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Diametral influence of deoxynivalenol (DON) and deepoxy-deoxynivalenol (DOM-1) on the growth of *Campylobacter jejuni* with consequences on the bacterial transcriptome

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Abstract

Background Deoxynivalenol (DON) is a type B trichothecene mycotoxin that is commonly found in cereals and grains worldwide. The presence of this fungal secondary-metabolite raises public-health concerns at both the agriculture and food industry level. Recently, we have shown that DON has a negative impact on gut integrity, a feature also noticed for *Campylobacter (C.) jejuni*. We further demonstrated that DON increased the load of *C. jejuni* in the gut and inner organs. In contrast, feeding the less toxic DON metabolite deepoxy-deoxynivalenol (DOM-1) to broilers reduced the *Campylobacter* load in vivo. Consequently, it can be hypothesized that DON and DOM-1 have a direct effect on the growth profile of *C. jejuni*. The aim of the present study was to further resolve the nature of this interaction in vitro by co-incubation and RNA-sequencing.

Results The co-incubation of *C. jejuni* with DON resulted in significantly higher bacterial growth rates from 30 h of incubation onwards. On the contrary, the co-incubation of *C. jejuni* with DOM-1 reduced the CFU counts, indicating that this DON metabolite might contribute to reduce the burden of *C. jejuni* in birds, altogether confirming in vivo data. Furthermore, the transcriptomic profile of *C. jejuni* following incubation with either DON or DOM-1 differed. Co-incubation of *C. jejuni* with DON significantly increased the expression of multiple genes which are critical for *Campylobacter* growth, particularly members of the Flagella gene family, frr (ribosome-recycling factor), PBP2 futA-like (Fe^{3+} periplasmic binding family) and PotA (ATP-binding subunit). Flagella are responsible for motility, biofilm formation and host colonization, which may explain the high *Campylobacter* load in the gut of DON-fed broiler chickens. On the contrary, DOM-1 downregulated the Flagella gene family and upregulated ribosomal proteins.

Conclusion The results highlight the adaptive mechanisms involved in the transcriptional response of *C. jejuni* to DON and its metabolite DOM-1, based on the following effects: (a) ribosomal proteins; (b) flagellar proteins; (c) engagement of different metabolic pathways. The results provide insight into the response of an important intestinal

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microbial pathogen against DON and lead to a better understanding of the luminal or environmental acclimation mechanisms in chickens.

Keywords Deoxynivalenol (DON), Deepoxy-deoxynivalenol (DOM-1), Prokaryotes, *Campylobacter jejuni*, RNA sequencing

Introduction

Food and feed safety is an important issue worldwide, and *Campylobacter (C.) jejuni*, being primarily associated with poultry, is the most important foodborne pathogen causing gastroenteritis in humans [1]. A high prevalence of campylobacteriosis in humans together with an increasing level of antimicrobial resistance became a serious problem in recent years [2–4]. Understanding how *Campylobacter* species, especially *C. jejuni*, establishes successful colonization in chickens remains a foremost research priority as this gastrointestinal pathogen not only overcomes the host's defense system, but also competes with the microbial community for nutrients and space. Therefore, further research on the pathogenesis of *C. jejuni* infections in chickens and counteracting strategies are needed. Colonization of the avian gut by *C. jejuni* is very complex and is influenced by many parameters. Dietary factors have been found to alter the resistance to infection in general and to influence the microbial dynamics of the gut [5–7]. It was also demonstrated that feed composition, age, as well as breed of birds, influenced the outcome of *C. jejuni* colonization, the immune system, and the gut microbiota [8–10]. Recently, in various studies, we were able to show that *C. jejuni*, contrary to the general belief, increases intestinal permeability or "leaky gut" and promotes not only the translocation of *C. jejuni* itself but also the spread of *Escherichia coli* to internal organs [10–12].

Contamination of animal feed with mycotoxins is a worldwide problem and the presence of mycotoxins in poultry feed compromises the health of birds in a multifaceted way. Deoxynivalenol (DON) is the most common trichothecene mycotoxin detected in feedstuffs worldwide [13, 14]. It is known that DON poses a health risk in livestock and can have consequences on production parameters. The toxicity of this mycotoxin is connected to the epoxide ring in the molecular structure that binds to ribosomes and inhibits protein synthesis [15]. This effect is particularly problematic in rapidly dividing cells of the intestine and the immune system. Beside the effect on protein synthesis, trichothecenes inhibit both RNA and DNA synthesis, presumably as a secondary effect of protein synthesis inhibition [16]. Inhibition of DNA synthesis also results in inhibition of mitosis [16]. In addition, trichothecenes trigger a ribotoxic stress response in various cell lines that stimulates mitogen-activated protein kinase (MAPK) components of a signal transduction pathway, which regulates cell survival and stress response [16–18]. Consequently, DON increases the susceptibility to diseases [19–21].

So far, few studies have investigated the interaction between DON and enteric pathogens. It was found that the co-exposure of pigs to DON and *Salmonella* Typhimurium promoted *Salmonella* invasion and translocation across the intestinal epithelium [19]. It has also been demonstrated that feeding of DON is a predisposing factor for the development of necrotic enteritis in broiler chickens [20]. In agreement with this we found that the co-exposure of broiler chickens to DON and *C. jejuni* supported *C. jejuni* colonization in the gut at certain time points post infection, revealing that DON might provide a favourable condition for *C. jejuni* growth [22].

To mitigate the toxicity of DON, various approaches have been formulated, with one of them being bacterial biotransformation which relies on the ability of microorganisms to produce metabolites of DON with decreased toxicity [23]. Biotransforming of DON occurs mainly through de-epoxidation, oxidation or isomerization. The de-epoxidation process consists of a reductive chemical transformation that breaks open the 12,13-epoxy ring, leading to the conversion of DON into its deepoxide derivative known as de-epoxy-deoxynivalenol (deepoxy-DON or DOM-1) [23]. Incubating DON with contents from the large intestine of hens showed complete transformation to deepoxy-DON [24]. Earlier investigations indicated that the deepoxidation of DON results in a loss of toxicity [25]. It has also been demonstrated in various studies that a microbial feed additive with de-epoxidation activity could neutralize the toxic effects induced by DON in chickens [26–30]. Formation of deepoxy-DON (DOM-1) in pigs, rats, mice, chickens, and cows has been shown in different studies [31–34]. Pierron [35] showed that administration of pure DOM-1 by gavage for 21 days in pigs does not have toxic effects on zootechnical parameters, intestinal histology, intestinal and inflammatory response and liver histology. The implications of co-exposure to DOM-1 in combination with a pathogen like *C. jejuni* or other species have not been studied. To our knowledge, we have shown for the first time that the feeding of DOM-1 reduced the colonization of *C. jejuni* NCTC 12744 in the intestine by approximately 1.5–3.0 $\log_{10}$ (CFU/g) within the first two weeks post infection compared with co-exposure of *C. jejuni* to DON [36]. Such findings highlight the need for further investigations of DON and DOM-1 effects on *C. jejuni* at the transcriptome level as a model prokaryote. Therefore,

co-incubation during in vitro studies followed by RNA sequencing were implemented to resolve the mycotoxin-bacteria interaction. Hence, a better understanding of the pathogen response to DON at the transcriptome level may also lead to the identification of novel detoxification mechanisms, which can be applied to overcome or reduce DON contamination.

Materials and methods

Campylobacter jejuni strains and growth curve analysis

Different *C. jejuni* (reference strains NCTC 12744 (*Campylobacter jejuni* subsp. *jejuni*, isolated from contaminated milk, Public Health England, UK) and ATCC 700819 (*Campylobacter jejuni* subsp. *jejuni* (Jones et al.) Veron and Chatelain ATCC 700819, isolated from human faeces, LGC Standards ATCC, UK), and field isolates (1303, isolated from broiler chicken flock, pooled faecal samples), and 969 (isolated from environmental sample, swab from anteroom). Both strains were identified as *C. jejuni* by PCR and PCR-RFLP at our clinic [37, 38], and were cultivated at 41.5 °C for 48 h under microaerophilic conditions (Genbox microaer, BioMerieux, Vienna, Austria) on *Campylobacter* Selective Agar (Campylosel agar, BioMerieux), which was used to determine bacterial counts in the samples.

In the first set of experiments (3 technical replicates in 3 biological replicates), the interaction of different *C. jejuni* strains with DON and DOM-1 was investigated in vitro by measuring the optical density at a wavelength of 690 nm using a microplate photometer (Micronaut-S Microdilution system, Merlin Diagnostika GmbH, Kleinstraße 14, Barnheim-Hersel, Germany) and determining the bacterial count at 48 h. Different strains of *C. jejuni* at 10^5 CFU/mL were grown in 100 µl of Mueller-Hinton-Broth (Merck KGaA, Darmstadt, Germany), 95 µl of bacterial suspension were inoculated into 96-well plates (Sarstedt AG&Co KG, Sarstedtstr.1, Nümbrecht, Germany). DON or DOM-1 was dissolved in PBS (10 mg/mL, stock solutions). In the 96-well plates, 5µl of PBS was added to the bacterial suspension as a control sample. The 96-well plates were then inoculated with either DON (5µl) or DOM-1 (5µl) (a total volume of 100 µl in one well) and incubated under microaerophilic conditions over 48 h. The DON concentration used in the in vitro model is relevant to the field situation and is also of practical relevance as the current guidance for the tolerated value of DON in poultry diets is set at 5 mg/kg feed [39]. In the second series of experiments, to confirm the results, the bacterial growth curve was measured with different DON concentrations 5 and 20 ppm (low and high levels) to find out whether there is a difference in the bacterial response when exposed to high DON concentrations. For this, we used only two strains (a reference strain and a field isolate as a representative) for characterizing the bacterial activity based on CFU counts over the period of time (24 h, 30 h, 36 h, 42 h, 48 h).

For RNA sequencing, we proceeded only with the reference strain *C. jejuni* NCTC 12744, which we used in the in vivo experiments. The optical density was determined at 30 h and 48 h and the experiment was repeated three times for each bacterial strain (3 biological replicates). For each biological replicate, we had three technical replicates for each treatment and time point. Bacteria were harvested at 30 h and 48 h, 3 wells per plate (3×100 µL) and pooled together. The bacterial cells were then centrifuged at 16,000 x g for 2 min for immediate RNA extraction as described below for RNA-sequencing analysis. For each time point, four pooled biological replicates (12 samples) were used as control for *C. jejuni*, and due to the workload and the availability of the mycotoxin metabolite (DOM-1), three pooled biological replicates (9 samples) each for *C. jejuni* + DON (5 µg/mL) and for *C. jejuni* + DOM-1 (5 µg/mL), respectively.

In parallel to the determination of the optical density, CFU counts (at 24 h and 48 h) were performed from each well as described by Ruhnau et al. [36]. For bacterial enumeration, serial 10-fold dilutions were made from each sample and 100 µL from each dilution were direct-plated in duplicate onto Campylosel agar (BioMérieux, Vienna, Austria). The plates were incubated under microaerobic conditions at 41.5°C for 48 h and typical *Campylobacter* colonies were enumerated by plate colony counter (ThermoFisher Scientific, USA) as colony-forming units. CFU counts were determined by calculating the mean value of both plates.

RNA extraction and bacterial RNA sequencing

Total RNA extractions were conducted with the RNAsnap method with slight modifications [40]. In short, the cell pellet was resuspended in 300 µL RNAsnap solution by shaking at 5000 rpm (2×20 s). Cells were lysed by incubating at 95 °C for 3 min, vortexed for 30 s, and incubation for 4 min at 95 °C. After pelleting the cell debris at 16,000 x g for 5 min, the supernatant was carefully transferred to a new tube for RNA cleanup. Cleanup was achieved with the Monarch RNA Cleanup kit (New England Biolabs, Frankfurt am Main, Germany) according to the manufacturer's instructions. Total RNA concentration was determined using a NanoDrop 2000 spectrophotometer (ThermoFisher Scientific, USA). Afterwards, the RNA samples were qualified for sequencing, and shipped to the sequencing platform provider (LGC Standards GmbH, Wesel, Germany). The provider further treated the RNA samples with DNase and performed rRNA depletion using the MICROB*Express*™ bacterial mRNA enrichment kit (ThermoFisher Scientific, USA). The RNA-Seq libraries were pooled and sequenced on Illumina NextSeq 500/550 V2 with 75 bp single reads.

In total, approximately 100 million reads were obtained across all 20 samples, with an average of 5.4 million of reads per sample (genome size of *C. jejuni* is 1.6 Mbp). The resulting reads were demultiplexed (Illumina bcl-2fastq 2.17.1.14 software), trimmed, and further filtered for rRNA sequences using RiboPicker 0.4.3. Alignment of RNA-Seq reads was performed with Bowtie 2, and against the annotated genome of *C. jejuni* 81–176 (NC_008787.1) showing 98.41% homology with our *C. jejuni* strain NCTC 12744 (microbial nucleotide BLAST, database prok_complete_genomes, NCBI). HTSeq was used for counting of TopHat-aligned reads, and gene expression levels were normalized as reads per kilobase of transcript per million mapped reads (RPKM).

Statistical analysis

For bacterial cell counts, the data are presented as means with standard error of mean (SEM). To evaluate the normality Kolmogorov-Smirnov´s test was utilized. A multivariate general linear model, ANOVA, Duncan´s multiple range test and LSD were applied to analyze differences between groups. Differences were considered significant at a level of $P \leq 0.05$. Data were analyzed by IBM SPSS Statistics 24 software for Windows (Chicago, IL, USA). For RNA-seq analysis, RPKM were used for fold change calculation between control and treatments (DON or DOM-1) at each time point (30–48 h). The mixed effects model approach was applied on the individual RPKM (reads per kilobase of transcript per million mapped reads) with treatment and time as two factors (Two-way ANOVA), followed by Sidak's multiple comparison test with *P* value. The principal component analysis (PCA) and heatmaps were conducted with the online tool ClustVis (https://biit.cs.ut.ee/clustvis/) and using the normalized gene expression levels (RPKM). The PCA was implemented to measure the strength of association between *Campylobacter* genes at both time points (30 h and 48 h). In addition, two-way ANOVA was done on the pooled normalized counts for flagellar genes (treatment and time factors), followed by Dunnett's multiple comparison test.

Results

Bacterial growth analysis

In the first set of experiments, to investigate a possible interference of DON with different *C. jejuni* strains, the in vitro co-incubation of *C. jejuni* NCTC 12744, *C. jejuni* ATCC 700,918, *C. jejuni 1303*, and *C. jejuni* 969 with DON or DOM-1 was investigated applying the Micronaut-Microdilution system. The results showed that that the presence of DON resulted in higher growth rates in all strains compared to the results when *C. jejuni* was grown alone (Fig. 1a, b). However, these findings were only significant for the reference strains NCTC 12744

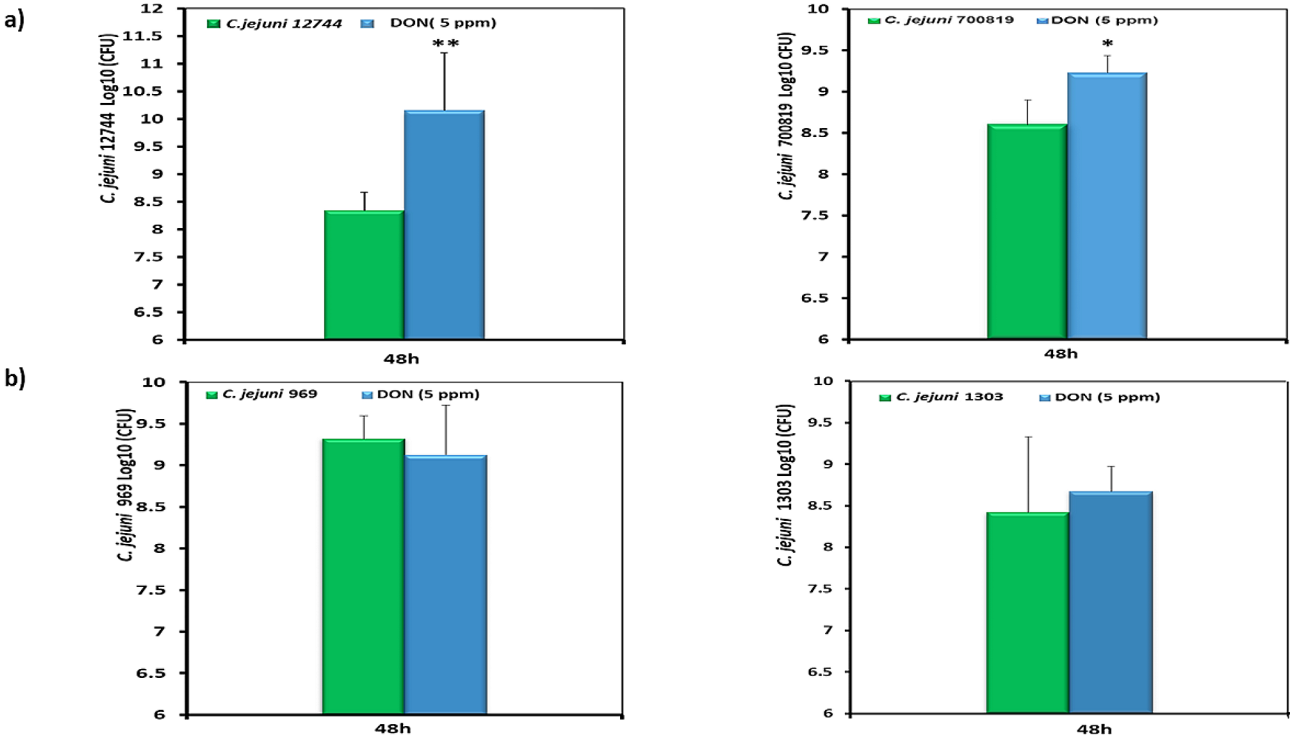

Fig. 1 Strain-specific differences **(a)** (Reference strains *C. jejuni* NCTC 12744 and *C. jejuni* ATCC 700819; **(b)** Field isolates (*C. jejuni* 969 and *C. jejuni* 1303) in *Campylobacter* proliferation in the presence of DON. Results are presented as mean values and standard error of mean (SEM) ($n = 9$). Asterisks mark differences with $P \leq 0.05$ (*), or $P \leq 0.01$ (**)

and ATCC 700,819 at 48 h and not for *C. jejuni* 969 and *C. jejuni* 1303 (Fig. 1b). For confirming the results, a bacterial growth curves were established to characterize the bacterial activity based on CFU counts over this period (Fig. 2a, b). This effect was statistically consistent and had good predictability, indicating a growth stimulation effect of the bacteria in the presence of DON (1.4×10^{10} CFU, Fig. 1a). On the contrary, co-incubation with DOM-1 resulted in a significant decrease in CFU counts for all *C. jejuni* strains investigated (2.3×10^{8} CFU, Fig. 3b).

Effect of DON on the transcriptome of *C. Jejuni* NCTC 12744 over time

Bacterial genes that were affected by DON at both time points, 30 h and 48 h, are presented in Fig. 4. A fold change of at least 1.5 was seen for those genes when DON was present compared to *C. jejuni* alone, either upregulating or downregulating their expressions. Interestingly, most of the genes showing lower expression were related to ribosomal RNAs, with 19 out of 32 downregulated genes mapping to the ribosome pathway (i.e. subunit rpl, 50 S ribosomal protein, and subunit rps, 30 S ribosomal protein, Fig. 5). Regarding the upregulated genes, the effects were more pronounced after 48 h of exposure to DON than after 30 h. This is for instance the case for the bacterial gene Acfc, accessory colonization factor, involved in infectivity, and exhibiting a significant 3.42-fold increase at 48 h. Furthermore, the expression of 6 genes (flgD, flgH, flgI, flgB, flgG, flgE), encoding bacterial motility proteins was increased in the *C. jejuni* samples treated with DON at 30 h (Fig. 6). However, after 48 h, the mRNA levels of those genes were either unchanged or reduced, showing a different profiling depending on the duration of DON exposure.

Effect of DOM-1 on the transcriptome of *C. Jejuni* over time

Like the approach with DON, the genes showing either a 1.5 fold increase or decrease at both time points were selected. Only 20 bacterial genes met these criteria at 30 h and 48 h. Interestingly, most of the genes showing lower expression were related to ribosomal RNAs, with 19 out of 32 downregulated genes mapping to the ribosome pathway (i.e. cje03010 in KEGG pathway analysis corresponding to subunit rpl, 50 S ribosomal proteins, and subunit rps, 30 S ribosomal proteins; Fig. 5 presents the expression of some of those ribosomal proteins). Furthermore, the expression of 6 genes, encoding bacterial motility proteins, was also decreased in the *C. jejuni* samples treated with DOM-1. These genes (flgD, flgH, flgI, flgB, flgG, flgE; not polycistronic mRNAs) are related to flagellar assembly in bacteria (i.e. cje02035 in KEGG pathway analysis corresponding to bacterial motility

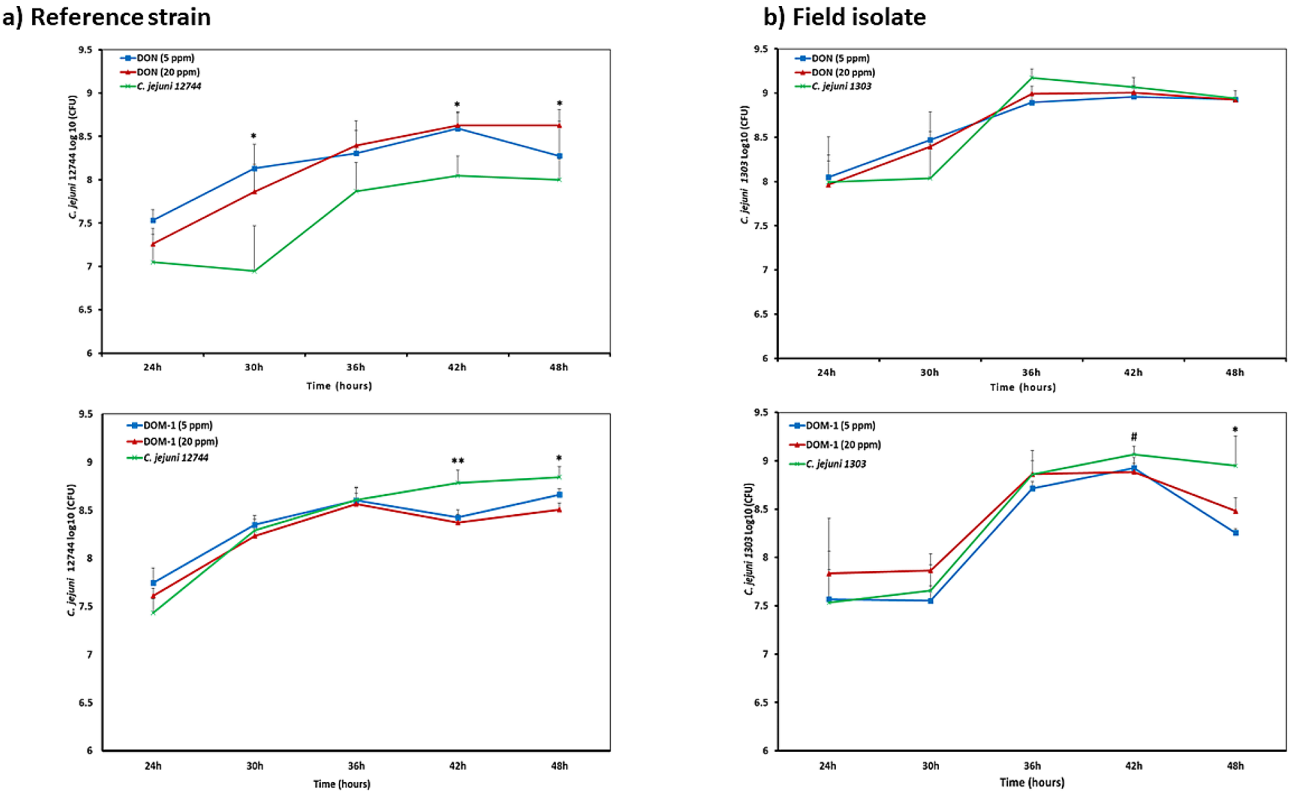

Fig. 2 Growth kinetics of **(a)** Reference strain (*C. jejuni* NCTC 12744); **(b)** Field isolate (*C. jejuni* 1303) in the presence of DON and DOM-1. Results are presented as mean values and standard error of mean (SEM). Asterisks mark differences with $P \leq 0.1$ (#), or $P \leq 0.05$ (*), or $P \leq 0.01$ (**)

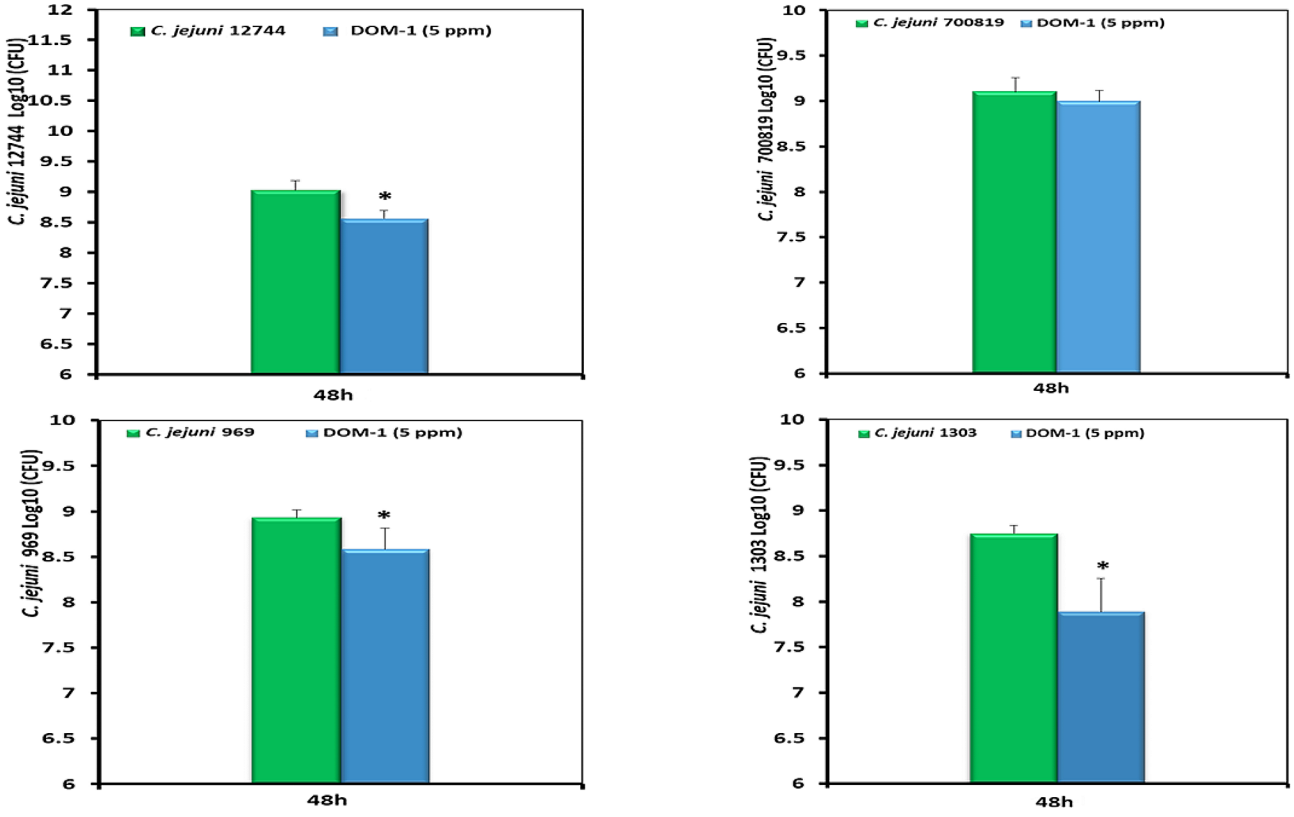

Fig. 3 Strain-specific differences **(a)** Reference strains (*C. jejuni* NCTC 12744 and *C. jejuni* ATCC 700819; **(b)** Field isolates (*C. jejuni* 969 and *C. jejuni* 1303) in *Campylobacter* proliferation in the presence of DOM-1. Results are presented as mean values and standard error of mean (SEM) ($n=9$). Asterisks mark differences with $P \leq 0.05$ (*)

proteins), and their expressions are presented in Fig. 6. Since these genes belong to the same pathway, their normalized counts were pooled to increase the number of data points. A significant effect of DON treatment on flagellar genes ($p=0.0031$) and, in contrast, a significant decrease in expression with DOM-1 as revealed by the multiple comparisons test ($p=0.084$ at 30 h, and $p=0.0021$ at 48 h).

Relationships among treatments were examined by Principal component analysis (PCA). The PCA showed no clear clustering of *Campylobacter* genes between control and DOM-1 treatment, especially at 48 h (Fig. 7). PCA plots also demonstrate that *Campylobacter* genes were more separated with DON treatment at both time points (Fig. 7). In addition, the fold changes of the genes sorted by time points of control, DON, and DOM-1 treatment of each biological replicate are shown in the heatmaps (Fig. 7). The gene similarity among all samples showed clear differences between treatments at both time points, indicating strong shifts in *Campylobacter* genes as a result of DON treatment.

Discussion

Chickens serve as a major source of human infections with *C. jejuni*, and therefore, infected birds remain a substantial problem for poultry production [41]. Likewise, contamination of food and feed with mycotoxins is a global problem and the presence of mycotoxins in poultry feeds is a significant economical factor. DON is the most common trichothecene mycotoxin detected in feedstuffs worldwide [14].

The effect of DON on prokaryote remains unclear, although the mechanisms and physiological disruption of this toxin in eukaryotes have been well characterized [42, 43]. It was reported that DON can alter the gut microbiota in humans and animals [44, 45]. Recently, we demonstrated that the co-exposure of broiler chickens to DON and *C. jejuni* increased the intestinal *C. jejuni* load, indicating that DON may represent a favorable prerequisite for *Campylobacter* multiplication [22]. Furthermore, the DON impact on *Campylobacter* growth can also be explained by the fact that this bacterium can rely on DON as a sole source of carbon [46]. This raises questions about the synergism between food contaminants and *Campylobacter* with regard to food-borne gastroenteritis. This, in turn, has led to a greater interest in understanding bacterial responses toward DON. Johnson

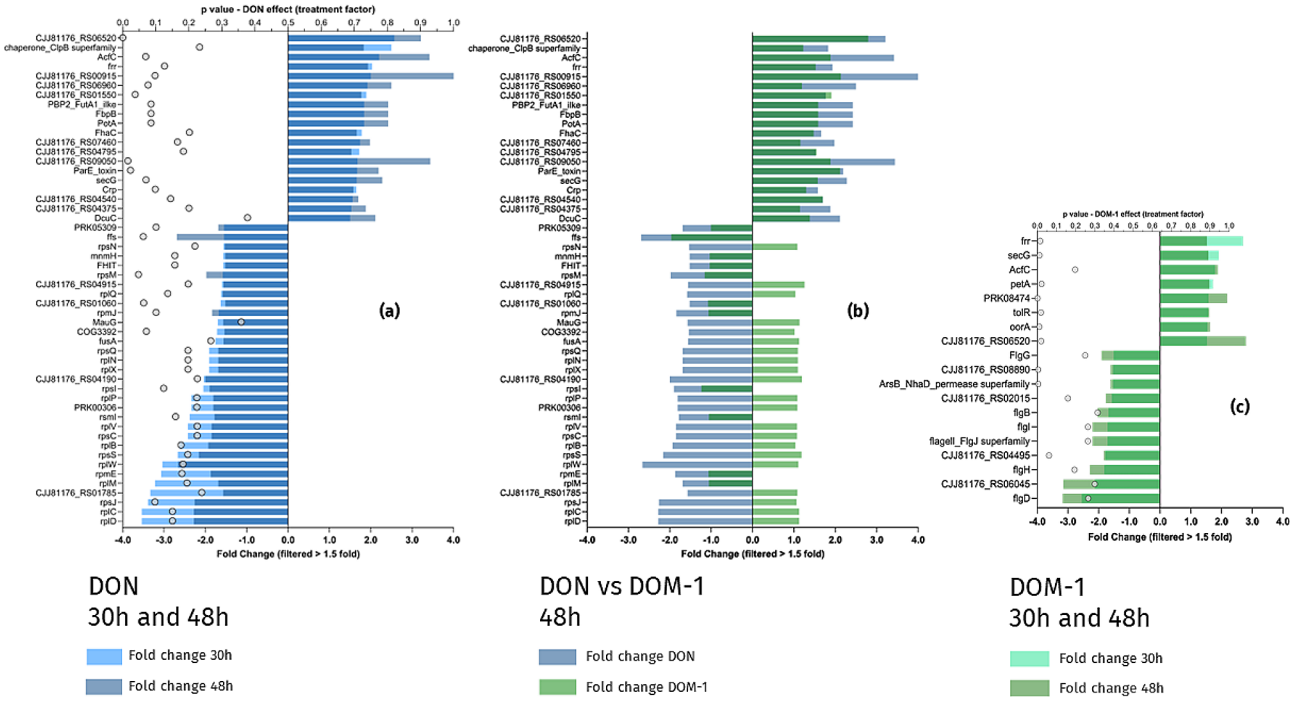

Fig. 4 Summary of the expression of genes of *C. jejuni* NCTC 12744 affected by DON and DOM-1 at both time points, 30 h and 48 h. The Y-axis represents the functional genes, and the X-axis represents the fold change with genes at least 1.5-fold decreased or increased compared to *C. jejuni* alone. **(a)** The blue color of bar represents the fold-change of up- or down-regulated genes for DON treatment at 30 h, and the grey color of bar represent the fold-change of up-or down-regulated genes for DON treatment at 48 h. *P*-values from the treatment effect (i.e. DON) following 2-way ANOVA (with treatment and time factors) were plotted as grey dots, and their values are seen through the extra X-axis on top of the bar chart. **(b)** Differences in gene transcription profiles between DON and DOM-1 on the genes affected by DON at 48 h. The blue color of bar represents the fold-change of up- or down-regulated genes for DON treatment, and the green color of bar represent the fold-change of the same genes for DOM-1 treatment. **(c)** The bright green color of bar represents the fold-change of up- or down-regulated genes for DOM-1 treatment at 30 h, and the grey color of bar represent the fold-change of up-or down-regulated genes for DOM-1 treatment at 48 h. *P*-values from the treatment effect (i.e. DOM-1) following 2-way ANOVA (with treatment and time factors) were plotted as grey dots, and their values are seen through the extra X-axis on top of the bar chart

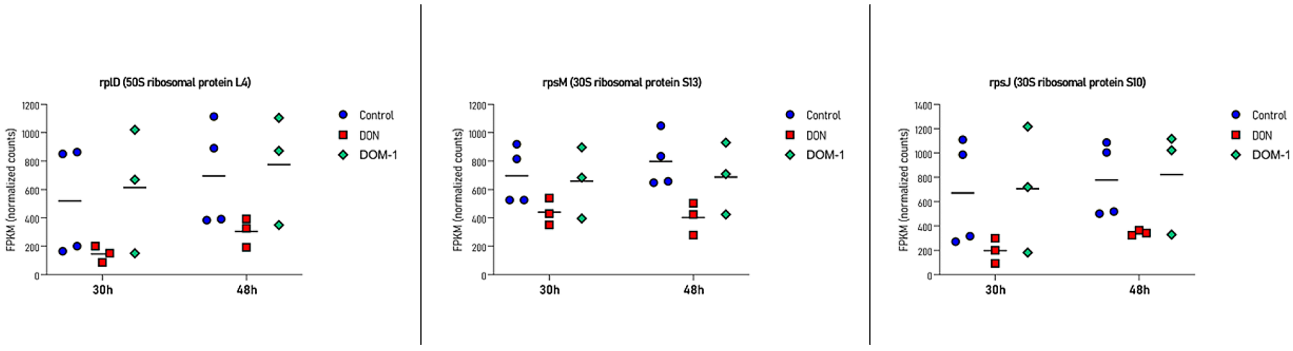

Fig. 5 Expression of *C. jejuni* NCTC 12744 ribosomal genes compared with the expression of the same genes of *C. jejuni* in the presence of DON at the two sampling points (30 h and 48 h) determined by RNA-seq. The functional analysis was done with KEGG pathway. The pathway enrichment analysis showed that many down-regulated genes mapped to the cje03010 ribosome pathway (i.e. cje03010 in KEGG pathway analysis corresponding to subunit rpl, 50S ribosomal proteins, and subunit rps, 30S ribosomal proteins)

et al. [47] reported that prokaryotic RNA-Seq analysis is challenging because most available RNA-Seq packages assume the input data reflect eukaryotic gene structures, which in many aspects differ from those of prokaryotes. Hence, RNA-Seq technology has been extensively used in studies of pathogenic bacteria to identify and quantify changes in gene expression among different samples from bacteria exposed to various conditions.

To resolve the nature of the influence of DON on the infection profile of *C. jejuni*, the direct interactions between *C. jejuni* and DON or a less-toxic DON-metabolite were investigated. Overall, the actual study demonstrated that the presence of DON resulted in significantly

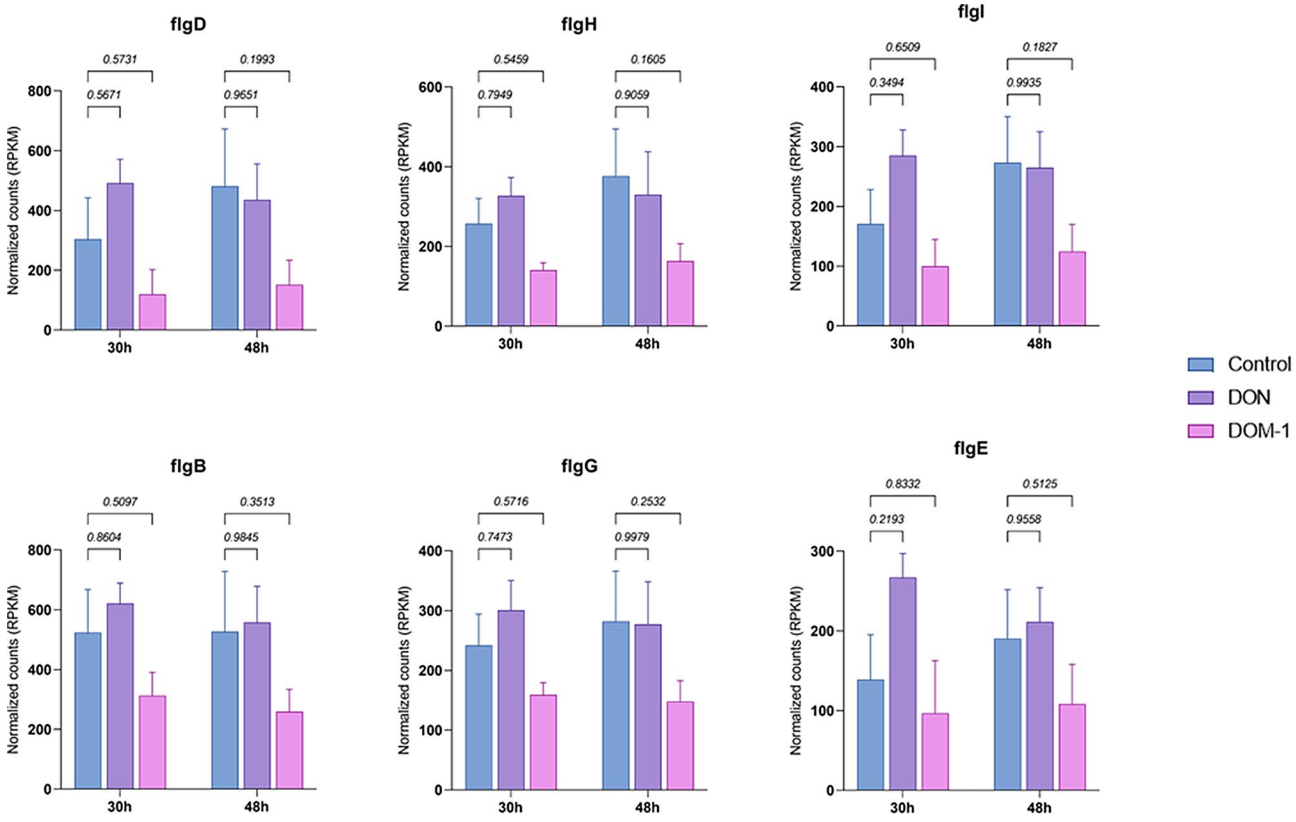

Fig. 6 Expression of *C. jejuni* NCTC 12744 flagella genes compared with the expression of the same genes of *C. jejuni* in the presence of DON or DOM-1 at the two sampling points (30 h and 48 h) determined by RNA-seq. Data are presented as the mean values and SD. The functional analysis was done with KEGG pathway (i.e. cje02035 in KEGG pathway analysis corresponding to bacterial motility proteins)

higher growth rates of *C. jejuni* from 30 h incubation onwards, confirming the in-vivo data using the same concentration, indicating growth-stimulating effects of the bacteria. However, none of the field strains showed stronger growth in the presence of DON. One explanation for this might be that the presence of DON could alter the activity of the reference strains and thus has an impact on growth. Thus, we employed RNA-seq technology to explore the changes in bacterial mechanisms in response to DON. Co-incubation of *C. jejuni* with DON increased the expression of Flagella gene family, frr (ribosome-recycling factor), PBP2 futA-like (Fe^{3+} periplasmic binding family), biosynthesis of amino acid and PotA (ATP-binding subunit), which are required for motility, biofilm formation, host cell interactions, and host colonization. The *C. jejuni* multiplication can be also explained by the fact that the bacterium evades DON toxicity by upregulating several ABC-dependent membrane transporters and efflux pumps that remove many undesirable toxins/chemicals (including DON) from the environment [48]. In line with that, we also found that DON increased the expression of efflux transporters (e.g. ATP-binding subunit). Together, co-incubation of *C. jejuni* with DON led to the development of a variety of mechanisms to compete environmental challenges.

It has been reported that mycotoxin deactivators can convert DON into the non-toxic metabolite DOM-1 through enzymatic biotransformation, thereby reducing DON burden in chickens [49]. The mechanisms of action of DOM-1 are activation of metabolic enzyme activity and feed digestibility, activation of the liver function as well as strengthening of the immune system [50]. Recently, we found that feeding of DOM-1 reduced intestinal *C. jejuni* load by 1.5-3.0 $\log_{10}$ (CFU/g) [36]. Furthermore, we hypothesized that DOM-1 might create a different intestinal environment to which *C. jejuni* could adapt [35, 51]. Therefore, based on these findings, the current study was conducted to explain how DOM-1 might directly affect a prokaryote such as *Campylobacter* in chickens. In the current experiments, we found that the presence of DOM-1 leads to a significant decrease in *C. jejuni* CFU counts of all strains. These results confirm our previous in vivo data, as we found that the dietary inclusion of DOM-1 reduced the intestinal load of *C. jejuni* at 7- and 14-days post infection [36]. The results showed that, in contrast to DON, DOM-1 downregulated bacterial motility genes (Flagella gene family) and

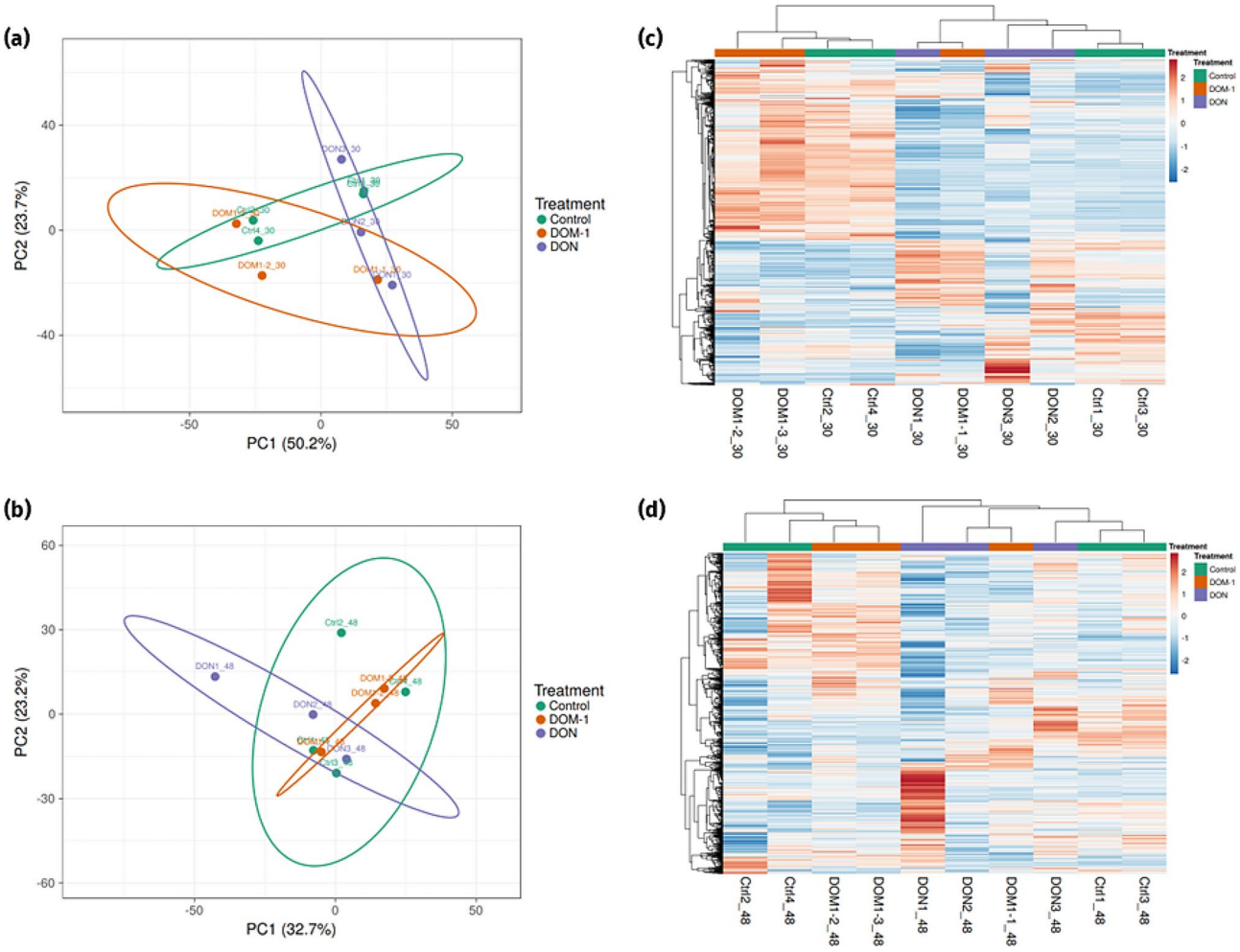

Fig. 7 The principal component analysis (PCA) and heatmaps were generated with the online tool ClustVis (https://biit.cs.ut.ee/clustvis/), and using the normalized gene expression levels (reads per kilobase of transcript per million mapped reads, RPKM). PCA were analysed for all samples at 30 h **(a)**, and at 48 h **(b)**. Green (control), dark orange (DOM-1), and purple (DON), with each dot indicating a biological replicate. Heatmaps were analysed for all samples at 30 h **(c)**, and at 48 h **(d)**

upregulated ribosomal proteins, implying reduced proliferation activity of this bacteria. The results showed that DOM-1 could threaten the survival of *Campylobacter*, indicating a specific response to the influx of DON and DOM-1 into bacterial cells through changes in the bacteria's metabolic pathways. However, the bacteria could be mitigating the effects of a metabolite targeting its ribosomal genes, allowing it to continue with protein synthesis. Overall, through a combination of our recent *in vivo* trial with the current results, we demonstrated that that mycotoxin-bacteria interactions can alter the virulence of *Campylobacter* in its hosts. The results may provide new insights into targets that can be used for future studies of molecular mechanisms underlying the *Campylobacter* response to DON and DOM-1. However, the specific functions of the identified genes in *Campylobacter* metabolic pathways should be confirmed by further molecular biological investigations.

Conclusion

The results of the present studies confirmed that DON interfered with the ribosomal proteins and upregulated the flagellar proteins. On contrast, it demonstrated that deepoxy-DON (DOM-1) did not activate these signaling pathways. These results expand the current knowledge of the toxicity of DON and beneficial effects of deepoxy-DON (DOM-1) and contribute to the evaluation of the efficacy of the microbial biotransformation strategies in the fight against mycotoxins. Finally, the study also demonstrated that DOM-1 has a substantial impact on the *C. jejuni* propagation and by this also on the colonization.

Abbreviations

DON	Deoxynivalenol
DOM-1	Deepoxy-deoxynivalenol
C.	*Campylobacter*
Frr	Ribosome-recycling factor
PBP2	futA-like Fe3 + periplasmic binding family
PotA	ATP-binding subunit

MAPK	Mitogen-activated protein kinase
CFU	Colony-forming units
RPKM	Reads per kilobase of transcript per Million mapped reads
PCA	Principal component analysis
FDR	False discovery rate correction

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Author contributions
All authors listed have made a substantial, direct and intellectual contribution to the work, and approved it for publication.WA and MH conceived and designed the project. DR performed the in vitro experiments and collected the data. BG performed the RNA-sequencing data analysis. WA, DR, CH, BG, DS, and MH discussed the results. WA and BG wrote the manuscript. All the authors contributed to manuscript revision and approved the final manuscript.

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Data availability
Sequencing data are available in the European Nucleotide Archive (ENA) database under the accession number GSE248277.

Declarations

Ethics approval and consent to participate
Not applicable.

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

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