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Reduction of the fitness cost of antibiotic resistance caused by chromosomal mutations under poor nutrient conditions

Wenfang Lin[a], Jie Zeng[a,b], Kun Wan[a,b], Lu Lv[c], Lizheng Guo[a,b], Xi Li[a,b], Xin Yu[a,*]

[a] Key Lab of Urban Environment and Health, Institute of Urban Environment, Chinese Academy of Sciences, Xiamen 361021, China
[b] University of Chinese Academy of Sciences, Beijing 100049, China
[c] Institute of Quality and Standard for Agricultural products, Zhejiang Academy of Agricultural Sciences, Hangzhou 310021, China

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ABSTRACT

The prevalence of antibiotic resistance in drinking water system is pressing public health risk. Antibiotic resistance conferred by chromosomal mutations often produces fitness cost, which may affect its spread and persistence. In this study, the rifampin-resistant strains were competed with their wild-type counterparts at different nutrient levels. It was observed that the ratio of the absolute number between resistant and wild-type cells quickly decreased under rich nutrient conditions, but it slowly reduced or remained stable in the poor nutrient medium. This finding suggested that poor nutrient conditions resulted in the reduction of fitness cost of antibiotic resistance, i.e. the resistant bacteria became more competitive. Implying mechanisms analysis found that the differences of metabolic activity between wild-type and rifampin-resistant strains was significant smaller ($P < 0.05$) at low nutrient levels. Additionally, distinguishable large colony size rifampin-resistant strains were observed during competition assay. DNA sequencing of RNA polymerase subunit genes further revealed that these colonies could be adaptive mutants from wild-type strain in *rpoB* gene. To our knowledge, this is the first study to reveal that the oligotrophic conditions facilitate the persistence of antibiotic resistance in drinking water by reducing the fitness cost of the resistant strains.

1. Introduction

Over the last decades, the continuous input of antibiotic compounds to the environment produces selective pressure for bacteria, thus developing resistance against antibiotics. This might be the main driver for the increasing antibiotic resistance found in the environment because of human activities (Gullberg et al., 2011; Jiang et al., 2013; Kolář et al., 2001). In addition, bacteria can also acquire antibiotic resistance by spontaneous mutations while adapting to external environments in the absence of antibiotics due to natural selection, i.e. adaptive mutations (Hershberg, 2017; Knöppel et al., 2017). The widespread of antibiotic resistant bacteria (ARB) and antibiotic resistance genes (ARGs) in aquatic environment, even in the finished and tap water, are raising considerable public health concerns (Baquero et al., 2008; Bouki et al., 2013; Su et al., 2018; Xi et al., 2009; Zhang et al., 2018). However, only trace level of antibiotic (1.2–200 ng/l) presents in source and finished drinking water (Benotti et al., 2009; Watkinson et al., 2009; Ye et al., 2007), which can hardly cause the pressure high enough to select the resistance. Therefore, it is still unclear why the bacterial antibiotic resistance can stably maintain in drinking water system with trace antibiotic concentrations.

Generally, antibiotic resistance can be intrinsic, can be conferred by horizontal gene transfer through mobile genetic elements (e.g. plasmids), or can be acquired through spontaneous mutations within chromosomal genes (Cox and Wright, 2013; Sharma et al., 2016). There were four main mechanisms leading to antibiotic resistance (Baker-Austin et al., 2006): reduction of membrane permeability to antibiotics (Delcour, 2009); antibiotic inactivation (Wright, 2005); rapid efflux of the antibiotics (Webber and Piddock, 2003); and alteration or bypass of cellular targets (Lambert, 2005). In many cases, chromosomal mutations that confer resistance to antibiotics engender structural or functional modifications in the cellular target (Reynolds, 2000), particularly, the resistance mutations in genes controlling DNA coiling, transcription, and protein synthesis. For instance, *rpoB* (RNA polymerase subunit β gene) mutations causing rifampin resistance (Reynolds, 2000; Wi et al., 2018); mutation in *gyrA* (DNA gyrase) inducing ciprofloxacin resistance (Eaves et al., 2004); and streptomycin resistance caused by mutations in *rpsL* gene (encoded 30S ribosomal protein S12) (Cuevas-Córdoba et al., 2013). Both plasmid and chromosomally conferred resistance could impair bacterial fitness or cause

* Corresponding author.
E-mail address: xyu@iue.ac.cn (X. Yu).

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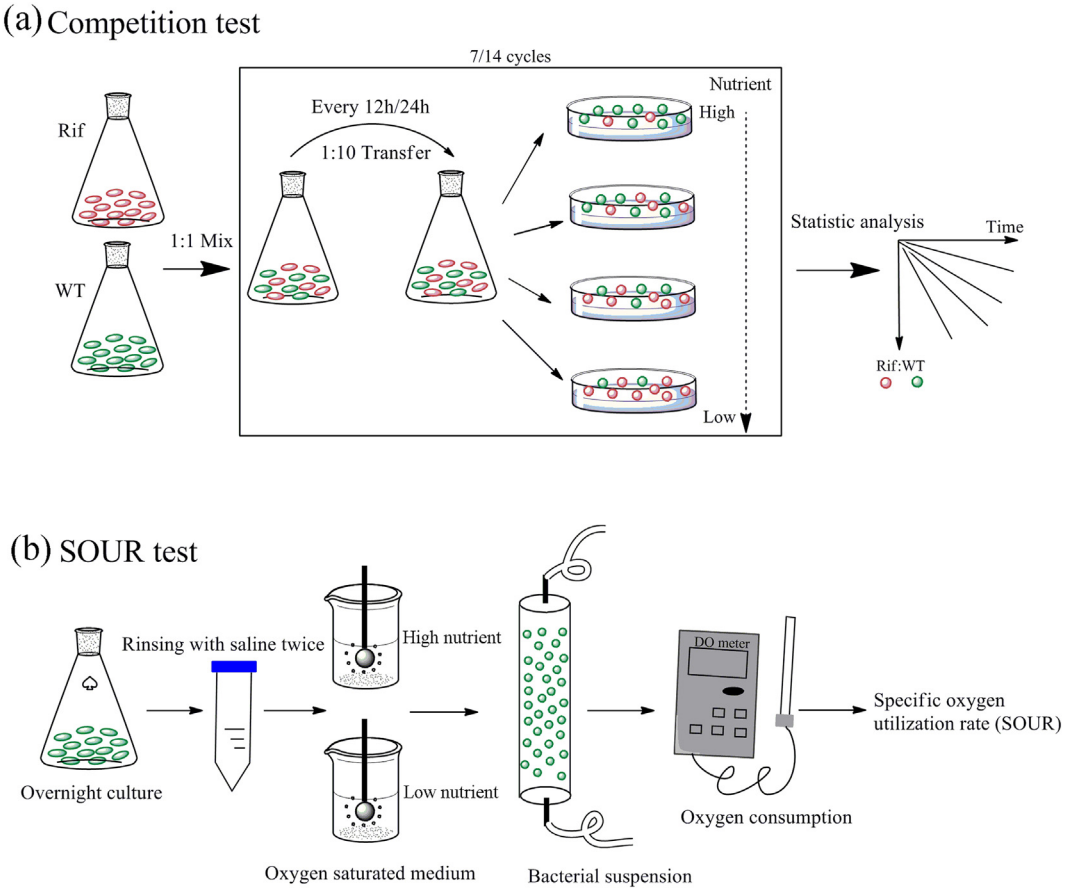

Fig. 1. Schematic depiction experimental approach. (a) Competition test, (b) SOUR test.

fitness cost (Andersson, 2006; Hershberg, 2017), such as a reduction in growth (Song et al., 2014), virulence, or transmission (Borrell and Gagneux, 2010). Bacterial fitness cost played a key role in its persistence under trace or no antibiotic conditions like drinking water system.

Environmental factors such as resource availability (including its type and concentration), growth condition, and temperature, have impacts on the fitness cost of resistance (Björkman et al., 2000; Gifford et al., 2016). For example, Hall et al. (2011) studies the effect of 41 different carbon sources on the fitness cost of rifampicin-resistant *Pseudomonas aeruginosa*, but the result found that no consistent effect of source types on bacterial fitness cost. In addition, σ^s factor (encoded by *rpoS* gene, a key regulator of many stationary-phase and stress-inducible genes) could also influence bacterial fitness cost under stress environments such as poor nutrient condition (Paulander et al., 2009). To inhibit the microbial regrowth in drinking water system, the concentration of AOC (Assimilable Organic Carbon) is limited at < 10 (without chlorination) or < 50 (with chlorination) μg/l (Van der Kooij, 1992). Therefore, oligotrophic condition may be a key environmental factor in drinking water system to affect the fitness cost of antibiotic resistance. Additionally, several studies have demonstrated that fitness cost can be compensated by second-site mutations without loss of resistance, which is called compensatory mutation (Andersson, 2006; Björkman et al., 2000; Brandis et al., 2012). For instance, it has revealed that the fitness of resistance to rifampin in bacteria is strongly associated with compensatory mutations in the different subunits of RNA polymerase (Hughes and Brandis, 2013). Furthermore, adaptive mutations that confer both antibiotic resistance and an adaptive effect, such as increasing growth rates, can affect the spread of antibiotic resistance (Hershberg, 2017; Wrande et al., 2008). Therefore, the persistence of antibiotic resistance in drinking water system is affected by complicated factors, including the rate of bacteria acquire resistance, the selective pressure for resistant bacteria, or the possibility of mutations compensating for the fitness cost of resistance.

Escherichia coli and *Pseudomonas aeruginosa* are commonly used model microorganisms, two kinds of the ubiquitous and metabolically versatile opportunistic pathogens. In this study, the rifampin-resistant *E. coli* K12 and *P. aeruginosa* PAO1 carrying representative chromosomal mutations, were used as research objects. Our aims were (i) to investigate the effect of carbon source concentrations as low as 0.05 mg/l the total organic carbon (TOC) on the fitness cost of the mutants, (ii) to explore the implying mechanisms on the change of fitness cost under poor nutrient conditions, (iii) to explain the persistence of bacterial antibiotic resistance under poor nutrient conditions like drinking water system from the perspective of fitness cost.

2. Materials and methods

2.1. Strains and growth conditions

A rifampin resistance strain *E. coli* K12 carried a mutant in *rpoB* gene (Ser574phe), was kindly gifted by Professor Junwen Li and Dr. Zhigang Qiu (Institute of Hygiene and Environmental Medicine, China). Additionally, rifampin-resistant *P. aeruginosa* PAO1 used in this study was induced a mutant in *rpoB* gene (Asp521Gly) by bromoacetamide (BAA) disinfection by-products (DBPs) (Lv et al., 2014). Bacteria were routinely activated in LB medium at 37 °C with shaking at 180 rpm overnight.

For growth experiments, *E. coli* K12 was grown in the M9 minimal medium. The following components were sterilized separately and then added (per liter of final M9 minimal medium): 2 ml of 1 M $MgSO_4$, 0.1 ml of 1 M $CaCl_2$, 20 ml 20% glucose (filter sterilized), 200 ml