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Research Article

Anti-Bacterial Mechanisms for Ag^{+}, Cu^{2+}, and Zn^{2+} Ion Solutions against *Staphylococcus Aureus* and *Escherichia Coli*

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Abstract:

Antibacterial mechanism of Ag^{+} ion solution against *S. aureus* had been found that **Ag^{+}-**induced *S. aureus* may inactivate PGN synthesis transglycosylase TG and transpeptidase TP. Bacteriolysis of *S. aureus* PGN cell wall, in which wall teichoic acids control PGN synthesis cross-linking TP, is due to the inhibition of PGN elongation by enhancing the activities of PGN autolysins; amidase AmiA and AmiE, and PGN hydrolase Lysostaphin-like endopeptidase (Glycine-Glycine bond cleavage). Against *E. coli,* the antibacterial mechanism of Ag^{+} ion solution had been found that bacteriolysis and destruction of *E.coli* cell wall by silver ions are caused by the destruction of outer membrane structure owing to the activation of endopeptidase of lipoprotein at C-, and N-terminals, and inhibition of PGN elongation due to the damage of PGN synthetic enzyme of silver-protein Amidase in periplasmic space, and PGN autolysins of Amidase, Peptidase, and Carboxypeptidase. Bacteriolysis and destruction of *E.coli* cell wall are due to the damage of LPS synthesis, destructing of outer membrane structure by degrading of lipoprotein at C-, N-terminals, owing to inhibition of PGN formations by inactivation of carboxypeptidase and TP-endopeptidase, and activities of PGN autolysins of amidase, peptidase and carboxypeptidase.

Bacteriolysis of *S.aureus* PGN cell wall by **Cu^{2+} ions** is thought to be due to inhibition of PGN elongation owing to the damages of PGN synthetic TG/TP and the activations of PGN autolysin, AmiA. Bacteriolysis of *E.coli* cell wall by **Cu^{2+} ions** occurs by destruction of outer membrane structure due to degradation of lipoprotein at N-, C-terminals, damage of TP enzyme and activations of PGN autolysins. Furthermore, deletion of PGN autolysin also becomes bacteriolytic factor.

It is thought that the activations of these PGN autolysins by **Zn^{2+} ions** could be enhanced the inhibitions of PGN elongation simultaneously, with bacteriolysis of *S. aureus* PGN cell wall. Bacteriolysis of *E. coli* cell wall by **Zn^{2+} ions** are due to destruction of outer membrane structure by degrading of lipoprotein at C-, Nterminals, owing to PGN formation inhibition by activities of PGN autolysins of amidase and carboxypeptidase-transpeptidase

Ag^{+},Cu^{2+},Zn^{2+} ions-induced ROS generation of O_2^{-} and H_2O_2 and ROS-mediated oxidative stress in bacterial cell lead to killing by stress damage for silver ions, cell membrane damages due to high reactive •OH and $OH^{-}$are formed by Haber-Weiss and Fenton reactions for Cu^{2+} ions, and DNA molecular damage for Zn^{2+} ions.

Keywords: Ag^{+}, Cu^{2+}, Zn^{2+} ions, Bacteriolysis, TG and TP, PGN autolysin, PGN elongation, Autolysin amidase, ROS-mediated oxidative stress.

Introduction

Silver, copper, and zinc of transition metals have highly antibacterial activities and are utilized as chemotherapy agents. The high antibacterial activities for these Ag^{+}, Cu^{2+}, Zn^{2+} ion solutions have the processes of bacteriolyses and destructions

of bacterial cell walls against *Staphylococcus aureus (S. aureus)* peptidoglycan (PGN) and *Escherichia coli* (*E.coli)* outer membrane cell walls.

Anti-bacterial activity of silver ions depends on bacteriolysis and destruction of bacterial cell walls that silver ions inhibit peptidoglycan elongation and peptidoglycan synthesis and autolysin activation **[1].**

The bacteriolytic mechanisms by copper (II) ions had been revealed that bacteriolysis of *S. aureus* PGN cell wall by Cu^{2+} ions is ascribed to the inhibition of PGN elongation due to the damages of PGN biosynthesis of transglycosylase (TG) and transpeptidase (TP), and the Cu^{2+} ions-induced activated PGN autolysins, whereas bacteriolysis of *E. coli* outer membrane cell wall by Cu^{2+} ions is attributed to the destruction of outer membrane structure and the inhibition of PGN elongation due to the damage of PGN biosynthesis TP and the activations of PGN autolysins **[2].**

On the other hand, in this study, zinc ions-induced anti-bacterial mechanism also may be clarified. It had been appeared that the anti-bacterial effects had the order of $Zn^{2+} > Cu^{2+} > Ag^{+} > Al^{3+}$ in metallic ion concentration 100 mL of the sulfate solution under the halo inhibitory tests, in which Zn^{2+} ion indicated to be the highest effect in the sulfates **[3].**

As described above, Ag^{+}, Cu^{2+}. Zn^{2+} ion solutions having very high antibacterial abilities call attention to potential treatments such as preventions of serious diseases, restriction of viral infection, and regulation of cancer tumor cells.

In addition, antibacterial Ag, Cu, Zn metallic ion solution materials are raised such as silver compound (silver chloride), silver nanoparticles for Ag^{+} ion solutions, copper sulfate, copper chelators, CuO nanoparticles for Cu^{2+} ion solutions, and zinc chloride, zinc sulfate, zinc pyrithione, zinc oxide for Zn^{2+} ion solutions.

In this research article, antibacterial mechanisms of silver(I), copper(II), and zinc(II) ions are clarified against from that relates the bacterial PGN elongation, PGN biosnthesis and autolysin, and metallic ions-induced autolysin activation against *S. aureus* and *E. coli.*

Molecular structures of bacterial cell walls and the action sites of PGN autolysins against *S. aureus* and *E. coli*

Bacterial PGN structure of both Gram-positive and Gram-negative bacteria comprises repeating disaccharide backbones of N-acetylglucosamine (NAG) and β-(1-4)-N-acetylmuramic acid (NAM) that are crosslinked by peptide stem chains attached to the NAM residues [**4]**. As shown in ***Fig. 1,*** **t**he action sites of bacterial autolysins are comprised that for *Staphylococcus aureus* (*S. aureus)* PGN layer cell wall, there are N-acetylmuramidase-L-alanine amidase and PGN chain cross-linkage DD-endopeptidase. The other, as shown in ***Fig. 2,*** for *Escherichia coli* (*E. coli)* cell wall**,** there are endopeptidase of degrading enzyme at lipoprotein of C- and N-terminals, and amidase, peptidase, and caboxypeptidase at thin PGN layer in periplasmic space [**5]**. The bacterial cell walls are a strong flexible mesh work of PGN that gives a bacterium structural integrity, in which to accommodate a growing cell, the walls are remodeled by PGN synthesis and PGN autolysin. PGN is the main constituent of bacterial cell walls and must be continuously synthesized and degraded to maintain the integrity and viability of the cells that bacterial cell wall hydrodases of amidase,gycosidase, and peptidase display a modular architecture combining multiple and different catalytic domains, including some lytic transglycosylases as well as cell wall binding domains [**6]**. Bacterial peptidoglycan autolysins against *S. aureus* and *E. coli* are summarily represented in ***Table 1*** that these autolysin sites are shown in Fig. 1 and Fig. 2. The *S. aureus* killing mechanism was more likely due to activation autolysins along with minimum membrane disruption **[7].** In these autolysins, zinc-dependent PGN autolysin of amidases chiefly may be enhanced and induced anti-bacterial activities.

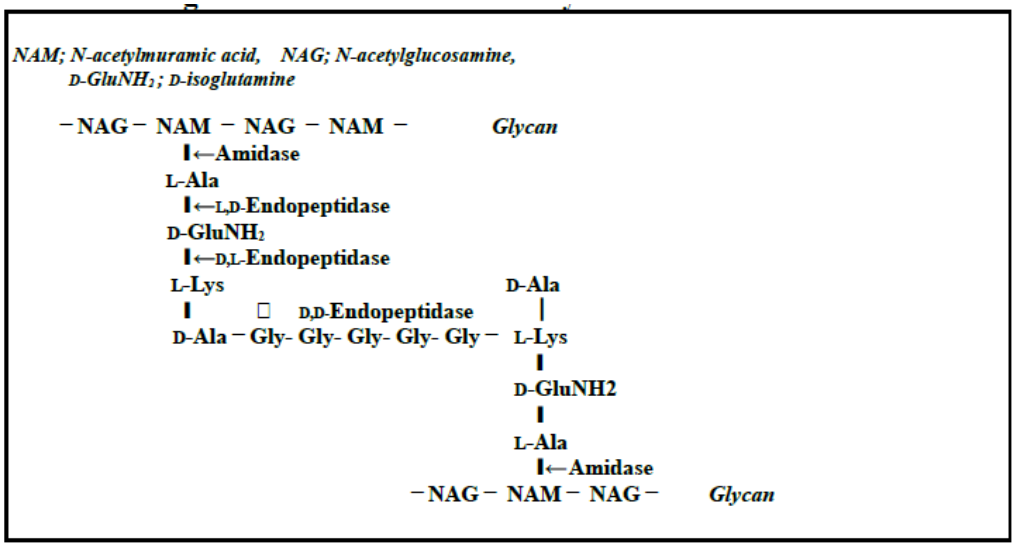

Figure (1): *PGN molecular structure and the action sites of PGN autolysins against S. aureus thick PGN layer*

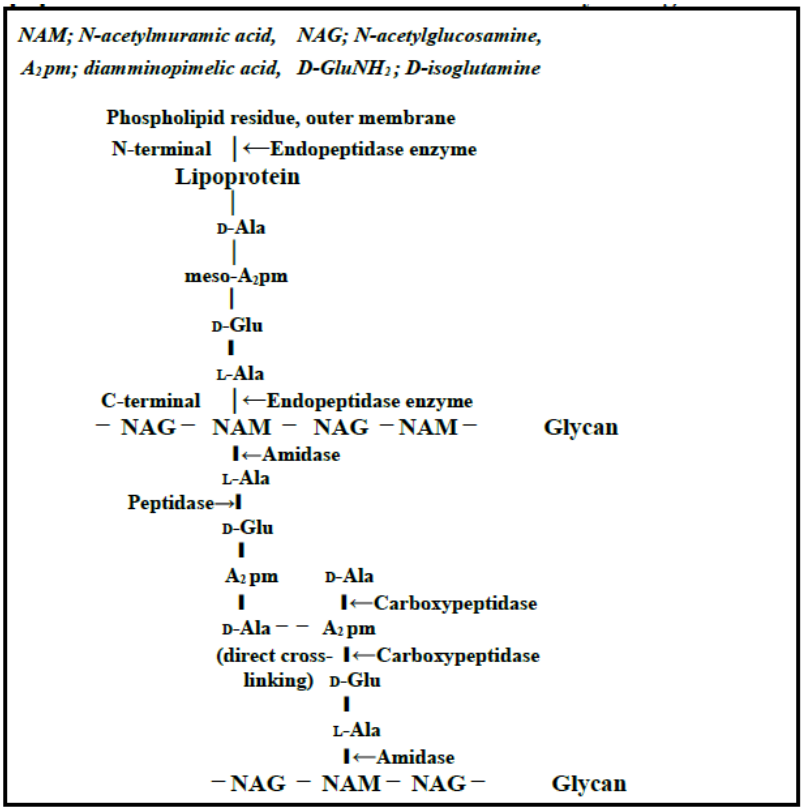

Figure (2): *Molecular structures of outer membrane lipoprotein and PGN layer in periplasmic space, and the action sites of degrading enzyme of lipoprotein at C- and N-terminals and PGN autolysins against E. coli*

Table1: *Bacterial PGN autolysins against S. aureus, and outer membrane lipoprotein degrading enzyme and PGN autolysins against E. coli*

Peptidoglycan autolysins against *S. aureus*	**Outer membrane lipoprotein degrading enzymes and peptidoglycan autolysins against *E. coli***
·**N-acetylmuramidase-L-alanine amidase.** ·**PGN chain cross-linkage DD-endopeptidase.**	·**Endopeptidase of degrading enzyme at lipoprotein of C- and N-terminals.** ·**PGN amidase, peptidase, and caboxypeptidase.**

Antibacterial mechanism of Silver (II) ions against *S. aureus* and *E. coli*

Ag^{+} ions-induced bacteriolysis of *S.aureus* PGN cell wall

The released Ag^{+} ions from $AgNO_3$ solution penetrate into bacterial cells, can inhibit the growth of Gram-positive B. subtilis bacterium which exerts toxicity by damaging cellular membrane, degrading chromosomal DNA, lowering reductase activity, and reducing protein expression. Wall teichoic acids are spatial regulators of PGN crosslinking biosynthesis of transpeptidase (TP), and silver ions could inhibit both transglycosylase (TG) and TP enzymes of the PGN that Ag^{+}-induced bacteria may inactivate PGN synthesis TG and TP. Lysostaphin-like PGN hydrolase and glycylglycine endopeptidase LytM may function as TP enzyme.

For the sake of growth of *S. aureus* thick PGN layer cell wall, there is necessarily required for the adequate balance between PGN synthesis and PGN autolysin. When the balance was broken to be imbalanced, bacteriolysis and destruction of the cell wall should occur. Hence, it became apparent that bacteriolysis of *S. aureus* PGN cell wall by Ag^{+} ions is caused by inhibition of PGN elongation due to inactivation of PGN TG or TP **[8]** and enhancement of activation of PGN autolysins of amidases **[9].**

Accordingly, antibacterial mechsnism against *S. aureus* had been found that Ag^{+}-induced *S. aureus* may inactivate PGN synthesis transglycosylase TG and transpeptidase TP. Bacteriolysis of *S. aureus* PGN cell wall, in which wall teichoic acids control PGN synthesis cross-linking TP, is due to the inhibition of PGN elongation by enhancing the activities of PGN autolysins; amidase Ami A and A mi E, and PGN hydrolase Lysostaphin-like endopeptidase (Glycine-Glycine bond cleavage). Bacteriolysis and destruction of *E.coli* cell wall are due to the damage of LPS synthesis, destructing of outer membrane structure by degrading of lipoprotein at C-, N-terminals, owing to inhibition of PGN formations by inactivation of carboxypeptidase and TP-endopeptidase, and activities of PGN autolysins of amidase, peptidase and carboxypeptidase.

Permeability of silver ions into *E.coli* cell wall

(1) Destruction of outer membrane structure of *E.coli* by hydrolases of lipoproteins at C- and N-terminals

Tol protein (Tol)–protein-associated lipoprotein (Pal) system is composed of five proteins that TolA, TolQ, and TolR are inner membrane proteins, TolB is a periplasmic protein, and Pal, the peptidoglycan associated lipoprotein, is anchored to the outer membrane. Ag^{+} ions induced Tol-Pal complex is antimicrobial agents widely used, it has recently been demonstrated to be essential for bacterial survival and pathogenesis that outer membrane structure may be destroyed **[10].** It is unclear whether both Amidase and Endopeptidase of lipoprotein at C-, and N-terminals are simultaneously activated by Ag^{+} ions. However, outer membrane may be considered to be destroyed probably by predominant activation of lipoprotein-endopeptidase.

(2) Damage of *E.coli* PGN synthetic enzyme of silver protein amidase in periplasmic space, and amidase, peptidase, and carboxypeptidase of PGN autolysins

Silver ions may be accumulated in *E.coli* periplasmic space, in which the silver ions are spent to the activation of bacteriolysis of the cell wall and efflux activity to extracellular cell. Then, lipoproteinendopeptidase may be degradative by Ag^{+} binding proteins. The other, it is unclear that the silver-induced PGN biosyntheses TG/TP should be inhibited by the silver ions. However, silver ions inactivate TP of endopeptidase by because of destructive observation of bacterial cell walls. Silver ions could activate *E.coli* PGN autolysins of amidase, peptidase, Carboxypeptidase, such as silver depending PGN autolysin, AmiC, AmiD, Muramidase, Amino acid amidase, Carboxypeptidase A, zinc metalloenzymes AmiD, Amidase zinc-containing amidase; AmpD, zinc-present PGLYRPs, Carboxypeptidase-degraded aldolase,

Carboxypeptidase, serve to be effective for the PGN autolysins. It is particularly worth noting that enhancement of the activities of autolysins is characterized on PGN carboxypeptidase and TP-endopeptidase, requiring divalent cations. Accordingly, the inhibition of PGN elongation had occurred by silver ion induced activities of PGN hydrolases and autolysins.

Thus, the antibacterial mechanism of Ag^{+} ion solution had been found that bacteriolysis and destruction of *E.coli cell* wall by silver ions are caused by the destruction of outer membrane structure owing to the activation of endopeptidase of lipoprotein at C-, and N-terminals, and inhibition of PGN elongation due to the damage of PGN synthetic enzyme of silver-protein Amidase in periplasmic space, and PGN autolysins of Amidase, Peptidase, and Carboxypeptidase.

(3) Ag^{+} ions-induced ROS generation in *S.aueus* and *E. coli*

For the penetration of Ag^{+} ions to *S. aureus* PGN cell wall, the ROS production such as superoxide anion radical O_2^{-}, hydroxyl radical ·OH, hydrogen peroxide H_2O_2 occurred from superoxide radical O_2^{-} molecular. O_2^{-} and H_2O_2 permeate into membrane and cytoplasm, and then, DNA molecular is damaged by oxidative stress **[11]**.

Silver ions react with -SH, and H^{+} in *E.coli* that free radicals O_2^{-}, OH^{-},·OH and H_2O_2 are formed as follows:

$$O_2 + e \rightarrow O_2^{-} \quad 2O_2^{-} + 2H^{+} \rightarrow H_2O_2 + O_2$$

$$O_2^{-} + H_2O_2 \rightarrow OH^{-} + \cdot OH + O_2.$$

In cell wall, reacting with polyunsaturated fatty acids

$$LH + OH\cdot \rightarrow L\cdot + HOH \qquad L\cdot + O_2 \rightarrow LOO\cdot$$

$$LH + LOO\cdot \rightarrow L\cdot + LOOH$$

Ag^{+}-containing peptidoglycan recognition proteins (PGRPs) induce ROS production of H_2O_2, O^{-}, $HO\cdot$, and then the ROS occur the oxidative stress, and killing by stress damage **[12]**.

Antibacterial mechanism for Copper (Ⅱ) ions against *S. aureus* and *E. coli*

(1) Bacteriolysis of *S.aureus* PGN Cell Wall by Cu^{2+} Ions

Bacteriolysis by balance deletion between biosynthesis enzyme and decomposition enzyme (autolysin) in PGN cell wall: For the sake of growth of *S.aureus* PGN cell wall, there is necessarily required for the adequate balance between PGN biosynthesis and PGN autolysin. When the balance is broken by Cu^{2+} penetration, Cu^{2+} ions are self-catalytically treated as coenzyme, that this is indicated that activation of autolysin is preceded, in which bacteriolysis and killing may result.

Hence, bacteriolysis of *S.aureus* PGN cell wall by Cu^{2+} ions is thought to be due to inhibition of PGN elongation owing to the damages of PGN synthetic TG/TP and the activations of PGN autolysin, AmiA

(2) Inhibition of Polymerization of Glycan Chains Bonding and Cross-Linking of Side Peptide

Cu^{2+} ions inhibit polymerization of glycan chains, forming copper complex in which is partial action sites of glycan saccharide chains **[2].** L is coordinated molecular.

$$Cu^{2+} + LH \rightarrow CuL^{+} + H^{+} \qquad CuL^{+} + LH \rightarrow CuL_2 + H^{+}$$

Copper-complexes on saccharide chains may be;

—NAG-(NAM-Cu-2O-2N-NAG)-NAM— .

The other, Cu^{2+} ions inhibit cross-linked reaction by peptide copper complex formation bonding to sidepeptide chains.

$$Cu^{2+} + 2LH \rightarrow CuL_2 + H^{+}$$

Peptide copper complex may be **3N-Cu-O, Cu (Gly-L-Ala) H_2O.** Specially, Cu^{2+} ions react with cross-molecular penta glycine $(Gly)_5$, copper-glycine complex may be formed.

Amino acid : $Cu^{2+} + Gly^{-} \rightarrow Cu(Gly)^{+}$, $Cu(Gly)^{+} + Gly^{-} \rightarrow Cu(Gly)_2$,

Peptido : $Cu^{2+} + GlyGly \rightarrow Cu(GlyGly)$, $Cu(GlyGly) + Gly^{-} \rightarrow Cu(GlyGlyGly)^{-}$.

(3) Bacteriolysis and Destruction of *E.coli* Outer Membrane Cell Wall by Cu^{2+} Ions

Inhibition of outer membrane cell wall: Cu^{2+} ions inactivate catalyst enzyme with forming Cu^{+} ions.

Cu^{2+} + –SH → –SCu(II) + H^{+}

By the penetration of Cu^{2+} ions, the activations of amidase enzyme of N-terminal and endopeptidase enzyme of C-terminal are enhanced.

Accordingly, the activations of decomposition at N-, C-terminals of lipoproteins may occur with the destruction of outer membrane structure. Hence, bacteriolysis of *E.coli* cell wall by Cu^{2+} ions occurs by destruction of outer membrane structure due to degradation of lipoprotein at N-, C-terminals, damage of TP enzyme and activations of PGN autolysins. Furthermore, deletion of PGN autolysin also becomes bacteriolytic factor.

(4) Cu^{2+} ions-induced ROS production in *S.aureus* and *E. coli*

Cu^{2+} ions-induced reactive oxygen species (ROS) O_2^- and H_2O_2 generated in the cell wall, and permeate into cell membrane and cytoplasm, in which in cell membrane high reactive •OH and OH^- are formed by Haber-Weiss and Fenton reactions.

Haber-Weiss reaction; $H_2O_2 + O_2^- \rightarrow .OH + OH^- + O_2$

Fenton reaction; $Cu^{+} + H_2O_2 \rightarrow .OH + OH^- + Cu^{2+}$

Furthermore, new ROS productions occur by Fenton-like type. L=Ligand

$LCu(II) + H_2O_2 \rightarrow LCu(I) + .OOH + H^{+}$
$LCu(I) + H_2O_2 \rightarrow LCu(II) + •OH + OH^-$

Production of reactive oxygen species (ROS) against *S. aureus.* O_2^- and H_2O_2 permeate into membrane and cytoplasm, that DNA molecular is damaged by oxidative stress [**13**]. By the penetration of copper ions into bacterial cell wall, productions of O_2^-, H^+, H_2O_2, $ONOO^-$ occurs. The other, in *E.coli* cell wall, the productions of O_2^-, H^+ in outer membrane, and H_2O_2, OH^-, ·OH in periplasmic space occur. These ROS and H_2O_2 damage the cell membrane and the DNA molecules by oxidase stress **[2]**.

Antibacterial mechanism for Zinc(II) ions against *S. aureus* and *E. coli*

(1) Bacteriolysis of *S. aureus* PGN Cell Wall by Zn^{2+} Ions against *S. aureus*

Zinc is redox-inert and has only one valence state of Zn(II). In proteins, the coordination is limited by His, Cys, Glu, and sulfur donors from the side chains of a few amino acids. In zinc sulfate solution, $ZnSO_4$ is dissociated into aqua zinc ion $[Zn(H_2O)_6]^{2+}$ and sulfuric ion $(SO_4)^{2-}$. aqua zinc ions are liable to be bound to ligand L having negative charge. The sulfuric ion has bactericidal inactivity **[14].**

$ZnSO_4 + 6H_2O \rightarrow [Zn(H_2O)_6]^{2+} + (SO_4)_2^-$

$[Zn(H_2O)_6]^{2+} + 2L^- \rightarrow [Zn(H_2O)L_2] + 5H_2O$

$Zn(H_2O)L_2 \rightarrow ZnL_2 + H_2O$

Zinc-proteins are formed by the reaction of Zn^{2+} ions with *S. aureus* surface, on the ground that is due to formation of S-atom containing Zn-cysteine complex in bacteria **[15],**

(2) PGN biosynthesis enzymes of transglycosylase TG and transpeptidase TP:

Wall teichoic acids are spatial regulators of PGN cross-linking biosynthesis TP **[16],** however, it is not explicit whether zinc ions could inhibit both TG and TP enzymes of the PGN, wherein is due to uncertain relation between wall teichoic acids biosynthesis and PGN biosynthesis. **[16]**

(3) Inhibition of PGN elongation due to the activations of autolysins:

Zn^{2+} binding Rv3717 showed no activity on polymerized PGN and but, it is induced to a potential role of N-Acetylmuramyl L-alanine Amidase **[17],** PGN murein hydrolase activity and generalized autolysis; Amidase MurA **[18],** Lytic Amidase LytA **[19],** enzymatically active domain of autolysin_LytM **[20],** Zinc-dependent metalloenzyme AmiE **[21]** as prevention of the pathogen growth, and Lysostaphin-like PGN hydrolase and glycylglycine endopeptidase LytM **[22].** It is thought that the activations of these PGN autolysins by Zn^{2+} ions could be enhanced the inhibitions of PGN elongation simultaneously, with bacteriolysis and destruction of *S. aureus* PGN cell wall

(4) Production of reactive oxygen species (ROS) against *S. aureus*

O_2^- and H_2O_2 permeate into membrane and cytoplasm, that DNA molecular is damaged by oxidative stress **[23].** For the penetration of zinc ions to PGN cell wall, the ROS production such as superoxide anion radical O_2^-, hydroxyl radical •OH, hydrogen peroxide H_2O_2 occurred from superoxide radical O_2^- molecular [**24].**

O_2^- and and H_2O_2 permeate into membrane and cytoplasm, and then, DNA molecular is damaged by oxidative stress **[23].**

$$O_2 + e^- + H^+ \rightarrow \bullet HO_2$$

$$\bullet HO_2 \rightarrow H^+ + O_2$$

$$H_2O_2 + e^- \rightarrow HO^- + \bullet OH$$

$$2H^+ + \bullet O_2^- + \bullet O_2^- \rightarrow H_2O_2 + O_2$$

$$H_2O \rightarrow \bullet OH + \bullet H + e^- \rightarrow H_2O_2$$

(5) Bacteriolysis and destruction of *E. coli* Cell Wall by Zn^{2+} Ions Permeability of Zinc Ions into *E. coli* Cell Wall

E. coli cell wall is constituted of lipopolysaccharide (LPS), lipoproteins (LPT), and PGN, thinner layer within periplasmic space. The first permeability barrier of zinc ions in the *E .coli* cell wall is highly anionic LPS with hydrophobic lipid A, core polysaccharide, O-polysaccharide, in which zinc ions may be possible for the inhibition of LPS biosynthesis, owing to that promotes formation of metal-rich precipitates in a cell surface[**25**]. In zinc ion uptake across the outer membrane, the lipoproteins of Omp A, Omp C, Omp F porins have a role for at least some of these proteins in Zn^{2+} uptake, in which the lipoproteins have metallic cation selective and hydrophilic membrane crossing pore, to be effective for zinc transfer [**26**]. Zinc (II) ions react with -SH base, and then H_2 generates. Zinc bivalent is unchangeable as -SZn—S— bond 4-coordinated.

$$Zn^{2+} + 2(\text{-SH}) \rightarrow \text{-SZn(II) –S–} + 2H^+$$

Destruction of outer membrane structure of *E. coli* cell wall by hydrolases of lipoproteins at C-, N-terminals

ZnPT (zinc pyrithione) and Tol (Tol proteins)-Pal (Proteinassociated lipoprotein) complex are antimicrobial agents widely used, however, it has recently been demonstrated to be essential for bacterial survival and pathogenesis that outer membrane structure may be destroyed [**27**, **28**].

Inhibition of PGN elongation due to the damage of PGN synthesis enzyme of zinc-protein amidase in periplasmic space, and the activities of PGN autolysins

The zinc-induced decrease of protein biosynthesis led to a partial disappearance of connexin-43 of protein synthesis in neurons **[29]**, but it is unknown whether PGN biosynthesis is inhibited. Further, it is also unclear whether the both TG/TP should be inhibited by the zinc ions **[30, 31**, **32**]. The other, zinc ions were accumulated in E. coli periplasmic space, in which the zinc ions are spent to the activation of bacteriolysis of the cell wall. Zinc depending PGN autolysin, amidase PGRPs [**33**], zinc metallo enzymes AmiD[**34**], zinc-containing amidase; AmpD [**35]**, zinc-present PGLYRPs[**36]** serve to be effective for the PGN autolysins. It is particularly worth noting that enhancement of the activities of autolysins is characterized on PGN carboxypeptidase-transpeptidase IIW [**37]** requiring divalent cations. Accordingly, the inhibition of PGN elongation had occurred by zinc ion induced activities of PGN hydrolases and autolysins. Thus, Bacteriolysis of *E. coli* cell wall by Zn^{2+} ions are due to destruction of outer membrane structure by degrading of lipoprotein at C-, N-terminals, owing to PGN formation inhibition by activities of PGN autolysins of amidase and carboxypeptidase-transpeptidase

ROS production and oxidative stress against *E. coli:* Zinc ions react with -SH, and H^+, ROS generate. In *E. coli,* free radicals O_2^-, OH^-, •OH) and H_2O_2 are formed as follows[**38]:**

$$O_2 + e \rightarrow O_2^- \qquad 2O_2^- + 2H^+ \rightarrow H_2O_2 + O_2$$
$$O_2^- + H_2O_2 \rightarrow OH^- + \bullet OH + O_2^-$$

In the cell wall, reacting with polyunsaturated fatty acids:

$$LH + OH\bullet \rightarrow L\bullet + HOH \qquad L\bullet + O_2 \rightarrow LOO\bullet \qquad LH + LOO\bullet \rightarrow L\bullet + LOOH$$

Zinc-containing Peptidoglycan Recognition Proteins (PGRPs) induce ROS production of H_2O_2, O_2^-, HO•, the ROS occur the oxidative stress, and killing by stress damage **[39].**

Ag^{+}, Cu^{2+}, Zn^{2+} ions-induced ROS generation and oxidative stress in *S. aureus* and *E. coli*

By penetration of Ag^{+} Cu^{2+}, Zn^{2+} ions to *S. aureus* PGN cell wall, the ROS production such as superoxide anion radical O_2^-, hydroxyl radical ·OH, hydrogen peroxide H_2O_2 occurred,

Ag^{+}-containing PGRPs induce ROS production of H_2O_2, O^-, HO·, and then the ROS occur the oxidative stress, and killing by stress damage,

Cu^{2+} ions-induced reactive oxygen species (ROS) O_2^- and H_2O_2 generated in the cell wall, and permeate into cell membrane and cytoplasm, in which in cell membrane high reactive •OH and OH^- are formed by Haber-Weiss and Fenton reactions.

Zn^{2+} ions-induced ROS generation O_2^- and H_2O_2 permeate into membrane and cytoplasm, that DNA molecular is damaged by oxidative stress**.** For the penetration of zinc ions to PGN cell wall, the ROS production such as superoxide anion radical O_2^-, hydroxyl radical •OH, hydrogen peroxide H_2O_2 occurred from superoxide radical O_2^- molecular**.**

Conclusions

Antibacterial mechanism of Ag^{+} ions against *S. aureus* have been found that Ag^{+}-induced *S. aureus* may inactivate PGN synthesis transglycosylase TG and transpeptidase TP. Bacteriolysis of *S. aureus* PGN cell wall, in which wall teichoic acids control PGN synthesis cross-linking TP, is due to the inhibition of PGN elongation by enhancing the activities of PGN autolysins; amidase Ami A and A mi E, and PGN hydrolase Lysostaphin-like endopeptidase (Glycine-Glycine bond cleavage). On the other hand, antibacterial mechanism of Ag^{+} ions against *E. coli* was found that bacteriolysis and destruction of *E.coli* cell wall by silver ions are caused by the destruction of outer membrane structure owing to the activation of endopeptidase of lipoprotein at C-, and N-terminals, and inhibition of PGN elongation due to the damage of PGN synthetic enzyme of silver-protein Amidase in periplasmic space, and activation of PGN autolysins of Amidase, Peptidase, and Carboxypeptidase.

Bacteriolysis of *S.aureus* PGN cell wall by Cu^{2+} ions is thought to be due to inhibition of PGN elongation owing to the damages of PGN synthetic TG/TP and the activations of PGN autolysin, AmiA. The other, bacteriolysis of *E.coli* cell wall by Cu^{2+} ions occurs by destruction of outer membrane structure due to degradation of lipoprotein at N-, C-terminals, damage of TP enzyme and activations of PGN autolysins. Furthermore, deletion of PGN autolysin also becomes bacteriolytic factor.

Antibacterial mechanism of Zn^{2+} ions against *S. aureus* was found that Zn^{2+} ions-induced PGN autolysin activation could be enhanced the inhibitions of PGN elongation simultaneously, with bacteriolysis snd destruction of *S. aureus* PGN cell wall. The other, antibacterial mechanism of Zn^{2+} ions against *E. coli* was found that bacteriolysis of *E. coli* cell wall by Zn^{2+} ions are due to destruction of outer membrane structure by degrading of lipoprotein at C-, N-terminals, owing to PGN formation inhibition by activities of PGN autolysins of amidase and carboxypeptidase-transpeptidase.

Ag^{+}, Cu^{2+}, Zn^{2+} ions-induced ROS generation of O_2^- and H_2O_2 and ROS-mediated oxidative stress in bacterial cell wall may lead to killing by stress damage for silver ions, cell membrane damages due to high reactive •OH and OH^- are formed by Haber-Weiss and Fenton reactions for Cu^{2+} ions, and killing stress and DNA molecular damages for Zn^{2+} ions.

References

[1] T. Ishida (2018); Antibacterial mechanism of Ag^{+} ions for bacteriolyses of bacterial cell walls via peptidoglycan autolysins, and DNA damage. MOJ Toxicol. 4(5):345–350.

[2] T. Ishida (2017); Mechanism of Antibacterial Activities of Cu (Ⅱ) Ions against *Staphylococcus aureus* and *Escherichia coli* on the Ground of Results

Obtained from Dilution Medium Method. Virology & Immunology Journal 1(3): 1-8.

[3] T. Ishida (2017); Halo inhibitory zone tests and antibacterial activities for metallic salts aqueous solutions. Journal of Preventive Medicine. 11(3): 93-99.

[4] T. Ishida (2016); Bacteriolyses of Cu^{2+} solution on bacterial cell walls/cell membrane and DNA base pairing damages, *Japanese Biomedical Research on Trace Elements,* 27, No.4:151-161.

[5] T. Ishida (2019); Comparative bacteriolytic mechanism for Ag^{+} and Zn^{2+} ions against *S.aureus* and *E. coli*: A review. *Annals of Microbiology and Infectious Diseases.* 2, Issue 1: 1-12.

[6] A. Vermassen, S. Leroy, Regine Talon et al (2019); Cell wall hydrolases in bacteria: Insight on the diversity of cell wall amidases, glycosidases and peptidases toward peptidoglycan. *Frontiers in Microbiology.* 10,article 331:1-27.

[7] Muhammad Yasir, Debarun Dutta, Mark D.P. Willcox; Mode of action of the antimicrobial peptide Mel4 is independent of *Staphylococcus aureus* cell membrane permeability. *bioRxiv preprint* 2019; 9 April: 1-25. doi: https://doi.org/10.1101 /603712.

[8] Baizman ER, Bransttrom SA, Longley CB, et al (2000). Antibacterial activity of synthetic analogues based on the disaccharide structure of moenomycin,an inhibitor of bacterial transglycosylase. Microbiology. 2000; 246: 3129– 3140.

[9] Mellroth P, Sandalova T. (2014); Structural and Functional Insights into Peptidoglycan Access for the Lytic Amidase LytA of *Streptococcus* pneumoniae. Mbio. 2014; 15:1–10.

[10] Slavin YN, Asnis J, Hafeli U, et al. (2017) Metal nanoparticles: understanding the mechanisms behind antibacterial activity. Journal of Nanobiotechnology. 15:15–65.

[11] R. Gaupp, N. Ledala and G. A.Somerville (2012); *Staphylococal* response to oxidative stress, *Frontiers in Cellular and Infection Microbiology, 2:1-8.*

[12] D. R. Kashyap, M. Kuzma, D.A. Kowalczyk, D. Gupta, and R. Dziarski (2017) Bactericidal peptidoglycan recognition protein induces oxidative stress in *Escherichia coli* through a block in respiratory chain and increase in central carbon catabolism *Mol Microbiol.* September ; 105(5): 755–776. doi:10. 11 11/mmi.13733.

[13] R Gaupp, N.Ledala, GA Somerville (2012). Staphylococal response to oxidative stress, *Frontiers in Cellular and Infection Microbiology.* 2(33):1-19. Doi:10.3389/ fcimb.2012.00033

[14] Faiz U, Butt T, Satti L, Hussain W, Hanif F. (2011) Efficacy zinc as an antibacterial agent against enteric bacterial pathogens, *J Ayub Med Coll Abbottabad.* 23(2):8-21.

[15] T. Ishida (2017) Bacteriolyses of Bacterial Cell Walls by Cu(II) and Zn(II) Ions Based on Antibacterial Results of Dilution Medium Method and Halo Antibacterial Test. *Journal of Advanced Research in Biotechnology* 2(2): 1-12.

[16] Atilano ML, Pereira PM, Yates J, Reed P, Veiga H, Pinho MG, Filipe SR. Teichoic acid are temporal and spatial regulators of peptidoglycan cross-linking in S.aureus, PNAS. 2010;107(44):18991-18996. Doi:10. 1073/pnas.1004304107

[17] Prigozhin DM, Mavrici D, Huizar JP, Vansell HJ, Alber T. (2013) Structural and Biochemical Analyses of Mycobacterium tuberculosis N-Acetylmuramyl-L-alanine AmidaseRv3717 Point to a Role in peptidoglycan Fragment Recycling, J Biol Chem. 288(44):31549- 31555. Doi:10.1074 /jbc.M113 .510792.

[18] Carroll SA, Hain T, Technow U, Darji A, Pashalidis P, Joseph SW, et al (2003). Identification and Characterization of a Peptidoglycan Hydrolase, MurA of Listeria monocytogenes, a Muramidase Needed for Cell Separation, J. Bacteriology. 2003; 185(23):6801-6808.

[19] Peter Mellrotha, Tatyana Sandalovab, Alexey Kikhneyc, Francisco Vilaplanad, Dusan Heseke, Mijoon Leee, et al (2014). Structural and Functional Insights into Peptidoglycan Access for the Lytic Amidase LytA of Streptococcus pneumonia. 2014; 5(1). Doi:10.1128/mBio.01120-13

[20] Elzbieta Jagielsksa, Olga Chojnacka, Izabela Sabata.(2016) LytM Fusion with SH3b-Like Domain Expands Its Activity to Physiological Conditions, Microbial Drug

Resistance.2016;22(6):461-469. Doi: org /10.1089/ mdr.2016.0053

[21] Sebastian Zoll, Bernhard Pätzold , Martin Schlag , Friedrich Götz, Hubert Kalbacher, Thilo Stehle (2010). Structural Basis of Cell Wall Cleavage by a Staphylococcal Autolysin. PloS Pathogens. 2010;6:1-13. Doi: org/10.1371/journal.ppat.1000807

[22] Ramadurai L1, Lockwood KJ, Nadakavukaren MJ, Jayaswal RK (1999). Characterization of a chromosomally encoded glycyglycine endopeptidase of S.aureus. Microbiology. 145(4):801-808. Doi: 10.1099/13500872-145-4-801

[23] R Gaupp, N.Ledala, GA Somerville.(2013) Staphylococal response to oxidative stress, Frontiers in Cellular and Infection Microbiology. 2:1-8. Doi:10.3389 /fcimb. 2012.00033

[24] F. Morina, M. Vidović, B. Kukavica, S. VeljovićJovanović (2015). Induction of peroxidase isoforms in the roots of two Verbascum Thapsus L. populations is involved in adaptive responses to excess Zn^{2+} and Cu^{2+}, *Botanica SERBICA*. 39(2);151-158.

[25] S Langley, TJ Beveridge.(1999) Effect of O-Side-Chain-LPS Chemistry on Metal Binding, *Appl Environ Microbiol.* 65(2):489-498.

[26] Claudia A Blindauer (2015). Advances in the molecular understanding of biological zinc transport. The Royal Society of Chemistry. 51:4544-4563. Doi:10.1039 /C4CC10174J

[27] A.J.Dinning, I.S.I.AL-Adham, P.Austin, M.Charlton and P.J.Collier (1998) Pyrithione biocide interactions with bacterial

[28] Godlewska R, Wiśniewska K, Pietras Z, Jagusztyn-Krynicka EK.(2009) Peptidoglycan-associated lipoprotein(Pal) of Gram-negative bacteria: function, structure, role in pathogenesis and potential application in immunoprophylaxis, *FEMS Microbiol Letter* ,298(1):1-11. Doi: 10.1111/j.1574-6968.2009.01659.x

[29] Alirezaei M, Mordelet E, Rouach N, Nairn AC, Glowinski J, Prémont J. (2002) Zinc-induced inhibition of protein synthesis and reduction of connexin-43 expression and intercellular communication in mouse cortical astrocytes. Eur J Neurosci. 16(6): 1037-1044.

[30] Alexander J F Egan, Jacob Biboy, Inge van't Veer, Eefjan Breukink, Waldemar Vollmer. (2015) Activities and regulation of peptidoglycan synthases. *Philos Trans R Soc Lond B Biol Sci.* 370(1679): 1-20. Doi: 10.1098/rstb.2015.0031

[31] Singh SK, SaiSree L, Amrutha RN, Reddy M.(2012) Three redundant murein endopeptidases catalyze an essential cleavage step in peptidoglycan synthesis of E.coli K12, Molecular Microbiology. 86(5): 1036-1051. Doi: 10.1111/ mmi. 12058

[32] Ramachandran V, Chandrakala B, Kumar VP, Usha V, Solapure SM, de Sousa SM.(2006) Screen for Inhibitors of the Coupled TransglycosylaseTranspeptidase of Peptidoglycan Biosynthesis in E.coli. Antimicrob Agents Chemother. 50(4): 1425-1432. Doi: 10.1128/ AAC.50.4.1425-1432.2006

[33] Rivera I, Molina R, Lee M, Mobashery S, Hermoso JA.(2016) Orthologous and Paralogous AmpD Peptidoglycan Amidases from Gram-Negative Bacteria, Microb Drug Resist. 22(6):470-476. Doi:10.1089/ mdr. 2016.0083

[34] Pennartz A, Généreux C, Parquet C, Mengin-Lecreulx D, Joris B.(2009) Substrate-Induced Inactivation of the E.coli AmiD N-AcetylmuramoylL-Alanine Amidase Highlights a New Strategy To Inhibit This Class of Enzyme, *Antimicrob Agents Chemother.* 53(7):2991-2997. doi: 10.1128 /AAC.01520-07

[35] Carrasco-López C, Rojas-Altuve A, Zhang W, Hesek D, Lee M, Barbe S, et al (2011). Crystal Structures of Bacterial Peptidoglycan Amidase AmpD and an Unprecedented Activation Mechanism. J Biol Chem. 286(36)9:31714-31722. Doi: 10.1074/jbc.M111.264366

[36] Wang M, Liu LH, Wang S, Li X, Lu X, Gupta D, et al (2007). Human Peptidoglycan Recognition Proteins Require Zinc to Kill Both Gram-Positive and Gram-negative Bacteria and Are Synergistic with Antibacterial Peptides J Immunol. 178(5): 3116-3125.

[37] DasGupta H Fan DP Fan (1979). Purification and Characterization of a Carboxypeptidase-Transpeptidase of Bacillus megaterium Acting on the Tetra

peptide Moiety of the Peptidoglycan, *J. Biologycal Chemistry.* 254(13): 5672-5682.

[38] ZN Kashmiri, SA Mankar (2014); Free radicals and oxidative stress in bacteria, *International J. of Current Microbio and Appl Scienses.* 3:34-40.

[39] D. R. Kashyap, A. Rompca, A. Gaballa, J. D. Helmann, J. Chan, C. J. Chang (2014); Peptidoglycan Recognition Proteins Kill Bacteria by Inducing Oxidative, Thiol,and Metal Stress. *PLOS Pathogen.* 10(7): 1-17. doi: 10.1371/journal.ppat.1004280.

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