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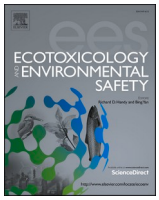

Impact of metal exposure on environmentally isolated *Serratia marcescens'* growth, oxidative-stress resistance, biofilm formation, and proliferation in eukaryotic co-culture models

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ABSTRACT

Environmental metals can be noxious to the surrounding biota, indirectly impact freshwater habitats, and also impact microbiological communities. In this study, zinc (Zn) (55.5 mg/kg), manganese (Mn) (863.4 mg/kg) and lead (Pb) (17.5 mg/kg) levels measured in Houston watershed flood plain soil samples were higher than environmental agencies' thresholds. To investigate the effects of metal exposures, an environmentally isolated *Serratia marcescens* (SME), etiological agent of endocarditis and respiratory infections, and its reference strain (SMR) were exposed to Pb, Zn, and Mn, and subsequent oxidative stress responses and biofilm production were measured. Not surprisingly, SME was less sensitive to all 3 metal exposures than was SMR. Interestingly, SME produced increased biofilm and was more resistant to oxidative stress in the presence of Zn and Pb than SMR. In a 6 h lung infection model using BAES-2B cells, SME exhibited greater proliferation than SMR in all metal challenges. Similarly, in our HT29 gut infection model, SME out-proliferated SMR when challenged with Pb and Mn following the 6 h infection. Taken together, SME was better able to withstand environmental stressors than SMR, suggesting increased virulence potential of this opportunistic human pathogen.

1. Introduction

Elevated concentrations of environmental metals can pose human health threats, and persistent environmental pollutants can be introduced into the environment via anthropogenic activities. Superfund sites are areas that require removal of hazardous materials released by local industry and often contain elevated levels of metals, which can negatively impact freshwater habitats and their microbiological communities (Gough and Stahl, 2011). Further, heavy metals, like Zn and Pb are typically found in high concentrations during metal analysis of superfund sites soil samples (Hussein and Joo, 2013). Consequently, bacteria have developed a variety of resistance mechanisms to counteract heavy metal exposures (Teitzel and Parsek, 2003). More specifically, bacteria may form or isolate metal complexes, reduce metals to a less toxic state, or expel the metal from within the cell (Ahemad, 2019; Prabhakaran et al., 2016). Heavy metals can also induce the production of reactive oxygen species within bacteria, which then cause an imbalance in the cellular oxidative status of bacteria and interfere with protein synthesis and function (Behera et al., 2014; Shahid et al., 2014). Elevated ROS can lead to the expression of bacterial detoxifying enzymes, like catalase, to neutralize H_20_2 and promote damage repair (Faulkner and Helmann, 2011).

Human diseases associated with metal exposures include lung damage, skin rashes, high blood pressure, memory loss, etc. (Mahurpawar, 2015; Rehman et al., 2018). More specifically, cadmium-chloride was shown to promote inflammation in the mouse gut but did not subsequently influence *Salmonella* infectivity (Breton et al., 2016). With regards to microbiota, the gut has the highest concentration of microbial organisms in the human body (Tchaptchet and Hansen, 2011). The microbial environment of the gut can promote dysbiosis and potentially lead to numerous diseases including: gastroenteritis, obesity, and irritable bowel syndromes (Cao et al., 2014; Khan et al., 2014). A number of these gut bacteria, like *Escherichia coli*, has been shown to cause different bowel dysfunction including chronic intestinal inflammation attributed to alterations in bacterial gene expression (Patwa et al., 2011).

In this study, an environmentally isolated *Serratia marcescens* (SME),

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opportunistic pathogen and member of the *Enterobacteriaceae* family, was exposed to metal toxicants found in Houston watersheds, and its various responses to abiotic stressors and eukaryotic cell co-culture were evaluated. *S. marcescens* has been shown to cause both lung (González-Juarbe et al., 2015) and gut infections (Ochieng et al., 2014) in humans, and, as a result, is an opportunistic pathogen of concern. More specifically, we isolated *S. marcescens* from both the Buffalo and Dickinson Bayous. Since elevated levels of Zn, Pb, and Mn were measured in our bayou samples, the aforementioned metals were used to challenge both SME as well as a reference strain (SMR) that we commercially acquired. Following metal challenge, growth kinetics, biofilm production, oxidative stress resistance, and interactions with human lung and gut cell lines were observed to determine whether the environmental isolate exhibited any beneficial adaptations.

2. Materials and methods

2.1. Bacteria strains, growth media, and metal stock solution preparation

S. marcescens SMR (Carolina item #: 155455), and SME (from the Buffalo Bayou, identified using the Biolog Microstation Gen III Microbial ID Assay with latitude 29.75948; longitude −95.33) were the bacterial strains used in this study. For all experiments, Luria Bertani (LB) broth (BD Difco™) medium was used to grow bacterial strains with agitation (250 rpm) at 37 °C. Growth on solid medium was achieved using LB agar (BD Difco™) plates. All absorbance readings were taken using a Bio-Tek™ Elx™800 microplate reader. Stock solutions were made from Pb $(NO_3)_2$ (Carolina item #: 10099–74–8), Zn $(C_2H_3O_2)_2.2\ H_2O$ (Carolina item #: 5970–45–6) and $MnSO_4.H_2O$ (Carolina item #: 10034–96–5) in parts per million (PPM) using sterile distilled water.

2.2. Eukaryotic cell lines

BEAS-2B (ATCC #: CRL-9609) normal human bronchial epithelial cells and HT29 (ATCC #: HTB38) human colon epithelial cells were cultured in T-75 flasks (Corning CLS431082).

using Dulbecco's modified Eagle medium (DMEM) (ATCC 30–2002) and Eagle minimal essential medium (EMEM) (ATCC 30–2003) supplemented with 10% fetal bovine serum (Thermo Fisher 16140071) and 5% penicillin-streptomycin cocktail (Thermo Fisher 15140122). Cells were incubated at 37 °C with 5% CO_2, and flask medium was changed every 3 days.

2.3. Measurements of metals

Concentrations of metal elements in soil samples were estimated by using inductively coupled plasma mass spectrometry (ICP-MS). Following treatment of 0.5 g of soil with 10 mL nitric acid (HNO_3), samples were placed into Mars 6 microwave vessels. Subsequently, they were digested using the EPA 3015a method (Link et al., 1999). Digested samples were then further digested for another 24 h. Finally, 0.2 μL of supernatant was diluted in water and analyzed by ICP-MS following calibrations with appropriate standards, and samples were statistically analyzed using MINITAB software (MINITAB Inc., State College, PA, USA).

2.4. Growth kinetic analysis

Previously described methods (Suraju et al., 2015; Bado et al., 2017, 2018) for growth analysis were employed with slight modifications. Briefly, $Pb(NO_3)_2$, Zn $(C_2H_3O_2)_2.2\ H_2O$, and $MnSO_4.H_2O$ were diluted in sterile, distilled water. Since 50 μg/mL and 500 μg/mL represent the environmental thresholds, concentrations of 10 μg/mL, 50 μg/mL, 100 μg/mL, 500 μg/mL and 1000 μg/mL of each were prepared. Saturated cultures of SME and SMR, grown in LB broth, were diluted to a starting optical density (OD) of 0.2 $OD_{600\ nm}$ in 96-well plates containing a volume of 200 μL of LB/well. Growth was monitored every 30 min for 9.5 h at 600 $_{nm}$. All growth assays were conducted in triplicate.

2.5. Oxidative stress assay

Previously described methods (Suraju et al., 2015; Bado et al., 2017) with slight modifications were employed. In short, stock solutions prepared for growth kinetic analysis (see above) were used. Dilutions of saturated cultures were prepared as described above for the growth kinetic analysis. Following 1 h of subculture growth, either 20 or 50 mM H_2O_2 was introduced. Oxidative stress sensitivity was monitored for 6 h by measuring $OD_{600\ nm}$. All experiments were carried out in triplicate, and a representative experiment is shown.

2.6. Crystal violet biofilm assay

Previously described methods (Suraju et al., 2015; Bado et al., 2017) were used with slight modifications. Briefly, saturated cultures of SME and SMR were grown in LB broth and diluted to 0.2 $OD_{595\ nm}$ in a 96-well plate (200 μL/well). Microtiter plates were incubated for 24 h with agitation (~ 100 rpm) at 37 °C, after which absorbance at $OD_{595\ nm}$ were measured. Wells were washed with water and incubated with 0.1% (vol/vol) crystal violet (125 μL/well) for 1 h at room temperature. Unbound crystal violet was removed by washing with water, and wells were dried overnight. Biofilm-bound crystal violet was dissolved in 250 μL of 30% acetic acid. Solubilized crystal violet was measured at $OD_{570\ nm}$. Biofilm production was normalized based on relative biomass (optical densities of biofilm formed/ optical density of terminal bacterial growth). All experiments were carried out in triplicate or quadruplicate where indicated. A representative experiment is shown.

2.7. MTT (3-(4, 5-dimethylthiazol-2-yl) −2, 5-diphenyltetrazolium bromide) assay

Previously described methods (Bado et al., 2018) were employed with slight modifications. In brief, BEAS-2B and HT29 cells were seeded in a 96-well plate at a density of 5000 cells/well 24 h preceding the experiment. The following day, wells were treated with 10, 50, or 100 μg/mL of Pb or Zn, or 100, 500, or 1000 μg/mL of Mn for 0, 3, 6, 8, 12, or 24 h. MTT *(3-(4, 5-dimethylthiazol-2-yl) − 2, 5-diphenyltetrazolium bromide)* reagent was added to each well, followed by a 4 h incubation at 37 °C with 5% CO_2, after which medium was gently removed from each well and replaced with 100 μL of DMSO (dimethyl sulfoxide). Cells were agitated in an orbital shaker for 5–10 min, and absorbencies were read at 570 $_{nm}$ with a reference filter of 630 $_{nm}$.

2.8. Bacterial co-culture with eukaryotic cells

Previously described methods (Bado et al., 2017) were employed with slight modifications. In brief, BEAS-2B and HT29 cells were seeded into 24-well plates at densities of ~ 1×10^5/well 24 h prior to bacterial infection, and monolayers were sub-confluent (~60–80% confluency) at the time of the experiment. Bacteria were grown to saturation in LB broth at 37 °C with agitation (~250 rpm), washed with 1X PBS, and diluted to $OD_{600\ nm}$ of 1.0 in DMEM + 10% FBS. Multiplicities of infection were ~ 10 whereby there were roughly 10 bacterial cells for every one eukaryotic cell during the co-culture. Following a 1 h attachment period, each well was washed with PBS, and DMEM containing either 10 μg/mL of Pb or Zn, or 100 μg/mL Mn was added to each well. Viable colony plate counts were enumerated for both the 0- and 6-h end points, and the fold-increases were calculated. All plates were incubated at 37 °C.

2.9. Statistical analysis

All experiments were carried out in triplicate or quadruplicate (when

noted) and averaged. Statistical analysis of the data was obtained using a two-tailed Student's T-test (unequal variance). Significant differences were considered with p-values less than or equal to 0.01 (**) or *p-values* $\leq$ to 0.05 (*).

3. Results

3.1. Metal contaminants in Houston watershed soils

In efforts of determining metal contaminant levels in Houston watershed soils, like Buffalo Bayou, we found that Pb (3.2 – 18.4 ppm), Zn (7.5 −85.3 ppm), and Mn (154–1056 ppm) ranges were all elevated and exceeded Texas Commission on Environmental Quality (TCEQ) threshold levels (15, 30, and 500 ppm, respectively). We observed similarly elevated levels of the three-aforementioned heavy metals exceeding both TCEQ and Environmental Protection Agency thresholds in several other Houston Watershed Soils (Table 1). Previously, we sought to evaluate bacterial loads in Houston watershed soil samples following flooding events and isolated the opportunistic pathogen *S. marsescens* from G58.4, G50, G49.4, and G6.1 location along Greens Bayou, B29.5 along Buffalo Bayou, HU20.7 along Hunting Bayou, HA28.5, HA24.7, and HA6.1 along Halls Bayou, W019.8, WO1.7, W00.1 along White Oak Bayou (collected during 2017), and CC58.1, CC49.2 and CC28.5 along Cypress Creek, MB56, MB48.8, and MB22.6 along Mustang Bayou, DKB 12, DKB9.4 and DKB0.1 along Dickinson Bayou, HB9.9, HB3.1 and HB0.1 along Horsepen Bayou (collected during 2018). The sample locations were named with a letter followed by a number as suffix where the letters stand for the name of the bayou, and the number represents the distance of the sample site, in km, from the mouth of the bayou. For example, G58.4 represents the sample site located at 58.4 km from the mouth of the Greens Bayou (Adedoyin et al., 2021). To determine whether, SME had adapted to increased metal exposure levels, we compared its growth to that of a commercially acquired SMR.

3.2. Impact of metal exposure on SME growth

When grown in the presence of 10, 50, or 100 μg/mL (ppm) of Zn, SME had significantly enhanced ($p < 0.01$) biomass at multiple time points (particularly between the 3–8 h time points) during a 9.5 h growth curve experiment than the reference strain (Fig. 1 A). Similarly, when challenged with 50 or 100 μg/mL Zn, SME had significantly greater biomass than the reference strain (Fig. 1B). Interestingly, 100 μg/mL of Zn treatment completely arrested SMR's growth while only diminishing SME's growth (compare Fig. 1 panels A to B). For reasons unexplained, growth of SMR was higher when challenged with 100 μg/mL of either Pb or Mn than at the lower 50 μg/mL concentration (Fig. 1). With regards to Mn treatment, SME had significantly greater biomass than SMR when challenged with either 100 or 500 μg/mL. However, for reasons unexplained, the highest concentration challenge (1000 μg/mL) yielded no difference between the SME's and SMR's biomasses (Fig. 1 C).

3.3. Impact of metal exposure on SME oxidative stress response and biofilm production

Following characterization of growth curves, we next sought to determine whether SME was better able to resist oxidative stress via H_2O_2 challenge, a stress commonly encountered when facing immune cells. In the same way that SME grew significantly better than SMR in the presence of metals (Fig. 1), SME grew significantly better than did SMR when challenged with 50 mM H_2O_2 (Fig. 2A). Moreover, SME's significantly greater (than SMR's) oxidative stress resistance to 50 mM H_2O_2 was maintained even during 10 μg/mL Zn (Fig. 2B), 10 μg/mL Pb (Fig. 2C), and 100 μg/mL Mn (Fig. 2C) exposures. These data suggest that environmental adaptations had occurred in SME.

In addition to oxidative stress resistance, another important virulence associated factor is biofilm production. Motivated by that understanding, we sought to determine whether SME, enhanced for oxidative stress–resistance (Fig. 2), was similarly able to produce more biofilm. Not only did SME produce ~1.3-fold significantly more biofilm than the reference strain in the absence of metal exposures (Fig. 3) but also was able to produce significantly more biofilm than SMR when challenged with 2 of our 3 metals at the lowest challenge concentration. More specifically, when challenged with either 10 μg/mL of Zn or Pb, significantly higher 1.3- and 1.2-fold, respectively, greater biofilm production was produced by the SME vs. SMR (Fig. 3A). Interestingly, challenge with the lowest concentration of Mn (100 μg/mL Pb) resulted in a 1.4-fold significantly reduced biofilm production in SME vs. SMR (Fig. 3A) for reasons that remain unclear. However, when evaluating 500 μg/mL and the highest 1000 μg/mL Mn challenge concentrations, SME had no significantly different biofilm production (Fig. 3B) and significantly greater 1.17-fold increased biofilm production (Fig. 3C), respectively. With regards to Zn and Pb challenges, SME exhibited greater biofilm production than SMR when challenged with intermediate (50 μg/mL) or high (100 μg/mL) concentrations (Fig. 3 panels B and C).

3.4. Influence of metal exposure on gut and lung models of SME infection

Since SME appeared to be armed with enhanced virulence associated properties, namely increased oxidative stress resistance and biofilm production, we sought to determine whether the SME was able to better colonize both lung and gut models. However, prior to characterizing co-culture models, we first had to determine whether metal exposures were toxic to the eukaryotic cell lines, to be used, in pure culture. Of all 3 metals evaluated, Pb induced the greatest cytotoxicity in BAES 2B lung epithelial cells (Fig. 4). More specifically, the lowest challenge dose of Zn (10 μg/mL) was the least cytotoxic to BAES 2B cells resulting in

Table 1
The soil background concentrations for heavy metals according to environmental agencies. The highest heavy metals recorded for the isolated watershed were lead (Pb), zinc (Zn), and manganese (Mn). They were above the set threshold for heavy metals in watersheds.

Metals	EPA standard	TCEQ standard	Halls	Buffalo	Greens	White Oak	Hunting	Dickinson
Arsenic	4.54	5.9	1.9 – 3.0	0.03 – 6.5	0.6 – 3.5	1.5 – 6.4	2.3–4	0.31 – 0.38
Copper	20.3	15	2.8 – 12.6	1.2 – 31.7	4.5 – 12.5	3.7 – 8.4	36.7 – 45.9	7.3 – 40.7
Chromium	43.2	30	13 – 68.2	2.7 – 16.1	16.5 – 24.1	9.4 – 24.2	35.7 – 48.1	7.1 – 49.9
Lead	32.6	15	3.2 – 18.4	1.3 – 36.8	7.3–17	2.4 – 16.1	53.1 – 95.2	9.2 – 26.7
Mercury	0.13	0.04	0.01 – 0.04	0.003 – 0.02	0.002 – 0.01	0.002 – 0.01	0.03 – 0.05	0.05 – 0.13
Zinc	43.1	30	32.7 – 90.5	7.5 – 85.3	38 – 82.8	33.3 – 66.8	317.1 – 388.3	16.5 – 96.5
Cadmium	1.74	8.75	0.12 – 0.28	0.1 – 1.1	0.09 – 0.7	0.09 – 0.38	0.62 – 1.06	0.29 – 0.87
Manganese	N/R	500	139–383	154 – 1056	118–794	152–979	209–747	35.7 – 101.2

N/R: Not Reported
TCEQ: Texas Commission on Environmental Quality
EPA: Environmental and Protection Agency
Table showing the soil background concentrations in **mg/kg (ppm)** for different metals in different watersheds sampled for 2017 compared to EPA, TCEQ Standards

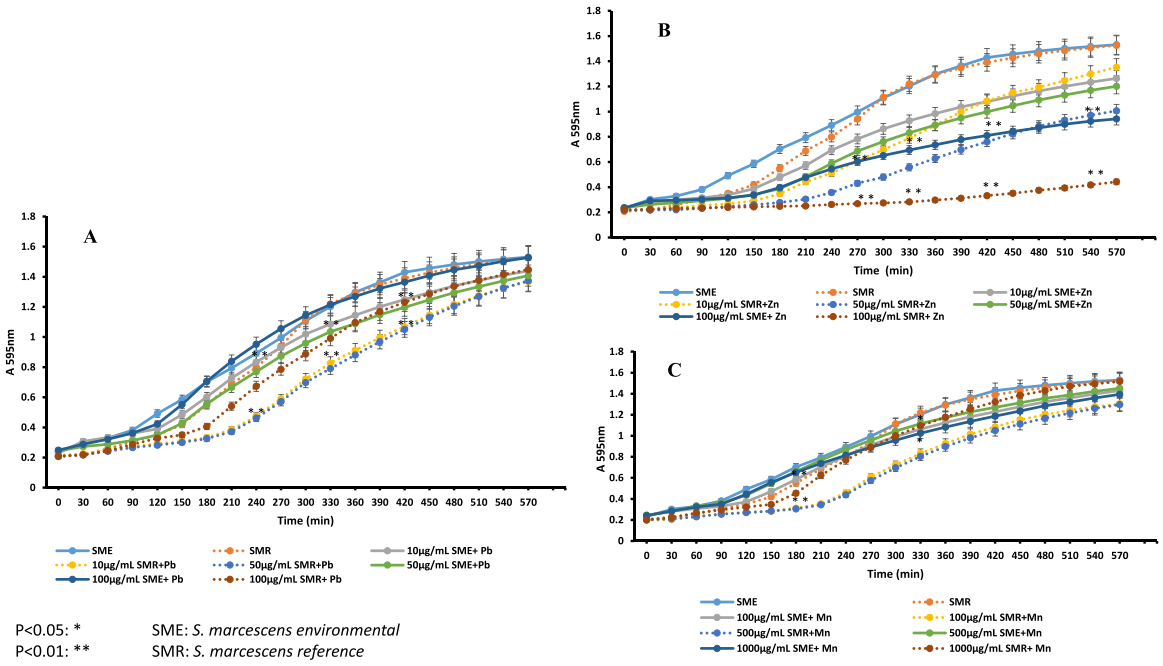

Fig. 1. Growth response of *Serratia marcescens* strains to heavy metal exposure. The growth curve of *Serratia marcescens* environmental isolate (SME) and *Serratia marcescens* reference strain (SMR) in response to Zn, Pb, and Mn exposure. This experiment was run in triplicate, and statistical analysis was determined using the Student's T-test, with $p < 0.05$ denoted by one asterisk and $p < 0.01$ denoted by two asterisks. The error bars shown are the standard error which is calculated by dividing the standard deviation of the sample by the square root of the sample size.

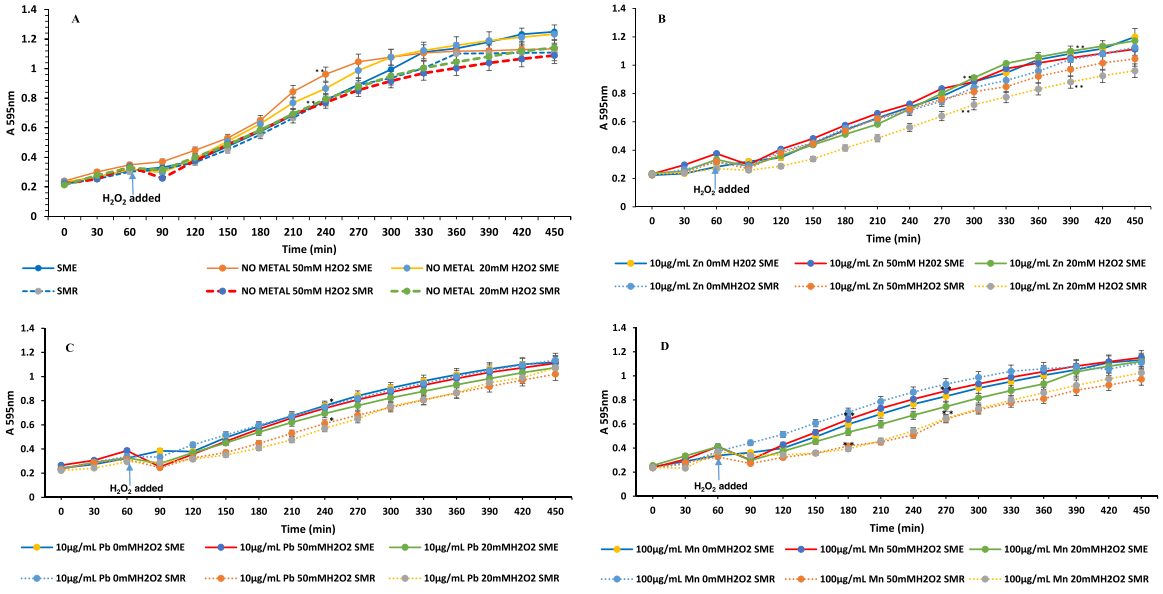

Fig. 2. Oxidative stress sensitivity of *Serratia marcescens* strains to heavy metal exposure. SME and SMR were exposed to 0, 20, and 50 mM H_2O_2 with and without 10 µg/mL, 50 µg/mL and 100 µg/mL of Zn, Pb and 100 µg/mL, 500 µg/mL and 1000 µg/mL of Mn. This experiment was run in triplicate, and statistical analysis was determined using the Student's T-test, with $p < 0.05$ denoted by one asterisk and $p < 0.01$ denoted by two asterisks.

68–56% viability over 6 h; viability dropped to 43% at 12 h at the lowest dose challenge while the highest dose challenge (100 µg/mL) resulted in ~ 95% cytotoxicity (Fig. 4A). As indicated earlier, Pb was the most toxic metal to BAES 2B cells, and the lowest challenge dose of (10 µg/mL) resulted in 25–20% viability over a 12 h period (Fig. 4B). Mn, of the 3 test metals, was the least toxic to BAES 2B cells; the lowest test concentration of 100 µg/mL-induced little cytoxicity resulting in 93–85% viability over a 12 h period.

In HT29 gut epithelial cells, Zn was the most toxic metal (Fig. 5), in sharp contrast with the BAES 2B cells, where Pb was the most toxic metal (Fig. 4). More specifically, at the lowest Zn challenge concentration (10 µg/mL), 31% cell viability was observed following a 3 h challenge compared to 51% and 58% viability following 3 h lowest concentrations challenges of Pb (10 µg/mL) and Mn (100 µg/mL), respectively (Fig. 5 compare panels A to B and C). Interestingly, unlike the BAES 2B cells with declining cell numbers (Fig. 4), HT29 cells proliferated over 3, 6, and 12 h time points when challenged with 10 and 50 µg/mL of Zn and all test concentrations of Pb and Mn (Fig. 5).

The above-mentioned findings informed our experimental design for the 6 h co-culture infection model, in which, the lowest metal

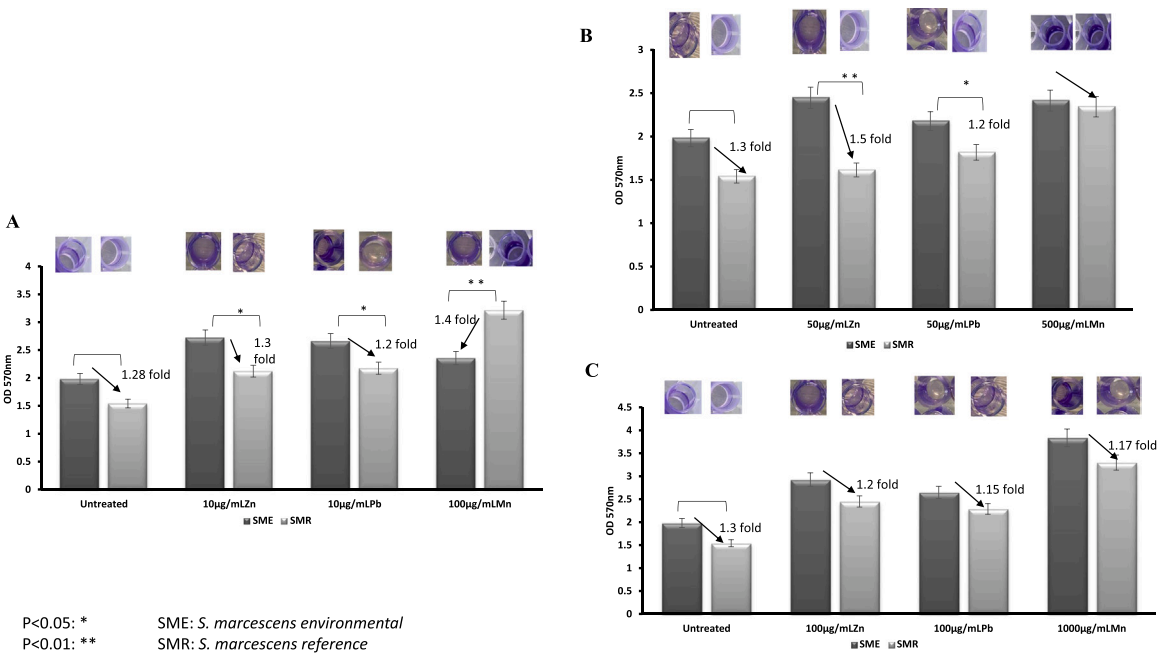

Fig. 3. Biofilm production of *Serratia marcescens* strains to heavy metal exposure. SME and SMR were exposed to 10 µg/mL, 50 µg/mL and 100 µg/mL of Zn, Pb and 100 µg/mL, 500 µg/mL and 1000 µg/mL of Mn. This experiment was run in triplicate, and statistical analysis was determined using the Student's T-test, with $p < 0.05$ denoted by one asterisk and $p < 0.01$ denoted by two asterisks.

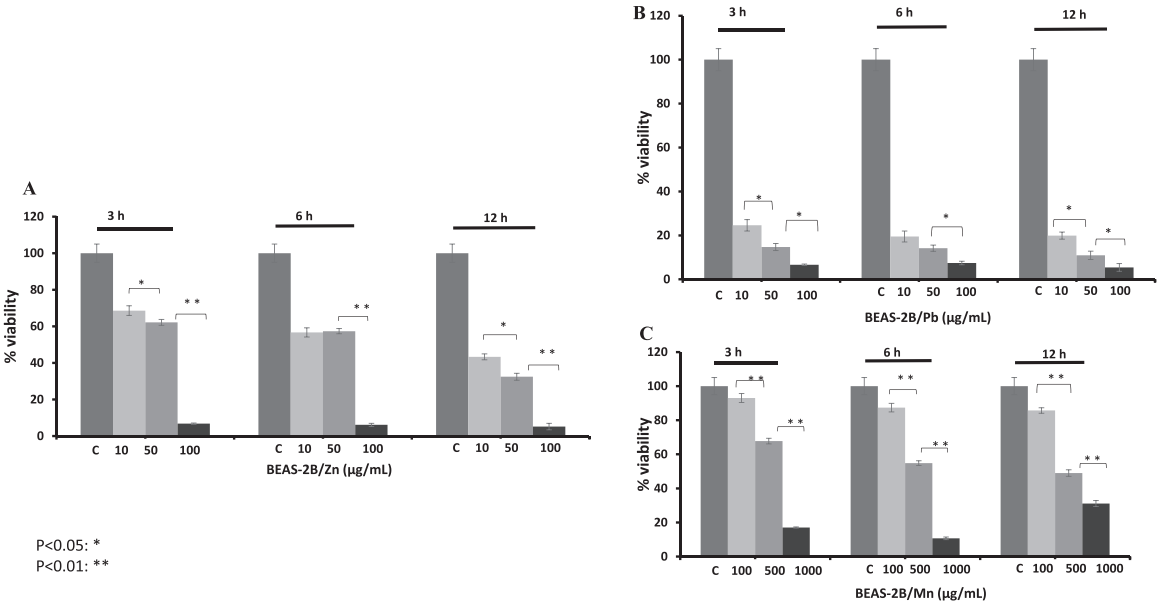

Fig. 4. Heavy metal exposure on human lung epithelial BEAS-2B cells. Viability assays were performed to determine cytotoxicity of 10, 50, and 100 µg/mL of Zn, Pb and 100, 500, and 1000 µg/mL of Mn on human BEAS-2B cells. This experiment was run in triplicate, and statistical analysis was determined using the Student's T-test, with $p < 0.05$ denoted by one asterisk and $p < 0.01$ denoted by two asterisks.

concentration of each challenge was used to avoid unnecessary eukaryotic cytotoxicity. In the lung infection model, SME exhibited a 1.22-fold significantly higher proliferation over 3 h compared to SMR (Fig. 6A) and increased to a 1.44-fold significantly higher proliferation at 6 h (Fig. 6B). Following a 3 h infection period, SME maintained significantly higher proliferation than SMR despite Zn (10 µg/mL), Pb (10 µg/mL), and Mn (100 µg/mL)-challenge, as seen by 1.30-, 1.12-, and 1.27-fold difference, respectively (Fig. 6A). Following a 6 h infection period, only the untreated and Pb-challenged (10 µg/mL) SME had significantly greater proliferation of 1.4- and 1.29-fold respectively (Fig. 6B).

To determine whether SME was similarly enhanced in its proliferation in the gut environment, HT29 cells were used for a co-culture infection. Unlike the lung infection model, in which SME exhibited significantly higher proliferation after 3 h in all challenge conditions, it only experienced significantly higher proliferation in two conditions in the gut model system. More specifically, significantly higher proliferation after a 3 h infection was only realized by SME in the untreated environment (1.16-fold) and Zn challenge (10 µg/mL) environment (Fig. 7A). Interestingly, those significantly enhanced differences were not maintained following a 6 h infection period; rather, significantly enhanced proliferation was only observed in the Pb (10 µg/mL) and Mn

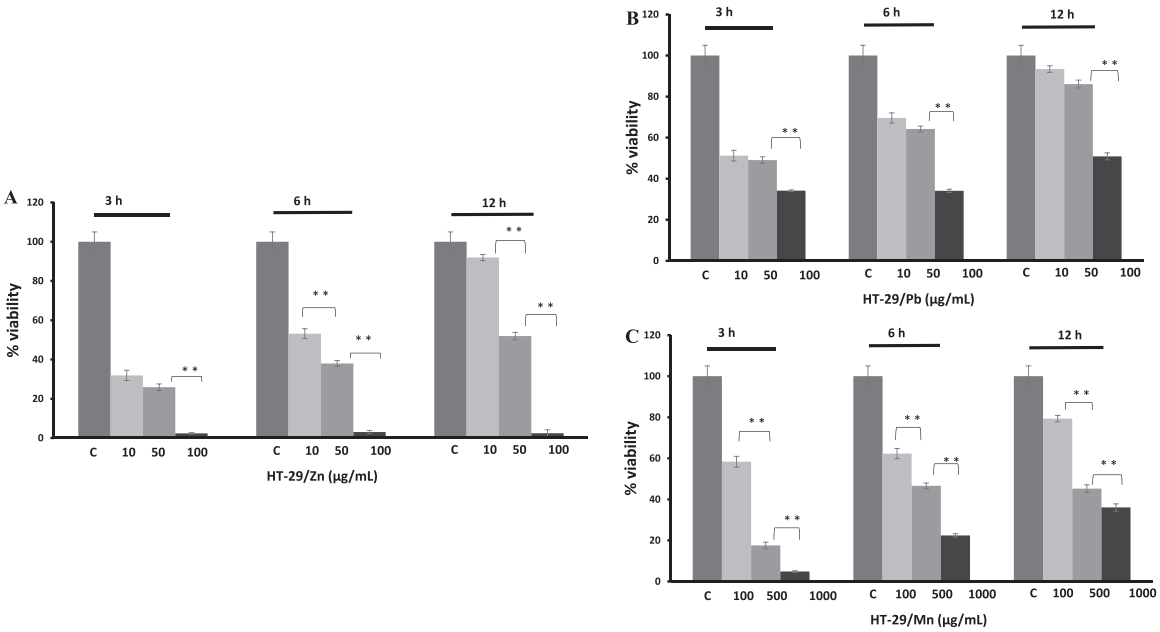

Fig. 5. Heavy metal exposure on human gut epithelial HT29 cells. Viability assays were performed to determine cytotoxicity of 10, 50, and 100 µg/mL of Zn, Pb and 100, 500, and 1000 µg/mL of Mn on human HT29 cells. This experiment was run in triplicate, and statistical analysis was determined using the Student's T-test, with $p < 0.05$ denoted by one asterisk and $p < 0.01$ denoted by two asterisks.

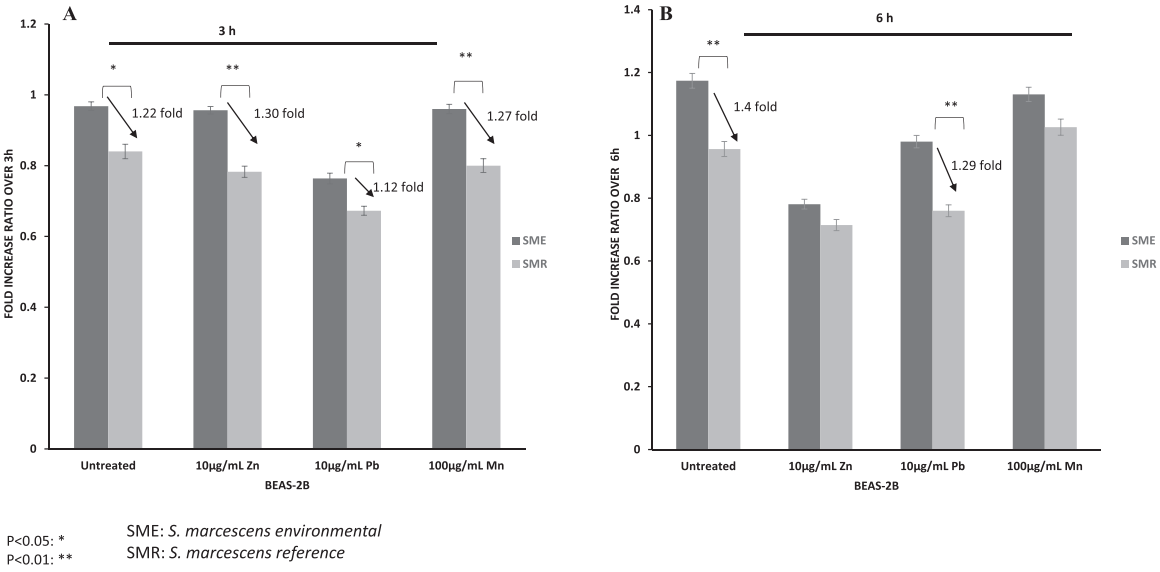

Fig. 6. Bacterial co-culture with human lung epithelial BEAS 2B cells. SME and SMR were co-cultured with BAES 2B cells in the presence of 10 µg/mL of Zn or Pb or 100 µg/mL of Mn to determine bacterial proliferation. This experiment was run in triplicate, and statistical analysis was determined using the Student's T-test, with $p < 0.05$ denoted by one asterisk and $p < 0.01$ denoted by two asterisks.

(100 µg/mL) challenge environments, evidenced by 1.24-fold and 1.19-fold differences (Fig. 7B). Taken together, depending on the infection period-length, SME was enhanced in its proliferative capability in both lung and gut tissues.

4. Discussion

S. marcescens is a formidable opportunistic pathogen capable of causing infections ranging from bacteremia to pneumonia, particularly in the nosocomial setting (González-Juarbe et al., 2015; Weakland et al., 2020). Previously, a *S. marcescens* isolate form the highly contaminated (mercury and uranium) Savannah River Site was found to have both increased metal and antibiotic resistance (Gendy et al., 2020). Such highly resistant opportunistic pathogens are more likely to cause disease outbreaks and could find their way into the nosocomial setting further challenging health workers already grappling with a laundry list of drug resistant organisms present. In evaluating Houston watershed soils, we also identified SME (Adedoyin et al., 2021) and sought to characterize its growth kinetics, oxidative stress resistance, biofilm production, and virulence potential in both lung and gut infection model systems. Consistent with earlier findings of increased metal resistance (Gendy et al., 2020), the Houston soil SME was similarly more resistant to Zn, Pb, and Mn challenge (chosen based on elevated levels measured in Houston soil samples) than SMR. Likely a result of environmental adaptation, the Houston SME was able to tolerate various challenge concentrations of the aforementioned metals and consistently

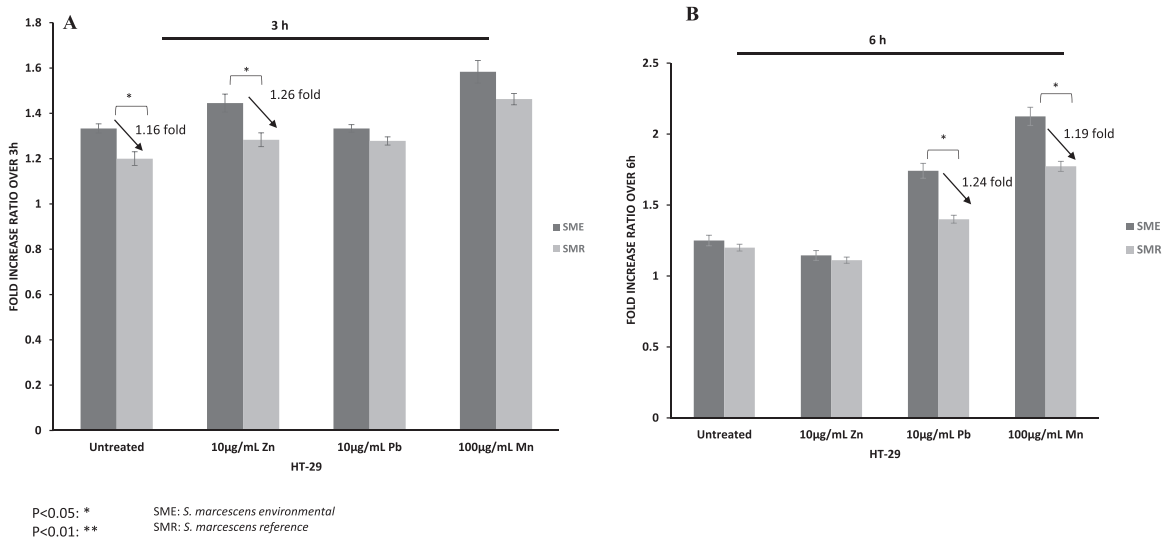

Fig. 7. Bacterial co-culture with human gut epithelial HT29 cells. SME and SMR were co-cultured with HT29 cells in the presence of 10 µg/mL of Zn or Pb or 100 µg/mL of Mn to determine bacterial proliferation. This experiment was run in triplicate, and statistical analysis was determined using the Student's T-test, with $p < 0.05$ denoted by one asterisk and $p < 0.01$ denoted by two asterisks.

outperformed SMR in growth curve assays, oxidative stress resistance experiments, biofilm production assays, and both lung and gut infection model systems.

Which, if any, specific metal resistance genes play (a) role(s) in subsequent challenges remains unknown; however, a recent proteomic study of Lauria-Bertani grown *S. marcescens* revealed some 15,000 unique peptides belonging to over 2500 protein groups. Several of these groups include chemotaxis genes as well as beta lactamase resistance genes (Gangadharappa et al., 2020). It is possible that several genes from either of the aforementioned groups could be playing a role during lung and/or gut infections/interactions, and that metal exposure results in their direct overexpression. In that same vein, a comparative genomic study of a Savannah River Site environmentally isolated *S. marcescens* strain and various reference strains revealed 360 distinct genes involved in drug and/or trace metal resistance (Gendy et al., 2020). It is possible that our Houston-area SME is also in possession of unique genes as well, enabling survival in the local, heavily polluted watershed soils. It has already been shown that glucose metabolism and capsule production drive virulence of *S. marcescens* during bacteremia in murine models (Anderson et al., 2017); therefore, it conceivable that some of those participating genes could also become upregulated in environmental strains challenged by metal exposure.

S. marcescens, an opportunistic pathogen increasingly causing nosocomial and community infections, can be found in local watersheds. In those environments, *S. marcescens* can become exposed to various environmental toxicants, including trace metals. Adaptations to these elevated toxicant levels can promote enhanced virulence. An adapted *S. marcescens* strain with enhanced biofilm production and oxidative stress resistance can cause increased virulence/disease burden and poses a significant threat to human health. These findings confirm that a Houston watershed soil SME had increased virulence potential (relative to SMR) and experienced enhanced proliferation in both lung and gut infection models. Taken together, routine bacterial surveillance of local watersheds is warranted and should be aimed at characterizing increased antibiotic resistance and virulence potential of environmentally isolated bacterial pathogens.

CRediT authorship contribution statement

Folasade T. Adedoyin carried out all experimental work in this manuscript and assisted with some of its writing. **Balaji Bhaskar Maruthi Sridhar** helped conceptualize the study and assisted with some of the writing. **Jason A. Rosenzweig** conceptualized the study, designed the research experiments and did the bulk of the manuscript writing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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References

Adedoyin, F.T., Bhaskar, M.S.B., Rosenzweig, J.A., 2021. Characterization of bacterial populations in urban and rural Houston watershed soil samples following a flooding event. Front. Environ. Microbiol. 7 (1), 22–34.

Ahemad, M., 2019. Remediation of metalliferous soils through the heavy metal resistant plant growth promoting bacteria: paradigms and prospects. Arab. J. Chem. 12 (7), 1365–1377.

Anderson, M.T., Mitchell, L.A., Zhao, L., Mobley, H.L.T., 2017. Capsule production and glucose metabolism dictate fitness during *Serratia marcescens* bacteremia. mBio 8 (3), e00740–17.

Bado, M., Kwende, S., Shishodia, S., Rosenzweig, J.A., 2017. Impact of dust exposure on mixed bacterial cultures and during eukaryotic cell co-culture infections. Appl. Microbiol. Biotechnol. 101 (18), 7027–7039.

Bado, M., Keita, D., Azu, N., Shishodia, S., Rosenzweig, J.A., 2018. Mixed bacterial responses to dust exposure in an A549 eukaryotic co-culture. Appl. Microbiol. Biotechnol. 102 (22), 9759–9770.

Behera, M., Dandapat, J., Rath, C.C., 2014. Effect of heavy metals on growth response and antioxidant defense protection in Bacillus cereus. J. Basic Microbiol. 54 (11), 1201–1209.

Breton, J., Daniel, C., Vignal, C., Body-Malapel, M., Garat, A., Plé, C., Foligné, B., 2016. Does oral exposure to cadmium and lead mediate susceptibility to colitis? The dark-and-bright sides of heavy metals in gut ecology. Sci. Rep. 6, 19200.

Cao, S., Feehley, T.J., Nagler, C.R., 2014. The role of commensal bacteria in the regulation of sensitization to food allergens. Fed. Eur. Biochem. Soc. (FEBS) Lett. 588 (22), 4258–4266.

Faulkner, M.J., Helmann, J.D., 2011. Peroxide stress elicits adaptive changes in bacterial metal ion homeostasis. Antioxid. Redox Signal. 15 (1), 175–189.

Gangadharappa, B.S., Rajashekarappa, S., Sathe, G., 2020. Proteomic profiling of *Serratia marcescens* by high-resolution mass spectrometry. Bioimpacts 10 (2), 123–135.

Gendy, S., Chauhan, A., Agarwal, M., Pathak, A., Rathore, R.S., Jaswal, R., 2020. Is long-term heavy metal exposure driving carriage of antibiotic resistance in environmental opportunistic pathogens: a comprehensive phenomic and genomic assessment using Serratia sp. SRS-8-S-2018. Front. Microbiol. https://doi.org/10.3389/fmicb.2020.01923.

González-Juarbe, N., Mares, C.A., Hinojosa, C.A., Medina, J.L., Cantwell, A., Dube, P.H., Orihuela, C.J., Bergman, M.A., 2015. Requirement for Serratia marcescens cytolysin in a murine model of hemorrhagic pneumonia. Infect. Immun. 83 (2), 614–624.

Gough, H.L., Stahl, D.A., 2011. Microbial community structures in anoxic freshwater lake sediment along a metal contamination gradient. Int. Soc. Microb. Ecol. (ISME) J. 5 (3), 543–558.

Hussein, K.A., Joo, J.H., 2013. Heavy metal resistance of bacteria and its impact on the production of antioxidant enzymes. Afr. J. Microbiol. Res. 7 (20), 2288–2296.

Khan, I., Yasir, M., Azhar, E.I., Kumosani, T., Barbour, E.K., Bibi, F., Kamal, M.A., 2014. Implication of gut microbiota in human health. CNS Neurol. Disord. Drug Targets 13 (8), 1325–1333.

Link, D.D., Walter, P.J., Kingston, H.M., 1999. Wastewater standards and extraction chemistry in validation of microwave-assisted EPA method 3015A. Environ. Sci. Technol. 33 (14), 2469–2473.

Mahurpawar, M., 2015. Effects of heavy metals on human health. Int. J. Res. Grant. 2394–3629. ISSN-23500530.

Ochieng, J.B., Boisen, N., Lindsay, B., Santiago, A., Ouma, C., Ombok, M., Fields, B., Stine, O.C., Nataro, J.P., 2014. *Serratia marcescens* is injurious to intestinal epithelial cells. Gut Microbes 5 (6), 729–736.

Patwa, L.G., Fan, T.J., Tchaptchet, S., Liu, Y., Lussier, Y.A., Sartor, R.B., Hansen, J.J., 2011. Chronic intestinal inflammation induces stress-response genes in commensal *Escherichia coli*. Gastroenterology 141 (5), 1842–1851.

Prabhakaran, P., Ashraf, M.A., Aqma, W.S., 2016. Microbial stress response to heavy metals in the environment. R. Soc. Chem. Adv. 6 (111), 109862–109877.

Rehman, K., Fatima, F., Waheed, I., Akash, M.S.H., 2018. Prevalence of exposure of heavy metals and their impact on health consequences. J. Cell. Biochem. 119 (1), 157–184.

Shahid, M., Pourrut, B., Dumat, C., Nadeem, M., Aslam, M., Pinelli, E., 2014. Heavy-metal-induced reactive oxygen species: phytotoxicity and physicochemical changes in plants. Rev. Environ. Contam. Toxicol. 232, 1–44.

Suraju, M.O., Lalinde-Barnes, S., Sanamvenkata, S., Esmaeili, M., Shishodia, S., Rosenzweig, J.A., 2015. The effects of indoor and outdoor dust exposure on the growth, sensitivity to oxidative-stress, and biofilm production of three opportunistic bacterial pathogens. Sci. Total Environ. 538, 949–958.

Tchaptchet, S., Hansen, J., 2011. The Yin and Yang of host-commensal mutualism. Gut Microbes 2 (6), 347–352.

Teitzel, G.M., Parsek, M.R., 2003. Heavy metal resistance of biofilm and planktonic Pseudomonas aeruginosa. Applied and Environmental Microbiololgy 69 (4), 2313–2320.

Weakland, D.R., Smith, S.N., Bell, B., Tripathi, A., Mobley, H.L.T., 2020. The *Serratia marcescens* siderophore serratiochelin is necessary for full virulence during bloodstream infection. Infect. Immun. 88 (8), e00117–e00120.