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Minireview: Bacterial Persistence Mechanisms to Escape Antibiotic Effects

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ABSTRACT

The ability of bacteria to sense and respond to their surrounding allows them to survive and grow under different types of stressful conditions. Resistance, tolerance, and persistence are survival strategies Bacteria possess to overcome stressful environment. The failure of Antibiotic treatments is attributed to these defense strategies. The misclassification of these three phenotypes as a result of poor characterization can result in treatments ineffectiveness. Unlike resistance, which is resulted from evolutions or mutations and external resistant genes acquisitions, that allows bacteria to reproduce under antibiotics, persistence allows a subpopulation of susceptible bacteria to escape antibiotic stress by entering a dormant non-replicative state. Several molecular mechanisms have been reported to implicate bacterial persistence to antibiotics. The two most substantial mechanisms of persistence in bacteria are Toxin- antitoxin system and stringent response. This review summarizes the role of some molecular mechanisms (Stringent response, SOS response, Phosphate metabolism, Sigma factor, and Toxin- antitoxin system) in bacterial persistence.

Keywords: Defense mechanism, persistence, Toxin- Antitoxin, Stringent response.

الخلاصة

قدرة البكتيريا على الإحساس والاستجابة لما حولها تمكنها من البقاء والنمو في ظل أنواع مختلفة من ظروف الاجهاد. المقاومة والتسامح والمثابرة هي استراتيجيات البقاء التي تمتلكها البكتيريا للتغلب على البيئات المجهدة. يُعزى فشل العلاجات بالمضادات الحيوية إلى استراتيجيات الدفاع هذه. يمكن أن يؤدي التصنيف الخاطئ لهذه الأنماط الظاهرية الثلاثة, نتيجتا لسوء التوصيف, إلى عدم فعالية العلاجات. على عكس المقاومة ، التي تنتج عن تطورات أو طفرات واكتساب جينات المقاومة الخارجية ، والتي تسمح للبكتيريا بالتكاثر تحت المضادات الحيوية، تسمح المثابرة لمجموعة سكانية فرعية من البكتيريا الحساسة بالهروب من إجهاد المضادات الحيوية عن طريق الدخول الى حالة السبات الغير تكاثرية. تم الإبلاغ عن العديد من الآليات الجزيئية ذات العلاقة بظاهرة المثابرة ضد المضادات الحيوية في البكتيريا. أهم آليتين للمثابرة والثباتية في البكتيريا هما نظام السموم و ضد السموم والاستجابة الصارمة. تلخص هذه المقالة دور بعض الاليات الجزيئية (الاستجابة الصارمة، استجابة SOS ، استقلاب الفوسفات، عامل سيغما، نظام السم وضد السم) في المثابرة البكتيرية.

الكلمات المفتاحية: اليات المقاومة، المثابرة، السم- ضد السم، الاستجابة الصارمة.

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INTRODUCTION

The excessive and un-appropriate use of antibiotics leads to the appearance of resistant bacteria. The ability of bacteria to escape various antibiotic treatments leads to a worldwide health problem [1]. Resistance is not the only cause of antibiotics treatment failure and infections relapse; persistence is another strategy that bacteria use to evade antibiotics. Persistence was first reported in 1942 [2- 4] when a small subpopulation of *Staphylococcus aureus* persisted the effect of penicillin for a longer time. Later on, studies have reported that antibiotics of different modes induce persistence in both Gram positive and Gram negative pathogenic bacteria [5-7]. Unlike resistance that can be acquired by gene transfer and mutations and can be identified through the high MIC level [8-10], Persistence can be identified by biphasic kill assay and it is a non-heritable phenomenon that enables subpopulation of susceptible bacteria to escape the killing effect of antibiotics by entering dormant state (non-growing state) and undergoing physiological changes [5, 11, 12].

Under antibiotic stress, single cell analyses and flowcytometry revealed that the majority of the un-killed cells in the subpopulation are dormant persister cells [13]. Additionally, persister cells can switch back to normal sensitive bacteria and revive after stress removal and re-suspending in nutrient rich environment [13, 14]. This phenomenon protects bacteria from extinction under harmful stress conditions [15-17]. In vitro studies have been conducted to measure the fraction of persister cells under antibiotic stress and have shown that unlike tolerance, Persister cells constitute a small fraction of the total wild type population with about 0.1% in early exponential phase and up to 1% in biofilm and stationary phase [13, 18, 19]. This fraction can be increased by mutations [20]. Persistence that is generated upon environmental stress signals, like starvation [21], oxidative stress [22], and antibiotics that cause DNA damage [23, 24] is known as triggered persistence [14]. While persistence naturally present in steady state exponential phase is known as spontaneous [14].

Different stress factors and conditions activate different stress responses, like Stringent or SOS responses, which control persistence mechanisms [25, 26]. In *Escherichia coli,* persister cells were formed upon TisB toxin production that reduces ATP production and targets membrane -proton motive force [23, 26]. The toxin production was under the control of SOS response. Dormancy is not the only mechanism of persistence; Toxin- antitoxin system (TA system), low energy production, and reduced metabolism are the major proposed mechanisms underlying persistence [23, 27- 31]. Studies on bacterial persistence suggested that this phenomenon may require all these mechanisms to work simultaneously to ensure bacterial survival. Although Persistence mechanisms are conserved among species, different bacterial species follow different molecular mechanisms underlying persistence. In bacteria like *E. coli* and *mycobacteria,* TA system was linked to persister cells formation, where a high level of persistence was concomitant with overexpression of Toxin [27, 33-36], While persister cells formation in *Staphylococcus*

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aureus was combined with low energy production and was not affected by the deletion of TA system [37].

Despite all the development in the field of bacterial persistence, still little information is available regarding pathways and mechanisms underlying spontaneous persistence. The grate body of evidence regarding the implication of bacterial persistence in antibiotic treatments failure and infections relapse scientists is now focusing on how to eliminate persistence either by investigating compounds with anti-persistence effect or by enhancing antibiotic uptake.

Stress responses control persistence in bacteria

1. Stringent response

In stress environments, the survival of bacteria is mediated by the activation of stress responses which will activate the bacterial persistence mechanisms, like toxins of TA system. Stringent response is under the control of stress alarmone, the guanosine tetra phosphate and pent phosphate (p) ppGpp [38, 39]. In *E. coli and P. aeruginosa*, starvation (amino acid and fatty acid starvation) activates RelA/SpoT homology RSH like protein family that is involved in (p) ppGpp synthesizing and hydrolyzing [38, 39]. The accumulation of high level of (p) ppGpp activates stringent response that causes: inhibition of cell division by regulating DNA replication and metabolic reduction by down regulating the synthesis of tRNA and rRNA, and up regulating stress related genes [40]. A high concentration of ppGpp has been implicated in the high level of persistence in biofilm [41] persistence mediated by ppGpp requires polyP, lon A, and active TA system. Any defect in stress signaling pathway results in defective persistence [42].

2. SOS response

Factors that can cause DNA damage, like antibiotics treatments, extreme pH, and oxidative stress induce SOS response through activate RecA protein [43]. RecA defective bacteria exhibited reduced persister cells fraction after exposure to DNA damage factors [26]. In *E. coli*, the SOS response regulates the TisB/IstR toxin antitoxin loci involved in persister cells formation [23]. This response also involved in persistence resuscitation by repairing the damaged DNA after antibiotic removal. With such observation, scientists suggesting targeting DNA repair system as a method to eliminate persistence [43].

3. Phosphate metabolism

One of the genes that have been reported to be implicated in bacterial persistence is the *PhoU* [44]. PhoU, a metal binding protein, is part of the phosphate regulon. This protein is a global regulator that negatively regulates many cellular metabolic processes including phosphate metabolism [45]. Compared to wild type, bacterial cells with deleted or mutated un functional *PhoU* gene, exhibited upregulation of genes involved in phosphate metabolism and energy

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production, and increased susceptibility to diverse stress factors with defect in persister formation [44]. Studying persistence in *E. coli* revealed that the level of phosphate was elevated after exposing to stress and that high level of phosphate led to growth- arresting toxin, HipA, liberation through the degradation of Hip B anti-toxin [27, 38]. This study concluded that metabolism of phosphate is implicated in persistence mechanisms of bacteria to stress factors [44].

4. Sigma factor RpoS

Activation of general stress response sigma factor by several stress factors, like, ppGpp, extreme pH, and oxidative stress results in genome instability [46, 47]. This factor induces mutations into DNA through inducing the expression of error –porn DNA polymerase IV independent on SOS pathway and suppressing the methyl directed mismatch repair pathway [48]. Beyond its role in general stress response, Sigma factor has been reported to be implicated in persister cell formation [49, 50]. Deletion of sigma factor increased *E. coli* persistence to Norflaxacin and Ampicillin, while an increase in bacterial susceptibility and defect persistence to gentamicin was observed [51].

5. Toxin-antitoxin system

TA systems are genetic modules that are highly abundant in bacterial chromosome, acquired through horizontal gene transfer, and plasmid [52- 54]. TA systems involved in bacterial persistence, post- segregation killing, and biofilms [55, 56]. This system comprised two elements within an operon, a toxin gene and antitoxin gene [57]. Toxin gene is located downstream of antitoxin gene and codes for a stable intercellular toxic protein or RNA, while antitoxin gene codes for unstable antitoxin that can be RNA or protein [58- 61]. TA modules are classified into eight classes (I- VIII). Toxins in type I-VII are proteins, where in type VIII toxins are small RNA. While antitoxins are non - coding RNA in type I, III, and VIII and toxins are proteins in the remaining TA Types [62]. Among these eight classified TA modules, type II is the most well studied one. Under normal conditions, the promoter of TA operon is negatively controlled by antitoxin protein and the toxicity of toxin is counteracted by direct or indirect interaction with its antitoxin [63, 64]. Under stress condition, three possible pathways have been proposed by which TA modules contribute in persister cells formation in *E. coli*. Under stress, the unstable type II antitoxins (mRNA endonucleases) are degraded by active ATP-dependent proteases pathway, Lon protease, CliPX, and CliPA [65, 66]. While type I antitoxins (antisense RNAs) are degraded by SOS response induction pathway, and Obg activation and ppGpp accumulation pathway [67, 68]. Antitoxins degradation results in toxin liberation. Once liberated and crossed certain threshold, toxin arrests cell growth by inhibiting cellular processes through targeting cell wall synthesis, membrane integrity, DNA replication, and translation [68- 71], resulting in cells entering non-growing dormant state and persister cells formation [72]. Transcriptomic analysis of persistence cells in *M. tuberculosis* and *E. coli* reveal an up regulation in TA modules. Studying persistence in *pseudomonas* concludes that number of persister cells correlates with the number of TA modules

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within the isolates [73]. Reduced level of persistence was observed after deletion of 10 Toxin-antitoxin loci in *E. coli* [74]. TA systems have been implicaten in persister cells formation in different pathogenic bacteria, like *Salmonella. Typhimurium*, and *M. tuberculosis.*

Conclusion

The phenomenon of persistence has been disregarded and persister cells have been misclassified. The increased incidence of persister cells infections and antibiotic treatment failure necessitated the need to investigate the mechanisms underlie the formation of persister cells and the resuscitation after stress removal. Multiple complex mechanisms are being used by bacteria to survive the stressful environments. Studying formation and resuscitation mechanisms can highlight pathways and factors that can be exploiting as a target to eliminate persisters and help in developing anti-persister therapeutics.

Conflict of interests.

There are non-conflicts of interest.

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