Colicin E2 expression in Escherichia coli biofilms: Induction and regulation revisited

Bihter Bayramoglu-Güven a,b, Lusine Ghazaryan a, David Toubiana a, Osnat Gillor a,∗

a Zuckerberg Institute for Water Research, Blaustein Institutes for Desert Research, Ben Gurion University of the Negev, 8499000 Midreshet Ben Gurion, Israel
b Yeditepe University, Faculty of Engineering, Department of Genetics and Bioengineering, Kayisdagi Cad., 34755, Istanbul, Turkey

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A B S T R A C T

Colicins, bacteriocins produced by the gram-negative bacterium Escherichia coli, are tightly regulated by the DNA damage response regulatory system (SOS), and are thus triggered at times of stress. Colicins’ regulation and expression profiles were primarily studied in suspended (planktonic) cultures yet, in their natural environments E. coli cells are sessile, assembled in biofilms. We hypothesized that colicin expression would differ between planktonic and biofilm E. coli cultures, even when induced by the same triggers. To test our hypothesis, we compared colicin E2 expression and SOS regulated genes in planktonic and biofilm cultures of E. coli, in response to DNA damaging agents and oxygen depletion. The results indicate that uninduced biofilms express more transcripts of the colicin operon than uninduced planktonic cells. Whole genome expression profiles confirmed that in uninduced biofilms, SOS genes are upregulated compared to planktonic cultures. However, DNA damaging agents and oxygen depletion augmented colicin expression in planktonic cells, while only marginal increase was recorded in biofilms. Our results suggest that the regulation of colicin E2 expression in E. coli biofilms considerably differ from planktonic cells, thus the induction of colicins in their host natural environment, i.e., the gastrointestinal tract, needs to be re-evaluated.

Introduction

In a mixed environment, bacterial populations use diverse competitive strategies in order to dominate the competition for nutrients and space (Pfeiffer et al., 2001; Schluter et al., 2015; Xavier and Foster, 2007). Among these strategies, one of the most common is bacteriocins. These are broad- or narrow-spectrum proteinaceous toxins that kill immediate adversaries, coupled with the secretion of special antitoxins that prevent self-poisoning (Abrudan et al., 2015; Ratcliff and Denison, 2011).

Colicins are the most extensively studied bacteriocins produced by the gram-negative bacterium Escherichia coli, and serve as a model system for gram-negative produced bacteriocins (Bosik et al., 2021; Cascales et al., 2007). These toxins are encoded on operons that carry two or three genes, namely a colicin-encoding gene (cxa, for colicin X). An immunity gene (cxi) which encodes a protein that provides the producing cell with specific protection against its own toxin. In addition, some colicins harbor an additional gene which encodes for a lysis protein (cxl) that facilitates the release of colicins through lysis of the cytoplasmic membrane of the producing cell (Cascales et al., 2007). Transcription of the colicin operons, as well as many Gram negative bacteriocins, is strongly regulated by the LexA and RecA proteins. These proteins are involved in the host’s SOS regulatory system and therefore, the transcription of these proteins mainly happens under stress conditions (Maslowska et al., 2019). Under ‘normal’ conditions, LexA blocks the transcription of the colicin operon (Llobés et al., 1988). Damage to the DNA by agents such as UV light, mitomycin C (MitC) (Hardy and Meynell, 1972), hydrogen peroxide (Goerlich et al., 1989) or ciprofloxacin (Jerman et al., 2005), activates RecA and prompts LexA auto-cleavage. This passage of events triggers SOS-regulated genes to repair the damaged DNA (Zgur-Bertok, 2013) along with the subsequent expression of the colicin operon (Gillor et al., 2008; Iwoff et al., 1952). In nuclease colicins, the colicin activity genes are tightly regulated by the SOS promoter. The immunity genes are regulated both by the SOS promoter their own constitutive promoter for protection (Nikaido, 2003; Thanassi and Hultgren, 2000). The third gene within the operon encoding a lysis protein, is regulated by the downstream SOS promoter. Thus, transcription of the lysis gene require the polymerase to transcribe...
through the entire operon which necessitate significant induction and prevent unnecessary lysis of the cell (Calcuttawala et al., 2022; Yin et al., 2019).

Colicin production is often dependent on host regulatory pathways, mainly the SOS regulation, and therefore principally occurs under times of stress (Masłowska et al., 2019). Under DNA-damage, increase in cell density, or nutrient depletion, a subset of the colicinogenic population is induced to produce colicins. These colicins are released into the extracellular environment and bind to specific cell-surface receptors on target cells membrane. The colicins then penetrate the target cells lacking the protective immunity proteins and kill them (Housden et al., 2010; Lazdunski and Pattus, 1991; Mulec et al., 2003). To date, 36 types of colicins have been identified (Cascales et al., 2007; Micenková et al., 2019; Rendueles et al., 2014), and despite variety among hosts, an average of 40% of the strains in any given population produce one or more colicins (Gordon and O’Brien, 2006; Gordon and Riley, 1999). In its natural environment, the gastrointestinal (GI) tract, E. coli is often in sessile form, frequently in an environment with structural characteristics of a multispecies biofilm (Costerton et al., 1995; Probert and Gibson, 2002). These biofilms are often assembled by extracellular polymeric substances (EPS) that forms structural scaffolds. One of the important properties of biofilms is enhanced resistance or tolerance to antibiotics and other antimicrobial agents compared to planktonic cultures (Yin et al., 2019). Antimicrobial tolerance in biofilms can be a product both of the properties of the EPS, through the entrapment or inactivation of antimicrobials, and of the slow growth that can occur in biofilms (Yin et al., 2019).

Most studies of colicin expression, ecology and evolution have been performed in planktonic environments. Yet, genome-wide analyses have demonstrated marked differences (5–10% of the genome) between the transcriptome of E. coli cultured under planktonic and sessile conditions (Beloin et al., 2008; Wood, 2009). Moreover, further studies evaluating colicin production in biofilms revealed an increase of 7–9% in production rates, while in planktonic cultures the rates were 0.5–3% (Majeed et al., 2015; Mulec et al., 2003; Riley and Wertz, 2002). In addition, although DNA-damaging agents are well known as key inducers to colicin production in planktonic environment, little is known about their impact in a biofilm (Jin et al., 2021). For instance, colicin R was shown to be expressed mainly in biofilms but was undetected under planktonic conditions (Rendueles et al., 2014). There are a few possible scenarios that could alter colicin expression in biofilm cells, including the putative increase in E. coli DNA damage response (Rendueles et al., 2014), the enhanced resistance of biofilm cells to antibiotics (Yin et al., 2019), or the nutrient and oxygen limited conditions of some cells within the biofilm (Beebout et al., 2019). To elucidate the regulators of colicin expression in biofilms, we examined colicin induction by DNA damaging agents and oxygen limitation in planktonic and biofilm E. coli cultures. We hypothesized that colicin expression would differ between planktonic and biofilm E. coli cultures, even when triggered by the same inducing agents.

Materials and methods

Bacterial strains and plasmid

All experiments were performed with E. coli strain MG1655 (F- lambda-dh5- rfb-50 rph-1) (Guyer et al., 1981) harboring pBR322-ColE2 and pSC303 plasmids (Ghazaryan et al., 2014; Kameşek et al., 2010).

Growth conditions

In the experiments described below biofilm and planktonic populations were cultured in M9 minimal medium (Sigma, St. Louis, MO, USA) supplemented with 4 g L⁻¹ Casein Digest (BD, Franklin Lakes, NJ, USA). The media was supplemented with 100 mg mL⁻¹ ampicillin (Sigma) and 50 mg mL⁻¹ kanamycin (Sigma) at 25 °C unless otherwise noted. For planktonic experiments, an overnight cultures of E. coli cells were refreshed by 1:100 dilution in fresh media and grown to early exponential phase (OD₆₀₀ of ~0.07). Duplicate 10 mL culture were placed in a 50 mL tube and cultivated 12 h with constant aeration in a shaking incubator (New Brunswick, Enfield, CT, USA) at 200 rpm to OD₆₀₀ of ~ 1. Induction experiments were performed by treating the cultures with antibiotics (see below) for 5 h. For biofilm cultures, E. coli cells were inoculated from overnight cultures (stationary phase cells) to fresh medium at 1:100 dilution and were cultured to early exponential phase (OD₆₀₀ of ~0.07). The biofilms were cultured as previously described (Majeed et al., 2015). Briefly, four glass cover-slides (Marienfeld, Lauda-Königshofen, Germany) size 24 × 40 mm were mounted in a unique flow-cell (Supplementary Figure 1) and placed vertically in grooves positioned in a custom-made polypropylene tub (63 × 38.5 × 50 mm). This set up provided homogeneous growth conditions, which would otherwise be possible only in four multi-channel flow-cells. The flow-cell was supplied with M9 medium at a constant flow rate of 1.5 mL min⁻¹ using a peristaltic pump (MasterFlex 07524-40, Vernon Hills, IL, USA) for 3 h. Then the medium flow was arrested, and the E. coli refreshed culture (90 mL) was injected into the flow-channel. After 1 h, the flow of the supplemented medium without bacteria was resumed at the same rate. Aerobic biofilms were cultivated for 3 days in an incubator.

Oxygen deprivation

Anaerobic 10 mL cultures were introduced in duplicates to a vinyl anaerobic chamber (Coy Laboratory Products, Grass Lake, MI, USA). The anaerobic chamber operated under an atmosphere of 90% N₂, 5% H₂ and 5% CO₂, was equipped with a palladium catalyst to remove excess oxygen. The oxygen levels in the chamber were equilibrated to 0–5 parts per million. The culture tube was placed in the anaerobic chamber, acclimated and then incubated for 30 h, until the cells reached OD₆₀₀ of ~ 1. Incubation period was determined during preliminary experiments (data not shown). In each experiment 10 mL of the media without the inoculated cells served as control. We note that we chose to use planktonic cells in stationary phase, rather than actively growing cells (in exponential phase), since this is closer to their environmental state (Finkel, 2006). The anaerobic biofilms were cultured for 6 days in the anaerobic chamber. Prior to the initiation of the anaerobic biofilm the growth medium and flow-cell were acclimated in the anaerobic hood overnight. Before beginning the biofilm cultivation experiment, the refreshed cell cultures were acclimated to the anaerobic chamber and then injected to the flow channel as described above. Initiation, inoculation, and cultivation of the anaerobic biofilm were all practiced in the anaerobic chamber where the oxygen levels were constantly monitored. At the end of the experiments the slides were removed and processed as described below.

Antibiotic treatment

For colicin induction experiments, the medium was supplemented with 50 ng mL⁻¹ mitomycin C (MitC; Sigma) and 1.25 × 10⁻⁴ M Cip rofloxacine (Sigma). For each treatment (see below) at least three biological replicates were conducted, each at least three technical replicates. The antibiotics were used at a sub-lethal concentration that yielded the highest fluorescence. E. coli cells were inoculated from overnight planktonic cultures (stationary phase cells) to fresh medium at 1:100 dilution and were cultured to early exponential phase (OD₆₀₀ of ~0.07). Planktonic cultures were treated with antibiotics for 5 h. Biofilm cultures were cultivated for 2 days and then treated with the antibiotic for 16 h.

Confocal laser-scanning microscopy (CLSM)

To visualize aerobic/anaerobic and induced biofilm cultures, the
flow-cells were opened and the slides were removed. The biofilm was visualized as previously described (Bayramoglu et al., 2017). Briefly, the removed slides were fixed in 4% formaldehyde, stained with DAPI and visualized as previously described (Bayramoglu et al., 2017). Briefly, the aerobic biofilm and 2.7

The qPCR primers of the respective targets (Table S1) were designed using Geneious (Kearse et al., 2012). All PCR amplifications were performed in iCycler thermos-cycler equipped with MyIO detection system (Bio-Rad, Hercules, CA, USA) and the data was processed using Bio-Rad CFX Manager 3.0 software (Bio-Rad). The reaction was adjusted to a final volume of 20 µl containing 5 µl cDNA (2.5 ng), 3 µl water (Sigma), 1 µl of each primer (400 nM) and 10 µl SYBR-Green PCR Master Mix (Thermo Fischer). All qPCR reactions were performed in duplicates under the following conditions: 95 °C for 5 min; 40 cycles of 94 °C for 30 s, 60 °C for 30 s, 72 °C for 30 s. To standardize the results, we used known amounts of the extracted plasmids pColE2 for colicin operon cloned into the vector plasmid pJET 1.2 (Fermentas, Burlington, Canada) containing the respective gene for SOS regulated genes.

RNA extraction, sequencing and analysis

Total RNA was extracted from two biological replicates of about 10^10 planktonic cells (OD600 ~ 1) and from the slides supporting 35 ± 5 µm^3 aerobic biofilm and 2.7 ± 0.3 µm^2 anaerobic biofilm per 1 µm^2 surface area. The slides were washed prior to the extraction to remove excess fluids and planktonic cells. Extractions were performed using MasterPure RNA purification kit (Epicentre, Madison, WI, USA) according to the manufacturer’s instructions. High-throughput sequencing of the mRNA was carried out by the Genome Center at the Institute of Technology as previously described (Brownstein et al., 2014). Briefly, the mRNA concentrations were estimated using Qubit Fluorometer (Thermo Fisher, Waltham, MA, USA) and TapeStation (Agilent, Santa Clara, CA, USA) and equal amounts were reverse transcribed. Library preparation was done using TruSeq RNA Library Preparation Kit v2 (Illumina, San Diego, CA, USA). The resulting library was then sequenced using a HiSeq 2000 Analyzer (Illumina). The sequence reads of all samples were deposited in NCBI’s Gene Expression Omnibus and are accessible through GEO Series accession number GSE72113. The reads were mapped to the genome of E. coli strain MG1655 (GenBank accession number U00096.2) and then each set of data was normalized by upper quartile normalization, thus enabling the comparison of gene expression levels between samples. Mapping of the mRNA reads to the E. coli strain MG1655 genome was performed with Rockhopper software (McClure et al., 2013) using default parameters. Around 24 million reads mapped uniquely to the strain’s genome from the raw sequence output of around 25 million reads. Rockhopper uses local regression to obtain an estimate of gene expression variances. For each transcript a statistical test is performed for the null hypothesis that the expression of the transcript is the same in different conditions. The Negative Binomial distribution is used as the statistical model to compute a p-value indicating the probability of observing a transcript’s expression levels in different conditions by chance. Because multiple tests are simultaneously performed across the transcripts, q-values are reported that are adapted p-values that control the false discovery rate using the Benjamini-Hochberg procedure. As suggested by the developers, changes in gene expression were considered statistically significant if the q-values were smaller or equal to 0.01. Significant differences in gene expression were considered only when expression changed at least 2.5-fold as previously suggested (Fitzgerald et al., 2014; Shao et al., 2015). Gene classification for SOS related genes were done by utilizing MultiFun software (Serres and Riley, 2000) that contains an enriched classification system for cellular functions of E. coli strain K-12 gene products. To analyze the difference in expression levels between environments, a moderated t-test was applied, integrated in the limma package (Ritchie et al., 2015) and the Biobase (Gentleman et al., 2004) in the R environment. In order to fit the microarray format used in the limma package, the voom transformation (Law et al., 2014), converting the counts of the RNA-Seq data to log-counts per million, was applied prior to the analysis data were log2-transformed for analyses of differential gene expression. The gene expression counts were used to determine fold change values in expression levels indicating the relative change in each transcript used to identify differentially expressed genes between two separate conditions. The fold change for a transcript is a positive number when the expression level increases and is a negative number when the relative expression level decreases.

Results

Colicin expression in planktonic and biofilm E. coli cultures

Colicinogenic cell cultures grown in planktonic and biofilm environments are depicted in Fig. 1. In each culture we estimated the fraction of colicin-expressing cells in planktonic cultures grown to stationary phase in three-day-old biofilms. While only 3.3 ± 1.7% of the planktonic cells expressed colicins, 12.1 ± 2.8% of the biofilm cells expressed colicins. These results suggest that in a biofilm environment colicin expression increased by approximately four-folds. To estimate colicin production, the expression of the activity, immunity and lysis genes in both planktonic and biofilm cultures were estimated (Fig. 2, black bars). The expression of each gene was measured in planktonic cultures grown to stationary phase and in three-days-old biofilms. Our qPCR results showed an approximate two-log increase in the colicin operon genes in biofilm settings (p < 0.05) (Fig. 2).

Expression of SOS related genes in planktonic and biofilm E. coli cultures

The expression activity and the downstream lysis genes are tightly regulated by the SOS response system. The immunity genes have two promoters, the SOS promoter of the colicin operon and their own constitutive promoter ensuring the cells from self-toxicification (Calcuttawala et al., 2022; Yin et al., 2019). We explored the expression of colicin and other SOS regulated genes in biofilm and planktonic cultures (Table S1). We focused on the SOS response system
that included 57 genes (Keseler et al., 2017) and we focused on those that differed in expression levels between planktonic and biofilm E. coli cultures (Table 1). It was reported that lexA was downregulated while recA and other SOS-regulated genes were upregulated in biofilms compared to planktonic cells cultivated under the same conditions. Table 1 demonstrates that 20 of the 57 SOS-regulated genes were upregulated in biofilm setting, while lexA alone was downregulated by two-folds. The upregulated SOS genes encode for DNA polymerase IV and V (dinB, umuCD), DNA damage induced proteins (dinD), recombination and repair protein (recN), membrane proteins (tsiB, ydfM, ymfE), toxin-antitoxins (yejNOP), regulatory protein (recX), recombination limiting protein (rnuC), cell division inhibitor (sulA) and toxic peptides (hokE, yafN) (Keseler et al., 2017; Maslowska et al., 2019). Moreover, downregulation of the repressor LexA allows for the transcription of the colicin activity and lysis genes, upregulating their expression in biofilms compared to planktonic cultures (Table 1).

Colicin expression response to antibiotics in planktonic and biofilm E. coli cultures

Colicin expression differed between planktonic and biofilm populations in response to sub-inhibitory concentrations of the DNA damaging agents MitC and ciprofloxacin. In planktonic cultures application of MitC and ciprofloxacin significantly increased (p < 0.05) colicin expression (Fig. 2A). However, when the two-day-old E. coli biofilms were treated with the same sub-lethal doses of the DNA damaging agents the response was not significant (p > 0.05) (Fig. 2B). The expression of the activity, immunity, and lysis genes of the colicin operon were compared showing higher expression levels in planktonic cells when induced with DNA-damaging agents compared to biofilm cultures (p < 0.05).

In comparison to uninduced controls, colicinogenic genes were upregulated by 1 – 1.5 orders of magnitude in planktonic cultures in response to both ciprofloxacin and MitC (Fig. 2). Yet, in biofilm culture the response to the mutagenic agents were reduced. Ciprofloxacin induced the colicin activity and lysis genes in planktonic cultures, yet in biofilms, induction was reduced by 0.5 – 1 orders of magnitude. However, in response to mitC colicin activity was not significantly induced, while immunity and lysis increased by less than one order of magnitude. Our results may suggest that biofilm culture moderate the response to mutagenesis inducers. We wondered whether the detected response is unique to colicins or shared by other SOS regulated genes. To answer this question, we monitored the expression of three key SOS regulated genes (toxR, rmuC, recN) and compared their response to DNA damaging agents in planktonic and biofilm cultures (Fig. 3). Our results suggest that the SOS genes were significantly upregulated in the induced biofilm (p < 0.05) but not in planktonic cells (p > 0.05).

Colicin expression in aerobic and anaerobic conditions

To test the effect of oxygen availability on colicin expression, colicin E2 operon expression in planktonic and biofilm cultures was monitored under aerobic and anaerobic conditions. Gene expression profiles were measured in anaerobic planktonic cultures grown to stationary phase and in six-day-old anaerobic biofilms (Fig. 4). Our results suggest that
when oxygen was depleted the expression of colicin activity, immunity and lysis decreased by 25, 10 and 10 times, respectively, in biofilms (Figs. 2 and 4).

We further tested SOS regulated genes expression under aerobic and anaerobic conditions expecting an increase in expression. However, oxygen depletion had negligible effect on the expression of SOS regulated genes in planktonic cultures (Table S2), while in biofilms the expression was generally reduced (Table 2; Table S3). Table 2 demonstrates that 10 of the 57 known SOS genes were downregulated in biofilm setting (Table S3). Lack of oxygen downregulated the expression of *lexA* and *recA* (Table 2) together with eight SOS genes encoding for the positive RecA modulator (*dinI*), a component of the 30S ribosomal subunit (*rpsU*), cell division inhibitor (*sulA*), DNA damage inducible protein (*ssb*), toxic peptide (*tisB*), in addition to two putative protein one acting as prophage and the other of unknown function (*ymfI* and *yebG*, respectively) (Keseler et al., 2017; Maslowska et al., 2019).

### Discussion

**Colicin expression in planktonic and biofilm E. coli cultures**

Many studies have explored colicin expression in planktonic cultures (Cascales et al., 2007). However, the characteristics of biofilms were shown to significantly differ from those of planktonic cultures in terms of gene expression (Becker et al., 2001; Beloin et al., 2008), stress response (Booth et al., 2011) and antibiotic resistance (de la Fuente-Núñez et al., 2013). In biofilm cultures colicin expression was shown to occur in 7–9% of cells (Majeed et al., 2015), while only 0.5–3% of planktonic clones expressed colicins (Majeed et al., 2015; Mulec et al., 2003; Riley and Wertz, 2002). In this study, we further characterized the regulation and expression of colicins in biofilm cultures and compared the results to the well-studied colicin-profiles in planktonic cells.

Since colicin expression is tightly regulated by the SOS response system (Gillor et al., 2008; Jin et al., 2021; Kamensk et al., 2010), we focused on the expression of genes associated with DNA damage in *E. coli* biofilm compared to planktonic cultures. It has been shown in planktonic cultures that LexA protein binds to the promoter area of SOS-regulated genes and represses their expression (Maslowska et al., 2019). However, when DNA damage occurs, RecA is activated and prompts LexA auto cleavage, therefore initiating the transcription of

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Fig. 3. Expression levels of the SOS genes *lexA*, *recA*, and *recN* in planktonic (A) and biofilm (B) colicinogenic E. coli populations. Expression levels are depicted in cells that were either induced with the DNA damaging agent, Mitomycin C, and the DNA fragmenting antibiotic, ciprofloxacin (white and grey bars) or uninduced (black bar). Expression levels were normalized to the depleted RNA template. Different letters signify significant difference at *p* < 0.05 between treatments using Tukey’s range test pairwise comparison test.

Fig. 4. Expression levels of colicin E2 genes *ce2a*, *ce2i* and *ce2l* in planktonic (A) and biofilm (B) colicinogenic *E. coli* populations. Expression levels are normalized to the depleted RNA template. Different letters signify significant difference at *p* < 0.05 between treatments using Tukey’s range test pairwise comparison test.

![Fig. 3](image1.png)

**Table 2**

List of differentially expressed SOS related genes in anaerobic compared to aerobic cultivated *E. coli* biofilms. Significant differences in gene expression were considered only when expression changed at least 2.5-fold and if the q-values were smaller or equal to 0.01. The negative values indicate downregulation.

| Gene Product                       | Fold change (Anaerobic/Aerobic) |
|------------------------------------|---------------------------------|
| *dinI* DNA damage-inducible protein I | ~6.34                           |
| *lexA* DNA-binding transcriptional repressor of SOS regulon | ~4.15                           |
| *lpxC* UDP-3-O-acyl N-acetylglucosamine deacetylase | ~4.00                           |
| *recA* DNA strand exchange and recombination protein with protease and nuclease activity | ~6.48                           |
| *rpsU* 30S ribosomal subunit protein S21 | ~5.76                           |
| *ssb* single-stranded DNA-binding protein | ~2.66                           |
| *sulA* SOS cell division inhibitor | ~4.83                           |
| *tisB* toxic membrane persister formation peptide, LexA-regulated | ~2.96                           |
| *yebG* conserved protein regulated by LexA | ~4.88                           |
| *ymfI* e14 prophage; putative protein | ~4.29                           |
genes involved in repairing the damaged DNA (Walker, 1995). Our results suggest that in biofilm settings lexA is downregulated which leads to an upregulation of DNA damage genes including the colicin operon (Table 1).

Colicin induction by antibiotics in planktonic and biofilm E. coli cultures

Following the link between colicin and SOS genes’ upregulation in biofilms (Majeed et al., 2015), we evaluated the role of DNA-damaging agents in biofilms and planktonic cells. Previous studies showed that at least 50% of the planktonic population bearing the colicin E2 plasmid were induced when treated with MitC (Hardy and Meynell, 1972) and up to 30% of planktonic populations bearing colicins E1, E7, K and A plasmids were induced when treated with ciprofloxacin (Jerman et al., 2005). To evaluate colicin induction in biofilms, two-day-old mature E. coli biofilms were treated with sub-lethal doses of the DNA-damaging agents MitC and ciprofloxacin. Unlike planktonic conditions, we found that a high increase in colicin expression was observed in the biofilm cultures (Fig. 2). This might be because the SOS response was already induced in biofilms under uninduced conditions (Fig. 2B) mitigating the effect of the DNA-damaging agents. Alternatively, it has been shown that bacteria within biofilms are 10 – 1000 times more resistant to antimicrobial agents than planktonic cells. Yet, pyocin activity was found to increase in biofilms, while colicin activity was shown to decrease under the inducing agent (Fig. 2). Both genes significantly increased in response to ciprofloxacin but not MitC (Figs. 2 and 3). However, colicin immunity and lysis as well as lexA and recN significantly responded to both agents (Figs. 2 and 3). This may indicate that colicin expression in biofilm cultures may be regulated not only by the SOS mechanism in response to different inducers.

Colicin induction by oxygen deprivation in planktonic and biofilm E. coli cultures

Anaerobic conditions were shown to upregulate colicin expression in planktonic cultures (Eraso and Weinstock, 1992; Kuhar and Zgur-Bertok, 1999). Likewise, the activity of pyocin, a bacteriocin produced by Pseudomonas aeruginosa, was enhanced in anaerobic biofilms (Waite and Curtis, 2009). Our results showed that strict anaerobic conditions reduced colicin E2 expression in biofilm compared to planktonic cultures (Fig. 4). Concomitantly, SOS-regulated genes were not affected by anaerobiosis in planktonic cultures (Table S3), but they were downregulated in biofilm cultures (Table 2 and S4). Likewise, the effect of oxygen availability was tested in planktonic and biofilm cultures of P. aeruginosa. The cells were grown under aerobic and anaerobic conditions (Alvarez-Ortega and Harwood, 2007) and similar to our study, anaerobic conditions significantly inhibited planktonic growth and biofilm development, altering gene expression profiles (Alvarez-Ortega and Harwood, 2007). Yet, pyocin activity was found to increase in slow-growing anaerobic P. aeruginosa biofilms (Waite and Curtis, 2009) while colicin activity was shown to decrease under the same conditions (Fig. 2). This could result from the compromised E. coli biofilm under anaerobic condition, while P. aeruginosa biofilm is more resilient to oxygen deprivation.

Conclusion

For over half a century, induction of colicinogenic E. coli by DNA-damaging agents was known to result in marked increase in colicin expression. Here, we have shown that this dogma does not hold in biofilms. The effect of DNA-damaging agents and oxygen depletion on colicin expression in E. coli biofilm was mild, probably due to the enhanced resistance to antibiotic agents, the differences in the cells’ expression patterns and the growth phase. Our results suggest that the dogmas associated with colicin regulation, function, and ecology are restricted to planktonic conditions and cannot be inferred to biofilm settings. We propose that the ruling dogmas in colicin induction require re-examination and verification in colicinogenic E. coli biofilm environments.

Author contributions

BB and OG designed the experiments that were performed by BB and LG. BB, DT and OG analysed the data. BB, DT, LG and OG wrote the manuscript.

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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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.crmicr.2022.100171.

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