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Efficacy of Zinc Pyrithione as A Novel Anti-Bacterial Coating Agent

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Abstract

Zinc pyrithione (ZPT) is used to prevent microbial degradation and deterioration of manufacturing starting materials such as plastics, polymers, and latexes. The main objective of this study was to evaluate the anti-bacterial properties of ZPT. Currently, there is insufficient data on the effect of ZPT on viability of commonly encountered bacterial pathogens. We tested the efficacy of ZPT manufactured in the form of film rolls as an anti-bacterial protective layer by using the ASTM–recommended protocol on growth of *Enterococcus faecalis* and *Escherichia coli.* The bacterial cultures were added to three materials provided by Cao Inc. containing either the base with no active ingredient, ZPT-A, or different amounts of active ingredient, ZPT-B (2.5%) and ZPT-C (5%). Following overnight incubation, bacterial growth was assessed by counting their colony forming units (CFUs). Growth of both *E. faecalis* and *E. coli* were strongly inhibited by ZPT-B and ZPT-C relative to growth on the control ZPT-A. Inhibition of *E. faecalis* was close to complete by ZPT-B and ZPT-C while *E. coli* growth was inhibited by greater than 95% in a dose dependent manner. This is the first report of zinc pyrithione, here in the form of thin film, inhibiting growth of common bacterial pathogens. ZPT rolls therefore show promise as an effective antibacterial

layer for use as a protective barrier, for example on door handles and counters, from clinical to global public health settings.

Keywords
Antibacterial; metal chelating; zinc pyrithione; mechanism

1. Introduction

1.1 Zinc Pyrithione (ZPT)

Zinc pyrithione (ZPT) is a zinc chelate of hydroxyl-pyrithione and exists in the monomeric form as two pyridine rings bound to a central zinc atom by bonds between the zinc atom and the sulphur and oxygen molecules of the pyridine ring structure, with a molecular weight of 317.7 g/mol (Figure 1) [1]. ZPT may also exist as a dimer. Because of its metal chelating properties, limited permeability and sparse water solubility, ZPT is ideal for deposition on the skin from rinse-off formulations, particularly in lipid rich regions such as the scalp. It has been widely used in the shampoo and cosmetic industry as an antifungal agent and preservative [2]. Its safety as a consumer product is well documented [3].

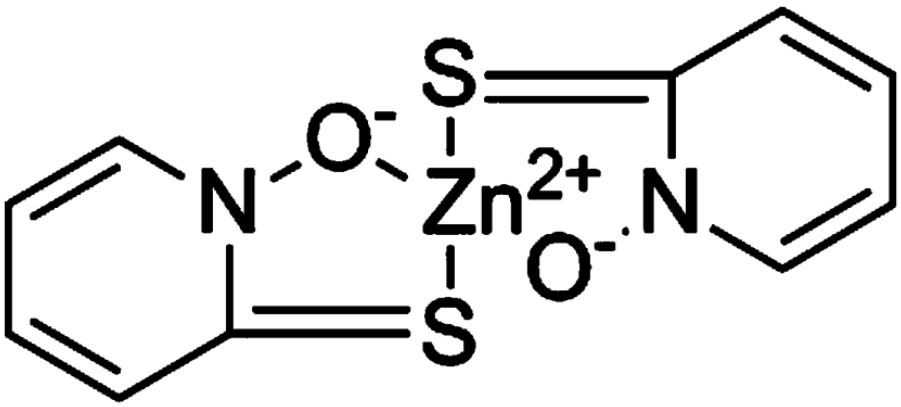


Figure 1 Zinc pyrithione (ZPT) is a zinc chelate of hydroxyl-pyrithione and exists in the monomeric form as two pyridine rings bound to a central zinc atom by bonds between the zinc atom and the sulphur and oxygen molecules of the pyridine ring structure.

Aside from the cosmetic industry, ZPT is used in many applications to prevent microbial degradation and deterioration of manufacturing starting materials such as in plastics, polymers, and latexes, and in a wide range of finished substances made from these starting materials. The chemical acts to prevent the growth of bacteria, fungi, mildew, and algae that can cause various types of deterioration such as discoloration, staining, odors, etc. Our goal was to evaluate ZPT in its potential antibacterial application from clinical to global public health settings.

From a clinical viewpoint, there have been reports of ZPT halting cancer cell proliferation [4]. Studies on anticancer activities in oral cancer cells identified ZPT in small molecule screens targeting inhibition of the ubiquitin-proteasome complex, a novel strategy for treating human cancer [5]. Additionally, ZPT was shown to exhibit cytotoxic effects against various cancer cell lines *in vitro* including selective killing of bone marrow cells from leukemia patients *ex vivo*, and efficiently inhibiting the growth of lung adenocarcinoma cancer cell xenografts in nude mice [5]. These studies have hence identified zinc pyrithione, a pharmacological agent approved by the Food and Drug Administration, as a proteasomal DUB (deubiquitylating enzyme) inhibitor with potential antitumor properties. A recent patent also cited application of ZPT in preparation of a drug used for treatment of lung cancer. ZPT is proposed to inhibit proliferation of lung cancer cells by multiple mechanisms such as inducing tumor cell apoptosis, inhibiting tumor cell cycle, and inhibiting tumor migration, thereby achieving the effect of inhibiting tumor growth or removing tumor [5].

Information on ZPT's application in dentistry is limited. One abstract reported the antibacterial effect of ZPT incorporated in a dental bonding agent applied on dentin disks [6]. A zone of inhibition test was used to compare the antibacterial efficacy of 1, 2 and 4% ZPT. Zones of inhibition were observed which were dose and time dependent. The testing was performed for 1 week, 1 month, 3 months and 6 months. The diameter of the zone diminished along the timeline with higher dose showing larger inhibition zones at earlier time points. ZPT displayed varying efficacy against different species of bacteria. It was most effective against *Streptococcus mutans, Streptococcus gordonii and Actinomyces viscocsis* and was less effective against *Lactobaccilus casei, Porphyromonas gingivalis* and *Fusobacterium nucleatum*, suggesting ZPT exerted higher inhibition on Gram-positive bacteria, which possess a thick peptidoglycan layer on the outer surface relative to Gram-negative bacteria.

Currently, there is a lack of data on the effect of ZPT against commonly encountered bacterial pathogens as well as on the long-term effect of ZPT on the barrier surfaces. Our *objective* was to investigate the efficacy of ZPT as an antibacterial surface, with the aim of using it as a plastic barrier such as on door handles in public areas (Figure 2). This type of cost-effective, globally available safe option with minimal side effects and simple application will aid in the primary goal of preventing the spread of pathogens, especially in a clinical setting. While we are reporting our results on two common bacterial pathogens, pilot studies on viral pathogens have also been conducted and will be reported separately, keeping in mind new preventive options against COVID-SARS 2 virus are urgently desired.

Figure 2 ZPT coated film can be utilized as a cost-effective and safe option with minimal side effects and simple application to fight off the COVID Pandemic.

Based on our data, this is the first report of a metal-ligand complex inhibiting growth of common bacterial pathogens.

2. Materials and Methods

2.1 Bacterial Strains and Growth Conditions

The two bacterial strains tested, *E. faecalis* from American Type Culture Collection (ATCC) strain #19433 and *E. coli* JM83, were grown on TYK agar plates (30 g/liter Trypticase soy broth, 5 g/liter yeast extract, and 1 mg/liter vitamin K3) [7]. Single colonies were inoculated into liquid TYK broth and grown for 18-24 hours in a 37°C shaker.

2.2 Usage of ASTM Protocol to Determine the Effect of ZPT on Bacterial Viability

The ASTM designated E2180–18 method "Standard Test Method for Determining the Activity of Incorporated Antimicrobial Agent(s) In Polymeric or Hydrophobic Materials" was used [8] with minor modifications as described below.

Overall, a pre-determined amount of bacterial culture was added to the three ZPT materials provided by Cao Group (West Jordan, UT) in the form of thin film provided as rolls. The three materials were [1] ZPT-A containing the base with no active ingredient, which served as a baseline negative control, [2] ZPT-B containing 2.5% zinc pyrithione and [3] ZPT-C, containing 5% zinc pyrithione. These were incubated overnight at room temperature, removed by sonication and vortexing and plated after serial dilution for the purpose of counting colony forming units (CFU).

All experiments were conducted three separate times for each bacterium, each with triplicate readings.

The three materials were first rolled out and the sticky side, opposite from the active side, was placed on transparency paper to attach it to a stiff surface and prevent wrinkling. This bi-layer was cut with a sharp scissor into 3.0 × 3.0 cm squares, placed in a sterile 15 × 100 mm petri dish, and sterilized by exposing to UV radiation using a hand-held 'UV-gun' (UVC 253.7 nm, power = 80 W, intensity = 65 mW/cm^2) for 10 sec.

Overnight bacterial cultures were quantitated using a spectrophotometer. The concentration for *E. faecalis* and *E. coli* were in the range of 1-5 × 10^9 cells/ml. Each culture was diluted serially 1:10 three times, the first two times in 1 ml PBS and the third time in 10 mls agar slurry to give a final concentration of 1-5 × 10^6 bacterial cells/mL. The agar slurry was prepared by adding 0.85 g NaCl and 0.3 g agar-agar in 100 mL water and heated while stirring on a hot plate until the agar dissolved. The agar slurry was sterilized by autoclaving and equilibrated in a 65°C hot air oven.

Bacterial slurry was next added to the ZPT square surfaces. Since the addition of saline did not facilitate spread of the bacterial slurry on the hydrophobic ZPT surfaces, and swabbing the ZPT-A control with 0.1% Tween-20, a surfactant, facilitated spread but led to growth inhibition of *E. coli*, we added 0.5 mL of agar bacterial slurry to the test and control ZPT samples in the form of drops directly over the entire surface (see picture, Figure 3, left). The surface area covered was ~40% of each square.

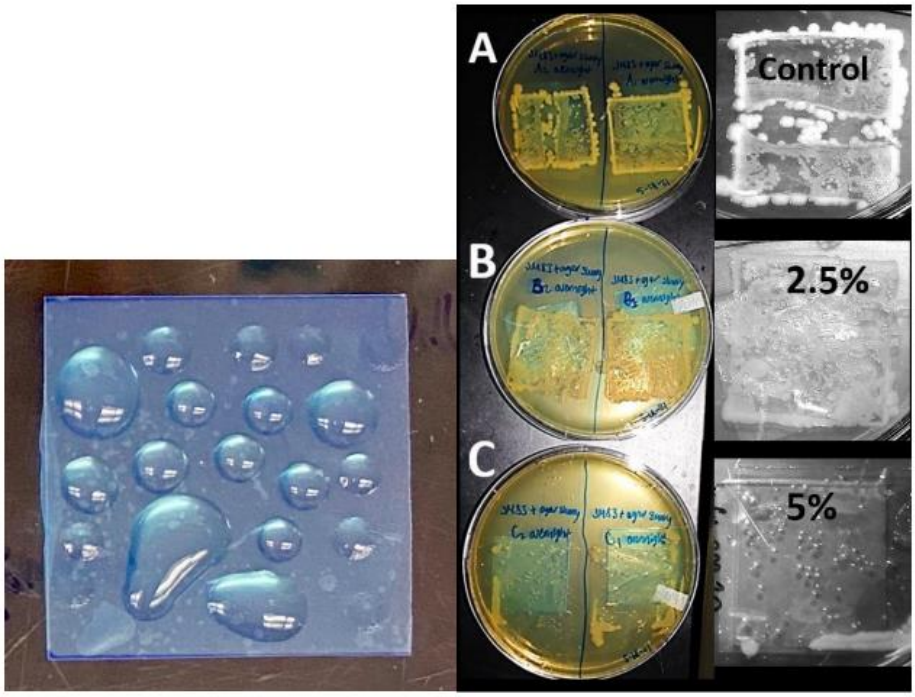


Figure 3 Antibacterial testing conforming to ASTM designated E2180–18 method. Left: 500 µl of bacterial culture-agar slurry mix was added in the form of drops on each ZPT surface. Right: The ZPT-A control surface revealed bacerial growth whereas ZPT-B and ZPT-C, containing 2.5% and 5% ZPT respectively, showed varying degrees of growth inhibition after the ZPT surfaces were implanted on agar plates following removal of the agar slurry-bacterial mix by vortexing and sonication.

The bacterial-agar slurry was allowed to solidify in sterile petri plates and incubated at room temperature, which served as the test temperature. Following contact time of 18-24 h, the control and treated samples were aseptically removed from the petri dishes to tubes containing 10 ml sterile PBS with the intent of forming an initial 1:20 (500 µl in 10 mls) dilution of the original inoculum. The three tubes containing the recovered test samples were placed in a non-cavitating sonic bath and sonicated for 1 min. Sonication was both preceded and followed by vigorous mechanical vortexing for 30 seconds. Vortexing and sonication together facilitated the release of the agar slurry from the sample. We imprinted the square ZPT materials onto TYK agar following sonication and vortexing to determine release efficiency of the inoculum from the treated surface.

Serial dilutions were performed of the 10 ml PBS solution containing released bacteria. For ZPT-A control, 1:10 serial dilutions were performed three times while one 1:10 dilution was performed for ZPT-B and ZPT-C.

The serial dilutions were spread plated, 100 µl each, on TYHK agar and incubated at 37°C overnight. Colony numbers (CFUs) were counted and recorded the next day. The average number of CFU and standard deviations were calculated for each experiment. Percent reduction in growth was calculated as described in the ASTM protocol.

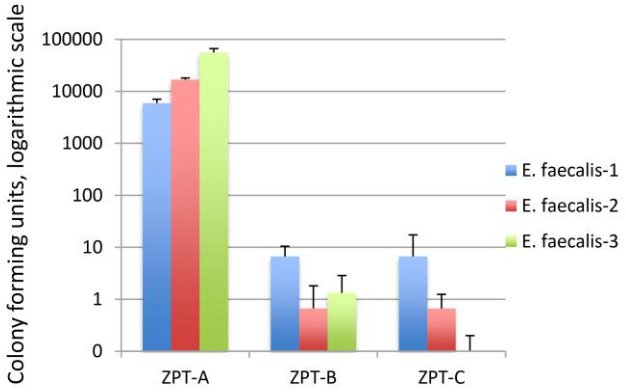
2.3 Inductively Coupled Plasma Analysis

We employed Inductively Coupled Plasma analysis (ICP) to confirm the presence of zinc and other trace elements within the samples. The coded plastic sheets were sent to an independent laboratory where the samples were liquefied and digested with nitric acid. A plasma torch was utilized to vaporize fine droplets of the sample. We focused on zinc as our major element but nearly all trace amounts elements within a sample were identified.

3. Results

3.1 Inhibition of E. faecalis by ZPT

As seen in Figure 4, growth of *E. faecalis* was strongly inhibited by both ZPT-B and ZPT-C materials, containing 2.5% and 5% zinc pyrithione respectively, relative to the control ZPT-A. Colony forming units (CFUs) from triplicate readings were plotted for each of three experiments. Percent reduction relative to ZPT-A is shown in the Table within Figure 4. Inhibition was close to complete for *E. faecalis* by both ZPT-B and ZPT-C, with <10 CFU compared to 10^3-10^4 CFU after growth on the control ZPT-A. Since the drop in CFU was severe, the data was plotted on logarithmic scale.



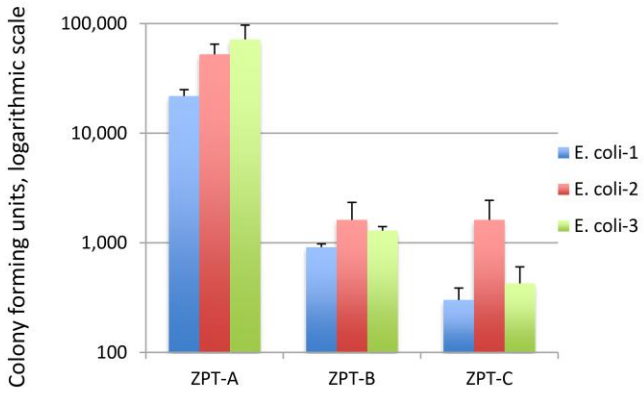
		Avg. # CFU	% survival
E. faecalis- Expt. 1	ZPT-A	5933.33	
	ZPT-B	6.67	0.1%
	ZPT-C	6.67	0.1%
E. faecalis- Expt. 2	ZPT-A	16766.67	
	ZPT-B	0.67	0.004%
	ZPT-C	0.67	0.004%
E. faecalis- Expt. 3	ZPT-A	55933.71	
	ZPT-B	1.33	0.002%
	ZPT-C	0	0%

Figure 4 Inhibition of *E. faecalis* by ZPT-B and ZPT-C. The y-axis shows average colony forming units (CFUs) of three experiments done in triplicate after growth on ZPT surfaces for 24 h, plotted on a logarithmic scale. The values and percent survival are shown in the table on the right.

A 10-100 fold difference in CFU count after growth on the ZPT-A control between experiments reflects variation in growth in combination with extent of removal of bacteria from ZPT surfaces. In each round, the three materials were treated in an identical manner, with the same starter culture and sonicated simultaneously for removal of bacteria. Degree of overnight growth of the starter culture could contribute to the variation as well.

3.2 Inhibition of E. coli by ZPT

ZPT-B and ZPT-C inhibited *E. coli* strongly as well, though not as completely as observed for *E. faecalis*. ZPT-C demonstrated a trend towards greater inhibition than ZPT-B as seen by the fewer number of CFUs in two out of three experiments (Figure 5).



		Avg. # CFU	% survival
E. coli- Expt. 1	ZPT-A	21833.33	
	ZPT-B	912.00	4.17%
	ZPT-C	301.33	1.38%
E. coli- Expt. 2	ZPT-A	52400.00	
	ZPT-B	1620.00	3.09%
	ZPT-C	1620.00	3.09%
E. coli- Expt. 3	ZPT-A	71733.33	
	ZPT-B	1293.33	1.8%
	ZPT-C	426.67	0.59%

Figure 5 Inhibition of *E. coli* by ZPT-B and ZPT-C. The y-axis shows average colony forming units (CFUs) after growth on ZPT surfaces for 24 h, plotted on a logarithmic scale. The values and percent survival are shown in the table on the right.

Squares that were imprinted on TYK agar following removal of bacteria by sonication/vortexing revealed that all bacteria were not removed, but the amount that remained was proportional to the CFU count. Visible growth was seen on ZPT-A after imprinting for 24 hours, but close to no growth was seen after ZPT-B and ZPT-C squares were imprinted on agar plates (Figure 3, right).

3.3 ICP Analysis

ICP, a powerful chemical analysis method, confirmed the relative zinc concentrations in Groups 2.5% and 5%. ICP confirmed that the ratio of Zn presence in 5% ZPT is double that of 2.5% (Table 1). Detectable metal elements include calcium, copper, magnesium, sodium, and sulphur. The presence of sulphur is to be expected since sulphur is a structural component of ZPT. Other detected trace metal elements may be structural components of the plastic film manufacturing starting materials.

Table 1 The amount of Zn^{2+} in 5% ZPT is almost double that of 2.5%. It is also noted that 0% control without ZPT loading also contains a trace amount of Zn^{2+}, probably as a structural element in the plastic film.

	% Zinc Pyrithione	Zn mg/L	Zn mg/Kg	Zn^{2+} %	Mean %
		0.859	211	0.021	0.02
Group A	0	0.813	200	0.020	
		0.81	199	0.019	
		6.72	1512	0.151	0.153
Group B	2.5	6.74	1516	0.152	
		6.95	1564	0.156	
		10.41	2680	0.27	0.263
Group C	5	10.01	2577	0.26	
		10.12	2605	0.26	
QC run		5.064			
QC true		5.000			

4. Discussion

Prior to the ASTM protocol, we performed pilot studies using zone of inhibition as a measure of ZPT disk diffusion, which confirmed the antibacterial effect of ZPT on *E coli*, *E. faecalis* and *Porphyromonas gingivalis.* We decided to use the ASTM protocol to better quantitate bacterial growth inhibition and follow an established testing method.

E. coli and *E. faecalis* were the commonly seen pathogens targeted in the current study on ZPT. *E. coli* can travel easily from person to person, especially when infected people don't wash their hands properly. *E. faecalis* infections spread from person to person often in hospital settings through patients exhibiting reduced immunity.

Our results demonstrated that Inhibition of *E. faecalis* by ZPT is more effective compared to *E. coli.* This is consistent with a previous report indicating ZPT has a greater potency for suppressing growth of Gram-positive cocci such as Streptococcus sp. than Gram-negative bacilli such as *P. gingivalis* and *F. nucleatum*. This is contrary to another report that demonstrated MIC-50 (minimal inhibitory concentration for inhibiting growth by 50%) is 10 ppm of ZPT for *E. coli* and 20 ppm for Enterobacter species, e.g. *E. faecalis* [9]. Regardless, this narrow antimicrobial range of ZPT is likely sufficient to include many pathogenic, opportunistic and saprophytic organisms. Inhibitory concentrations are often achieved at 40 ppm or less for numerous pathogenic species [9].

Aside from the antibacterial efficacy, ZPT has been shown to have persistence characteristics [9]. A previous study revealed that 4% ZPT incorporation in a dentin bonding agent maintained antibacterial properties even after 6 months [6]. Using an “expanded floral test”, which measures the ability of an agent to suppress a dense and flourishing population of organisms, ZPT was shown to have a prolonged suppression of the skin bacterial flora obtained at 24-and 48-hour sample points [9]. Such persistence characteristic is ideal for use in its application in prolonged protection of surfaces.

Like zinc-containing compounds, silver has been used as an antibacterial agent for a long time. Silver nanoparticles have been used in the emerging field of nanomedicine such as in wound dressings, catheters, and various household products due to their antimicrobial activity. Polymethyl methacrylate loaded with nano-silver is considered as bone cement and the nano-silver can improve the antimicrobial activity. Ultrahigh molecular weight polyethylene fabricated with silver nanoparticles are used in joint replacement components to reduce the wear and tear of the polyethylene polymer. Silver nanoparticle coated polypropylene are used for surgical meshes due to its antimicrobial and anti-inflammatory properties.

During cleaning operations, as well as in the presence of humidity, silver ions are released from the top layer of the film which, upon encountering bacteria, block their metabolism and interrupt their proliferation, leading to their lack of survival. PVC film manufactured by Hexis has silver ions encapsulated in a glass matrix distributed over the film in a uniform manner. This concept is similar to the ZPT rolls manufactured by Cao Group that we are investigating.

Despite decades of successful use to treat human scalps, little is understood about the antifungal mechanism of action of ZPT. It was shown that ZPT inhibits fungal growth through increased cellular levels of copper that damages iron–sulphur clusters of proteins essential for fungal metabolism [8]. Pyrithione acts as an ionophore, interacting nonspecifically with the plasma membrane and shuttling copper into the cell. The authors speculate that pyrithione mediates copper transport across intracellular membranes, enabling copper to disperse throughout the cell and gaining access to intracellular organelles such as mitochondria. There is a precedent for pyrithione-mediated ionophore activity across intracellular membranes, as was reported of pyrithione mediated zinc transport across vacuole vesicles in vitro [2].

Antifungal and antibacterial mechanisms may not be the same. ZPT forms stable interactions with the bacterial membrane phospholipid phosphatidylethanolamine [1]. This suggested the mode of action for the pyrithione biocides is not dissimilar to that of quaternary ammonium

compounds (QACs) which act through binding and breakdown of cell membranes to form pores. QACs are well known potent antimicrobials used to prevent the spread of pathogenic bacteria.

Previous work performed suggests that both zinc and copper pyrithiones induce the leakage of intracellular material from exposed cultures of *Escherichia coli* and *Pseudomonas aeruginosa* as well as affect nutrient uptake in bacteria due to inhibition of membrane-bound metabolic processes [2]. Mangion et al discussed the anti-fungal mechanism of action [10]. We summarized and projected the mechanisms for bacteria in Figure 6.

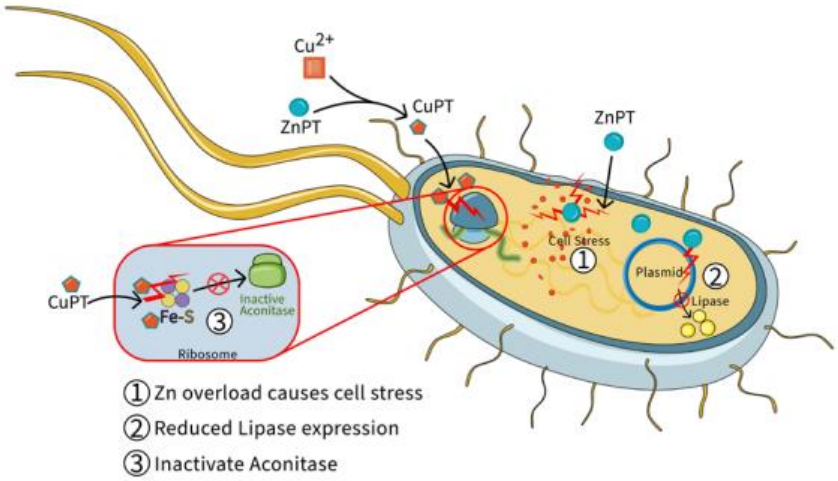


Figure 6 Proposed antibacterial mechanisms within E. *coli*. 1. Zinc overloading induced cell stress. 2. Reduced lipase expression and 3. Copper pyrithione (CuPT) Inactivation of aconitase enzyme in bacterial cytosol.

A relatively novel approach to an antibacterial strategy is to use coatings that are designed to provide an ongoing reduction of infectivity long after the coating is added. Copper-based coatings have already been reported to inactivate SARS-CoV-2. The virus has also been reported to be less stable on nanostructured aluminum surfaces and light-activated titanium dioxide. Zinc oxide was used as an alternative ingredient that has the ability to inactivate SARS-CoV-2 [11]. ZnO has a different color (white) compared to copper compounds and has different stability and toxicity. Zn^{2+} ions and ZnO nanoparticles have recently been shown to have activity against SARS-CoV-2 [12]. Our own pilot data also demonstrated ZPT to have some efficacy against Pseudo virus.

Interestingly, our ICP analysis detected trace amounts of copper and sulphur in the ZPT plastic film structural material. Copper-based coatings have already been reported to inactivate SARS-CoV-2. How the trace amount of copper and sulfur affected the reduction in our present study is presently unknown.

Recent market analysis reports have shown that the global market for antibacterial coating was growing rapidly even before the COVID-19 pandemic. Although ZPT lacks a dramatic quick kill action compared to quick-acting antiseptics such as alcohol, it has a prolonged suppression effect. This makes it perfect for the application of using as an alternative tool for controlling harmful

microorganisms. It is likely that additional uses for ZPT as a safe and effective antimicrobial will be found as we struggle with ever increasing resistance to current antibiotics and antimicrobial agents.

Author Contributions

DCNC conceived, designed, and wrote the article. SJ designed, acquired the data and drafted the article. NK and YP conducted the experiments to acquire the data. DC conceived and critically revised the article. All authors contributed to the article and approved the submitted version.

Competing Interests

The authors have declared that no competing interests exist.

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