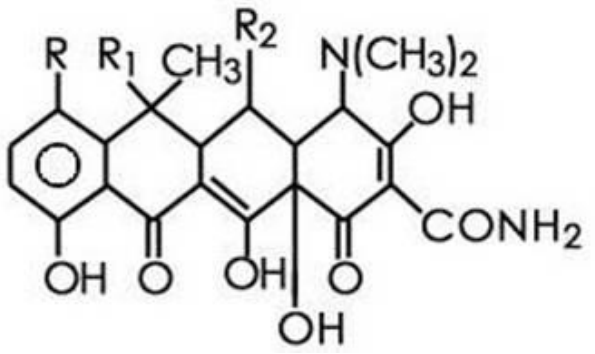
Background

- ribosome-targeting antibiotics

研究结果启示:有大量的抗生素能够与核糖体结合行使功能。例如：与核糖体50S 亚基相结合的抗生素有大环内酯类、酮内酯类、链阳菌素、截短侧耳素、苯丙素类、噁唑烷酮类; 与核糖体30S 亚基相结合的有氨基糖苷类、四环素类、伊短菌素类、密旋霉素类、奇霉素类及其他抗生素。由于现有药物细菌耐药性的不断出现，对这些药物阻碍蛋白质合成机制的更好理解和深入研究，将对以结构为基础新型抗生素的设计有很大的启发和帮助。

以核糖体为靶点的抗生素虽然具有不同的作用目标，但也具有共同点。核糖体抗生素都结合于核糖体功能相关区域，以阻止生物合成循环中的某一关键步骤。这些作用主要包括: 引起错译，抑制翻译起始，干扰tRNA 分子底物与解码中心结合，阻碍tRNA 底物进入肽基转移酶中心，阻止核糖体再循环因子的相互作用及阻断蛋白出口通道等。





Materials and Method

- Antibiotics

From Fisher Scientific

- Growth media

MOPS

- Strains

Escherichia coli K12 strain MG1655

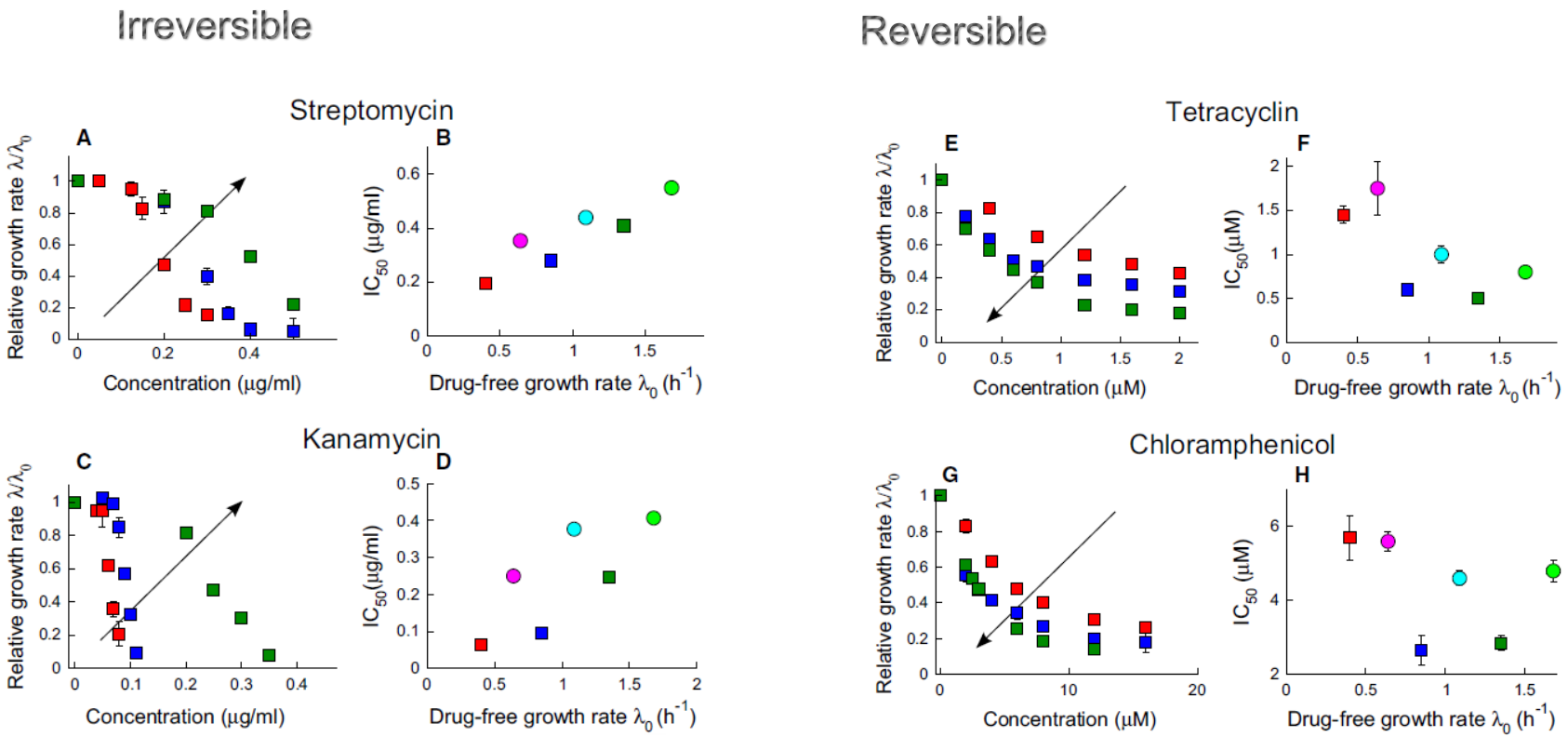
- Protein and RNA extraction

Lowry method / cold perchloric acid precipitation

- Data fits

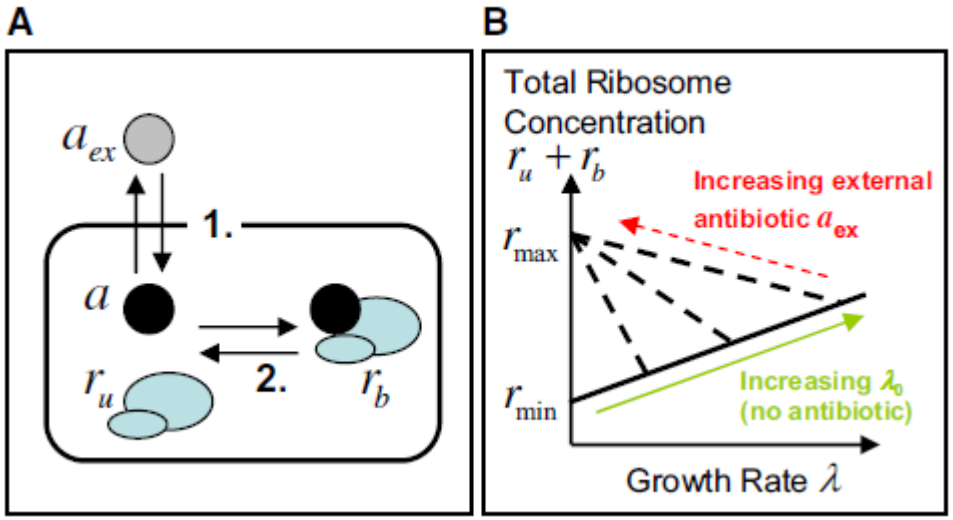
Powell ‘s method

1、Antibiotic efficacy depends on growth rate



	Gly	Glu	Gly_{CAA}	Glu_{CAA}	Gly_{RDM}	Glu_{RDM}
λ_0(/h)	0.40	0.64	0.85	1.09	1.35	1.68

2、Mathematical model



抗生素吸收及排出：$J(a_{ex},a) = P_{in}a_{ex} - P_{out}a$

核糖体与抗生素的结合及分离：$f(r_u,r_b,a) = -k_{on}a(r_u-r_{min}) + k_{off}r_b$,

2、Mathematical model

$$\frac{da}{dt} = -\lambda a + f(r_u, r_b, a) + J(a_{ex}, a), \quad (1)$$

$$\frac{dr_u}{dt} = -\lambda r_u + f(r_u, r_b, a) + s(\lambda), \quad (2)$$

$$\frac{dr_b}{dt} = -\lambda r_b + f(r_u, r_b, a). \quad (3)$$

This model is coupled to cell physiology via the empirical relations of Scott et al (2010), which link the growth rate λ and ribosome synthesis rate s(λ) to the ribosome concentration; these act as constraints on the dynamical equations (1–3).

$$r_u = \lambda/\kappa_t + r_{\min}. \quad (4)$$

the unbound ribosome content r_u and the growth rate λ are linearly proportional

2、Mathematical model

$$r_{\mathrm{tot}} = r_u + r_b = r_{\max} - \lambda\Delta r\left(\frac{1}{\lambda_0} - \frac{1}{\kappa_t \Delta r}\right), \tag{5}$$

the total ribosome content
r_{tot} increases linearly, reaching a fixed maximal value
r_{max} = 65.8

$$s(\lambda) = \lambda r_{\mathrm{tot}} = \lambda\left[r_{\max} - \lambda\Delta r\left(\frac{1}{\lambda_0} - \frac{1}{\kappa_t \Delta r}\right)\right]. \tag{6}$$

Adding together equations (2) and (3) at steady state ($dr_u/dt = dr_b/dt = 0$) shows that the ribosome synthesis rate $s(\lambda)$ is the product of growth rate and total ribosome content

3、Model results for growth inhibition curves

Solving the model equations (1–3) at steady state, together with the physiological constraints, equations (4) and (5), produces a universal equation that links the steady state relative growth rate k/k0 to the extracellular antibiotic concentration a_{ex}

$$0 = \left(\frac{\lambda}{\lambda_0}\right)^3 - \left(\frac{\lambda}{\lambda_0}\right)^2 + \left(\frac{\lambda}{\lambda_0}\right)\left[\frac{1}{4}\left(\frac{\lambda_0^*}{\lambda_0}\right)^2 + \frac{a_{ex}}{2IC_{50}^*}\left(\frac{\lambda_0^*}{\lambda_0}\right)\right] - \frac{1}{4}\left(\frac{\lambda_0^*}{\lambda_0}\right)^2. \quad (7)$$

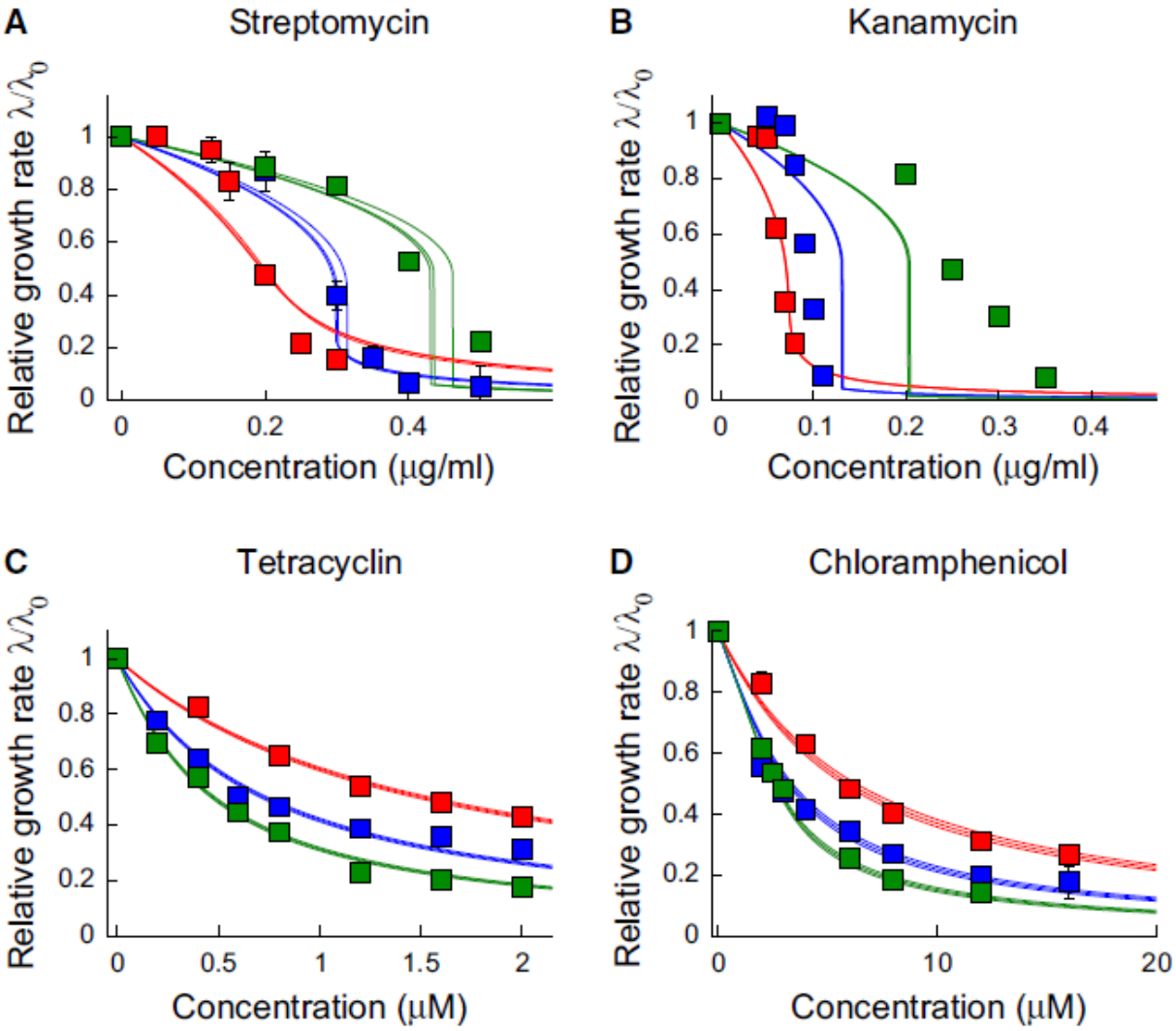
The first parameter combination is a rate λ_0^*, which characterizes the reversibility of ribosome-targeting antibiotic transport and binding:

$$\lambda_0^* = 2\sqrt{P_{out}\kappa_t K_D}, \quad (8)$$

The second parameter combination is a concentration scale

$$IC_{50}^* = \frac{\Delta r \lambda_0^*}{2P_{in}}. \quad (9)$$

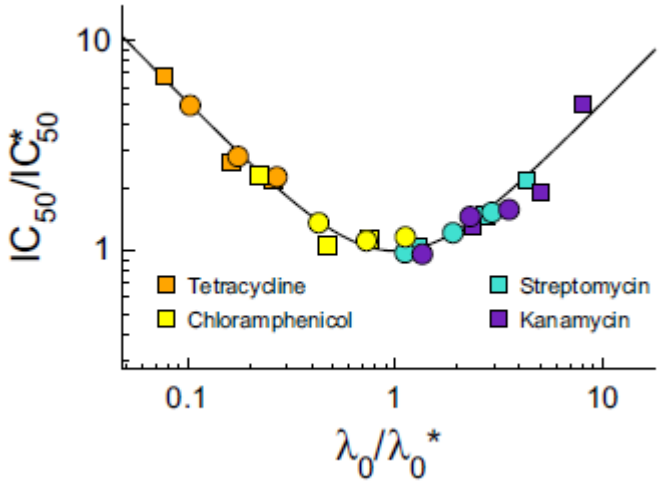
3、Model results for growth inhibition curves



4、Universal growth-dependent antibiotic susceptibility curve

Substituting $a_{ex} = IC_{50}$ and $\lambda = \lambda_0/2$ into equation (7),we find that, for all antibiotics, the growth rate dependence of the half-inhibition concentration IC50 is predicted to fall onto a universal growth dependent susceptibility curve

$$\frac{IC_{50}}{IC_{50}^*} = \frac{1}{2}\left[\frac{\lambda_0}{\lambda_0^*} + \frac{\lambda_0^*}{\lambda_0}\right]. \qquad (10)$$

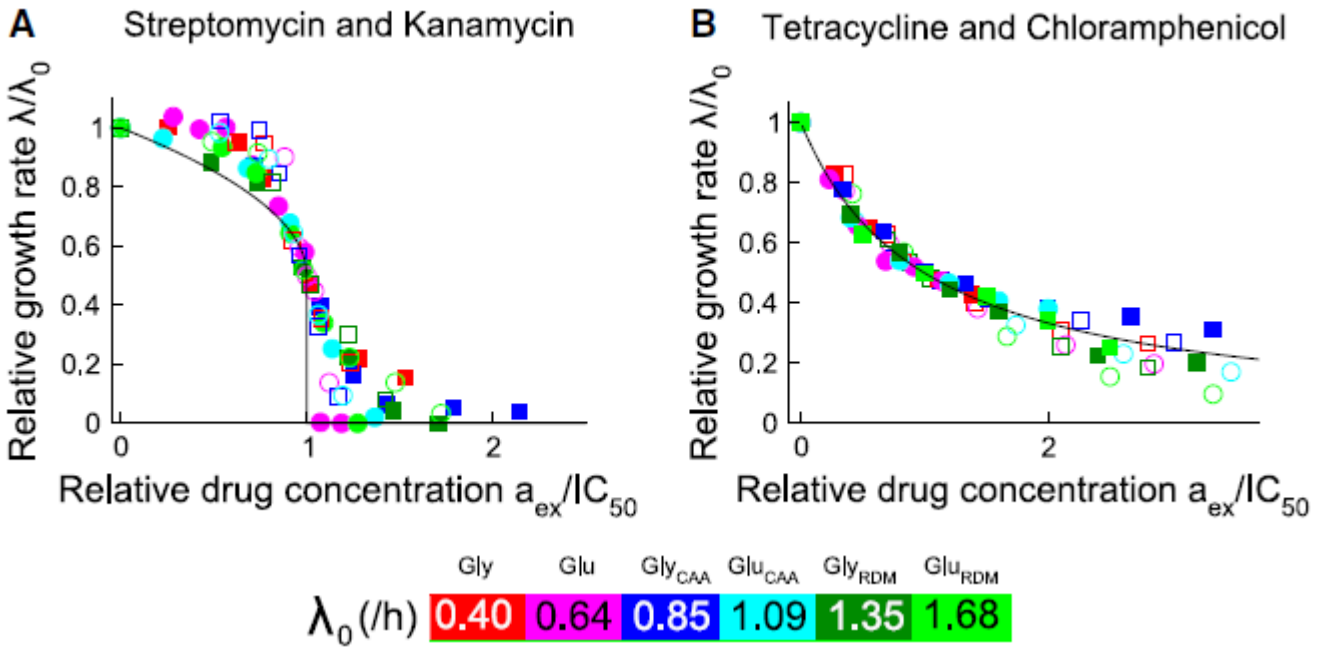


5、Simple predictions in the reversible and irreversible limits

$$\frac{\lambda}{\lambda_0} = \frac{1}{2}\left[1 + \sqrt{1 - \frac{a_{ex}}{IC_{50}}}\right], \qquad (11)$$

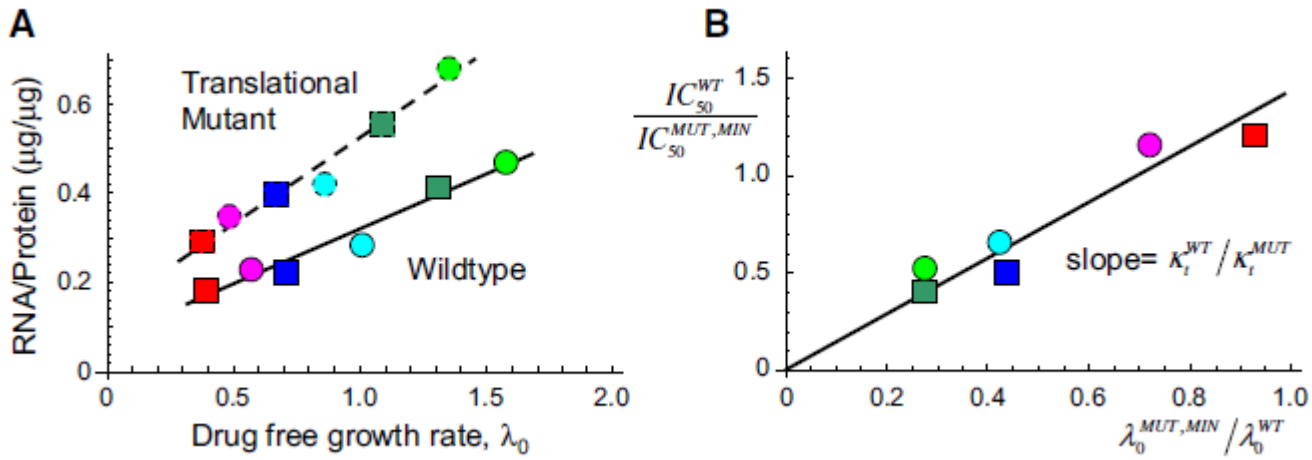
$$\frac{\lambda}{\lambda_0} = \frac{1}{1 + a_{ex}/IC_{50}}, \qquad (12)$$

In the limiting cases of very large or very small λ_0*, that is the limits in which antibiotic transport and binding is either fully reversible or fully irreversible, the model leads to simple predictions for the growth inhibition curve and growth rate dependence of the half-inhibition concentration IC_{50}.

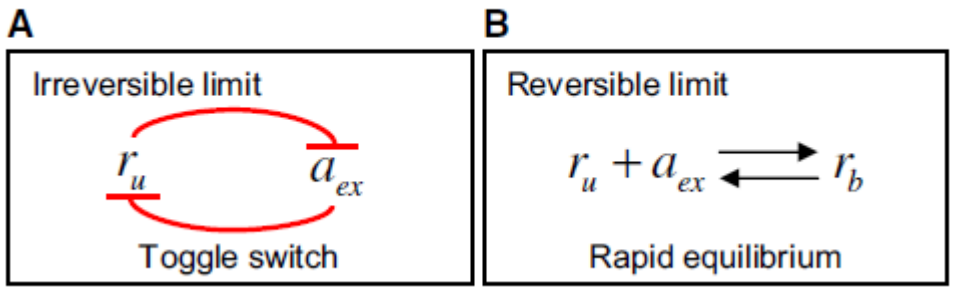


6、Testing the model predictions for a translation mutant strain of E. coli

In our model, the key parameters λ_0 *and IC_{50}* (defined in equations8 and 9) depend on the translational capacity jt. To test the predictions of the model, we used a strain of E. coli MG1655



7、Mechanistic link between reversibility timescale and growth-dependent susceptibility



A In the limit that either transport or binding is irreversible (as is the case for streptomycin and kanamycin), the system exhibits a 'toggle-switch' topology, leading to a steep inhibition curve (equation 11).

B In the limit of fully equilibrated transport and binding (as is the case for tetracycline and chloramphenicol), the model predicts more gradual inhibition (equation 12).

8、conclusion

1、主要结论

细菌对以核糖体为靶标的抗生素的敏感性对生长速率有强烈的依赖关系，并且不同类型的抗生素有不同的表现。

2、启发

对抗生素作用机制有更深度的研究，可以从控制生长速率来改变细菌对抗生素的敏感性，并对新型抗生素的设计提供了指导。在对其他动态过程进行研究的过程中，可以建立一个类似的数学模型进行分析。

3、不足

对于生长速率的控制可以利用除碳源外的其他物质，以排除碳源的影响。在得到相应的实验结论之后用其他的一些抗生素来验证实验结论。

Thank You!