2025,Volume 2 , Issue 6 , PSIJK (48-52)

Pure Sciences International Journal of Kerbala

Environmental Stress Response and Toxin-Antitoxin Systems Influence bacterial Persister Formation

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P A P E R I N F O

Received: 14 February 2025
Accepted: 24 February 2025
Published: 30 June 2025

Keywords:
Toxin–antitoxin, Persister Cells, Antitoxin, Intracellular

Abstract

Bacterial respond to extreme environment stress let them to survive and grow. One bacterial strategy to survive is persister state. Persistence enables a subset of small cells population to become a dormant, non-replicative state and avoid stress. Bacterial persister has been linked to a number of biological processes. The toxin-antitoxin system(TA) is one of the most significant persistence mechanisms in bacteria. Thus, it has been determined that bacterial toxin–antitoxin complexes are a suitable target for treatment. Through two gene, protein is encoded. toxin that targets a vital cellular function besides an antitoxin that suppresses the activation of the toxin. Toxin expression can have a Bactericide effect; they could be regarded as "intracellular molecular bombs" that may cause their host cells to be eliminated. Therefore, current review discusses the functions of TA modules in development persister cell and the effects of environmental stress on persister cells.

1. INTRODUCTION

More effective antimicrobial medicines. For microorganisms to survive and compete in harsh conditions, Metabolic changes are necessary, for example, when antibiotics are present or the host is sick. TA Systems that contain a toxin that has the ability to modify metabolism and a nearby antitoxin that counteracts it are important components of the bacterial stress defense. [1] A toxin section and its corresponding antitoxin section make up the ubiquitous gene of toxin-antitoxin modules, which are loci found in bacteria. Antitoxin neutralizes the toxin's toxicity under normal physiological conditions. A large number of the toxins are proteinaceous and interfere with DNA replication or translation. However, antitoxins immediate interaction, mostly neutralizes its cognate toxin., but also aids in the organization of the TA module with the assistance of other signaling elements. TAs are several groups the antitoxin is categorized based on its molecular bases and how it interacts with its cognate toxin [2]. These "two" gene loci are arranged in operons, and the two (antitoxin and toxin–antitoxin complexes) closely control their expression [1]. Persistence is condition that arises when tiny subpopulations of bacterial cells momentarily exhibit a recognizable, also not inherited and stress-resistant phenotype [3]. Physiological programs conferring increasing fitness, also, are one of the essential properties of a few metabolic states known as persister that may rise as a result of toxin activity [4]. One method of preventing phage infection is Persistence, bacteriostatic efficacy inhibiting the making of mature visions during bacterial cloning [5]. However, absent plasmids TA-encoding, the accumulation of stable toxins causes the intracellular level of antitoxins to rapidly drop, which ultimately results in significant metabolic restriction [6]. Because TAs lessen the competitiveness of cells that did not inherit TA-encoded plasmids, they generate a significant selective pressure for their maintenance [7]. The current review discusses the effects of environmental stress on persister cells and the functions of TA modules in formation of persisted cell.

2. BACTERIAL PERSISTENCE

2.1 Bacterial persistence Ancient History

Staphylococcus aureus was found to exhibit persister cells. First, when discovered that penicillin could break down ninety-nine present of the target bacteria by Hobby and colleagues (1942). "One in a million" according to Bigger that *Staphlococcus aureus* cells are still alive during exposure to penicillin. Two years after this discovery, he called these cells persisters

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[8]. Additionally, the subpopulation of bacterial cells survived while the member of alive bacteria was regrown and subjected to antibiotics [8]. Before making this discovery, researchers believed that the populations of bacteria in planktonic cultures were uniform [9]. Bacterial phenotypes were recognized to be different within the same population because of the development and accessibility of instruments to study bacterial cell physiology [10]. Interestingly, a different bacterial phenotype known as "viable but non-culturable" (VBNC) was found by Xu and associates in 1982. Where the total number of cells remined unchanged in direct "fluorescent microscopic analysis", that is a subpopulation stayed viable but could not grow on the offered medium, in the (culture-based method), which including plate counting and most probable number determination. Escherichia coli was exposed to stressful situations (5–25% NaC1) during 2 weeks showed a rapid decrease in the total bacterial count when E. coli was exposed to stressful conditions (5–25% NaC1) for two weeks [11]. The recognizing of living cells that might be cultivated also retrieved under normal growing conditions. This served as foundation for experimental microbiology prior to this discovery, which was in line with ideas put forth by Robert Koch more than 150 years earlier [11].

2.2. VBNC Cells and Persister Cells

Persister & VBNC are cells found in a dormant condition, where a non-growing condition of hibernation the cells have been encouraged to enter in. The extent to which cells are led to enter the persisted as well as VBNC condition is thought to be largely determined by the length and severity of the relevant stress exposure [12]. Furthermore, three distinctive phases: initiation, resting, and revival have been classified to bacterial dormancy by Lennon & Jones (2011)

2.2.1 Initiation phase

Changes in environmental state the bacteria enter a persisted stage, for example temperature [13], osmotic pressure, oxygen concentration, pH level outside the optimal growth state. Nutrient starvation can be one of the factors that causes microorganisms to go into dormancy, primarily in the natural environment, which is an entry into the initiation phase. [14]

2.2.2 Resting phase

Microorganisms go through a rest period after commencement. During this phase, cells react to adverse circumstances by changing their metabolic processes and, on occasion, by changing their phenotype or structure, cell size decreases, these phenotypic changes include changes to the 18 concentrations of proteins, lipids, fatty acids, and nucleic acids; modifications to general stoichiometry; and rises in the quantities of survival-related storage compounds. Signoretto and associates (2002) monitored the *E. coli* during stress exposure entry into a persister state, there has been evidence that persister cells contain less ribosomes than non-persisted cells [15].

2.2.3 Revival phase

Bacterial strains differ in how long the resting stage lasts, ranging from a few days to years. However, as soon as favorable conditions and reduced stress allow for ongoing growth, cells invariably enter the revival stage. The first recorded instance of revival was when VBNC Salmonella cells were reported to have returned in 1984 by Roszak and associates, when bacterial cells emerge from hibernation and regain the capacity to establish colonies on nutritive media, they undergo intricate cellular transformations. Restoring metabolic competence, lowering oxidative stress, and controlling toxin-antitoxin ratios are the goals of these modifications [12].

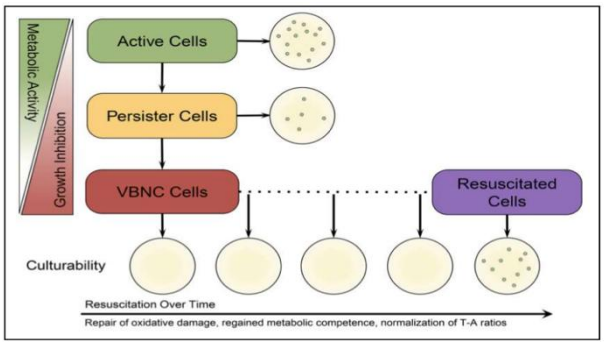

Figure 1. The VBNC cell concept and the encouragement of persister [12].

3. CLASSIFICATION of TOXIN ANTITOXIN SYSTEM

The TAs can be classified according to antitoxins interact with toxins or how antitoxins inhibit toxins [16]. The toxins are short RNA in type VIII TA modules, while proteins within type I to type VII modules [17]. In the case of type, I, type III and type VIII TA modules, antitoxins are small noncoding RNAs while in type II, type IV, type V, type VI and type VII TA modules are small proteins, **Table 1** [18].

4. FORMING PERSISTER CELL and THE ROLES of TA MODULES

Antibiotic-related research revealed that a tiny percentage of cells remained latent and exhibited a mechanism distinct from conventional antibiotic resistance [19]. "Persistence" refers to the small, genetically homogeneous cells' capacity to withstand stress by briefly becoming a dormant state. The primary traits of persistence are decreased cell growth and metabolic activity [20]. This "persister cells" consider phenotypic variants but not Mutants in genes [21]. The important roles that TA modules play in persister cell development have been unequivocally established. [22]. remains unknown TA systems are crucial for the persistence of bacteria. Other investigations confirm the importance of "Toxin Antitoxin systems" for persistence of bacteria [23]. in "dinJ/yafQ" Toxin Antioxin module has been implicated in cephalosporin and aminoglycoside antibiotic tolerance in another investigation [24]. According to a study on uropathogenic strains of E. coli, there is more evidence linking "type II" TA modules to the persistence of bacteria [25]. Before being used as antimicrobial targets, it is critical to ascertain if TA activation affects persister production because the overall mechanism of persistence is yet unknown. It has been demonstrated that persistence in E. coli is mediated by TA systems other than the "type II" TA modules. Although employing TAs as antibacterial targets may result in persistence, these systems can be also used in anti persister tactics. [26]. "Conlon and colleagues" [27] think about how a substance that can target dormant cells can destroy the persister cell. Protease "ClpP" is activated by acyldepsipeptide antibiotics (ADEP4) [28] then demonstrated that by damaging more than four hundred protein, that force the cells to (self-digest), thus becomes totally non-nominated and kills persisters [29]. Bacterial genomes have been shown to include over 90 Type (II) Toxin Antitoxin modules. In addition, it had been demonstrated that these modules are crucial in regulating persisted cell development [2]. TA modules are thought to play a role in persister cell production, but the exact mechanisms are still unknown. [30] Furthermore, HipA-hipB, the persisted phenomenon, and the first TA module are among the most well-known type-II TA modules. [31] It had been demonstrated that a rise in persistence in Escheria coli "K-12" is caused by the hipA and hipB genes. Both genes are found in the hip operon; a very stable toxin are encoded by the "hipA" gene, while the unstable antitoxin encoded by "hipB" gene. HipA forms the stable, non-toxic complex HipBA by forming a strong bond with hipB. However, when they attach to the DNAThrough an indirect method, HipB and maybe HipA can inhibit the hip operon's transcriptional activity. [32], colleagues & Rotem discovered, when the "hipB" (antitoxin gene) was cancelled in the Escherichia coli, the cells entered the VBNC condition, which stops the cells from forming colonies on medium, as a result of "hipA" overexpression [33]. HipA's toxicity and its direct and indirect regulatory effects on the expression of other proteins have been connected to its function in persister cell development. In 2018, Goormaghtigh & colleagues investigated how the presence of ampicillin and ofloxacin affected the generation of persisters after deleting ten Type-II TA modules. in comparison to the "wild-type" strain, they discovered that the deletion of 10 TA modules had no effect on the development of persister cells, indicating that Toxin A ntitoxin modules donot promote formation persister cell [34]. In summary, TA modules may contribute to persister cell formation; however, a thorough understanding is needed because it is difficult to analyze the connections between persistence and TA modules because of the complexity and diversity of TA modules as well as the ways in which

TABLE 1. Biological characteristics of types I to VIII toxin-antitoxin (TA) systems

TA types	Toxin	Antitoxin	Examples	Mode of action of antitoxin
I	Protein	RNA	*hok*/*sok*; SymE/SymR	Antitoxin binds to toxin mRNAs, thereby inhibiting toxin expression
II	Protein	Protein	*ccdA*/*ccdB*; *higB*/*higA*	Antitoxin and toxin form a protein–protein complex to neutralize its toxicity
III	Protein	RNA	*toxI*/ToxN; *antiQ*/AbiQ	Antitoxin binds directly to toxin, thereby inhibiting toxicity
IV	Protein	Protein	CbeA/CbtA; AbiEi/AbiEii	Antitoxin binds to the targets of toxin, thereby protecting such targets
V	Protein	Protein	GhoT/GhoS	Antitoxin is an RNase that specifically cleaves toxin mRNA
VI	Protein	Protein	SocA/SocB	Antitoxin acts as a proteolytic adaptor that promotes toxin degradation
VII	Protein	Protein	Hha/TomB; TglT/TakA	Antitoxin is an enzyme that neutralizes toxin via post-translational modification
VIII	RNA	RNA	SdsR/RyeA; CreT/CreA	RyeA and SdsR form a duplex that can be degraded; CreA forms a complex with Cascade and guide it to repress CreT transcription

they are expressed in bacteria. There is a knowledge vacuum about the potential effects of the various types of TA systems on bacterial persistence because most research has focused on Type-II TA.

5. EFFECT of ENVIRONMENTAL STRESS on PERSISTER CELL

5.1 Thermal Impact

Increase Antitoxin Degradation to TA Transcription. Several conditions that promote TA transcription heat -shock donot halt "protein synthesis", in contrast to chlor and SHX medications. The stress accelerating the breakdown of antitoxins must be the cause of the observed transcriptional activation in these circumstances. They investigated antitoxin stability after a transition from 30C to 45C using pulse chase analysis in order to test this theory. In fact, in line with earlier research, the heat -shock markedly accelerated the amount of "YefM" breakdown. [35]. Thus, heat shock does not inhibit translation, or enhanced breakdown of "YefM" & the ensuing alleviation of autorepression stimulate antitoxin transcription, which in turn should increase the quantities of antitoxin proteins.

5.2 Osmosis

One of fundamental part of cell functions is "Osmosis"; osmotic pressure influences the rate of cell growth, turgor and transport phenomena across the cell & its surroundings [36]**.** Study the effect of osmolytes & pH in *E. coli* strain "MG1655" which induce persistence state. The "LB broth" was used (no NaCl) in cell cultures to avoid any further osmotic effects that may occur. A study has demonstrated that the absence of "NaCl (1%)" from the standard LB broth has no significant effect on cell viability, cell growing average.

5.3 Alkaline and acidity

According to [37], they use a plasmid- encoded "fluorescent pH sensor", microfluidics, and time-lapse imaging to assess the intracellular pH dynamics of susceptible Escherichia coli, VBNC, and persister cells after being treated with ampicillin. They discovered that low degree of intracellular "pH" in persisters cell compared to cells of susceptible &VBNC. even before giving antibiotics. Tryptophanase activity, which is encoded by tnaA, is linked to the reported changes in pH control in persister Escherichia. coli cells, according to later research into the molecular mechanisms behind these variations. In fact, we found that the intracellular pH did not differ between susceptible, "VBNC", persister *E.coli* cells on DtnaA strain. In "DtnaA" strain lowered important "pH" homeostasis ways in addition to tryptophan metabolism, according to whole-genome transcriptome study. These pathways included the reaction to various carboxylic acid catabolism processes, oxidation reduction**,** and pH, in contrast to expression levels in the parental strain.

6.CONCLUSION

There is more than evidence that refer to contribution of the environmental stress and TA system in formation of persister cell. As a result, the bacterial cell will experience a transitory phenotypic transformation that allows surviving via a persistence occurrence in the presence of stress that led to limited availability and accessibility to the 'cellular target'.

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Arabic Abstract

تستجيب البكتيريا للإجهادات البيئية المختلفة مما يساعدها على البقاء والنمو. إحدى الاستراتيجيات التي تعتمدها البكتيريا للبقاء هي حالة "المثابرة". هذه المثابرة تمكّن مجموعة من خلايا البكتيريا الصغيرة من الدخول في حالة خاملة غير تكاثرية مما يساعدها على تجنب الإجهاد. تم ربط البكتيريا المثابرة بعدد من العمليات البيولوجية. ويعد نظام السم-المضاد أحد أهم آليات المثابرة في البكتيريا. ولذلك، تم تحديد أن انظمة السم-المضاد البكتيرية تعد هدفًا مناسبًا للعلاج. يحتوي النظام على جينين يشفران بروتينا، السم الذي يستهدف وظيفة خلوية أساسية، والمضاد الذي يثبط نشاط السم. يمكن أن يؤدي التعبير عن السم إلى تأثيرات قاتلة على البكتيريا؛ حيث يمكن اعتبارها "قنابل جزيئية داخل الخلايا" قد تؤدي إلى القضاء على الخلايا المضيفة. لذلك، تناقش المراجعة الحالية وظائف انظمة السم-المضاد في تكوين خلايا المثابرة وتأثيرات الإجهاد البيئي على خلايا المثابرة.