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Original article

Interference of phosphane copper (I) complexes of β-carboline with quorum sensing regulated virulence functions and biofilm in foodborne pathogenic bacteria: A first report

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ABSTRACT

Foodborne pathogens are one of the major cause of food-related diseases and food poisoning. Bacterial biofilms and quorum sensing (QS) mechanism of cell–cell communication have also been found to be associated with several outbreaks of foodborne diseases and are great threat to food safety. Therefore, In the present study, we investigated the activity of three tetrahedrally coordinated copper(I) complexes against quorum sensing and biofilms of foodborne bacteria. All the three complexes demonstrated similar antimicrobial properties against the selected pathogens. Concentration below the MIC i.e. at sub-MICs all the three complexes interfered significantly with the quorum sensing regulated functions in *C. violaceum* (violacein), *P. aeruginosa* (elastase, pyocyanin and alginate production) and *S. marcescens* (prodigiosin). The complexes demonstrated potent broad-spectrum biofilm inhibition in *Pseudomonas aeruginosa*, *E. coli*, *Chromobacterium violaceum*, *Serratia marcescens*, *Klebsiella pneumoniae* and *Listeria monocytogenes*. Biofilm inhibition was visualized using SEM and CLSM images. Action of the copper(I) complexes on two key QS regulated functions contributing to biofilm formation i.e. EPS production and swarming motility was also studied and statistically significant reduction was recorded. These results could form the basis for development of safe anti-QS and anti-biofilm agents that can be utilized in the food industry as well as healthcare sector to prevent food-associated diseases.

1. Introduction

Foodborne pathogenic bacteria are the most frequent cause found associated with the foodborne diseases and food poisoning and hence pose a potential danger to both the food safety and human health (Oliver et al., 2005). There are several types of bacteria implicated for contamination of raw and processed food items such as *E. coli*, *Salmonella*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Serratia*, *Clostridium* and *Listeria monocytogenes* (Zhao et al., 2017). In recent times, significant morbidity and mortality is observed because of the foodborne diseases making it a serious public health problem (Zhao et al., 2016). Foodborne bacteria not only threaten human health but also cause enormous economic losses to the food industry (Zhao et al., 2014; Zhao et al., 2017). The food industry has been facing a major problem of food spoilage due to the biofilm formation that has been found responsible for various outbreaks of foodborne infection (Aarnisalo et al., 2007). Biofilms are the hydrated matrix of extracellular polymeric substances produced by bacteria that adhere them to the surface help-

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ing the bacterial communities to live in a sessile and protected environment (O'Toole et al., 2000). Foodborne pathogens exist in natural environment and they form biofilms on food and food contact surfaces (Kusumaningrum et al., 2003). These biofilms provide bacteria the resistance against antibiotics, chemical agents and environmental changes as well as helps bacteria to overcome the host immune responses (Zhao et al., 2017). Consequently, removal of these foodborne bacteria and their resistant biofilm is a major challenge for the food industry.

Quorum sensing (QS) is used both by Gram-positive and Gram-negative bacteria including foodborne pathogens for communication through the production of autoinducers, which are small signal molecules (Bai and Rai, 2011). QS regulates the expression of several genes in response to population density and controls the production of various enzymes that are directly or indirectly involved in food spoilage such as proteolytic, lipolytic, chitinolytic, and pectinolytic enzymes (Williams, 2007). Furthermore, QS is also involved in the activation of certain genes in bacteria to secrete extracellular matrix like EPS and proteins and hence, helps in the formation of biofilm that is resistant to drugs and other adverse environmental factors (Greenberg, 2003). In recent years, detection of quorum sensing signal molecules in spoiled food items has added a new dimension in the study of food spoilage and its control. Thus, there is an urgent need to develop quorum-sensing inhibitors that target the synthesis of autoinducer molecules or block the signaling systems resulting in reduced biofilm formation to prevent the food spoilage. A number of strategies are currently being investigated to develop QS inhibitors that can interfere with biofilm formation by foodborne bacteria so that spoilage of food can be prevented.

Currently, various compounds and complexes containing metals like copper, silver, aluminium, zinc are in use in food industry to control the bacterial growths. Various investigators have reported antibacterial properties of copper against many foodborne pathogenic bacteria such as *Salmonella entrica*, *Camplylobacter jejuni*, *E. coli*, *Listeria monocytogenes*, and *S. aureus* (Faundez et al., 2004; Ibrahim et al., 2008; Gyawali et al., 2011). Further, the use of metallic copper surfaces in food processing operations have also been reported inhibitory to various foodborne pathogens (Faundez et al., 2004). Bacteria come across numerous host-derived antimicrobial agents, through the passage of infection. Microbes are known to get exposed to copper by macrophages. A specific protein ATP7A facilitates the influx of copper which repositions to the phagosomal membrane from the golgi compartment (White et al., 2009). Moreover, virulence in several pathogenic bacteria like *Listeria monocytogenes*, and *Pseudomonas aeruginosa* is allied to copper homeostasis and resistance mechanisms (Francis and Thomas, 1997; Schwan et al., 2005). In a study, paints incorporated with copper have been found to inhibit bacterial biofilm formation and hence may be exploited as anti-biofilm agent (Cooney and Tang, 1999). It is reported that the repression of genes vital for biofilm formation, and virulence is lead by copper stress in pathogenic bacteria (Baker et al., 2010). Coordination of complexes of metals with ligands are attractive molecules with known antimicrobial properties. In these complexes, metals act as a framework for the ligands, creating a 3D structure that interacts with the target (Beeton et al., 2014). The copper complex formation is usually beneficial since its biological activity is significantly greater compared with ligands or metal alone (Ng et al., 2013). Consequently, a number of copper complexes have been synthesized and screened for their biological activities and such coordination complexes could be more beneficial in delivering Cu ions to the target tissues (Szymanski et al., 2012). Several researchers have reported Cu complexes with different ligands to possess antimicrobial activities and hence the approach of employing bactericidal or fungicidal agents as ligands in Cu complexes to increase their antimicrobial potential have been widely exploited (Singh et al., 2009; Patel et al., 2010; Ng et al., 2016). However, most of these studies have focused on the evaluation of antibacterial activities of complexes on planktonic cells.

In the present study, we investigated the activity of copper(I) complexes against bacterial biofilms specifically on foodborne pathogens that are capable of forming stable biofilms. Here we report the anti-quorum sensing and antibiofilm activity of tetrahedrally coordinated Cu(I) having the ligand Hnor, triphenylphosphane as a co-ligand (to stabilize Cu ion) and Halides Cl^-, Br^- and I^- (as anions) against *Pseudomonas aeruginosa*, *E. coli*, *Chromobacterium violaceum*, *Serratia marcescens*, *Klebsiella pneumoniae* and *Listeria monocytogenes*. The choice of the ligands for selecting these copper(I) complexes was based on their high importance in coordination, bioinorganic and supramolecular chemistry. The investigated mixed-ring heterocyclic compounds belongs to alkaloid family, which are known to possess pharmacological properties whereas halides like chlorine and iodine are used in disinfection and sanitization.

2. Materials and methods

2.1. Strains and growth conditions

Bacterial strains used in the study were *Pseudomonas aeruginosa* PAO1, *Chromobacterium violaceum* ATCC 12472, *E. coli*, *Serratia marcescens*, *Klebsiella pneumoniae* and *Listeria monocytogenes* (laboratory strains). All strains were maintained on Luria Bertani (LB) broth solidified with 1.5% agar (Oxoid).

2.2. Synthesis of compounds

CuCl, triphenylphosphine (PPh_3), DL-tryptophan, formaldehyde and other reagents were purchased from Sigma-Aldrich. Solvents were used as received from the Sigma-Aldrich without further purification. We have synthesized these three copper compounds 1–3 as described previously (Khan et al., 2016).

The synthesis of the three copper complexes, i.e. $[(PPh_3)_2Cu(Hnor)Cl]$ C1, $[(PPh_3)_2Cu(Hnor)Br]$ C2 and $[(PPh_3)_2Cu(Hnor)I]$ C3 were performed by the procedure adopted by Khan et al. (2016) (Fig. 1). However, in breif, the CuCl, Triphenylphospane (PPh_3), Hnor (ligand) were mixed in 1:2:1 acetonitrile/methanolic solution for 4 h and filtered, washed and recrystallized on slow evaporation of the mother liqour.

2.3. Minimum inhibitory concentration

Minimum inhibitory concentration (MIC) values for the complexes were determined using concentrations ranging from 256 to 0.0625 μg/ml by broth microdilution method as described by Andrews (2001). Concentrations below MIC i.e. Sub-MICs were selected for virulence assays.

2.4. Effect of Cu(I) complexes on violacein production in *Chromobacterium violaceum* 12472

Chromobacterium violaceum ATCC 12472 (CV12472) is a wild-type strain that produces purple colored violacein pigment in response to cognate N-butanoyl and N-hexanoyl homoserine lactone molecules. CV12472 was incubated for 16–18 h and inoculated into flasks containing LB broth amended with synthesized complexes. The flasks were incubated at 27 °C with 150 rpm in a shaker incubator (Husain et al., 2016). Violacein production in treated and untreated CV12472 was quantified by the method described by Blosser and Gray (2001).

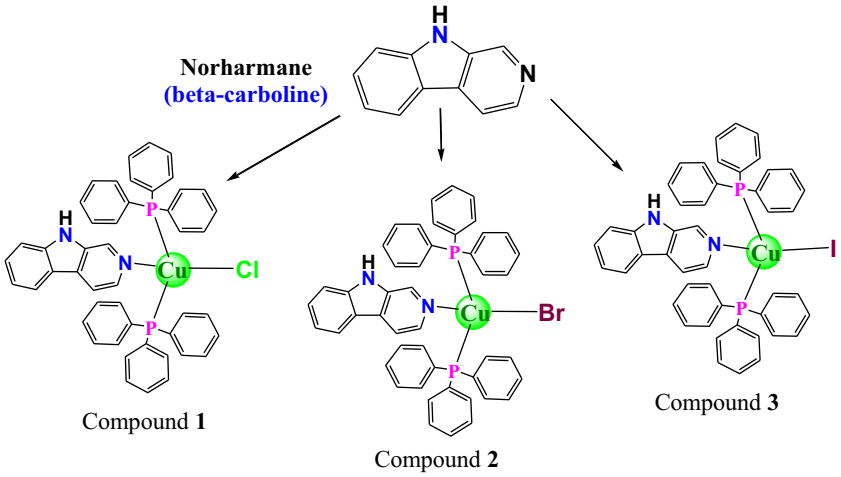

Fig. 1. Schematic representation of the structures of the ligand, Norharmane (a β-carboline) and the compounds 1–3.

2.5. Effect on QS regulated functions in Pseudomonas aeruginosa PAO1

Effect of sub-inhibitory concentrations of Cu(I) complexes on quorum sensing regulated virulence factors of PAO1 (elastase, pyocyanin and alginate production) was determined using previously described protocols (Al-Shabib et al., 2016).

2.6. Effect on prodigiosin production in Serratia marcescens

Prodigiosin production in *S. marcescens* was determined using the method of Morohoshi et al., (2007). Briefly, 1% of *S. marcescens* cells (0.4 OD at 600 nm) were inoculated into 2 ml of fresh LB medium and incubated with and without sub-MICs of Complexes C1–C3. In late stationary phase the cultures were collected and centrifuged at 10,000 rpm for 10 min. Prodigiosin from the cell pellet was extracted with acidified ethanol solution (4% 1 M HCl in ethanol) and absorbance was read at 534 nm using a UV–visible spectrophotometer (Labomed).

2.7. Assay for extraction and quantification of exopolysaccharides (EPS) and swarming motility

Exopolysaccharides from test pathogens were extracted and quantified by the method described previously (Al-Shabib et al., 2016). Swarming migration in treated and untreated pathogens was determined using the protocol of Husain and Ahmad (2013).

2.8. Effect of Cu(I) complexes on biofilm formation in microtitre plates

The effect of Cu(I) complexes C1-C3 on biofilm formation was tested at respective ½ × MICs of the test pathogens, using polystyrene flat-bottomed 96 well microtitre plates (NunC). Biofilms of the test pathogens were grown in tryptone soy broth (TSB) without shaking at 30 °C for 24 h except *Pseudomonas aeruginosa* and *E. coli* that were incubated at 37 °C. The biofilms formed in the microtiter plates were stained with 0.1% crystal violet solution and quantified by solubilizing the dye in ethanol and measuring the absorbance at OD_{470} (O'Toole and Kolter, 1998; Al-Shabib et al., 2017).

2.9. Scanning electron microscopy (SEM) of biofilms

Sub-MICs of complex C3 was used to treat bacterial cells while untreated bacteria served as controls. Wells containing glass coverslips were inoculated with test pathogens (10^6 CFU/ml) and incubated at 30 °C for 24 h. Cover slips were removed from the wells and washed three times in sterile phosphate buffer saline (PBS). Formaldehyde (2%) and 2.5% glutaraldehyde were used to fix the samples. The samples were again rinsed with PBS (3 times), followed by a series of ethanol dehydration. Samples were then dried completely, gold coated and observed using scanning electron microscope (JEOL, Japan).

2.10. Visualization of biofilms using confocal laser scanning microscopy (CLSM)

Biofilms of the treated and untreated bacteria on coverslips were allowed to form as described in the previous section. The glass coverslips were removed from the wells, washed thrice in sterile PBS, and stained with acridine orange for 15 min in dark. The coverslips were again washed with sterile PBS and mounted onto glass slides with nail varnish. Stained cover slips were visualized with CLSM (Zeiss Spinning disk confocal microscope, Zeiss, Germany) equipped with an excitation filter 515–560 and magnification at 40×.

2.11. Statistical analysis

All studies were performed in triplicate, and the data obtained from experiments were presented as mean values, and the difference between control and test were analyzed using Student's t-test.

3. Results and discussion

The MIC values of Cu(I) complexes 1–3 were determined against foodborne bacteria *viz. Pseudomonas aeruginosa* PAO1, *Chromobacterium violaceum* ATCC 12472, *E. coli*, *Serratia marcescens*, *Klebsiella pneumoniae* and *Listeria monocytogenes* (Table 1). Overall, all the three tested copper complexes showed significant antimicrobial activity against all selected test pathogens with MIC values ranging from 32 to 128 μg/ml. These results are in accordance with MIC values previously determined for Cu(II) complexes with 2-acetylpyridine- and 2-benzoylpyridine-derived hydrazones (Despaigne et al., 2012) and Cu(I) complexes containing phenanthroline-type ligands (Starosta et al., 2011). The sub-MICs of complexes (C1-C3) were selected for the study of quorum sensing and biofilm inhibition.

Table 1
Minimum inhibitory concentrations (MICs) of Cu(I) complexes against test pathogens.

Strains	Minimum inhibitory concentration (μg/ml)		
	C1	C2	C3
P. aeruginosa	128	128	128
E. coli	64	32	32
C. violaceum	64	64	64
S. marcescens	32	32	32
L. monocytogenes	64	32	32
K. pneumoniae	128	64	128

3.1. Effect of Cu(I) complexes on violacein production in CV12472

Wild-type *C. violaceum* 12472 produces a characteristic purple pigment called violacein in response to signaling molecules acyl-homoserine lactones (AHLs). Based on this observable pigmentation, *C. violaceum* 12472 is used to detect interference with AHL-mediated quorum sensing. Reduction in violacein pigment in our assay indicated quorum-sensing inhibition. Complex C1 showed 41–77% reduction in violacein, while C2 and C3 showed 38–81% and 31–75% decrease respectively at sub-MICs ranging from 4 to 32 μg/ml (Fig. 2). Azithromycin, which is a known quorum sensing inhibitor, was used as positive control for violacein inhibition. Our results are in accordance with the results observed with five Cu(II) complexes having aromatic nitrogen-containing heterocycles, which reduced violacein production in *C. violaceum* (Glisic et al., 2013).

3.2. Effect on QS-regulated functions in Pseudomonas aeruginosa PAO1

Bacterial cell–cell communication system, i.e. QS plays an important role in the modulation of virulence gene expression in *P. aeruginosa* (Williams, 2007). *P. aeruginosa* is an important opportunistic human pathogen that has been the subject of intensive investigations and is one of the model organisms in search of QS inhibitors. A sophisticated hierarchy of QS network operates in this *P. aeruginosa*, which consists of *las*, *iqs*, *pqs* and *rhl* systems (Lee and Zhang, 2015). LasR is a key regulator in the expression of *lasB* gene encoding for elastase. Elastase causes degradation of elastin, collagen and other matrix proteins in the host (Wolz, 1994; Yanagihara et al., 2003). In the present study, Complex C1 reduced elastase production significantly (23–69%) at concentrations ranging from 8-64 μg/ml. Similar concentration dependent effects of complex C2 and C3 were observed as shown in Fig. 3. Highest decrease of 58% and 72% were observed at 64 μg/ml with complexes C2 and C3 respectively.

Pyocyanin and its precursor molecule have crucial roles in the establishment of infection. The expression of pyocyanin, which is a secondary metabolite, is controlled by the QS. It also enhances the colonization of pathogen that leads to the invasion of the host immune system (Lee and Zhang, 2015). Therefore, we examined the effect of sub-MICs (8–64 μg/ml) of Cu(I) complexes C1-C3 on the pyocyanin production in PAO1. Complexes C1, C2, and C3 induced reduction of pyocyanin production in PAO1 in a concentration dependent manner with 18–58%, 31–76%, and 27–66% decrease was observed respectively as depicted in Fig. 4.

Alginate is one of the major components of the exopolysaccharide of biofilm formed by PAO1 and is responsible for conferring resistance to *P. aeruginosa* against antimicrobial agents (Gopu et al., 2015). The effect of sub-MICs of complexes C1-C3 was studied for their efficacies to reduce the production of alginate. The obtained results demonstrated that the alginate production was reduced significantly with increasing concentration of all the three complexes. At concentrations ranging from 8 to 64 μg/ml, C1 inhibited alginate production by 32–82%, C2 showed a decrease of 17–74% and PAO1 treated with C3 reduced alginate by 15–79% over untreated control (Fig. 5). This is an important finding as alginate confers resistance to the pathogens against antimicrobial agents and reduction in alginate production would decrease the rate of resistance among bacteria and make them susceptible to the drugs.

All the three Cu(I) complexes demonstrated statistically significant reduction in QS-regulated virulence factors (elastase, pyocyanin and alginate production) in PAO1 in a concentration dependent manner. To our best of knowledge, this is probably the first report on the interference of QS-controlled virulence function in PAO1 by sub-MICs of Cu(I) complexes.

3.3. Effect on prodigiosin production in Serratia marcescens

In *S. marcescens* the major virulence factor is prodigiosin, a reddish pigment. The production of this pigment is controlled by the

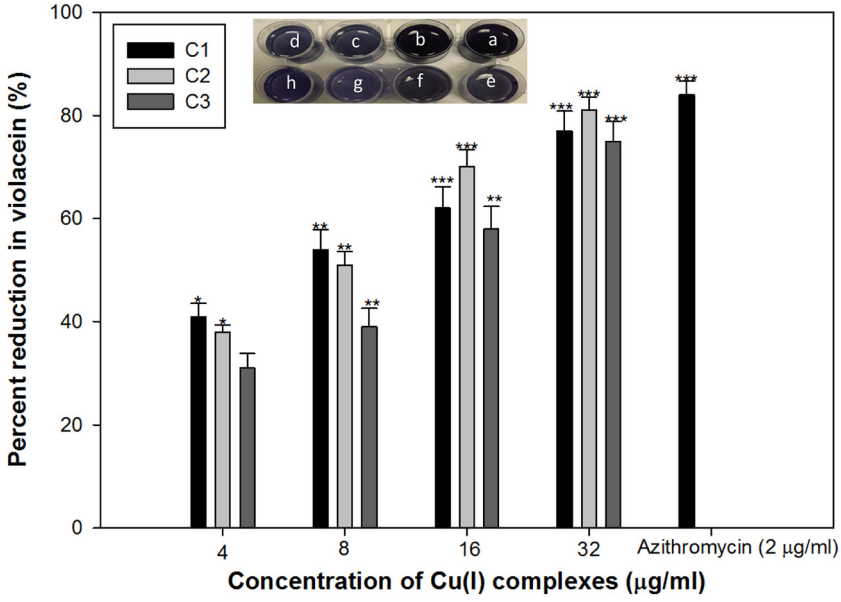

Fig. 2. Inhibition of violacein production in *C. violaceum* 12472 by the sub-MICs of investigated copper (I) compounds. Inset shows the Effect of the complex on CV12472 violacein production in. (a–b) Untreated control; (c–f) complex treated cultures showing reduction in the violacein production at the 32 μg/ml concentration of C1, C2 and C3.

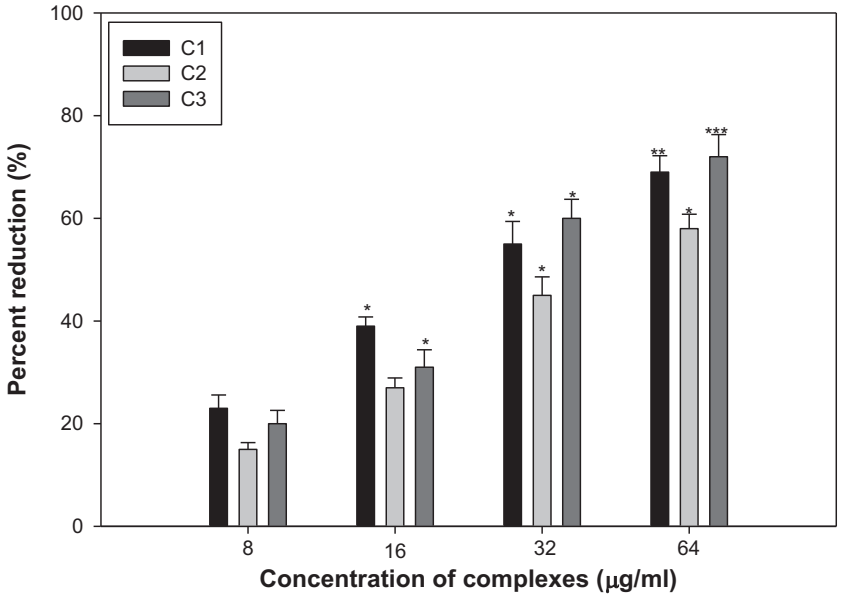

Fig. 3. Effect of sub-MICs of compound C1, C2 and C3 on elastase production in *P. aeruginosa*. All of the data are presented as mean ± standard deviation. *, $p \leq 0.05$, **, $p \leq 0.005$, ***, $p \leq 0.001$.

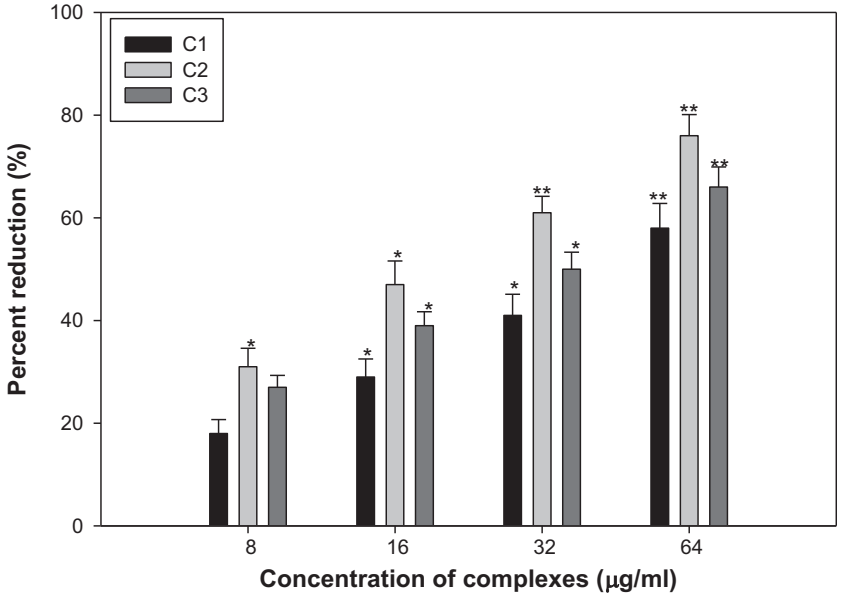

Fig. 4. Effect of sub-MICs of compound C1, C2 and C3 on pyocyanin production in *P. aeruginosa*. All of the data are presented as mean ± standard deviation. *, $p \leq 0.05$, **, $p \leq 0.005$.

phenomenon of quorum sensing (Morohoshi et al., 2007). Hence, inhibition of prodigiosin is an important strategy to reduce the pathogenicity of *S. marcescens*. We observed prodigiosin production in *S. marcescens* was reduced considerably on treating with sub-inhibitory concentrations (2–16 μg/ml) of complexes C1-C3. Reduction of 16–69%, 24–71% and 29–71% was recorded in prodigiosin produced by *S. marcescens* treated with C1, C2 and C3, respectively (Fig. 6). We for the first time have targeted the QS regulated production of prodigiosin in *S. marcescens* using three Cu(I) complexes, though phytocompounds, marine sponges and bacterial supernatant were previously reported for the inhibition of prodigiosin (Packiavathy et al., 2014; Annapoorani et al., 2012; Nithya et al., 2010).

3.4. Effect on EPS production and swarming motility

Exopolysaccharides (EPS) are an integral component of biofilms as it maintains biofilm architecture and helps in microcolony formation. EPS confers resistance to pathogens in biofilm mode by preventing the entry of antibiotics into bacterial cells (Fux et al., 2005). Further, increased EPS secretion means increased resistance to antimicrobials due to altered biofilm architecture (Yildiz and Schoolnik, 1999). Hence, reduction in EPS production will expose bacterial cells in biofilms and thus, help in its eradication. Considering the vital role played by EPS in biofilm formation, we assessed the effect of C1-C3 on EPS production by the test pathogens. EPS extracted from treated and untreated cultures of test pathogens

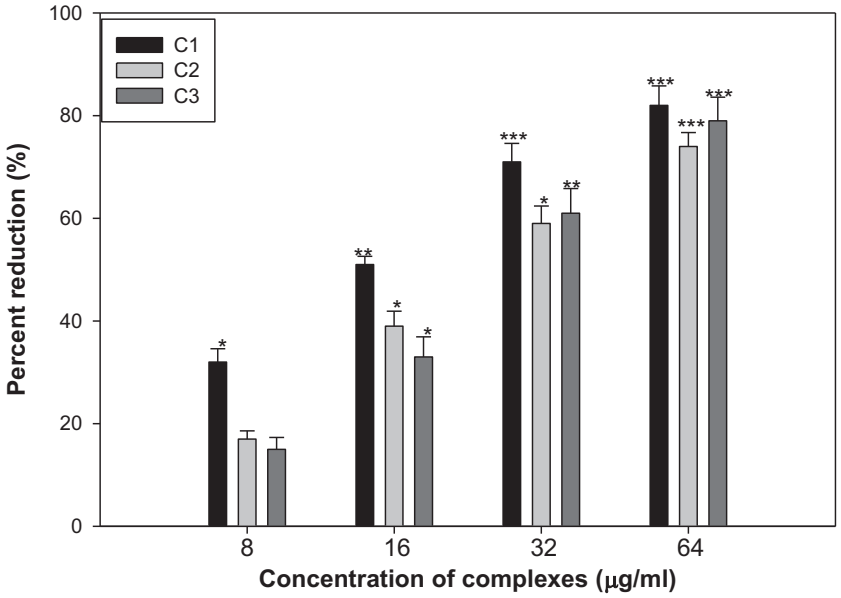

Fig. 5. Effect of sub-MICs of compound C1, C2 and C3 on alginate production in *P. aeruginosa*. All of the data are presented as mean ± standard deviation. *, $p \leq 0.05$, **, $p \leq 0.005$, ***, $p \leq 0.001$.

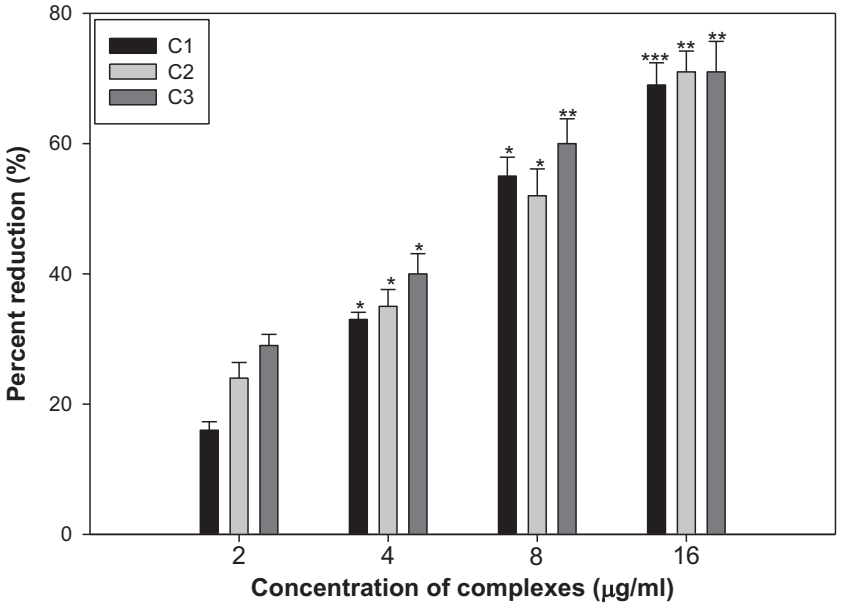

Fig. 6. Effect of sub-MICs of compound C1, C2 and C3 on prodigiosin production in *S. marsecens*. All of the data are presented as mean ± standard deviation. *, $p \leq 0.05$, **, $p \leq 0.005$, ***, $p \leq 0.001$.

were analyzed spectrometrically. EPS production in each test pathogen was reduced considerably at respective ½ × MIC of C1, C2 and C3 as shown in Fig. 7. C1 at respective ½ × MICs exhibited 71, 68, 69, 74 and 74% decrease in EPS production in *C. violaceum* 12472, *P. aeruginosa* PAO1, *E. coli, S. marcescens, K. pneumoniae* and, *L. monocytogenes,* respectively. C2 and C3 also demonstrated significantly reduced EPS production in all test pathogens at their respective ½ × MIC as depicted in Fig. 7.

Swarming motility is an important virulence factor that contributes to the biofilm formation by the bacterial pathogens. Swarming behavior helps in the initial attachment of the bacteria to the surfaces. Therefore, disrupting swarming motility is a suitable approach to inhibit biofilm-forming capability of bacteria (Pratt and Kolter, 1998). The effect of sub-inhibitory concentrations of complex C1-C3 in reducing swarming migration by *C. violaceum*, *P. aeruginosa*, *E. coli*, *S. marcescens, K. pneumoniae*, and *L. monocytogenes,* is shown in Fig. 8. Swarming motility in *C. violaceum* was reduced by 72, 65 and 77% after treatment with 32 μg/ml of C1, C2 and C3, respectively. The complex C3 at 16 μg/ml impaired the swarming migration of *E. coli*, *S. marcescens* and *L. monocytogenes* by 81, 84 and 80%, respectively over untreated control. A significant decrease in swarming motility was also observed in all test pathogens when complex C2 was tested at respective ½ × sub-MICs (Fig. 8).

3.5. Effect of Cu(I) complexes on biofilm formation

Biofilm formation is not only an emergent public health issue but also become one of the main food hygiene issue. Foodborne pathogens and their biofilms exist in the natural environments as

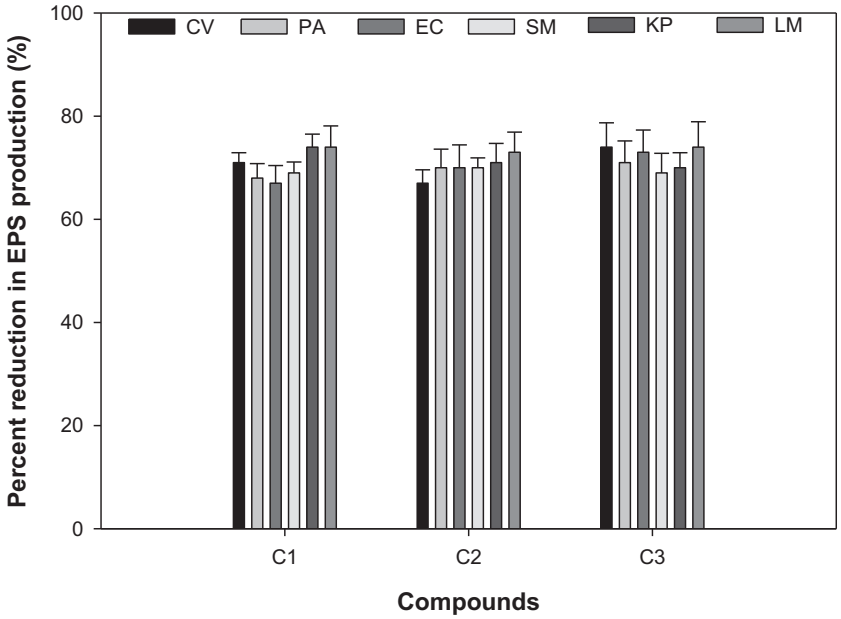

Fig. 7. Effect of ½ × MICs of compound C1, C2 and C3 on the EPS production in the selected pathogens. All of the data are presented as mean ± standard deviation. *, $p \leq 0.05$, **, $p \leq 0.005$, ***, $p \leq 0.001$.

well as on the biological materials and food contact surfaces (Kusumaningrum et al., 2003). These biofilms confer resistance to bacteria against antimicrobial agents as well as helps bacteria to evade host immune system (Zhao et al., 2017). Thus, foodborne bacteria and their biofilms can affect the health of consumers and make maintenance of food hygiene difficult. Effective anti-biofilm agents are needed to prevent and remove biofilms on surfaces of food as well as on food contact surfaces.

In the current investigation, sub-inhibitory concentrations (1/2 × MIC) of C1-C3 were tested against biofilm formation of six foodborne bacterial pathogens *viz*, *P. aeruginosa* PAO1, *E. coli*, *L. monocytogenes*, *K pneumonia*, *S. marcescens* and *C. violaceum* 12472. C1 reduced biofilm formation in all pathogens ranging from 70-80% while biofilm formation dwindled by 73–84% after treatment with respective sub-MICs of C3 (Fig. 9A). Further, *L. monocytogenes* biofilm was visualized with or without treatment using SEM and CLSM and results of the microtitre plate assay were confirmed as significant reduction in the microcolony formation was observed (Fig. 9B). Our results are in accordance with the previous studies conducted with copper (II) complexes against biofilm formed by *E. coli* and *P. aeruginosa* (Usman et al., 2016; Glišić et al., 2013). However, this is probably the first report on the broad-spectrum biofilm and QS inhibition by Cu(I) complexes against foodborne pathogens.

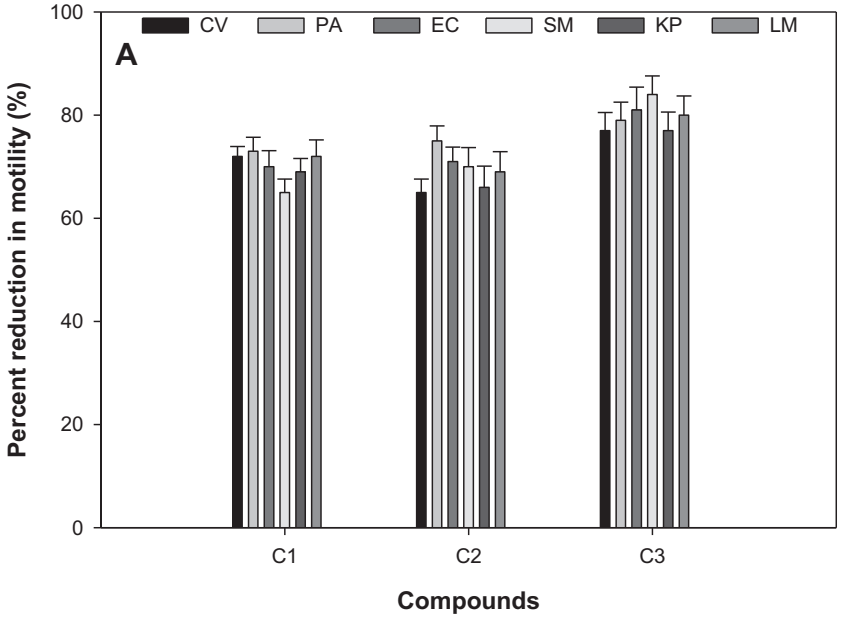

Fig. 8. Effect of ½ × MICs of compound C1, C2 and C3 on the swarming motility in the selected pathogens. All of the data are presented as mean ± standard deviation. *, $p \leq 0.05$, **, $p \leq 0.005$, ***, $p \leq 0.001$.

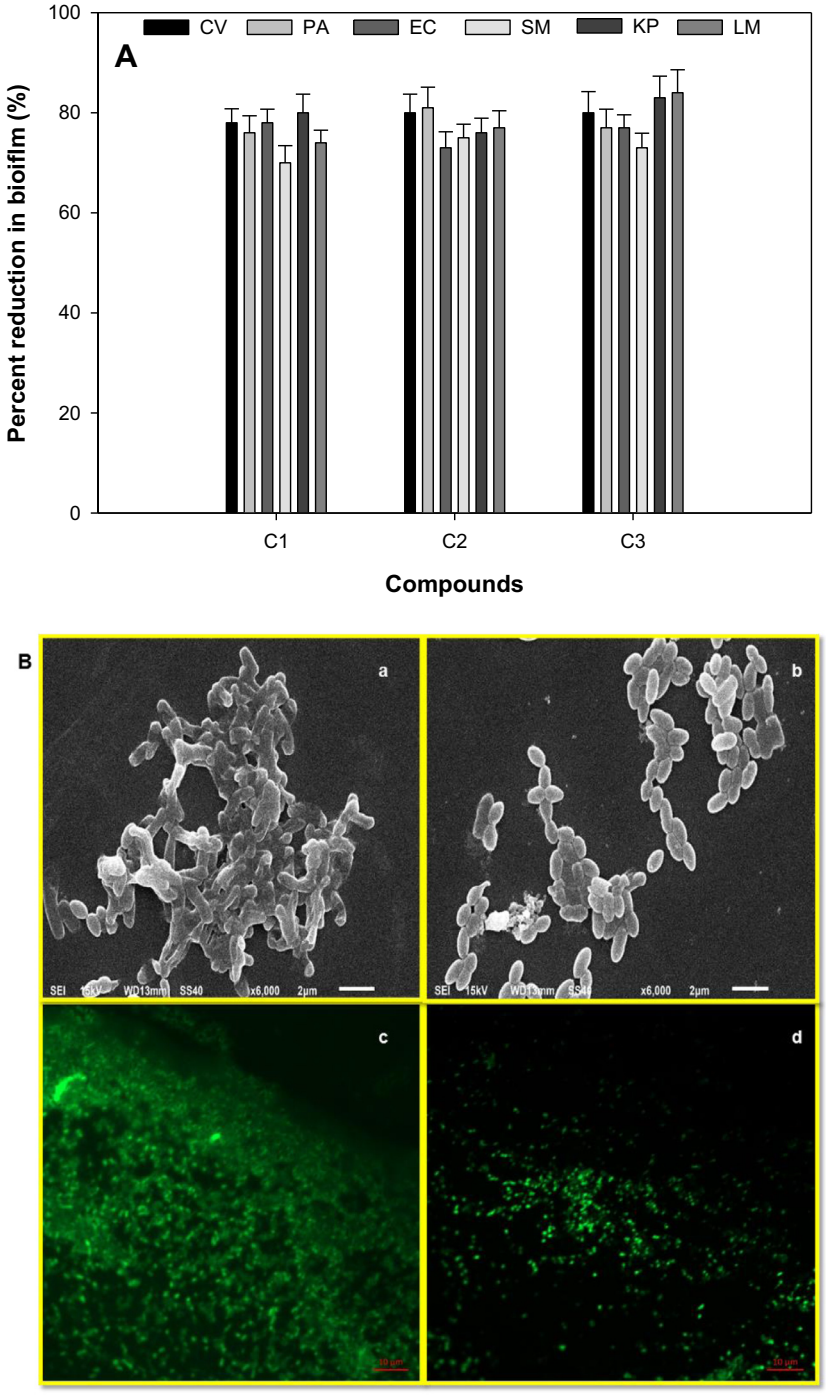

Fig. 9. A. Activity of ½ × MICs of Cu (I) compounds C1, C2 and C3 against biofilms of selected foodborne pathogens using microtitre plate assay. B. Microscopic images of biofilm reduction in foodborne pathogen *L. monocytogenes*. a. SEM image of *L. monocytogenes* that were untreated; b. treated with 16 μg/ml of C3; c. CLSM image of untreated *L. monocytogenes* and d. treated with 16 μg/ml of C3.

4. Conclusion

In summary, three tetrahedrally coordinated copper(I) complexes having the ligand Hnor, triphenylphosphane as a co-ligand and Halides Cl^-, Br^- and I^- as anions were synthesized. All the three complexes demonstrated significant antimicrobial properties against the selected test pathogens. At concentration below the MIC all, the three complexes interfered significantly with the quorum sensing regulated functions in *C. violaceum* (violacein), *P. aeruginosa* (elastase, pyocyanin and alginate production) and *S. marcescens* (prodigiosin). Beside the action of the copper(I) complexes on the EPS production and swarming motility, they also demonstrated potent broad-spectrum inhibition of biofilm formed by *Pseudomonas aeruginosa*, *E. coli*, *Chromobacterium violaceum*, *Serratia marcescens*, *Klebsiella pneumoniae* and *Listeria monocytogenes*. These findings could pave the way for further complexation of Cu (I) with known QS inhibitors to develop novel anti-infective agents that attenuate the virulence without effecting the growth, thus lowering the risk for development of resistance. Moreover, our results on QS and biofilm inhibition against six foodborne pathogens may form the basis for future research work to address biofilm control and removal in the food industry.

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