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Original article

Naringin inhibits the biofilms of metallo-β-lactamases (MβLs) producing *Pseudomonas* species isolated from camel meat

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ABSTRACT

Food producing animals harbouring bacteria carrying drug resistance genes especially the metallo-beta-lactamase (MBL) pose high risk for the human population. In addition, formation of biofilm by these drug resistant pathogens represents major threat to food safety and public health. In this study, metallo-β-lactamases (MβLs) producing *Pseudomonas* spp. from camel meat were isolated and assessed for their biofilm formation. Further, *in vitro* and *in silico* studies were performed to study the effect of flavone naringin on biofilm formation against isolated *Pseudomonas* spp. A total of 55% isolates were found to produce metallo-β-lactamase enzyme. Naringin mitigated biofilm formation of *Pseudomonas* isolates up to 57%. Disturbed biofilm architecture and reduced the colonization of bacteria on glass was observed under scanning electron microscope (SEM) and confocal laser scanning microscope (CLSM). The biofilm related traits such as exopolysaccharides (EPS) and alginate production was also reduced remarkably in the presence of naringin. Eradication of preformed biofilms (32–60%) was also observed at the respective 0.50 × MICs. Molecular docking revealed that naringin showed strong affinity towards docked proteins with binding energy ranging from −8.6 to −8.8 kcal mol^{-1}. Presence of metallo-β-lactamase producers indicates that camel meat could be possible reservoir of drug-resistant *Pseudomonas* species of clinical importance. Naringin was successful in inhibiting biofilm formation as well as eradicating the preformed biofilms and demonstrated strong binding affinity towards biofilm associated protein. Thus, it is envisaged that naringin could be exploited as food preservative especially against the biofilm forming foodborne *Pseudomonas* species and is a promising prospect for the treatment of biofilm based infections.

1. Introduction

Diseases associated with food are one of the major threat to the public health both in the developing and developed nations of the world (The European Union summary report on trends and sources of zoonoses, zoonotic agents and food-borne outbreaks in 2017, 2018). Microbial contamination of food can occur at any time during production and or consumption leading to disease outbreaks, illness, hospitalization and mortality (Srey et al., 2013). Gram negative bacteria (GNB) are the common cause of food spoilage and are responsible for foodborne infections due to the production of extracellular enzymes and toxins (Martinović et al., 2016; Rawat, 2015). Further, food producing animals harbouring drug resistance genes especially the metallo-beta-lactamase (MBL) pose high risk for the human population. MBLs confer resistance to GNB against β-lactam antibiotics and have put the use of carbapenemases to treat infections under serious threat. MBL producing drug resistant GNB amongst animals and poultry birds are a constant source for the dissemination of multi-drug resistant (MDR) pathogenic bacteria among humans (Chika et al., 2017). In addition, formation of biofilm by these drug resistant pathogens represents another major threat to food safety as it is found responsible for food spoilage, infections and economic loses (Alvarez-Ordóñez et al., 2019).

Biofilms have emerged as an enormous challenge for the food industry as they facilitate the bacteria to adhere to diverse surfaces

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including plastic, glass, stainless steel, rubber and even food products in a short span of few minutes and form a spatially organized community in a self-secreted matrix comprising of exoplymeric substances (EPS) (Flemming and Wingender, 2010; Hall-Stoodley et al., 2004). Biofilm provides protection to the bacteria against the action of chemicals, disinfectants, antimicrobials and stressful environmental conditions that they encounter in various habitats including the food processing environments (Alvarez-Ordóñez et al., 2019). Several food spoiling bacteria including *Pseudomonas*, *Serratia*, *Acinetobacter*, *Enterococcus*, *E. coli*, *Shewanella* have been reported to form biofilms by adhering to food contact surfaces (Vilanova et al., 2015; Yuan et al., 2018). In US, 80% of the bacterial infections have been linked with foodborne pathogens living in biofilm mode (Srey et al., 2013). Biofilms have proved problematic in various food industries including meat processing. Organic residues in meat processing provide a niche for the accumulation of bacteria and biofilm formation and are a source of cross-contamination also (Sofos and Geornaras, 2010; Srey et al., 2013).

Gram negative bacteria *Pseudomonas* is known to occupy various ecological niches and often constitutes the indigenous microbiota of the foods. These bacteria easily contaminate and spoil food items as they are capable of utilizing diverse nutrients for growth and are psychrotrophic possessing lipolytic and proteolytic properties (Remenant et al., 2015). In the meat processing industry, *Pseudomonas* sp. poses a serious threat as it can adhere to the surfaces, form biofilm, allowing these bacteria to persist and survive stress conditions (Sterniša et al., 2019). The bacteria not only spoil the food products but also combine with other pathogens to develop multispecies biofilms, causing cross-contamination of other food products, reducing shelf life and increasing the foodborne transmission of diseases (Bridier et al., 2015). Therefore, control of biofilm formation by *Pseudomonas* on foods (especially meat) and contact surfaces requires urgent attention. Safe, nontoxic and stable antibiofilm agents are needed to combat the menace.

Plant based phenolic compounds have been well documented to inhibit bacterial growth and biofilm (Al-Yousef et al., 2017; Gopu et al., 2015; Husain et al., 2018; Saleem et al., 2010). Naringin is a flavone glycoside produced from naringenin and neohisperidose and is a constituent of citrus and grape fruits, cherries, cocoa and oregano. It has been widely exploited in the Chinese herbal medicine and is known to be anti-inflammatory and potent antioxidant (Chen et al., 2016). Few reports are also available on the antimicrobial and biofilm inhibitory potential of naringin against pathogenic bacteria (Céliz et al., 2011; Dey et al., 2020; Vikram et al., 2010).

Camel meat is consumed on a regular basis in the middle east. It is one of the best meat and is not very harmful to the human heart as it contains low percentage of fat as compared to other red meat (Kadim, 2009). Fresh camel meat is a product in demand and by 2022 it is predicted to constitute 22% of the total market share. In Europe, Middle east and Africa camel meat makes up for 45% of the market and it is predicted that by 2022 in addition to these regions, Asia pacific region will also be a huge market for camel meat (Osman et al., 2019). The demand for camel meat is ever increasing in Kingdom of Saudi Arabia. Previously it was believed that camels were not affected by most diseases that were common to livestock. However, recent reports have indicated towards the susceptibility of camels to various disease-causing pathogens. Camels are now considered as a reservoir and carrier of various diseases of animals and zoonoses (Graveland et al., 2011).

Most of the studies till date have focussed on the prevalence of drug-resistant, biofilm forming *Pseudomonas* in camel meat (Elhariri et al., 2017; Osman et al., 2019; Rhouma et al., 2018); the strategies to curb the menace of biofilm formed by foodborne pathogens present in camel meat is still unexplored. Keeping in mind the importance and consumption of camel meat in Saudi Arabia and the scarcity of data about the colonization and distribution of drug-resistant *Pseudomonas* in camel meat in this region, we isolated metallo-β-lactamases (MβLs) producing *Pseudomonas* spp. from camel meat. The isolates were screened for biofilm formation on polystyrene plates under *in vitro* conditions. Further, the biofilm inhibitory activity of naringin was studied against the strong biofilm forming isolates of *Pseudomonas* spp *in vitro* and visualized under microscope. The ability of naringin to eradicate the preformed biofilms was also assessed. Finally, molecular docking of naringin with biofilm associated proteins of *P. aeruginosa* was performed to obtain a closer insight.

2. Material and methods

2.1. Isolation of Pseudomonas spp. from camel meat

A total of 45 retail camel meat samples (25 g pieces) were bought from domestic market in Riyadh under aseptic conditions. The samples in sterile Stomacher bags containing 300 ml maximal recovery diluent (Oxoid, Basingstoke, UK) were homogenized and 100 μl of serially diluted homogenate specimens were placed on Cetrimide agar. *Pseudomonas* isolated were identified by Gram's-staining, and biochemical tests like catalase, oxidase and urease activity, casein and starch hydrolysis, citrate and indole utilization, and Methyl Red–Voges Proskauer and gelatin liquefaction tests, using standard microbial techniques. Growth with or without the production of pigment was determined on various growth media, *Pseudomonas* F medium (King B medium; fluorescein or pyoverdin, a green/yellow pigment) or *Pseudomonas* P medium (King A medium; pyocyanin, a blue/green pigment) at 37 °C. In the absence of visible pigment, colonies were examined using UV illumination (Osman et al., 2019).

2.2. Detection of metallo-β-lactamases (MBL)

Isolated bacteria were further screened for the detection of MBL. Disc potentiation assay using EDTA impregnated imipenem and meropenem discs were used for the detection of MBL (Zubair et al., 2011).

2.3. Biofilm formation by bacterial isolates

The isolates were first tested for their biofilm forming ability in polystyrene microtitre plate. Briefly, the bacteria were cultured in LB broth in a 96-well microtitre plate at 37 °C for 24 h. The growth media was decanted and the wells of microtitre plate were washed with sterile phosphate buffer. Biofilm was then stained for 20 min with crystal violet (0.1% w/v). The wells were again washed to remove the excess dye. The staining dye was then dissolved in ethanol (90%) and the optical density was recorded at 600 nm using microtitre plate reader (Husain et al., 2017). Bacteria were classified as strong and weak biofilm formers using the criteria described previously (Al-Shabib et al., 2017).

2.4. Determination of MIC and MBC

Microbroth dilution method using growth indicator dye (2,3,5-triphenyltetrazolium chloride) was used for determining the minimum inhibitory concentrations (MIC) and minimum bactericidal concentrations (MBC) of naringin against isolated *Pseudomonas* spp. (Qais et al., 2019). Bacteria were cultured overnight in the absence and presence of different concentrations of naringin at 37 °C. Growth indicator TTC (20 μl) was added to each well and incubated for 20 min. The metabolically active cells were indicated

by the development of pink/red color in the wells. The absence of the color at the lowest concentration was taken as MIC.

2.5. Inhibition of biofilm by naringin

The ability of naringin to inhibit the biofilm formation was assessed in polystyrene microtitre plate using crystal violet (O'Toole and Kolter, 1998). Briefly, bacteria were cultured in the absence and presence of sub-MICs (0.25 × MIC and 0.50 × MIC) of naringin under static conditions at 37 °C for 24 h. Biofilm formation was determined as described in the previous section. Percent reduction was calculated using the formulae: $[(ODcontrol - ODtreated) \div ODcontrol] \times 100$.

2.6. Microscopic analysis of biofilm inhibition

2.6.1. Scanning electron microscopy

The effect of naringin on biofilm inhibition was validated through scanning electron microscopy (Maheshwari et al., 2019). Briefly, naringin treated and untreated bacteria were grown on sterile glass coverslips in 24 well polystyrene culture plates for 24 h under static condition. The coverslips were removed from the wells and loosely adhered cells were removed by washing. Biofilm was fixed overnight with glutaraldehyde (2.5%) at 4 °C. Following fixation, the biofilm was dehydrated by treating with gradient of ethanol (10, 30, 50, 80, and 100%) for 15 min each. The coverslips were dried in air completely and sputter coated with gold just before visualization under SEM (JEOL-JSM, 6510LV).

2.6.2. Confocal laser scanning microscopy (CLSM)

For confocal microscopy, biofilms of test bacteria were developed on glass coverslips as mentioned above. The coverslips were gently washed, air-dried and stained with acridine orange (0.1%) in dark for 15 min. Excess dye was removed by washing and air-dried coverslips were visualized under CLSM (Zeiss, Germany).

2.7. Assessment of exopolysaccharides (EPS) production

The inhibition EPS production by naringin was determined by quantifying the sugars in cell supernatant of treated bacteria (Huston et al., 2004). The naringin (0.25 × MIC and 0.50 × MIC) treated and untreated bacteria were grown for 18 h and supernatant was obtained by centrifugation. The EPS was precipitated from the supernatant by adding chilled ethanol (3 volumes) and incubating overnight at 4 °C. Concentration of EPS was quantified by Dubois method (DuBois et al., 1956).

2.8. Assessment of alginate production

The effect of sub-inhibitory concentrations of naringin on alginate production was assessed by standard procedure as described earlier (Gopu et al., 2015). Bacterial cultures were grown for 18 h under shaking conditions. Test bacteria (70 µl) was added to 600 µl of boric acid-sulphuric acid solution and kept on ice bath for proper mixing. Further, 20 µl of carbazole was added to the mixture. The reaction mixture was vortexed and incubated at 55 °C for half an hour and the absorbance was recorded at 530 nm.

2.9. Disruption of mature biofilms

Naringin was also tested for its ability of disrupt the mature biofilms using previously described method (Qais et al., 2018). Briefly, the bacteria were grown in 96-well microtitre plate for 24 h to form the biofilms. The media was decanted and washed gently to remove loosely bound cells. Wells were filled with fresh media containing respective 0.50 × MICs of naringin and incubated overnight. The media was removed and wells were stained with crystal violet as mentioned above. Absorbance was recorded at 600 nm.

2.10. Molecular docking

Molecular docking was performed by analyzing the binding of naringin with biofilm associated proteins *viz.* Pil family proteins (PilT and PilY1). AutoDock vina was used for docking calculations as it is known to do more accurate calculations (Pandya et al., 2014; Trott and Olson, 2009). The 3D structure of PilT [PDB: 3JVV] and PilY1 [PDB: 3HX6] were obtained from RCSB Protein Data Bank. All ions, water molecules and ligands if any, in the receptor molecules were removed and only monomer of each protein was selected for docking. Non-polar hydrogens atoms were merged followed by addition of Kollman charges and the coordinate file was saved into PDBQT format using MGL Tools-1.5.6 (Morris et al., 1998). The 3D structure of naringin was downloaded from PubChem (https://pubchem.ncbi.nlm.nih.gov) in SDF format and converted to PDB format using Chimera 1.14. The ligand was made flexible by detecting roots and finally saved in PDBQT format. The size and centre for each receptor proteins is mentioned in Supplementary Table S1. The conformation with lowest biding energy was selected for analysis using Discovery Studio 2020, Maestro 12.3, and PyMOL 2.3.3.

2.11. Statistical analysis

Experiments were performed in triplicates and the data are presented as average ± SD. Student's *t*-test was employed to determine the level of significance between control and treated groups.

3. Results

3.1. Isolation of Pseudomonas spp. from camel meat

A total of 18 *Pseudomonas* spp. were isolated from the tested camel meat samples. All the 18 isolated bacteria were screened for the production of metallo-β-lactamase enzyme and 55% (10) isolates were found to be positive for MBL production.

3.2. Biofilm forming ability of isolates

The MBL producing *Pseudomonas* spp. isolated from camel milk were vetted for biofilm formation under *in vitro* conditions on polyvinyl microtiter plates. Based on $OD_{600\ nm}$ of crystal violet extracted from formed biofilms of test bacteria, a varying degree of biofilm formation was observed (Fig. 1). Five isolates *viz.* CAP9, CAP31, CAP32, CAP37, and CAP41, were found to be strong biofilm formers with OD more than 0.85. Other isolates showed either moderate or weak biofilm forming potential.

3.3. MIC and MBC determination

The MIC and MBC of naringin against test isolates were determined before assessing the antibiofilm activity. The MIC of naringin ranged from 128 to 512 µg/ml while, MBC was recorded in the range of 128–1024 µg/ml (Supplementary Table S2). All antibiofilm and related experiments were carried out at the respective 0.25 × MICs and 0.50 × MICs. The antibiofilm activity was only assessed against the isolates exhibiting strong biofilm forming ability.

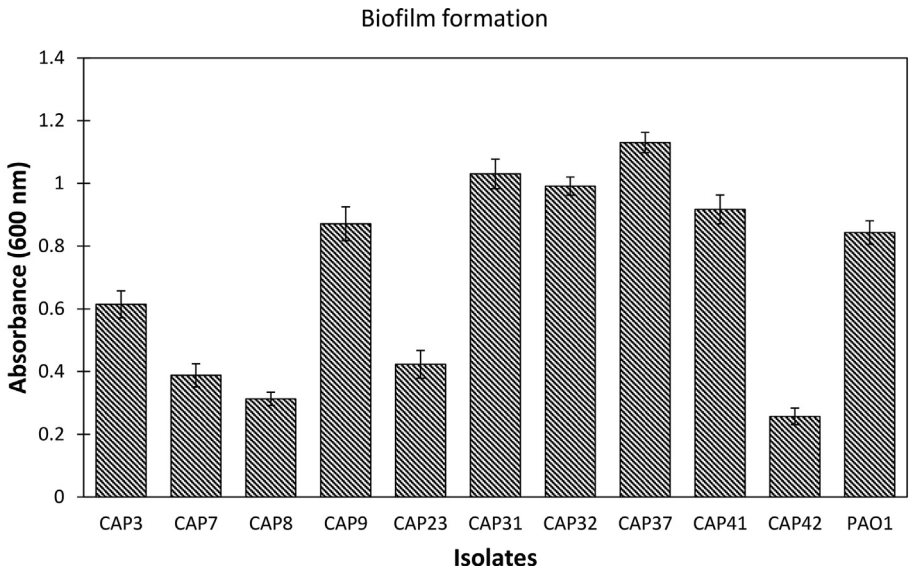

Fig. 1. The biofilm forming ability of metallo-β-lactamases producing *Pseudomonas* spp. Isolated from the camel meat. The data presented is average ± SD of three replicates.

3.4. Inhibition of biofilm formation by naringin

A varying levels of biofilm inhibition by naringin was recorded against the test isolates (Fig. 2). There was 40.07 and 48.06% inhibition of biofilms of CAP9 in the presence of 0.25 × MIC and 0.50 × MIC, respectively. More than 60% reduction in biofilm formation of CAP31 was observed by the treatment of 0.50 × MIC. Similarly, the presence of the respective 0.50 × MICs of CAP32 and CAP37 decreased the biofilms by 43.03 and 39.06% compared to their respective controls. A similar trend for biofilm inhibition of CAP41 was also recorded with 57.06% inhibition at 0.50 × MIC.

The quantitative biofilm data was further verified microscopically. The biofilm inhibition of one strong biofilm forming *Pseudomonas* isolate (CAP31) by naringin was explored. As evident from the SEM images (Fig. 3), the untreated *Pseudomonas* isolate (CAP31) formed a mat like structure with dense matrix of exopolysaccharides. The treatment with naringin (0.25 × MIC and 0.50 × MIC) resulted in decreased biofilm formation. Moreover, the cells were scattered indicating the lesser adherence of bacterial cells to glass coverslips. The content of exopolysaccharides was also reduced. Similar observations were observed with images of confocal microscopy (Fig. 4). Bacterial cells were present in thick clusters in the untreated slides that got remarkably

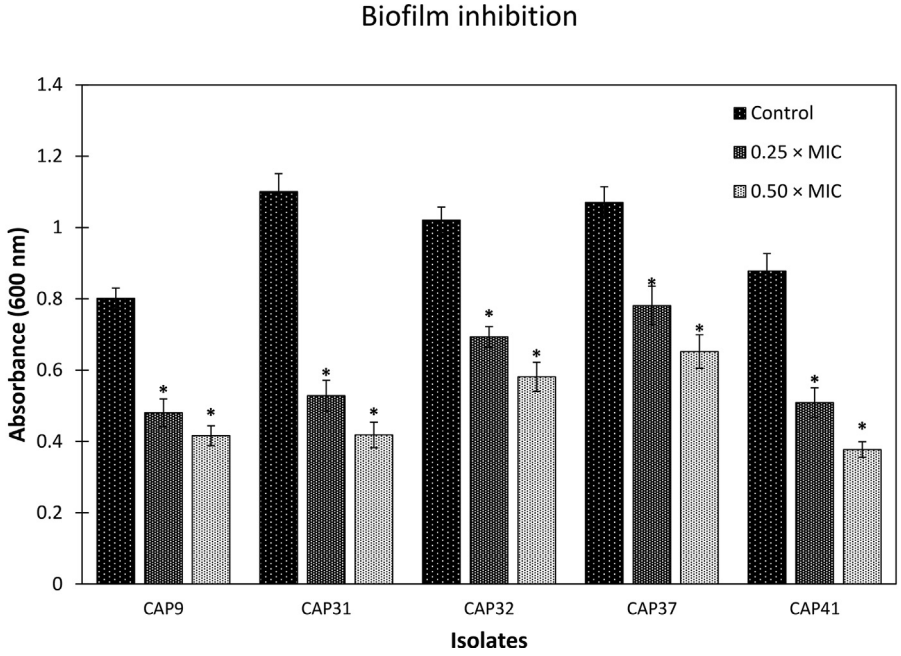

Fig. 2. Effect of naringin on the biofilm formation of metallo-β-lactamases producing *Pseudomonas* spp. The data presented is average ± SD of three replicates. * indicates P value ≤ 0.05.

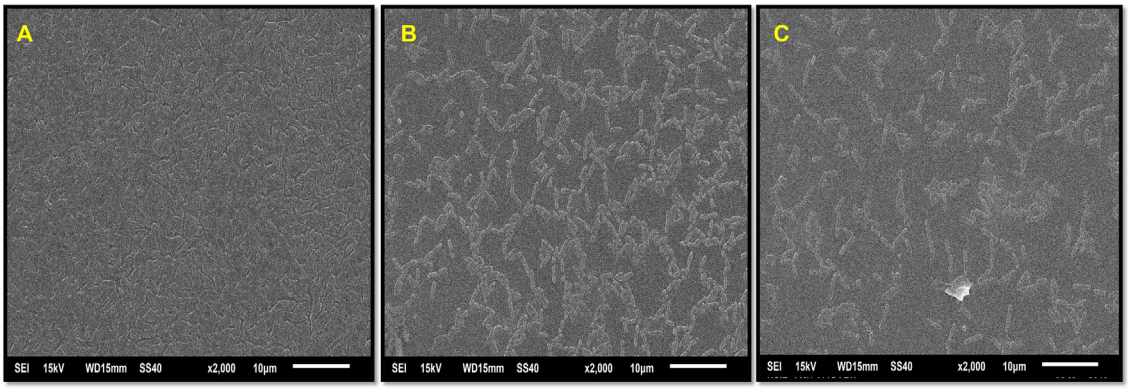

Fig. 3. Scanning electron microscopic analysis of naringin treated biofilm of CAP31. (A) Untreated control; (B) 0.25 × MIC and; (C). 0.5 × MIC.

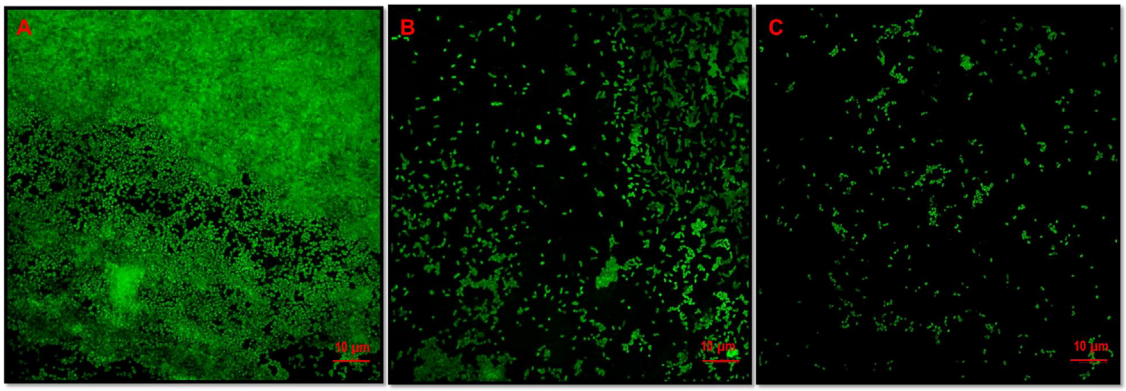

Fig. 4. Confocal laser scanning microscopic analysis of naringin treated biofilm of CAP31. (A) Untreated control; (B) 0.25 × MIC and; (C). 0.5 × MIC.

reduced in the presence of sub-inhibitory concentrations of naringin.

3.5. Inhibition of EPS production by naringin

Based on the positive correlation between EPS production and biofilm formation, exopolysaccharides (EPS) extracted from naringin treated and untreated cultures of *Pseudomonas* spp. was evaluated spectrophotometrically and results obtained are presented in Fig. 5A. The EPS production of CAP9 was reduced by 24.08 and 39.00% by the treatment of 0.25 × MIC and 0.50 × MIC, respectively. At 0.50 × MIC, the EPS production of CAP31 was inhibited by more than 50%. Similarly, the supplementation of naringin (respective 0.50 × MICs) in culture media reduced the EPS production of CAP32 and CAP37 by 55.03 and 65.10, respectively. Same trend was also observed for the EPS of CAP41 with more than 55% inhibition at 0.50 × MIC.

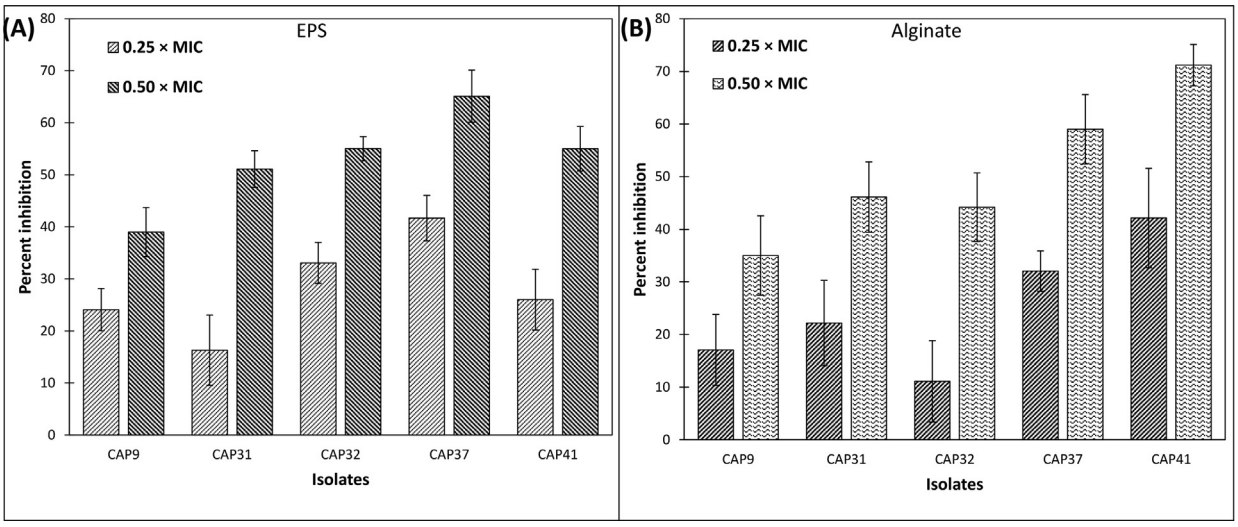

Fig. 5. Effect of naringin on the (A) EPS production of *Pseudomonas* spp; (B) alginate production of *Pseudomonas* spp. The data presented is average ± SD of three replicates.

3.6. Inhibition of alginate production by naringin

Alginate is an integral component of the EPS of *Pseudomonas* biofilm. Treatment of sub-MICs of naringin resulted in a dose-dependent inhibition of alginate production in tested isolates (Fig. 5B). There was 17.08 and 35.01% inhibition of alginate production in CAP9 by the treatment of 0.25 × MIC and 0.50 × MIC, respectively. At highest sub-MIC (0.50 × MIC), the alginate production of CAP31 was reduced by 46.12%. Similarly, 46.12 and 44.20% reduction were observed in CAP31 and CAP32 in the presence of respective 0.50 × MICs. The maximum inhibition of alginate production by 72.20% was recorded in CAP41 by naringin (0.50 × MIC).

3.7. Disruption of mature biofilms by naringin

Naringin was assessed for its ability to eradicate mature biofilms of isolates at their respective 0.50 × MIC and the results obtained are presented in Fig. 6. A moderate level of biofilm eradication (33.02 and 32.07%) was observed in CAP9 and CAP37, respectively. The mature biofilms of CAP31, CAP32, and CAP41 were reduced by 55.05, 60.00, and 58.09% in the presence of respective 0.50 × MICs of naringin.

3.8. Molecular docking

The binding energy for the interaction of naringin with PilT and PilY1 were found to be −8.6 and −8.8 kcal mol^{-1}, respectively. Naringin formed hydrogen bonds with Ser16, Ser134, Lys136, and Arg276 of PilT (Fig. 7). Moreover, the complex was also stabilized by the hydrophobic interactions between Leu109, Leu268, and Ala278 of PilT and naringin. Similarly, the naringin interacted with Gln652, Thr792, and Arg848 of PilY1 by hydrogen bonds with bond length as 2.53, 2.69, and 3.01 Å, respectively, as shown in Fig. 8. Additionally, other amino acids such as Val734, Lys790, Ala794, Leu849, and Ala858 were also involved in the interaction by hydrophobic forces.

4. Discussion

Biofilms are the organized communities of microbes which remain enclosed in a EPS matrix in which polysaccharides are the major component (Flemming and Wingender, 2010). Bacteria under biofilms are more resistant to antimicrobials and are also capable of tolerating adverse conditions. We observed that 50% of the MBL producing isolates formed strong biofilm. Many studies have demonstrated that bacteria related to food spoilage form biofilms on abiotic surfaces. *Pseudomonas lundensis* isolated from Chinese pork meat has been documented to form strong biofilms (Liu et al., 2015). Moreover, in a study on fresh food products, 22 isolated bacterial strains were capable of forming biofilms (Bae et al., 2014). Once the biofilms are formed, it is tough to get rid of such food spoiling bacteria by chemical means. Therefore, we have studied the biofilm removal strategy of the isolated bacteria by naringin in the next section of this study.

Naringin demonstrated statistically significant reduction in the biofilm formed by the drug-resistant *Pseudomonas* species isolated from the camel meat (Fig. 2). The findings of the microtiter plate (MTP) assay were further validated by SEM and CLSM analysis as decreased aggregation and disturbed biofilm architecture was observed in sub-MICs treated cells (Figs. 3 and 4). The food industry standard nitric acid (75 °C, 1.8%, 30 min) and caustic (75 °C, 2%, 30 min) treatments at Reynolds number >2000 has been reported to eradicate films of *A. flavithermus* from cold-rolled 316 stainless steel (Parkar et al., 2004). Conversely, it has also been reported that 1% NaOH and 1.0% nitric acid treatment did not ensure the reproducible elimination of polymicrobial biofilm (Bremer et al., 2006). Moreover, the food itself can't be treated with such hazardous chemicals to remove biofilms. Therefore, an alternative strategy is needed to inhibit or reduce the colonization of bacteria by inhibiting their biofilms. Naringin successfully inhibited the biofilms of isolated bacteria and can be exploited as biofilm inhibitor in the food processing.

Exopolysaccharides not only help in the maintenance of the biofilm architecture but also protect the biofilm from harsh environments. Moreover, biofilms also confer resistance to microbes

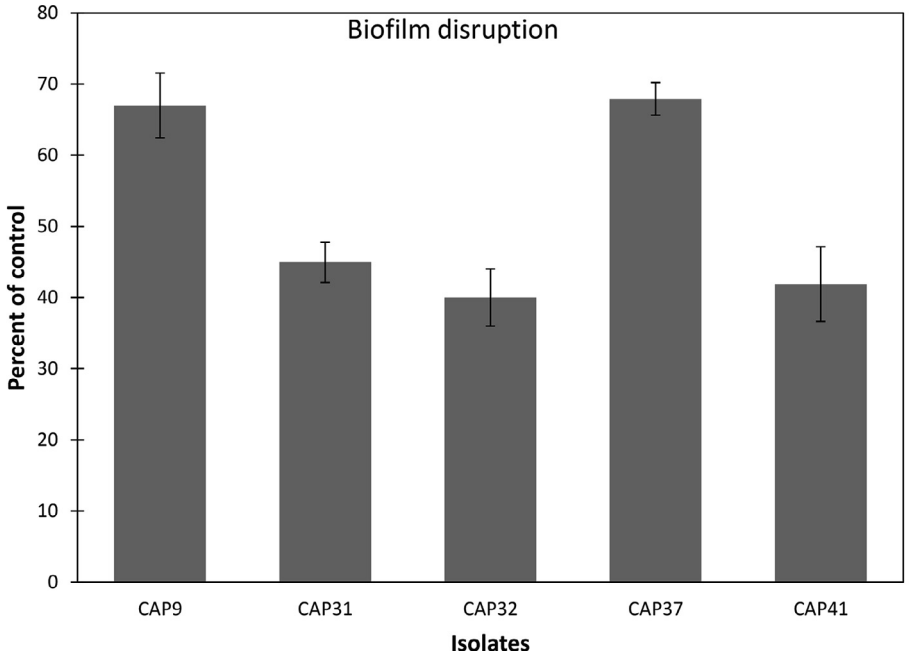

Fig. 6. Effect of naringin (0.5 × MIC) on the disruption of preformed biofilms of metallo-β-lactamases producing *Pseudomonas* spp. The data presented is average ± SD of three replicates.

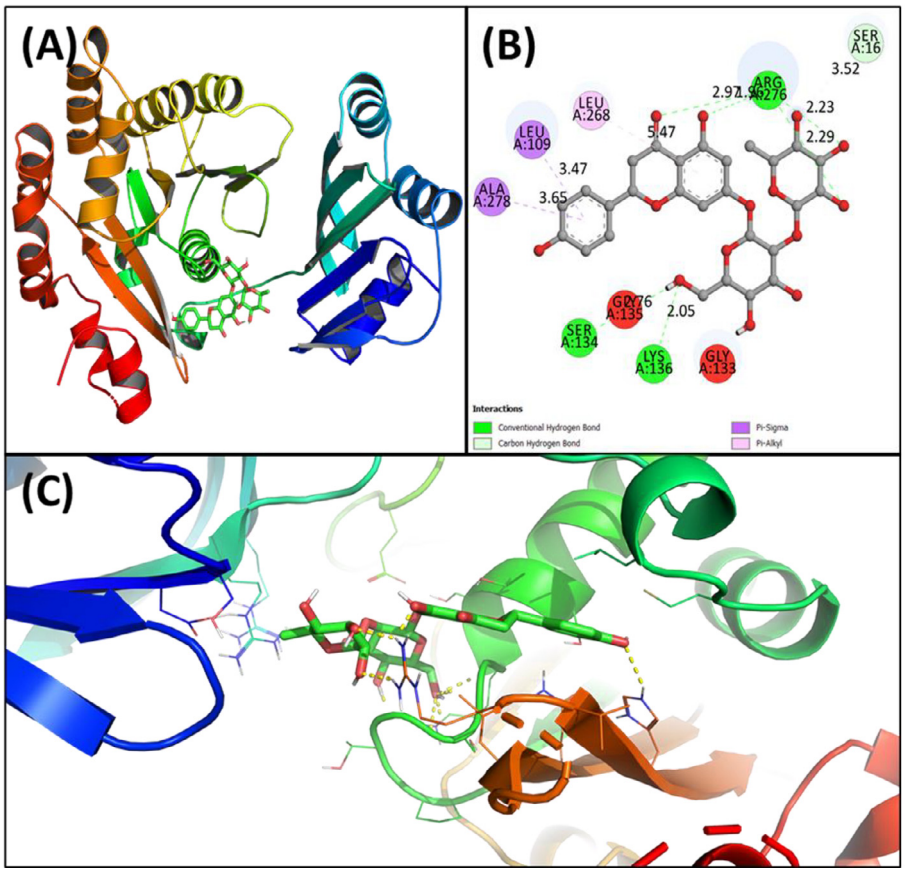

Fig. 7. Molecular docked pose of PilT complexed with naringin. (A) Full view of the docked pose of the complex, protein is shown as ribbons and naringin is shown as green sticks. (B) 2-dimensional view by Discovery Studio 2020. (C) Naringin is shown with the interacting amino acids of PilT.

encapsulated in biofilms by limiting the entry of antibiotics inside the matrix (Fux et al., 2005). Therefore, any interference with EPS production will expose the biofilm cells to the antimicrobials leading to eradication of biofilm (Yildiz and Schoolnik, 1999). Considerable decrease in EPS production ranging from 39% to 65% was recorded in the isolated strains at the highest tested concentration (0.5xMIC) of naringin (Fig. 5A). The findings of this study corroborate well with the results reported from our lab on the inhibition of single and mixed biofilm of drug resistant bacteria isolated from ready to eat food by rutin (Al-Shabib et al., 2017).

Changing environmental conditions are chiefly responsible for the alginate synthesis by *Pseudomonas aeruginosa*. Alginate plays dual role of protecting the cells from hostile external conditions and boosts the adhesion of bacteria to the substratum. Thus, alginate is vital for the biofilms formed by *Pseudomonas* (Boyd and Chakrabarty, 1995). Naringin effectively reduced the production of alginate in all test isolates at sub-MICs (Fig. 5B). Considering the importance of alginate production in biofilm maintenance any interference in its production will adversely affect biofilm formation.

Bacteria growing in biofilm mode are more several folds more tolerant to antibiotics and other chemotherapeutic agents as compared to their planktonic forms (Khan et al., 2014). This makes eradication of already formed biofilms a herculean task. The findings clearly demonstrate the ability of naringin to eradicate mature biofilms of bacterial isolates. The results are supported by our previous findings where azorubine disrupted the mature biofilms of *C. violaceum* ATCC 12472, *E. coli* O157:H7, *P. aeruginosa* PAO1, *L. monocytogenes* ATCC 19114, and *S. marcescens* ATCC 13880 (Al-Shabib et al., 2020). Based on the results of alginate and EPS production inhibition, it may be concluded that naringin penetrates the biofilm and eliminates the preformed mature biofilm of the test bacteria.

Molecular docking analysis was done to obtain the binding site of naringin in the proteins involved in biofilm formation of *P. aeruginosa* (Burrows, 2012). Naringin exhibited strong binding with biofilm associated proteins, *viz.* Pil family proteins PilY1 and PilT. Previously, it has been reported by molecular docking that the binding energy for the interaction of plumbagin to PilY1 and PilT were −4.2 and −5.8 kcal mol^{-1}, respectively (Gupta et al., 2017). Moreover, the binding energy of phytocompounds of green tea with these two proteins ranged from −4.9 to −7.0 kcal mol^{-1} (Qais et al., 2019). The strong binding affinity between naringin and such proteins could be responsible for the biofilm inhibitory action of the flavone as recorded by *in vitro* assays.

5. Conclusion

In conclusion, nearly 55% of *Pseudomonas* spp. isolated from camel meat of domestic market of Riyadh were metallo-β-lactamase producers. Present investigation demonstrates that camel meat is a reservoir of drug-resistant *Pseudomonas* species of clinical importance. Further, the study also demonstrates the antibiofilm activity of naringin against MβL producing *Pseudomonas* spp. Microscopic studies validated the biofilm inhibitory property of naringin. Production of EPS and alginate that are key to the maintenance and dispersal of biofilm was also reduced remarkably. Naringin was also successful in eradicating the preformed biofilms of test bacteria. Molecular docking revealed that naringin showed strong binding affinity towards the protein

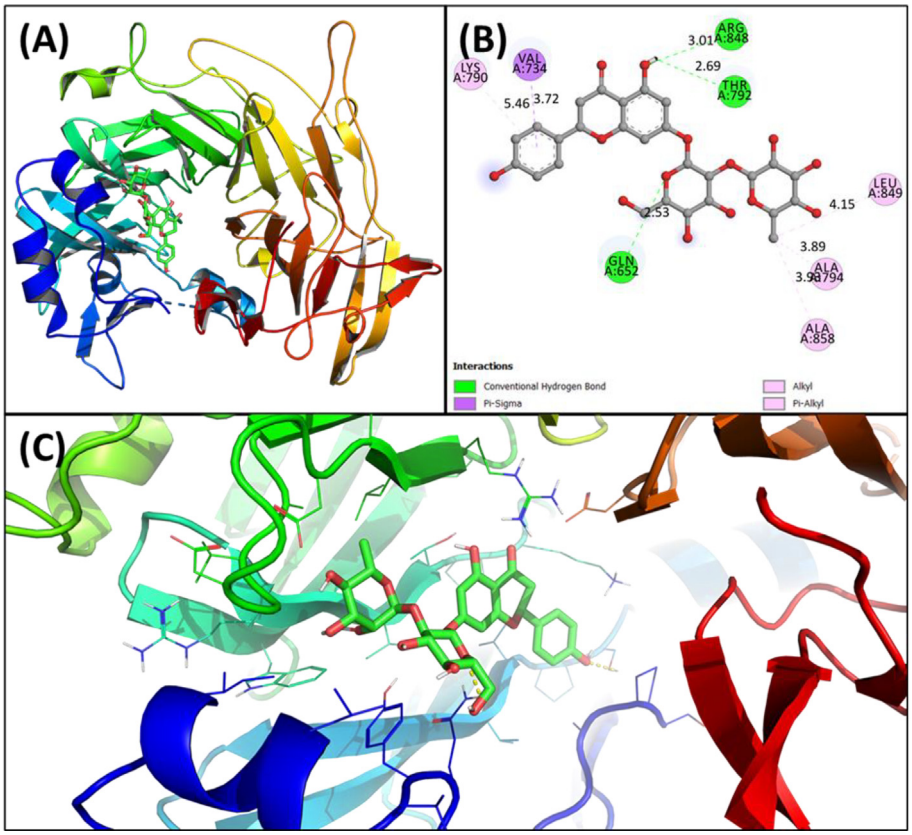

Fig. 8. Molecular docked pose of PilY1 complexed with naringin. (A) Full view of the docked pose of the complex, protein is shown as ribbons and naringin is shown as green sticks. (B) 2-dimensional view by Discovery Studio 2020. (C) Naringin is shown with the interacting amino acids of PilY1.

associated with biofilm formation. Outcomes of the present investigation indicate the possible use of naringin as food preservative especially against the biofilm forming food-borne *Pseudomonas* species and is a promising prospect as therapeutic agent to treat biofilm based persistent infections.

Declaration of Competing Interest

The authors declare no conflict of interest.

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Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.sjbs.2020.10.009.

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