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Samara Paula Mattiello Drescher

CARACTERIZAÇÃO DA FORMAÇÃO DE CÉLULAS PERSISTENTES EM *Salmonella enterica*

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Pontifícia Universidade Católica
do Rio Grande do Sul

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Tese de Doutorado apresentada ao Programa de Pós-Graduação em Biologia Celular e Molecular, da Escola de Ciências da Pontifícia Universidade Católica do Rio Grande do Sul.

Orientadora: Dra. Sílvia Dias de Oliveira

Coorientador: Dr. Carlos Alexandre Sanchez Ferreira

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“We are just an advanced breed of monkeys on a minor planet of a very average star.

But we can understand the Universe. That makes us something very special.”

(Stephen Hawking em entrevista à revista alemã Der Spiegel, 1988).

RESUMO

A *Salmonella enterica* é uma importante bactéria zoonótica, associada a doenças transmitidas por alimentos, devido ao consumo de alimentos contaminados, especialmente os de origem animal. Diferentes sorovares de *S. enterica* não-tifóide são considerados patógenos adaptados para infectar e sobreviver no interior de células fagocíticas, desencadeando quadros de gastroenterites em animais, incluindo humanos, porém, na maioria das vezes, autolimitante. O uso de terapia antimicrobiana só se faz necessário nos casos de salmonelose grave, sendo as fluoroquinolonas e as cefalosporinas de terceira geração os fármacos de escolha. Entretanto, a prevalência de isolados multirresistentes de *S. enterica* em amostras de diferentes origens têm sido cada vez mais reportada, o que poderia levar a falhas no tratamento de infecções com antimicrobianos. Por outro lado, o insucesso terapêutico e a recalcitrância de infecções também podem ser associados à presença de células *persisters*. Diante disso, esse trabalho se propôs a avaliar os níveis de células *persisters* em isolados de *S. enterica* expostos aos antimicrobianos ciprofloxacina e ceftazidima, bem como a influência da exposição prévia a aditivos alimentares animal na tolerância à ciprofloxacina. Adicionalmente, buscou-se identificar transcritos diferencialmente expressos em células *persisters* de diferentes sorovares de *S. enterica* expostas à ciprofloxacina e à ceftazidima em cultivo planctônico. Para tanto, foram avaliados 10 isolados de *S. enterica*, que se mostraram fracos formadores biofilme em superfície de poliestireno e suscetíveis à ciprofloxacina, ceftazidima e colistina. Todos os isolados foram capazes de formar frações distintas de células *persisters* após a exposição a 100X o valor da Concentração Inibitória Mínima (CIM) para ciprofloxacina ou ceftazidima em cultivo planctônico e em biofilme. Os níveis de *persisters* em biofilmes foram superiores àqueles encontrados em cultivo planctônico para ambos os fármacos, bem como foi possível observar uma heterogeneidade nesses níveis entre os isolados de *S. enterica* frente a um mesmo desafio. Adicionalmente, foi constatada a presença de *small colony variants* (SCV) em meio às células sobreviventes após as exposições à ciprofloxacina em todos os isolados de *S. enterica*. Contudo, o fenótipo SCV mostrou-se instável, uma vez que foi observada a reversão para o fenótipo de colônia normal (FCN) quando foram realizados sub-cultivos derivados destas colônias na ausência do agente estressor, mesmo após repetidos ciclos de exposição à ciprofloxacina. Da mesma forma, foi possível verificar que foram encontrados níveis semelhantes de *persisters* em um isolado de *S. enterica* após os sucessivos ciclos de exposição ao mesmo fármaco, não

ocorrendo seleção de um fenótipo altamente persistente, o que demonstra o caráter não-herdável da condição de *persisters*. Também foi verificada heterogeneidade nos níveis de *persisters* frente a fármacos com mecanismos de ação diferentes, não indicando a persistência como um fenótipo de multitolerância. Estes achados estão de acordo com os padrões heterogêneos de expressão gênica encontrados frente às exposições à ciprofloxacina e à ceftazidima. As células oriundas de SCVs e FCNs, obtidas de cultivo planctônico e de biofilme expostos à ciprofloxacina foram avaliadas por meio de microscopia eletrônica de varredura, sendo observado que os dois fenótipos apresentaram forma e tamanho semelhantes, independentemente da condição de cultivo analisada. Entretanto, foi visualizada a presença de septo de divisão e de filamentação em todos os morfotipos e condições de cultivo analisados. Cultivos planctônicos de um subgrupo de seis isolados de *S. enterica* também foram expostos a concentrações acima da CIM de colistina, tendo sido encontrado um isolado de *S. Agona* incapaz de formar *persisters* frente a esse fármaco. Nos demais isolados não só foram detectadas células sobreviventes ao tratamento com colistina, como, interessantemente, após 48 h de exposição, foi verificada a retomada do crescimento na presença de concentrações do fármaco similares às iniciais. A seleção de mutantes resistentes e de hetero-resistentes estáveis foi descartada nesta população sobrevivente que se multiplicou na presença da colistina. Além disso, foi verificada que a exposição prévia a concentrações subinibitórias de ácidos orgânicos, colistina e, até mesmo, de ciprofloxacina não influenciou nos níveis de células *persisters* após a exposição a concentrações letais deste último fármaco. Desta forma, estes resultados sugerem que os antimicrobianos testados, que foram ou ainda são empregados como aditivos alimentares adicionados à ração ou água de bebida em criação animal, não induziram a tolerância a antimicrobianos nem selecionaram mutantes altamente persistentes. De uma maneira geral, os achados deste trabalho sugerem que além da possível presença de várias estratégias adaptativas para a sobrevivência frente a estressores antimicrobianos entre isolados de *S. enterica*, um único isolado pode originar populações fisiologicamente distintas de *persisters*, onde células que vivenciam condições estressoras diferentes possam adotar estratégias de sobrevivência variadas e talvez complementares.

Palavras-chave: células *persisters*, *small colony variants*, ciprofloxacina, ceftazidima, colistina, ácidos orgânicos, biofilme, tolerância a antimicrobianos.

ABSTRACT

Salmonella enterica is an important zoonotic pathogen associated with foodborne diseases due to the consumption of contaminated foods, especially those derived from animal origin. Different non-typhoid *S. enterica* serovars are considered pathogens adapted to infect and survive inside phagocytic cells, triggering gastroenteritis in animals, including humans, but usually self-limiting. The use of antimicrobial therapy is only necessary in cases of severe salmonellosis, being fluoroquinolones and third-generation cephalosporins the drugs of choice. However, prevalence of multiresistant isolates of *S. enterica* in samples from different origins has been increasingly reported, which could lead to failures in the antimicrobial treatment against infections. On the other hand, therapeutic failure and recalcitrant infections may also be associated with persister cells. Therefore, this study aimed to evaluate the persister cell levels in *S. enterica* isolates exposed to ciprofloxacin and ceftazidime, as well as the influence of previous exposure to animal feed additives on tolerance to ciprofloxacin. Additionally, it identified differentially expressed transcripts in persister cells from different *S. enterica* serovars exposed to ciprofloxacin and ceftazidime in planktonic culture. For this, 10 *S. enterica* isolates were evaluated and characterized as weak producers of biofilm on polystyrene surface and susceptible to ciprofloxacin, ceftazidime, and colistin. All isolates were able to form distinct fractions of persister cells after exposure to 100X the Minimum Inhibitory Concentration (MIC) value of ciprofloxacin or ceftazidime in planktonic culture and biofilm. The levels of persisters in biofilms were higher than those found in planktonic culture for both drugs, and it was possible to observe heterogeneity in these levels among *S. enterica* isolates against the same challenge. In addition, *small colony variants* (SCV) were found among surviving cells after exposure to ciprofloxacin in all *S. enterica* isolates. Nevertheless, the SCV phenotype showed to be unstable, since reversion to the normal colony phenotype (NCP) was observed when sub-cultures derived from these colonies were performed in the absence of the stressor, even after repeated cycles of exposure to ciprofloxacin. Likewise, it was possible to verify that similar persister levels were found in a *S. enterica* isolate after successive cycles of exposure to the same drug, with no selection of a highly persistent phenotype, demonstrating the non-inheritable condition of the persisters. We also found heterogeneity in persister levels following exposure to drugs with different mechanisms of action, indicating that persistence is not a multitolerant phenotype. These findings are in agreement with the heterogeneous

patterns of gene expression found on exposure to ciprofloxacin and ceftazidime. Cells from SCVs and NCPs, obtained from planktonic culture and biofilm exposed to ciprofloxacin were evaluated by scanning electron microscopy, and it was observed that the two phenotypes presented similar shape and size, regardless of the culture condition analyzed. However, division septum and filamentous cells were found in all morphotypes and culture conditions analyzed. Planktonic cultures of a subgroup of six *S. enterica* isolates were also exposed to concentrations above the MIC of colistin, and one *S. Agona* isolate was unable to form persisters against this drug. The other isolates not only presented surviving cells after colistin treatment, but, interestingly, after 48 h of exposure, a resumption of growth was observed in the presence of the drug. The possible selection of resistant mutants and stable hetero-resistant cells was discarded in this surviving population that was able to grow in the presence of colistin. Furthermore, a previous exposure to sub-inhibitory concentrations of organic acids, colistin, and even ciprofloxacin did not influence persister cell levels after exposure to lethal concentrations of ciprofloxacin. Thus, these results suggest that the antimicrobials tested, which were or are still employed as feed additives added to animal feed or drinking water, did not induce antimicrobial tolerance nor select highly persistent mutants. Overall, the findings of this work suggest that, in addition to the possible presence of several adaptive strategies for survival against antimicrobial stressors among *S. enterica* isolates, a single isolate may originate physiologically distinct populations of persisters, where cells growing under distinct stress conditions may adopt different and perhaps complementary survival strategies.

Key words: persister cells, small colony variants, ciprofloxacin, ceftazidime, colistin, organic acids, biofilm, antimicrobial tolerance.

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LISTA DE ABREVIATURAS E SIGLAS

(p)ppGpp – Guanosina tetra- ou pentafofato

AMP – Adenosina 3',5'-monofosfato cíclico

Asp87 – Ácido aspártico na posição 87

ATP – Trifosfato de adenosina (*Adenosine Triphosphate*)

ATR – Resposta de tolerância ao ácido (*acid tolerance response*)

CAT – Catalase

CDC – Centro de controle e prevenção de doenças (*Centers for Disease Control and Prevention*)

DTA – Doenças Transmitidas por Alimentos

EPS – Matriz polimérica extracelular (*Extracellular Polymeric Substance*)

ERN – Espécies reativas de nitrogênio

ERO – Espécies reativas de oxigênio

ESBL – β -lactamases de espectro estendido (*Extended-Spectrum Beta-Lactamase*)

F – Antígeno flagelar

FACS – Separação de células mediada por fluorescência (*Fluorescence-Activated Cell Sorting*)

Fe-S – Ferro e enxofre

GltX – Glutamil-RNAt sintase

H₂O₂ – Peróxido de hidrogênio

H₂S – Ácido sulfídrico

hip – Mutante altamente persistente (*High Persister*)

HipA – Proteína serina quinase

KatG – Catalase-peroxidase

LPS – Lipopolissacarídeo

MAPA – Ministério da Agricultura, Pecuária e Abastecimento

MDR – Resistência a múltiplos fármacos (*Multidrug Resistance*)

MDT – Multitolerância

NADH – Nicotinamida adenina dinucleotídeo

NTS – *Salmonella* não tifoide (*Non-typhoid Salmonella*)

O – Antígeno somático

OH· – Radical hidroxila

OMS – Organização Mundial da Saúde (*World Health Organization*)

PBP – Proteína de ligação à penicilina (*Penicillin Binding Protein*)

pH – Potencial hidrogeniônico

PMF – Força próton-motiva

PMQR – Plasmídeos mediadores de resistência às quinolonas (*Plasmid Mediated Quinolone Resistance*)

PPK – Polifosfato quinase

PPX – Exopolifosfatase

QRDR – Regiões determinantes de resistência às quinolonas (*Quinolone Resistance Determining Region*)

QS – Percepção de quórum (*Quorum Sensing*)

SCV – Colônia variante pequena (*Small Colony Variant*)

Ser83 – Serina na posição 83

ShpAB – *Salmonella* altamente persistente (*Salmonella High Persister*)

SOD – Superóxido dismutase

SPI – Ilhas de patogenicidade de *Salmonella* (*Salmonella Pathogenicity Island*)

SR – Resposta à privação nutricional (*Stringent Response*)

T3SS – Sistema de secreção do tipo III

TA – Sistema toxina-antitoxina

Thr57 – Treonina na posição 57

VapBC – Virulência associada às proteínas B e C (*Virulence-Associated Proteins B and C*)

Vi – Antígeno de virulência

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Capítulo 1

Introdução

Objetivos

1.1 Introdução

Bactérias do gênero *Salmonella* pertencem à família Enterobacteriaceae, e são referidas como bacilos Gram negativos, intracelulares facultativas e não formadoras de esporos. Bioquimicamente é descrita como anaeróbia facultativa, oxidase negativa, produtora de ácido sulfídrico (H₂S), fermentadora de glicose e não fermentadora de lactose, malonato ou sacarose. Do mesmo modo, não exibem a capacidade de hidrolisar a ureia ou produzir indol, além de serem catalase positiva e fazerem a descarboxilação da lisina e ornitina. Apresentam flagelos peritríquios, conferindo-lhes a capacidade de locomoção, com exceção da *Salmonella Pullorum* e da *Salmonella Gallinarum* (1). Estes microrganismos apresentam pH ótimo para seu crescimento de 6,5 a 7,0 e são capazes de crescer em temperaturas que podem variar de 8 a 45°C, com temperatura ótima de crescimento a 37°C. No entanto, são termossensíveis, sendo inativados a temperaturas superiores a 60°C (2,3).

Taxonomicamente, esse gênero é constituído de apenas duas espécies, *Salmonella enterica* (*S. enterica*), mais comumente isolada do homem e de outros animais de sangue quente, e *Salmonella bongori* (*S. bongori*), geralmente isolada de animais de sangue frio. *Salmonella enterica* é dividida em seis subespécies: *S. enterica* subsp. *enterica* (I), *S. enterica* subsp. *salamae* (II), *S. enterica* subsp. *arizonae* (IIIa), *S. enterica* subsp. *diarizonae* (IIIb), *S. enterica* subsp. *houtenae* (IV) e *S. enterica* subsp. *indica* (VI) (4). As salmonelas apresentam uma complexa nomenclatura proposta por Kauffmann-White (1981), baseada na sua estrutura antigênica – antígenos somáticos (O), flagelares (F) e no antígeno de virulência (Vi) (5) –, sendo descritos até o momento 2.659 sorovares compondo a espécie *S. enterica* (6). Esse patógeno pode ser naturalmente encontrado habitando locais distintos na natureza, como solo, sedimento, água (7-9), e até mesmo

integrando a microbiota do trato digestório de diversas espécies de animais, incluindo mamíferos, aves, répteis e insetos (10).

A capacidade de adaptação às condições do organismo hospedeiro e a patogenicidade resultante dependem do sorovar envolvido. Alguns sorovares são altamente adaptados a um hospedeiro específico, como *S. Typhi* e *S. Paratyphi* A, B e C ao homem, *S. Dublin* aos bovinos, *S. Choleraesuis* e *S. Typhisuis* aos suínos, e *S. Pullorum* e *S. Gallinarum* às aves (11,12). Infecções por esse microrganismo são classicamente separadas em tifoide e não tifoide, de acordo com a natureza do agente envolvido. *Salmonella Typhi* e *S. Paratyphi* A, B e C pertencem ao grupo das tifoides, causando a febre tifoide e a febre entérica, respectivamente (13). O quadro clínico é caracterizado pela presença de sintomas severos, como diarreia sanguinolenta, vômito, dor abdominal e febre, podendo evoluir para morte. Uma fração pequena, porém importante, da população torna-se portadora crônica assintomática, excretando o patógeno no ambiente por longos períodos (14), contaminando água e alimentos, o que torna a rota de transmissão fecal-oral a mais comum (15). A febre tifoide ainda é prevalente no mundo, principalmente nos países em desenvolvimento, que apresentam áreas com condições precárias de saneamento básico, afetando cerca de 21,5 milhões de pessoas a cada ano (15). No Brasil, essa enfermidade ocorre sob a forma endêmica, com superposição de epidemias, especialmente nas regiões Norte e Nordeste, refletindo as condições de vida de suas populações (16). Dessa forma, o saneamento básico, o preparo adequado dos alimentos e a higiene pessoal são as principais medidas de prevenção. A vacinação como medida profilática é indicada para pessoas que pretendam viajar para zonas de alta endemicidade, uma vez que apresenta um alto poder imunogênico de curta duração (15,16). O tratamento baseia-se no uso de terapia antimicrobiana para evitar a evolução para infecções sistêmicas severas. No entanto, cepas resistentes a diferentes classes de

antimicrobianos têm sido cada vez mais encontradas, limitando a eficácia do tratamento (17).

Em contrapartida, o grupo das salmonelas não tifoides (NTS), formado pelos demais sorovares de *S. enterica*, é associado à salmonelose, que é apontada como uma importante zoonose de distribuição mundial (18). Essa enfermidade é responsável por casos de gastroenterite, normalmente autolimitante, rotineiramente adquirida pelo consumo de alimentos contaminados, principalmente os de origem animal, tais como ovos, carne, laticínios e até mesmo frutas e verduras contaminadas com dejetos de animais (19). Apesar da grande diversidade de sorovares encontrados nas diferentes fontes de surtos alimentares, *S. Enteritidis*, *S. Typhimurium* e *S. Heidelberg* têm sido consideradas os principais patógenos causadores de doenças transmitidas por alimentos (DTA) em todo o mundo (18-20). Segundo dados publicados pelo *Centers for Disease Control and Prevention* (CDC), nos Estados Unidos, são reportados aproximadamente 1,2 milhões de casos de salmonelose humana, com 450 mortes todos os anos (18). Ainda que no Brasil, nem todas as unidades federativas disponham de dados minuciosos de vigilância epidemiológica quanto às DTAs, estima-se que no período de 2000 até 2017, tenham ocorrido cerca de 12.660 surtos de DTAs, sendo que desses, 35% foram relacionados com algum dos diferentes sorovares de *S. enterica* (21).

Após a ingestão do alimento contaminado, as salmonelas aderem-se na mucosa intestinal com auxílio de fímbrias e iniciam o processo de multiplicação, invasão e disseminação pelo intestino e órgãos linfoides secundários, causando diarreia em animais, incluindo humanos (22). Na maioria dos indivíduos infectados, os quadros causados por NTS são caracterizados por diarreia branda, com recuperação do paciente após alguns dias, não necessitando auxílio terapêutico. Contudo, a disseminação linfática e a implantação de uma infecção sistêmica grave podem ser observadas em algumas

situações, sobretudo quando há acometimento de crianças, idosos e pacientes imunodeprimidos, fazendo-se necessária a utilização de terapia antimicrobiana (23-25). A presença de *S. enterica* veiculada por alimentos carreando genes que conferem resistência às fluoroquinolonas e às cefalosporinas de terceira geração (26-30), que são os fármacos de escolha utilizados para o tratamento de salmonelose grave, tem sido progressivamente reportada (18,19).

As fluoroquinolonas são antimicrobianos que atuam na replicação de DNA bacteriano, bloqueando a atividade da DNA girase, codificada pelos genes *gyrA* e *gyrB*, e na topoisomerase IV, codificada pelos genes *parC* e *parE* (31). São inúmeros os mecanismos de resistência associados a essa classe de fármacos, entre eles mutações nos genes supracitados, que ocasionam alterações nos sítios de ligação do antimicrobiano, chamados de regiões determinantes de resistência a quinolonas (QRDR) (26-30). As substituições de aminoácidos nas posições Asp87 e Ser83 em *gyrA*, e Thr57 em *parC* têm sido apontadas como a principal causa da ocorrência de *Salmonella* spp. com drástica redução na suscetibilidade às fluoroquinolonas (26-28,30,32). Além das alterações cromossômicas, existe a preocupação com a ampla disseminação de plasmídeos que medeiam a resistência às quinolonas (PMQR) por carregarem os genes *qnr* (*qnrA*, *qnrB*, *qnrC*, *qnrD*, *qnrS* e *qnrVC*), os quais codificam para uma proteína que confere proteção à DNA topoisomerase (30,32,33). Da mesma forma, a resistência a estes fármacos pode ser devida à hiperexpressão de bombas de efluxo, como a QepA (30) e a OqxAB (28), ambas codificadas por genes carregados por plasmídeos, que também podem conter o gene *aac(6')-Ib-cr*, que codifica uma acetiltransferase de fluoroquinolonas (33).

As cefalosporinas, que pertencem à classe dos β -lactâmicos, são os fármacos de escolha para o tratamento de salmonelose grave em crianças e nos casos de resistência às fluoroquinolonas (23,25). Os β -lactâmicos atuam basicamente inibindo a síntese do

peptideoglicano pela ligação e inibição das proteínas de ligação à penicilina (PBP) (34). A resistência às cefalosporinas de terceira geração, bem como aos β -lactâmicos de modo geral, tem emergido em bactérias associadas a alimentos de origem animal (27,35,36). A resistência de *S. enterica* a essa classe de fármacos se deve a diversos mecanismos, mas tem sido especialmente associada à produção de β -lactamases (36). No entanto, também pode ser atribuída à diminuição da permeabilidade de membranas externas, provavelmente ocasionada pela perda ou modificação das porinas, especialmente OmpC e OmpF (37), alteração da afinidade das PBPs (38) e pela hiperexpressão da bomba de efluxo AcrAB-TolC (39).

As β -lactamases são codificadas por genes que podem estar inseridos no cromossomo bacteriano ou em plasmídeos, o que facilita a rápida disseminação deste mecanismo de resistência (40). De acordo com a classificação de Ambler, estas enzimas são divididas em quatro classes (A, B, C e D), baseando-se nas suas sequências de aminoácidos (41). Inúmeras β -lactamases de interesse clínico já foram descritas em membros da família Enterobacteriaceae: NDM, IMP, VIM, SPM, SIM e GIM (metalo- β -lactamases – classe B); AmpC e CMY (classe C); oxacilinas (classe D); e as de classe A, como *Klebsiella pneumoniae* carbapenemases (KPCs) e as β -lactamases de espectro estendido (ESBLs) TEM, SHV e CTX-M, sendo que essa última está entre as principais responsáveis pela resistência de *Salmonella* spp. às cefalosporinas (29,36,40,42,43).

A crescente incidência de *S. enterica* oriunda de alimentos de origem animal, carreando diferentes mecanismos de resistência (26-29,36), constitui um importante problema de saúde pública. Décadas de uso indevido e excessivo de antimicrobianos contribuíram para a evolução da resistência a antibióticos nos principais patógenos, resultando em uma verdadeira crise de falhas terapêuticas, onde as infecções facilmente curáveis estão se tornando uma séria ameaça à saúde humana. Alguns autores defendem

a ideia de que o intenso uso de antimicrobianos de forma profilática na alimentação animal possa favorecer a seleção de populações mutantes resistentes a estes fármacos, além da possibilidade de co-seleção de resistência em isolados carregando elementos genéticos móveis com diversos genes de resistência (44-47). O aumento de cepas multirresistentes (MDR) fez com que a Comunidade Europeia interviesse banindo a utilização não terapêutica de antimicrobianos como promotores de crescimento na produção animal (48). Visto isso, a Organização Mundial da Saúde (OMS) publicou recentemente diretrizes com recomendações estritas sobre o uso de antimicrobianos na produção animal, incluindo a restrição completa do uso desses fármacos como aditivos zootécnicos. Além disso, a OMS também sugere que os antimicrobianos identificados como de importância crítica para a medicina humana não sejam usados em animais de produção, a menos que o teste de suscetibilidade demonstre que o fármaco em questão seja a única opção de tratamento (49). No entanto, o Brasil lança mão desse artifício para aumentar a produtividade animal, utilizando fármacos como a bacitracina de zinco, avilamicina, lincomicina, monensina e tilosina. Esses antimicrobianos são liberados pelo Ministério da Agricultura, Pecuária e Abastecimento (50) para serem utilizados como aditivos zootécnicos para a promoção do crescimento, sendo empregados em pequenas dosagens de modo contínuo junto à ração. O mecanismo pelo qual os antimicrobianos atuam no trato digestório dos animais com esta finalidade ainda não foi completamente elucidado. No entanto, é proposto que ocorra a redução de bactérias da microbiota intestinal e, conseqüentemente, a inflamação (51-54). O sulfato de colistina integrava a lista de substâncias permitidas como promotores de crescimento; no entanto, no final de 2016, o MAPA lançou uma instrução normativa retirando e proibindo o uso desse antimicrobiano com a finalidade de aditivo zootécnico em todo o território nacional (55). Contudo, a colistina (polimixina E), um polipeptídeo catiônico, continua sendo

extensivamente utilizada na medicina veterinária para o controle de infecções causadas por membros da família Enterobacteriaceae, principalmente na suinocultura (56). O mecanismo de ação desse fármaco está relacionado com a ligação ao lipopolissacarídeo (LPS) da membrana externa, especificamente ao lipídeo A, e desligar de forma competitiva cátions divalentes, como cálcio (Ca^{2+}) e magnésio (Mg^{2+}), que normalmente estabilizam o LPS. Na sequência, a colistina leva a um aumento da permeabilidade da membrana externa, pela formação de áreas desestabilizadas, levando à morte celular pelo extravasamento do conteúdo intracelular (57,58). A colistina também é capaz de impedir a indução do choque via endotoxina ao se ligar ao lipídeo A do LPS (59). Outro mecanismo de ação proposto para a colistina é a produção de radicais hidroxila, que geram danos oxidativos, resultando em morte celular (60). Além disso, a inibição da enzima respiratória NADH-quinona oxidoreductase na membrana interna bacteriana tem sido descrita como outro mecanismo de ação exercido pelas polimixinas (61). A resistência à colistina em isolados de *S. enterica* e *Escherichia coli* é basicamente atribuída a mutações que resultam na superexpressão das proteínas PmrA e PmrB (62), bem como perda ou inativação do lipídeo A devido a mutações nos genes *ipxA*, *ipxC* e *ipxD* (63). Entretanto, recentemente, foi descrita a presença de genes para fosfoetanolamina transferase (*mcr*) em plasmídeos, que vêm se disseminando rapidamente por inúmeros países (64,65), incluindo no Brasil, onde foram encontrados em isolados de *S. Typhimurium* provenientes de carne de varejo (66).

Ao longo dos anos, pesquisas têm se concentrado no desenvolvimento de alternativas antibióticas para manter ou melhorar a saúde e o desempenho das aves, e, da mesma maneira, controlar doenças bacterianas de considerável impacto econômico, como a salmonelose (67-71). A salmonelose aviária é considerada a principal doença bacteriana relacionada à queda na produção de ovos, à perda de peso devido à baixa conversão

alimentar e à mortalidade dos lotes (72-74). As doenças causadas por esse patógeno são divididas em três grupos: (1) pulorose, causada pela *S. Pullorum*; (2) tifo aviário, causado por *S. Gallinarum* e (3) paratifo aviário, causado por outros sorovares de *S. enterica* (74,75). Sabe-se relativamente pouco sobre como e por que *S. enterica* persiste no intestino das aves por meses, sem desencadear sinais clínicos (76). A colonização crônica do trato intestinal é um aspecto importante da infecção persistente por esse patógeno, pois resulta em uma propagação silenciosa de bactérias no meio devido à impossibilidade de isolar os animais contaminados (77).

Desde que *S. Enteritidis* emergiu na indústria avícola brasileira na década de 90 (78), os esforços foram direcionados para o controle da salmonelose aviária, bem como para a redução da disseminação de cepas MDR na cadeia de produção de alimentos. A utilização de vacinas tem sido adotada como estratégia para reduzir os níveis de colonização por *S. enterica* nas aves de produção e, conseqüentemente, gerar menores taxas de infecções em humanos (71,79). Além do controle da salmonelose, há uma preocupação por parte das indústrias com o desempenho zootécnico desses animais. Para contornar esse problema, as empresas têm preconizado a adição de agentes como prebióticos, probióticos (69,80) e ácidos orgânicos (ácido láctico, cítrico, málico, fórmico e propiônico) (68,70,81,82) junto à formulação de ração para aves, bem como em água de consumo. O mecanismo de ação dos ácidos orgânicos em geral está relacionado com a redução do pH citoplasmático da célula bacteriana (83). Um dos objetivos da acidificação da dieta (semelhante ao gerado pela utilização dos antimicrobianos) é a inibição de bactérias intestinais competindo com o hospedeiro pelos nutrientes disponíveis, melhorando a saúde intestinal e, conseqüentemente, o desempenho zootécnico das aves (68,70,81,82,84). No entanto, embora a utilização dos ácidos orgânicos como aditivos em ração de aves tenha sido proposta como estratégia para

combater patógenos intestinais, o seu efeito se mostrou limitado quando testado em patógenos específicos, bem como não mostrou influência nas sucessivas mudanças no microbioma cecal de frangos durante 42 dias de crescimento (85). Uma possível explicação para a essa limitação pode ser o desenvolvimento de mecanismos de sobrevivência ao pH ácido (84,86,87).

A capacidade de adaptação a diversas condições ambientais permite que a *S. enterica* possa permanecer no ambiente, possibilitando a contaminação cruzada e formação de biofilme (88). Biofilmes são definidos como comunidades estruturadas de células que têm a capacidade de adesão a superfícies bióticas ou abióticas (89,90), envolvidas por uma matriz polimérica extracelular (EPS). A EPS de biofilmes formados por sorovares de *S. enterica* é composta principalmente por proteínas, polissacarídeos, DNA extracelular (91), fimbrias e celulose (92). A capacidade de formação do biofilme é dependente de inúmeros fatores, como estado fisiológico das células, tempo de contato com a superfície, propriedades estruturais do material, pH, temperatura e presença de matéria orgânica (93-96). Uma vez iniciado o processo de colonização, *Salmonella* começa a se multiplicar, produzir EPS e a expressar em maior número fimbrias com capacidade agregativa, formando um microambiente que facilita a adesão das células bacterianas às superfícies (97). Em um estágio inicial, esses microrganismos ainda podem ser facilmente removidos com ação química de desinfetantes (98). Porém, se não houver a remoção desses agregados, novos microrganismos podem ser recrutados e aderirem-se a esta estrutura, formando até mesmo biofilmes polimicrobianos (99,100). Quando esta estrutura está completamente organizada, formada por microcolônias bacterianas de uma ou mais espécies é reconhecida como biofilme maduro (91,100). Nesse estágio, a matriz extracelular desempenha um importante papel na persistência dos biofilmes (101), pois permite a transferência de moléculas de comunicação celular relacionadas ao *quorum*

sensing (QS), que regulam principalmente a ação do biofilme maduro (100). A sinalização exerce um papel fundamental no controle da atividade metabólica das células em resposta à demanda nutricional e à densidade populacional, liberando as bactérias do biofilme quando a população se torna alta (93). Como consequência, ocorre a dispersão das células no ambiente a fim de formar novos agregados em diferentes superfícies (102). Além disso, a matriz extracelular confere proteção às células contra a ação do sistema imune do hospedeiro (103), resistência contra a dessecação (104) e proteção contra antimicrobianos e desinfetantes (105-108).

Apesar da matriz extracelular exercer, de certa forma, uma ação protetora para as células presentes no biofilme, a capacidade de sobrevivência das bactérias presentes nesse ambiente, tem sido cada vez mais associada à formação de uma pequena subpopulação, conhecida como *persisters* (109,110). Esse fenômeno já havia sido observado logo após a introdução do primeiro antimicrobiano, há aproximadamente 70 anos, quando Joseph Bigger constatou que a penicilina rompia a maioria das células de *Staphylococcus aureus* (anteriormente chamado de *Staphylococcus pyogenes aureus*) em crescimento. No entanto, uma pequena fração (menos de 0,001% da população inicial), que não era classificada como mutante resistente, se mantinha viável e não se dividia, propondo-se então que essas células eram persistentes e que poderiam entrar em um estado de dormência (111). Somente mais tarde, nos anos 80, Harris Moyed estudou esse fenótipo em culturas de *E. coli* tratadas com aplicações intermitentes de altas concentrações de ampicilina, proporcionando o isolamento de um mutante altamente persistente, conhecido como *hip* (*high persister*), a partir de uma população homogênea (112). Durante todos esses anos, as pesquisas focaram em descrever mecanismos genéticos relacionados à resistência aos antimicrobianos, além do desenvolvimento de novos fármacos para combater infecções causadas por bactérias MDR. Somente na última década, os

pesquisadores começaram a tentar elucidar os mecanismos por trás da formação e manutenção da persistência.

Muitas das informações obtidas até o momento corroboraram e expandiram os achados de Bigger. Classicamente, a persistência pode ser definida como a formação de variantes fenotípicas transitórias a partir de uma população isogênica e geneticamente suscetível, que apresentam a capacidade de tolerar concentrações letais de diferentes estressores, incluindo antibióticos bactericidas, sem transmitir sua tolerância à progênie (113-116). A persistência tem sido demonstrada por meio de uma curva de morte bifásica após a adição de doses letais de um fármaco bactericida, onde a grande maioria das células é eliminada, mas uma subpopulação sobrevive a esse estresse (117). Esta pequena fração retoma o crescimento na medida em que o estressor for removido, e quando a população for exposta novamente ao mesmo agente originará frações similares de células tolerantes (118,119). Diferentemente, as células resistentes possuem a capacidade de se multiplicar na presença do antimicrobiano devido à aquisição de mecanismos genéticos relacionados com a incapacidade dos fármacos antimicrobianos de atuarem (113,116,117).

A recidiva de doenças como a tuberculose, (120) infecções do trato urinário (121) e até mesmo candidose (122), além de falhas na terapia contra microrganismos relacionados à fibrose cística (119,123), podem estar relacionadas com a presença desse fenótipo tolerante. As *persisters* foram identificadas em diversos microrganismos, tais como: *S. enterica* (124), *E. coli* (121), *S. aureus* (125), *Mycobacterium tuberculosis* (120), *Pseudomonas aeruginosa* (123), *Acinetobacter baumannii* (126), *Borrelia burgdorferi* (127), *Candida albicans* (122) e Archaea (128). Porém, acredita-se que quase todas as espécies microbianas sejam capazes de formar células *persisters*, que podem estar associadas com a recalcitrância de doenças, devido a falha terapêutica, apesar dessas células serem geneticamente suscetíveis aos antimicrobianos. Entretanto, modelos

matemáticos têm sugerido a possível seleção de populações mutantes resistentes, carreando mecanismos genéticos de resistência, devido à contínua exposição a elevadas doses de antimicrobianos para eliminar as *persisters* (129,130). Adicionalmente, tem sido sugerido que a contínua exposição a concentrações subinibitórias (sub-MIC) de antimicrobianos ou de outro agente estressor – como o paraquat (indutor de estresse oxidativo) – poderia promover a indução de populações persistentes, o que resulta em um aumento dramático no número de células *persisters* (131-133).

As últimas descobertas aumentaram a nossa percepção acerca do tema, mas também trouxeram novos desafios para o entendimento da fisiologia por trás da formação e manutenção desse fenótipo. As células *persisters* são formadas estocasticamente dentro de uma população bacteriana, devido a uma perturbação (*noise*, em inglês) em um pequeno número de moléculas impactando em alguns processos biológicos (116,134). Além disso, sabe-se que sua formação também pode ser induzida por situações entendidas pelas células como estresse, tais como exposição a agentes antimicrobianos, alteração nas condições de oxigênio, pH, fontes de carbono, bem como privação nutricional (*stringent response* – SR) (135-140). Dessa forma, Balaban e colaboradores (2004) propuseram que as *persisters* seriam basicamente divididas em dois grupos: *persisters* tipo I, formadas na fase estacionária em resposta a diferentes estímulos estressores, e *persisters* tipo II, formadas continuamente, porém em menor número, durante a fase exponencial, de maneira puramente estocástica (141). A proposta de que essas células não se dividiam e entrariam em um estado de dormência, no qual a expressão de genes essenciais para o metabolismo bacteriano estaria inibida, tem sido amplamente descrita (111,112,142-145). No entanto, em *Mycobacterium smegmatis* foi reportado um balanço dinâmico entre células mortas e sobreviventes, que possivelmente estariam em divisão quando expostas ao fármaco isoniazida (146), gerando indícios de que o fenótipo de persistência não é

necessariamente associado ao metabolismo inativo encontrado no estado de dormência, como previamente suposto. A partir de *fluorescence-activated cell sorting* (FACS) para separar as células com metabolismo ativo daquelas com metabolismo inativo ou reduzido em culturas de *E. coli* expostas à ampicilina ou ofloxacina, foi sugerido que o estado de dormência não é suficiente como única explicação para a persistência, e que muitas nuances precisam ser levadas em consideração (147). Recentemente, foi demonstrado que durante a infecção em macrófagos, as *persisters* de *S. Typhimurium* mantêm um estado metabolicamente ativo, pois conseguem transcrever, traduzir e translocar efetores capazes de reprogramar os fagócitos, inativando a resposta imune pró-inflamatória, permitindo, assim, a sua sobrevivência na célula hospedeira. Este mecanismo foi associado à expressão de diversos fatores de virulência, encontrados nas ilhas de patogenicidade de *Salmonella* (SPI) do tipo II e secretados pelo sistema de secreção do tipo III (T3SS) (148).

Diante disso, diversos mecanismos têm sido propostos com o intuito de explicar a formação de células *persisters*, especialmente os relacionados com a interferência em processos essenciais para a manutenção celular. Nesse contexto, encontram-se a expressão dos sistemas toxina-antitoxina (TA), produção de adenosina trifosfato (ATP), síntese e degradação proteica, reparo e proteção do DNA (resposta SOS), sinalização celular QS, atividade de efluxo e alterações nas vias relacionadas com o metabolismo microbiano (118,135,137,139,148,149-156). Acredita-se que mecanismos moleculares possam operar de forma independente e em paralelo, ou sobrepostos na formação de *persisters* (113,114,139), inibindo a expressão de sítios importantes para a atividade dos inúmeros antimicrobianos, e, assim, configurando um fenótipo de multitolerância (MDT) (135). No entanto, culturas idênticas expostas a diferentes agentes estressores têm apresentado padrões divergentes nos níveis de *persisters* formadas (121,126,140,151). Isso sugere aos pesquisadores que essas células compreendem uma população

extremamente dinâmica e heterogênea, cada qual com mecanismos distintos para tolerar efeitos letais de diferentes agentes estressores como uma estratégia de adaptação e sobrevivência em ambientes inóspitos (134). Além disso, especula-se que o fenótipo de persistência como uma estratégia evolutiva possa estar ligado a uma combinação de herança epigenética (transmissão de fatores não genéticos da célula mãe para as células descendentes, como níveis de expressão de genes, RNA e outras biomoléculas) associada ao “*noise*” celular, acarretando alterações na expressão de certos genes de forma estocástica ou induzida (116).

O conhecimento atual sobre persistência é muito fragmentado e questões importantes referentes aos mecanismos responsáveis pela formação e manutenção desse fenótipo permanecem inexploradas. A grande maioria dos aspectos moleculares relacionados com a fisiologia das *persisters* foram obtidos a partir de modelos usando *E. coli*. Inicialmente, o mais importante mecanismo proposto para a regulação da formação de *persisters* foram os sistemas TAs, os quais são constituídos por uma toxina estável, tipicamente agindo na inibição de processos biológicos importantes na célula, como transcrição, replicação e síntese de parede celular, e uma antitoxina instável, que interage neutralizando a ação da toxina (157,158). Sistemas TAs estão organizados em operons, e foram originalmente identificados em plasmídeos, associados com a viabilidade celular e manutenção plasmidial (159). No entanto, a maioria dos sistemas TAs importantes para o metabolismo celular são encontrados no cromossomo bacteriano (160), os quais são basicamente caracterizados de acordo com a natureza da antitoxina e seu modo de regulação. Aparentemente, os sistemas TAs que estão relacionados com a indução do fenótipo de persistência são os pertencentes ao tipo II (161,162), presumindo-se que devido ao seu mecanismo de ação, esses sistemas poderiam estar diretamente

relacionados com o baixo- ou não-crescimento apresentado por essas células (139,147,157,158).

O primeiro sistema TA *bona fide*, associado à formação de células *persisters*, foi o sistema HipAB (112), onde a toxina HipA é uma proteína serina-quinase, pertencente ao sistema TA tipo II, que fosforila a glutamyl-RNAt sintase (GltX), levando ao acúmulo de RNAt(Glu) na célula. Esse sistema normalmente é ativado devido a uma SR em decorrência da privação nutricional, como carbono, aminoácidos e ferro (162-164), principalmente em bactérias associadas ao biofilme, inibindo a tradução e induzindo a persistência (138,165,166). A SR é desencadeada por uma série de sinais vinculados à ativação do segundo mensageiro alarmônio guanosina tetra- ou pentafosfato – (p)ppGpp (167). Nesse modelo, o acúmulo intracelular de (p)ppGpp é ocasionado em resposta à ativação de *relA* e/ou *spoT*, levando à inibição de exopolifosfatase (PPX), o que resulta em aumento dos níveis de polifosfato quinase (PPK), que, por sua vez, ativa a protease Lon dependente de ATP. A maioria das toxinas ativadas por Lon são RNA endonucleases que corrompem processos de tradução, interrompendo o crescimento celular e promovendo a sobrevivência das células (150,168-170). Esse desenho foi descrito associado a pelo menos 14 sistemas TAs relacionados com aumento da persistência *in vivo* em *S. Typhimurium* após internalização por macrófagos (139). As antitoxinas são substratos da protease Lon, resultando na impossibilidade de neutralização das toxinas, especialmente aquelas componentes dos sistemas ShpAB (*Salmonella high persister*) (171), RelBE e VapBC (172). Recentemente, Rycroft e colaboradores também demonstraram a ação de três toxinas acetiltransferases (TacT, TacT2 e TacT3) em *S. Typhimurium* e *S. Enteritidis*, causando a acetilação de moléculas de aminoacil-RNAt, o que levou à inibição da tradução e indução do estado de persistência (124). Além disso, o sistema TA Hha-TomB foi relacionado com a formação de *persisters* em *S.*

Typhimurium, inibindo a morte celular programada sob estresse causado pelo fármaco gentamicina (173).

Outro sistema igualmente importante descrito em *E. coli*, porém envolvendo a regulação da resposta SOS, é o TisB/IstR pertencente ao sistema TA do tipo I (174). Acredita-se que antibióticos bactericidas como β -lactâmicos e fluoroquinolonas desencadeiam uma resposta SOS em reação ao dano ocasionado ao DNA. Este efeito é mediado pela ativação de RecA, que, quando ativada, induz a autoproteólise do repressor LexA, estimulando a expressão de proteínas envolvidas no reparo, como SulA, DinG, UvrABCD, RecABCD e RuvABC (149). A toxina TisB, por sua vez, é ativada e forma canais na membrana celular, acarretando um desbalanço da força próton-motiva (PMF), diminuindo os níveis de ATP celular (174) e promovendo a perda da atividade dos antimicrobianos nos alvos (151,153). Outros sistemas TAs também foram associados à persistência, como MazEF e RelBE em *S. mutans* e *E. coli* (118,175,176), DinJ/YafQ e MqsRA em *E. coli* (177,178) e, recentemente, AbkAB em *A. baumannii* (179). Ao longo dos anos, assumiu-se que os sistemas TAs desempenhavam o papel principal na formação de células *persisters*. Há muito tem-se refletido sobre o verdadeiro papel desses sistemas na persistência, sobretudo após observações de que diferentes tipos de estresse ativam a expressão dos sistemas TAs, mas não necessariamente induzem a persistência (153). Além disso, foi constatado que mutantes com deleção combinada ou não desses sistemas apenas reduzem o número de *persisters*, mas não ocasionava a completa erradicação desse fenótipo (135,139,150,151,155,166,174). Também foi demonstrado em biofilmes de *E. coli* que a tolerância à ofloxacina é independente dos sistemas TAs induzidos pela resposta SOS (166), bem como em *P. aeruginosa* (113). Em adição aos achados anteriores, Goormaghtigh e colaboradores passaram a sugerir que não há ligação direta entre a ativação dos sistemas TAs e a indução do fenótipo de persistência (170). Isto

deveu-se especialmente à descoberta de infecção por bacteriófagos lisogênicos em cepas de *E. coli* usadas como referência por vários laboratórios para estudos desse fenótipo, afetando fortemente os resultados obtidos com esses isolados (180).

O estresse oxidativo enfrentado pelas bactérias dentro de fagócitos é considerado um dos estresses mais impactantes, além de apresentar grande importância para patógenos, como *Salmonella* spp., que podem causar infecções persistentes no ambiente vacuolar de macrófagos (139). Esses patógenos produzem enzimas antioxidantes para reparar o dano oxidativo e protegê-los tanto da resposta imune, como da terapia antimicrobiana (181). As enzimas antioxidantes catalase-peroxidase (KatG), superóxido dismutase (SOD) e a peroxidase dependente de NADH são sintetizadas pelas bactérias sob a influência dos reguladores SoxRS, OxyR e RpoS para neutralizar a ação das espécies reativas de nitrogênio (ERN) e espécies reativas de oxigênio (ERO) produzidas no fagolisossomo (131,182). Acredita-se que o tratamento com antibióticos bactericidas também possa resultar em aumento do estresse oxidativo, gerando danos nas células bacterianas devido à toxicidade ocasionada pelos produtos da reação de Fenton, e assim, induzir a resposta SOS. Essa reação promove a geração de radicais hidroxila (OH^\cdot) altamente tóxicos devido à combinação de ferro (Fe^{2+}) ou cobre (Cu^{2+}) com o peróxido de hidrogênio (H_2O_2) (183). Recentemente, foi demonstrado em *E. coli* uropatogênica que a ausência do AMP cíclico leva à diminuição de EROs e ao reparo do dano oxidativo ao DNA gerado pelos radicais OH^\cdot e, como resultado, a bactéria consegue superar o efeito tóxico, dependente de uma resposta SOS, induzindo o fenótipo de persistência (184). Além disso, a tolerância em biofilmes de *C. albicans* está diretamente ligada à atividade de ERO, e acredita-se que a utilização de inibidores da SOD pode potencializar a atividade do miconazol nessas células no biofilme (185). Da mesma forma, foi evidenciado em *P. aeruginosa* que a SOD confere tolerância na fase estacionária

associada ao (p)ppGpp, e que a deleção do gene que codifica SOD aumenta significativamente a permeabilidade da membrana bacteriana, proporcionando a internalização dos fármacos e redução do estado de persistência (186).

Wu e colaboradores demonstraram que o pré-tratamento com um pró-oxidante indutor de estresse oxidativo (paraquat) resultou em uma elevação nos níveis de *persisters* após tratamento com fluoroquinolonas, devido a um aumento da expressão da bomba de efluxo AcrAB-TolC (131). Devido a esse achado, foi estabelecido um possível elo entre a atividade das bombas de efluxo e tolerância a antimicrobianos. Além disso, foi recentemente constatado, por meio de análise do proteoma de *E. coli*, que as proteínas de membrana, incluindo proteínas de transporte, estavam superexpressas, indicando que elas podem ser importantes para a tolerância apresentada pelas *persisters* (156). Paralelamente, foi demonstrado em biofilme de *P. aeruginosa* que a expressão do ativador transcricional MerR ativa genes que codificam vários sistemas de transporte ABC, além de genes de bombas de efluxo, contribuindo para a tolerância aos fármacos (187). Visto isso, especula-se que as atividades de efluxo possam desempenhar um papel importante no mecanismo de formação e manutenção da persistência bacteriana, especialmente as relacionadas com a hiperexpressão da proteína de membrana TolC, que auxilia na extrusão de antibióticos, diminuindo a quantidade de fármacos no citoplasma bacteriano e favorecendo assim a sobrevivência celular (152).

Moléculas envolvidas em QS produzidas em situações de alta densidade populacional, como crescimento em final de fase exponencial, em fase estacionária e em biofilmes, também foram associadas com o fenótipo de persistência (118,188,189). A acil-homosserina-lactona e a piocianina são exemplos de moléculas sinalizadoras de QS que mostraram aumentar a formação de *persisters* em *P. aeruginosa* (188). Além disso, foi demonstrado que a piocianina induz um efeito protetor contra o estresse oxidativo em

A. baumannii, além de aumentar de forma considerável os níveis de células *persisters*, podendo ser um problema na clínica no caso de coinfeções com *A. baumannii* e *P. aeruginosa* (190). O mesmo fato foi observado em *S. Typhi*, mostrando que quando expostas à ciprofloxacina e ampicilina, na presença de bile, a *S. Typhi* foi capaz de aumentar três vezes os níveis de *persisters*. A bile, por sua vez, leva à geração de ERO e, em resposta, a *S. Typhi* produz enzimas antioxidantes como a SOD e a catalase (CAT). No entanto, o QS regula os níveis dessas enzimas, ajudando a *S. Typhi* no manejo do estresse oxidativo e no aprimoramento da persistência bacteriana dentro da vesícula biliar (189).

Outro mecanismo proposto para a indução e manutenção do estado de persistência é a diminuição dos níveis intracelulares de ATP, preditivo da redução da atividade dos alvos antibióticos e da sobrevivência bacteriana (155). Inicialmente, foi considerado que células *persisters* de *S. aureus* são induzidas devido à entrada estocástica na fase estacionária de crescimento acompanhada de uma queda nos níveis de ATP intracelular e tolerância bacteriana aos antibióticos (151). Usando técnicas de mutagênese por transposons em *P. aeruginosa* expostas a fluoroquinolonas, foi demonstrado que o rompimento do gene *carB* (que codifica a subunidade maior da carbamoil fosfato sintetase – CPSase – envolvida na síntese de pirimidina e arginina), resultou em acúmulo de ATP intracelular. No entanto, quando arsenato foi utilizado, o mesmo reduziu os níveis de ATP, restaurando o perfil de tolerância a antibióticos do mutante para níveis semelhantes aos observados com o tipo selvagem, o que demonstrou a importância do ATP intracelular na formação de células *persisters* (155). Além disso, foi constatado por meio de FACS que o promotor *rrnB* marcado com *gfp*, pode ser um indicador de persistência regulado pelo ATP independente da ativação de sistemas TAs em *E. coli*. A diminuição do nível de ATP retarda a tradução e evita a formação de quebras de fita dupla

de DNA após o tratamento com fluoroquinolonas, causando tolerância a esses fármacos (153). No entanto, recentemente, foi observado que a toxina HokB se insere na membrana citoplasmática, onde forma poros, resultando no extravasamento do ATP intracelular. Quando essa toxina é reprimida na presença de um bloqueador de canal, ocorre a inibição da formação de células *persisters*, demonstrando assim, uma ligação direta entre a toxina HokB e a formação de poros na membrana que causam o vazamento do ATP intracelular e a indução de persistência (191). Adicionalmente a achados anteriores, Pu e colaboradores sugeriram que cada célula apresenta diferentes “profundidades de dormência”, como é o caso das “células viáveis não cultiváveis”, sugerindo que essas células apresentam um grau profundo de dormência. Além disso, os autores estabeleceram que uma coleção de agregados de proteínas endógenas é um importante indicador do estado de persistência, cuja formação é promovida pela diminuição do nível de ATP celular (192).

Adicionalmente às células *persisters*, existe outra variante fenotípica também associada à sobrevivência a estresses, e que pode fazer parte do ciclo de vida de algumas bactérias, conhecida por ser formadora de *small colony variants* (SCV) (193-199). As células que compõem as SCVs apresentam crescimento lento, formando colônias com quase um décimo do tamanho em relação as colônias normais (197). As SCVs possuem uma variedade de características, tais como: alterações no metabolismo de carbono, diminuição na produção de toxinas e enzimas líticas, suscetibilidade reduzida a alguns antimicrobianos e, principalmente, estão associadas com aumento da persistência intracelular em diferentes quadros clínicos de infecções prolongadas ou recorrentes (196). Essas células são capazes de emergir de forma espontânea em meio a uma população homogênea e de rápido crescimento (197), ou serem induzidas por fármacos como estreptomicina (200), gentamicina (201) ou sulfametoxazol combinado ao trimetoprim

(202). É importante ressaltar que em algumas situações, as SCVs são colônias instáveis e auxotróficas, pois apresentam a capacidade de reversão para o fenótipo normal e de rápido crescimento quando subcultivadas com o metabólito necessário em meio livre de estressores (198,200,203). Essa reversão do fenótipo pode explicar a recorrência de infecções por SVCs após períodos de aparente remissão (197). Em contrapartida, têm sido descritas SCVs estáveis carreando mutações nos genes para a biossíntese de hemina (*hemA*) e menadiona (*menA*), bem como no gene que codifica para a timidilato sintetase (*thyA*), resultando na diminuição na cadeia de transporte de elétrons e redução da síntese de ATP (193,201,203). Aparentemente, essas alterações também podem estar associadas com a redução da suscetibilidade aos aminoglicosídeos e β -lactâmicos (193,195). Além disso, mutação pontual no gene *relA*, também associado ao fenótipo de persistência, foi reportada como mediadora da origem do fenótipo SCV (204), indicando a possibilidade de uma conexão na formação e manutenção entre os dois fenótipos de persistência bacteriana.

A mudança fenotípica para o estado de SCV sob condições distintas, especialmente em ambientes hostis, como dentro da célula hospedeira, tem sido reconhecida como uma estratégia de sobrevivência, bem como tolerância a antimicrobianos (196,197). Dessa forma, relatos apontam que o pH ácido, como o do fagolisossomo, pode favorecer a formação de SCVs em *S. aureus*, refletindo na persistência encontrada na clínica médica (205). O mesmo fato foi observado em isolados de *S. Typhimurium* infectando fibroblastos por um tempo prolongado (206). Embora isolados de *S. enterica* não sejam foco de muitos estudos com SCV, tem sido proposto que a redução no crescimento dessas células possa estar associada à mutação em genes como: *hemL*, *lpd*, *aroD*, *prfB*, *ubiE* e *glnA* (198,200,206). Foi demonstrado que o mutante *glnA*-SCV em *S. Typhimurium* apresentou uma severa diminuição da expressão de genes

relacionados aos flagelos e vários fatores de virulência da SPI tipo 1 (SPI-1) (198), confirmando que a atenuação da virulência da SCV está diretamente associada com a capacidade de persistir na célula hospedeira.

Células *persisters* e SCVs são consideradas subpopulações altamente dinâmicas, constituindo estratégias adaptativas que permitem que uma pequena porcentagem da população sobreviva após a exposição a um agente estressor (134,196,197). Estes dois fenótipos compartilham uma série de características, incluindo crescimento lento, persistência intracelular e, principalmente, apresentam uma ligação com falhas na terapia antimicrobiana, resultando em cronicidade e recalcitrância de infecções (113,114,197,198). A seleção desse fenótipo em infecções recorrentes tem sido associada principalmente com o uso de antimicrobianos, pois as SCVs são rapidamente formadas devido às mudanças das condições ambientais (198,200,201,206). Diante desse contexto, é extremamente importante a elucidação das vias envolvidas na formação e manutenção dessas estratégias adaptativas desenvolvidas em *S. enterica* e, por conseguinte, estratégias que possam auxiliar no combate de infecções causadas por esses fenótipos.

1.2 Objetivos

1.2.1 Objetivo Geral

Este trabalho teve como objetivo avaliar a capacidade de *S. enterica* em formar células *persisters* frente à exposição a antimicrobianos, bem como avaliar a influência da exposição prévia a promotores de crescimento utilizados na produção animal na tolerância à ciprofloxacina. Além disso, buscou-se identificar transcritos diferencialmente expressos em células *persisters* frente à exposição à ciprofloxacina e à ceftazidima.

1.2.2 Objetivos Específicos

1.2.2.1 Determinar a concentração mínima de ciprofloxacina, ceftazidima e colistina para inibir o crescimento de isolados de *S. enterica*;

1.2.2.2 Caracterizar os isolados de *S. enterica* quanto à capacidade de formar biofilme em superfície de poliestireno;

1.2.2.3 Avaliar a capacidade de isolados de *S. enterica* em formar células *persisters* em estado planctônico, bem como na condição de biofilme, frente à ciprofloxacina e à ceftazidima;

1.2.2.4 Avaliar a capacidade de formação de células *persisters* em isolados de *S. enterica* na condição planctônica frente à ciprofloxacina mediante exposição prévia aos ácidos fórmico e láctico;

1.2.2.5 Verificar a capacidade de formação de células *persisters* em isolados de *S. enterica* na condição planctônica frente à ciprofloxacina mediante exposição prévia a concentrações subinibitórias de ciprofloxacina ou colistina;

1.2.2.6 Avaliar a morfologia de colônias de células *persisters* de *S. enterica* formadas mediante exposição de cultivo planctônico à ciprofloxacina e à ceftazidima;

- 1.2.2.7 Determinar a estabilidade na formação de células *persisters* nos diferentes tipos morfológicos encontrados frente à ciprofloxacina;
- 1.2.2.8 Sequenciar o genoma total de isolados de *S. enterica* capazes de formar diferentes frações de células *persisters* frente à exposição à ciprofloxacina e à ceftazidima em cultivo planctônico;
- 1.2.2.9 Identificar genes diferencialmente transcritos em células *persisters* oriundas de cultivo planctônico de *S. enterica* expostas à ciprofloxacina e à ceftazidima.

Capítulo 2

Artigo Científico 1

***Salmonella enterica* persister cells form unstable small colony variants after *in vitro* exposure to ciprofloxacin**

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Salmonella enterica persister cells form unstable small colony variants after *in vitro* exposure to ciprofloxacin

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Persistence phenotype and small colony variants (SCVs) can be part of a bacterial bet-hedging strategy for survival under environmental stresses, such as antimicrobial exposure. These phenotypes are of particular concern in persistent and relapsing infections, since cells resume to normal growth after cessation of the stressful condition. In this context, we found persisters and unstable SCVs as phenotypic variants of *Salmonella enterica* that were able to survive ciprofloxacin exposure. A high heterogeneity in persister levels was observed among *S. enterica* isolates grown under planktonic and biofilm conditions and exposed to ciprofloxacin or ceftazidime, which may indicate persistence as a non-multidrug-tolerant phenotype. Nevertheless, a comparable variability was not found in the formation of SCVs among the isolates. Indeed, similar proportions of SCV in relation to normal colony phenotype (NCP) were maintained even after three successive cycles of ciprofloxacin exposure testing colonies from both origins (SCV or NCP). Additionally, we found filamentous and dividing cells in the same scanning electron microscopy images from both SCV and NCP. These findings lead us to hypothesize that besides variability among isolates, a single isolate may generate distinct populations of persisters, where cells growing under distinct conditions may adopt different and perhaps complementary survival strategies.

Salmonella enterica comprises pathogens adapted to infect and survive inside human and animal epithelial and phagocytic cells^{1,2}, including some non-host adapted serovars that are among the most important zoonotic pathogens worldwide. *Salmonella enterica* infection can result in diseases that range from gastroenteritis to enteric fevers. In the midst of this scenario, millions of foodborne outbreaks caused by *S. enterica* are reported every year, wherein the majority are due to consumption of food derived from animals³. Enteric fevers are life-threatening febrile illnesses requiring antibiotic therapy⁴, and fluoroquinolones, especially ciprofloxacin, are the chosen drugs. However, fluoroquinolones block DNA replication by inhibiting DNA gyrase and topoisomerase IV⁵ and are not suitable to treat infections in children and pregnant women⁴. Thus, in those cases, the treatment is performed using third-generation cephalosporins, such as ceftazidime, whose mechanism of action is the inhibition of peptidoglycan synthesis⁶.

Most *S. enterica* serovars are able to adhere to abiotic surfaces and persist in the environment for long periods, especially when growing as biofilms⁷. In fact, biofilms are recognized as major contributors to food processing cross-contamination due to the difficulty in removing them from contaminated surfaces. This makes them an important public health concern⁸. *In vivo*, biofilms can also prevent antimicrobial diffusion and block the entry of immune system components⁹. In addition, the higher bacterial survival levels in biofilms could be explained by the presence of persister cells¹⁰, a non-heritable phenotype that comprises a small subpopulation of cells derived from an isogenic bacterial culture, which displays high antibiotic tolerance by entering in a transient slow or non-growth state^{10–12}. It is postulated that all bacteria can form persisters¹³, including *S. enterica*^{14–17}, as well as archaea¹⁸ and fungi¹⁹. Persister cells can be stochastically formed in a microbial population, or induced by stressors such as antimicrobials. Indeed, persisters can survive exposure even to high levels of bactericidal

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Isolates (ID)	Origin	MIC ($\mu\text{g/ml}$)	
		CIP	CAZ
<i>S. Agona</i> (S48)	Feathers meal	0.01	2
<i>S. Agona</i> (S79)	Meat meal	0.005	1
<i>S. Enteritidis</i> (152)	Ready-to-eat-food	0.005	0.5
<i>S. Enteritidis</i> (192)	Poultry carcass	0.01	1
<i>S. Enteritidis</i> (393)	Food handler	0.01	1
<i>S. Enteritidis</i> (45A)	Porcine faeces	0.005	1
<i>S. Enteritidis</i> (S45)	Meat meal	0.005	2
<i>S. Infantis</i> (S02)	Meat meal	0.01	2
<i>S. Infantis</i> (S67)	Viscera meal	0.03	1
<i>S. Schwarzengrund</i> (S58)	Flesh and bones meal	0.005	1

Table 1. *Salmonella enterica* isolates and minimum concentration of ciprofloxacin (CIP) and ceftazidime (CAZ) required to inhibit their growth. MIC, minimum inhibitory concentration. MIC breakpoints for CIP: $\leq 0.06 \mu\text{g/ml}$, susceptible; $0.12\text{--}0.5 \mu\text{g/ml}$, intermediate; $\geq 1 \mu\text{g/ml}$, resistant. MIC breakpoints for CAZ: $\leq 4 \mu\text{g/ml}$, susceptible; $8 \mu\text{g/ml}$, intermediate; $\geq 16 \mu\text{g/ml}$, resistant (CLSI Document M100-S28)³².

antibiotics without undergoing any genetic change, unlike drug resistant cells²⁰. Thus, eradication of persisters has become a challenge to avoid recurrent treatment failures and recalcitrance of chronic infections¹⁰. Molecular mechanisms behind persister cell formation have been studied, but they have not yet been fully elucidated. The trigger for persisters phenotype formation may involve a down or up-regulation of molecules related to stringent response²¹, energy production^{22,23}, phosphate metabolism²⁴, SOS response²⁵ and toxin-antitoxin (TA) systems¹⁷, acting whether alone or overlapped^{15,20}. *Salmonella* may form persisters in host macrophages when induced by vacuolar acidification and nutritional deprivation, and TA systems are presumed to be responsible for this micro-organism's physiological state¹⁵.

Another phenotypic switching found in response to harsh environments are the small colony variants (SCV)²⁶. SCVs are characterized as slow-growing cells forming pin-prick-sized colonies²⁷ that can revert to wild-type-like colonies^{28,29}, or even be stably kept²⁶, which enable survival to diverse environmental pressures, such as antimicrobial exposure²⁹ and intracellular host defense²⁶. Therefore, isogenic bacterial populations may present heterogeneous phenotypes such as persisters and SCVs.

We found persisters and unstable SCVs as phenotypic variants of *S. enterica* that were able to survive ciprofloxacin exposure. In addition, a high heterogeneity in the levels of persisters was observed among *S. enterica* isolates cultured under planktonic and biofilm conditions after ciprofloxacin or ceftazidime exposure, therefore not indicating persistence as a multidrug-tolerant phenotype. However, a similar variability was not found in the proportion of SCVs formed among the isolates, which was maintained even after successive treatments. Importantly scanning electron microscopy analysis allowed us to observe division septum and filamentous cells from both SCV and normal colony phenotype (NCP) images. Thus, our findings contribute to the characterization of these adaptive strategies to survive stressful environments, and may help to explain treatment failure and relapsing infections.

Experimental Procedures

Bacterial isolates. *Salmonella enterica* isolated between 1995 and 2012 from poultry by-product meals, poultry carcass, food, porcine faeces and food handler in Southern Brazil were used in this study as follows: *Salmonella* Schwarzengrund ($n = 1$), *Salmonella* Agona ($n = 2$), *Salmonella* Infantis ($n = 2$) and *Salmonella* Enteritidis ($n = 5$) (Table 1). All isolates were stored at -80°C in Trypticase Soy Broth (TSB) (BioBras, São Paulo, Brazil) with 20% glycerol.

Antimicrobial susceptibility. The ciprofloxacin (CIP) and ceftazidime (CAZ) (Sigma-Aldrich, St Louis, USA) minimum inhibitory concentrations (MIC) were determined by broth microdilution method, in triplicate³⁰. The cut-off values were interpreted according to the Clinical and Laboratory Standards Institute guidelines³¹.

Biofilm assay. All *S. enterica* isolates were evaluated with regard to biofilm formation in 96-well polystyrene plates. 1- μl aliquots of overnight cultures of each strain were adjusted to approximately 10^6 colony-forming units per millilitre (CFU/ml). These were added in triplicate to wells containing 200 μl of fresh Luria Bertani (LB) broth [10 g/l tryptone (Kasvi, Roseto degli Abruzzi, Italy), 5 g/l yeast extract (Himedia, Mumbai, India) and 5 g/l NaCl (Nuclear, Diadema, Brazil), pH 7.2], and incubated for 48 h at 37°C . Afterwards, wells were washed twice with phosphate-buffered saline (PBS) [8 g/l NaCl (Nuclear), 0.2 g/l KCl (Nuclear), 1.44 g/l Na_2HPO_4 (Nuclear) and 0.24 g/l KH_2PO_4 (Nuclear)] to remove planktonic cells, dried at 60°C for 15 min, and then the biofilms were stained with 0.1% crystal violet for 5 min. After washing twice with PBS, wells were dried at 60°C for 1 h and incubated with absolute ethanol for 15 min at room temperature. Wells containing only 200 μl of LB broth were used as negative control. Adherent cells were measured using a SpectraMax[®] 190 microplate reader (Molecular Devices, Sunnyvale, USA) at 570 nm. *Salmonella enterica* isolates were classified according to Stepanovic *et al.*³² as non-biofilm producers ($\text{OD} \leq \text{OD}_c$), weak biofilm producers ($\text{OD}_c < \text{OD} \leq 2\text{OD}_c$), moderate biofilm producers ($2\text{OD}_c < \text{OD} \leq 4\text{OD}_c$), and strong biofilm producers ($4\text{OD}_c < \text{OD}$). OD_c is the cut-off OD which was the mean

OD plus three times the negative control standard deviations. *Salmonella* Typhimurium ATCC 14028 was used as a positive control for biofilm formation.

Persister cell levels. Persister cell levels were determined in planktonic and biofilm cultures after exposure to ciprofloxacin or ceftazidime according to the protocol described by Gallo *et al.*³³, with some modifications (Fig. 1). To evaluate persister levels in planktonically growing cells, overnight cultures in LB broth were diluted 1:30 and incubated at 37 °C for 2 h 30 min until the mid-exponential growth phase (approximately 10⁸ CFU/ml) (Supplementary Fig. S1). Before antimicrobial exposure, the initial cell density was determined by diluting a 100- μ l aliquot until 10⁻⁶ in 0.85% saline and spotting 10 μ l of each dilution in triplicate on nutrient agar (Oxoid, Hampshire, England), which was then incubated at 37 °C for 24 h. Afterwards, the mid-exponential growth phase cultures were exposed to antimicrobials at 100-fold MIC for each isolate at room temperature for 72 h (see Table 1). In order to determine the surviving fractions at 6, 12, 24, 48 and 72 h of antimicrobial exposure, 1 ml-aliquots were removed at each time, centrifuged at 7,200 rpm for 7 min, and the supernatants were discarded. The pellets were washed with 1 ml of 0.85% saline to remove antimicrobial residues. After washing, the pellets were resuspended in 1 ml of 0.85% saline that was diluted until 10⁻⁶, and 10 μ l of each dilution were spotted on nutrient agar (Oxoid).

To determine the persister levels in biofilm, *S. enterica* isolates were grown in LB broth for 48 h at 37 °C using 96-well polystyrene plates. After this period, the culture medium containing non-adherent cells was removed and the biofilm was washed twice with PBS. The initial biofilm population density was evaluated by adding 200 μ l of 0.85% saline to each well with subsequent disruption by an ultrasonic water bath (Ultrasonic Cleaner 1400 A, Unique, Indaiatuba, Brazil) for 10 min. For the determination of persistence levels, 200 μ l of fresh LB broth containing 100-fold MIC of ciprofloxacin or ceftazidime were added to the 48 h-biofilms and incubated at room temperature until 72 h. At 6, 24, 48, and 72 h of exposure (evaluated in independent microplates), wells were washed twice with PBS, and 200 μ l of 0.85% saline was added. Biofilms were disrupted by an ultrasonic water bath for 10 min. The supernatant containing dissociated adherent cells was removed and their quantification was performed as described for the planktonic cultures.

The survival cell fractions were calculated by dividing the number of remaining colonies counted by the number of colonies found before the antibiotic treatment. After a 72-h exposure to high concentrations of ciprofloxacin or ceftazidime, the MIC of each antimicrobial was determined again by broth microdilution³⁰ in the surviving cells to exclude the selection of mutant resistant. All assays were performed in biological triplicate, and CFU count data were the means of three replicates.

***Salmonella enterica* small colony variant (SCV).** Colonies formed by surviving cells after exposure to 100-fold MIC of ciprofloxacin or ceftazidime both in planktonic and biofilm cultures were morphologically analysed at all-time points (Fig. 1). *Salmonella enterica* SCVs were characterized by a maximum diameter of 0.5 mm, contrasting with around 2 mm diameter of the NCP on nutrient agar after 48-h incubation (Fig. 2). The cells from SCVs were also evaluated with regard to susceptibility to ciprofloxacin by broth microdilution³¹. Furthermore, to confirm SCVs as *S. enterica*, genomic DNA from each colony was extracted by boiling for 10 min³⁴ and used as template for PCR targeting the *invA* gene³⁵.

The ability of SCVs to revert to a normal phenotype was evaluated by sub-culturing colonies from all isolates in a fresh nutrient agar without antimicrobials. Likewise, two isolates (*S. infantis* S02 and *S. enteritidis* 393) were used to investigate the stability of SCV phenotype after exposure to ciprofloxacin. For this, overnight cultures of each isolate were diluted 1:30 with fresh LB broth and cultured at 37 °C for 2 h 30 min until the mid-exponential growth phase. Afterwards, cultures were incubated with ciprofloxacin at 100-fold MIC for 72 h at room temperature, and spotted on nutrient agar. Surviving cells from one NCP and one SCV were separated into independent experiments. Each colony was grown separately overnight in a fresh LB broth, diluted 1:30 with fresh LB broth, cultured at 37 °C for 2 h 30 min, exposed again to ciprofloxacin at 100-fold MIC for 72 h at room temperature, and spotted on nutrient agar (cycle 1). Surviving cells from one NCP and one SCV were again separated into independent experiments and the assay was repeated two more times (cycles 2 and 3) as described for cycle 1. All tests were performed in three independent biological replicates. This same assay used to evaluate one colony of each morphology was performed using a pool of ten each of NCPs or SCVs. In each cycle, susceptibility to ciprofloxacin was re-evaluated by broth microdilution³⁰.

Scanning electron microscopy (SEM). SEM was employed to analyse *S. enteritidis* 393 cells from normal and small colonies cultured under planktonic and biofilm conditions exposed to ciprofloxacin. As mentioned above, after exposure to antimicrobials for 72 h, aliquots of cultures grown under each condition were removed, plated on nutrient agar, and grown at 37 °C for 24 h. Afterwards, NCPs and SCVs, 10 of each, were gently collected, inoculated in 1 ml of 0.85% saline, and centrifuged at 7,200 rpm for 7 min. The supernatants were removed and the pellets were immediately fixed by immersion in 2.5% glutaraldehyde and 0.1 M phosphate buffer (pH 7.2–7.4) for one week. Then, cells were adhered on 18-mm glass coverslips previously coated with poly-L-lysine. The material was washed thrice with phosphate buffer, dehydrated with acetone, and desiccated to remove the acetone, followed by gold metallization. The images were observed with a Field Emission Scanning Electron Microscope (Inspect F50, FEI Company Inspect, Eindhoven, Netherlands) at the Central Laboratory of Microscopy and Microanalysis (LabCEMM) of PUCRS.

Statistical analysis. Surviving fractions from planktonic or biofilm cultures after treatment with antimicrobials for 72 h were compared using an analysis of variance (ANOVA) with permutations (9,999 bootstrap iterations in all tests), and repeated measures ANOVA when applicable. Analyses were carried out with pooled mean values of all isolates, and considering each isolate separately. The same analyses were performed to compare the

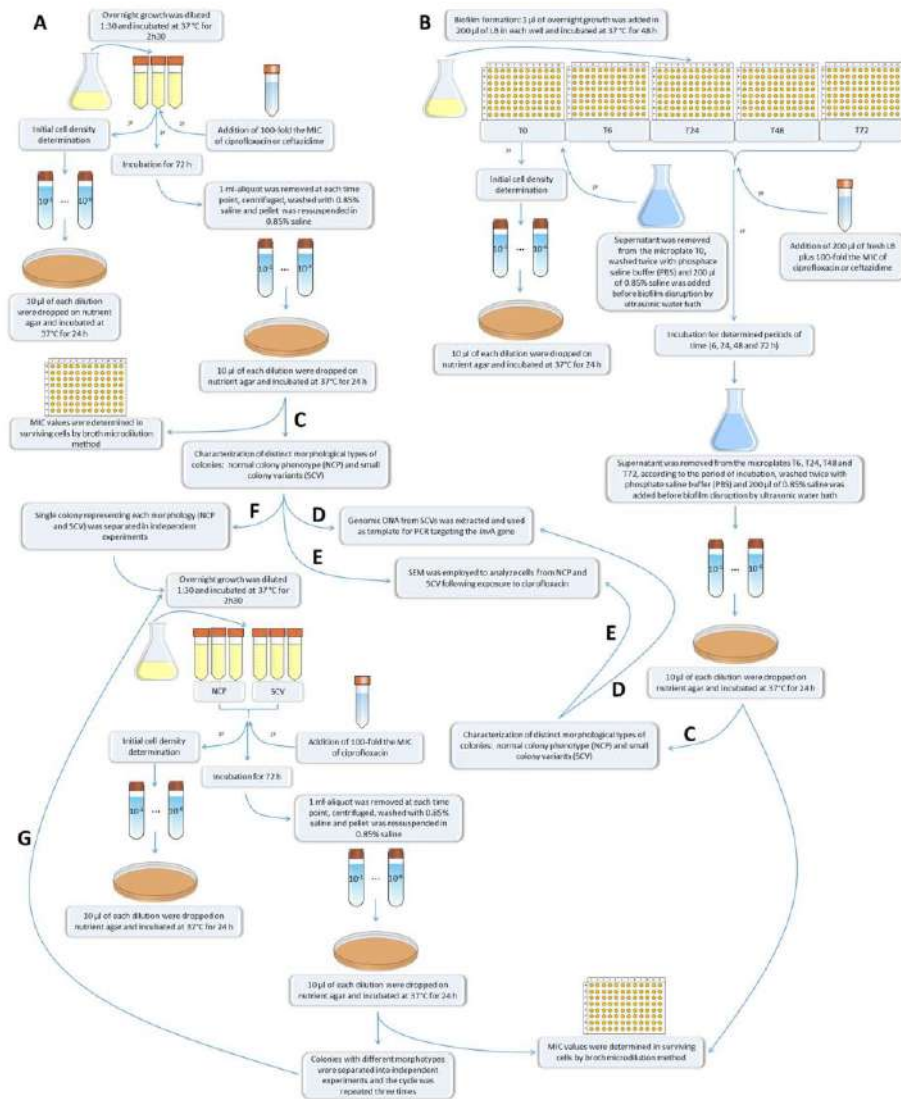


Figure 1. Flow diagram of experimental design for the *in vitro* evaluation of persistence in *Salmonella enterica*. (A) Evaluation of persisters levels in planktonic culture at mid-exponential growth phase following exposure to ciprofloxacin or ceftazidime for 72 h. For the measurement of the surviving fractions, before addition of the antimicrobial, the initial cell density was determined by dilution and count of colonies on agar plate. After removal of the aliquot to determine initial cell density, cultures were exposed to 100-fold MIC of ciprofloxacin or ceftazidime for 72 h at room temperature, and 1 ml-aliquots were taken at 6, 12, 24, 48, and 72 h following the antimicrobial exposure for the count of colonies formed by the surviving cells. The minimum inhibitory concentration (MIC) of each antimicrobial was evaluated in the surviving cells by microdilution broth according to CLSI (2012). (B) Persisters levels were also determined in biofilm after exposure to ciprofloxacin or ceftazidime. Firstly, isolates were grown as 48 h-biofilm on independent microplates according to the time point to be evaluated after the antimicrobial was added. Prior to the initial cell density determination, cells not adherent to the microplate T0 were discarded and biofilms were washed to be disrupted by ultrasonic water bath. After removal of the aliquot for the initial density determination, antimicrobial was added and cultures were incubated for 72 h at room temperature. At each time point (T6, T24, T48 and T72), biofilms were washed by ultrasonic water bath, and the following steps were performed according to described for the planktonic cultures. (C) Morphology of the colonies formed by persisters from both planktonic and biofilm cultures were analysed, and after exposure to ciprofloxacin were found small colony variants (SCV) in addition to the normal colony phenotype (NCP). (D) SCVs from both planktonic and biofilm growth were confirmed as *Salmonella* spp. by PCR targeting the *invA* gene. (E) Morphology of the cells forming NCP and SCV from both planktonic and biofilm growth was analysed by scanning electron microscopy (SEM). (F) Stability of

the SCV phenotype among persisters surviving after exposure to ciprofloxacin was evaluated, separately, in overnight cultures derived from both NCP and SCV. For this, the same procedures described for the evaluation of persisters in planktonic growth were employed and repeated for three consecutive cycles (G). The same procedures used to analyse stability of SCV originated from single colonies (SCV or NCP) were also employed using a pool of colonies derived from SCV or NCP. All assays were performed in three biological replicates, and data of CFU counts represent the mean of three replicates. Visual representations were taken from a free online source (clker.com) with the exception of microplates that were designed by the co-author S.P.M.D.

SCV ratios found for both culture conditions and antimicrobial exposures and to evaluate the stability of the SCV phenotype during successive cycles of exposure to ciprofloxacin. Pairwise comparisons between persister fractions obtained from different serovars, as well as the SCV ratios found in different serovars, were employed using Tukey's post-hoc test after ANOVA with permutations. All analyses were conducted in the statistical platform R³⁶ using 'ImPerm' package³⁷. We considered p -values ≤ 0.05 as significant.

Results

Biofilm intensity and minimum inhibitory concentration to ciprofloxacin and ceftazidime. All *S. enterica* isolates were characterized as weak biofilm producers after growth in polystyrene microplates for 48 h, and cell densities ranged from 6.1×10^6 to 3.9×10^7 CFU (Supplementary Tables S1 and S2). The MIC values ranged from 0.005 to 0.01 $\mu\text{g/ml}$ and 0.5 to 2 $\mu\text{g/ml}$, for ciprofloxacin and ceftazidime, respectively (Table 1), which characterized all isolates as susceptible to both antimicrobials.

Different persister levels were found in *S. enterica* isolates after ciprofloxacin or ceftazidime exposure. Persister cells were detected in all *S. enterica* isolates after 72-h exposure to high concentrations of ciprofloxacin or ceftazidime in both planktonic and biofilm cultures. In order to assure the presence of *S. enterica* persisters and not of antibiotic-resistant mutants, a new susceptibility test was performed after all persister assays with the remaining 72-h cells and no difference in MIC values was detected.

Treatments with 100-fold MIC of ciprofloxacin or ceftazidime for 72 h resulted in distinct persister fractions ($p < 0.05$) in planktonically grown cells ranging from 0.0020% to 0.2252% (Fig. 3A,C and Supplementary Table S1) and 0.1466% to 1.6755% (Fig. 3B,C and Supplementary Table S2), respectively. In the same context, persister fractions from biofilms after a 72-h treatment with ciprofloxacin or ceftazidime ranged from 0.0694% to 0.9378% (Fig. 3A,D and Supplementary Table S1) and 0.6076% to 1.5869% (Fig. 3B,D and Supplementary Table S2), respectively. All *S. enterica* isolates, except for three *S. Enteritidis* (192, 45A, and S45) grown as biofilms, had significantly different persister levels ($p < 0.05$) when exposed to the distinct antimicrobials (Supplementary Table S3). Furthermore, a high heterogeneity in persister levels was found among *S. enterica* isolates when cultured under the same conditions and exposed to a same antimicrobial, especially planktonically grown cells exposed to ciprofloxacin (Fig. 3A,C and Supplementary Tables S1 and S2).

Biofilms presented higher persister levels than planktonic cultures. Taking together the persister fractions from all isolates, it was possible to notice higher levels of persisters in biofilms compared to planktonic cultures, and in both of those exposed to ciprofloxacin ($p < 0.001$) or ceftazidime ($p < 0.05$) (Fig. 4). Indeed, in some isolates, the persister levels found in biofilms after ciprofloxacin exposure were up to 140-fold higher than those detected in planktonic counterparts (Supplementary Table S1).

Levels of persisters after ceftazidime exposure were not affected by serovar regardless of culture condition. Persister levels in isolates from different serovars cultured under both conditions after exposure to ceftazidime or ciprofloxacin were compared, and no difference ($p > 0.05$) was found among serovars after ceftazidime treatment, as well as in biofilms exposed to ciprofloxacin (Supplementary Table S4). On the other hand, persister levels from planktonic cultures exposed to ciprofloxacin varied depending on the serovar ($p < 0.01$), except when *S. Enteritidis* was compared with *S. Infantis* ($p = 0.7364$). In addition, we found significantly different persister levels among *S. Enteritidis* isolates when comparing both culture conditions regardless of the antimicrobial used; this was also observed among *S. Infantis* isolates ($p < 0.05$) (Supplementary Table S5).

SCVs were found among *S. enterica* tolerant to ciprofloxacin. After ciprofloxacin exposure, colonies formed by surviving cells were morphologically analysed and SCVs could be seen from all *S. enterica* isolates (Fig. 2). All isolates showed similar ratios of SCVs in relation to the total number of colonies formed by persisters ($p > 0.05$) (Supplementary Fig. S2). However, when comparing colonies of persisters from all isolates in planktonic cultures with biofilms, SCVs were observed in higher proportion in planktonic cultures (Supplementary Fig. S3) ($p < 0.05$). On the other hand, similar ratios of SCVs were detected in both culture conditions for the same isolate (Supplementary Table S6). All SCVs reverted to a wild-type-like phenotype after sub-culturing in a medium without an antimicrobial. SCVs were confirmed as *Salmonella* spp. by the presence of the *invA* gene (Supplementary Fig. S4), and susceptibility to ciprofloxacin was maintained, since no difference between MIC values from NCPs and SCVs were detected. In groups of isolates from the same serovar, we did not find significant differences between each group in the ratios of SCVs to total colony numbers formed by persisters (Supplementary Fig. S5). Culture conditions also did not have a significant influence on the ratio of SCVs formed in each serovar (Supplementary Table S7). SCVs could not be observed in ceftazidime assays even after 48-h incubation.

Throughout three cycles, regardless if the analysis was performed from a single colony or from a pool of ten colonies, or whether originating from SCVs or NCPs, there was no significant difference between persister fractions forming SCVs after 72-h exposure to ciprofloxacin ($p > 0.05$). The same findings were observed in isolates

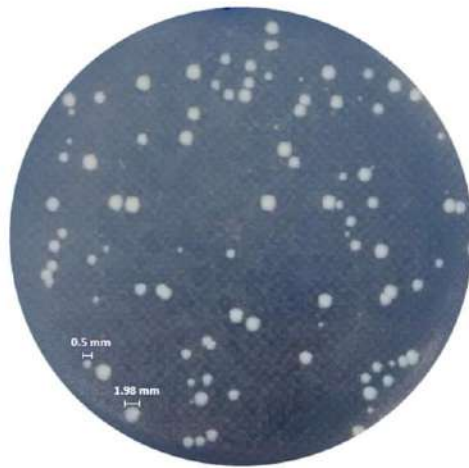


Figure 2. *Salmonella enterica* colony morphologies. After 72-h exposure to 100-fold MIC of ciprofloxacin, two different colony morphotypes were observed on nutrient agar during 24-h incubation at 37 °C, normal colony phenotype (NCP) and pinpoint colonies with reduced size, called small colony variants (SCV). The diameter of the colonies was measured using ImageJ software 1.8.0, represented here by an NCP of 1.98 mm and SCV of 0.5 mm.

belonging to different serovars (*S. Infantis* and *S. Enteritidis*). Therefore, a stable SCV phenotype was not selected throughout three cycles. Interestingly, when analysing the persister levels during 72-h ciprofloxacin exposure in each cycle for both isolates, we detected similar fractions from cells growing as SCVs or NCPs, regardless of their source (Fig. 5 and Table 2).

Cells from SCV and NCP showed similar size, division septum and filamentation. SEM was employed to evaluate morphology of cells from SCV and NCP grown in planktonic and biofilm conditions (Fig. 6). Regardless of the culture condition, a similar size was observed in cells from both SCVs and NCPs (Fig. 6A,C,E,G). Interestingly, in both SCVs and NCPs cultured in planktonic and biofilm condition, we found filamentous cells (Fig. 6B,D,F,H) concurrent with cells showing septum division (Fig. 6A,C,E,G). Furthermore, an extracellular substance was noticed circumventing SCVs cells obtained from planktonic culture (Fig. 6C,D), and filamentous cells were observed in SCVs and NCPs from both planktonic and biofilm cultures.

Discussion

Bacteria can continuously face unpredictable stresses, such as host immune defence; starvation; temperature, oxygen, and pH alterations, and antimicrobial action³⁸. The phenotypic switching that occurs in a small number of individuals within isogenic populations can be an essential adaptability strategy that is adopted by many microorganisms to survive different challenges^{28,38}. Persisters and SCVs comprise phenotypic variants able to survive a hostile environment and can resume normal growth after the stressful condition has ceased^{10,28}.

In this paper, we showed that isolates from four distinct *S. enterica* serovars were able to generate persisters after exposure to both antimicrobials tested. However, we did not find correlations between persister levels and *S. enterica* serovars, especially when ceftazidime was employed; however, a fluctuation in persister fractions among serovars was noticed in planktonic cultures exposed to ciprofloxacin. Nevertheless, different persister levels were found after exposure of isolates to antimicrobials with distinct action mechanisms. These findings led us to hypothesize that a single isolate generates distinct populations of persisters, each one with particular mechanisms to tolerate the lethal effects of different bactericidal antibiotics. Thus, the classical paradigm of a multidrug-tolerance phenotype because of antimicrobial ineffectiveness^{12,39} may not be present in all persister cells.

S. enterica persister levels also varied with regard to the culture conditions. Indeed, higher levels of surviving cells were detected in biofilms when compared to their planktonic counterparts, especially after ciprofloxacin exposure. It is important to highlight that the planktonic cells evaluated here were from a mid-exponential phase, since levels of persisters from bacterial cells growing in a stationary phase have been described as similar to or even higher than those found in biofilms⁴⁰. Bacteria growing in biofilms can face stressful conditions related to persistence, such as starvation⁴¹, oxygen deprivation⁴² and limited metabolic flux⁴³, triggering a stringent response, which, in turn, may activate the SOS response⁴⁴. The SOS response allows survival after exposure to β -lactams and fluoroquinolone antibiotics^{25,45}, and has been proposed as necessary for biofilm ofloxacin tolerance⁴⁶.

Quorum sensing had also been associated with persister formation in biofilms⁴⁷; however, we found that both biofilm and planktonic cultures with higher initial densities did not have the highest persister levels (Supplementary Tables S1 and S2), which we had previously reported in *Acinetobacter calcoaceticus-baumannii*⁵³. Additionally, initial cell density, i.e., the population before antibiotic exposure, was higher in planktonic cultures than in biofilms. Therefore, we were not able to corroborate that quorum sensing is playing a major role in the generation of persisters in biofilms; once we detected up to a 10-fold variation in biofilm persister levels when

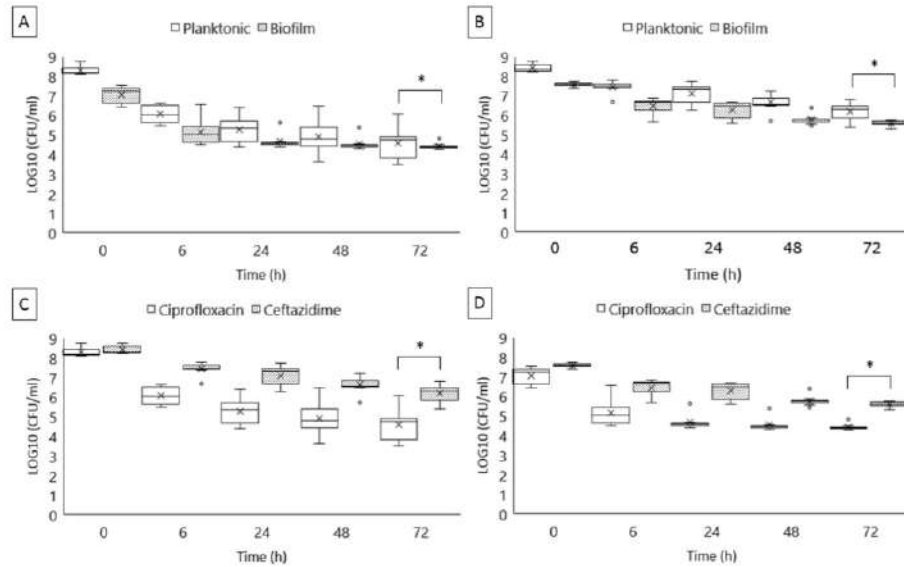


Figure 3. Persister fractions of *Salmonella enterica* after exposure to antimicrobials for 72 h. Box plots representing the average and variance of all isolates ($n = 10$) cultured under planktonic and biofilm conditions at each time evaluated after exposure to (A) 100-fold MIC of ciprofloxacin or (B) ceftazidime, as well as the persister fractions after exposure to ciprofloxacin or ceftazidime found in the isolates growing as (C) planktonic culture and (D) biofilm. Surviving fractions after 72 h-exposure to antimicrobials were compared by ANOVA with permutation, considering p -values ≤ 0.05 (*) as significant.

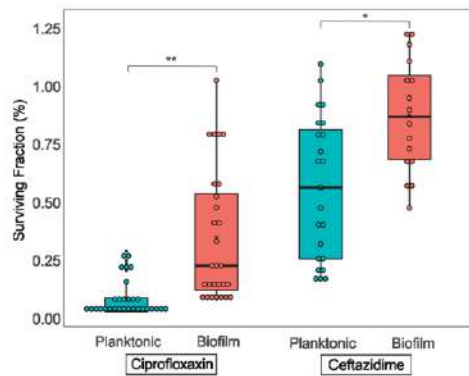


Figure 4. Comparison among persister fractions obtained from all *Salmonella enterica* isolates in planktonic and biofilm cultures exposed to ceftazidime or ciprofloxacin. In each box, bold horizontal lines and 'x' letters represent medians and mean values, respectively. Results from the analysis of variance with permutation are represented as p -values ≤ 0.05 (*) and ≤ 0.001 (**).

initial cell densities were similar. Indeed, an important aspect to take into account is the physiological states of cells growing in different conditions, which would be involved with the ability to respond to stresses and transport substances across membranes⁴⁸.

In addition to isolates with distinct behaviours that are related to persister levels when exposed to different antimicrobials and/or cultured under different conditions, heterogeneity was observed among isolates facing a same situation, which highlights a wide individual variation in antimicrobial tolerance, as also described in other bacteria^{33,41,49,50}.

Phenotypic switching to SCVs has also been recognized as a strategy for antimicrobial tolerance⁵¹. Here, we reported SCVs among persisters surviving after ciprofloxacin treatment in all *S. enterica* isolates, and a stable SCV phenotype was not found even in three consecutive cycles of ciprofloxacin exposure, regardless of whether the origin of the colony was small or normal, since all SCVs reverted to the wild-type-like phenotype when

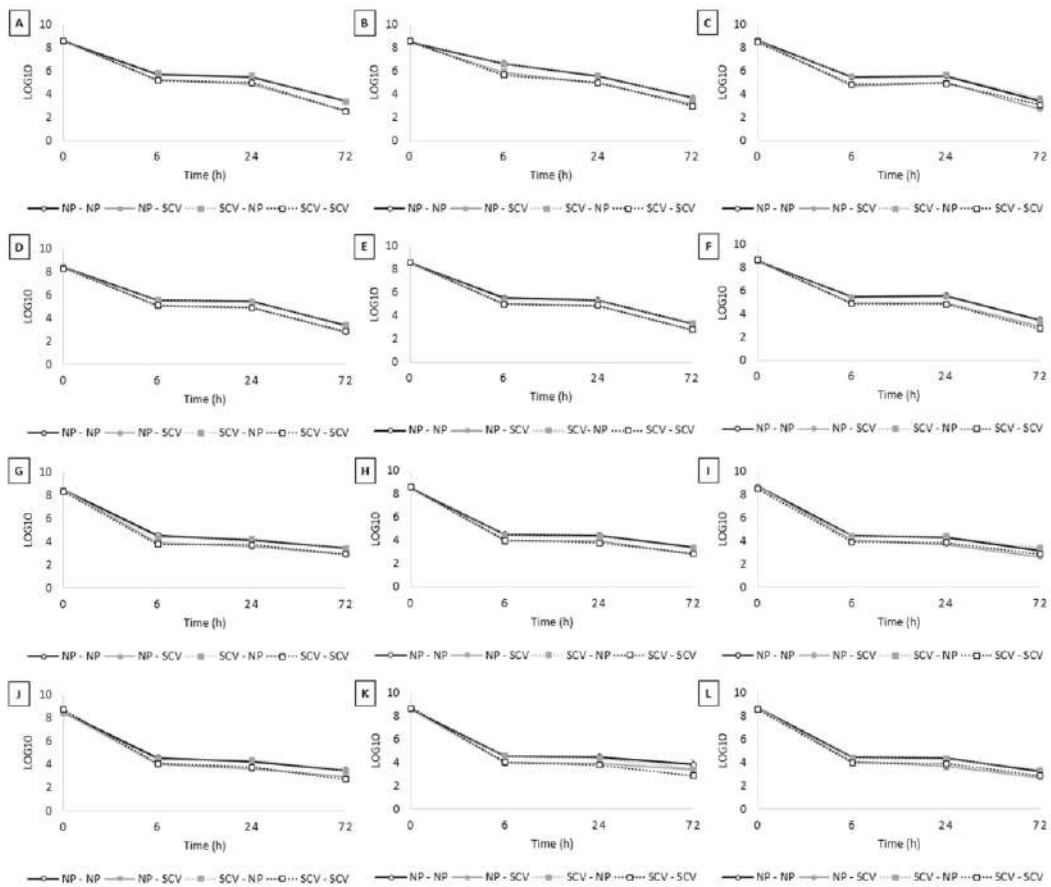


Figure 5. Small colony variants (SCV) phenotype evaluation throughout three consecutive cycles. Overnight culture was diluted 1:30, grew until mid-log phase and treated with ciprofloxacin 100-fold MIC for 72-h. The surviving cells forming SCV and normal colony phenotype (NCP) were separated in different experiments. The procedure was repeated three times and at the end of each cycle, SCV was obtained from NCP (NCP-SCV) or SCV (SCV-SCV) and NCP was also obtained from NCP (NCP-NCP) or SCV (SCV-NCP). (A–F) *Salmonella* Infantis: cycle one to three performed with (A–C) only one colony or (D–F) pool of colonies. (G–L) *Salmonella* Enteritidis: cycle one to three performed with (G–I) only one colony or (J–L) pool of colonies. The values are average of three biological with three technical replicates and bars indicate the standard error.

sub-cultured under stress-free conditions. This can indicate that SCVs, like persisters, represent a transient phenotype originating from stress responses and possibly coordinated by epigenetic changes^{52,53}. Furthermore, we also confirmed that the formation of persisters in *S. enterica* is a non-heritable mechanism, since the fractions of persisters remained approximately the same during repeated cycles.

All unstable SCVs we detected maintained the same ciprofloxacin MIC values of their ancestors, as already described in other studies^{24,27}. However, SCVs have been reported to be less susceptible to aminoglycosides and β -lactams antimicrobials^{34–56}, especially in stable SCVs, which may be due to mutations in genes involved in pathways required for the antimicrobial actions independent of those involved in the small colony size phenotype⁵⁷.

Despite the wide variation of persister levels found between the isolates, the ratios of SCV:total colonies were not different among them. Another important aspect to be highlighted was the detection of more SCVs in planktonic cultures than in biofilms, unlike what is usually found in persisters, which allow us to speculate that cells growing under distinct conditions may adopt different and perhaps complementary survival strategies. Nevertheless, no fluctuation in SCV rates among serovars could be seen, leading us to assume that serovars do not influence in SCV rates.

We also investigated the cell morphology of cells forming SCVs and NCPs, since SCVs from *Staphylococcus* spp. have been described as cells with different sizes, smaller or larger, when compared to those of NCPs^{52,58}. However, we observed similar shapes and sizes when comparing all cells, regardless of the culture conditions (Fig. 6A,C,E,G). Nevertheless, filamentous cells were seen among cells forming both SCVs and NCPs. These have been reported in *E. coli* after exposure to ciprofloxacin due to the inhibition of cell division resulting from

Isolate	Single colony or pool of colonies	Source (NCP or SCV)	Cycle	SCV persist fraction (%)
S. Infantis (S02)	Single colony	SCV	1	0.00008
	Single colony	SCV	2	0.00026
	Single colony	SCV	3	0.00040
	Single colony	NCP	1	0.00010
	Single colony	NCP	2	0.00045
	Single colony	NCP	3	0.00011
	Pool	SCV	1	0.00037
	Pool	SCV	2	0.00015
	Pool	SCV	3	0.00011
	Pool	NCP	1	0.00090
	Pool	NCP	2	0.00018
	Pool	NCP	3	0.00030
S. Enteritidis (393)	Single colony	SCV	1	0.00043
	Single colony	SCV	2	0.00019
	Single colony	SCV	3	0.00024
	Single colony	NCP	1	0.00030
	Single colony	NCP	2	0.00022
	Single colony	NCP	3	0.00009
	Pool	SCV	1	0.00036
	Pool	SCV	2	0.00017
	Pool	SCV	3	0.00021
	Pool	NCP	1	0.00030
	Pool	NCP	2	0.00074
	Pool	NCP	3	0.00010

Table 2. Small colony variants (SCV) persist fractions obtained from cells growing as SCVs or as normal colony phenotype (NCP) during three ciprofloxacin exposure cycles derived from a single colony or a pool of ten colonies.

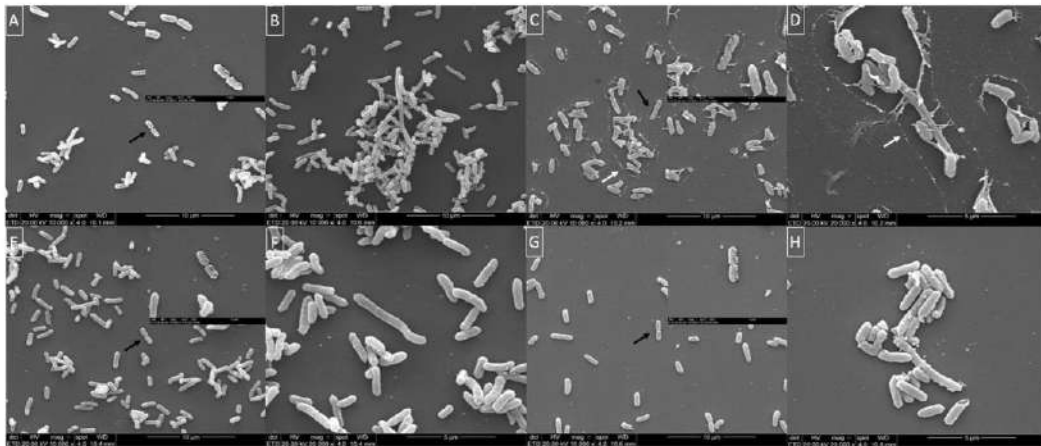


Figure 6. Scanning electron microscopy of *Salmonella* Enteritidis (393) forming small colony variants (SCV) and wild-type-like phenotype colonies after exposure to 100-fold MIC of ciprofloxacin for 72 h. In cells from both (A,B,E,F) wild-type-like phenotype and (C,D,G,H) SCV derived from (A–D) planktonic and (E–H) biofilm cultures were observed septum division (insets), (B,D,F,H) filamentation, and similar size between wild-type-like phenotype and SCV (A) (1.129–1.155 μm), (C) (1.285–1.327 μm), (E) (1.057–1.041 μm) and (G) (1.275–1.158 μm). (C,D) White arrow indicates the extracellular substance in SCVs obtained from planktonic culture and black arrows indicate septum division.

the induction of SOS response and raise of DNA-repair capability^{59,60}. In opposite, several cells showed septum division, which lead us to suggest that different behaviours can be found among cells forming distinct colonies morphologies, where cells may exhibit metabolic activity at different levels resulting in different growing speeds.

Both SCV and persisters are thought to be part of a bacterial bet-hedging strategy for the survival under stress. So, could SCVs comprise a phenotypic variant of persisters characterized by slow growth? If we consider these phenotypes as independent variants in *S. enterica* that randomly generate unstable SCV regardless of antibiotic exposure, we should also have found SCV after exposure to ceftazidime, which did not happen. Can we postulate that the diversity of strategies may be greater or that there is an overlap of physiological strategies depending on the challenging stress? Thus, elucidating the mechanisms involved in phenotypic switching of *S. enterica* isolates in an isogenic population is essential for the development of methods for an effective treatment of chronic infections, which may be of special concern in infections caused by invasive *Salmonella* serovars.

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Author Contributions

S.D. performed the experiments, analysed the data, and wrote the manuscript. S.G. and C.F. analysed the data and revised the manuscript. P.F. performed the statistical analysis and revised the manuscript. S.O. conceived and designed the experiments, analysed the data and wrote the manuscript.

Additional Information

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***Salmonella enterica* persisters cells form unstable small colony variants after *in vitro* exposure to ciprofloxacin**

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Supplementary Table 1. Colony forming units (CFU) from *Salmonella enterica* isolates after exposure to ciprofloxacin for 72 h in planktonic and biofilm cultures.

Isolate	Planktonic						Biofilm					
	0h*	6h	24h	48h	72h	Persister fractions (%)	0h	6h	24h	48h	72h	Persister fractions (%)
<i>S. Agona</i> (S48)	1.28E+08 [†] ±	3.78E+06±	3.11E+05±	1.36E+05±	2.22E+05±	0.1737	1.44E+07±	6.66E+04±	4.22E+04±	2.55E+04±	1.89E+04±	0.1353
	1.73E+06 [‡]	1.17E+06	8.40E+04	1.03E+04	5.08E+04		1.96E+06	5.77E+03	5.08E+03	3.87E+03	6.97E+03	
<i>S. Agona</i> (S79)	1.32E+08±	6.11E+05±	2.58E+05±	3.11E+05±	7.22E+04±	0.0552	2.67E+06±	3.11E+04±	2.33E+04±	2.45E+04±	1.78E+04±	0.6849
	8.54E+06	1.68E+05	2.15E+04	6.97E+04	1.26E+04		3.35E+05	6.97E+03	3.35E+03	6.93E+03	5.08E+03	
<i>S. Enteritidis</i> (152)	1.47E+08±	3.33E+05±	2.78E+04±	5.67E+03±	4.67E+03±	0.0032	3.33E+07±	3.33E+05±	4.56E+04±	3.44E+04±	2.67E+04±	0.0892
	2.08E+07	1.21E+05	5.08E+03	1.34E+03	3.35E+02		1.34E+07	5.77E+04	1.50E+04	5.10E+03	3.35E+03	
<i>S. Enteritidis</i> (192)	1.61E+08±	4.34E+05±	2.44E+04±	4.11E+03±	3.22E+03±	0.0020	2.11E+07±	3.78E+06±	4.22E+05±	2.33E+05±	6.89E+04±	0.4007
	1.15E+07	1.53E+05	5.10E+03	7.68E+02	3.87E+02		1.35E+07	1.65E+06	1.26E+05	6.65E+04	7.68E+03	
<i>S. Enteritidis</i> (393)	1.54E+08±	3.00E+05±	4.00E+04±	2.22E+04±	1.67E+04±	0.0108	2.33E+07±	1.56E+05±	4.33E+04±	2.89E+04±	2.45E+04±	0.1251
	2.31E+06	6.70E+04	6.70E+03	5.08E+03	3.35E+03		1.00E+07	1.96E+04	1.34E+04	8.40E+03	3.87E+03	
<i>S. Enteritidis</i> (4SA)	2.89E+08±	2.33E+06±	2.00E+05±	4.11E+04±	4.44E+04±	0.0165	2.67E+06±	4.56E+04±	3.56E+04±	2.33E+04±	2.44E+04±	0.9378
	1.02E+08	3.35E+05	8.83E+04	1.26E+04	1.96E+03		3.35E+05	1.26E+04	5.10E+03	6.65E+03	5.10E+03	
<i>S. Enteritidis</i> (S45)	4.89E+08±	4.22E+06±	1.53E+06±	8.00E+05±	8.33E+04±	0.0188	3.66E+06±	3.78E+05±	3.11E+04±	2.89E+04±	2.55E+04±	0.7879
	1.65E+08	7.74E+05	2.31E+05	3.30E+04	3.35E+03		1.15E+06	7.74E+04	5.10E+03	9.64E+03	1.07E+04	
<i>S. Infantis</i> (S02)	1.30E+08±	4.78E+05±	5.78E+04±	5.00E+04±	3.22E+03±	0.0025	3.22E+07±	1.55E+05±	4.11E+04±	3.11E+04±	2.22E+04±	0.0694
	8.89E+06	1.02E+05	6.93E+03	1.15E+04	6.93E+02		6.93E+06	3.87E+04	5.10E+03	1.34E+04	8.40E+03	
<i>S. Infantis</i> (S67)	1.27E+08±	1.78E+06±	5.78E+05±	7.44E+04±	6.67E+04±	0.0530	2.33E+07±	4.11E+04±	3.44E+04±	2.00E+04±	2.22E+04±	0.0989
	5.77E+06	8.40E+05	5.08E+04	5.10E+03	1.20E+04		6.65E+06	1.26E+04	5.10E+03	3.30E+03	8.40E+03	
<i>S. Schwarzengrund</i> (S58)	5.45E+08±	4.44E+06±	2.48E+06±	2.92E+06±	1.21E+06±	0.2252	6.45E+06±	3.11E+04±	2.33E+04±	2.45E+04±	1.78E+04±	0.5195
	6.93E+07	1.96E+05	1.50E+05	1.01E+05	7.21E+04		1.57E+06	6.97E+03	3.35E+03	6.93E+03	5.08E+03	

*CFU counts from culture before adding antimicrobial. The persister fractions at each time point should take into account the value of CFU in 0h for each culture condition.

[†] Data of CFU counts represent the average of three biological and three technical replicates.

[‡] Standard Deviation

Supplementary Table 2. Colony forming units (CFU) from *Salmonella enterica* isolates after exposure to ceftazidime for 72 h in planktonic and biofilm cultures.

Isolate	Planktonic						Persister fractions (%)	Biofilm					
	0h*	6h	24h	48h	72h	0h		6h	24h	48h	72h	Persister fractions (%)	
<i>S. Agona</i> (S48)	1.78E+08 [†] ± 5.08E+07 [‡]	2.67E+07± 8.79E+06	4.33E+06± 8.79E+05	3.00E+06± 3.30E+05	5.55E+05± 6.93E+04	0.3279	3.67E+07± 1.34E+07	4.22E+06± 5.08E+05	1.44E+06± 5.10E+05	3.56E+05± 1.26E+05	3.56E+05± 1.26E+05	1.0613	
<i>S. Agona</i> (S79)	1.78E+08± 1.91E+07	5.00E+07± 1.20E+07	2.45E+07± 6.93E+06	1.66E+07± 5.77E+06	3.00E+06± 6.70E+05	1.6755	2.33E+07± 3.35E+06	4.55E+05± 1.07E+05	3.78E+05± 1.02E+05	4.33E+05± 1.00E+05	3.00E+05± 3.30E+04	1.3097	
<i>S. Enteritidis</i> (152)	4.56E+08± 1.39E+08	6.11E+07± 6.97E+06	5.44E+07± 1.26E+07	1.67E+07± 3.35E+06	6.11E+06± 1.02E+06	1.4639	4.44E+07± 8.36E+06	5.00E+06± 1.76E+06	2.44E+06± 8.36E+05	6.00E+05± 8.83E+04	5.11E+05± 8.40E+04	1.2007	
<i>S. Enteritidis</i> (192)	1.67E+08± 3.35E+07	4.55E+06± 6.93E+05	1.89E+06± 5.10E+05	5.11E+05± 5.10E+04	2.44E+05± 5.10E+04	0.1466	5.44E+07± 1.02E+07	6.44E+06± 1.17E+06	3.67E+06± 1.21E+06	2.44E+06± 8.36E+05	5.45E+05± 1.35E+05	1.0574	
<i>S. Enteritidis</i> (393)	5.56E+08± 1.17E+08	2.78E+07± 8.40E+06	2.67E+07± 8.79E+06	3.11E+06± 5.10E+05	2.33E+06± 6.65E+05	0.4275	5.45E+07± 1.35E+07	7.11E+06± 1.26E+06	4.33E+06± 6.65E+05	5.67E+05± 6.65E+04	3.22E+05± 6.93E+04	0.6076	
<i>S. Enteritidis</i> (4SA)	1.78E+08± 3.87E+07	2.44E+07± 5.10E+06	5.44E+06± 1.54E+06	3.67E+06± 6.65E+05	1.44E+06± 5.10E+05	0.8056	3.56E+07± 8.36E+06	1.33E+06± 3.35E+05	5.44E+05± 1.02E+05	4.89E+05± 1.07E+05	4.22E+05± 1.02E+05	1.2078	
<i>S. Enteritidis</i> (S45)	3.89E+08± 3.81E+07	3.89E+07± 5.10E+06	1.89E+07± 6.97E+06	3.67E+06± 1.00E+06	2.89E+06± 5.10E+05	0.7450	4.11E+07± 8.40E+06	5.11E+06± 6.97E+05	4.45E+06± 1.07E+06	7.11E+05± 4.99E+04	5.00E+05± 8.83E+04	1.2283	
<i>S. Infantis</i> (S02)	1.89E+08± 1.91E+07	2.22E+07± 9.58E+06	4.25E+06± 1.55E+06	3.66E+06± 5.77E+05	3.78E+05± 8.40E+04	0.1989	3.11E+07± 7.68E+06	5.00E+06± 1.20E+06	4.78E+06± 1.02E+06	5.78E+05± 6.93E+04	3.45E+05± 3.87E+04	1.2471	
<i>S. Infantis</i> (S67)	2.22E+08± 6.93E+07	4.00E+07± 8.83E+06	3.11E+07± 8.40E+06	7.11E+06± 3.81E+05	1.78E+06± 5.08E+05	0.8027	2.45E+07± 6.93E+06	5.11E+05± 1.68E+05	4.33E+05± 3.35E+04	2.78E+05± 5.08E+04	2.00E+05± 3.32E+04	0.8756	
<i>S. Schwarzengrund</i> (S58)	3.45E+08± 1.07E+08	3.00E+07± 8.83E+06	2.67E+07± 6.65E+06	7.22E+06± 8.40E+05	4.78E+06± 6.93E+05	1.4668	3.56E+07± 5.10E+06	4.67E+06± 8.75E+05	3.44E+06± 8.36E+05	5.89E+05± 8.40E+04	5.67E+05± 1.21E+05	1.5869	

*CFU counts from culture before adding antimicrobial. The persister fractions at each time point should take into account the value of CFU in 0h for each culture condition.

[†] Data of CFU counts represent the average of three biological and three technical replicates.

[‡] Standard Deviation

Supplementary Table 3. Analysis of variance with permutation (PERM-ANOVA) of persister fractions obtained from each *Salmonella enterica* isolate in planktonic and biofilm cultures exposed to ceftazidime or ciprofloxacin.

Ciprofloxacin vs Ceftazidime						
Isolate	Biofilm			Planktonic		
	Sum of squares	F-statistic	p-value	Sum of squares	F-statistic	p-value
152	0.00018	21.52000	0.00974	0.00032	16.84000	0.01481
192	0.00006	5.35700	0.08163	0.00001	1639.00000	0.00001
393	0.00003	29.01000	0.00574	0.00002	34.23000	0.00425
4SA	0.00001	1.34200	0.31120	0.00009	61.76000	0.00141
S02	0.00020	20.73000	0.01039	0.00001	128.10000	0.00034
S45	0.00002	2.18100	0.21380	0.00008	76.54000	0.00094
S48	0.00012	12.04000	0.02560	0.00001	7.98600	0.04754
S58	0.00017	36.40000	0.00380	0.00023	20.20000	0.01087
S67	0.00009	17.34000	0.01410	0.00008	1642.00000	0.00001
S79	0.00005	7.88600	0.04841	0.00039	132.40000	0.00036

Supplementary Table 4. Comparison between persister fractions from each culture conditions and antimicrobial exposure in each group of *Salmonella enterica* serovars employing Tukey's test.

		Agona	Enteritidis	Infantis	Schwarzengrund
Biofilm Ceftazidime	Agona		0.88820	0.93190	0.40860
	Enteritidis	1.01800		1.00000	0.12090
	Infantis	0.84510	0.00824		0.19110
	Schwarzengrund	2.23000	3.27100	2.92000	
Planktonic Ceftazidime	Agona		0.70360	0.39820	0.62630
	Enteritidis	1.52900		0.84150	0.15580
	Infantis	2.25700	1.16800		0.08126
	Schwarzengrund	1.71100	3.08100	3.55400	
Biofilm Ciprofloxacin	Agona		0.98610	0.39540	0.97120
	Enteritidis	0.48170		0.13530	0.99560
	Infantis	2.26500	3.18800		0.32150
	Schwarzengrund	0.62010	0.32530	2.46900	
Planktonic Ciprofloxacin	Agona		0.00001	0.00128	0.00079
	Enteritidis	8.64700		0.73640	0.00000
	Infantis	6.02100	1.45000		0.00000
	Schwarzengrund	6.28300	13.63000	11.20000	

Supplementary Table 5. Comparison of persister levels from different culture conditions after exposure to ceftazidime or ciprofloxacin by group of serovars using ANOVA.

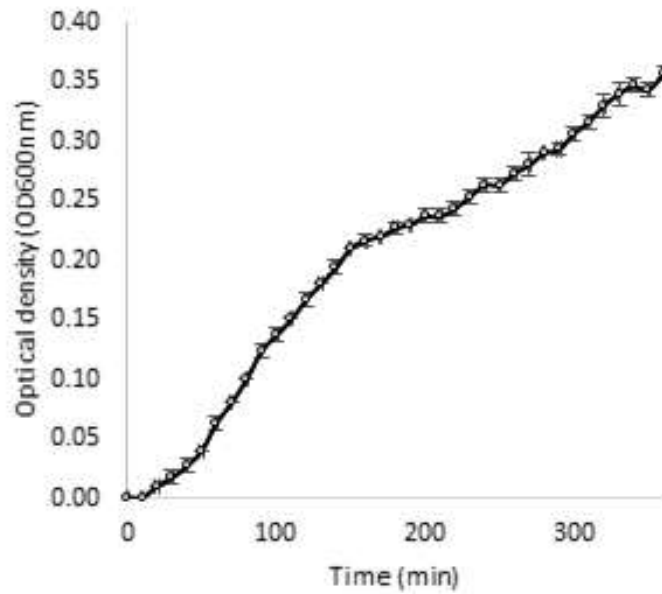
Serovar	Biofilm vs Planktonic					
	Sum of squares	Ceftazidime		Sum of squares	Ciprofloxacin	
		F-statistic	p-value		F-statistic	p-value
Agona	0.00001	0.28830	0.60310	0.00002	4.12600	0.06965
Enteritidis	0.00009	4.37500	0.04567	0.00015	17.54000	0.00025
Infantis	0.00009	6.91800	0.02515	0.00001	9.28500	0.01231
Schwarzengrund	0.00001	0.16140	0.70840	0.00001	4.75000	0.09480

Supplementary Table 6. Comparison between SCVs levels from different culture conditions in the same *Salmonella enterica* isolate using ANOVA.

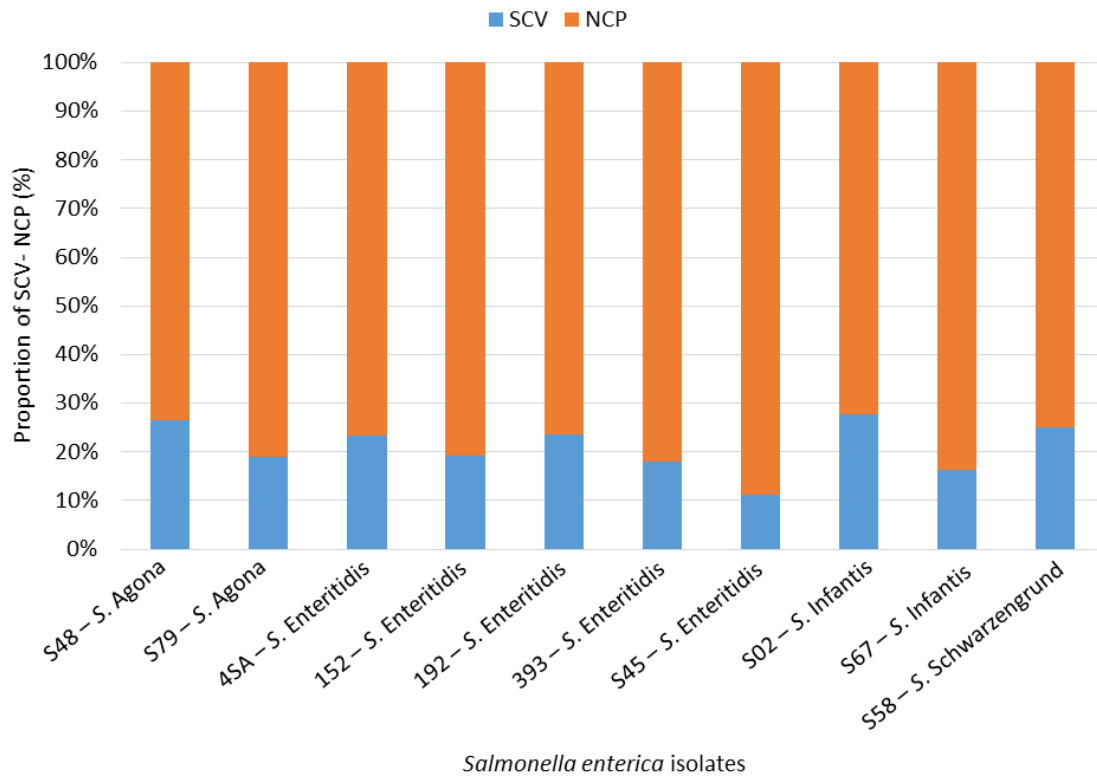
Biofilm vs Planktonic			
Isolate	Sum of squares	F-statistic	p-value
152	0.02570	0.60890	0.47880
192	0.02154	0.16310	0.70690
393	0.01773	0.44960	0.53920
4SA	0.13481	2.16400	0.21530
S02	0.07014	1.12900	0.34800
S45	0.01048	0.74610	0.43640
S48	0.05711	0.93460	0.38840
S58	0.01174	0.08805	0.78140
S67	0.00017	0.00655	0.93940
S79	0.00490	0.11320	0.75350

Supplementary Table 7. Comparison between ratios of SCVs from different culture conditions after exposure to ciprofloxacin by group of serovars using ANOVA.

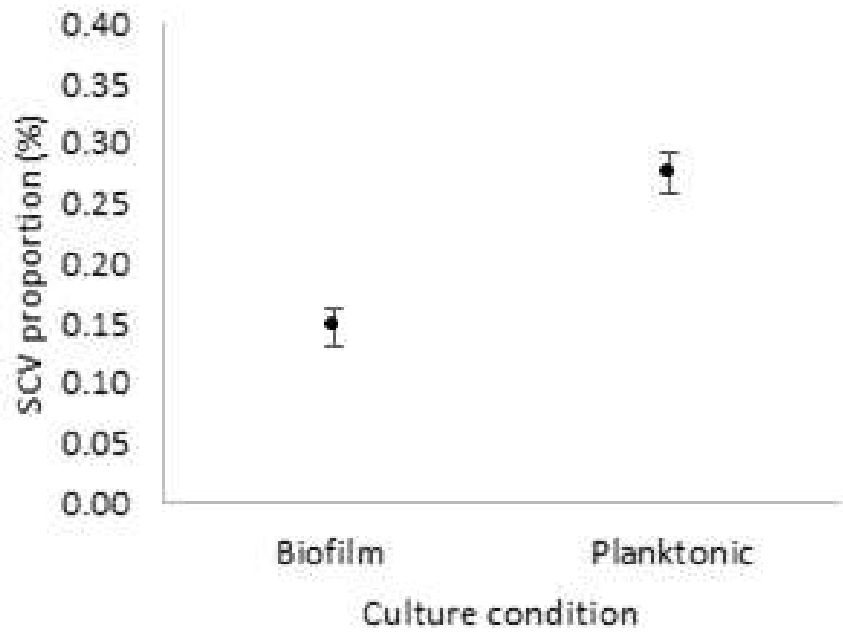
Biofilm vs Planktonic			
Serovar	Sum of squares	F-statistic	p-value
Agona	466.11600	2.33600	0.15740
Enteritidis	0.02896	0.26920	0.60790
Infantis	0.04496	0.80250	0.39140
Schwarzengrund	935.43500	0.99280	0.37540



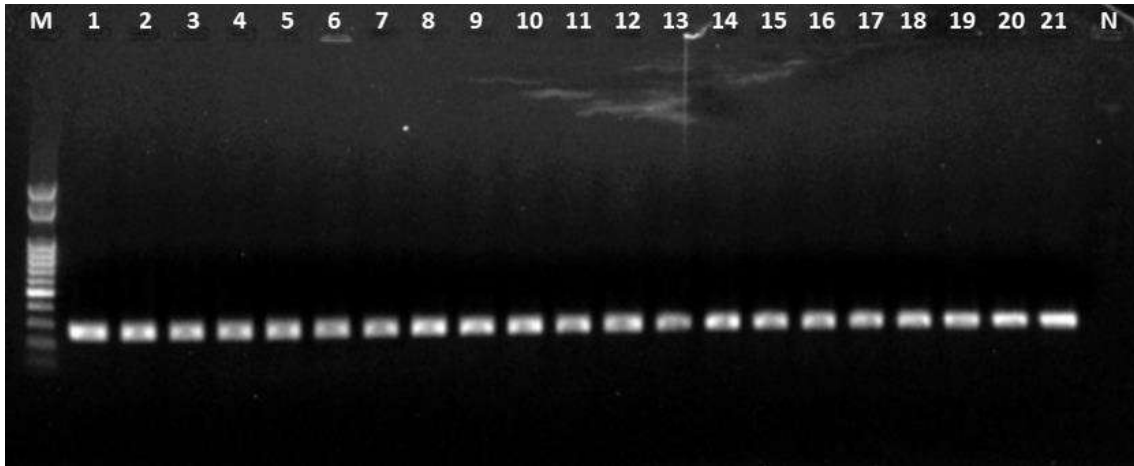
Supplementary Figure S1. Graphical representation of *Salmonella* Infantis growth curve in Luria-Bertani broth (LB) medium. The isolate was diluted 1:30 and the growth at 37 °C was monitored by measuring the optical density (OD_{600nm}) every 10 min in a SpectraMax® 190 microplate reader. Plotted points represent the mean ± standard deviation of three replicates. The curve fitted show $r^2 = 0.92$ and a half-life of 6 h.



Supplementary Figure S2. Proportions of small colony variants (SCVs) and normal colony phenotypes (NCPs) in each isolate of *Salmonella enterica* exposed to 100-fold MIC of ciprofloxacin. Tukey’s test was employed for the statistical analysis and similar ratios were found (p -value > 0.05).



Supplementary Figure S3. Graphical representation of the small colony variants (SCV) proportion found between both culture conditions. The values represent means of three replicates from all isolates and the bars indicate the error standard.



Supplementary Figure S4. Agarose gel electrophoresis of *invA* gene amplicons from SCV. Lane M: 100 bp DNA Ladder; Lanes 1-21: 284 bp *invA* amplicons; Lane N: negative control (water was used as sample).

Capítulo 3

Artigo Científico 2

Pre-exposure to poultry feed additives at sub-inhibitory concentrations may not influence persister cell levels

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1 **Pre-exposure to poultry feed additives at sub-inhibitory concentrations may not**
2 **influence persister cell levels**

3

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16

17 **Abstract**

18 Organic acids and other antimicrobials have been used in poultry feed and water to
19 improve growth performance by optimizing the balance of gastrointestinal tract
20 microbiota and reducing pathogen colonization. However, stressful conditions can
21 induce a bacterial phenotypic switching triggered by common regulatory networks.
22 Therefore, we aimed to evaluate whether a prior exposure to feed additives (such as
23 organic acids and colistin) or even to ciprofloxacin is able to influence persister levels
24 among *Salmonella enterica* isolates subsequently exposed to high concentration of
25 ciprofloxacin. *S. enterica* isolates (*S. Enteritidis*, *S. Infantis*, *S. Agona* and *S.*
26 *Schwarzengrund*) were exposed to 5-fold the minimum inhibitory concentration (MIC)
27 of colistin for 48 h, presenting persister levels ranging from 0.0186% to 0.2577%, with
28 the exception of the *S. Agona* isolate that was not able to form persisters. Additionally,
29 most isolates showed a significant resumption of growth after colistin treatment without
30 selection of a resistant mutant, hetero-resistance phenotype or antimicrobial
31 degradation. Exposure to formic and lactic acids resulted in a substantial reduction in
32 the number of surviving cells in most isolates, and did not seem to induce an acid
33 tolerance response. Furthermore, exposure to colistin, organic acids or even
34 ciprofloxacin at sub-MICs followed by treatment with 100-fold the MIC of
35 ciprofloxacin did not affect the persister fractions when compared to cultures exposed
36 only to 100-fold the MIC of ciprofloxacin. Therefore, our results may suggest that the
37 feed additives evaluated could not induce antimicrobial tolerance neither select highly
38 persistent mutants.

39 **Keywords:** Organic acids, colistin, ciprofloxacin, tolerance induction, *Salmonella*
40 *enterica*, persisters.

41 **1. Introduction**

42 The increasing concern about the use of antibiotics in poultry production has
43 been changing the way in which producers manage poultry health. Antimicrobials have
44 been used at sub-therapeutic doses to improve growth, feed conversion efficiency and to
45 prevent intestinal infections. However, this practice may be linked to the intense
46 development of antimicrobial resistance among pathogenic bacteria. Thus, many
47 countries have banned the use of antimicrobials as feed additives, forcing the poultry
48 industry to develop alternatives to replace antibiotic growth promoters in feed (Millet
49 and Maertens, 2011; Brown et al., 2017; Broom, 2018).

50 The organic acids such as formic, lactic, propionic, citric, sorbic and phosphoric
51 acids have been used in poultry diets and drinking water for decades and seem to elicit a
52 positive response in growth performance. In addition, they are considered safe, with no
53 involvement in antimicrobial resistance, as well as residues in the meat usually cannot
54 get over into the human food chain. Organic acids optimize the balance of
55 gastrointestinal tract microbiota and are able to reduce *Salmonella enterica* colonization
56 by lowering the pH and protecting especially young chickens from intestinal infections
57 (Biggs and Parsons, 2008; Dittoe et al., 2018; Hamid et al., 2018).

58 On the other hand, *S. enterica* is an adaptable microorganism able to respond to
59 diverse acid stresses, inducing different levels of acid tolerance response (ATR) that are
60 dependent on pH concentration, time exposure and growth phase (Ye et al., 2019). The
61 acid stress tolerance in *S. enterica* is of particular importance because it's a major
62 human zoonotic pathogen causing salmonellosis, which is related to foodborne
63 infections mainly due to consumption of poultry meat and eggs products (CDC, 2018).
64 Since *Salmonella* can be found intracellularly, ATR may become even more challenging

65 by allowing intravacuolar survival, which could result in persistent infections (Kenney
66 et al., 2018; Stapels et al., 2018).

67 Persistent infections can be mediated by persisters, which are slow or non-
68 growing cells, stochastically formed and/or induced by stressors, such as acids and
69 antimicrobials. Although persisters arise from an isogenic population susceptible to
70 antimicrobials, they are able to tolerate lethal doses of antibiotics, hindering the
71 treatment and causing relapsing infections (Lewis, 2012). Persistence is described as a
72 non-heritable phenotype; however, different stressful conditions, such as pre-exposure
73 to sub-inhibitory concentrations of several antimicrobial classes, can significantly
74 increase persister levels (Johnson and Levin 2013; Cui et al., 2018). Likewise, pre-
75 treatment with sub-inhibitory doses of paraquat (oxidative stress inducer) promoted a
76 dramatic increase in the number of persisters surviving challenge with fluoroquinolone
77 antibiotics (Wu et al., 2012). Taking that into account, we aimed to evaluate whether a
78 previous exposure to feed additives (such as organic acids and colistin) or even to
79 ciprofloxacin (drug of choice for severe salmonellosis treatment in humans) is able to
80 influence on persister levels among *S. enterica* isolates later exposed to ciprofloxacin.

81

82 **2. Materials and methods**

83 *2.1. Bacterial isolates and growth conditions*

84 Three *S. enterica* isolates from poultry by-product meals (*S. Infantis*, *S.*
85 *Schwarzengrund*, and *S. Agona*), and three *S. Enteritidis* isolates from poultry carcass,
86 ready-to-eat-food and food handler were used in this study (Table 1). Samples were
87 grown overnight in Trypticase Soy Broth (TSB) (BioBras, São Paulo, Brazil) at 37°C,
88 and stored at -80°C with 20% glycerol.

89

90 2.2. *Minimum inhibitory concentrations*

91 Colistin (Sigma-Aldrich, St Louis, USA) and organic acids (OA) (formic and
92 lactic acids – 4,096 µg/ml/4,698 µg/ml) (Oligo Basics Agroindustrial, Paraná, Brazil)
93 minimum inhibitory concentrations (MIC) were determined by broth microdilution
94 method (CLSI, 2012). All assays were performed in triplicate. The colistin breakpoints
95 were interpreted according to the European Committee on Antimicrobial Susceptibility
96 Testing (EUCAST, 2018) guidelines.

97

98 2.3. *Persister cell assays*

99 2.3.1. Colistin

100 Initially, the formation of persisters following exposure to colistin was evaluated
101 in both *S. Enteritidis* (152) and *S. Agona* (S79) isolates employing concentrations of 10,
102 5 and 2.5-fold the MIC. For this, overnight cultures in Luria-Bertani broth (LB) were
103 diluted 1:30 and incubated at 37°C for 2 h 30 min until mid-exponential growth phase,
104 achieving 10⁸ colony-forming units per milliliter (CFU/ml). The cell densities of these
105 cultures were determined by removing 100 µl-aliquots of the cultures, diluting to 10⁻⁶ in
106 0.85% saline, spotting 10 µl of each dilution on nutrient agar (Oxoid, Hampshire,
107 England), in triplicate, and then incubating at 37°C for 24 h (Drescher et al., 2019
108 submitted). Afterward, to access persister cells, the cultures at mid-exponential growth
109 phase were exposed to colistin at room temperature for 6 h. The surviving cell fractions
110 were determined at every hour after drug exposure. One ml-aliquots from each time
111 point were removed, centrifuged at 7,200 rpm for 7 min, diluted to 10⁻⁴ in 0.85% saline,
112 10 µl of each dilution were spotted on nutrient agar, in triplicate, and incubated at 37°C
113 for 24 h. The same procedure was performed exposing five isolates (S02, S58, 152, 192
114 and 393) to 5-fold the MIC of colistin for 48 h, and determining the persister fractions at

115 6, 12, 24 and 48 h. The persister cell fractions were measured by dividing the number of
116 remaining colonies by the number of colonies found before the antibiotic treatment. All
117 assays were performed in biological triplicate, and data of CFU counts represent the
118 mean of three technical replicates. After the persistence assays, the remaining colonies
119 were submitted to a new broth microdilution test in order to confirm non-selection of
120 resistant mutants.

121

122 2.3.2. Organic acids

123 Survival of all *S. enterica* isolates was determined after exposure to OA at 2.5-
124 fold the MIC for 72 h at room temperature, as described above. The time points used to
125 access the surviving cells were 6, 12, 24, 48 and 72 h of exposure to OA. A culture of *S.*
126 Enteritidis (152) isolate at mid-exponential phase was employed for the evaluation of
127 the acidification of the medium provided by OA. Thus, pH of the culture added of OA
128 was measured every 10 min for 30 min, followed by hourly measurements up to 6 h, as
129 well as at 12, 24, 48 and 72 h at room temperature incubation.

130

131 2.3.3. Pre-exposure to sub-inhibitory concentrations of antimicrobials

132 The effect of a pre-exposure to sub-inhibitory concentrations of ciprofloxacin,
133 colistin and OA in the formation of persister cells before exposure to high concentration
134 of ciprofloxacin (100-fold the MIC) was evaluated in all *S. enterica* isolates. The first
135 steps of adjusting the culture to the mid-exponential growth phase were performed as
136 described above. Subsequently, the cultures were exposed to ciprofloxacin, colistin or
137 organic acid at 0.5-fold the MIC, in separate assays, for 30 min at room temperature.
138 The culture-containing vials were then centrifuged at 10,000 rpm for 10 min and
139 washed once with phosphate-buffered saline (PBS). The pellets were resuspended in 10

140 ml of fresh LB-containing 100-fold the MIC of ciprofloxacin, which was incubated at
141 room temperature. Pre-treatment effect was measured by accessing the CFU/ml at
142 designated time points (30 min, 6, 12 and 24 h after exposure to antimicrobials at sub-
143 MIC), as described above.

144 One *S. Enteritidis* (393) isolate was evaluated by successive exposures to
145 colistin at 0.5-fold the MIC for 30 min, once a day. For this, cultures were grown in LB
146 broth overnight at 37°C, diluted 1:30 and again incubated 37°C for 2 h 30 min. At this
147 time, colistin was added at 0.5-fold the MIC and incubated at room temperature for 30
148 min. After this period, the cultures were centrifuged at 10,000 rpm for 10 min, washed
149 once with PBS, a fresh LB was added without colistin and the cultures were further
150 incubated overnight at 37°C. This procedure was repeated for four subsequent days. At
151 the end of the fifth day of repeated exposure to 0.5-fold the colistin, the culture was
152 centrifuged, washed once with PBS, and the pellet was resuspended in LB containing a
153 100-fold the MIC of ciprofloxacin. The levels of persisters were determined as
154 described above.

155

156 *2.4. Evaluation of colistin hetero-resistance*

157 Hetero-resistant colistin subpopulation from *S. Enteritidis* (192) isolate was
158 determined by population analysis profile (PAP), as previously described (El-Halfawy,
159 2015). The isolate was grown on nutrient agar during 24 h and the colonies were diluted
160 in 0.85% saline until turbidity of 0.5 MacFarland standard (approximately 10^8 CFU/ml).
161 After the initial inoculum is adjusted, it was serially diluted to 10^{-8} and 100 μ l of each
162 dilution was spread, in duplicate, on Mueller Hinton agar (MHA) surface containing 0,
163 1, 2, 4, 8 and 16 μ g/ml of colistin and plates were incubated at 37°C for 48 h. The
164 frequency of hetero-resistant subpopulations was calculated by dividing the number of

165 colonies (more than 20 CFU/ml) grown at the highest drug concentration by the colony
166 counts from the same bacterial inoculum plated onto antibiotic-free plates. The obtained
167 colonies were sub-cultured for five days in MHA without colistin and the MIC values
168 were assessed again in order to evaluate whether this resistance was stable.

169

170 *2.5. Liquid chromatography coupled to mass spectrometry in tandem (LC-MS/MS)*

171 LC-MS/MS analysis was employed in order to verify the colistin content during
172 the persistence assay in *S. Enteritidis* (192), at final concentration of 5 µg/ml. At time
173 points of 0, 6, 24 and 48 h after exposure to colistin, 1-ml aliquots were removed and
174 stored at -20 °C until analysis.

175 Colistin has a number of amino groups that can generate multiple charged
176 molecular ions in the LC-MS/MS electrospray ionization source (ESI) either by
177 protonation or deprotonation of these groups. In order to achieve stable and intense
178 signals for colistin A and colistin B, acid formic was added in both sample and LC-
179 MS/MS mobile phases. In this condition, colistin fractions A and B form double and
180 triple charged molecular ions. The samples preparation before LC-MS/MS analysis
181 consisted in thawing and removing of 100 µl of each sample into a new tube with 400-
182 µl acetonitrile. After, the mixture was vortexed and centrifuged at 5,500 rpm for 5 min,
183 being the supernatant transferred to a glass vial and injected (3 µl) into LC-MS/MS.

184 LC-MS/MS analysis was performed on an Agilent 1290 liquid chromatograph
185 coupled to an Agilent 6460 triple quadrupole mass spectrometer (Agilent Technologies,
186 Paolo Alto, CA, USA) equipped with an ESI source operated in positive mode. The
187 chromatography was carried out on a reverse phase Phenyl-Hexyl C18 column (50 x 4.6
188 mm, 1.8 µm, Agilent, Santa Clara, USA) preceded by a guard column with the same
189 packing material. Separation of colistin fractions A and B was performed in gradient

190 mode, at flow rate of 0.7 ml/min, at 30 °C. Mobile phases were consisted of (solvent A)
191 0.1% formic acid in water, and (solvent B) 0.1% formic acid in acetonitrile. The
192 gradient was set to start with 5% of solvent B and then linearly increased to 95% after 2
193 min, which was maintained for 2 min before returning to the start condition. ESI was set
194 with the following parameters: a dry gas temperature of 350 °C, a dry gas flow rate of
195 10 l/min, a nebulizer pressure of 35 psi, a sheath gas temperature of 320 °C, a sheath
196 gas flow rate of 11 l/min, a nozzle voltage of 500 V, and a capillary voltage of 3500 V.
197 Mass spectrometer was operated in multiple-reaction monitoring (MRM) to detect both
198 colistin A and colistin B with the parameters summarized in Table S1. Analyte
199 concentrations were calculated by external standardization, using matrix-matched
200 calibration curves of five concentration levels of colistin in bacterial culture medium
201 (from 1 to 10 µg/ml) (Fig. S1). Colistin concentrations were determined as the sum of
202 their fractions A and B. All data were acquired and processed by using Agilent
203 MassHunter (Agilent Technologies Paolo Alto, CA, USA).

204

205 2.6. Statistical analysis

206 Surviving cell fractions after treatments were compared using analysis of
207 variance (ANOVA) with permutation (9,999 bootstrap iterations in all tests), followed
208 by Tukey's post-hoc test for the pairwise comparisons. Analyzes were carried out with
209 pooled mean values of all isolates and considering each isolate separately. All analyzes
210 were performed in the statistical platform R (R Core Team, 2016) using package
211 'ImPerm' (Wheeler and Torchiano, 2016). We considered p -values < 0.05 as
212 significant.

213

214

215 3. Results

216 The MIC values of colistin ranged from 1 to 2 µg/ml (Table 1), characterizing all
217 isolates as susceptible to this antimicrobial. The MICs of formic/lactic acids were
218 512/587 µg/ml for all isolates.

219 Initially, *S. Enteritidis* (152) and *S. Agona* (S79) isolates were exposed to 2.5, 5
220 and 10-fold the MIC of colistin, and, interestingly, *S. Agona* (S79) was not able to form
221 persisters at any concentration tested even after 1 h of exposure to colistin. Conversely,
222 *S. Enteritidis* (152) produced persisters, and there was no significant difference among
223 their levels after exposure to the two highest concentrations ($p > 0.05$) (Table S2).
224 Therefore, 5-fold the MIC of colistin was employed in the further assays with all
225 isolates, and the persister levels after 48 h-exposure ranged from 0.0186% to 0.2577%.
226 *S. Enteritidis* (152) isolate was able to produce more persisters after 6-h exposure to
227 colistin when compared to the other isolates, whilst the comparison among persister
228 fractions from all isolates after 48 h showed that the lowest fraction was found for *S.*
229 *Enteritidis* (192). Additionally, after 48-h colistin exposure, all isolates but *S. Enteritidis*
230 (152) showed a significant increase of populations when compared to those from 6 h of
231 exposure ($p < 0.05$). A resumption of growth, although non-significant, can also be
232 observed in *S. Enteritidis* (152) (Fig. 1).

233 Colonies formed by persisters were tested again by broth microdilution, and no
234 change in the previous MIC values was observed. Additionally, taking into account the
235 resumption of growth, it was investigated hetero-resistance as a possible explanation for
236 the increase in the number of surviving cells. Therefore, the PAP assay was performed,
237 but a stable resistance pattern was not detected. In order to verify colistin integrity
238 during persistence assay, LC-MS/MS analysis was employed and it was not detected
239 degradation of colistin after 6, 24 and 48 h of exposure (Table S3).

240 The addition of organic acids at 2.5-fold the MIC in the culture provided an
241 immediate acidification (pH 4.0) that was maintained throughout the experimental
242 procedure. Exposure to organic acids resulted in a substantial reduction in the number
243 of surviving cells even in 6 h, and the population was undetectable in 48 h for four
244 isolates (*S. Infantis* was not detected in 24 h). However, around 10^2 cells from the *S.*
245 *Enteritidis* (152) survived even after 72 h of organic acid exposure (Fig. S2).

246 We investigated if a pre-exposure to colistin, organic acids or ciprofloxacin at
247 sub-MICs could induce an increase of persister levels before exposure to a lethal
248 concentration of ciprofloxacin. However, we found that pre-exposure to these
249 antimicrobials at sub-MICs during 30 min did not affect the persister fractions after
250 exposure to 100-fold the MIC of ciprofloxacin at any time point evaluated. Taking
251 together the results from all isolates, we found that regardless of the sub-MIC treatment
252 and isolate evaluated, there was no significant difference in the persister cell fractions
253 when compared to the cultures exposed only to 100-fold the MIC of ciprofloxacin ($p >$
254 0.05) (Fig. 2). The analysis of the results from each isolate showed that exposure to sub-
255 MIC of ciprofloxacin or colistin in the 192, 393, S79 and S02 isolates resulted in lower
256 population densities at some time points than those exposed to sub-MIC of organic
257 acids (data not shown). It was also possible to notice that multiple exposures to sub-
258 MIC of colistin followed by the treatment with 100-fold the MIC of ciprofloxacin did
259 not influence the persister levels obtained when compared to control ($p > 0.05$) (Fig. 3)

260

261 **4. Discussion**

262 *Salmonella* spp. are constantly faced with different stressful conditions, both in
263 the animal production environment and inside the host, where they need to survive the
264 presence of bile salts, gastrointestinal tract pH, high osmolarity, low oxygen tension and

265 macrophages intracellular environment. As a consequence, *S. enterica* presents various
266 regulatory networks in order to properly sense and respond to stress (Álvarez-Ordóñez
267 et al., 2012). In this study, we evaluated if levels of persister cells would be affected by
268 a prior exposure to sub-inhibitory concentrations of products used as feed additives in
269 poultry feed. In this sense, we firstly tested the ability of *S. enterica* isolates to form
270 persister cells against different concentrations of colistin (antimicrobial previously used
271 as feed additives in Brazil) (MAPA, 2018). Interestingly, *S. Agona* (S79) was unable to
272 form persisters at any concentration tested. Although the absence of this phenotype in
273 bacterial populations seems to be uncommon (Lewis, 2012), a similar finding was
274 previously reported by our research group in *A. baumannii* exposed to polymyxin B and
275 tobramycin (Barth et al., 2013), leading to speculation that mechanisms associated with
276 bacterial persistence could be absent or silenced. However, all other isolates formed
277 persisters at similar levels when exposed to clinically relevant lower concentrations (5
278 and 10 µg/ml), in agreement with Cui et al. (2016), who found no difference in persister
279 levels after exposure to these concentrations, but detected that, at higher doses, colistin
280 acts depending on its concentration. Among the characteristics of killing curves from
281 the *S. enterica* isolates exposed to colistin is that they significantly resumed their
282 growth, which is in disagreement with the behavior expected for the persisters (Lewis,
283 2012). A possible explanation for the apparent discrepancy would be the selection of a
284 hetero-resistant population. Thus, in order to evaluate this possibility, a PAP assay was
285 employed, and did not find a stable resistance pattern, since the MIC values obtained for
286 the isolates were the same after and before exposure to the drug. Another possible
287 interpretation for this finding would be antimicrobial degradation throughout the time of
288 exposure, but HPLC analysis showed that the levels of colistin remained at similar
289 levels during the experiment. However, it's also important to consider that a mechanism

290 of extrusion of the drug, such as efflux pumps, may be involved in this tolerance
291 accompanied by growth. Regulation of efflux pumps has already been associated with
292 persistence in *Escherichia coli*, but not implying in resumption of growth (Pu et al.,
293 2016).

294 Organic acids used as feed and/or water additives to reduce pathogens that can
295 contaminate broilers (Biggs and Parsons, 2008; Dittoe et al., 2018) could potentially
296 lead to a development of an acid tolerance response (ATR) in *S. enterica* (Ye et al.,
297 2019). Bacterial ATR can be an important concern, since this response involves genes
298 that may also participate in pathways common to virulence and protection against other
299 environmental challenges, such as oxidative stress, heat, osmolarity, and DNA damage
300 (Hu et al., 2018). In this study, although the cultures achieved a pH 4.0 after the
301 addition of organic acids, we couldn't detect a pattern to be assumed as ATR among
302 most of the isolates.

303 Taking into account that the pre-exposure to stressors could increase persister
304 levels (Wu et al., 2012; Johnson and Levin, 2013; Cui et al., 2018), we attempt to
305 investigate if organic acids, as well as colistin, would influence the persister fractions of
306 *S. enterica* isolates. Therefore, *S. enterica* cultures were treated with sub-inhibitory
307 concentrations of colistin or OA for 30 min before the addition of a lethal concentration
308 of ciprofloxacin. Conversely to the increase in persister levels described for exposure to
309 paraquat prior to fluoroquinolone (Wu et al., 2012), both antimicrobials were not able to
310 produce the same effect here. However, this effect should still be evaluated in the *S.*
311 *Enteritidis* (152) isolate, which presented higher tolerance to acid organics.
312 Furthermore, considering persistence as a non-multidrug-tolerant phenotype, where a
313 population can be formed by cells with distinct ability to survive different stressors
314 (Van den Bergh et al., 2017), we tested a prior exposure to a same stressor

315 (ciprofloxacin), but variation on persister levels were again not observed. These
316 findings may indicate a major role of stochastic origin for persisters instead as from
317 induction when a stressor is faced. In addition, prior and multiple exposures to
318 antimicrobials were employed to investigate the possible induction/selection of a
319 persistent mutant, as found in *E. coli* after intermittent ampicillin applications (Moyed
320 and Bertrand, 1983). These authors described a high persistent mutant (*hipA7*) able to
321 increase the level of persisters in 1,000-fold. Likewise, a highly persistent mutant was
322 also found in *S. Typhimurium*, imparting a 3- to 4-fold increase in survival after
323 ampicillin exposure, which was attributed to a nonsense mutation at the 3' end of the
324 *shpB* gene encoding an antitoxin from the TA module ShpAB (Slattery et al., 2013).
325 However, we did not detect a high persistent mutant (*hip*) in *S. enterica* isolates under
326 pre-exposure to colistin, organic acids or even ciprofloxacin.

327 In conclusion, the model described as persisters arising from a stochastic switch
328 in a microbial population seems to fit with our results, since we were not able to induce
329 an increase on persister fractions by pre-exposure to antimicrobials or even by multiple
330 exposures. Additionally, the results presented here suggest that antimicrobial tolerance
331 mediated by highly persistent mutants may not be selected by feed additives.

332

333 **Competing interests**

334 The authors declare that they have no competing interests.

335

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341

342 **Author contributions**

343 SD performed the experiments, analyzed the data and wrote the manuscript. CF

344 analyzed the data and revised the manuscript. PF performed the statistical analysis and

345 revised the manuscript. SO conceived and designed the experiments, analyzed the data

346 and wrote the manuscript.

347

348 **Appendix A. Supplementary data**

349 Supplementary material related to this article can be found, in the online version, at doi:

350

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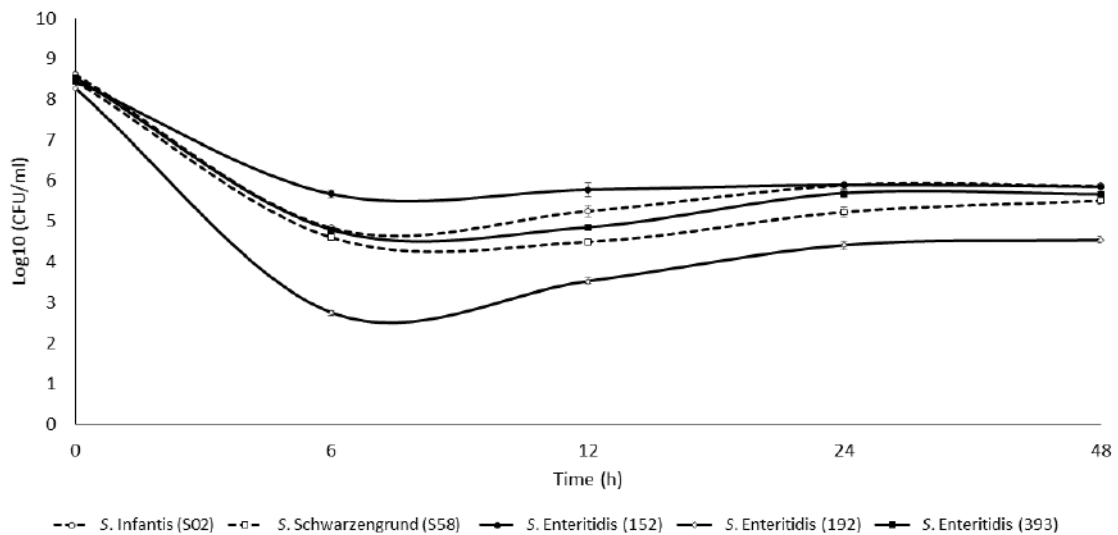
488 **Table 1**

489 Minimum concentration of colistin required to inhibit the growth of *Salmonella enterica*
 490 isolates from different serovars and origins.

Isolates (ID)	Origin	MIC^a (µg/ml)
<i>S. Agona</i> (S79)	Meat meal	2
<i>S. Enteritidis</i> (152)	Ready-to-eat-food	2
<i>S. Enteritidis</i> (192)	Poultry carcass	1
<i>S. Enteritidis</i> (393)	Food handler	1
<i>S. Infantis</i> (S02)	Meat meal	1
<i>S. Schwarzengrund</i> (S58)	Flesh and bones meal	1

491 ^aMIC: minimum inhibitory concentration.

492



493

494 **Fig. 1.** Killing curve of *Salmonella enterica* isolates after exposure to colistin for 48 h.

495 Cultures were grown until mid-log phase and exposed to 5-fold the MIC of colistin. At

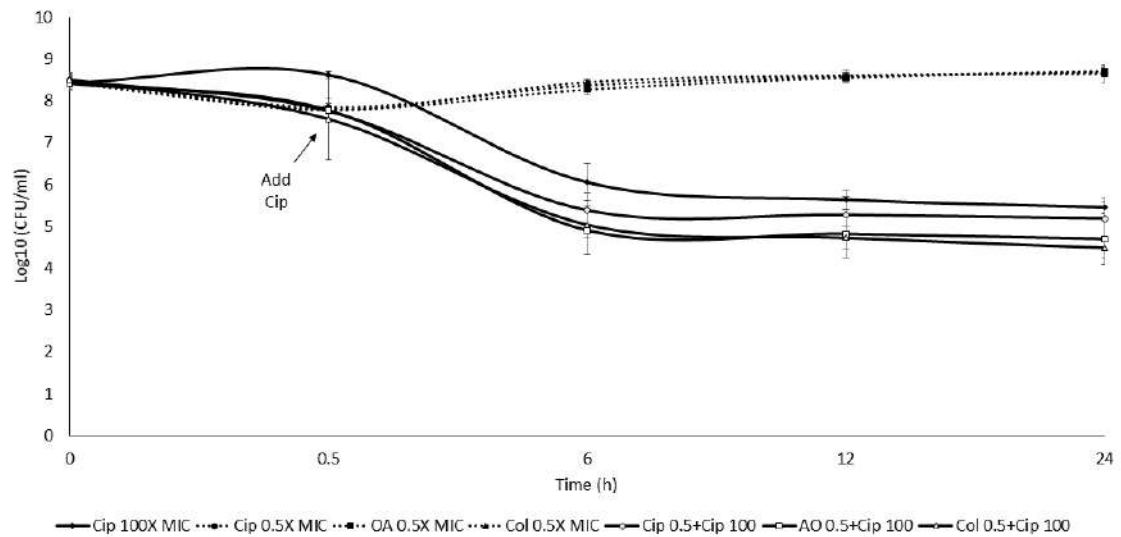
496 each time point, aliquots were removed to determine the surviving cell counts. Plotted

497 values are the mean of three biological replicates measurements and error bars represent

498 standard deviation (\pm SD).

499 CFU: Colony-forming unit.

500

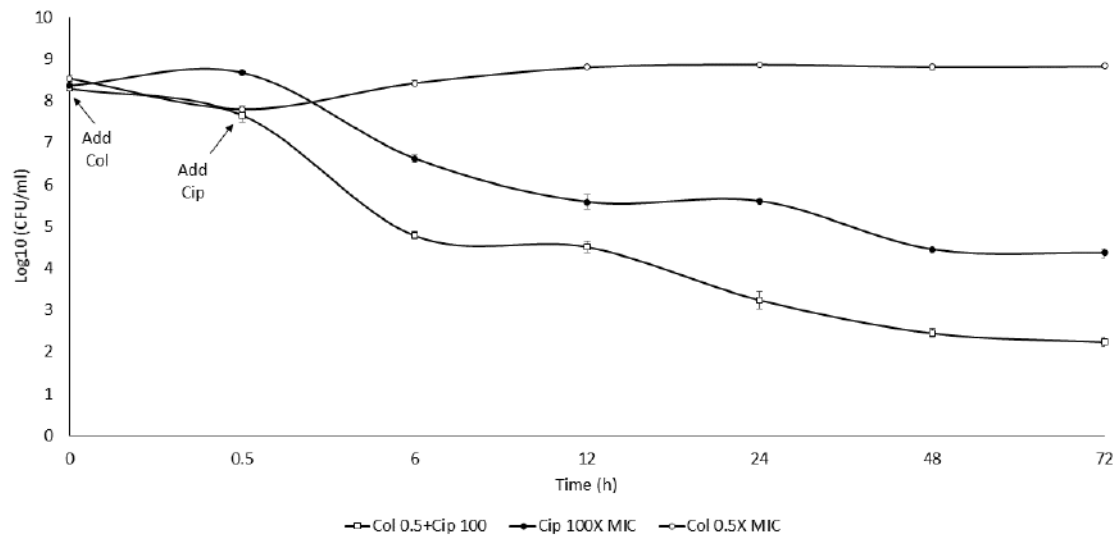


501

502 **Fig. 2.** Killing curves of *Salmonella enterica* isolates exposed to sub-MIC (0.5-fold the
 503 MIC) of colistin (Col), organic acids (OA) or ciprofloxacin (Cip) before exposure to
 504 100-fold the MIC of ciprofloxacin for 24 h. Arrow indicates the addition of 100-fold the
 505 MIC of ciprofloxacin. At each time point, aliquots were removed to determine the
 506 surviving cell counts. Plotted values are the mean of all isolates measurements and error
 507 bars represent standard deviation (\pm SD).

508 CFU: Colony-forming unit.

509



510

511 **Fig. 3.** Killing curve of *Salmonella enterica* isolates exposed four times to sub-MIC of
 512 colistin (Col) followed by addition of 100-fold the MIC of ciprofloxacin for 72 h (white
 513 squares). Cultures were grown until mid-log phase and exposed to 0.5-fold the MIC of
 514 Col during 30 min (four consecutive days) (the first three exposures to Col at sub-MIC
 515 are not represented in this figure). Thereafter, 100-fold the MIC of ciprofloxacin was
 516 added as indicated by the arrow. Two other curves represent exposure to only
 517 ciprofloxacin (100-fold the MIC) (black circle) or colistin (0.5-fold the MIC) (white
 518 circle). At each time point, aliquots were removed to determine the surviving cell
 519 counts. Plotted values are the mean of all isolates measurements and error bars represent
 520 standard deviation (\pm SD).

521 CFU: Colony-forming unit.

522

523 **Supplementary material**

524

525 **Supplementary Table 1.** Retention times (Rt) and parameters of mass spectrometry for

526 the target analytes.

Compound name	Formula	Rt (min)	FV (V)	CE (V)	Precursor ion (m/z)	Production ^a	
						Q1 (m/z)	Q2 (m/z)
Colistin B	C ₅₂ H ₉₈ N ₁₆ O ₁₃	4.14	100	10	386	101.0	374
Colistin A	C ₅₃ H ₁₀₀ N ₁₆ O ₁₃	3.98	100	10	391	101.1	385

527 ^aTwo transitions were used for multiple-reaction monitoring (MRM). The first one was used for

528 quantification, and the second one was used for confirmation. Rt, retention time; FV, fragmentor voltage;

529 CE, collision energy.

530

531 **Supplementary Table 2.** Persisters (colony-forming units-CFU) from *Salmonella*
 532 Enteritidis isolate (152) exposed to different colistin concentrations

Time of exposure (h)	Colistin ($\mu\text{g/ml}$)*		
	5	10	20
0 [#]	2.11E+08	2.22E+08	2.44E+08
1	3.33E+06 ^{a†}	4.67E+05 ^b	4.78E+05 ^b
2	5.67E+05 ^a	2.45E+05 ^a	2.44E+05 ^a
3	3.67E+05 ^a	2.11E+05 ^a	1.67E+05 ^a
4	3.11E+05 ^a	1.67E+05 ^a	5.22E+04 ^a
5	1.78E+05 ^a	6.11E+04 ^b	2.33E+04 ^b
6	1.56E+05 ^a	5.55E+04 ^b	1.66E+04 ^b

533 *Colistin concentrations of 5, 10 and 20 $\mu\text{g/ml}$ correspond to 2.5, 5 and 10-fold the MIC for the isolate,
 534 respectively.

535 [#] CFU values before colistin exposure.

536 [†]Different superscript lowercase letters in the same line indicate significant difference ($p < 0.05$).

537

538 **Supplementary Table 3.** Concentration of colistin A and colistin B throughout the
 539 persister cell assay evaluated by liquid chromatography coupled to mass spectrometry in
 540 tandem.

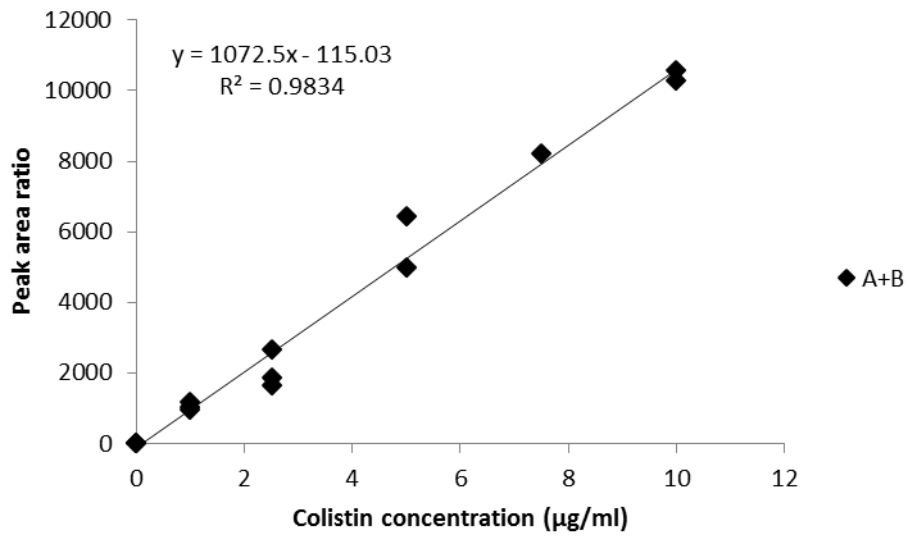
Sample ID	A†	B†	A+B†	Colistin(µg/ml)*
Withe	0	0	0	0
T0a	899	3939	4838	4.61820979
T0b	1033	3647	4680	4.470890443
T0c	948	3728	4676	4.467160839
T6a	1066	3887	4953	4.725435897
T6b	1960	3943	5903	5.611216783
T6c	1038	3841	4879	4.656438228
T24a	1021	3114	4135	3.962731935
T24b	1278	3642	4920	4.694666667
T24c	930	3422	4352	4.165062937
T48a	1009	3404	4413	4.221939394
T48b	1274	3615	4889	4.665762238
T48c	966	3613	4579	4.376717949

541 † Area ratio of colistin compounds

542 * Concentration found after addition of 5 µg/ml of colistin.

543 Different letters on sample ID represent biological replicates.

544

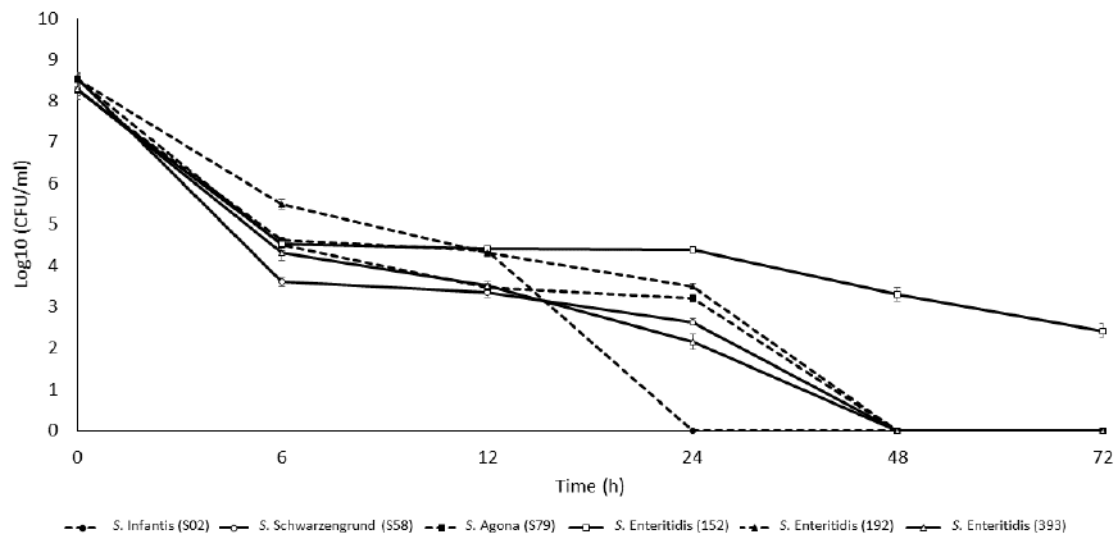


545

546 **Supplementary Fig. 1.** HPLC calibration curve of analytes using the peak area ratio of

547 colistin A and colistin B for internal standard.

548



549

550 **Supplementary Figure 2.** Killing curve of *Salmonella enterica* isolates after exposure
 551 to organic acids for 72 h. Cultures were grown until mid-log phase and exposed to 2.5-
 552 fold the MIC of organic acids. At each time point, aliquots were removed to determine
 553 the survival cell counts. Plotted values are the mean of three biological replicates
 554 measurements and error bars represent standard deviation (\pm SD).

555 CFU: Colony-forming unit.

556

Capítulo 4

Resultados Preliminares

Análise do transcrito (RNA-seq) de isolados *Salmonella enterica* formadores de diferentes frações de células *persisters* expostas à ciprofloxacina ou à ceftazidima

Resultados preliminares que irão compor artigos a serem submetidos a periódicos científicos.

Análise do transcritoma (RNA-seq) de isolados *Salmonella enterica* formadores de diferentes frações de células *persisters* expostas à ciprofloxacina ou à ceftazidima

Introdução

Os diferentes sorovares de *Salmonella enterica* não tifoide são responsáveis por casos de gastroenterite, normalmente autolimitante, associada com o consumo de alimentos contaminados de origem animal (1). A *S. enterica* é considerada um patógeno intracelular facultativo e, apresenta a habilidade para sobreviver no interior de células fagocitárias. A capacidade de adaptação para persistir em ambientes inóspitos é compatível com a formação de um fenótipo altamente tolerante a antibióticos devido à presença de células *persisters*, que são descritas como responsáveis pela recalcitrância de infecções (2).

Células *persisters* são variantes fenotípicas transitórias originadas a partir de uma população isogênica e geneticamente suscetível a antimicrobianos, com a capacidade de tolerar concentrações letais de diferentes estressores, incluindo antibióticos bactericidas, sem transmitir sua tolerância à progênie (3-6). Essas células apresentam um metabolismo reduzido devido ao baixo ou não-crescimento e podem ser formadas estocasticamente dentro de uma população bacteriana (7) ou induzidas por situações que resultem em um estresse celular (2,5,8,9).

Diversos mecanismos foram propostos com o intuito de explicar a formação do fenótipo de persistência, tais como: alterações nas vias relacionadas com a redução do metabolismo microbiano, sistema toxina-antitoxina (TA), produção de adenosina trifosfato (ATP), síntese e degradação proteica, reparo e proteção do DNA (resposta SOS), sinalização celular QS e atividade de efluxo (2,8,-18). No entanto, a forma como esses mecanismos moleculares operam na formação e manutenção das *persisters* ainda

não foi completamente elucidada. Este contexto, somado à importância da *S. enterica* na cadeia produtiva de alimentos de origem animal, enfatiza a necessidade de identificar a constituição fisiológica e metabólica de células *persisters* de diferentes sorovares de *S. enterica*. Assim, neste trabalho buscou-se identificar genes diferencialmente expressos em células *persisters* de diferentes sorovares de *S. enterica* em cultivo planctônico expostos à ciprofloxacina ou à ceftazidima por meio do sequenciamento de alto desempenho do DNA complementar (dsDNA, Illumina RNA-seq).

Material e Métodos

Isolados bacterianos

Dois isolados de *S. enterica* sorovar Enteritidis provenientes de fezes de suínos (785-4SA) e de carcaça de frango (182-192), e um isolado de *S. Schwarzengrund* (796-S58) oriunda de farinha de carne e ossos foram selecionados a partir de ensaios anteriores, baseando-se na capacidade de formar diferentes frações de células *persisters* quando expostas à ciprofloxacina ou ceftazidima em cultivo planctônico. Os isolados foram mantidos a -80 °C em meio Luria-Bertani (LB) (Sigma-Aldrich, Saint Louis, USA) com adição de DMSO a 20%. As análises genômicas e transcritômicas empregando esses isolados foram realizadas durante o período de doutorado sanduíche no, Laboratório de Investigação e Diagnóstico de Doenças Animais (ADRDL) no Departamento de Veterinária e Ciências Biomédicas da Universidade do Estado da Dakota do Sul (SDSU), coordenado pelo prof. Dr. Joy Scaria e, financiado pela CAPES pelo período de seis meses.

Isolamento do DNA genômico e sequenciamento pela plataforma Illumina

Os isolados de *S. enterica* foram cultivados *overnight* a 37 °C em LB. Alíquotas de 1 mL foram removidas para isolamento do DNA genômico usando o *kit* Qiagen DNeasy (Qiagen, Valencia, CA, EUA) de acordo com o protocolo estabelecido pelo fabricante, seguindo eluição em 50 µL de água livre de nucleases. A qualidade do DNA foi analisada utilizando NanoDrop™ One (Thermo Scientific™, DE), quantificada com o fluorímetro Qubit® 3.0 (Thermo Fisher Scientific Inc., MA), sendo, subsequentemente, armazenados a -20 °C. O sequenciamento do genoma completo foi realizado utilizando a plataforma Illumina Miseq V2 com 2x250 *paired-end*. A concentração do DNA genômico foi ajustada para 0,3 ng/µL para a preparação das bibliotecas com Nextera XT DNA *Sample Prep kit* (Illumina Inc., San Diego, CA). As bibliotecas foram normalizadas, reunidas em volume único, desnaturadas e o sequenciamento prosseguiu usando o reagente Miseq versão 2 (Illumina, Inc.).

Montagem e anotação do genoma

Os arquivos de dados brutos com os genomas dos isolados de *S. Enteritidis* (192 e 4SA) e *S. Schwarzengrund* (S58) foram convertidos em arquivos FASTQ usando o Casava v.1.8.2. (Illumina, Inc.) e montados usando o método *de novo* com o *software* CLC Genomics workbench 9.4 (Qiagen Bioinformatics, CA) (Tabela – 01). A anotação dos genomas de cada isolado foi realizada usando o *software* Prokka que, posteriormente, foi empregada como referência para mapear as leituras obtidas no RNA-seq.

Tabela 01. Parâmetros obtidos no sequenciamento e montagem do genoma dos isolados de *Salmonella enterica*.

Isolado	Número de <i>contigs</i>	Tamanho do genoma (pb)
<i>S. Enteritidis</i> (192)	68	4.711.380
<i>S. Enteritidis</i> (4SA)	117	4.770.518
<i>S. Schwarzengrund</i> (S58)	106	4.616.765

Ensaio de persistência, extração de RNA de células persisters e enriquecimento do RNAm

Para a análise do transcrito das células *persisters* de *S. enterica*, os isolados foram cultivados *overnight* a 37 °C. As culturas foram diluídas na proporção 1:30 em meio LB, incubadas por 2 h e 30 min a 37 °C, o que correspondeu ao meio da fase exponencial, e expostas a 100 X o valor da concentração inibitória mínima (CIM) para ciprofloxacina e ceftazidima, separadamente. Nos tempos de 0, 6 e 48 h após a exposição aos fármacos, alíquotas de 5 mL foram removidas e imediatamente tratadas por 5 min sob refrigeração com RNAlater (Invitrogen, CA, USA) mantido a 4 – 8 °C. As amostras foram centrifugadas a 10.000 rpm durante 5 min a 4 °C e o *pellet* obtido foi lavado uma vez com solução tamponada de salina-fosfato (PBS) mantida a 4 – 8 °C. As células remanescentes foram lisadas com lisozima (Qiagen) (0,5 mg/mL) e dodecil sulfato de sódio (SDS a 10%) refrigerados (4 – 8 °C) com subsequente incubação por 10 min em temperatura ambiente. Na sequência, foi adicionado UltraPure™ fenol:clorofórmio:álcool isoamílico (PCA) (25:24:1) (Invitrogen, Cat 15593031) mantido a 4 – 8 °C, seguido por homogeneização por 1 min e incubação por 10 min a 65 °C (homogeneização a cada minuto). Após esse período, as amostras foram imediatamente incubadas em gelo por 5 min e centrifugadas a 14.000 rpm por 10 min a 4 °C. A fase aquosa foi cuidadosamente transferida para um novo tubo, adicionando-se 400 µL de clorofórmio mantido a 4 – 8 °C, o que foi homogeneizado e novamente centrifugado a

14.000 rpm por 10 min a 4 °C (essa etapa foi repetida duas vezes). Subsequentemente, foi adicionado 10% do volume total obtido da fase aquosa de acetado de sódio (NaOAc) a 3 M e adicionado de etanol absoluto em quantidade suficiente para completar 1 mL. Os tubos foram cuidadosamente homogeneizados e centrifugados a 14.000 rpm durante 10 min a 4 °C. O sobrenadante foi removido e o *pellet* foi lavado duas vezes com etanol a 70% e seco em temperatura ambiente durante 5 min. O RNA total obtido foi eluído em 50 µL de água livre de RNase e armazenados a -80 °C. Em cada um dos tempos designados, três replicatas biológicas, cada uma constituída de três replicatas técnicas, foram utilizadas para a extração do RNA.

A concentração total do RNA foi medida utilizando NanoDrop™ One (Thermo Scientific™, DE) e a integridade do mesmo foi verificada por meio de eletroforese em gel de agarose com formaldeído, mediante observação de bandas intactas correspondentes aos RNAr 16S e 23S. Na sequência, o RNA total (5 µg) foi submetido à depleção do RNAr empregando o *kit* RiboZero™ (Epicenter), que contém esferas magnéticas específicas para a depleção do RNAr e enriquecimento do RNAm em bactérias Gram-negativas. O RNA obtido foi purificado com RNeasy Power Clean™ (Qiagen) e armazenado a -80 °C.

Preparação da biblioteca do DNAs e sequenciamento do transcrito com Illumina

O RNAm obtido após a depleção do RNAr foi usado como molde para a confecção da primeira fita do DNAc empregando *primers* randômicos ancorados ao dT (dT₂₃VN) (S1330S) (BioLabs, New England) associado a uma transcriptase reversa recombinante (M-MuLV) usando ProtoScript® II Reverse Transcriptase (M0368L) (BioLabs, New England), de acordo com as especificações do fabricante. Na sequência, a segunda fita do DNA (DNAs) foi sintetizada usando NEBNext® Ultra™ II *Non-directional RNA*

second strand synthesis module (E6111L) (BioLabs), de acordo com as especificações do fabricante. O DNAs obtido foi purificado empregando esferas magnéticas Agencourt AMPure XP (Illumina, Inc.) e armazenado a -20 °C. Posteriormente, 0,3 ng/μL do DNAs foi tagmentado (fragmentação enzimática com transposase e ligação de adaptadores ao final de cada sequência) usando o *kit* Nextera XT DNA™ Library Prep Kit (Illumina, Inc.). Subsequentemente, as bibliotecas foram montadas usando os indexadores i7 e i5, seguindo-se uma PCR de 12 ciclos de amplificação para o enriquecimento e ligação dos indexadores aos adaptadores no final dos fragmentos obtidos, gerando amplicons com \geq 300 pb. Uma etapa de purificação foi realizada com esferas magnéticas Agencourt AMPure XP (Illumina, Inc.) para a remoção de fragmentos pequenos. A normalização e quantificação das bibliotecas foi realizada de acordo com o protocolo estabelecido pela Illumina MiSeq® (Illumina, Inc.). A qualidade das bibliotecas obtidas foi medida e ajustada para 0,3 ng/μL de DNAs em cada amostra com Qubit® 3.0 (Thermo Fisher Scientific Inc.). Para o sequenciamento, todas as amostras foram reunidas em um volume único, desnaturadas e adicionada em uma *flow cell* com reagentes específicos para o Miseq versão 2 com 2×250 *paired-end* (Illumina, Inc.).

RNA-seq e análise dos dados

A análise de expressão gênica diferencial (DEG) entre os tempos e tratamentos foi determinada usando o *software* CLC, juntamente com a normalização das leituras *Reads per Kilobase per Million* (RPKM), seguido da transformação em \log_2 . A análise da DEG foi realizada empregando o ajuste do valor de *p* pela aplicação da *False Discovery Rate* (FDR) e relação de proximidade entre os diferentes grupos de genes expressos, obtidos nos diferentes isolados e tempos de exposição aos fármacos foi avaliada pela Análise de Componentes Principais (PCA). A ontologia gênica (GO) foi

realizada usando a base de dados *Kyoto encyclopedia of genes and genomes* (KEGG pathway) e a complementação com a função dos genes foi realizada empregando a base de dados *on-line* Uniprot. Os *heatmaps* foram gerados com o programa R, empregando o pacote *heatmap.2*, com o método de agrupamento padrão (distância euclidiana).

Coloração Live/Dead

Para avaliar a presença de células mortas nas culturas de *S. enterica* após os tratamentos com ciprofloxacina e ceftazidima (0, 6 e 48 h) como possíveis contaminantes para análise do transcrito, foi retirada uma alíquota de 1 mL do ensaio de persistência (descrito acima), a qual foi centrifugada a 8.000 rpm por 5 min. O *pellet* obtido foi lavado uma vez com solução salina a 0,85% para a remoção de restos celulares e ressuspensão em 1 mL de solução salina a 0,85%. Para a coloração diferencial, foi empregado o *kit* de marcação de viabilidade bacteriana LIVE/DEAD® *BacLight*TM (*Bacterial Viability Kit*; Life Technologies, Carlsbad, CA, USA) na concentração de 1,5 µM de SYTO 9 e 1,5 µM de iodeto de propídio em cada amostra e incubada por 15 min no escuro. As amostras foram examinadas sob um microscópio Olympus BX51 com filtro WBI, excitação a 460-495 nm e emissão a 510 nm. Foi estabelecida a contagem das células em 10 campos aleatórios, sendo que bactérias vivas foram coradas pelo SYTO 9 com emissão de fluorescência verde e bactérias mortas (ou com membrana danificada) foram coradas pelo iodeto de propídio com emissão de fluorescência vermelha.

Resultados Preliminares

As imagens de microscopia de fluorescência permitiram a observação de que as alíquotas removidas nos tempos em que a análise transcritômica foi realizada eram compostas por células vivas sobreviventes à exposição aos fármacos (Figura – 01). Após

a análise dos diferentes campos de imagem, um número muito pequeno ou até mesmo a ausência de células mortas ou com a membrana danificada pode ser visto (Figura – 01 B, D, F, H, J) quando comparado com a presença de células vivas (Figura – 01 A, C, E, G, I) após 6 e 48 h de exposição à ciprofloxacina ou ceftazidima.

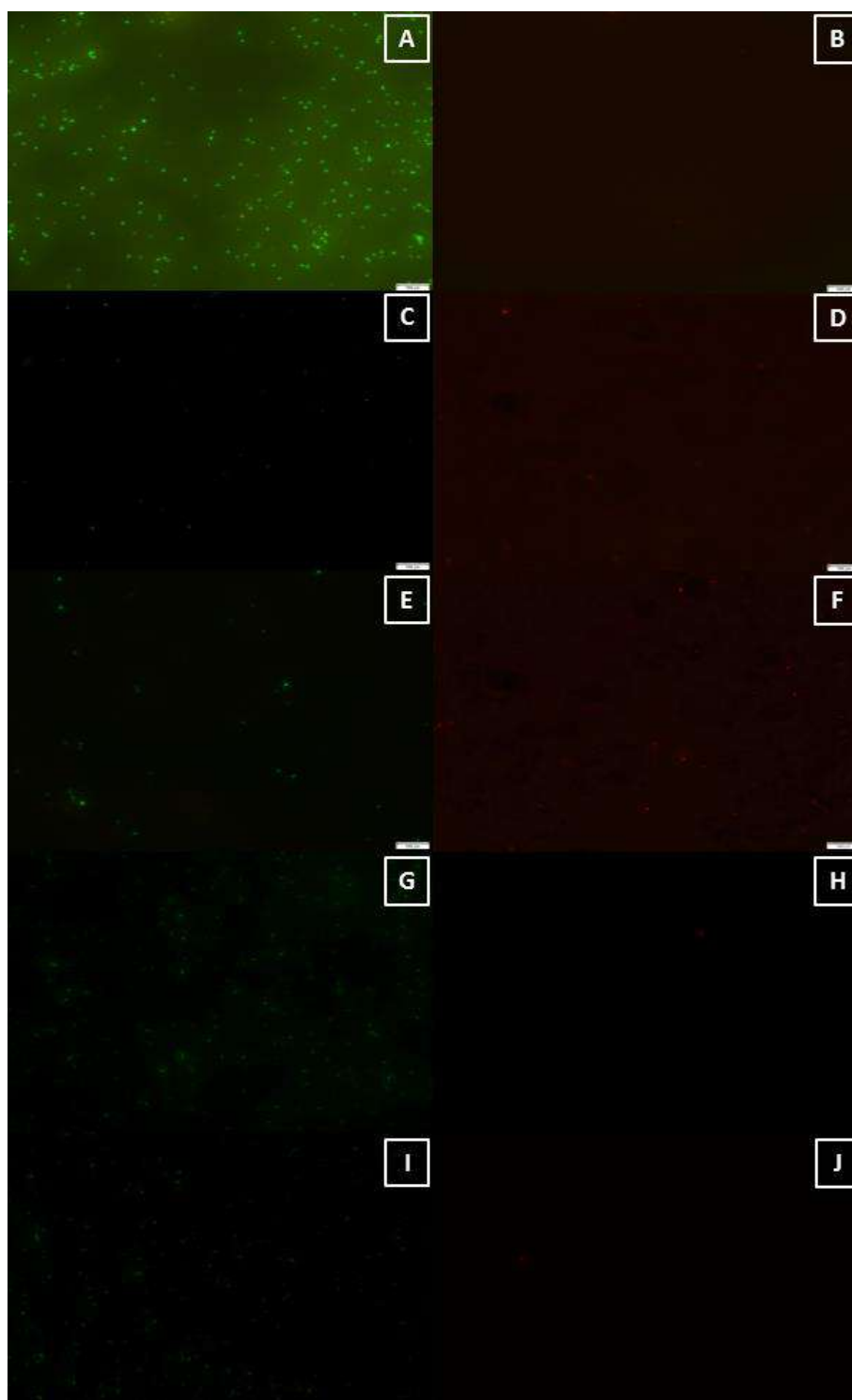


Figura – 01. Microscopia de imunofluorescência empregando o *kit* Live/Dead® Baclight™ (*Bacterial Viability Kit*; Life Technologies) em cultura de *Salmonella* Enteritidis (4SA) no meio de fase exponencial do isolado antes da exposição aos fármacos (A-B), 6 h (C-D) e 48 h (E-F) após a exposição a 100X o valor da concentração inibitória mínima (CIM) para ceftazidima e, 6 h (G-H) e 48 h (I-J) após a exposição a 100X o valor da CIM para ciprofloxacina. Bactérias coradas em vermelho pelo iodeto de propídio estão mortas (ou com membrana danificada) (B, D, F, H, J), bactérias vivas foram coradas pelo SYTO 9 com emissão de fluorescência verde (A, C, E, G, I).

A variação encontrada no conjunto de dados relativos à expressão gênica foi avaliada pela análise dos componentes principais (PCA) em cada isolado nos diferentes tempos (0, 6 e 48 h) para cada um dos fármacos (Figura – 02). A análise de PCA permitiu observar que o padrão de expressão agrupou de forma homogênea as replicatas biológicas dos experimentos, com exceção de uma replicata do isolado de *S. Schwarzengrund* tratada por 6 h com ciprofloxacina. Analisando o componente principal (PC1), observou-se que o tratamento com ceftazidima mostrou um padrão transcricional mais definido na comparação entre antes (0 h) e após a exposição (6 h e 48 h) do que o encontrado para o tratamento com ciprofloxacina, especialmente nos isolados de *S. Enteritidis* (Figura – 02A, 02C e 02E). Desta forma, pode-se observar uma variação evidente no padrão transcricional das células *persisters* avaliadas após a exposição à ceftazidima, quando comparadas às células não expostas ao tratamento. Por outro lado, a exposição à ciprofloxacina resultou em uma variação no padrão transcricional ao longo do tempo, considerando as diferenças encontradas entre 6 h e 48 h (Figura – 02B, 02D e 02F).

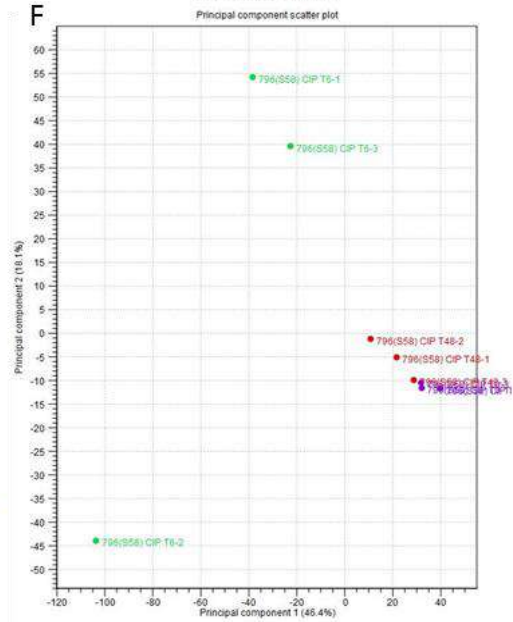
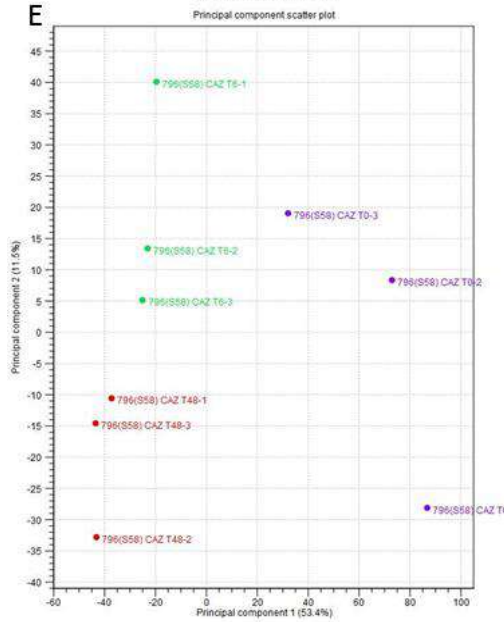
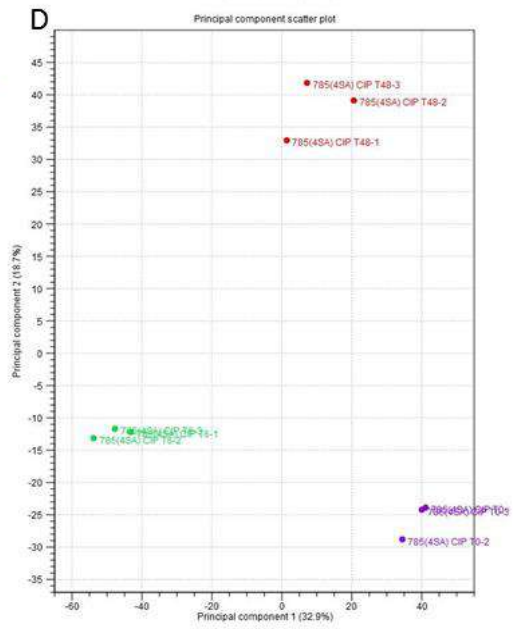
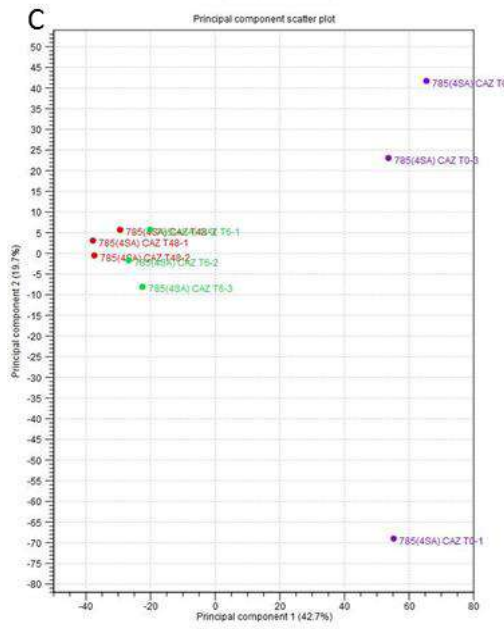
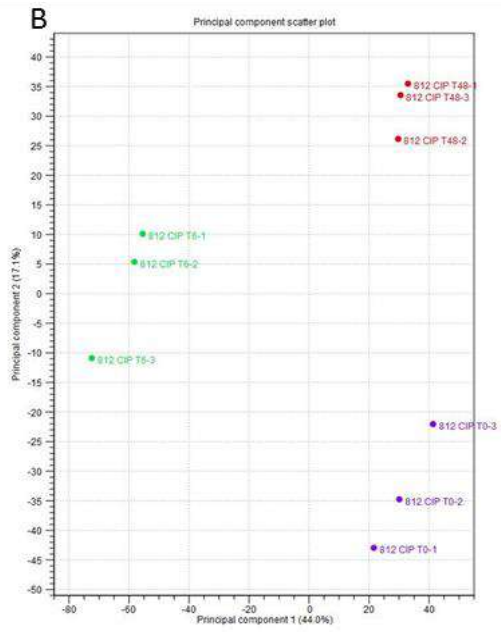
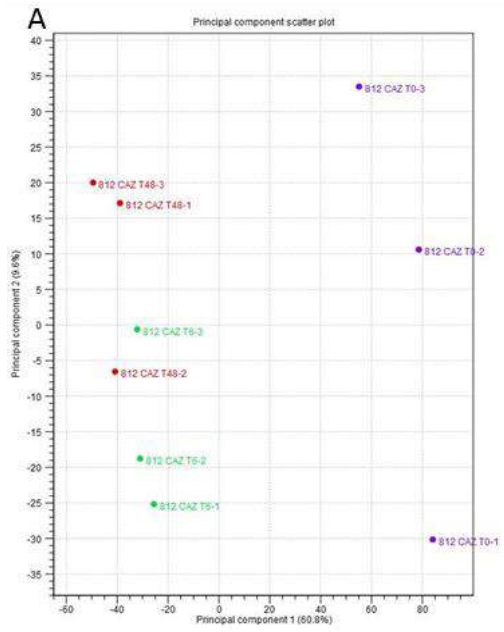


Figura – 02. Agrupamento das amostras não tratadas com antibiótico e das tratadas com ceftazidima (A, C e E) ou ciprofloxacina (B, D e F) baseado nos dados de transcriptoma pela análise dos componentes principais (PCA) nos isolados *S. Enteritidis* (192) (A e B), *S. Enteritidis* (4SA) (C e D) e *S. Schwarzengrund* (S58) (E e F). Replicatas biológicas são representadas pela mesma cor, sendo a cor roxa para amostras não expostas aos antimicrobianos, verde para exposição por 6 h e vermelho para exposição por 48 h. A análise por PCA foi realizada usando o *software* CLC Genomics Workbench 9.4 (CLC Bio).

Apesar dos recentes estudos acerca do tema, ainda se sabe muito pouco sobre os mecanismos de formação e regulação envolvidos no fenótipo de persistência. Diante disso, este trabalho procurou avaliar o diferencial de expressão gênica ($q \leq 0,05$), que nos mostrou, de uma forma geral, que a maioria dos genes avaliados nas células *persisters* expostas à ciprofloxacina ou ceftazidima apresentou níveis de expressão mais baixos quando comparados àqueles de células não expostas aos antimicrobianos (Tabela – 02). No entanto, comparando os diferentes tempos de exposições e fármacos, pode-se observar que, independentemente do isolado, o maior nível de expressão diferencial foi encontrado 6 h após a exposição à ciprofloxacina (Tabela – 02, Figura – 03 e Anexo – 01). É importante ressaltar que as análises dos transcritos obtidos foram conduzidas apenas com os genes que se apresentaram, no mínimo, com expressão duas vezes maior ou menor quando comparados ao padrão encontrado antes da exposição aos fármacos.

Tabela 02. Número de genes diferencialmente expressos nos isolados de *Salmonella enterica* após 6 e 48 h de exposição à ciprofloxacina (CIP) ou à ceftazidima (CAZ).

Tratamento	Nível de expressão (<i>fold change</i>)	<i>S. Enteritidis</i> (192)	<i>S. Enteritidis</i> (4SA)	<i>S. Schwarzengrund</i> (S58)
T6 - CIP	≥ 2	988	567	797
	≥ 4	422	198	356
	≤ 2	649	684	736
	≤ 4	92	163	110
T48 - CIP	≥ 2	350	326	348
	≥ 4	138	86	92
	≤ 2	305	376	188
	≤ 4	63	100	39
T6 - CAZ	≥ 2	108	110	79
	≥ 4	13	14	27
	≤ 2	747	245	370
	≤ 4	254	42	103
T48 - CAZ	≥ 2	159	96	45
	≥ 4	10	5	40
	≤ 2	829	349	4
	≤ 4	321	109	0

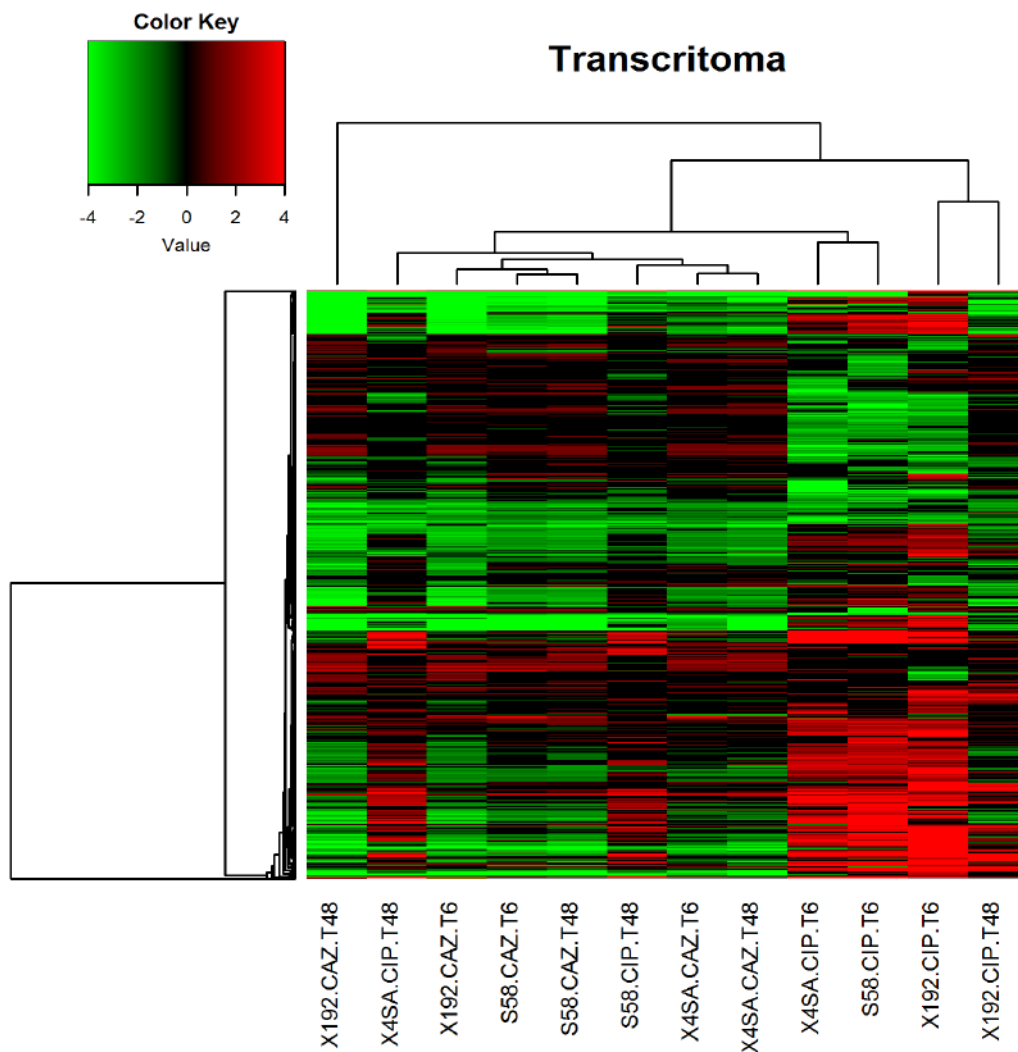


Figura – 03. *Heat map* de todos os 1.519 genes diferencialmente expressos nos isolados de *S. enterica* (*S. Enteritidis* -192 e 4SA- e *S. Schwarzengrund* -S58) expostos a 100X o valor da CIM de ciprofloxacina (CIP) ou ceftazidima (CAZ) por 6 e 48 h (T6 e T48). Os padrões de expressão gênica de cada amostra são agrupados pelo dendrograma apresentado acima, e os genes diferencialmente expressos são agrupados pelo dendrograma apresentado na lateral esquerda. O código de cores mostrado na legenda indica as leituras normalizadas transformadas em \log_2 . Níveis de expressão maior ou menor são indicados pelas cores vermelho e verde, respectivamente. O mapa e os dendrogramas foram construídos na plataforma R com o pacote *heatmap.2*, com o método de agrupamento padrão (distância euclidiana).

Empregando o banco de dados KEGG, foram identificadas as funções moleculares dos genes transcritos, bem como os processos biológicos nos quais estão implicados (Anexo – 01). Dessa forma, foi observado que um elevado número de genes codificadores para proteínas ribossomais e aqueles envolvidos nos processos de transcrição e tradução *down-regulated*, especialmente quando os diferentes isolados de *S. enterica* foram expostos à ceftazidima. Entretanto, especialmente após 6 h de exposição à ciprofloxacina foi detectado um padrão mais heterogêneo, com vários genes *up-regulated* (Anexo – 01). Em uma visão mais global, podem ser destacados alguns genes que tiveram sua expressão *down-regulated* em todos os isolados independentemente do fármaco utilizado, tais como: *era* (regulação do ciclo celular, metabolismo energético, bloqueio do início da tradução e redução nos seus níveis de expressão leva à interrupção temporária do crescimento celular), *pheS* (pertence à família aminoacil-RNAt sintetase de classe II), *pheT* (pertence à família de subunidades beta da fenilalanil-RNAt sintetase), *rpoC* (transcrição), *rpsQ* (liga-se à extremidade 5' do RNAr durante a tradução) e *trmH* (metilação 2'-O da guanosina na posição 18 em RNAt). Por outro lado, não foi possível identificar genes relacionados a esses processos biológicos que estivessem *up-regulated* em todos os isolados independentemente do fármaco. Após a exposição à ciprofloxacina, em todos os isolados, foram observados níveis maiores de expressão nos seguintes genes: *fmt* (acopla um grupo formil ao grupo amino livre de metionil-RNAt (fMet), desempenhando o seu reconhecimento por IF2), *greA* (necessária para o eficiente alongamento da transcrição pela RNA polimerase), *miaA* (catalisa a transferência de um grupo dimetilalilo para a adenina na posição 37 do RNAt, a qual lê códons que começam com uridina, levando à formação de N6- (dimetilalil) adenosina), *queG* (catalisa a conversão de epoxiqueuosine em queuosine, que é uma base hipermodificada de RNAt (Asp, Asn, His e Tyr), *raiA* (inibe o alongamento da tradução, bloqueando o sítio A),

rapA (ativa a transcrição em condições de estresse), *rlmE* (metila a uridina na posição 2552 do RNAr 23S), *rpoH* (fator sigma envolvido na regulação da expressão de genes de choque térmico, incluindo transcrição de reguladores globais e genes envolvidos na manutenção da funcionalidade da membrana e homeostase), *trmJ* (catalisa a formação de citidina 2'O-metilada (Cm32) ou uridina 2'O-metilada (Um32) na posição 32 do RNAt), *tsaA* (formação de pseudouridina nas posições 38, 39 e 40 no tronco do anticódon e alça de RNAt) e *yhbY* (montagem do ribossomo).

A maioria dos reguladores transcricionais encontraram-se *up-regulated*, especialmente quando os isolados foram expostos à ciprofloxacina (Anexo – 01), tais como: *fabR* (reprime a transcrição de *fabA* e *fabB*, envolvidos na biossíntese de ácidos graxos insaturados), *iscR* (regula a transcrição de vários operons e genes envolvidos na biogênese de Fe-S), *nhaR* (regula de forma positiva *nhaA* Na^+/H^+), *phoB* (regula de forma positiva o operon PhoBR quando o fosfato é limitado), *ygaV* (repressor de transcrição) e *yqjI* (reprime a expressão de YqjH que está envolvida na homeostase do ferro sob excesso de níquel). Por outro lado, os genes *cspA* (estimula a transcrição dos promotores de indução por choque térmico) e *dauR* (reprime o operon *dauBAR* relacionado como um pré-requisito para a utilização da D-arginina como única fonte de carbono e nitrogênio através de vias catabólicas de L-arginina) foram observados *down-regulated* em todos os isolados expostos à ceftazidima. Contudo, o gene *pspC* (desempenha um papel na competição pela sobrevivência em condições limitadas de nutrientes ou energia) foi observado *up-regulated* em praticamente todos os tempos de exposição a ambos os fármacos.

De modo geral, a maioria dos genes que coordenam os processos de replicação, recombinação homóloga e reparo do DNA nos isolados de *S. enterica* apresentaram-se *up-regulated*, especialmente 6 h após a exposição à ciprofloxacina e *down-regulated* após

a exposição à ceftazidima (Anexo – 01). Após exposição à ceftazidima, níveis menores de expressão foram observados nos seguintes genes: *dnaE* (DNA polimerase III responsável pela maior parte da replicação, também exibe uma atividade de exonuclease de 3'a 5'), *dnaX* (parte do complexo do grampo necessário para a pré-iniciação da replicação do DNA), *hupA* (proteína de ligação ao DNA semelhante à histona, que é capaz de envolver o DNA para estabilizá-lo e, assim, evitar sua desnaturação sob condições ambientais extremas), *priB* (liga o DNA de fita simples no local de montagem do primossomo), *recR* (envolvido em um processo de recombinação independente de RecBC no reparo de DNA) e *rnhB* (endonuclease que degrada especificamente o RNA de híbridos de RNA-DNA). Contudo, genes como *dnaA* (desempenha um papel fundamental na iniciação e regulação da replicação cromossômica), *gyrA* e *gyrB* (topoisomerases tipo II que regulam os níveis de supertorção negativa do DNA), *recA* (necessário para recombinação homóloga de danos ao DNA pela resposta SOS), *recN* (envolvido no reparo do DNA danificado), *recX* (modula a atividade da RecA e tem um papel regulador durante a resposta do SOS) e *uvrB* (o sistema de reparo UvrABC catalisa o reconhecimento e processamento de danos no DNA) encontraram-se *up-regulated* quando os isolados foram expostos à ciprofloxacina.

Genes que codificam proteínas necessárias para o processo de divisão celular foram observados *down-regulated*, principalmente após a exposição à ceftazidima (Anexo – 01). Os genes como *ftsW* (polimerização do peptidoglicano essencial para a divisão celular) e *murG* (organização da parede celular durante a divisão celular) foram observados *down-regulated* em todos os isolados, independentemente do fármaco. Além disso, destacam-se os genes *cpoB* (media a coordenação da síntese de peptidoglicano e a constrição da membrana externa durante a divisão celular), *damX* (liga peptidoglicanos nos septos e é necessário para direcionar a DamX para o anel do septo de divisão), *ftsB*

(essencial para a divisão celular), *ftsK* (proteína essencial da divisão celular que coordena o processo de divisão e a segregação cromossômica), *ftsQ* (controla a montagem correta do divisomo), *mreB* (forma filamentos associados à membrana que são essenciais para a forma celular, atua através da regulação da síntese da parede, alongamento e forma celular), *mreC* (formação e manutenção da forma da célula, contribui para a regulação da formação de proteínas de ligação à penicilina) e *rseP* (protease intramembrana) por se apresentarem *down-regulated* em todos os isolados quando expostos à ceftazidima. O gene *sulA*, que atua no sistema SOS, bem como inibe a divisão celular levando a uma parada rápida da divisão celular e ao aparecimento de filamentos longos não-septados), foi observado *up-regulated* quando os isolados foram expostos à ciprofloxacina.

A maioria dos transcritos associados à glicólise, gliconeogênese, ciclo do ácido tricarboxílico e fosforilação oxidativa foram observados *up-regulated* frente a exposição à ciprofloxacina, especialmente após 6 h, e *down-regulated* quando os isolados foram expostos à ceftazidima (Anexo – 01). Entretanto, alguns genes tiveram sua expressão *down-regulated* em todos os isolados independentemente do fármaco, tais como: *aceF* (componente do complexo piruvato desidrogenase (PDH), que catalisa a conversão global do piruvato em acetil-CoA e CO₂), *atpD* (produz ATP a partir de ADP na presença de um gradiente de prótons através da membrana), *nuoH*, *nuoI_1*, *nuoJ*, *nuoK* e, *nuoL* (NDH-1 transporta elétrons do NADH, via centros de ferro-enxofre (Fe-S), para quinonas na cadeia respiratória). Além disso, é possível observar expressão diferencial de genes envolvidos na biossíntese do folato, apenas quando os isolados foram expostos à ciprofloxacina, sendo esses *up-regulated* em sua maioria.

De modo geral, a maioria dos genes associados a sideróforos e metabolismo do ferro e enxofre em *S. enterica* não apresentaram uma expressão diferencial significativa quando os isolados foram expostos à ceftazidima ou à ciprofloxacina (Anexo – 01).

Entretanto, foram observados *up-regulated* nos isolados de *S. enterica* expostos à ciprofloxacina os genes *fdnH_2* (unidade de transferência de elétrons contendo 4 grupos de Fe-S, que serve como um canal para os elétrons que são transferidos a partir da oxidação do formato), *fdoH* (permite o uso de formato como principal doador de elétrons durante a respiração aeróbica), *fdx* (proteínas Fe-S que transferem elétrons em uma ampla variedade de reações metabólicas), *frdB* (responsável pela catálise da interconversão de fumarato e succinato, sendo a fumarato redutase usada no crescimento anaeróbico e a succinato desidrogenase no crescimento aeróbico), *fur* (atua como um elemento de controle negativo global, empregando Fe²⁺), *iscA_2* (capaz de transferir grupos de Fe-S para apo-ferredoxina, recruta ferro livre intracelular), *iscS* (fornece enxofre a vários membros envolvidos na montagem de Fe-S, para a modificação de RNAt ou biossíntese de cofatores), *iscU* (auxilia na montagem do grupo Fe-S) e *yfeX* (promove extração de ferro a partir de fonte exógena de heme), assim como o gene *pspE* (catalisa a reação de transferência de enxofre do tiosulfato para o cianeto, para formar sulfito e tiocianato) quando os isolados foram expostos à ceftazidima.

Em sua maioria, genes responsáveis pela formação do flagelo encontraram-se *down-regulated* quando os isolados de *S. enterica* foram expostos à ceftazidima e, eventualmente, à ciprofloxacina (Anexo – 01). Entretanto, os genes *flgK*, *flgL*, *flhC*, *flhD*, *fliD*, *fliS*, *fliT*, *fliV*, *fliZ* (composição do flagelo), *motA* e *motB* (necessários para rotação do motor flagelar), apresentaram-se *up-regulated* especialmente após 6 h de exposição à ciprofloxacina.

Genes que codificam proteínas associadas à virulência, tais como fímbrias, *pilli* e sistemas de secreção, não apresentaram um padrão de expressão homogêneo (Anexo – 01). Entretanto, alguns genes apresentaram maiores níveis de expressão, especialmente após 6 h de exposição à ciprofloxacina, destacando-se: *cheA* (envolvido na transmissão

de sinais sensoriais dos quimiorreceptores para os motores flagelares), *cheB* (parte de um sistema de transdução de sinal que modula a quimiotaxia em resposta a vários estímulos), *cheR* (metila as proteínas quimiotáticas ligadas à membrana), *cheV* e *cheY* (transmissão de sinais sensoriais dos quimiorreceptores para os motores flagelares), *secB* (exportação de proteínas do citoplasma da célula), *secE* (subunidade essencial do canal de translocação de proteínas SecYEG), *tar* (medeia a taxia através de uma interação com a proteína periplasmática de ligação à maltose.), e *tsr_1* e *tsr_2* (transdução de sinal do lado de fora para o interior da célula). Os genes *secB* e *secY* (subunidade central do canal de translocação de proteínas SecYEG) apresentaram-se *down-regulated* quando os isolados de *S. enterica* foram expostos à ceftazidima em todos os tempos analisados. Da mesma forma, não foi visualizado um padrão de expressão diferencial dos genes envolvidos em *quorum sensing*. Contudo, alguns genes apresentaram-se *up-regulated* 6 h após a exposição à ciprofloxacina (Anexo – 01).

Poucos genes associados com proteínas e transportadores de membrana apresentaram-se diferencialmente expressos (Anexo – 01). Entretanto, quando os isolados de *S. enterica* foram expostos à ceftazidima, alguns genes encontraram-se *down-regulated*, tais como: *bamA* e *bamB* (parte do complexo de montagem de proteína de membrana externa (Bam), que está envolvido na montagem e inserção de proteínas de beta-barril na membrana externa), *glpT* (componente integral da membrana responsável pela captação de glicerol-3-fosfato), *lamB* (transporte de maltodextrinas, também atua como um receptor para vários bacteriófagos, incluindo lambda), *lspA* (catalisa especificamente a remoção de peptídeos sinalizadores), *malE* (parte do complexo transportador ABC MalEFGK envolvido na importação de maltose), *malK* (parte do complexo transportador ABC MalEFGK envolvido na importação de maltose e responsável pelo acoplamento de energia ao sistema de transporte), *ompW* (proteína de

membrana externa) e *pta* (excreção de acetato em troca de ATP) No entanto, níveis maiores de expressão foram observados nos genes *osmW* e *osmX* (parte do complexo transportador OsmU ABC, que está envolvido na captação de osmoprotetores, como colina-O-sulfato e glicina-betaína) quando os isolados de *S. enterica* foram expostos à ceftazidima. Da mesma forma, alguns genes mostraram-se *up-regulated* após exposição à ciprofloxacina: *alaE* (exportação de L-alanina), *copA* (ATPase tipo P, exportadora de cobre), *macA_1* (parte do sistema de efluxo MacAB-TolC, responsável pela resistência a macrolídeos), *modB* (parte do sistema de transporte dependente de proteína de ligação para o molibdênio, provavelmente responsável pela translocação do substrato através da membrana), *pstS* (parte do complexo transportador ABC PstSACB envolvido na importação de fosfato), *ygaP* (proteína integral de membrana) e *yebE* (proteína de membrana interna). Por outro lado, efeito contrário foi observado após a exposição a esse fármaco nos genes *lolD_1* (parte do complexo transportador ABC LolCDE envolvido na translocação de lipoproteínas maduras dirigidas à membrana externa), *proV* e *proW* (parte do complexo transportador ProU ABC envolvido na captação de glicina betaína e prolina betaína, provavelmente responsável pelo acoplamento de energia ao sistema de transporte).

A maioria dos transcritos envolvidos na biossíntese de lipopolissacarídeo e petideoglicano encontraram-se *down-regulated*, especialmente quando os isolados de *S. enterica* foram expostos à ceftazidima (Anexo – 01). Os genes *mraY*, *murC*, *murD* e *murG* (envolvidos na formação de parede celular) encontraram-se *down-regulated* em todos os isolados quando expostos à ceftazidima e à ciprofloxacina. Entretanto, os genes *ampD* (envolvido na reciclagem de peptideoglicanos da parede celular e na indução de beta-lactamase) e *lpxC* (biossíntese do lipídeo A) apresentaram-se *up-regulated* quando os isolados foram expostos à ciprofloxacina.

Os transcritos que atuam nas vias de estresse oxidativo apresentaram-se em sua maioria *up-regulated*, especialmente quando os isolados foram expostos à ciprofloxacina (Anexo – 01). Os genes *katG* (enzima bifuncional com atividade de catalase-peroxidase), *sodB* (detoxifica radicais superóxido) e *ahpC* (catalisa a redução de peróxido de hidrogênio e hidroperóxidos orgânicos em água e álcoois), encontraram-se *up-regulated* quando os isolados foram expostos à ciprofloxacina, exceto no isolado de *S. Enteritidis* (192) 48 h após a exposição a mesma.

Pode ser observado um aumento dos níveis de expressão dos genes pertencentes a módulos TAs, especialmente quando os isolados foram expostos à ciprofloxacina (Anexo – 01). Dentro deste contexto, destacaram-se: *bssS* (reprime a formação de biofilme em meios que contém glicose, parece atuar como um regulador global de vários genes envolvidos na repressão catabólica e resposta ao estresse e na regulação da captação e exportação de vias de sinalização), *bhsA_2* e *bhsA_3* (redução da permeabilidade da membrana externa ao cobre, parecem estar envolvidos na regulação negativa da formação de biofilme) e *tisB* (componente tóxico de um sistema toxina-antitoxina do tipo I, cuja superexpressão leva à parada do crescimento e indução da resposta ao estresse, além de inibir a síntese de ATP).

De modo geral, os genes associados a múltiplas vias metabólicas como biossíntese de purinas e pirimidinas e degradação de metabólitos secundários, encontraram-se *down-regulated*, especialmente quando os isolados foram expostos à ceftazidima e *up-regulated* frente à ciprofloxacina, especialmente após 6 h (Anexo – 01). Diante disso, os seguintes genes encontraram-se *down-regulated* em todos os isolados e tempos analisados após a exposição à ceftazidima: *accA*, *accB* e *accC* (componentes do complexo acetil-coenzima A carboxilase), *ackA* (catalisa a formação de acetilfosfato a partir de acetato e ATP), *acpP_1* (envolvida na biossíntese de ácidos graxos), *aroB* (catalisa a conversão de 7-

fosfato de 3-desoxi-D-arabino-heptuloseonato em desidroquinato), *aroK* (catalisa a fosforilação específica do grupo 3-hidroxila do ácido chiquímico usando ATP como um co-substrato), *fabH* (catalisa a primeira reação de condensação que inicia a síntese de ácidos graxos e pode, portanto, desempenhar um papel no controle da taxa total de produção de ácidos graxos), *fabZ* (envolvido na biossíntese de ácidos graxos insaturados), *fdhF_1* (decompõe o ácido fórmico em hidrogênio e dióxido de carbono sob condições anaeróbicas na ausência de receptores de elétrons exógenos), *glmS_2* (catalisa o primeiro passo no metabolismo da hexosamina, convertendo a frutose-6-P em glucosamina-6-P usando glutamina como fonte de nitrogênio), *gph* (participa na dissimilação do 2-fosfoglicolato intracelular formado durante o reparo do DNA), *gpsA* (metabolismo do glicerofosfolípido), *hybO* (uma das três hidrogenases sintetizadas em resposta a diferentes condições fisiológicas), *ispD* (biossíntese de terpenóide), *ispF* (biossíntese de isopentenil difosfato e dimetilalil difosfato, dois principais blocos de construção de compostos isoprenóides), *malQ* (quebra da maltose), *nrdA* e *nrdB* (fornecem os precursores para a síntese de DNA), *pal* (desempenha um papel na invaginação da membrana externa durante a divisão celular e é importante para manter a integridade da membrana externa), *pflB* (sintetiza formato a partir de piruvato), *plsX* (utiliza acil-ACP como doador de acil graxo, mas não acil-CoA), *prc* (pode estar envolvido na proteção da bactéria contra estresses térmicos e osmóticos), *prs* (biossíntese do metabólito central fosfo-alfa-D-ribosil-1-pirofosfato através da transferência do grupo pirofosforilo do ATP para 1-hidroxilo ribose-5-fosfato), *sdaB* (biossíntese de carboidratos), *sdaC* (envolvido na importação de serina para a célula), *tktA_2* (catalisa a transferência de um grupo cetona de dois carbonos de um dador de cetose para um aceitador de aldose, através de um intermediário covalente com o cofactor pirofosfato de tiamina) e *treC* (hidrólise de trealose-6-fosfato para glicose e glicose-6-fosfato). No entanto, frente a este mesmo

fármaco níveis maiores de expressão diferencial foram observados nos genes *otsA* (essencial para a viabilidade das células a baixas temperaturas e com elevada força osmótica) e *otsB* (remove o fosfato da trealose 6-fosfato para produzir trealose livre). Por outro lado, em todos os isolados e tempos analisados após a exposição à ciprofloxacina, observou-se níveis menores de expressão diferencial de *aspA* (catálise da reação de L-aspartato em fumarato + NH₃), *lexA_1* (reprime vários genes envolvidos na resposta SOS, incluindo *recA*), *nhaA* (expulsa sódio em troca de prótons externos), *ptrB* (cliva as ligações peptídicas no lado C-terminal dos resíduos de lisil e arginil) e *ubiF* (participa da via de biossíntese da ubiquinona).

Alguns genes não foram incluídos em vias reconhecidas pelo KEGG nos isolados de *S. enterica*, estando a maioria destes *down-regulated* quando os isolados foram expostos à ceftazidima, e *up-regulated* frente à exposição à ciprofloxacina, especialmente após 6 h (Anexo – 01). Níveis menores de expressão em todos os isolados e tempos analisados após a exposição à ceftazidima foram encontrados especialmente em: *apaG* (função não conhecida, mutações fornecem um fenótipo de resistência a baixo nível de CO₂, também associado à diminuição do efluxo de Mg²⁺), *fabD* e *fabF* (envolvidos na biossíntese de ácidos graxos), *fkpB* (aceleração do dobramento de proteínas), *focA* (envolvido no transporte bidirecional de formato), *glmU* (catalisa as duas últimas reações sequenciais na via biossintética de novo para UDP-N-acetilglucosamina), *grcA* (acetiltransferase tendo formato como substrato), *grxC* (redução de algumas ligações dissulfeto em um sistema acoplado com a glutatona redutase), *hscA* (chaperona envolvida na maturação de proteínas contendo grupo ferro-enxofre), *kbl* (catalisa a clivagem de 2-amino-3-cetobutirato em glicina e acetil-CoA), *proQ* (pode regular a atividade de ProP através de um mecanismo pós-transcricional), *skp* (chaperona molecular que interage especificamente com as proteínas da membrana externa,

mantendo assim a solubilidade dos intermediários de dobramento precoce durante a passagem pelo periplasma), *surA* (chaperona envolvida no correto dobramento e montagem de proteínas da membrana externa, como OmpA, OmpF e LamB), *tig* (atua como uma chaperona ao manter as proteínas secretoras e não secretoras recém-sintetizadas em uma conformação aberta), *ybaB* (liga-se ao DNA e altera sua conformação, podendo estar envolvido na regulação da expressão gênica, organização do nucleoide e proteção do DNA) e *yceD* (desempenha um papel na síntese, processamento e/ou estabilidade do RNAr 23S). Por outro lado, os seguintes genes encontraram-se *up-regulated* em todos os isolados e tempos analisados após a exposição à ceftazidima: *spy* (chaperona periplasmática independente de ATP, diminui a agregação de proteínas e ajuda a redobrar as proteínas), *pspD* (faz parte do operon da proteína de choque do fago - *pspABCDE* - pode desempenhar um papel significativo em condições limitadas de nutrientes ou energia), *osmE* e *osmB* (fornecimento de resistência ao estresse osmótico, podem ser importantes para a sobrevivência em fase estacionária) e *dps* (durante a fase estacionária, liga-se ao cromossomo de forma não específica, formando um co-cristal dps-DNA altamente ordenado e estável, dentro do qual o DNA cromossômico é condensado e protegido de danos). Em todos os isolados e tempos analisados após a exposição à ciprofloxacina, os seguintes genes foram observados *up-regulated*: *cueO* (provavelmente envolvido na desintoxicação periplasmática do cobre oxidando Cu^+ a Cu^{2+} e impedindo sua captação no citoplasma), *hscB* (co-chaperona envolvida na maturação de proteínas contendo grupo ferro-enxofre), *hypB*, *hypC* e *hypD* (envolvidos na maturação de hidrogenases níquel-ferro), *uspE* (necessário para resistência a agentes prejudiciais ao DNA), e *ywIC* (necessário para a formação de um grupo de treonil-carbamoil em adenosina na posição 37 em RNAs que leem códons começando com adenina). Adicionalmente, o gene *pspA* (função descrita acima no gene *pspD*) foi

encontrado *up-regulated* em todos os isolados e tempos analisados após a exposição a ambos os fármacos.

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Anexo 01. Função biológica de cada gene diferencialmente expresso ($q \leq 0.05$), bem como seu nível de expressão, nos isolados de *Salmonella enterica* (S. Enteritidis – 192 e 4SA– e S. Schwarzengrund – S58) expostos a 100X o valor da CIM para ciprofloxacina (CIP) - ou ceftazidima (CAZ) por 6 e 48 h (T6 e T48).

			Escala de cores dos valores de expressão											
			≤ -4	≤ -2	-1.99	-0.199	0.199	≥ 2	≥ 4					
Processos biológicos	Gene	Anotação funcional	192-CAZ-T6	192-CAZ-T48	4SA-CAZ-T6	4SA-CAZ-T48	S58-CAZ-T6	S58-CAZ-T48	192-CIP-T6	192-CIP-T48	4SA-CIP-T6	4SA-CIP-T48	S58-CIP-T6	S58-CIP-T48
	<i>aat</i>	Leucyl/phenylalanyl-tRNA--protein transferase	1.38	1.44	1.65	1.61	0	0	0	1.72	-3.73	0	0	0
	<i>ala S</i>	Alanine--tRNA ligase	-4.45	-3.71	-2.13	-2.64	-3.08	-3.51	0	1.65	-1.43	-2.16	-1.45	-2.48
	<i>arg S</i>	Arginine--tRNA ligase	-1.37	-1.38	0	0	0	-1.32	1.64	-1.78	-1.65	0	-1.59	0
	<i>asn S</i>	Asparagine--tRNA ligase	-2.17	-2.08	-1.46	-1.46	-2.2	-2.38	1.19	0	0	0	0	0
	<i>asp S</i>	Aspartate--tRNA ligase	-3	-3.68	-1.65	-2.13	-2.04	-2.79	6.33	-1.74	1.55	-1.68	2.33	-1.61
	<i>asp T_1</i>	Aspartate/alanine antiporter	0	-1.33	0	0	1.35	1.5	0	-1.48	-1.45	0	-3.54	0
	<i>asp T_2</i>	Aspartate/alanine antiporter	1.5	1.47	0	0	0	0	1.54	-2.97	-2.36	2.13	-1.45	-1.39
	<i>cmo A</i>	Carboxy-S-adenosyl-L-methionine synthase	0	0	0	0	0	0	-1.83	0	0	-2.63	0	0
	<i>cmo B</i>	tRNA L34 carboxymethyltransferase	0	0	0	0	0	0	-1.8	-1.43	0	-2.04	0	0
	<i>cys S</i>	Cysteine--tRNA ligase	-2.03	-2.04	-1.48	-1.55	-1.38	-1.52	2.99	-1.5	2.02	2.05	2	0
	<i>dea D</i>	ATP-dependent RNA helicase DeaD	-6.86	-8.36	-1.95	-1.64	-2.88	-2.74	9.14	0	0	2.05	2.1	1.97
	<i>der</i>	GTPase Der	-3.75	-4.41	-1.76	-2.08	-2.13	-2.44	0	2.93	0	-2.09	0	0
	<i>dks A</i>	RNA polymerase-binding transcription factor DksA	-1.92	-1.95	0	0	0	-1.32	6.05	-1.96	2	-2.09	4.79	0
	<i>dtc</i>	D-aminoacyl-tRNA deacylase	-2.13	-2.42	-1.46	-1.48	-1.35	-1.65	0	0	0	0	0	0
	<i>dus A</i>	tRNA-dihydrouridine(20/20a) synthase	0	0	0	0	0	0	-1.78	1.33	-2.65	0	-2.33	0
	<i>dus C</i>	tRNA-dihydrouridine(16) synthase	1.6	1.67	0	1.37	1.37	1.42	0	-1.83	0	0	-2.05	0
	<i>efp</i>	Elongation factor P	-2.7	-2.97	-1.35	-1.35	-1.78	-2.07	4.57	-2.04	1.75	0	0	0
	<i>end A</i>	Endonuclease-1	0	0	0	0	0	0	-1.95	0	-2.26	0	-2.21	0
	<i>epm A</i>	Elongation factor P--(R)-beta-lysine ligase	-2.01	-1.99	0	0	0	-1.38	3.08	-1.71	-1.86	0	0	0
	<i>epm C</i>	Elongation factor P hydroxylase	-1.3	-1.33	0	0	0	0	0	-1.51	-2.06	0	0	0
	<i>ero</i>	GTPase Era	-3.23	-3.4	-2.03	-2.03	-2.09	-2.3	-3.6	-3.13	-2.1	-2.32	-2.05	-2.28
	<i>ett A</i>	Energy-dependent translational throttle protein EttA	-2.12	-2.45	-1.5	-1.57	-1.77	-2.18	5.84	0	2.11	0	2.8	0
	<i>fmt</i>	Methionyl-tRNA formyltransferase	-1.2	-2.5	-1.57	-1.78	-2.19	-2.1	2.95	1.74	2.15	3.61	2.23	2.02
	<i>frr</i>	Ribosome-recycling factor	-3.02	-3.55	-1.74	-1.95	-1.96	-2.41	1.45	-3.26	-1.79	0	1.69	0
	<i>fus A</i>	Elongation factor G	-14.02	-14.14	-9.17	-9.17	-8.79	-13.75	4.95	-2.23	0	-3.18	0	-4.02
	<i>gln S</i>	Glutamine--tRNA ligase	-3.08	-3.31	-1.77	-1.91	-2.24	-2.69	6.55	2.27	1.9	0	1.72	0
	<i>glu Q</i>	Glutamyl-Q-tRNA(Asp) synthetase	0	0	0	0	0	0	-3.33	0	-2.06	-1.78	-1.99	0
	<i>gly Q</i>	Glycine--tRNA ligase alpha subunit	-2.8	-3.72	-1.5	-1.73	-1.74	-1.94	2.08	-1.48	1.33	0	1.37	-1.43
	<i>gly S</i>	Glycine--tRNA ligase beta subunit	-2.71	-3.59	-1.65	-1.88	-1.86	-2.19	2.27	-1.63	0	0	0	-1.67
	<i>gre A</i>	Transcription elongation factor GreA	-2.52	-2.53	-1.6	-1.47	-1.56	-1.95	2.1	2.33	2.4	3.16	12.73	4.62
	<i>gro L</i>	60 kDa chaperonin	-2.04	-3.81	0	0	-1.58	-1.85	16	0	15.82	3.62	12.42	0
	<i>hem A</i>	Glutamyl-tRNA reductase	-3.3	-2.94	-1.84	-1.81	-2.56	-2.13	1.39	-1.64	0	1.58	1.39	0
	<i>hfl X</i>	GTPase HflX	-2.48	-3.55	-1.92	-2.22	-1.93	-2.32	2.13	0	1.38	0	2.14	0
	<i>his S</i>	Histidine--tRNA ligase	-4.18	-5.53	-2.16	-2.2	-2.91	-2.97	1.99	-1.81	0	-1.39	0	0
	<i>hpf_1</i>	Ribosome hibernation promoting factor	0	0	-1.35	-1.95	-1.87	-2.25	-1.53	0	3.36	0	3.54	1.81
	<i>hpf_2</i>	Ribosome hibernation promoting factor	-2.33	-3.3	1.48	1.55	0	0	3.13	0	-1.79	0	2.11	0
	<i>ile S</i>	Isoleucine--tRNA ligase	-3.16	-4.48	-1.96	-2.47	-2.7	-3.25	3.23	0	0	0	1.48	0
	<i>inf A</i>	Translation initiation factor IF-1	2.84	2.02	0	0	-1.82	-1.58	1.9	0	-4.39	0	0	2.41
	<i>inf B</i>	Translation initiation factor IF-2	-6.11	-11.71	-1.91	-3.97	-4.84	-6.56	1.13	0	2.03	0	1.87	-1.57
	<i>inf C</i>	Translation initiation factor IF-3	-8.2	14.07	-2.27	-3.87	-2.85	-4.62	3.18	0	1.67	-1.89	3.88	0
	<i>lep A</i>	Elongation factor 4	-2.7	-3.16	-1.42	-1.46	-2.06	-2.03	2.19	0	1.54	1.44	1.65	0
	<i>leu S</i>	Leucine--tRNA ligase	-2.56	-3.22	-1.59	-1.89	-1.94	-2.27	9.3	0	1.77	0	1.85	0
	<i>lys S</i>	Lysine--tRNA ligase	-3.76	-5.56	-1.75	-2.45	-3.02	-3.83	3.9	-1.51	1.91	0	2.2	-1.76
	<i>met G</i>	Methionine--tRNA ligase	-2.27	-2.48	-1.29	-1.32	-1.7	-1.72	2.87	-1.63	2.06	1.42	1.71	0
	<i>mia A</i>	tRNA dimethylallyltransferase	-1.48	-2.26	0	-1.33	0	0	3.16	0	2.14	2.83	3.73	2.01
	<i>mia B</i>	tRNA-2-methylthio-[N(6)] dimethylallyladenosine synthase	-1.34	-1.51	0	0	0	0	3.84	-1.83	2.95	1.81	3.66	0
	<i>mmE</i>	tRNA modification GTPase MmmE	-1.51	-1.74	-1.45	-1.41	-1.32	-1.43	-3.5	-1.84	-2.34	-2.22	-2.21	-1.74
	<i>mmG</i>	tRNA uridine 5-carboxymethylaminomethyl modification enzyme MmmG	-2.56	-2.38	-1.46	-1.43	-2.06	-2.23	0	-1.65	0	1.83	0	0
	<i>nus A</i>	Transcription termination/antitermination protein NusA	-4.97	-10.38	-1.78	-3.15	-3.46	-4.64	3.74	0	2.97	1.57	2.99	0
	<i>nus B</i>	N utilization substance protein B	-3.98	-5.59	-1.72	-1.77	-2.13	-2.61	3.75	-1.94	2.73	1.88	2.72	0
	<i>nus G</i>	Transcription termination/antitermination protein NusG	-3.04	-3.93	-1.35	-1.5	-2.18	-2.69	3.07	-2.8	2.8	0	3.83	0
	<i>obg</i>	GTPase Obg	-4.38	-4.65	-1.77	-1.63	-2.11	-2.19	1.53	-2.88	0	0	0	0
	<i>phe S</i>	Phenylalanine--tRNA ligase alpha subunit	-5.72	-6.33	-2.89	-3.48	-3.59	-3.86	0	-2.91	-3.9	-5.54	-1.94	-2.99
	<i>phe T</i>	Phenylalanine--tRNA ligase beta subunit	-5.21	-7.35	-2.68	-3.77	-3.81	-4.61	1.67	-2.93	-2.88	-5.35	2.2	-4.28
	<i>pmr D</i>	Signal transduction protein PmrD	-4.29	-5.49	0	0	0	0	-3.66	-11.16	0	0	0	0
	<i>pnp</i>	Polyribonucleotide nucleotidyltransferase	-6.64	-12.47	-2.44	-4.38	-3.6	-5.94	0	-7.31	-1.46	-3.55	0	-3.49
	<i>prf A</i>	Peptide chain release factor RF1	-3.85	-3.61	-2.08	-2.18	-2.81	-2.92	3.07	-1.66	1.45	1.74	0	0
	<i>prf B_1</i>	Peptide chain release factor RF2	-4.99	-6.92	-2.73	-3.03	0	0	2.23	-1.51	0	-1.41	0	0
	<i>prf B_2</i>	Peptide chain release factor RF2	0	0	0	0	-3.72	-4.95	0	1.48	0	0	1.64	-1.64
	<i>prf C</i>	Peptide chain release factor RF3	-2.16	-2.31	-1.26	-1.32	-1.53	-1.61	4.83	0	1.56	0	1.88	0
	<i>prm B</i>	50S ribosomal protein L3 glutamine methyltransferase	-1.68	-1.77	0	0	0	0	2.97	0	2.35	1.69	2.28	0
	<i>prm C</i>	Release factor glutamine methyltransferase	-3.1	-3.04	-2.01	-1.86	-2.73	-2.55	2.68	-1.65	0	0	0	-1.9
	<i>pro S_1</i>	Proline--tRNA ligase	-2.24	-2.25	0	1.7	0	0	2.1	-1.66	0	0	0	0

<i>pro S_2</i>	Proline-tRNA ligase	1.83	2.01	0	0	1.47	1.66	0	1.57	-3.61	0	-3.87	0
<i>pro S_3</i>	Proline-tRNA ligase	0	0	-1.33	-1.37	-1.73	-1.84	-4.09	0	0	0	0	0
<i>que A_2</i>	S-adenosylmethionine:tRNA ribosyltransferase-isomerase	-1.73	-1.8	0	0	-1.36	-1.34	0	0	-2.04	0	0	0
<i>que G</i>	Epoxyqueuosine reductase	0	0	0	0	0	0	4.88	0.41	1.75	4.74	1.59	2.69
<i>rai A</i>	Ribosome-associated inhibitor A	1.44	0	0	0	2.39	2	4.11	0	13.17	8.39	25.38	16.73
<i>rap A</i>	RNA polymerase-associated protein RapA	-1.65	-1.75	0	0	0	0	6.95	0.87	3.74	3.38	2.27	1.71
<i>rbf A</i>	30S ribosome-binding factor	-5.81	-7.22	-2.04	-2.74	-2.79	-3.39	0	-2.92	0	-2.11	0	-2.29
<i>rec Q</i>	ATP-dependent DNA helicase RecQ	-1.97	-2.16	-1.36	-1.33	-1.52	-1.57	0	-1.57	0	0	0	0
<i>rhl B</i>	ATP-dependent RNA helicase RhlB	-3.82	-4.5	-1.92	-2.23	-2.64	-2.72	0	-3.12	1.55	0	1.49	0
<i>rho</i>	Transcription termination factor Rho	-4.38	-5.4	-1.78	-1.46	-2.13	-2.08	2.13	1.74	0	1.72	0	1.66
<i>rim K</i>	Ribosomal protein S6-L-glutamate ligase	0	0	0	0	0	0	2.06	0.23	0	0	0	0
<i>rim L</i>	Ribosomal-protein-serine acetyltransferase	1.49	1.84	1.4	1.56	1.54	1.99	2.05	0	1.63	2.05	2.75	0
<i>rim M</i>	Ribosome maturation factor RimM	-8.86	-23.09	-3.2	-6.26	-6.38	-10.13	1.59	-4.18	1.67	-1.64	1.17	0
<i>rim O_1</i>	Ribosomal protein S12 methylthiotransferase RimO	0	0	1.37	1.41	0	0	-1.34	0	-1.68	-2.12	-3.28	0
<i>rim O_2</i>	Ribosomal protein S12 methylthiotransferase RimO	1.43	1.37	0	0	0	0	1.88	0	-2.49	0	-2.47	0
<i>rim O_3</i>	Ribosomal protein S12 methylthiotransferase RimO	0	0	0	0	0	0	-1.52	0	-3.2	0	-2.04	-1.66
<i>rim P</i>	Ribosome maturation factor RimP	-5.72	-9.53	-2.01	-2.97	-3.18	-3.47	2.53	-1.86	1.94	1.37	-4.43	2.73
<i>rim A</i>	23S rRNA (guanine[745]-N(1))-methyltransferase	0	0	0	1.34	0	0	-3.39	0	0	-2.07	-2.17	0
<i>rim B_1</i>	23S rRNA (guanosine-2'-O)-methyltransferase RimB	-3.51	-4.38	-1.3	-1.63	-1.41	-1.86	0	-1.93	-1.83	-1.68	0	0
<i>rim B_2</i>	23S rRNA (guanosine-2'-O)-methyltransferase RimB	-1.4	-1.48	0	0	0	0	2.57	-2.09	1.69	0	2.2	0
<i>rim C</i>	23S rRNA (uracil[747]-C(5))-methyltransferase RimC	0	1.34	0	0	0	1.29	-1.87	0	-4.26	0	-2.18	0
<i>rim D</i>	23S rRNA (uracil[1939]-C(5))-methyltransferase RimD	-2.55	-2.54	0	0	0	1.41	-1.6	2.64	2.05	1.75	1.6	0
<i>rim E</i>	Ribosomal RNA large subunit methyltransferase E	-2.71	-3.58	-1.63	-1.73	-2.22	-2.29	12.87	0.87	4.12	4.36	7.33	4.83
<i>rim F</i>	Ribosomal RNA large subunit methyltransferase F	-1.36	0	0	0	0	0	1.52	1.81	0	0	2.26	0
<i>rim G</i>	Ribosomal RNA large subunit methyltransferase G	-1.65	-2.06	0	0	0	-1.31	-2.76	-2.02	2.61	1.57	2.16	0
<i>rim H</i>	Ribosomal RNA large subunit methyltransferase H	-2.15	-1.96	-1.76	-1.54	-1.66	-1.52	0	0	1.85	1.91	0	0
<i>rim I</i>	Ribosomal RNA large subunit methyltransferase I	0	0	0	1.26	1.25	1.27	2.89	0	2.24	1.6	1.52	0
<i>rim L</i>	Ribosomal RNA large subunit methyltransferase K/L	-2.13	-2.2	-1.32	0	-1.53	-1.47	0	-1.51	-1.6	-1.69	-2.02	-1.71
<i>rim M</i>	Ribosomal RNA large subunit methyltransferase M	-2.61	-2.59	0	0	-1.42	-1.51	0	-1.46	-1.72	-1.75	-1.82	-1.6
<i>rim N</i>	Dual-specificity RNA methyltransferase RimN	-1.97	-1.96	0	0	-1.45	-1.41	1.82	-1.6	2.81	0	3.38	0
<i>rlu A</i>	Ribosomal large subunit pseudouridine synthase A	-1.44	-1.47	0	-1.3	-1.37	-1.29	4.83	3.18	1.61	1.64	1.58	1.41
<i>rlu B</i>	Ribosomal large subunit pseudouridine synthase B	-2.19	-1.97	0	0	0	0	5.22	2.05	2.91	0	2.5	0
<i>rlu C</i>	Ribosomal large subunit pseudouridine synthase C	0	0	0	0	0	0	-1.72	-2.23	0	0	0	0
<i>rlu D</i>	Ribosomal large subunit pseudouridine synthase D	-4.42	-5.37	0	0	0	0	2.27	5.15	0	-1.58	0	0
<i>rlu E</i>	Ribosomal large subunit pseudouridine synthase E	1.32	1.61	1.53	1.73	0	0	-2	0	0	0	-3.68	0
<i>rlu F</i>	23S rRNA pseudouridine(2604) synthase	0	0	0	0	0	0	-2.27	0	0	0	0	0
<i>rnb</i>	Exoribonuclease 2	-1.62	-1.59	0	0	0	0	-1.61	-1.32	-2.31	0	-5.48	-1.89
<i>rng</i>	Ribonuclease G	-2.21	-2.53	-1.82	-1.91	-1.66	-1.98	-1.54	-2.04	-1.77	-2.06	0	0
<i>rnt</i>	Ribonuclease T	0	0	0	0	0	1.36	2	1.7	0	0	0	0
<i>rox A</i>	50S ribosomal protein L16 3-hydroxylase	-2.17	-2.22	0	0	-1.38	-1.27	0	-1.96	0	0	0	0
<i>rpl A</i>	50S ribosomal protein L1	-9.32	-24.91	-2.63	-5.04	-5.76	-7.65	0	-7.3	0	0	2.06	0
<i>rpl B</i>	50S ribosomal protein L2	11.34	51.96	-2.44	-10.59	-9.15	-17.64	2.51	0	-1.73	-6.81	0	-4.35
<i>rpl C</i>	50S ribosomal protein L3	-7.84	-31.19	-2.18	-7.75	-6.43	-12.83	2.69	0	-1.59	-3.15	1.57	-3.3
<i>rpl D</i>	50S ribosomal protein L4	-9.17	-36.25	-2.39	-8.37	-7.32	-13.48	7.71	0	-1.56	-3.9	0	-3.49
<i>rpl E</i>	50S ribosomal protein L5	10.13	35.32	-2.4	-6.11	-6.39	-9.4	3.86	0	0	0	1.98	-1.69
<i>rpl F</i>	50S ribosomal protein L6	-12.86	-52.23	-2.56	-9.25	-8.68	-13.01	2.65	0	0	-2.01	1.72	-2.41
<i>rpl I</i>	50S ribosomal protein L9	-5.57	-7.11	-1.46	-1.92	-2.03	-2.59	0	3.94	0	0	2.13	0
<i>rpl J</i>	50S ribosomal protein L10	-16.27	-39.46	-2.35	-4.77	-6.68	-7.21	0	-16.04	1.4	0	3.15	0
<i>rpl K</i>	50S ribosomal protein L11	-9.38	-24.57	-3.04	-5.12	-5.02	-6.93	2	-8.63	0	0	2.56	0
<i>rpl L</i>	50S ribosomal protein L7/L12	15.01	23.01	-2.96	-5.38	-4.83	-5.1	0	-10.38	0	-1.56	0	0
<i>rpl M</i>	50S ribosomal protein L13	-10.41	-17.57	-2.33	-3.37	-4.6	-5.28	2.76	-7.86	0	0	5.34	1.92
<i>rpl N</i>	50S ribosomal protein L14	-10.17	-33.23	-2.38	-5.92	-5.16	-7.69	16.2	0	0	0	2.3	0
<i>rpl O</i>	50S ribosomal protein L15	-20.07	71.67	-4.45	-15.88	-9.96	-18.76	3	0	0	-2.67	0	-5.34
<i>rpl P</i>	50S ribosomal protein L16	-18.52	-60.89	-2.98	-9.84	-8.67	-15.68	2.53	0	-2.15	-6.23	0	-7.89
<i>rpl Q</i>	50S ribosomal protein L17	-5.25	-9.03	-1.92	-3.2	-3.55	-4.26	1.64	-2.23	0	-1.49	1.74	0
<i>rpl R</i>	50S ribosomal protein L18	-15.6	-61.34	3.1	-11.75	-8.72	-15.13	2.7	0	0	-2.34	1.52	-3.12
<i>rpl S</i>	50S ribosomal protein L19	-6.03	-8.8	-1.98	-3.35	-4.17	-5.69	2.47	-2.96	0	0	4.01	0
<i>rpl T</i>	50S ribosomal protein L20	-5.24	-5.81	0	-1.81	-2.77	-2.57	5.63	0	0	0	3.4	0
<i>rpl U</i>	50S ribosomal protein L21	-6.48	-8.08	-1.7	-1.99	-2.83	-3.64	6.33	-7.78	3.15	2.23	9.34	-3.16
<i>rpl V</i>	50S ribosomal protein L22	-15.81	-52.88	-2.91	-11.34	-9.01	-17.77	2.65	0	-1.71	-4.41	0	-6.19
<i>rpl W</i>	50S ribosomal protein L23	-8.85	-38.58	-2.62	-10.06	-8.14	-14.25	3.1	0	-1.44	-3.79	0	-3.66
<i>rpl X</i>	50S ribosomal protein L24	-9.48	-31.63	-2.48	-6.11	-5.9	-8.44	4.04	-1.88	1.5	0	2.02	-2.12
<i>rpl Y</i>	50S ribosomal protein L25	-1.76	-1.45	0	0	0	0	0	0	0	0	2.69	0
<i>rpm A</i>	50S ribosomal protein L27	-3.34	-3.9	-1.5	-1.53	-2.07	-2.7	6.43	-5.31	3.15	2.06	6.94	3.77
<i>rpm B</i>	50S ribosomal protein L28	-6.19	-7.15	0	0	-2.24	-2.56	1.69	-6.73	1.83	0	4.56	0
<i>rpm C</i>	50S ribosomal protein L29	-16.14	-37.19	-2.71	-8.11	-6.81	-10.35	2.43	0	-2.35	-5.98	0	-5.64
<i>rpm D</i>	50S ribosomal protein L30	20.17	80.29	-3.71	-14.62	-9.87	-14.42	3.13	0	0	-2.74	0	-4.78
<i>rpm E2</i>	50S ribosomal protein L31 type B	0	1.72	0	1.8	0	2.18	0	0	0	0	2.73	0.63

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<i>rpm F</i>	50S ribosomal protein L32	-5.68	-6.13	-1.62	-2.26	-3.29	-3.79	2.66	-7.3	1.95	0	2.3	0
<i>rpm G</i>	50S ribosomal protein L33	-3.8	-3.83	0	0	0	0	0	-2.48	0	0	0	0
<i>rpm I</i>	50S ribosomal protein L35	-12.6	18.05	0	-2.55	-4.12	-4.7	1.55	0	0	0	3.06	-2.01
<i>rpm J</i>	50S ribosomal protein L36	-24.94	-87.14	-7.69	-18.25	-6.92	-12.47	7.11	0	0	0	0	0
<i>rpo A</i>	DNA-directed RNA polymerase subunit alpha	-13.39	40.16	-2.8	-7.75	-7.78	-14.12	1	-1.89	0	-2.77	1.68	-2.62
<i>rpo B</i>	DNA-directed RNA polymerase subunit beta	6.93	18.64	-1.99	-5.01	-3.96	6.61	4.81	0	1.64	0	1.71	-2.14
<i>rpo C</i>	DNA-directed RNA polymerase subunit beta'	-9.87	-19.23	-2.58	-4.61	-3.86	-5.8	0	-3.19	-2.2	-4.54	-1.63	-4.6
<i>rpo D</i>	RNA polymerase sigma factor RpoD	-3.35	-3.81	-1.5	0	-2.36	-2.3	-4.08	0	2.54	1.56	3.19	0
<i>rpo E</i>	ECF RNA polymerase sigma-E factor	-4.01	-3.72	-2.8	-2.1	-3.1	-2.47	0	-2.04	0	1.77	4.1	2.63
<i>rpo H</i>	RNA polymerase sigma factor RpoH	-3.08	-2.98	-1.51	0	-2.03	-1.75	-20.91	1.88	3.74	2.41	2.98	-3.98
<i>rpo N</i>	RNA polymerase sigma-54 factor	-2.98	-3.42	-1.99	-2.18	-2.16	-2.54	1.59	0	1.39	1.33	1.64	0
<i>rpo S</i>	RNA polymerase sigma factor RpoS	0	-1.9	1.62	1.99	1.97	0	0	-3.82	3.42	2.48	4.01	0
<i>rpo Z</i>	DNA-directed RNA polymerase subunit omega	-4.18	-5	-1.79	-2	-2.37	-2.74	0	-3.99	0	0	0	-2.07
<i>rps A_1</i>	30S ribosomal protein S1	12.08	25.84	-3.42	-5.79	-5.91	8.32	1.96	-2.41	-1.41	-2.45	0	-2.52
<i>rps B</i>	30S ribosomal protein S2	-11.15	-23.7	-2.76	-4.7	-5.24	-7.15	4	-4.55	0	-1.72	2.48	0
<i>rps C</i>	30S ribosomal protein S3	-17.35	-65.63	-2.89	-10.72	-9.28	-18.38	2.91	0	-1.98	-5.03	0	-5.71
<i>rps D</i>	30S ribosomal protein S4	12.69	-46.3	-3.01	-8.74	-7.53	-14.63	3.39	0	0	-2.09	1.61	-2.85
<i>rps E</i>	30S ribosomal protein S5	-16.61	-59.88	-3.58	-13.95	-9.05	-16.47	3.09	0	0	-2.52	0	-3.59
<i>rps F</i>	30S ribosomal protein S6	-8.28	-18.49	0	-2.66	-3.28	-4.44	0	-6.67	0	-1.83	1.51	0
<i>rps G</i>	30S ribosomal protein S7	-11.74	30.18	-2.63	-6.1	-6.18	-8.19	3.37	2.48	0	-2.44	1.72	-2.52
<i>rps H</i>	30S ribosomal protein S8	-11.22	-42.39	-2.72	-8.67	-7.6	-11.77	2.57	0	0	-1.68	1.8	-2.21
<i>rps I</i>	30S ribosomal protein S9	-5.02	-5.57	-1.5	-2.03	-2.62	-2.59	2.92	-6.42	0	0	4.83	1.95
<i>rps J</i>	30S ribosomal protein S10	-7.59	-32.28	-2.17	-6.8	-5.77	-11.46	3.41	0	-1.71	-3.54	1.75	-2.48
<i>rps L</i>	30S ribosomal protein S12	-11.89	-27.47	-2.26	-5	-5.63	-7.08	3.1	-2.48	0	-2.97	2.04	0
<i>rps M</i>	30S ribosomal protein S13	-12.2	-44.99	-3.12	-7.54	-6.76	-14.83	3.86	-1.29	0	-1.91	1.97	-2.26
<i>rps N</i>	30S ribosomal protein S14	-9.2	-33.31	-2.5	-6.71	-6.83	-10.08	2.29	0	0	0	1.82	-1.98
<i>rps O</i>	30S ribosomal protein S15	-3.59	-4.67	-1.56	-1.82	-1.68	-2.05	0	-7.73	0	-3.07	0	0
<i>rps P</i>	30S ribosomal protein S16	-9.71	-21.7	-2.75	-5.67	-5.51	-8.05	3.29	-3.01	1.48	-2.04	3.7	0
<i>rps Q</i>	30S ribosomal protein S17	-13.32	-27.64	-2.17	-4.9	-6.61	-8.69	0	-2.16	-2.68	-7.41	-1.6	-5.86
<i>rps R</i>	30S ribosomal protein S18	-7.49	-15.01	0	-2.89	-2.49	-3.7	0	-6.85	0	-1.73	2.38	0
<i>rps S</i>	30S ribosomal protein S19	-14.6	-60.17	-2.66	-11.74	-9.04	-20.01	2.36	0	-1.64	-5.62	0	-4.88
<i>rps T</i>	30S ribosomal protein S20	0	0	0	0	-2.33	-2.08	0	0	0	0	5.6	2.3
<i>rps U</i>	30S ribosomal protein S21	-5.13	-4.73	-1.64	-1.68	-2.59	-2.66	0	-4.85	0	0	2.19	0
<i>rra A</i>	Regulator of ribonuclease activity A	-1.89	-1.64	0	0	0	0	7.83	0	2.14	0	2.69	-2.34
<i>rra B</i>	Regulator of ribonuclease activity B	-1.7	-1.78	0	-1.37	-1.45	-1.48	3.25	0	2.43	2.28	1.84	1.84
<i>rse A</i>	Anti-sigma-E factor RseA	-3.67	-3.18	-3.34	-2.37	-2.89	-2.29	0	0	0	1.67	3.67	2.99
<i>rse B</i>	Sigma-E factor regulatory protein RseB	-2.82	-2.85	-2.82	-2.15	-2.8	-2.41	0	0	0	1.39	1.83	0
<i>rsg A</i>	Small ribosomal subunit biogenesis GTPase RsgA	-1.75	-1.87	-1.34	-1.29	0	-1.4	0	0	1.62	2.03	1.52	0
<i>rsm A</i>	Ribosomal RNA small subunit methyltransferase A	-4.28	-4.81	-2.76	-3.07	-3.59	-3.42	1.42	-1.7	-1.46	0	0	0
<i>rsm B</i>	Ribosomal RNA small subunit methyltransferase B	-2.09	-2.12	0	-1.52	-1.52	-1.69	0	0	0	1.84	-1.54	0
<i>rsm C</i>	Ribosomal RNA small subunit methyltransferase C	-2.04	-2.12	-1.3	0	0	0	0	-1.53	0	0	-1.72	0
<i>rsm D</i>	Ribosomal RNA small subunit methyltransferase D	0	0	0	0	0	0	0	0	-2.11	0	0	-2.02
<i>rsm F</i>	Ribosomal RNA small subunit methyltransferase F	0	1.36	0	1.33	0	0	-1.75	0	-1.8	0	-2.14	0
<i>rsm G</i>	Ribosomal RNA small subunit methyltransferase G	-2.44	-2.48	-1.38	-1.63	-2.39	-2.04	-2.05	-1.67	0	0	0	0
<i>rsm H</i>	Ribosomal RNA small subunit methyltransferase H	-2.78	-2.81	-1.47	-1.48	-1.83	-1.82	1.68	0	0	0	1.87	0
<i>rsm I</i>	Ribosomal RNA small subunit methyltransferase I	0	0	0	0	0	0	0	1.73	-2.18	-1.96	-2.16	-1.67
<i>rsm J</i>	Ribosomal RNA small subunit methyltransferase J	0	0	1.64	0	0	0	0	0	0	0	-2.75	0
<i>sel B</i>	Selenocysteine-specific elongation factor	0	-1.45	-1.3	-1.36	0	0	5.91	1.4	1.55	1.39	1.62	0
<i>sel U</i>	tRNA 2-selenouridine synthase	0	0	0	0	0	0	1.63	1.55	-3.4	0	0	0
<i>ser S</i>	Serine-tRNA ligase	-2.47	-3.35	-1.5	-1.62	-1.62	-1.97	1.88	2.04	2.11	0	2.81	1.58
<i>sro</i>	Stationary-phase-induced ribosome-associated protein	0	0	0	0	0	2.88	0	0	0	0	16.8	0
<i>ssp A</i>	Stringent starvation protein A	-3.1	-3.63	0	-1.42	-1.55	-1.8	1.91	-3.03	4.53	4.18	8.04	4.38
<i>ssp B</i>	Stringent starvation protein B	-2.32	-2.4	0	0	-1.45	-1.54	2.91	-2.4	3.48	3.51	4.91	3.47
<i>tcd A</i>	tRNA threonylcarbamoyladenosine dehydratase	-1.31	0	0	0	0	0	0	0	-2.07	0	-2	0
<i>tgt</i>	Queuine tRNA-ribosyltransferase	-1.13	-3.94	-1.41	-1.66	-2.18	-2.21	3.31	-2.27	0	0	2.05	0
<i>thr S</i>	Threonine-tRNA ligase	-3.87	-8.1	-1.7	-3.17	-1.87	-3.37	7.43	1.72	3.51	0	7.3	0
<i>til S</i>	tRNA(Ile)-lysine synthase	0	0	0	0	0	0	-1.83	0	-1.55	0	-2.52	0
<i>trm D</i>	tRNA (guanine-N(1)-)methyltransferase	-9.4	24.84	-3.19	-6.63	-7.25	-10.03	1.85	-3.37	1.48	-1.61	3.17	0
<i>trm H</i>	tRNA (guanosine(18)-2'-O)-methyltransferase	-2.92	-3.56	-1.76	-2	-2.15	-2.29	-2.25	-1.72	-2.83	-2.26	-2.5	-2.05
<i>trm J</i>	tRNA (cytidine/uridine-2'-O)-methyltransferase TrmJ	-1.57	-1.47	0	0	0	0	11.33	10.98	0	2.81	1.88	2.3
<i>trm L</i>	tRNA (cytidine(34)-2'-O)-methyltransferase	0	0	0	0	0	0	-2.69	0	-2.04	-2.29	-2.39	0
<i>trm O</i>	tRNA (adenine(37)-N6)-methyltransferase	0	0	0	0	0	0	-2.91	0	0	0	-3.08	0
<i>trp S</i>	Tryptophan-tRNA ligase	-2.14	-2.5	-1.5	-1.81	-2.16	-2.47	1.7	-1.75	0	-1.67	0	-1.52
<i>tru A</i>	tRNA pseudouridine synthase A	-1.69	-1.94	0	0	0	0	2.23	0	1.49	-2.44	0	0
<i>tru B</i>	tRNA pseudouridine synthase B	-5.11	-5.99	-2.2	-2.81	-2.78	-2.95	0	-3.31	-1.52	-3.35	0	-2.25
<i>tru C</i>	tRNA pseudouridine synthase C	-1.9	-1.9	-1.65	-1.52	0	0	2.69	0	0	-2.06	0	0
<i>tru D</i>	tRNA pseudouridine synthase D	-2.67	-3.08	-2.14	-2.44	-2.06	-2.15	1.92	-1.46	0	0	1.82	0
<i>tso A</i>	putative peroxiredoxin	0	-2.11	1.55	1.42	0	0	24.95	1.66	10.6	2.91	14.82	3.05

<i>tso B</i>	IRNA threonylcarbamoyladenosine biosynthesis protein TsaB	-2.55	-2.73	-1.75	-1.55	-1.99	-1.59	1.74	-2.02	1.52	0	1.73	0
<i>tso C</i>	Threonylcarbamoyl-AMP synthase	-2.37	-2.47	-1.6	-1.53	-1.68	-1.77	1.73	0	0	0	2.22	2.22
<i>tso E</i>	IRNA threonylcarbamoyladenosine biosynthesis protein TsaE	0	-1.34	0	0	0	0	1.57	1.65	1.96	0	0	2.4
<i>tsf</i>	Elongation factor Ts	-10.23	-18.4	-2.89	-4.56	-5.16	-7.35	1.24	-6.95	0	-1.98	1.88	-2.11
<i>tufA_1</i>	Elongation factor Tu 1	-14.9	-44.48	-2.47	-3.72	0	0	5.38	-3.59	7.84	0	0	0
<i>tufA_2</i>	Elongation factor Tu 1	-6.21	-11.71	0	0	0	0	7.5	-7.81	0	0	0	0
<i>tyr S</i>	Tyrosine-tRNA ligase	-2.54	-2.96	-1.49	-1.72	-1.83	-2.17	6.06	-1.41	1.96	0	2.9	0
<i>val S</i>	Valine-tRNA ligase	-2.81	-3.52	-1.58	-1.75	-1.77	-1.88	7.18	-1.71	0	-1.39	0	-1.58
<i>yba K</i>	Cys-tRNA(Pro)/Cys-tRNA(Cys) deacylase YbaK	0	0	0	1.38	0	0	-2.58	-1.69	0	0	-5.52	0
<i>ybe Y</i>	Endoribonuclease YbeY	-1.57	-1.47	-1.32	0	-1.37	-1.34	0	0	1.57	1.59	2.64	0
<i>yca O</i>	Ribosomal protein S12 methyltransferase accessory factor YcaO	-1.56	-1.53	0	0	1.4	1.44	1.72	-1.53	0	0	-2.2	0
<i>ych F</i>	Ribosome-binding ATPase YchF	-1.99	-1.62	-1.63	0	-1.73	-1.63	4.81	0	1.27	3.33	5.39	3.74
<i>yda F</i>	Putative ribosomal N-acetyltransferase YdaF	-1.53	-1.37	-1.45	-1.39	0	0	7.28	-1.75	1.64	0	2.64	0
<i>yef P</i>	Elongation factor P-like protein	0	0	0	0	0	0	7.53	0	0	0	0	0
<i>yfi C</i>	tRNA1(Val) (adenine(37)-N6)-methyltransferase	0	1.35	0	0	0	0	0	0	0	0	-2.05	0
<i>yhb Y</i>	RNA-binding protein YhbY	-2.72	-3.13	-1.82	-2.01	-1.94	-2.17	11.24	-7.84	3.14	7.53	3.95	3.95
<i>yfg O</i>	50S ribosomal protein L36 2	0	0	0	0	0	6.85	0	0	0	0	0	12.78
<i>acr R_2</i>	HTH-type transcriptional regulator AcrR	0	0	0	0	0	0	0	0	0	0	0	2.34
<i>ada</i>	Bifunctional transcriptional activator/DNA repair enzyme Ada	0	0	0	0	0	0	0	0	0	0	0	-2.23
<i>ail R</i>	HTH-type transcriptional repressor AilR	0	0	0	1.26	0	0	1.48	0	2	0	0	0
<i>ail S_1</i>	HTH-type transcriptional activator AilS	0	0	0	0	0	1.34	-3.84	0	-4.38	0	-4.68	0
<i>ail S_2</i>	HTH-type transcriptional activator AilS	1.27	1.26	0	1.29	0	0	0	0	-2.92	0	0	0
<i>arg R_1</i>	Arginine repressor	0	0	0	0	0	0	0	0	0	0	2.63	0
<i>arg R_2</i>	Arginine repressor	-1.41	-1.37	0	0	0	0	1.54	0	0	1.9	6.17	7.7
<i>asn C</i>	Regulatory protein AsnC	0	0	1.62	0	0	0	2.05	0	0	2.19	2.43	2.59
<i>bad R</i>	Transcriptional activatory protein BadR	0	0	1.67	2.12	0	1.88	0	2.23	0	0	0	4.16
<i>bae R</i>	Transcriptional regulatory protein BaeR	0	0	0	1.35	1.38	1.39	0	0	0	0	-2.55	0
<i>bas R</i>	Transcriptional regulatory protein BasR	-3.06	-3.65	-1.92	-2.37	-1.94	2.09	0	-2.04	0	-1.79	0	0
<i>bas S</i>	Sensor protein BasS	-2.57	-2.87	-1.9	-1.79	-1.85	-1.81	-1.75	-1.65	0	0	-1.66	-1.45
<i>bdc R</i>	HTH-type transcriptional repressor BdcR	1.98	2.08	1.54	1.92	1.46	1.51	0	1.78	0	0	0	0
<i>ben M</i>	HTH-type transcriptional regulator BenM	1.56	1.98	1.65	1.93	0	1.48	-3.22	1.4	5.74	0	-2.2	0
<i>ben M_2</i>	HTH-type transcriptional regulator BenM	0	-1.37	0	0	0	-1.34	3.99	0	0	0	0	0
<i>bet I</i>	HTH-type transcriptional regulator BetI	1.64	1.86	1.81	2.28	1.74	2.1	0	1.91	1.92	0	0	0
<i>bgJ</i>	Transcriptional activator protein BgJ	0	0	0	0	0	0	2.9	0	0	0	3.09	0
<i>cod C</i>	Transcriptional activator CodC	0	0	0	0	0	0	0	-2.05	0	0	0	1.6
<i>cda R_1</i>	Carbohydrate diacid regulator	1.27	0	1.33	1.34	0	0	0	1.46	0	0	0	0
<i>cda R_2</i>	Carbohydrate diacid regulator	0	0	-1.25	0	0	0	0	0	-2	0	-2.67	0
<i>chb R</i>	HTH-type transcriptional regulator ChbR	0	0	0	1.38	1.39	1.4	0	-2.03	1.96	0	1.68	0
<i>cit B</i>	Transcriptional regulatory protein CitB	0	0	0	0	0	0	0	-2.96	0	-2.81	0	0
<i>cmp R</i>	HTH-type transcriptional activator CmpR	0	0	0	0	0	0	2.42	0	2	0	1.69	0
<i>com R</i>	HTH-type transcriptional repressor ComR	1.41	1.44	1.53	1.56	1.54	1.58	5.35	7.38	1.82	2.36	0	2.12
<i>csg D</i>	CsgBAC operon transcriptional regulatory protein	0	0	2.08	1.89	1.77	0	0	0	0	0	0	0
<i>csp A</i>	Cold shock protein CspA	-11.1	-9.7	-5.58	-6.22	-5.19	-3.08	0	-2.28	5.74	-4.71	0	0
<i>csp D</i>	Cold shock-like protein CspD	-1.8	-1.9	0	0	0	0	0	0	2.04	0	8.08	7.8
<i>csp J</i>	Cold shock-like protein CspJ	-3.07	-4.47	-2.2	-1.84	-2.88	-1.83	0	0	0	0	0	0
<i>cue R</i>	HTH-type transcriptional regulator CueR	-1.72	-1.48	0	0	0	-1.28	0	-2.2	0	0	0	0
<i>cyn R_2</i>	HTH-type transcriptional regulator CynR	0	1.4	0	0	0	1.34	0	-2.29	-1.83	0	-1.98	0
<i>cyn R_3</i>	HTH-type transcriptional regulator CynR	0	0	1.61	0	0	1.54	4.7	0	0	0	0	0
<i>cys L</i>	HTH-type transcriptional regulator CysL	-2.09	-1.92	0	0	0	1.63	-1.8	-1.94	0	0	-2.54	1.84
<i>dau R</i>	Transcriptional regulator DauR	-3.07	-4.37	-2.72	-3.06	-2.57	-2.48	1.86	-1.77	0	0	1.85	0
<i>dec R</i>	DNA-binding transcriptional activator DecR	0	0	0	1.39	0	0	10.98	3.93	0	0	0	1.83
<i>deo R_1</i>	Deoxyribose operon repressor	0	0	0	-1.29	0	0	0	0	-2.56	0	0	0
<i>dml R_1</i>	HTH-type transcriptional regulator DmlR	0	0	0	1.23	1.48	1.85	0	0	-2.14	0	0	0
<i>dml R_2</i>	HTH-type transcriptional regulator DmlR	0	0	1.41	1.68	0	0	-1.75	0	0	0	-4.3	0
<i>dml R_3</i>	HTH-type transcriptional regulator DmlR	1.44	0	0	0	0	0	0	0	-2.39	0	0	0
<i>dml R_5</i>	HTH-type transcriptional regulator DmlR	0	0	0	0	0	0	2.86	0	0	0	-2.5	0
<i>dml R_6</i>	HTH-type transcriptional regulator DmlR	0	0	0	1.42	0	0	-1.87	0	-2.28	0	-1.87	0
<i>dml R_7</i>	HTH-type transcriptional regulator DmlR	0	1.48	0	0	0	1.35	2.63	0	-2.42	-2.16	-2.69	0
<i>dpl A</i>	Transcriptional regulatory protein DplA	-1.33	0	0	0	0	0	2.22	0	-3.01	-2.85	-1.85	-1.59
<i>ecp R</i>	HTH-type transcriptional regulator EcpR	0	0	0	0	0	0	0	0	0	0	0	2.74
<i>fab R</i>	HTH-type transcriptional repressor FabR	-1.39	-1.67	0	0	0	0	3.34	0	2.35	2.8	1.05	3.11
<i>frm R</i>	Transcriptional repressor FrmR	-1.5	0	0	0	0	0	2.22	0	0	0	0	0
<i>gab R_2</i>	HTH-type transcriptional regulatory protein GabR	1.51	1.51	0	1.26	0	0	-2.01	1.8	-3.55	0	0	0
<i>gad X_2</i>	HTH-type transcriptional regulator GadX	-1.78	0	0	0	0	0	1.57	0	0	0	0	0
<i>gal S_1</i>	HTH-type transcriptional regulator GalS	1.28	1.59	1.28	1.42	1.36	1.61	-2.19	0	0	0	0	0
<i>gbp R_1</i>	HTH-type transcriptional regulator GbpR	1.76	1.79	1.33	1.44	0	1.64	0	1.66	2.48	0	0	0
<i>gbp R_2</i>	HTH-type transcriptional regulator GbpR	1.81	1.96	0	1.45	0	1.38	0	0	0	0	0	0
<i>acv A</i>	Glycine cleavage system transcriptional activator	-1.82	-1.72	0	0	0	0	0	0	-5.89	0	0	0

	<i>glc_R_2</i>	HTH-type transcriptional repressor GlcR	0	1.48	0	0	-1.25	-1.42	2.51	0	0	0	2.06	1.58	
	<i>glc_R_3</i>	HTH-type transcriptional repressor GlcR	0	0	0	0	0	0	0	0	0	0	2.74	0	
	<i>glc_R_4</i>	HTH-type transcriptional repressor GlcR	-1.39	-1.38	0	0	0	0	-5.34	0	0	0	0	0	
	<i>glp_R_2</i>	Glycerol-3-phosphate regulon repressor	-1.5	-1.68	-1.42	-1.5	-1.38	-1.56	-1.99	-1.49	-1.64	2.14	0	0	
	<i>glp_R_3</i>	Glycerol-3-phosphate regulon repressor	0	0	0	1.34	0	0	0	0	0	0	-2.42	0	
	<i>gir_R_1</i>	Transcriptional regulatory protein GirR	0	0	0	0	-1.43	-1.41	-1.71	0	-1.69	0	-2.32	-1.55	
	<i>gir_R_2</i>	Transcriptional regulatory protein GirR	-1.65	-1.67	-1.25	0	0	0	0	0	-1.95	-2.26	0	0	
	<i>glt_C_1</i>	HTH-type transcriptional regulator GltC	-2.36	-2.33	-1.56	-1.43	-1.42	0	-1.98	0	0	0	-2.75	0	
	<i>glt_C_2</i>	HTH-type transcriptional regulator GltC	-3.65	-3.56	0	0	0	0	0	-4.78	6.17	5.01	10.42	3.12	
	<i>glt_C_3</i>	HTH-type transcriptional regulator GltC	0	0	0	0	0	0	0	0	-2.01	0	-2.58	0	
	<i>glt_R_1</i>	HTH-type transcriptional regulator GltR	0	1.43	1.82	2.46	0	1.52	0	0	0	2.14	0	0	
	<i>glt_R_2</i>	HTH-type transcriptional regulator GltR	0	0	1.34	1.3	0	0	1.7	1.51	0	2.54	0	0	
	<i>gnt_P</i>	High-affinity gluconate transporter	1.44	1.55	0	0	1.41	1.33	-2.29	-1.41	0	0	0	0	
	<i>gnt_R_1</i>	HTH-type transcriptional regulator GntR	0	0	0	0	0	0	1.64	0	-2.17	0	-2.89	0	
	<i>gnt_R_2</i>	HTH-type transcriptional regulator GntR	-1.39	-1.49	0	-1.23	0	0	1.39	-1.57	1.62	1.67	-4.08	0	
	<i>gnt_R_3</i>	HTH-type transcriptional regulator GntR	0	-1.37	0	0	0	0	-1.35	-2.16	0	0	0	0	
	<i>hca_R_1</i>	Hca operon transcriptional activator HcaR	0	0	0	0	0	0	0	1.42	0	0	-3.3	0	
	<i>hca_R_2</i>	Hca operon transcriptional activator HcaR	1.69	1.74	1.37	1.39	0	0	-1.77	0	-2.64	0	-5.64	-1.56	
	<i>iscR</i>	HTH-type transcriptional regulator IscR	-2.1	0	0	0	-2.32	0	6.45	6.28	6.91	11.85	8.11	1.81	
	<i>kdgR</i>	Transcriptional regulator KdgR	0	0	1.57	1.55	0	1.47	0	-1.63	2.19	0	2.64	0	
	<i>kdpE</i>	KDP operon transcriptional regulatory protein KdpE	0	0	0	0	0	1.54	0	-2.08	0	0	0	0	
	<i>leuO_2</i>	HTH-type transcriptional regulator LeuO	0	0	1.33	0	1.47	1.6	0	0	0	0	0	4.88	
	<i>licR</i>	putative licABCH operon regulator	0	0	0	0	0	0	-2.78	0	-2.07	0	-3.07	0	
	<i>lrp_2</i>	Leucine-responsive regulatory protein	-2.63	-2.61	0	-1.5	0	0	1.53	-2.93	-3.67	0	0	0	
	<i>lsrR</i>	Transcriptional regulator LsrR	-1.37	-1.38	0	0	0	0	0	0	0	0	3.72	0	
	<i>lutR_1</i>	HTH-type transcriptional regulator LutR	0	0	0	0	0	0	3.91	1.73	0	0	0	0	
	<i>malR</i>	HTH-type transcriptional regulator MalR	0	0	0	0	1.45	0	-2.24	0	-1.81	0	-1.96	0	
	<i>mcbR</i>	HTH-type transcriptional regulator McbR	0	1.85	2.17	2.54	1.49	1.76	0	0	0	0	0	0	
	<i>metJ</i>	Met repressor	0	0	0	0	0	0	3.08	0	0	0	0	0	
	<i>metR</i>	HTH-type transcriptional regulator MetR	0	0	0	0	0	0	0	0	0	0	-2.24	0	
	<i>modE</i>	Transcriptional regulator ModE	0	0	0	0	0	0	2.78	0	1.82	1.13	2.68	0	
	<i>mprA</i>	Transcriptional repressor MprA	-2.08	-2.21	0	-1.34	0	-1.45	0	0	0	1.55	1.76	1.98	
	<i>mraZ</i>	Transcriptional regulator MraZ	-2.9	-3.09	0	0	-1.76	-1.54	1.56	-1.86	1.76	1.54	2.76	0	
	<i>murR</i>	HTH-type transcriptional regulator MurR	0	1.25	1.29	0	0	0	3.79	-1.57	-4.05	0	-3.3	0	
	<i>nag_C_1</i>	N-acetylglucosamine repressor	0	1.42	1.44	1.54	0	1.34	-4	0	-4.18	1.34	-2.08	-2.08	
	<i>nag_C_2</i>	N-acetylglucosamine repressor	0	0	0	0	0	0	1.52	1.74	2.91	1.67	1.43	0	
	<i>narW</i>	putative nitrate reductase molybdenum cofactor assembly chaperone NarW	1.91	2.3	1.49	1.71	1.62	1.88	-1.84	1.41	0	0	-2.06	0	
	<i>nemR</i>	HTH-type transcriptional repressor NemR	0	1.44	1.37	1.59	0	0	2.52	2.23	0	2.08	0	0	
	<i>nhaR</i>	Transcriptional activator protein NhaR	-1.57	-1.57	0	0	0	0	18.55	17.98	1.72	5.03	2.61	2.58	
	<i>nimR</i>	HTH-type transcriptional regulator NimR	0	1.51	0	1.64	1.47	1.57	0	0	-2.99	0	-2.15	0	
	<i>nrpR</i>	Transcriptional repressor NrpR	-1.6	-1.8	0	0	0	0	3.22	1.87	3.41	2.01	4.78	1.79	
	<i>nsrR</i>	HTH-type transcriptional repressor NsrR	-6.13	-6.46	0	-1.49	0	-1.43	0	-2.36	0	1.74	2.36	1.72	
	<i>pdh_R_1</i>	Pyruvate dehydrogenase complex repressor	-6.58	-8.01	0	1.32	1.33	1.41	-2.42	-6.98	0	0	0	0	
	<i>pdh_R_3</i>	Pyruvate dehydrogenase complex repressor	-1.68	-1.62	-2.88	-3.71	-4.07	-4.34	0	0	2.64	0	2.86	0	
	<i>perR</i>	HTH-type transcriptional regulator PerR	0	0	0	0	0	0	-2.04	1.62	-2.06	0	-3.53	-1.72	
	<i>phoB</i>	Phosphate regulon transcriptional regulatory protein PhoB	-1.58	-1.82	1.53	1.51	0	0	4.88	0	5.91	7.48	4.13	6.08	
	<i>prpR</i>	Propionate catabolism operon regulatory protein	1.5	1.6	0	0	1.46	1.41	-1.77	2.01	0	0	-2.29	0	
	<i>pspC</i>	Phage shock protein C	1.94	3.43	3.04	3.98	5.88	3.86	0	3.35	0	0	3.98	2.34	4.08
	<i>pspF</i>	Psp operon transcriptional activator	1.6	1.84	1.54	2.01	1.58	1.65	0	1.83	0	0	0	0	
	<i>puuR</i>	HTH-type transcriptional regulator PuuR	0	0	0	0	0	1.55	0	0	0	0	-3.09	0	
	<i>rcdA</i>	HTH-type transcriptional regulator RcdA	0	1.36	1.51	1.6	0	1.32	-2.4	0	0	0	0	0	
	<i>rciR_1</i>	RCS-specific HTH-type transcriptional activator RciR	1.66	1.6	0	0	0	0	-2.12	0	-2.16	0	-1.99	0	
	<i>rciR_2</i>	RCS-specific HTH-type transcriptional activator RciR	0	0	0	1.42	0	0	2.47	17.95	0	0	-2.24	0	
	<i>rcnR</i>	Transcriptional repressor RcnR	0	0	1.84	0	0	0	2.22	0	0	0	0	0	
	<i>rcsB_2</i>	Transcriptional regulatory protein RcsB	0	1.76	0	0	1.34	1.37	0	2.99	0	0	0	0	
	<i>rcsB_3</i>	Transcriptional regulatory protein RcsB	-2.88	0	0	0	0	0	7.26	0	0	0	0	0	
	<i>rcsB_4</i>	Transcriptional regulatory protein RcsB	0	1.48	0	0	0	0	0	2.89	0	0	0	0	
	<i>rhaS</i>	HTH-type transcriptional activator RhaS	0	0	0	0	1.33	1.35	0	0	-5.08	0	-2.48	0	
	<i>rhmR_1</i>	putative HTH-type transcriptional regulator RhmR	1.65	1.72	0	0	1.36	1.37	-2.49	0	0	0	0	0	
	<i>rhmR_2</i>	putative HTH-type transcriptional regulator RhmR	0	0	0	1.63	0	0	2.27	1.85	0	0	0	0	
	<i>rocR</i>	Arginine utilization regulatory protein RocR	1.3	1.43	0	1.31	0	1.34	1.3	1.47	-3.4	0	-2.26	0	
	<i>rspR_2</i>	HTH-type transcriptional repressor RspR	0	0	0	0	0	0	0	0	3.08	0	0	0	
	<i>rstA</i>	Transcriptional regulatory protein RstA	0	0	1.41	1.78	0	0	-3.59	-2.49	-18.16	0	-3.97	0	
	<i>rutR</i>	HTH-type transcriptional regulator RutR	0	1.41	0	0	1.48	1.42	0	0	0	0	-12.23	0	
	<i>sgrR_1</i>	HTH-type transcriptional regulator SgrR	1.46	1.45	0	0	0	0	0	0	0	0	-3	0	
	<i>sgrR_2</i>	HTH-type transcriptional regulator SgrR	1.52	1.61	0	0	0	0	-1.77	1.47	-2.06	-1.63	-2.1	0	
	<i>sgrR_3</i>	HTH-type transcriptional regulator SgrR	0	0	1.25	1.29	0	0	-2.87	-1.64	-1.7	0	0	0	
	<i>slyA_1</i>	Transcriptional regulator SlyA	1.4	1.69	0	0	0	0	0	1.76	3.11	1.94	6.19	3.8	

Reguladores
transcripcionais

<i>slyA_2</i>	Transcriptional regulator SlyA	-1.87	-2.36	0	-2.05	1.63	1.69	-4.06	0	0	0	0	0	0
<i>srfR_1</i>	Glucitol operon repressor	0	1.37	1.36	1.67	1.54	1.76	-2.06	0	0	0	0	0	0
<i>srfR_2</i>	Glucitol operon repressor	1.92	-2.27	1.41	1.69	0	1.41	0	0	0	0	0	0	0
<i>srfR_3</i>	Glucitol operon repressor	0	0	0	0	0	0	-4.19	1.54	-2.04	-2.07	0	0	0
<i>srfR_4</i>	Glucitol operon repressor	0	0	0	0	0	0	1.59	1.45	1.82	0	-4.06	1.66	0
<i>sutR_1</i>	HTH-type transcriptional regulator SutR	1.73	-2.03	0	0	0	0	0	0	0	1.65	0	0	0
<i>sutR_2</i>	HTH-type transcriptional regulator SutR	1.37	1.78	0	1.57	0	1.82	-2.64	-0.88	0	0	0	0	0
<i>tauR</i>	HTH-type transcriptional regulator TauR	1.43	1.55	0	0	1.38	1.43	0	0	-2.09	-2.25	-2.99	0	0
<i>tctD</i>	Transcriptional regulatory protein tctD	1.35	1.41	0	0	0	1.42	-3.87	0	0	0	0	0	0
<i>torR</i>	TorCAD operon transcriptional regulatory protein TorR	0	-1.53	0	0	0	0	-1.6	-1.59	-2.74	0	-2.32	0	0
<i>treR</i>	HTH-type transcriptional regulator TreR	1.27	0	0	0	0	0	0	-2.95	-2.19	0	-3.95	0	0
<i>tyrR</i>	Transcriptional regulatory protein TyrR	0	0	0	1.35	0	1.31	-2.65	1.61	1.43	0	-2.06	1.54	0
<i>uhpA</i>	Transcriptional regulatory protein UhpA	-1.49	-1.36	0	0	0	0	-2.47	0	-3.77	0	-2.31	-1.74	0
<i>ulaR</i>	HTH-type transcriptional regulator UlaR	0	0	0	0	0	0	-1.82	0	-2.14	0	-1.72	0	0
<i>xyfR</i>	Xylose operon regulatory protein	0	0	0	0	0	0	-2.3	1.75	-1.88	0	-2.11	0	0
<i>ybdO_2</i>	putative HTH-type transcriptional regulator YbdO	0	0	-2.21	0	-2.61	0	0	0	0	0	0	0	0
<i>ybdO_3</i>	putative HTH-type transcriptional regulator YbdO	1.38	1.43	-2.68	0	0	0	-3.23	0	0	0	0	0	0
<i>ybdO_4</i>	putative HTH-type transcriptional regulator YbdO	0	0	1.54	0	0	0	-2.92	0	0	0	-2.31	-1.5	0
<i>ydeO_1</i>	HTH-type transcriptional regulator YdeO	-8.38	-7.7	0	-1.93	-3.31	-2.06	0	-2.55	0	0	0	0	0
<i>ydeO_2</i>	HTH-type transcriptional regulator YdeO	-2.3	-2.19	1.31	0	1.53	0	-2.91	0	-2.44	1.61	-2.99	0	0
<i>ydgT</i>	Transcription modulator YdgT	-2.69	-2.45	0	0	0	0	0	0	0	0	0	0	0
<i>yebC</i>	putative transcriptional regulatory protein YebC	-3.14	-3.81	0	-1.34	-2.22	-2.37	0	-3.07	0	-2.42	1.59	-1.68	0
<i>yedW</i>	putative transcriptional regulatory protein YedW	0	0	0	1.38	0	1.39	0	0	-2.33	0	0	0	0
<i>yehT</i>	Transcriptional regulatory protein YehT	-1.39	-1.36	0	0	-1.35	-1.46	-1.75	-1.47	-2	0	0	0	0
<i>ygaV</i>	putative HTH-type transcriptional regulator YgaV	0	0	0	0	1.59	1.69	-3.02	-2.52	-3.1	-3.19	-4.24	0	0
<i>yhaJ_1</i>	HTH-type transcriptional regulator YhaJ	0	1.53	1.3	0	1.37	1.54	1.48	0	0	0	-3.21	-1.53	0
<i>yhbB_2</i>	Putative HTH-type transcriptional regulator YhbB	0	0	1.49	1.41	0	0	0	0	-2.91	-2.04	0	0	0
<i>yidZ</i>	HTH-type transcriptional regulator YidZ	0	0	0	0	0	0	-1.75	0	-2.37	0	-3.31	0	0
<i>yjiE_2</i>	HTH-type transcriptional regulator YjiE	0	0	0	0	0	0	0	0	-2.92	0	0	0	0
<i>yqjI</i>	Transcriptional regulator YqjI	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>yvoA_1</i>	HTH-type transcriptional repressor YvoA	0	0	0	0	0	0	-2.78	-2.28	1.73	-5.28	-2.68	-2.58	0
<i>yvoA_3</i>	HTH-type transcriptional repressor YvoA	0	0	0	0	0	0	-2.16	-2.3	1.84	1.82	0	0	0
<i>zraR</i>	Transcriptional regulatory protein ZraR	0	0	-1.37	-1.32	0	0	0	0	0	0	-4.6	0	0
<i>cho</i>	Excinuclease cho	1.54	1.49	1.41	0	1.71	1.53	-3.66	-3.23	0	0	0	0	0
<i>dam</i>	DNA adenine methylase	1.37	1.52	0	0	-2.85	-3.45	0	1.51	0	0	-1.7	-1.58	0
<i>dam_2</i>	DNA adenine methylase	-3.77	-4.79	-2.02	-2.37	0	0	0	-1.77	-2.31	-2.03	0	0	0
<i>dinB</i>	DNA polymerase IV	0	0	0	0	0	0	0	0	0	0	0	0	1.62
<i>dinB1</i>	DNA polymerase IV 1	1.58	1.57	0	0	1.39	1.48	2.4	-13.78	0	0	-2.56	-2.29	0
<i>dinI_1</i>	DNA damage-inducibile protein I	0	0	0	0	0	0	-2.05	0	0	0	0	0	-3.18
<i>dnaA</i>	Chromosomal replication initiator protein DnaA	-1.92	-2.61	-1.68	-1.67	-1.58	-1.82	-4.65	0	-3.71	-2.94	-3.47	-2.47	0
<i>dnaB</i>	Replicative DNA helicase	-2.07	-2.3	-1.48	-1.36	0	0	-2.13	-1.7	0	0	-3.06	-4.58	0
<i>dnaC</i>	DNA replication protein DnaC	-1.4	-1.6	0	0	0	-1.31	-3.83	0	1.7	1.77	1.8	1.53	0
<i>dnaE</i>	DNA polymerase III subunit alpha	-3.47	-4.02	-2.18	-2.3	-2.77	-3.06	0	-1.96	-2.33	-3.19	-2.28	-1.75	0
<i>dnaG</i>	DNA primase	-3.09	-3.92	-1.79	-1.87	-2.52	-2.38	0	-1.7	0	0	0	0	0
<i>dnaK_1</i>	Chaperone protein DnaK	0	-1.95	0	1.4	1.36	1.46	-4.43	0	0	0	0	0	0
<i>dnaK_2</i>	Chaperone protein DnaK	0	0	0	0	0	0	1.09	0	-4.94	3.11	-5.08	1.66	0
<i>dnaN</i>	DNA polymerase III subunit beta	-1.66	-2.08	-1.67	-1.94	-1.63	-1.94	-2.36	0	3.1	2.29	3.43	1.54	0
<i>dnaQ</i>	DNA polymerase III subunit epsilon	0	0	0	0	0	0	-2.16	0	0	0	-10.56	0	0
<i>dnaT</i>	Primosomal protein 1	0	-1.5	-1.51	-1.47	1.53	1.56	-3.23	0	1.94	-2.28	1.81	0	0
<i>dnaX</i>	DNA polymerase III subunit tau	-3.74	-3.63	-2.23	-2.01	-2.71	-2.77	1.61	-1.56	1.45	0	0	0	0
<i>exoX</i>	Exodeoxyribonuclease 10	0	0	1.57	1.65	1.43	1.62	-12.84	-42.25	0	0	-2.58	0	0
<i>fis</i>	DNA-binding protein Fis	-4.87	-4.93	0	0	0	0	-2.05	-6.93	-3.07	-3.34	0	0	0
<i>gyrA</i>	DNA gyrase subunit A	-3.03	-4.23	-1.49	-1.94	-1.7	-2.22	-13.93	0	5.33	3.4	6.97	3.08	0
<i>gyrB</i>	DNA gyrase subunit B	-2.26	-3.08	-1.51	-1.8	-1.74	-2.12	-3.51	-4.08	4.6	3.75	4.31	-2.01	0
<i>hns_1</i>	DNA-binding protein H-NS	0	0	0	0	0	0	0	-6.89	-1.1	0	0	0	0
<i>hslB</i>	DNA polymerase III subunit delta'	-2.34	-2.04	-1.43	-1.28	-1.45	-1.34	0	-1.46	-2.32	0	0	0	0
<i>hslD</i>	DNA polymerase III subunit psi	-1.71	-1.69	0	-1.56	-1.72	-1.42	-2.07	0	1.84	1.68	1.63	0	0
<i>hslE</i>	DNA polymerase III subunit theta	0	0	0	0	0	0	1.45	-15.08	0	0	0	0	0
<i>hupA</i>	DNA-binding protein HU-alpha	-4.73	-6.6	-1.81	-2.58	-2.72	-3.41	3.38	-2.04	0	-2.39	-4.96	0	0
<i>hupB</i>	DNA-binding protein HU-beta	0	-1.98	0	-1.76	-1.76	-1.95	-3.57	0	0	-6.4	0	0	0
<i>ihfA</i>	Integration host factor subunit alpha	-2.9	-3.93	0	-1.59	0	0	1.88	-3.52	0	0	-2.39	0	0
<i>ihfB</i>	Integration host factor subunit beta	-2.22	-1.81	0	0	0	0	-2.96	0	-2.14	0	0	0	0
<i>mutH</i>	DNA mismatch repair protein MutH	0	0	0	1.37	0	0	0	0	0	0	-2.12	0	0
<i>mutL</i>	DNA mismatch repair protein MutL	-1.6	0	-1.63	-1.59	-1.45	-1.58	-2.49	0	1.55	-2.06	1.58	0	0
<i>mutT</i>	8-oxo-dGTP diphosphatase	0	0	0	0	0	0	-2.35	0	0	0	0	0	0
<i>nfi</i>	Endonuclease V	-1.26	-1.39	0	0	0	-1.42	-3.17	0	3.93	0	-3.28	1.52	0
<i>parC</i>	DNA topoisomerase 4 subunit A	-1.98	-2.05	-1.31	-1.48	-1.52	-1.63	0	-1.45	1.52	0	0	0	0
<i>parE</i>	DNA topoisomerase 4 subunit B	-2.12	-2.54	-1.85	-2.01	-1.91	-2.03	1.65	1.39	1.47	0	1.7	0	0

Replicação, Recombinação Homóloga e Reparo do DNA	<i>phr B</i>	Deoxyribodipyrimidine photo-lyase	1.4	1.67	1.29	1.54	1.56	1.66	2.78	3.58	0	0	1.52	0	0	
	<i>pol A</i>	DNA polymerase I	-1.7	-2.11	-1.36	-1.51	-1.32	-1.44	-2.07	0	-2.06	-1.82	0	-1.38	0	0
	<i>pol B</i>	DNA polymerase II	0	0	0	0	0	0	0	6.3	3.58	1.74	-3.74	1.55	-3.18	0
	<i>pri B</i>	Primosomal replication protein N	-6.78	-16.21	-1.5	-3.2	-3.58	-4.32	0	0	-7.4	0	-1.62	2.18	0	0
	<i>rad A</i>	DNA repair protein RadA	0	0	-1.36	0	0	0	0	-2.28	7.64	0	-1.92	-1.79	0	0
	<i>rdg C_2</i>	Recombination-associated protein RdgC	-1.4	-1.29	0	0	0	0	1.45	0	2.13	0	0	0	0	0
	<i>rec A</i>	Protein RecA	-2.54	-3.18	-1.49	-1.53	0	-1.71	174.1	0.98	23.6	15.32	44.32	3.78	0	0
	<i>rec B</i>	RecBCD enzyme subunit RecB	-1.64	-1.93	-1.5	-1.59	-1.66	-1.77	0	-1.56	-3.63	-2.22	-3.19	-2.05	0	0
	<i>rec C</i>	RecBCD enzyme subunit RecC	0	0	0	0	0	0	-1.81	0	-1.62	-1.53	-2.31	-1.48	0	0
	<i>rec D</i>	RecBCD enzyme subunit RecD	0	0	0	-1.31	0	-1.4	-5	-1.51	-5.54	-2.54	-16.64	-1.92	0	0
	<i>rec E_1</i>	Exodeoxyribonuclease 8	1.8	1.89	0	0	1.54	1.65	-3.54	0	0	0	-1.5	0	0	0
	<i>rec E_2</i>	Exodeoxyribonuclease 8	1.72	1.8	0	0	1.42	1.6	0	0	0	0	0	-2.34	0	0
	<i>rec F_1</i>	DNA replication and repair protein RecF	-1.75	-2.17	1.26	1.29	-1.64	-1.78	0	0	0	0	0	0	0	0
	<i>rec F_2</i>	DNA replication and repair protein RecF	0	0	-1.64	-1.97	0	0	-2.43	-1.42	1.45	1.44	0	0	0	0
	<i>rec G</i>	ATP-dependent DNA helicase RecG	-2.15	-2.28	-1.36	-1.44	-1.57	-1.73	-2.82	-1.8	-3.84	-2.18	-3.08	-1.96	0	0
	<i>rec J</i>	Single-stranded-DNA-specific exonuclease RecJ	-2.14	-2.53	-1.91	-2.42	-1.98	-2.1	2.51	0	0	-1.81	1.78	-1.73	0	0
	<i>rec N</i>	DNA repair protein RecN	1.26	1.24	0	0	0	0	10.69	4.28	3.57	6.38	5.68	3.75	0	0
	<i>rec O</i>	DNA repair protein RecO	-1.9	-1.87	-1.51	-1.5	-1.8	-1.49	-2.39	-1.93	-1.95	-2.43	-1.78	-2.13	0	0
	<i>rec R</i>	Recombination protein RecR	0	-3.19	-2.33	-2.33	-2.28	-2.73	1.75	-1.5	0	0	0	-1.79	0	0
	<i>rec X</i>	Regulatory protein RecX	0	0	0	0	0	0	20.6	9.78	3	2.29	2.09	0	0	0
	<i>rep_1</i>	ATP-dependent DNA helicase Rep	0	-1.27	0	0	0	0	-2.23	-1.65	0	0	0	0	0	0
	<i>rep_2</i>	ATP-dependent DNA helicase Rep	0	0	0	0	0	0	-1.69	0	-3.03	0	0	0	0	0
	<i>rnh B</i>	Ribonuclease HII	-8.82	-4.38	-2.2	-2.28	-3.12	-3.35	0	-2.37	0	-2.63	-2.72	-2.24	0	0
	<i>ruv A</i>	Holliday junction ATP-dependent DNA helicase RuvA	0	-1.34	0	0	0	0	2.95	0	0	0	3.42	0	0	0
	<i>ruv B</i>	Holliday junction ATP-dependent DNA helicase RuvB	0	-1.32	0	0	-1.28	-1.32	2.48	3.04	0	0	1.58	0	0	0
	<i>ruv C</i>	Crossover junction endodeoxyribonuclease RuvC	-2.47	-2.34	0	0	-1.69	-1.72	-1.92	-2.06	-1.76	-3.99	0	-2.03	0	0
	<i>sbm C</i>	DNA gyrase inhibitor	0	0	0	0	1.44	1.52	11.93	1.21	16.3	1.92	4.84	1.74	0	0
	<i>seq A</i>	Negative modulator of initiation of replication	-2.86	-2.78	-1.71	-1.86	-2.53	2.47	3.18	0	2.38	1.67	3.47	0	0	0
	<i>ssb</i>	Single-stranded DNA-binding protein	-1.8	-1.91	0	0	0	0	12.29	0	6.99	3.41	0	0	0	0
	<i>ssb_1</i>	Single-stranded DNA-binding protein	0	0	0	0	0	-1.5	0	0	0	0	0	0	0	0
	<i>ssb_2</i>	Single-stranded DNA-binding protein	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	<i>topA_1</i>	DNA topoisomerase I	-2.66	-3.17	0	0	-1.75	-1.71	3	0	0	-1.66	0	-1.94	0	0
	<i>topA_2</i>	DNA topoisomerase I	-2.5	-2.52	-1.81	-1.75	-1.72	-1.68	1.55	0	0	0	2.92	2.46	0	0
	<i>tus</i>	DNA replication terminus site-binding protein	1.53	2.13	1.82	1.97	1.52	1.88	1.13	3.21	1.87	1.92	2.4	1.91	0	0
	<i>uvr A</i>	UvrABC system protein A	0	0	0	0	0	0	5.4	0	3.39	3.34	2.45	0	0	0
	<i>uvr B</i>	UvrABC system protein B	-1.53	-1.48	0	0	0	-1.34	6.29	1.64	1.88	6.18	3.98	3.08	0	0
<i>uvr D</i>	DNA helicase II	-1.94	-2.2	-1.45	-1.42	-1.58	-1.87	1.67	-1.45	1.66	1.69	1.59	0	0	0	
<i>vsr</i>	Very short patch repair protein	0	0	0	0	0	0	-3.17	0	0	0	0	0	0	0	
<i>xse A</i>	Exodeoxyribonuclease 7 large subunit	0	0	0	0	0	0	-2.55	0	-1.76	0	0	0	0	0	
<i>xse B</i>	Exodeoxyribonuclease 7 small subunit	-1.42	0	0	0	0	0	2.39	0	2.78	0	0	0	0	0	
<i>ygb T</i>	CRISPR-associated endonuclease Cas1	1.33	0	0	0	0	0	0	0	-2.41	0	2.12	0	0	0	
<hr/>																
Divisão celular	<i>cbp A</i>	Curved DNA-binding protein	0	0	0	1.26	1.56	1.41	2.36	0	2.7	2.18	1.87	1.62	0	0
	<i>cbp M</i>	Chaperone modulatory protein CbpM	1.58	1.61	0	1.68	2.1	2.33	1.93	0	3.13	0	2.88	0	0	0
	<i>clp X_2</i>	ATP-dependent Clp protease ATP-binding subunit ClpX	-2.88	-3.18	-1.94	-1.83	-1.9	-1.97	4.67	-1.92	2.73	1.79	4.08	2.04	0	0
	<i>cpo B</i>	Cell division coordinator CpoB	3.7	-3.81	-2.53	-2.88	-3.35	3.22	-1.44	-3.3	-1.93	-3.05	0	-2.02	0	0
	<i>dam X</i>	Cell division protein DamX	-4.49	-6.17	-2.2	-2.72	-2.77	-3.23	1.98	0	1.37	0	1.66	0	0	0
	<i>ded D</i>	Cell division protein DedD	-1.97	-2.02	-1.54	-1.57	-1.79	-1.89	0	-1.49	0	0	-1.99	-2	0	0
	<i>dia A</i>	DnaA initiator-associating protein DiaA	-2.34	-2.86	0	-1.34	0	0	1.46	1.74	0	0	0	0	0	0
	<i>eng B</i>	putative GTP-binding protein EngB	-1.33	-1.3	0	0	0	-1.39	2.93	-1.62	1.95	1.79	1.96	1.69	0	0
	<i>env C</i>	Murein hydrolase activator EnvC	-1.99	-2.24	-1.66	-1.56	-1.52	-1.67	0	0	0	0	0	0	0	0
	<i>fts A_1</i>	Cell division protein FtsA	-2.68	-3.37	-2.12	-2.9	3.98	1.31	2.08	0	-1.41	0	0	-1.58	0	0
	<i>fts A_2</i>	Cell division protein FtsA	2.47	1.64	3.29	0	-2.28	-2.53	-2.84	0	-3.61	0	0	0	0	0
	<i>fts B</i>	Cell division protein FtsB	-4.95	-5.5	-2.52	-2.31	-2.79	-2.98	2.15	-1.69	2	0	3.34	-1.91	0	0
	<i>fts E</i>	Cell division ATP-binding protein FtsE	-1.83	-2.07	0	-1.37	-1.45	-1.56	3.08	0	1.54	1.59	0	0	0	0
	<i>fts H</i>	ATP-dependent zinc metalloprotease FtsH	-3.03	-4.41	-1.8	-2.08	0	0	10.66	-3.82	1.61	3.91	0	0	0	0
	<i>fts H_1</i>	ATP-dependent zinc metalloprotease FtsH	0	0	0	0	0	0	0	0	0	0	6.4	2.25	0	0
	<i>fts H_2</i>	ATP-dependent zinc metalloprotease FtsH	0	0	0	0	-2.38	-2.99	0	0	0	0	4.28	2.15	0	0
	<i>fts K</i>	DNA translocase FtsK	-3.2	-3.42	-1.72	-2.05	-2.26	-2.41	1.51	-1.79	-1.53	-2.54	0	-1.87	0	0
	<i>fts L</i>	Cell division protein FtsL	-2.73	-2.99	-1.83	-1.62	-1.77	-1.92	0	0	-1.86	0	0	0	0	0
	<i>fts Q</i>	Cell division protein FtsQ	-2.68	-3.48	-2.41	-2.59	-2.53	-2.51	0	-1.42	-2.53	-2.24	-1.89	-1.95	0	0
	<i>fts W</i>	putative peptidoglycan glycosyltransferase FtsW	-2.79	-3.92	-2.3	-2.45	-2.74	2.93	-1.77	-2.94	-5.13	-3.93	-3.29	-2.14	0	0
	<i>fts X</i>	Cell division protein FtsX	-1.53	-1.73	0	0	-1.42	-1.42	2.91	0	0	0	0	0	0	0
	<i>fts Z</i>	Cell division protein FtsZ	-2.06	-3.9	-1.36	-2.18	-1.67	-2.32	4.17	0	1.82	0	3.09	1.57	0	0
	<i>lon_1</i>	Lon protease	-2.54	-1.59	-1.62	-1.53	-1.76	-1.63	-1.59	-1.56	-2.13	-1.7	-2.14	0	0	0
	<i>lon_2</i>	Lon protease	-1.65	-2.18	0	0	0	-1.5	1.57	-1.71	4.04	1.83	4.49	0	0	0
	<i>lrp</i>	Leucine-responsive regulatory protein	1.57	1.68	0	-1.5	-1.81	-1.95	1.53	1.78	0	0	2.28	0	0	0
	<i>min C</i>	Septum site-determining protein MinC	-2.21	-2.21	-1.59	-1.8	-1.86	-1.8	0	2.97	-1.7	-2.5	0	0	0	0
	<i>min D</i>	Septum site-determining protein MinD	-2.16	-2.15	0	-1.43	-1.45	-1.72	1.91	-3.04	0	-2.36	0	-1.85	0	0

<i>min E</i>	Cell division topological specificity factor	0	0	0	0	0	0	0	-6.38	0	0	0	0
<i>mrd B</i>	Peptidoglycan glycosyltransferase MrdB	-1.37	-1.3	1.36	1.28	0	0	-2.3	0	-1.87	0	-1.65	0
<i>mre B</i>	Rod shape-determining protein MreB	-2.86	-3.35	-2.35	-2.48	-2.4	-2.68	2.02	-1.83	0	0	2.02	0
<i>mre C</i>	Cell shape-determining protein MreC	-2.82	-3.12	-2.52	-2.52	-2.38	-2.74	0	-1.75	-1.88	0	-1.61	-1.41
<i>mre D</i>	Rod shape-determining protein MreD	-2.64	-3.19	-1.82	-1.81	-1.97	-2.19	0	0	-2.02	-2.04	-2.38	-1.68
<i>muk B</i>	Chromosome partition protein MukB	-3.05	-3.33	-1.84	-1.92	2.23	2.31	0	0	-2.36	0	0	-2.15
<i>muk E</i>	Chromosome partition protein MukE	-2.9	-3.17	-1.74	-1.76	-2.2	-2.45	2.35	2	1.53	0	1.81	0
<i>muk F</i>	Chromosome partition protein MukF	-2.04	-2.16	-1.63	-1.42	-1.68	-1.8	2.29	1.65	1.56	0	1.62	0
<i>mur G</i>	UDP-N-acetylglucosamine-N-acetylmuramyl- (pentapeptide) pyrophosphoryl-undecaprenol	-2.9	-4.13	-2.58	-2.58	-2.55	-3.24	-2.2	-2.41	-3.54	-4.1	-3.03	-3.1
<i>rob_2</i>	Right origin-binding protein	-1.85	-1.94	0	0	0	0	2.92	0	2.43	1.89	1.81	0
<i>rse P</i>	Regulator of sigma-E protease RseP	-3.85	-4.95	-3.31	-3.87	-3.35	-3.77	1.76	-1.63	0	-1.49	1.46	0
<i>slm A</i>	Nucleoid occlusion factor SlmA	-2.41	-2.12	0	-1.26	-1.6	-1.7	3.73	0	3.08	2.33	1.84	0
<i>stp A</i>	DNA-binding protein StpA	-1.71	0	0	0	0	0	0	2.54	0	0	0	0
<i>sul A</i>	Cell division inhibitor SulA	-1.5	0	0	0	0	1.3	30.63	11.18	-6.87	11.83	14.01	10.11
<i>xer C</i>	Tyrosine recombinase XerC	-1.86	-2.12	-1.79	-1.81	-1.85	-2.2	0	-1.53	-1.65	-2.18	0	0
<i>xer D_1</i>	Tyrosine recombinase XerD	-1.42	-1.43	-1.31	-1.46	0	0	5.01	6.28	2.05	0	39.16	7.48
<i>xer D_2</i>	Tyrosine recombinase XerD	-1.81	-1.96	0	0	0	0	1.65	1.57	0	0	0	0
<i>xer D_3</i>	Tyrosine recombinase XerD	0	0	0	0	-1.58	-1.71	0	0	0	0	3.34	0
<i>yej K</i>	Nucleoid-associated protein YejK	-1.89	-1.85	-1.38	0	0	0	3.28	-1.52	1.81	1.52	2.69	1.66
<i>zap A</i>	Cell division protein ZapA	-3.91	-4.08	0	0	-1.89	2.01	1.65	-2.85	1.7	1.96	2.45	1.86
<i>zap B</i>	Cell division protein ZapB	0	-1.36	0	0	0	0	2.35	0	1.87	0	0	0
<i>zap C</i>	Cell division protein ZapC	0	0	0	1.5	0	0	-2.23	0	0	0	0	0
<i>zap D</i>	Cell division protein ZapD	0	-1.31	0	0	0	-1.22	-2.59	0	0	0	0	0
<i>zap E</i>	Cell division protein ZapE	-1.94	-2.16	-1.47	-1.49	-1.59	-1.58	2.43	-1.39	1.51	1.47	2.1	0
<i>zip A</i>	Cell division protein ZipA	-2.39	-2.64	-1.45	-1.38	-1.7	-1.66	1.71	-2.28	0	-2.36	0	0
<i>ace E</i>	Pyruvate dehydrogenase E1 component	-14.3	-39.88	-6.66	-25.94	-8.92	-18.3	0	-2.81	0	-5.3	0	-3.92
<i>ace F</i>	Dihydropyridoxyl-lysine-residue acetyltransferase component of pyruvate dehydrogenase complex	-14.42	-76.68	-7.65	-17.88	-8.06	-15.47	-2.08	-3.51	-1.9	6.79	-1.85	-2.52
<i>acn A</i>	Aconitate hydratase A	0	1.92	1.81	1.86	1.9	1.81	0	0	3.8	12.38	1.57	-3.57
<i>acn B</i>	Aconitate hydratase B	0	-1.48	0	0	0	-1.72	0	-1.55	2.76	1.72	1.23	0
<i>acs_1</i>	Acetyl-coenzyme A synthetase	0	0	0	0	0	0	-3.28	-1.39	0	0	0	-1.41
<i>acs_2</i>	Acetyl-coenzyme A synthetase	0	0	1.23	1.18	0	0	-1.56	-0.88	-1.65	0	-1.68	0
<i>adh E_1</i>	Aldehyde-alcohol dehydrogenase	-1.57	-1.84	0	1.34	0	0	2.94	0	-6.04	0	0	-1.5
<i>adh E_2</i>	Aldehyde-alcohol dehydrogenase	2.11	1.91	0	1.42	2.65	0	2.89	0	0	0	0	-1.51
<i>adh E_3</i>	Aldehyde-alcohol dehydrogenase	2.15	1.88	0	1.27	0	0	1.51	5.4	1.59	-1.48	2.14	-3.95
<i>adh E_4</i>	Aldehyde-alcohol dehydrogenase	3.54	1.45	3.63	0	1.52	1.51	-2.99	0	0	-2.82	-2.57	-1.58
<i>adh P</i>	Alcohol dehydrogenase, propanol-preferring	1.73	1.37	3.69	1.86	1.57	1.48	1.81	0	0	0	0	-1.77
<i>agg_1</i>	Glucose-1-phosphatase	1.83	2.11	0	0	0	0	0	0	5.23	1.94	0	0
<i>agg_2</i>	Glucose-1-phosphatase	1.37	1.47	0	0	0	0	1.49	0	0	0	0	0
<i>app X</i>	Oxidative phosphorylation; Two-component system	0	0	0	8.42	0	0	0	0	0	0	0	0
<i>atp A</i>	ATP synthase subunit alpha	-8.67	-12.04	-2.24	-5.9	-3.71	-7.12	3.58	0	2.36	-1.56	3.71	-2.29
<i>atp B</i>	ATP synthase subunit a	-3.85	-5.52	-2.08	-2.83	-2.47	-2.9	-1.44	-2.6	2.26	0	4.42	0
<i>atp C</i>	ATP synthase epsilon chain	-1.92	-2.67	-1.72	-1.96	-1.46	-1.68	0	2.51	0	0	0	-3.08
<i>atp D</i>	ATP synthase subunit beta	-8.71	-8.22	-1.94	-3.62	-3.49	-5.21	1.83	-2.61	0	-4.36	1.48	-2.62
<i>atp E</i>	ATP synthase subunit c	-4.45	-10.84	-1.51	-1.88	-2.97	-4.06	3.24	-1.98	3.19	0	6.64	0
<i>atp F</i>	ATP synthase subunit b	-4.28	-10.5	-1.74	-3.93	-3.31	5.15	1.61	-1.68	4.03	0	5.96	0
<i>atp G</i>	ATP synthase gamma chain	-4.27	-9.61	-2.17	-4.89	-3.42	-5.51	2.21	-1.85	0	-3.21	1.88	-2.61
<i>atp H</i>	ATP synthase subunit delta	-4.48	-12.1	-2.32	-5.4	-3.88	-6.7	4.83	0	3.24	0	4.75	-1.74
<i>atp I</i>	ATP synthase protein I	-3.84	-4.89	-1.6	-1.9	-2.28	-2.42	-2.05	-3.59	1.47	0	4.56	2.63
<i>chb F</i>	6-phospho-beta-glucosidase	1.63	1.43	1.48	1.46	1.42	1.41	2.51	0	2.81	0	2.81	0
<i>clp A</i>	ATP-dependent Clp protease ATP-binding subunit ClpA	-1.53	-2.02	0	0	0	0	4.57	1.5	5.27	3.09	9.44	4.54
<i>clp P</i>	ATP-dependent Clp protease proteolytic subunit	-2.22	-2.98	0	-1.61	-1.4	-1.64	4	-1.62	1.75	2.08	5.94	1.86
<i>clp S</i>	ATP-dependent Clp protease adapter protein ClpS	-2.87	-3.43	0	0	-1.44	0	0	0	1.48	6.74	7.77	6.28
<i>clp X_2</i>	ATP-dependent Clp protease ATP-binding subunit ClpX	-2.88	-3.18	-1.94	-1.83	-1.9	-1.97	4.67	-1.92	2.23	1.79	4.06	2.01
<i>crp</i>	PTS system glucose-specific EIIA component	-2.19	-2.61	-1.57	-1.51	0	-1.44	13.5	-1.7	5.94	1.86	13.98	1.86
<i>cyd A</i>	Cytochrome bd-I ubiquinol oxidase subunit 1	-3.35	-6.75	0	-2.99	-1.54	-2.9	6.85	0	7.91	-2.05	7.19	0
<i>cyd B</i>	Cytochrome bd-I ubiquinol oxidase subunit 2	-2.62	-4.98	0	-2.02	-1.46	-2.19	5.34	0	2.94	-3.06	6.11	-1.82
<i>cyo A</i>	Cytochrome bo(3) ubiquinol oxidase subunit 2	-2.92	-2.94	0	0	-2.37	-1.91	-1.73	-2.43	5.43	-3.12	5.34	1.85
<i>cyo B</i>	Cytochrome bo(3) ubiquinol oxidase subunit 1	0	0	0	0	-2.73	-2.66	-1.99	-3.53	3.74	1.96	2.98	0
<i>cyo C</i>	Cytochrome bo(3) ubiquinol oxidase subunit 3	-4.66	-5.82	-1.6	-2.26	-2.78	-3.15	2.51	-4.39	2.39	0	1.75	0
<i>cyo D</i>	Cytochrome bo(3) ubiquinol oxidase subunit 4	-3.61	-4.39	-1.5	-1.88	-2.12	-2.04	-3.15	-3.65	2.3	0	0	0
<i>cyo E</i>	Protoheme IX farnesyltransferase	-2.2	-2.12	0	0	-1.48	-1.51	2.89	-3.23	1.54	0	0	-2.05
<i>eno</i>	Enolase	-4.71	-8.24	-2.44	-3.64	-2.89	-3.54	17.13	0	2.64	0	3.7	-1.6
<i>fba B</i>	Fructose-bisphosphate aldolase class 1	3.08	2.93	1.91	1.86	2.97	2.79	-1.62	0	0	1.57	0	0
<i>fbp</i>	Fructose-1,6-bisphosphatase class 1	0	0	1.76	0	1.87	1.56	7.53	0	7.12	-5.18	9.13	2.6
<i>frd A</i>	Fumarate reductase flavoprotein subunit	-1.83	-3.53	-2.07	-3.75	-1.65	-3.5	60.7	3.35	4.28	0	11.64	1.77
<i>frd B</i>	Fumarate reductase iron-sulfur subunit	0	-3.13	-1.9	-3.92	-1.49	-3.23	47.14	6.36	3.72	0	8.14	0
<i>frd C</i>	Fumarate reductase subunit C	0	-2.31	-1.86	-3.04	-1.5	-3.33	39.21	4.08	3.28	0	8.09	0
<i>frd D</i>	Fumarate reductase subunit D	0	-1.44	-1.43	-1.72	0	-2.5	35.4	3.13	1.93	0	6.26	0

	<i>fum A</i>	Fumarate hydratase class I, aerobic	1.39	0	0	-1.4	0	0	1.07	0	26.26	7.31	32.03	3.11
	<i>fum B_1</i>	Fumarate hydratase class I, anaerobic	0	-1.39	1.82	1.78	0	0	1.93	0	0	0	0	0
	<i>fum C</i>	Fumarate hydratase class II	1.67	1.74	0	0	0	0	0	0	13.76	3.36	15.64	2.65
	<i>gal M</i>	Aldose 1-epimerase	0	0	-1.64	-1.69	0	0	4.69	3.02	6.43	2.32	4.94	1.71
Metabolismo	<i>gap A_1</i>	Galactarate dehidratase (L-three-forming]	-2.59	-4.9	-1.73	-2.01	1.38	-2.04	75.29	0	1.4	-2.67	10.61	0
	<i>glp X</i>	Fructose-1,6-bisphosphatase 1 class 2	-1.58	-1.64	-1.94	-1.87	-1.89	-2.06	4.89	0	1.71	0	0	0
	<i>glt A</i>	Citrate synthase	1.5	1.55	2.04	2.17	1.63	1.4	0	0	13.52	13.35	5.68	1.58
	<i>gpm A</i>	2,3-bisphosphoglycerate-dependent phosphoglycerate mutase	-2.32	-2.9	0	0	0	-1.63	1.97	-1.82	1.81	1.71	2.08	0
	<i>icd</i>	Isoctrate dehidrogenase [NADP]	0	-1.72	1.66	0	0	0	6.31	0	17.59	5.06	15	2.65
	<i>lpd A</i>	Dihydrolipoyl dehidrogenase	-10.85	-18.97	-4.54	-7.47	-4.91	-8.4	0	-6.08	1.8	0	2.47	-3.03
	<i>mdh _1</i>	Malate dehidrogenase	-1.94	-2.39	0	0	3.27	1.38	10.34	0	3.24	2.04	0	0
	<i>mdh _2</i>	NAD-dependent methanol dehidrogenase	0	0	3.36	0	-1.54	-2.47	2.26	1.38	-2.3	0	8.46	0
	<i>mdh _3</i>	NAD-dependent methanol dehidrogenase	-2.97	1.76	0	0	0	0	-3.51	0	3.34	1.69	0	0
	<i>ndh</i>	NADH dehidrogenase	-6.65	-7.53	-2.62	-3.57	-4.76	-5.32	4.53	0	2.95	0	3.5	1.81
	<i>ndh C</i>	NAD(P)H-quinone oxidoreductase subunit 3	-1.78	-2.2	0	0	-1.54	-1.9	0	0	1.67	1.68	5.13	0
	<i>ndh I</i>	NAD(P)H-quinone oxidoreductase subunit I, chloroplastic	0	1.3	1.37	1.44	0	0	-4.31	1.41	0	0	0	0
	<i>nifj</i>	Pyruvate-flavodoxin oxidoreductase	0	1.44	0	1.59	1.53	1.72	1.47	-2.02	3.25	2.24	2.23	1.68
	<i>nuo B</i>	NADH-quinone oxidoreductase subunit B	-1.9	-2.78	-1.48	-1.83	-1.48	-1.87	1.51	0	6.58	0	4.73	0
	<i>nuo C</i>	NADH-quinone oxidoreductase subunit C/D	-1.84	-3.32	0	-2.48	-1.63	-2.5	0	0	1.84	0	2.75	-2.16
	<i>nuo E</i>	NADH-quinone oxidoreductase subunit E	-2.06	-3.58	-1.76	-3.42	-2	-3.35	0	0	1.83	-2.04	0	-4.12
	<i>nuo F</i>	NADH-quinone oxidoreductase subunit F	-2.39	-3.86	-1.8	-3.5	-2.01	-3.26	-1.91	-1.62	1.69	-2.53	0	-2.82
	<i>nuo G</i>	NADH-quinone oxidoreductase subunit G	-3.04	-4.97	-1.94	-3.81	-2.17	-3.68	-2.62	-2.36	-1.85	-8.22	-1.64	-5.06
	<i>nuo H</i>	NADH-quinone oxidoreductase subunit H	-3.36	-5.57	-2.03	-3.22	-2.46	-4.07	-4.6	-3.41	-2.42	-9.79	-2.11	-3.72
	<i>nuo I_1</i>	NADH-quinone oxidoreductase subunit I	-3.27	-3.73	-1.79	-2.8	-2.03	-2.88	-3.77	-3.07	-4.38	-9.97	-2.14	-5.34
	<i>nuo I_2</i>	NADH-quinone oxidoreductase subunit I	0	-2.12	-3.39	-4.7	-3.19	-4.57	-4.54	0	0	-3.24	0	-4.35
	<i>nuo J</i>	NADH-quinone oxidoreductase subunit J	-3.02	-4.18	-1.92	-3.15	-1.9	-2.59	-4.81	-2.51	-3.26	-30.56	0	-2.58
	<i>nuo K</i>	NADH-quinone oxidoreductase subunit K	-3.18	-4.31	-2.19	-3.33	-1.92	-2.93	-4.44	-3.11	-7.38	-4.6	-2.03	-3.05
	<i>nuo L</i>	NADH-quinone oxidoreductase subunit L	-2.57	-3.23	-1.79	-2.88	-1.96	-2.59	-4.31	-3.04	-7.27	-10.89	-2.39	-2.86
	<i>nuo M</i>	NADH-quinone oxidoreductase subunit M	-1.97	-2.39	-1.6	-2.03	-1.7	-2.31	-6.06	-2.4	-18.25	-5.76	-3.27	-3.95
	<i>nuo N</i>	NADH-quinone oxidoreductase subunit N	-1.44	-1.58	0	-1.46	0	0	7.49	-2.69	-2.43	-3.52	-2.4	-2.23
	<i>pck A</i>	Phosphoenolpyruvate carboxykinase (ATP)	0	-1.56	0	-1.62	0	-1.35	4	0	16.95	4.34	13.35	1.78
	<i>pfk A</i>	ATP-dependent 6-phosphofructokinase isozyme 1	-2.26	-2.42	0	0	0	0	8.34	3.4	1.66	2.09	2.52	2.06
	<i>pfk B</i>	ATP-dependent 6-phosphofructokinase isozyme 2	-2.8	-2.84	1.89	2	2.44	2.12	0	0	3.83	2.14	2.3	0
	<i>pgi</i>	Glucose-6-phosphate isomerase	-1.46	-1.96	0	-1.41	0	-1.73	5.29	0	2.74	1.95	2.49	0
	<i>pgk</i>	Phosphoglycerate kinase	-2.56	-3.86	-1.53	-1.95	0	-1.99	10.68	1.77	7.2	3.94	14.47	-4.02
	<i>pgm</i>	Phosphoglucomutase	-2.11	-2.55	-1.35	-1.79	-1.67	-2.13	9.79	0	3.05	1.43	4	0
	<i>ppa</i>	Inorganic pyrophosphatase	-2.39	-2.51	0	-1.61	-1.47	-1.84	9.85	-2.23	2.3	0	5.94	2.07
	<i>pts G</i>	PTS system glucose-specific EIICB component	-2.83	-2.51	0	0	-1.67	-1.6	1.6	-4.05	-2.79	-2.68	0	0
	<i>pyk A</i>	Pyruvate kinase II	-1.62	-2.11	-1.83	-1.81	-1.65	-2.37	9.43	0	0	-2.2	2.38	-1.77
	<i>pyk F</i>	Pyruvate kinase I	-2.7	-5.72	-1.66	-2.28	-1.42	-2.32	14.13	0	0	-2.48	1.66	0
	<i>sdh A</i>	Succinate dehidrogenase flavoprotein subunit	0	-1.77	0	-1.79	-2.28	-2.98	2.94	0	34.98	5.11	15.96	2.77
	<i>sdh B</i>	Succinate dehidrogenase iron-sulfur subunit	0	0	0	-1.66	-1.77	2.62	2.19	0	18.95	8.36	10.17	0
	<i>sdh C</i>	Succinate dehidrogenase cytochrome b556 subunit	0	0	0	0	-2.17	-1.89	0	0	19.3	10.32	15	3.64
	<i>sdh D</i>	Succinate dehidrogenase hydrophobic membrane anchor subunit	0	0	0	-1.34	-2.86	-2.56	2.04	0	44.87	32.73	18.72	3.45
	<i>sdh E</i>	FAD assembly factor SdhE	0	0	0	0	0	0	0	0	0	0	1.63	0
	<i>suc A</i>	2-oxoglutarate dehidrogenase E1 component	-2.95	-4.56	-2.03	-3.38	-2.1	-3.98	7	0	3.63	-1.53	3.17	-2.72
	<i>suc B</i>	Dihydrolipoyllysine-residue succinyltransferase component of 2-oxoglutarate dehidrogenase	-2.43	-5.38	-1.47	-6.52	-1.51	-3.81	2.29	0	2.13	-2.75	2.74	-2.85
	<i>suc C</i>	Succinate-CoA ligase (ADP-forming) subunit beta	-1.89	-3.31	0	-4.74	0	-2.48	1.94	0	0	-3.04	1.9	-2.44
	<i>suc D_1</i>	Succinate-CoA ligase (ADP-forming) subunit alpha	1.5	1.55	0	0	0	-1.78	-2.86	0	0	0	2.74	-1.89
	<i>suc D_2</i>	Succinate-CoA ligase (ADP-forming) subunit alpha	-1.48	-2.09	0	-3.21	0	0	1.63	0	0	-4.63	0	0
	<i>tpi A</i>	Triosephosphate isomerase	-2.27	-3.28	-1.97	-2.2	-1.64	-2.4	9.25	-1.43	3.89	0	5.04	1.6
	<i>yih X</i>	Alpha-D-glucose 1-phosphate phosphatase YihX	-1.62	-2.19	0	0	0	0	0	0	0	0	0	0
	<i>fol B</i>	Dihydropteroin aldolase	-3.01	-3.39	0	0	0	0	0	-2.08	0	0	0	0
	<i>ahr</i>	Aldehyde reductase Ahr	1.45	0	0	0	1.43	1.34	0	0	-2.12	0	0	0
	<i>fol E</i>	GTP cyclohidrolase 1	0	0	1.33	1.3	0	0	0	0	1.54	6.75	0	0
	<i>fol K</i>	2-amino-4-hydroxy-6-hydroxymethylidihydropteridine pyrophosphokinase	0	0	0	0	0	2.57	0	0	-9.43	0	0	0
	<i>fol P</i>	Dihydropterate synthase	-1.75	-1.83	-1.64	-1.68	-1.45	-1.48	2.13	1.99	0	0	0	0
	<i>moa A</i>	GTP 3',beta-cyclase	1.39	1.48	0	0	0	1.39	1	0	1.9	1.68	2.01	0
	<i>moa B</i>	Molybdenum cofactor biosynthesis protein B	1.52	1.42	0	0	1.72	1.59	3.81	1.41	3.8	3.28	4.38	1.63
	<i>moa C</i>	Cyclic pyranopteroin monophosphate synthase	1.5	1.4	0	0	1.56	1.45	3.19	0	2.43	0	2.72	0
	<i>moa E</i>	Molybdopteroin synthase catalytic subunit	1.3	1.38	0	0	1.51	1.58	3.17	0	1.98	0	2.06	0
	<i>mob A</i>	Molybdenum cofactor guanylyltransferase	0	1.67	1.39	1.58	0	0	0	0	-2.53	0	0	0
	<i>moe A</i>	Molybdopteroin molybdenumtransferase	-1.32	-1.3	0	0	0	0	3.98	0	2.48	1.57	1.52	0
	<i>pab A</i>	Aminodeoxychorismate synthase component 2	0	0	0	0	0	0	0	0	0	2.7	0	0
	<i>pab B</i>	Aminodeoxychorismate synthase component 1	0	0	0	0	0	1.33	0	0	0	2.7	-2.77	0
	<i>pab C</i>	Aminodeoxychorismate lyase	0	0	0	0	1.36	0	0	0	0	-2.29	0	0
	<i>rib A</i>	GTP cyclohidrolase-2	0	0	1.71	1.9	0	0	0	0	0	-2.86	0	0
	<i>apb C</i>	Iron-sulfur cluster carrier protein	-1.64	-1.63	0	0	0	0	3.64	-1.58	2.86	0	2.53	0

<i>asr A</i>	Anaerobic sulfite reductase subunit A	0	1.47	1.78	1.71	1.42	1.57	0	-4.17	0	-3.03	0	
<i>asr B</i>	Anaerobic sulfite reductase subunit B	1.79	1.72	0	0	1.64	1.59	-2.6	-1.52	0	-23.86	0	
<i>ass T_2</i>	Arylsulfate sulfotransferase AssT	1.31	0	0	0	0	0	-1.44	0	-2.21	0	-2.65	0
<i>ass T_4</i>	Arylsulfate sulfotransferase AssT	0	0	0	0	0	0	-2.11	0	0	0	0	0
<i>ass T_5</i>	Arylsulfate sulfotransferase AssT	0	0	0	0	0	0	0	0	0	0	-2.02	0
<i>ats B</i>	Anaerobic sulfatase-maturing enzyme	1.29	1.3	0	0	0	0	2.29	0	0	0	0	0
<i>bet C_2</i>	Choline-sulfatase	0	0	1.27	1.23	0	0	-1.53	1.99	-3.42	0	0	0
<i>chu R_3</i>	Anaerobic sulfatase-maturing enzyme	-1.45	-1.38	0	0	0	0	-2.46	0	0	0	-2.41	0
<i>chu R_4</i>	Anaerobic sulfatase-maturing enzyme	1.45	0	0	0	0	0	0	0	-2.2	-1.8	0	0
<i>csd A</i>	Cysteine desulfurase CsdA	0	0	-1.29	0	0	-1.29	1.48	1.63	1.87	1.52	-2.13	1.36
<i>cys A_1</i>	Sulfate/thiosulfate import ATP-binding protein CysA	0	0	0	0	0	1.34	1.93	1.21	1.14	1.67	-2	0
<i>cys A_2</i>	Sulfate/thiosulfate import ATP-binding protein CysA	1.32	1.35	0	1.21	0	0	-2.77	0	-2.22	-3.35	1.86	0
<i>cys B</i>	HTH-type transcriptional regulator CysB	0	0	0	1.29	0	1.37	1.24	0	0	0	-2.23	1.08
<i>cys C</i>	Adenylyl-sulfate kinase	0	0	0	0	0	0	0	0	-4.83	0	-2.33	0
<i>cys E</i>	Serine acetyltransferase	-2.26	-2.72	-2.24	1.56	1.76	1.62	0	-1.67	0	0	0	0
<i>cys E_2</i>	Serine acetyltransferase	1.76	1.8	-2.24	-2.38	-1.9	-2.1	0	1.48	-1.83	0	0	0
<i>cys H_1</i>	Phosphoadenosine phosphosulfate reductase	1.43	1.47	1.53	1.64	1.35	1.41	-2.68	0	-3.67	0	0	0
<i>cys I</i>	Sulfite reductase [NADPH] hemoprotein beta-component	0	0	0	0	0	0	-2.79	0	-2.53	0	-1.92	0
<i>cys I_1</i>	Sulfite reductase [NADPH] flavoprotein alpha-component	1.39	1.32	0	0	-1.72	-1.54	-1.7	0	-3.44	0	1.83	-1.72
<i>cys I_2</i>	Sulfite reductase [NADPH] flavoprotein alpha-component	0	0	0	0	0	0	1.85	0	0	-3.08	-2.47	0
<i>cys I_3</i>	Sulfite reductase [NADPH] flavoprotein alpha-component	-2.11	-1.91	0	0	0	0	0	0	0	1.18	0	0
<i>cys K</i>	Cysteine synthase A	0	0	1.3	1.26	1.47	1.44	1.48	0	-1.06	1.58	1.49	0
<i>cys L_1</i>	HTH-type transcriptional regulator CysL	-2.09	-1.92	0	0	0	1.63	-1.8	-1.94	0	0	0	1.84
<i>cys L_2</i>	HTH-type transcriptional regulator CysL	0	0	0	0	0	0	0	0	0	0	-2.54	0
<i>cys M</i>	Cysteine synthase B	-1.38	0	0	0	0	0	3.78	-1.67	-4.5	-2.56	-2.4	-1.48
<i>cys N</i>	Sulfate adenylyltransferase subunit 1	0	1.3	0	0	0	0	0	0	-1.9	0	-2.37	0
<i>cys P</i>	Thiosulfate-binding protein	1.53	1.57	0	1.26	0	0	0	1.5	-2.73	0	-1.91	0
<i>cys Q</i>	3(2'),5'-bisphosphate nucleotidase CysQ	0	0	0	0	0	0	1.87	1.44	-6.41	1.63	-3.66	-3.06
<i>cys T</i>	Sulfate transport system permease protein CysT	1.64	1.65	0	0	0	1.38	0	0	-2.41	0	-2.67	0
<i>cys W_2</i>	Sulfate transport system permease protein CysW	1.38	1.55	0	0	0	0	-2.85	0	0	0	0	0
<i>cys Z</i>	Sulfate transporter CysZ	0	0	1.37	1.39	0	1.51	0	0	0	0	-2.3	0
<i>dms A_2</i>	Dimethyl sulfoxide reductase DmsA	1.4	1.43	0	0	0	0	-4.24	0	0	0	1.66	1.72
<i>dms B_2</i>	Anaerobic dimethyl sulfoxide reductase chain B	1.39	1.5	0	0	0	0	2.47	0	0	0	2.18	0
<i>dms B_3</i>	Anaerobic dimethyl sulfoxide reductase chain B	0	0	0	0	0	0	-2.34	0	-3.09	0	1.7	0
<i>dms B_5</i>	Anaerobic dimethyl sulfoxide reductase chain B	1.46	1.52	0	0	1.64	1.46	1.92	0	0	-2.86	-3.64	0
<i>dms C_1</i>	Anaerobic dimethyl sulfoxide reductase chain C	1.54	0	0	0	0	0	-2.4	0	0	0	1.77	0
<i>dms C_2</i>	Anaerobic dimethyl sulfoxide reductase chain C	0	0	0	0	0	0	3.18	1.58	0	0	0	0
<i>dms C_4</i>	Anaerobic dimethyl sulfoxide reductase chain C	1.5	1.58	0	0	0	1.49	1.83	0	0	0	-1.92	0
<i>dms C_5</i>	Tat proofreading chaperone DmsD	0	0	0	0	1.39	1.37	0	0	0	0	-2.69	0
<i>dsb A</i>	Thiol:disulfide interchange protein DsbA	-1.57	-2.22	0	-1.27	0	-1.39	0	0	0	1.51	0	0
<i>dsb B_1</i>	Disulfide bond formation protein B	-1.6	-1.45	0	0	0	1.74	-4.3	0	0	0	0	0
<i>dsb B_2</i>	Disulfide bond formation protein B	0	0	1.58	2	0	1.67	0	0	0	0	-2.54	0
<i>dsb C</i>	Thiol:disulfide interchange protein DsbC	-1.97	-2.77	-1.39	-1.92	-1.74	-1.89	1.92	0	1.9	0	-2.7	0
<i>dsb D_1</i>	Thiol:disulfide interchange protein DsbD	0	1.45	0	1.33	0	0	-1.73	0	0	0	-2.36	0
<i>dsb E</i>	Thiol:disulfide interchange protein DsbE	0	0	0	0	0	0	5.13	-2.1	-1.71	-2.63	3.2	-2
<i>dsb I</i>	Protein-disulfide oxidoreductase DsbI	0	0	0	1.42	0	0	-2.13	0	-2.55	0	-2.93	0
<i>dsr F</i>	Intracellular sulfur oxidation protein DsrF	-2.61	-3.21	-2.37	-2.5	-2.19	-2.56	0	0	0	0	0	0
<i>ent B</i>	Enterobactin synthase component B	0	0	0	0	1.31	1.34	-2.09	-1.41	-3.36	-2.39	-2.18	0
<i>ent E</i>	Enterobactin synthase component E	0	0	0	0	1.32	1.39	-2.1	0	-3.18	0	0	0
<i>erp A</i>	Iron-sulfur cluster insertion protein ErpA	-1.77	-1.79	0	0	-1.46	0	1.53	0	1.98	1.66	2.04	1.93
<i>fdh D</i>	Sulfurtransferase FdhD	0	-1.29	0	0	0	0	-2.18	-1.59	-1.63	0	0	0
<i>fdh H_2</i>	Formate dehydrogenase, nitrate-inducible, iron-sulfur subunit	-1.98	-2.65	-3.79	-4.53	-2.52	-3.28	0.89	0.68	1.76	0	8.08	1.91
<i>fdh H</i>	Formate dehydrogenase-O iron-sulfur subunit	-4.03	-6.39	-1.67	-2.78	-2.1	-3.21	-1.69	-2.52	7.64	1.54	2.53	0
<i>fdx</i>	2Fe-2S ferredoxin	-2.08	-2.26	-2.26	-2.62	-1.9	-1.9	1.34	0.82	2.63	0	-3.17	0
<i>fep A</i>	Ferrienterobactin receptor	0	0	1.3	1.41	0	1.47	-1.39	1.53	-1.98	0	-2.01	0
<i>fep C</i>	Ferric enterobactin transport ATP-binding protein FepC	0	0	0	0	0	0	0	0	-1.95	0	-2.45	0
<i>fep D</i>	Ferric enterobactin transport system permease protein FepD	0	0	0	0	0	1.38	0	0	-4.58	0	-2.19	0
<i>fep E</i>	Ferric enterobactin transport protein FepE	0	0	0	0	0	0	0	0	0	2.05	0	1.95
<i>fep G</i>	Ferric enterobactin transport system permease protein FepG	0	0	0	0	0	1.37	0	0	-2.37	0	-2.55	0
<i>fet B</i>	putative iron export permease protein FetB	0	0	0	0	0	0	-2.88	0	0	-3.69	-3.47	0
<i>fhu A_1</i>	Ferrichrome-iron receptor	-4.18	-3.65	0	1.53	0	1.33	-1.76	-2.82	-2.48	0	0	0
<i>fhu A_2</i>	Ferrichrome-iron receptor	0	0	0	0	-1.84	0	-1.52	0	-1.82	0	-3.35	0
<i>fhu B</i>	Iron(3+)-hydroxamate import system permease protein FhuB	-1.5	-1.46	0	0	0	0	-5.69	-2.45	-4.02	-1.94	-3.35	-1.73
<i>fhu C</i>	Iron(3+)-hydroxamate import ATP-binding protein FhuC	-2.79	-2.72	0	0	0	0	-6.01	-2.58	-5.45	0	-4.1	-1.92
<i>fhu D</i>	Iron(3+)-hydroxamate-binding protein FhuD	-2.03	-1.98	0	0	0	0	-3.31	-1.99	-5.7	-2.29	-3.87	-1.6
<i>fhu F</i>	Ferric iron reductase protein FhuF	0	0	1.35	1.29	0	0	-2.31	0	-3.88	0	-2.17	0
<i>fje F</i>	Ferrous-iron efflux pump FjeF	-1.9	-2.13	0	0	0	1.63	0	0	0	1.72	1.67	1.25
<i>fix X_1</i>	Ferredoxin-like protein FixX	0	0	3.06	3.23	1.78	2.17	-5.38	0	0	0	0	0

Sideróforos e
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<i>ftn_A_1</i>	Bacterial non-heme ferritin	0	0	0	0	1.42	1.43	1.89	0	3.1	0	3.01	0	
<i>ftn_A_2</i>	Bacterial non-heme ferritin	1.42	1.76	1.52	1.55	1.43	1.7	0	0	3.21	0	0	1.99	
<i>fur</i>	Ferric uptake regulation protein	0	0	0	0	0	0	2.77	0	7.89	7.89	8.26	4.77	
<i>gip_E_1</i>	Thiosulfate sulfurtransferase GipE	0	0	-2.28	-2.63	-1.38	-1.43	-3.35	0	2.02	0	0	1.67	
<i>gip_E_2</i>	Thiosulfate sulfurtransferase GipE	-3.59	-4.69	0	0	-2.4	-2.62	4.33	-2.6	0	2.84	4.04	0	
<i>hem_H</i>	Ferrochelatase	0	0	0	0	0	0	0	0	0	8.9	0	1.83	
<i>isc_A_1</i>	Iron-binding protein IscA	1.72	1.98	-2.29	-2.77	0	1.57	0	0	0	2.89	0	0	
<i>isc_A_2</i>	Iron-binding protein IscA	-2.86	-2.62	-1.59	-2.28	-2.77	-2.36	11.31	17.87	5.45	5.13	7.56	5.97	
<i>isc_S</i>	Cysteine desulfurase IscS	-1.65	0	0	0	-2.22	-1.57	9.59	11.37	11.64	10.1	10.47	7.48	
<i>isc_U</i>	Iron-sulfur cluster assembly scaffold protein IscU	-2.3	-2.21	-1.44	-1.91	-2.41	-1.97	14.86	12.61	3.98	2.62	2.42	-1.11	
<i>met_A</i>	Homoserine O-succinyltransferase	0	0	1.34	1.37	0	1.36	-2.8	0	-2.53	0	0	0	
<i>moa_A</i>	GTP 3',8-cyclase	1.39	1.48	0	0	0	1.39	0	0	1.9	1.68	2.02	0	
<i>moa_D</i>	Molybdopterin synthase sulfur carrier subunit	0	0	0	0	1.69	0	2.02	0	0	0	2.69	0	
<i>moe_B</i>	Molybdopterin-synthase adenylyltransferase	0	0	0	0	0	0	4.56	0	-2.3	0	0	0	
<i>moe_Z</i>	putative adenylyltransferase/sulfurtransferase MoeZ	0	1.46	1.42	1.6	0	1.53	-3.29	-1.73	0	0	0	0	
<i>msr_A</i>	Peptide methionine sulfoxide reductase MsrA	0	0	1.62	1.3	0	0	0	0	-3.34	0	-2.05	0	
<i>msr_AB</i>	Peptide methionine sulfoxide reductase MsrA/MsrB	1.84	2.28	0	0	1.57	1.67	0	0	0	0	0	0	
<i>msr_B</i>	Peptide methionine sulfoxide reductase MsrB	0	0	0	1.41	0	1.43	0	0	0	10.68	5.83	8.15	1.63
<i>msr_C</i>	Free methionine-R-sulfoxide reductase	-4.36	-3.51	-1.75	-1.88	-2.33	-2.08	0	-1.88	-1.55	-2.32	0	0	
<i>nfu_A</i>	Fe/S biogenesis protein NfuA	0	0	0	0	0	0	3.04	0	0	0	1.76	0	
<i>pfe_A</i>	Ferric enterobactin receptor	1.32	1.38	1.36	1.36	0	1.32	-1.43	0	-2.85	0	-1.68	0	
<i>psa_C</i>	Photosystem I iron-sulfur center	0	1.56	0	0	0	1.39	0	0	8.04	0	-2.89	-2.11	
<i>psp_E</i>	Thiosulfate sulfurtransferase PspE	0	1.76	2.06	2.75	2.85	2.5	6.29	2.63	1.87	0	6.69	4.27	
<i>psr_A</i>	Polysulfide reductase chain A	2.01	1.66	0	-1.38	0	0	1.08	1.85	8.83	0	14.25	0	
<i>sbn_A</i>	putative siderophore biosynthesis protein SbnA	0	1.28	1.24	1.34	1.23	1.33	-1.56	0	0	2.56	0	0	
<i>sbp</i>	Sulfate-binding protein	0	0	0	0	0	0	1.58	1.59	0	0	-1.58	0	
<i>sse_A_1</i>	Type III secretion system chaperone SseA	0	1.59	0	1.82	0	1.95	0	0	0	0	0	2.03	
<i>sse_A_2</i>	3-mercaptopyruvate sulfurtransferase	0	0	0	0	0	0	4.82	0	3.72	1.73	9.1	0	
<i>suf_S</i>	Cysteine desulfurase	2.14	2.19	1.72	1.79	1.39	1.79	-1.72	1.56	0	0	0	0	
<i>suy_B</i>	(ZR)-sulfolactate sulfo-lyase subunit beta	-2.23	1.68	1.42	1.27	1.53	1.48	0	0	0	0	3.37	0	
<i>thi_F</i>	Sulfur carrier protein ThiS adenylyltransferase	0	0	0	0	0	0	-2.04	0	0	0	-5.24	0	
<i>thi_I</i>	tRNA sulfurtransferase	-2.86	-2.78	-1.88	-1.72	-1.96	-1.95	1.58	-2.35	0	0	0	0	
<i>thi_S</i>	Sulfur carrier protein ThiS	0	0	0	0	0	0	5.12	0	0	0	0	0	
<i>ttr_A</i>	Tetrathionate reductase subunit A	1.94	2.27	1.3	1.55	1.56	1.72	0	0	0	0	0	0	
<i>ttr_B</i>	Tetrathionate reductase subunit B	1.75	2.32	0	1.68	1.5	1.64	-1.83	0	0	0	0	0	
<i>ttr_C</i>	Tetrathionate reductase subunit C	1.9	2.27	1.51	1.7	1.57	1.9	0	0	0	0	0	0	
<i>tus_B</i>	Protein TusB	0	0	-2.45	-2.67	0	0	0	0	0	0	0	0	
<i>tus_D</i>	Sulfurtransferase TusD	-2.46	-3.71	-2.45	-2.68	-2.56	-2.4	1.51	0	0	0	0	0	
<i>ydh_X_1</i>	putative ferredoxin-like protein YdhX	0	0	0	0	0	0	-4.65	0	7.4	0	-5.91	0	
<i>ydh_X_2</i>	putative ferredoxin-like protein YdhX	1.81	1.55	0	0	0	0	2.42	2.84	-5.77	0	-12.02	0	
<i>yfc_G_1</i>	Disulfide-bond oxidoreductase YfcG	0	1.36	1.56	1.54	0	0	-3.41	0	-6.72	0	-1.95	0	
<i>yfc_G_2</i>	Disulfide-bond oxidoreductase YfcG	0	0	0	0	1.43	1.49	0	0	0	0	10.41	0	
<i>yfe_X</i>	putative deferrochelatase/peroxidase YfeX	-1.53	-1.66	0	0	0	-1.41	86	1.72	6.29	2.24	6.26	3.31	
<i>yih_P_1</i>	Putative 2,3-dihydroxypropane-1-sulfonate exporter	1.43	1.43	0	0	0	0	0	0	8.91	-2.83	0	-1.83	0
<i>yih_P_2</i>	Putative 2,3-dihydroxypropane-1-sulfonate exporter	0	0	0	0	0	0	3.25	0	-1.81	-1.92	-2.02	0	
<i>yih_P_3</i>	Putative 2,3-dihydroxypropane-1-sulfonate exporter	0	0	0	0	0	0	-3.38	-1.58	0	0	0	0	
<i>yih_P_4</i>	Putative 2,3-dihydroxypropane-1-sulfonate exporter	0	0	0	0	0	0	0	0	0	0	0	0	
<i>yih_Q</i>	Sulfoquinovosidase	0	0	0	0	0	0	-3.13	0	-2.96	0	-2.07	0	
<i>yih_S</i>	Sulfoquinovose isomerase	0	0	0	0	0	0	-3.24	0	-2.28	-1.6	-3.32	0	
<i>yih_T</i>	Sulfofructosephosphate aldolase	0	0	0	0	0	0	-2.75	-1.47	-3.9	-2.69	-4.05	0	
<i>yih_U</i>	3-sulfolactaldehyde reductase	0	0	1.29	1.31	0	0	-2.37	0	-3.99	0	-2.34	-1.47	
<i>yih_V</i>	Sulfofructose kinase	0	0	0	0	0	0	-2.67	0	0	0	0	0	
<i>yjc_S</i>	Putative alkyl/aryl-sulfatase YjcS	2.3	2.47	0	0	0	0	-1.77	1.62	0	0	0	0	
<i>ynf_E_1</i>	Putative dimethyl sulfoxide reductase chain YnfE	1.34	1.43	0	0	1.62	1.64	9.98	3.75	2.26	0	2.48	0	
<i>ynf_E_2</i>	Putative dimethyl sulfoxide reductase chain YnfE	0	1.31	0	1.31	0	1.38	-2.23	0	-1.99	0	-1.7	0	
<i>ynf_F</i>	putative dimethyl sulfoxide reductase chain YnfF	1.43	1.38	0	0	1.45	1.33	2.65	2.31	2.21	-1.82	1.59	0	
<i>yqj_H</i>	NADPH-dependent ferric-chelate reductase	0	0	0	0	0	0	0	0	0	1.94	-6.09	0	
<i>ytf_E</i>	Iron-sulfur cluster repair protein YtfE	0	0	0	0	0	0	0	0	-3.68	0	-2.28	0	
<i>yus_V</i>	putative siderophore transport system ATP-binding protein YusV	1.38	1.6	1.35	1.58	1.3	1.4	2.32	0	-2.12	0	3.35	0	
<i>flg_A</i>	Flagella basal body P-ring formation protein FlgA	-2.85	-2.33	-1.57	-1.32	-2.21	-2.21	1.81	-1.74	0	-4.71	0	-1.8	
<i>flg_B</i>	Flagellar basal body rod protein FlgB	-10.97	-10.8	-5.09	-4.59	-8.73	-8.36	7.93	0	0	-4.09	0	-4.74	
<i>flg_C</i>	Flagellar basal-body rod protein FlgC	-18	-19.16	-6.86	-6.7	-15.58	-16.81	7.6	0	0	-8.91	0	-12.16	
<i>flg_D</i>	Basal-body rod modification protein FlgD	-18.49	-20.09	-9.61	-9.08	-18.78	-15.79	7.71	0	0	-12.41	0	-8.14	
<i>flg_E</i>	Flagellar hook protein FlgE	-18.93	-22.19	-10.86	-3.26	-18.45	-20.88	7.86	0	0	-23.36	0	-14.4	
<i>flg_F</i>	Flagellar basal-body rod protein FlgF	-14.14	-14.39	-9.02	-7.3	-14.32	-14.88	6.45	0	-1.4	-20.46	-1.42	-17.43	
<i>flg_G</i>	Flagellar basal-body rod protein FlgG	-14.34	-13.49	-6.82	-6.36	-13.09	-12.06	5.08	0	-2.1	-12.03	-1.79	-9.54	
<i>flg_H</i>	Flagellar L-ring protein	-8.86	-9.74	-4.8	-4.65	-8.31	-8.27	1.77	-2.89	-4.43	13.34	-4.56	-6.35	
<i>flg_I</i>	Flagellar P-ring protein	-8.77	-7.9	-4.18	-3.45	-6.54	-5.81	0	-1.65	-6.17	-10.21	-5.15	-5.68	

	<i>pip B</i>	Secreted effector protein PipB	0	1.51	0	1.57	0	1.85	0	2.08	0	0	0	2.36	0
	<i>pip B2</i>	Secreted effector protein PipB2	-1.59	-1.97	1.43	1.36	0	1.39	-2.09	-1.58	0	0	0	0	1.55
	<i>prg K</i>	Lipoprotein PrgK	-4.73	-6.78	2.16	0	1.54	0	0	0	0	0	0	0	0
	<i>prf E_2</i>	Type I secretion system membrane fusion protein PrfE	1.58	1.66	0	0	1.25	1.32	-1.63	3.24	0	0	0	-3.36	0
	<i>sec A</i>	Protein translocase subunit SecA	-2.68	-4.28	-1.75	-2.27	-2.34	-2.89	2.77	6.98	0	0	0	0	0
	<i>sec B</i>	Protein-export protein SecB	-8.81	-6.14	-2.91	-3.82	-3.2	-3.56	7.4	-1.75	2.3	0	0	3.65	0
	<i>sec E</i>	Protein translocase subunit SecE	-3.44	-4.45	-1.33	-1.66	-2.43	-2.97	2.62	-3.79	2.52	1.5	0	3.59	0
	<i>sec G</i>	Protein-export membrane protein SecG	-3.2	-3.46	-1.74	-2.01	-2	-1.97	0	-3.84	0	0	0	2.21	0
	<i>sec M</i>	Secretion monitor	-2.8	-2.9	-1.67	-1.89	-1.6	-1.44	4	12.11	0	0	0	0	0
	<i>sec Y</i>	Protein translocase subunit SecY	-19.99	-69.92	-4.23	-13.98	-9.48	-19.26	3.18	0	-1.42	-3.11	0	-3.68	0
	<i>sef A</i>	Fimbrial protein	0	0	0	0	0	0	3.11	2.31	0	0	0	0	0
Virulência (Fimbrias, Pili), Invasão e Sistemas de secreção)	<i>sfm C_1</i>	putative fimbrial chaperone SfmC	0	0	0	0	0	0	0	-2.23	0	0	-2.89	0	0
	<i>sfm C_2</i>	putative fimbrial chaperone SfmC	1.36	1.5	-1.62	-1.74	0	0	-2.98	0	0	0	-2.28	0	0
	<i>sfm H_2</i>	putative fimbrial-like protein SfmH	1.42	1.46	0	0	0	1.34	-1.77	0	-2.56	0	-2.75	0	0
	<i>sif A</i>	Secreted effector protein SifA	0	1.69	2.07	1.84	0	1.75	0	0	0	0	0	0	0
	<i>sif A_2</i>	Secreted effector protein SifA	0	1.59	1.97	0	0	2.61	0	0	0	0	0	3.9	0
	<i>sip A</i>	Cell invasion protein SipA	-2.62	-2.83	1.37	1.28	0	0	0	0	-1.81	0	-1.68	0	0
	<i>sip B</i>	Cell invasion protein SipB	-2.19	-3.24	0	0	1.45	0	0	0	1.59	0	1.56	0	0
	<i>sip C</i>	Cell invasion protein SipC	-2.51	-3.83	1.56	0	1.38	0	0	0	1.64	0	0	0	0
	<i>sip D</i>	Cell invasion protein SipD	-2.32	2.7	1.6	0	0	0	0	0	2.23	0	0	0	0
	<i>slr P_1</i>	E3 ubiquitin-protein ligase SlrP	-1.56	0	1.54	1.48	0	0	0	0	-2.63	0	0	0	0
	<i>slr P_2</i>	E3 ubiquitin-protein ligase SlrP	0	0	0	0	0	0	0	0	-3.67	0	0	0	0
	<i>slr P_3</i>	E3 ubiquitin-protein ligase SlrP	0	0	1.89	1.46	0	0	-2.86	0	0	3.43	0	0	0
	<i>sop B</i>	Inositol phosphate phosphatase SopB	0	0	1.36	1.47	1.7	1.74	0	0	0	2.26	0	0	0
	<i>sop D</i>	Secreted effector protein SopD	0	0	0	0	0	0	0	0	0	0	3.46	2.95	0
	<i>sop D2</i>	Secreted effector protein sopD2	0	0	1.69	1.8	0	1.6	2.45	0	0	0	0	0	0
	<i>spo O</i>	Surface presentation of antigens protein SpaO	-2.62	-2.66	0	0	0	0	0	0	0	0	-1.88	0	0
	<i>spl C</i>	Salmonella pathogenicity island 2 protein C	0	1.85	3.67	2.23	0	1.89	0	0	0	0	0	2.81	0
	<i>spt P</i>	Secreted effector protein SptP	-1.9	-2.1	1.38	0	0	0	1.64	2.75	-1.71	0	0	0	0
	<i>spv B</i>	Mono(ADP-ribosyl)transferase SpvB	1.61	1.68	0	0	0	0	1.89	2.26	0	0	0	0	0
	<i>spv C</i>	MAPK phosphothreonine lyase	1.55	1.54	0	0	0	0	2.78	2.44	0	0	0	0	0
	<i>ssa N</i>	putative secretion system apparatus ATP synthase SsaN	1.88	2.17	1.49	1.81	1.6	1.7	0	0	0	0	-2	0	0
	<i>ssa V</i>	Secretion system apparatus protein SsaV	1.87	2.16	1.41	1.59	1.53	1.85	-1.74	0	0	0	0	0	0
	<i>sse J</i>	Secreted effector protein SseJ	0	1.75	2.17	2.06	0	1.93	0	0	0	5.6	0	0	0
	<i>sse L</i>	Deubiquitinase SseL	0	0	0	0	0	2.01	3.25	0	0	6.31	0	0	0
	<i>ssp H2_1</i>	E3 ubiquitin-protein ligase SspH2	0	1.48	1.41	0	0	0	-2.65	0	-3.76	0	0	0	0
	<i>ssr A</i>	E3 ubiquitin-protein ligase SspH2	-2.88	-2.68	0	0	0	0	17.3	3.15	0	0	0	0	0
	<i>ste C</i>	Secreted effector kinase SteC	0	0	1.96	1.97	0	0	2.28	0	0	0	0	0	0
	<i>tad A</i>	Sec-independent protein translocase protein Tata	0	0	0	0	0	0	-3.55	-2.18	-15.61	0	-3.8	0	0
	<i>tar</i>	Methyl-accepting chemotaxis protein II	-1.93	-2.1	0	1.48	0	0	3.77	0	6.58	3.43	4.3	0	0
	<i>tsr_1</i>	Methyl-accepting chemotaxis protein I	0	-1.46	1.59	0	-2.23	-2.92	1.64	0	6.2	2.11	4.72	0	0
	<i>tsr_2</i>	Methyl-accepting chemotaxis protein I	-4.03	-5.45	-1.54	-1.9	0	0	6.6	0	6.31	1.83	3.73	0	0
	<i>vir B</i>	Virulence regulon transcriptional activator VirB	0	1.46	0	0	0	0	4.9	11.31	0	0	0	0	0
	<i>vsd E</i>	Virulence protein vsdE	0	0	0	0	0	0	8.13	6.93	0	0	0	0	0
	<i>yad K</i>	putative fimbrial-like protein YadK	0	0	0	0	1.39	0	0	0	0	0	-2.02	0	0
	<i>yad M</i>	putative fimbrial-like protein YadM	0	0	0	0	0	0	0	0	0	0	-4.01	0	0
	<i>yad N</i>	putative fimbrial-like protein YadN	0	0	0	0	1.43	0	0	0	0	0	0	2.35	0
	<i>yad V</i>	putative fimbrial chaperone YadV	1.55	1.71	0	0	1.47	1.55	-4.16	0	0	0	0	0	0
	<i>yad V_3</i>	putative fimbrial chaperone YadV	0	0	0	0	1.37	1.43	0	0	0	0	-9.54	0	0
	<i>ybg D</i>	putative fimbrial-like protein YbgD	0	0	0	0	0	0	-1.91	0	0	0	-2.43	0	0
	<i>ycb V</i>	putative fimbrial-like protein YcbV	1.46	1.5	0	0	0	0	-2.4	0	0	0	0	0	0
	<i>yeh A</i>	putative fimbrial-like protein YehA	0	1.51	1.66	1.62	1.58	2.08	0	0	0	0	0	1.87	0
	<i>yeh C</i>	putative fimbrial chaperone YehC	0	0	3	0	0	1.92	0	0	0	3.83	0	0	0
	<i>yeh D</i>	putative fimbrial-like protein YehD	0	0	1.8	0	1.52	1.97	2.56	0	0	0	3.85	0	0
	<i>yfc P_1</i>	putative fimbrial-like protein YfcP	1.6	1.57	0	0	0	0	-2.4	0	0	0	0	0	0
	<i>yfc Q_1</i>	putative fimbrial-like protein YfcQ	1.48	1.52	0	0	0	0	2.47	0	0	0	0	0	0
	<i>yfc Q_2</i>	putative fimbrial-like protein YfcQ	1.44	1.53	0	0	0	0	-2.59	0	0	0	0	0	0
	<i>yfc R_2</i>	putative fimbrial-like protein YfcR	1.53	1.66	0	0	0	0	-2.54	0	0	0	0	0	0
	<i>yfc S_1</i>	putative fimbrial chaperone YfcS	1.48	1.4	0	0	0	0	2.03	0	0	0	0	0	0
	<i>yfc S_2</i>	putative fimbrial chaperone YfcS	1.57	1.51	0	0	0	0	-2.42	0	0	0	0	0	0
	<i>ysc J</i>	Yop proteins translocation lipoprotein J	1.47	1.76	2.29	2.23	0	1.98	0	0	0	0	0	0	0
	<i>aro F</i>	Phospho-2-dehydro-3-deoxyheptonate aldolase, Tyr-sensitive	-1.4	-1.46	-1.35	-1.31	0	0	1.4	0	-2.38	0	-1.91	0	0
	<i>aro G</i>	Phospho-2-dehydro-3-deoxyheptonate aldolase, Phe-sensitive	-1.49	-1.49	0	0	0	0	-3.66	-1.66	-2.9	0	-2.17	0	0
	<i>aro H</i>	Phospho-2-dehydro-3-deoxyheptonate aldolase, Trp-sensitive	0	1.34	1.31	1.56	1.26	1.45	0	0	-2.07	0	0	0	0
	<i>crp</i>	cAMP-activated global transcriptional regulator CRP	-1.34	-1.49	0	0	0	0	1.73	1.49	4.07	2.56	3.28	1.69	1.69
	<i>ffh</i>	Signal recognition particle protein	-2.51	-3.2	-1.79	-1.75	-1.82	-2.08	3.23	1.61	2.58	2.48	3.57	1.96	1.96
	<i>flh D</i>	Flagellar transcriptional regulator FlhD	-1.63	-1.58	-1.91	-1.44	-1.81	-1.52	3.43	-2.04	4.24	0	3.63	3.41	0
	<i>fts Y</i>	Signal recognition particle receptor FtsY	-1.96	-2.08	-1.4	-1.33	-1.36	-1.6	2.08	0	1.97	2.21	1.74	0	0

<i>hfq</i>	RNA-binding protein Hfq	-2.33	-3.53	-1.41	-1.74	-1.6	-1.83	4.87	0	2.68	1.67	5.16	1.84
<i>kdp E</i>	KDP operon transcriptional regulatory protein KdpE	0	0	0	0	0	1.54	0	-2.05	0	0	0	0
<i>lep B</i>	Signal peptidase I	-2.36	-2.35	-1.31	-1.44	-1.51	-1.57	1.54	-1.93	0	0	0	-1.61
<i>liv F</i>	High-affinity branched-chain amino acid transport ATP-binding protein LivF	0	0	0	0	0	0	-3.59	0	-4.6	0	-5.76	0
<i>liv H</i>	High-affinity branched-chain amino acid transport system permease protein LivH	0	0	0	0	0	0	-2.82	0	-4.2	0	-4.01	-1.66
<i>liv K</i>	Leucine-specific-binding protein	0	0	0	0	0	0	-1.73	0	0	0	-4.82	0
<i>lux S</i>	S-ribosylhomocysteine lyase	-1.81	-2.17	0	0	0	-1.39	1.42	0	0	0	2.78	0
<i>mpp A</i>	Periplasmic murein peptide-binding protein	-1.49	-1.5	0	0	0	0	1.8	0	2.05	1.8	2.02	1.55
<i>opp A</i>	Periplasmic oligopeptide-binding protein	0	0	1.43	1.46	1.31	1.4	0	0	2.73	0	3.08	0
<i>opp B</i>	Oligopeptide transport system permease protein OppB	0	0	0	0	1.44	1.49	-2.18	0	0	0	-2.25	0
<i>opp C</i>	Oligopeptide transport system permease protein OppC	-1.8	-1.61	0	1.3	0	1.36	-2.33	-2.28	0	0	0	0
<i>opp D_1</i>	Oligopeptide transport ATP-binding protein OppD	-1.85	-1.94	0	1.44	1.51	1.37	-2.19	-2.01	0	0	0	0
<i>opp D_2</i>	Oligopeptide transport ATP-binding protein OppD	1.39	1.49	0	0	0	0	2.12	1.69	0	0	0	0
<i>opp D_3</i>	Oligopeptide transport ATP-binding protein OppD	0	0	0	0	0	0	-2.17	0	0	0	-1.6	0
<i>opp F_1</i>	Oligopeptide transport ATP-binding protein OppF	-1.3	0	0	0	0	0	-1.56	-2.33	0	0	0	0
<i>opp F_3</i>	Oligopeptide transport ATP-binding protein OppF	0	0	0	0	0	0	-2	0	-2.23	0	-1.65	0
<i>rcs A_1</i>	Transcriptional regulatory protein RcsA	-1.49	0	2.38	0	0	0	3.36	0	0	0	-5.71	0
<i>rcs A_2</i>	Transcriptional regulatory protein RcsA	0	0	1.45	1.27	2	2.55	-2.9	0	0	0	0	0
<i>rib A</i>	GTP cyclohydrolase-2	0	0	1.71	1.9	0	0	0	0	0	2.88	0	0
<i>rib D</i>	Riboflavin biosynthesis protein RibD	-3.02	-4.05	-1.35	-1.45	-1.7	-1.81	1.65	0	3.28	1.85	3.96	0
<i>sdi A</i>	Regulatory protein SdiA	1.29	1.28	0	0	0	0	-3.91	0	2.34	0	2.46	0
<i>sec A</i>	Protein translocase subunit SecA	-2.68	-4.28	-1.75	-2.27	-2.34	-2.89	2.72	0.98	0	0	0	0
<i>sec B</i>	Protein-export protein SecB	-6.84	-6.14	-2.91	-3.82	-3.2	-3.56	2.4	-1.75	2.3	0	3.65	0
<i>sec E</i>	Protein translocase subunit SecE	-3.44	-4.45	-1.33	-1.66	-2.43	-2.97	2.62	-3.79	2.52	1.5	1.59	0
<i>sec G</i>	Protein-export membrane protein SecG	-3.2	-3.46	-1.74	-2.01	-2	-1.97	0	-3.84	0	0	2.21	0
<i>sec Y</i>	Protein translocase subunit SecY	-19.99	-69.92	-4.23	-13.98	-9.48	-19.76	3.18	0	-1.42	-3.11	0	-3.68
<i>trp E</i>	Anthranilate synthase component 1	1.76	1.99	1.48	1.72	1.56	1.71	0	0	-2.36	0	-2.01	0
<i>ycd Z</i>	Inner membrane protein YcdZ	1.87	2.11	0	1.48	1.38	1.45	0	1.98	0	0	0	0
<i>yid C</i>	Membrane protein insertase YidC	-8.72	-6.81	-2	-2.41	-3.71	-3.84	3.27	-3.12	-1.51	-1.62	0	0
<i>zur</i>	Zinc uptake regulation protein	-1.41	-1.5	0	0	0	0	1.85	3.71	0	0	0	0
<i>aae A_1</i>	p-hydroxybenzoic acid efflux pump subunit AaeA	1.41	1.78	1.68	1.92	1.45	1.93	0	2.82	0	0	0	0
<i>aae A_2</i>	p-hydroxybenzoic acid efflux pump subunit AaeA	0	0	0	0	0	0	1.78	1.89	0	0	-3.8	0
<i>aae B_1</i>	p-hydroxybenzoic acid efflux pump subunit AaeB	1.63	1.91	0	1.48	1.47	1.53	0	2.8	-2.52	-1.59	-1.57	0
<i>aae B_2</i>	p-hydroxybenzoic acid efflux pump subunit AaeB	0	0	1.28	1.29	0	0	0	1.35	-2.12	0	-4.6	0
<i>acr A</i>	Multidrug efflux pump subunit AcrA	-2.77	-4.8	0	-1.67	-1.87	-2.65	2.33	-1.48	0	-2.67	0	-2.69
<i>acr B_1</i>	Multidrug efflux pump subunit AcrB	-2.67	-4.31	0	1.36	1.37	1.34	2.39	-1.55	-1.66	0	-1.82	0
<i>acr B_2</i>	Multidrug efflux pump subunit AcrB	1.37	1.42	0	-1.92	-1.83	-2.54	-2.15	0	-1.66	-4.11	0	-2.56
<i>acr E</i>	Multidrug export protein AcrE	1.46	1.44	0	0	0	1.32	-5.28	0	-4.24	0	-4.76	0
<i>acr F</i>	Multidrug export protein AcrF	0	0	0	0	0	0	-3.13	0	-2.54	-1.42	-2.77	0
<i>ala E</i>	L-alanine exporter AlaE	0	0	1.46	0	0	1.8	5.04	3.82	12.35	10.13	10.42	3.41
<i>alx</i>	Inner membrane protein Alx	0	0	1.25	0	0	0	-1.69	-1.5	-2.22	0	-3.14	0
<i>app A_1</i>	Oligopeptide-binding protein AppA	1.43	1.5	1.3	1.44	1.55	1.48	30.08	2.72	-2.67	0	10.25	1.7
<i>ara E</i>	Arabinose-proton symporter	1.34	1.31	0	0	0	0	-2.17	0	-2.72	-3.37	-3.27	0
<i>arg O</i>	Arginine exporter protein ArgO	0	0	1.37	0	0	0	1.4	1.66	2.07	2.94	0	0
<i>bam A</i>	Outer membrane protein assembly factor BamA	-4.49	-9.05	-3.02	-5.55	-3.7	-5.19	2.98	-1.96	0	-2.08	1.76	-1.85
<i>bam B</i>	Outer membrane protein assembly factor BamB	-3.41	-4.73	-1.97	-2.77	-2.82	-3.26	1.8	-2.32	0	-1.76	1.45	-1.91
<i>bam C</i>	Outer membrane protein assembly factor BamC	-2.42	-3.48	-1.98	-2.65	-1.9	-2.83	2.64	-1.89	3.25	1.46	5.8	0
<i>bam D</i>	Outer membrane protein assembly factor BamD	-2.18	-2.51	0	-1.46	-1.51	-1.63	2.53	-2.64	1.67	1.6	3.53	0
<i>bam E</i>	Outer membrane protein assembly factor BamE	0	0	0	0	0	0	3.4	1.46	0	0	2.09	1.79
<i>bep C</i>	Outer membrane efflux protein BepC	1.5	1.56	0	0	1.31	0	0	3.04	0	-2.38	-2.23	0
<i>bic</i>	Outer membrane lipoprotein Bic	0	0	0	0	0	0	-1.95	0	-2.12	0	-2.94	0
<i>ccm A</i>	Cytochrome c biogenesis ATP-binding export protein CcmA	0	0	0	0	0	0	-2.31	-1.72	-3.01	0	0	0
<i>ccm B</i>	Heme exporter protein B	0	0	0	0	0	0	-4.23	-1.81	0	0	-2.72	0
<i>ccm C</i>	Heme exporter protein C	0	0	0	0	0	0	-2.66	-1.86	-2.6	-2.58	-2.29	0
<i>cop A</i>	Copper-exporting P-type ATPase A	0	0	0	0	0	0	10.61	10.27	2.13	14.8	1.82	3.58
<i>cor C_1</i>	Magnesium and cobalt efflux protein CorC	-1.72	-1.64	0	0	0	0	5.1	2.07	0	0	0	0
<i>cor C_2</i>	Magnesium and cobalt efflux protein CorC	-1.58	-1.5	0	1.43	0	0	-1.75	-1.7	-1.8	0	0	2.05
<i>cor C_4</i>	Magnesium and cobalt efflux protein CorC	2.41	-2.24	0	0	-1.99	-1.89	0	-1.71	-2.09	0	-3.13	0
<i>cre D</i>	Inner membrane protein CreD	0	0	0	0	0	0	-2.1	0	-1.6	0	-1.87	0
<i>dgo T_1</i>	D-galactonate transporter	1.96	2.3	0	0	0	0	-1.81	0	0	0	0	0
<i>eam A</i>	putative amino-acid metabolite efflux pump	0	1.44	0	1.48	1.31	1.56	0	0	0	0	-2.21	0
<i>eam B</i>	Cysteine/O-acetylserine efflux protein	0	-1.36	0	0	0	0	0	2.14	-4.22	0	0	0
<i>ecf T</i>	Energy-coupling factor transporter transmembrane protein EcfT	1.38	0	0	0	0	0	2.52	0	-3.19	0	0	0
<i>emr A</i>	Multidrug export protein EmrA	-2.11	-2.26	-1.72	-1.71	-1.47	-1.43	0	0	-2.13	0	-1.76	0
<i>emr B_1</i>	Multidrug export protein EmrB	1.39	1.32	0	0	0	0	-2.07	0	-2.42	0	-1.61	0
<i>emr B_2</i>	Multidrug export protein EmrB	-1.65	-1.72	-1.28	-1.28	0	0	-1.89	0	-4.3	-2.62	0	0
<i>emr D</i>	Multidrug resistance protein D	0	0	0	0	0	0	1.03	3.28	0	2.11	0	0
<i>emr E</i>	Multidrug transporter EmrE	1.9	2.14	0	1.69	0	1.49	0	0	0	0	0	0

<i>ent 5</i>	Enterobactin exporter Ent5	0	0	1.41	1.58	1.39	1.42	-1.66	0	-5.95	0	-2.27	0
<i>feb B</i>	Ferrienterobactin-binding periplasmic protein	0	0	0	0	0	0	0	0	-3.72	0	0	0
<i>fim D_1</i>	Outer membrane usher protein FimD	1.55	1.52	0	0	0	1.35	-2.8	1.46	-3.04	0	0	0
<i>fim D_2</i>	Outer membrane usher protein FimD	1.6	1.47	0	0	0	0	1.7	2.02	-2.25	0	0	0
<i>fli Y_2</i>	L-cystine-binding protein FliY	0	1.86	0	0	-1.66	-1.8	2.06	0	2.37	0	-2.72	0
<i>fsr</i>	Fosmidomycin resistance protein	0	0	1.43	1.53	0	1.3	0	0	-3.01	0	0	0
<i>gal P</i>	Galactose-proton symporter	0	0	-1.84	-1.87	0	0	0.33	0	0	0	0	0
<i>gln M</i>	putative glutamine ABC transporter permease protein GlnM	1.6	1.65	0	1.4	1.43	1.57	0	0	0	0	-2.77	0
<i>glp T</i>	Glycerol-3-phosphate transporter	-1.99	-2.74	-13.86	-16.01	-12.65	-14.35	-20.67	0	3.3	-21.87	-4.14	-3.02
<i>gsi C</i>	Glutathione transport system permease protein GsiC	0	0	0	1.26	1.35	0	-2.89	0	-2.41	0	-3.56	-1.72
<i>gsi D</i>	Glutathione transport system permease protein GsiD	0	0	1.56	0	0	0	-4.06	0	-2.16	0	-2.21	0
<i>gud P</i>	putative glucarate transporter	0	0	0	0	1.26	0	-2.81	-1.54	-2.31	0	-2.89	0
<i>hco T</i>	putative 3-phenylpropionic acid transporter	0	0	0	1.24	0	0	2.32	0	2.13	1.82	0	-1.47
<i>his J</i>	Histidine-binding periplasmic protein	-1.41	-1.41	0	0	0	0	2.48	-1.49	-2.63	0	3.8	0
<i>htr E</i>	Outer membrane usher protein HtrE	0	0	0	0	0	0	0	0	0	0	-2.25	0
<i>ics A_1</i>	Outer membrane protein IcsA autotransporter	0	0	0	0	0	0	-2.11	0	-1.44	0	-2.09	0
<i>ics A_2</i>	Outer membrane protein IcsA autotransporter	1.52	1.59	0	1.23	0	0	-2.41	0	-1.99	0	-1.96	0
<i>kef C_1</i>	Glutathione-regulated potassium-efflux system protein KefC	0	0	0	0	0	0	-3.12	0	-2.64	0	-2.83	0
<i>kgt P</i>	Alpha-ketoglutarate permease	0	0	0	0	1.33	1.31	-3.81	-1.41	-1.92	0	-1.74	0
<i>lac G</i>	Lactose transport system permease protein LacG	1.36	0	0	0	0	0	-2.5	0	-2.58	0	-2.85	0
<i>lam B</i>	Maltoporin	-7.31	-7.74	-10.9	-12.46	-10.52	-11.43	-13.65	0	-11.38	-18.04	-4.3	-2.74
<i>lpl A</i>	Outer-membrane lipoprotein carrier protein	-2.64	-3.51	0	-1.84	-1.5	-1.77	1.6	0	0	-1.82	1.61	-2.59
<i>lpl C</i>	Lipoprotein-releasing system transmembrane protein LolC	-1.32	0	0	0	-1.28	0	-2.48	-2.04	-2.92	-1.99	0	0
<i>lpl D_1</i>	Lipoprotein-releasing system ATP-binding protein LolD	-1.91	-1.72	-1.61	-1.46	-1.79	-1.39	-3.5	-2.45	-3.83	-2.18	-3.83	-2.06
<i>lpl E</i>	Lipoprotein-releasing system transmembrane protein LolE	-1.99	-2.11	-1.36	-1.4	-1.88	-1.68	-2.15	-2.32	-4.3	-2.15	0	-1.79
<i>lpl T</i>	Lysophospholipid transporter LplT	0	0	0	0	0	0	0	0	0	0	-2.26	-1.83
<i>lpp</i>	Major outer membrane lipoprotein Lpp	-1.14	-1.99	0	-0.63	0	-4.02	10.33	-2.14	0	0	19.36	13.81
<i>lpt A</i>	Lipopolysaccharide export system protein LptA	-2.67	-3.48	-1.74	-2.17	-2.2	2.64	2.1	0	2.46	0	2.52	0
<i>lpt B_2</i>	Lipopolysaccharide export system ATP-binding protein LptB	-2.46	-3.1	-1.95	-2.3	0	0	2.03	-1.37	1.93	0	-4.3	-1.54
<i>lpt C</i>	Lipopolysaccharide export system protein LptC	-2.64	-3.07	-1.59	-1.94	-1.94	-2.01	1.92	0	1.86	0	1.66	1.38
<i>lpt F</i>	Lipopolysaccharide export system permease protein LptF	-1.79	-1.83	0	0	-1.37	-1.31	2.89	0	1.42	1.36	1.4	0
<i>lpt G</i>	Lipopolysaccharide export system permease protein LptG	-1.62	-1.61	0	0	0	0	2.34	0	0	0	0	0
<i>ltp A</i>	Lipoprotein signal peptidase	-2.86	-3.73	-1.85	-2.3	-2.26	-2.21	2.72	0	0	0	1.67	0
<i>ltxB_1</i>	Leukotoxin export ATP-binding protein LtxB	-1.59	0	0	0	0	0	2.63	0	0	0	0	-1.16
<i>ltxB_2</i>	Leukotoxin export ATP-binding protein LtxB	0	1.68	0	0	0	0	-2.28	3.02	-2.38	-1.64	0	0
<i>lys O</i>	Lysine exporter LysO	0	1.39	1.41	1.7	1.41	1.8	0	0	-3.87	0	0	0
<i>mac A_1</i>	Macrolide export protein MacA	0	-1.38	0	0	0	0	3.29	2.81	4.23	3.84	2.76	1.91
<i>mac A_2</i>	Macrolide export protein MacA	0	1.32	1.25	0	0	0	0	0	-2.22	-1.56	0	0
<i>mac A_3</i>	Macrolide export protein MacA	-3.15	-4	1.34	1.46	1.77	1.68	-4.05	-2.33	0	0	2.13	0
<i>mac B</i>	Macrolide export ATP-binding/permease protein MacB	-1.89	-2.27	0	0	0	0	-1.79	0	-3.09	0	-1.88	0
<i>mal E</i>	Maltose-binding periplasmic protein	-3.75	-3.95	-6.01	-7.29	-6.14	-7.27	9.28	0	-5.89	-11.85	-7.04	-2.54
<i>mal K</i>	Maltose/maltodextrin import ATP-binding protein MalK	-3.98	-4.34	-5.02	-5.55	-3.69	-4.05	3.04	0	12.73	-6.04	-3.19	-2.63
<i>mar A_2</i>	Multiple antibiotic resistance protein MarA	-2.67	-2.34	2.27	2.33	1.84	1.57	3.74	2.23	0	2.02	0	3.53
<i>mdf A</i>	Multidrug transporter MdfA	0	0	1.39	1.54	0	0	0	0	0	0	-4.76	-1.91
<i>mdt H_1</i>	Multidrug resistance protein MdtH	1.7	1.82	0	1.4	1.54	1.65	0	0	0	0	-2.01	0
<i>mdt H_2</i>	Multidrug resistance protein MdtH	0	0	0	0	0	0	-1.65	-1.4	-1.79	0	-2.67	0
<i>mdt J</i>	Spermidine export protein MdtJ	1.56	2.21	1.66	1.9	0	1.69	0	0	0	0	0	0
<i>mdt L</i>	Multidrug resistance protein MdtL	0	0	0	0	0	0	-3.8	0	-5.48	0	-5.07	0
<i>met P</i>	Methionine import system permease protein MetP	0	1.39	0	0	1.45	1.35	0	0	-2.84	0	0	0
<i>mia C</i>	putative phospholipid-binding protein MiaC	-1.78	-2.68	0	-1.27	0	-1.5	1.03	-1.49	-3.75	1.38	2.56	0
<i>mit A</i>	Membrane-bound lytic murein transglycosylase A	-1.41	-1.5	0	0	0	0	-1.72	-2.1	-2.48	0	0	0
<i>mit B</i>	Membrane-bound lytic murein transglycosylase B	0	0	0	0	0	0	1.49	0	0	0	0	0
<i>mit F</i>	Membrane-bound lytic murein transglycosylase F	0	0	0	1.28	0	0	-2.59	-1.46	-3.1	0	0	0
<i>mnt B_1</i>	Manganese transport system membrane protein MntB	0	0	0	0	0	0	-2.63	0	-3.21	0	-2.3	0
<i>mod A</i>	Molybdate-binding periplasmic protein	-1.35	-1.61	0	1.3	0	0	1.21	1.53	2.93	2.77	4.28	1.62
<i>mod B</i>	Molybdenum transport system permease protein ModB	0	-1.46	0	0	0	0	2.12	2.03	5.09	2.52	2.67	0
<i>mxl D</i>	Outer membrane protein MxlD	-2.73	-2.65	1.42	1.26	0	0	0	0	0	1.41	0	0
<i>nan T</i>	Putative sialic acid transporter	1.8	0	1.3	0	1.46	0	1.78	0	0	0	13.86	0
<i>nep I</i>	Purine ribonucleoside efflux pump NepI	0	-1.36	0	0	0	-1.61	0	0	-2.07	0	-1.78	0
<i>omp A</i>	Outer membrane protein A	-4.22	-10.05	1.48	-1.56	0	-2.69	4.79	0	1.51	-2.31	9.3	0
<i>omp C_2</i>	Outer membrane protein C	-12.41	-28.74	-2.02	-3.95	-4.25	-6.71	1.61	9.3	0	-14.64	1.66	-5.83
<i>omp D</i>	Outer membrane porin protein OmpD	-18.52	-25.66	-5.73	-12.58	-10.8	-23.79	19.88	0	5.35	-16.52	12.21	-12.34
<i>omp N_1</i>	Outer membrane protein N	1.44	1.72	0	1.34	1.49	1.75	-2.05	0	0	0	-3.25	0
<i>omp N_2</i>	Outer membrane protein N	1.45	1.76	1.55	1.69	1.49	1.79	0	0	-2.12	0	-2.82	0
<i>omp R</i>	Transcriptional regulatory protein OmpR	-1.36	-1.56	0	0	0	0	1.75	0	0	0	-4.11	2.71
<i>omp W</i>	Outer membrane protein W	-2.52	-3.43	-2.02	-3.64	-1.63	-2.86	8.95	0	0	0	22.95	1.62
<i>omp X</i>	Outer membrane protein X	-1.57	-2.39	4.39	2.43	2.18	1.37	5.03	-1.76	0	-2.74	4.63	0
<i>osm V</i>	Osmoprotectant import ATP-binding protein OsmV	2.76	1.69	2.3	1.79	2.24	1.81	1.88	2.01	0	0	-2.66	0

Proteínas e transportadores de membrana

<i>osm W</i>	Osmoprotectant import permease protein OsmW	2.77	1.76	2.6	2.19	2.41	2.09	0	2.2	0	0	0	-2.11
<i>osm X</i>	Osmoprotectant-binding protein OsmX	2.36	2.15	2.13	2.24	2.26	2.4	0	1.95	-2.22	0	-4.46	0
<i>pap N</i>	Outer membrane protein PapN	0	0	1.66	1.58	0	1.48	0	0	-2.13	0	0	1.69
<i>pap C</i>	Outer membrane usher protein PapC	0	0	0	0	0	0	0	0	0	-1.63	-2.36	0
<i>pap C_1</i>	Outer membrane usher protein PapC	1.53	1.38	0	1.25	0	0	-2.4	0	-4.11	0	0	0
<i>pap C_2</i>	Outer membrane usher protein PapC	1.38	1.42	0	1.29	0	0	2.44	0	-2.6	0	0	0
<i>pap C_3</i>	Outer membrane usher protein PapC	1.51	1.42	1.22	0	0	0	-2.45	0	0	-1.6	0	0
<i>phn S</i>	Putative 2-aminoethylphosphonate-binding periplasmic protein	1.66	1.61	0	0	0	0	-2.42	0	-4.86	0	-3.35	0
<i>phn T</i>	Putative 2-aminoethylphosphonate import ATP-binding protein PhnT	1.53	1.54	0	1.28	1.39	0	-2.08	0	-8.71	0	-2.12	0
<i>pot A_2</i>	Spermidine/putrescine import ATP-binding protein PotA	1.54	1.63	1.57	1.63	0	1.39	-3.25	0	-2.2	0	-5.4	-1.72
<i>pot H</i>	Putrescine transport system permease protein PotH	1.68	1.87	1.51	1.66	1.67	1.63	-3.3	0	-3.95	0	-3.43	0
<i>pro V</i>	Glycine betaine/proline betaine transport system ATP-binding protein ProV	-3.01	-3.24	-1.48	-1.39	-2.05	-1.75	-5.61	-3.78	-9.95	-6.56	-3.82	0
<i>pro W</i>	Glycine betaine/proline betaine transport system permease protein ProW	-2.14	-2.22	-1.37	-1.42	0	-1.41	-5.24	-2.2	-7.62	-5.1	-2.22	0
<i>pst S</i>	Phosphate-binding protein PstS	2.47	1.44	1.89	2.33	1.85	1.8	2.14	0	-4.75	6.11	2.03	2.15
<i>pta</i>	Phosphate acetyltransferase	-4.4	-6.43	-2.06	-2.14	-2.73	-3.82	-7.6	0	-1.96	-5.04	0	-4.53
<i>rci C</i>	Inner membrane protein RciC	1.45	1.56	0	1.43	0	1.34	-2.63	0	-3.31	0	-6.38	0
<i>rcn A</i>	Nickel/cobalt efflux system RcnA	1.4	1.45	0	1.27	0	0	-1.7	0	-2.88	0	-1.91	-1.7
<i>rhm T</i>	Inner membrane transport protein RhmT	1.58	1.57	1.4	1.4	1.35	1.43	-3.18	-1.56	0	0	0	0
<i>rht C_2</i>	Threonine efflux protein	0	0	0	0	0	0	-2.4	0	0	0	0	0
<i>rob_1</i>	Right origin-binding protein	1.69	1.86	1.64	1.52	1.52	2.38	0	0	0	0	0	3.78
<i>sad B</i>	Inner membrane lipoprotein SadB	-1.64	0	2.1	0	0	0	0	0	0	0	0	3.43
<i>sfm D</i>	Outer membrane usher protein SfmD	1.41	1.52	-1.37	-1.48	0	0	-2.1	0	-1.67	0	-2.09	0
<i>sly B</i>	Outer membrane lipoprotein SlyB	-3.49	-3.53	0	0	0	0	1.69	0	0	0	-4.43	-8.71
<i>smv A</i>	Methyl viologen resistance protein SmvA	1.86	2.22	1.71	2.02	1.61	2.02	1.42	1.76	0	2.4	-3.31	0
<i>suf C</i>	putative ATP-dependent transporter SufC	2.05	2.25	0	1.38	1.8	1.67	0	0	0	2.31	-2.57	0
<i>tol C_2</i>	Outer membrane protein TolC	0	-1.66	0	0	0	0	2.92	0	0	0	2.42	1.69
<i>ttu B_1</i>	Putative tartrate transporter	1.67	1.76	1.32	1.55	1.61	1.65	-2.95	0	0	0	0	0
<i>ttu B_2</i>	Putative tartrate transporter	0	0	0	0	1.39	1.88	0	0	-3.8	0	0	0
<i>ttu B_3</i>	Putative tartrate transporter	0	0	1.41	1.65	0	0	-2.03	0	-3.38	0	0	0
<i>ugp C</i>	sn-glycerol-3-phosphate import ATP-binding protein UgpC	0	0	0	0	0	0	3.09	0	-2.42	-1.86	-2	-1.45
<i>uhp C_1</i>	Membrane sensor protein UhpC	0	0	1.54	1.49	1.39	1.55	-2.07	0	0	0	0	0
<i>uup</i>	ABC transporter ATP-binding protein uup	-2.8	-2.84	-1.34	0	-1.85	-1.84	-1.42	-1.61	-1.79	-1.88	-2.1	-2.69
<i>xap B</i>	Xanthosine permease	1.51	1.72	1.27	1.39	1.35	1.46	2.49	0	-2.41	0	-2.34	0
<i>yab I</i>	Inner membrane protein YabI	0	0	0	0	0	0	2.06	0	0	0	-1.81	0
<i>yad H</i>	Inner membrane transport permease YadH	0	0	0	0	0	0	3.4	1.63	1.57	0	0	0
<i>yaj R</i>	Inner membrane transport protein YajR	0	0	0	0	0	0	-2.34	0	-2.15	-1.75	-2.14	-1.67
<i>yba L</i>	Inner membrane protein YbaL	-2.02	-1.98	-1.28	-1.21	0	0	0	-1.67	1.52	1.52	-1.65	0
<i>yba N</i>	Inner membrane protein YbaN	0	0	0	0	0	0	-7.3	0	0	0	0	0
<i>ybc I</i>	Inner membrane protein YbcI	1.36	0	0	0	1.63	1.67	0	0	0	0	-2.63	0
<i>ybh L_2</i>	Inner membrane protein YbhL	0	0	1.69	1.55	0	0	2.52	-2.39	1.63	0	-6.6	1.63
<i>ybh N</i>	Inner membrane protein YbhN	1.76	1.68	1.6	1.66	1.47	1.41	-2.04	0	-2.1	0	-2.85	0
<i>ybh Q</i>	Inner membrane protein YbhQ	0	0	0	0	0	0	0	0	-3.17	0	0	0
<i>ybh R</i>	Inner membrane transport permease YbhR	1.33	1.42	1.43	1.42	1.28	1.28	-1.81	0	0	-3.14	-2.67	-1.97
<i>ybi R</i>	Inner membrane protein YbiR	0	0	0	0	0	0	0	0	0	0	-2.06	-1.59
<i>ybj J</i>	Inner membrane protein YbjJ	0	0	0	0	0	0	-1.6	1.33	-1.85	0	-2.02	0
<i>ybj M</i>	Inner membrane protein YbjM	0	1.61	0	1.59	0	1.5	0	2.83	0	2.4	0	0
<i>ybj O</i>	Inner membrane protein YbjO	0	0	0	0	0	0	0	0	0	0	-5.87	0
<i>yca D</i>	putative MFS-type transporter YcaD	1.56	1.74	1.56	1.61	1.49	1.62	-6.16	0	0	-2.19	-4.6	0
<i>yca M</i>	Inner membrane transporter YcaM	1.63	1.91	1.53	1.6	0	1.41	-2.34	-1.45	-2.39	0	-4.88	0
<i>ycc S_2</i>	Inner membrane protein YccS	0	0	0	0	0	0	2.26	0	-2.01	0	-1.74	0
<i>ydc O</i>	Inner membrane protein YdcO	1.6	2	1.6	1.85	1.52	1.72	1.88	16.88	0	0	0	0
<i>ydc V_1</i>	Inner membrane ABC transporter permease protein YdcV	-1.76	-1.41	0	0	0	0	4.3	0	1.96	0	2.48	0
<i>ydc V_2</i>	Inner membrane ABC transporter permease protein YdcV	1.76	1.75	0	1.33	1.48	1.57	-2.05	0	-2.4	0	-3.51	0
<i>ydc Z</i>	Inner membrane protein YdcZ	1.87	2.11	0	1.48	1.38	1.45	1.72	1.98	0	0	0	0
<i>ydh C</i>	Inner membrane transport protein YdhC	0	1.46	0	1.63	0	0	0	0	-2.39	-2.26	-2.76	0
<i>ydh P_1</i>	Inner membrane transport protein YdhP	0	1.3	0	0	0	0	-1.91	0	-2.23	0	-3.17	0
<i>ydh P_2</i>	Inner membrane transport protein YdhP	1.37	1.33	0	0	0	0	-5.64	0	-5.46	0	0	0
<i>ydi M</i>	Inner membrane transport protein YdiM	1.44	1.91	1.98	2	0	1.62	0	0	0	0	-1.97	1.59
<i>ydi N</i>	Inner membrane transport protein YdiN	1.64	1.93	1.76	1.8	1.3	1.65	0	0	-3.06	0	-2.31	0
<i>yeb E</i>	Inner membrane protein YebE	2.17	1.94	1.85	2.17	2.24	2.07	30.02	124.83	6.08	18.74	19.23	19.41
<i>yeb S</i>	Inner membrane protein YebS	-1.53	-1.34	1.3	1.39	0	1.23	-2.05	-1.78	-1.87	0	-2.19	0
<i>yeb Z</i>	Inner membrane protein YebZ	-1.74	-1.7	0	0	-1.41	-1.33	0	3.18	0	0	0	0
<i>yed A_2</i>	putative inner membrane transporter YedA	-2.47	-1.85	0	0	1.52	2.04	0	-2.64	0	0	0	0
<i>yed I</i>	Inner membrane protein YedI	1.38	1.43	0	1.32	1.54	1.62	0	0	0	-2.5	-3.16	0
<i>yed R</i>	Inner membrane protein YedR	1.51	1.62	0	0	0	0	0	2.33	0	0	0	2.3
<i>yee A</i>	Inner membrane protein YeeA	0	0	0	1.39	0	0	11.85	3.12	3.19	1.84	-4.21	0
<i>yeh Y</i>	Glycine betaine uptake system permease protein YehY	2.05	1.88	1.31	1.34	1.68	1.74	0	0	0	1.51	0	0
<i>yeh Z</i>	Glycine betaine-binding protein YehZ	2.09	2.17	1.49	1.47	1.99	1.72	0	0	0	0	0	0

<i>yej B</i>	Inner membrane ABC transporter permease protein YejB	0	0	0	1.24	0	0	-1.92	2.74	-2.17	0	-1.67	0
<i>yej E</i>	Inner membrane ABC transporter permease protein YejE	0	0	0	0	1.35	1.38	0	3.84	-2.2	0	-3.14	0
<i>yej M</i>	Inner membrane protein YejM	0	0	0	0	0	0	-1.46	0	-1.9	0	-2.86	0
<i>yfa C</i>	Inner membrane protein YfaC	0	1.68	1.41	1.55	1.39	1.67	-1.76	0	0	0	-2.67	0
<i>yga P</i>	Inner membrane protein YgaP	1.52	0	0	0	1.53	0	8.85	3.11	1.64	3.98	-8.91	3.47
<i>yga Z</i>	Inner membrane protein YgaZ	0	-1.36	0	0	0	0	-1.83	-1.41	0	0	-3.31	0
<i>ygb E</i>	Inner membrane protein YgbE	0	0	0	0	0	0	-4.39	0	-12.15	0	0	0
<i>ygc S</i>	Inner membrane metabolite transport protein YgcS	1.48	1.43	0	0	0	0	-1.75	0	0	0	-2.1	0
<i>ygf X</i>	Inner membrane protein YgfX	-1.36	-1.65	0	0	0	-1.43	2.86	0	2.59	0	-1.72	0
<i>ygh B</i>	Inner membrane protein YghB	0	0	0	0	0	0	2.88	-11.28	0	0	-2.31	0
<i>yha H</i>	Inner membrane protein YhaH	0	0	0	0	0	0	0	0	0	0	-3.67	-2.86
<i>yhb E</i>	putative inner membrane transporter YhbE	-4.55	-5.44	-1.72	0	-2.74	-2.61	1.5	-2.34	0	-1.44	0	1.6
<i>yhc B</i>	Inner membrane protein YhcB	-1.88	-2.16	0	0	0	-1.35	2.7	-1.91	4.8	2.88	-5.44	1.59
<i>yhe H</i>	putative multidrug resistance ABC transporter ATP-binding/permease protein YheH	0	1.31	0	0	0	0	-2.12	0	-2.12	0	-3.84	-1.84
<i>yhe I</i>	putative multidrug resistance ABC transporter ATP-binding/permease protein YheI	0	0	0	0	0	0	7.35	4.13	0	0	0	0
<i>yhh J</i>	Inner membrane transport permease YhhJ	0	0	0	0	0	0	1.55	0	0	-2.39	-1.73	-1.84
<i>yhh Q</i>	Inner membrane protein YhhQ	0	0	0	0	0	0	-1.76	0	-3.89	0	-2.73	0
<i>yhj D</i>	Inner membrane protein YhjD	0	0	0	0	0	0	-1.96	0	-1.98	0	-2.83	0
<i>yhj E</i>	Inner membrane metabolite transport protein YhjE	0	0	0	0	0	0	-2.28	0	-1.65	0	-3.29	0
<i>yhj V</i>	Inner membrane transport protein YhjV	0	0	0	0	0	0	0	-1.86	0	0	-2.47	0
<i>yic J_1</i>	Inner membrane symporter YicJ	0	0	1.38	0	0	0	-2.25	1.47	-2.66	0	0	0
<i>yic J_2</i>	Inner membrane symporter YicJ	0	0	0	0	0	0	-1.99	0	-2.05	-1.56	-1.81	1.54
<i>yic J_3</i>	Inner membrane symporter YicJ	0	0	0	0	0	0	-2.74	0	0	0	-2.27	0
<i>yic L</i>	putative inner membrane transporter YicL	-1.42	-1.63	0	0	0	0	-2	0	-1.68	0	1.77	1.44
<i>yid G</i>	Inner membrane protein YidG	-1.69	-1.74	0	0	0	-1.38	-2.59	-1.78	0	0	0	0
<i>yij D</i>	Inner membrane protein YijD	-1.42	-1.37	0	0	0	-1.44	1.9	0	1.82	1.92	2.66	0
<i>yjc H</i>	Inner membrane protein YjcH	1.53	1.38	0	0	0	0	0	0	0	0	-2.69	0
<i>yje M</i>	Inner membrane transporter YjeM	0	0	0	0	0	0	1.62	0	-4.2	0	-1.96	0
<i>yjg N</i>	Inner membrane protein YjgN	0	0	0	0	0	1.33	0	0	-4.13	0	-2.52	0
<i>yji G</i>	Inner membrane protein YjiG	1.84	0	0	-1.63	1.48	0	0	0	17.84	0	19.06	0
<i>yji Y</i>	Inner membrane protein YjiY	0	0	-1.41	-1.42	0	0	6.96	2.34	-1.61	-1.63	-1.77	0
<i>yla C</i>	Inner membrane protein YlaC	0	0	1.52	1.48	0	1.77	0	0	0	0	2.23	2.23
<i>ynf M</i>	Inner membrane transport protein YnfM	1.68	1.84	1.36	1.55	1.4	1.56	-2.08	1.82	-1.93	0	-1.8	0
<i>yoh K_1</i>	Inner membrane protein YohK	0	0	0	1.35	0	0	2.39	-1.53	0	0	2.41	0
<i>yoh K_2</i>	Inner membrane protein YohK	0	0	0	0	1.45	1.56	2.64	3.25	-4.13	0	0	0
<i>ypj D</i>	Inner membrane protein YpjD	0	-1.38	0	0	0	0	-2.94	0	-1.85	0	0	0
<i>yqa A</i>	Inner membrane protein YqaA	-2.29	-2.33	-1.51	-1.55	-1.35	-1.67	-1.77	0	0	0	0	0
<i>yqe G</i>	Inner membrane transport protein YqeG	0	0	1.3	1.3	0	0	-1.73	0	0	0	-3.36	0
<i>yqi K</i>	Inner membrane protein YqiK	0	0	0	0	0	0	-1.45	0	0	0	-2.25	-1.4
<i>yqi A</i>	Inner membrane protein YqiA	-2.93	-2.65	0	0	0	0	0	0	0	1.91	0	1.68
<i>yqi E</i>	Inner membrane protein YqiE	0	0	1.94	1.44	2.51	1.85	3.38	0	3.05	0	1.77	1.83
<i>yqi F</i>	Inner membrane protein YqiF	0	0	0	0	0	0	0	0	0	0	-3.28	-2.84
<i>ytf F</i>	Inner membrane protein YtfF	0	0	0	0	0	0	-2.65	0	0	1.6	-2.36	0
<i>znu C_2</i>	High-affinity zinc uptake system ATP-binding protein ZnuC	0	0	1.49	1.36	0	0	-2.42	0	-2.5	0	-3.93	0
<i>amp D</i>	1,6- α -hydro-N-acetylmuramyl-L-alanine amidase AmpD	-1.31	-1.4	0	0	0	0	3.37	0	4.9	3.29	8.1	3.35
<i>amp H</i>	D-alanyl-D-alanine- carboxypeptidase/endopeptidase AmpH	0	0	0	1.24	0	0	-1.89	0	-3.67	0	-1.65	0
<i>cpt A</i>	Phosphoethanolamine transferase CptA	-2.61	-2.82	-1.56	-1.71	-1.33	-1.5	0	0	-2.33	-1.9	0	0
<i>dac B</i>	D-alanyl-D-alanine carboxypeptidase DacB	0	0	0	0	0	0	-1.55	0	-2.45	0	-2.4	0
<i>dac C</i>	D-alanyl-D-alanine carboxypeptidase DacC	0	0	1.71	1.48	1.64	1.77	0	2.6	0	0	0	0
<i>dac D</i>	D-alanyl-D-alanine carboxypeptidase DacD	1.46	1.63	0	1.37	1.51	1.36	2.44	1.88	0	0	0	0
<i>dal A</i>	D-alanine-D-alanine ligase A	0	0	0	0	0	0	3.17	3.11	1.46	1.47	1.99	1.36
<i>dal B</i>	D-alanine-D-alanine ligase B	-2.83	-4.06	-2.18	-2.67	-2.28	-2.7	0	-1.67	-3.42	-2.86	-1.99	-2.34
<i>fts I_1</i>	Peptidoglycan D,D-transpeptidase FtsI	-1.98	-2.26	0	0	0	1.3	0	0	-3.68	-2.54	-3.93	0
<i>fts I_2</i>	Peptidoglycan D,D-transpeptidase FtsI	0	1.39	-1.52	-1.48	-1.41	-1.45	-5.03	0	-1.58	-1.36	-1.51	0
<i>hid D</i>	ADP-L-glycero-D-manno-heptose-6-epimerase	-2.67	-3.24	-1.49	-1.88	-1.7	-1.97	3.28	-1.81	3.32	2.31	3.62	1.75
<i>kds A</i>	2-dehydro-3-deoxyphosphooctonate aldolase	-2.72	-2.83	-1.36	-1.45	-1.87	-2.08	4.37	2.35	1.54	-1.69	0	0
<i>kds B</i>	3-deoxy-manno-octulosonate cytidyllyltransferase	-1.3	0	0	0	0	0	8.67	2.27	3.02	3.01	1.99	0
<i>ldc A</i>	Murein tetrapeptide carboxypeptidase	0	1.39	1.3	1.64	0	1.34	-3.03	0	0	0	0	0
<i>lpx A</i>	Acyl-[acyl-carrier-protein]-UDP-N- acetylglucosamine O-acyltransferase	-4.76	-6	-2.03	-2.4	-2.5	-3.02	3.7	3.71	0	-1.72	0	-1.76
<i>lpx B</i>	Lipid-A-disaccharide synthase	-2.54	-2.76	-1.88	-1.85	-2.03	-2.43	-1.65	-2.18	0	-1.61	-1.66	0
<i>lpx C</i>	UDP-3-O-acyl-N-acetylglucosamine deacetylase	-2.84	-3.68	0	-1.37	-1.58	-1.56	3.97	3.95	2.72	3.16	3.9	4.38
<i>lpx D_1</i>	UDP-3-O-[3-hydroxymyristoyl]glucosamine N-acyltransferase	-7.21	10.01	1.51	1.83	1.45	1.74	-1.54	-4.65	0	0	0	0
<i>lpx D_2</i>	UDP-3-O-[3-hydroxymyristoyl]glucosamine N-acyltransferase	1.87	2.04	-3.7	-5.51	-5.05	-6.97	0	1.72	-2.16	-6.25	0	-3.13
<i>lpx H</i>	UDP-2,3-diacylglucosamine hydrolase	-1.39	-1.37	0	0	0	0	0	0	0	0	-2.1	0
<i>lpx K</i>	Tetraacyldisaccharide 4'-kinase	-1.91	-1.86	-1.31	0	0	0	-1.31	0	-1.78	-1.92	-1.69	-2.36
<i>lpx P</i>	Lipid A biosynthesis palmitoleoyltransferase	0	0	0	0	0	1.34	0	0	0	2.13	0	0
<i>lpx T</i>	Lipid A 1-diphosphate synthase	-1.54	0	0	0	0	0	-2.9	0	0	0	0	0
<i>mep M</i>	Murein DD-endopeptidase MepM	-2.06	-2.07	-1.55	-1.39	-1.31	0	0	-1.48	-2.54	-2.31	-1.57	0

Biosynthesis_ria

Lipopolissacarídeo e Petideoglicano	<i>mepS_1</i>	Murein DD-endopeptidase MepS/Murein LD-carboxypeptidase	0	1.4	0	-1.75	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
	<i>mraY</i>	Phospho-N-acetylmuramoyl-pentapeptide- transferase	-2.82	-3.78	-2.11	-2.43	-2.27	-2.97	0	-2.7	-2.19	-3.56	-2.28	-2.03	0	0	0	0	0	0	
	<i>mrcA</i>	Penicillin-binding protein 1A	0	-1.35	0	0	0	0	0	0	-1.71	0	-1.94	-1.4	0	0	0	0	0	0	
	<i>mrcB</i>	Penicillin-binding protein 1B	-1.67	-1.8	-1.47	-1.45	-1.34	-1.33	3.87	0	0	-2	0	0	0	0	0	0	0	0	
	<i>mrdA_1</i>	Peptidoglycan D,D-transpeptidase MrdA	0	1.42	1.32	1.43	1.31	1.38	-2.8	0	-3.33	0	-2.71	0	0	0	0	0	0	0	
	<i>mrdA_2</i>	Peptidoglycan D,D-transpeptidase MrdA	-1.44	-1.41	0	0	0	0	-1.85	0	-1.62	0	-1.58	0	0	0	0	0	0	0	
	<i>mtgA</i>	Biosynthetic peptidoglycan transglycosylase	0	0	0	0	0	0	0	0	-1.88	0	-1.75	0	0	0	0	0	0	0	
	<i>murA</i>	UDP-N-acetylglucosamine 1-carboxyvinyltransferase	-2.61	-3.11	-1.29	-1.48	-1.55	-1.89	4	-1.9	1.53	0	1.82	0	0	0	0	0	0	0	
	<i>murB</i>	UDP-N-acetylenolpyruvoylglucosamine reductase	-1.62	-1.67	0	0	0	-1.3	-2.38	0	0	1.62	0	0	0	0	0	0	0	0	
	<i>murC</i>	UDP-N-acetylmuramate-L-alanine ligase	-3.46	-4.66	-2.38	-2.59	-2.51	-3.04	-1.57	-2.15	-4.62	-3.66	-2.9	-2.57	0	0	0	0	0	0	
	<i>murD</i>	UDP-N-acetylmuramoylalanine-D-glutamate ligase	-2.83	-3.95	-2.41	-2.71	-2.78	-3.29	0	-2.58	-3.44	-3.58	-2.54	-2.28	0	0	0	0	0	0	
	<i>murE</i>	UDP-N-acetylmuramoyl-L-alanyl-D-glutamate-2, 6-diaminopimelate ligase	-2.6	-3.44	-1.73	-2.08	-1.8	-2.24	1.43	-1.78	0	-1.85	0	-1.68	0	0	0	0	0	0	
	<i>murF</i>	UDP-N-acetylmuramoyl-tripeptide-D-alanyl-D- alanine ligase	-2.6	-3.7	-1.76	-2.23	-1.96	-2.58	1.52	-1.9	-1.36	-2.49	-1.45	-1.79	0	0	0	0	0	0	
	<i>murG</i>	UDP-N-acetylglucosamine-N-acetylmuramyl- (pentapeptide) pyrophosphoryl-undecaprenol	-2.9	-4.13	-2.58	-2.58	-2.55	-3.24	-2.2	-2.41	-3.54	-4.1	-3.03	-3.1	0	0	0	0	0	0	
	<i>murJ</i>	putative lipid II flippase MurJ	0	0	0	1.25	0	0	-2.14	0	-2.58	-1.86	0	0	0	0	0	0	0	0	
	<i>pbpC</i>	Penicillin-binding protein 1C	1.46	1.42	0	0	0	1.31	-1.77	0	-1.95	-2.18	-2.3	-1.83	0	0	0	0	0	0	
	<i>rfaC</i>	Lipopolysaccharide heptosyltransferase 1	-2.01	-2.33	-1.75	-2.03	-1.67	-1.84	-1.52	-1.36	-1.48	-1.51	0	0	0	0	0	0	0	0	
	<i>rfaF</i>	ADP-heptose-LPS heptosyltransferase 2	-2.41	-2.87	-1.89	-2.1	-1.76	-2.45	1.76	-1.37	1.36	0	1.98	0	0	0	0	0	0	0	
	<i>rfaG</i>	Lipopolysaccharide core biosynthesis protein RfaG	-1.73	-2.07	0	-1.31	-1.43	-1.37	0	0	1.51	1.74	1.62	0	0	0	0	0	0	0	
	<i>rfaP</i>	Lipopolysaccharide core heptose(i) kinase RfaP	-1.91	-2.17	0	-1.36	0	-1.49	0	-1.43	2.92	1.8	2.27	0	0	0	0	0	0	0	
<i>rfaQ</i>	Lipopolysaccharide core heptosyltransferase RfaQ	-1.47	-1.64	0	0	0	-1.37	0	-1.41	1.7	2.19	1.78	0	0	0	0	0	0	0		
<i>rfaY</i>	Lipopolysaccharide core heptose(ii) kinase RfaY	-2.63	-2.73	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0		
<i>rfbV</i>	Abequosyltransferase RfbV	-6.97	-8.11	0	0	-3.79	-3.29	3.92	0	0	0	1.93	0	0	0	0	0	0	0		
<i>uppP</i>	Undecaprenyl-diphosphatase	-2.23	-2.24	0	0	0	0	-1.49	-2.06	-1.68	0	0	0	0	0	0	0	0	0		
<i>ybjG</i>	Putative undecaprenyl-diphosphatase YbjG	0	0	0	1.33	0	1.44	0	2.14	-8.11	7.75	0	0	0	0	0	0	0	0		
<i>ynhG</i>	putative L,D-transpeptidase YnhG	1.68	1.86	1.5	1.52	1.4	1.53	0	0	0	0	-2.13	0	0	0	0	0	0	0		
Estresse oxidativo	<i>katE</i>	Catalase HPII	3.11	3.22	1.93	1.99	2.1	2.95	-1.63	0	0	0	0	0	0	0	0	0	0	0	
	<i>katG</i>	Catalase-peroxidase	2.22	-1.45	0	-1.65	1.66	0	17.89	0	13.18	29.91	13.69	3.74	0	0	0	0	0	0	
	<i>oxyR_1</i>	Hydrogen peroxide-inducible genes activator	0	0	-1.48	-1.65	-1.37	-1.65	0	-1.77	3.6	0	0	0	0	0	0	0	0	0	
	<i>oxyR_3</i>	Hydrogen peroxide-inducible genes activator	-2.11	-2.67	0	1.32	0	0	6.93	0	-3.62	0	1.47	0	0	0	0	0	0	0	
	<i>oxyR_4</i>	Hydrogen peroxide-inducible genes activator	1.69	1.83	0	0	0	0	-2.05	0	0	1.59	0	0	0	0	0	0	0	0	
	<i>sodA</i>	Superoxide dismutase [Mn]	-1.49	-1.6	0	0	0	0	2.27	0	2.27	2.33	0	0	0	0	0	0	0	0	
	<i>sodB</i>	Superoxide dismutase [Fe]	0	-2.64	1.59	-1.66	0	-2.19	14.61	-1.96	13.96	2.01	12.66	4.56	0	0	0	0	0	0	
	<i>sodC</i>	Superoxide dismutase [Cu-Zn]	1.73	1.61	0	0	1.78	1.79	2.01	0	0	0	1.9	0	0	0	0	0	0	0	
	<i>sodC1</i>	Superoxide dismutase [Cu-Zn]	0	-2.88	0	0	0	0	3.44	-3.94	0	0	0	0	0	0	0	0	0	0	
	<i>ahpC</i>	Alkyl hydroperoxide reductase subunit C	0	-3.62	0	-3.17	-1.68	-3.07	3.98	-3.7	3.99	3.66	9.7	4.1	0	0	0	0	0	0	
	<i>soxS_1</i>	Regulatory protein SoxS	0	1.64	2.06	1.73	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
	<i>soxS_2</i>	Regulatory protein SoxS	0	0	0	0	0	1.82	0	0	2.61	2.97	0	0	0	0	0	0	0	0	
	Sistemas TA	<i>bhsA_1</i>	Multiple stress resistance protein BhsA	0	0	1.98	2.34	1.89	2.12	0	0	0	3.36	0	0	0	0	0	0	0	0
		<i>bhsA_2</i>	Multiple stress resistance protein BhsA	0	0	0	0	1.74	0	14.31	9.79	0	3.32	5.42	4.46	0	0	0	0	0	0
<i>bhsA_3</i>		Multiple stress resistance protein BhsA	0	0	0	0	0	0	3.95	2.74	0	3.94	0	3.14	0	0	0	0	0	0	
<i>bssS</i>		Biofilm regulator BssS	0	0	2.26	1.96	2.13	1.86	9.97	3.15	15.07	3.69	24.55	14.66	0	0	0	0	0	0	
<i>ccdA_1</i>		Antitoxin CcdA	0	0	0	0	0	0	9.99	6.03	0	0	0	0	0	0	0	0	0	0	
<i>ccdB_1</i>		Toxin CcdB	-1.58	-1.74	0	0	0	0	10.4	9.98	0	0	0	0	0	0	0	0	0	0	
<i>hha</i>		Hemolysin expression-modulating protein Hha	-2.14	0	1.84	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3.53	
<i>higA</i>		Antitoxin HigA	-1.61	-1.66	0	0	0	0	0	0	-7.76	0	0	0	0	0	0	0	0	0	
<i>higA-2_1</i>		Antitoxin HigA-2	0	0	0	0	0	0	0	0	3.31	0	0	0	0	0	0	0	0	0	
<i>ldrD_1</i>		Small toxic polypeptide LdrD	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1.91	
<i>ortT</i>		Orphan toxin OrtT	-2.63	0	0	0	0	0	20.23	4.91	0	0	0	0	0	0	0	0	0	0	
<i>parD1</i>		Antitoxin ParD1	0	0	0	0	0	0	0	0	0	0	2.39	3.88	0	0	0	0	0	0	
<i>parE4</i>		Toxin ParE4	0	0	0	0	0	0	0	0	0	0	0	3.94	0	0	0	0	0	0	
<i>tabA_2</i>		Toxin-antitoxin biofilm protein TabA	0	0	0	0	0	0	-5.8	0	0	0	1.82	0	0	0	0	0	0	0	
<i>tabA_3</i>		Toxin-antitoxin biofilm protein TabA	0	0	1.43	0	0	0	0	0	2.51	0	0	0	0	0	0	0	0	0	
<i>tabA_4</i>		Toxin-antitoxin biofilm protein TabA	1.93	0	0	0	0	0	-3.81	-1.49	0	0	0	0	0	0	0	0	0	0	
<i>tisB</i>		Small toxic protein TisB	-2.8	0	0	0	0	0	1,000,000	180,31	29,87	28,6	1,912,83	4,897,21	0	0	0	0	0	0	
<i>tomB</i>		Hha toxicity modulator TomB	0	0	0	0	0	0	2.52	0	2.25	0	0	0	0	0	0	0	0	0	
<i>vapB</i>	Antitoxin VapB	-1.43	-1.47	0	0	0	0	2.56	2.04	0	0	0	0	0	0	0	0	0	0		
<i>yafN</i>	Antitoxin YafN	0	0	0	0	1.58	2.02	0	0	0	0	0	0	0	0	0	0	0	0		
<i>yafQ</i>	mRNA Interferase YafQ	0	0	0	0	0	0	2.43	3.83	0	0	0	0	0	0	0	0	0	0		
	<i>accA</i>	Acetyl-coenzyme A carboxylase carboxyl transferase subunit alpha	-3.61	-3.56	-2.06	-2	-2.34	-2.56	1.78	-3.47	0	-3.42	0	-1.65	0	0	0	0	0		
	<i>accB</i>	Biotin carboxyl carrier protein of acetyl-CoA carboxylase	-9.8	11.22	-2.8	-3.16	-4.38	4.86	0	-3.37	-4.1	-5.52	0	-3.02	0	0	0	0	0	0	
	<i>accC</i>	Biotin carboxylase	-6.12	-7.34	-2.43	-2.54	-3.53	-3.83	0	-3.05	-2.82	-3.27	0	-2.36	0	0	0	0	0	0	
	<i>ackA</i>	Acetate kinase	-5.73	-7.32	-2.04	-2.22	-3.87	-4.71	13.61	0	-1.31	-5.84	1.52	-3.19	0	0	0	0	0	0	
	<i>acp_P_1</i>	Acyl carrier protein	-10.36	-14.85	-2.83	-4.7	-4.45	-5.68	4.36	-2.79	1.59	-1.73	1.78	0	0	0	0	0	0	0	
	<i>acul</i>	putative acrylyl-CoA reductase Acul	0	0	0	-1.38	0	0	37.7	1.69	3.71	1.51	10.59	1.89	0	0	0	0	0	0	
	<i>adiC</i>	Arginine/arginine antiporter	0	0	0	0	0	0	-2.08	0	-2.66	0	-2.54	0	0	0	0	0	0	0	
	<i>ansB_2</i>	L-asparaginase 2	0	0	-1.39	-1.6	0	0	10.26	3.37	36.41	0	76.19	0	0	0	0	0	0	0	
	<i>arcA_1</i>	Arginine deiminase	1.45	1.34	0	0	2.4	0	-1.56	0	4.17	6.11	8.78	0	0	0	0	0	0	0	

<i>arc A_2</i>	Aerobic respiration control protein ArcA	-1.39	-1.43	1.52	0	0	0	0	11.7	3.05	3.58	0	3.79	3.95
<i>arg E</i>	Acetylornithine deacetylase	0	-1.38	0	0	0	0	0	1.25	0	2.02	0	2.52	0
<i>arg I</i>	Ornithine carbamoyltransferase subunit I	0	0	0	0	0	0	0	5.05	0	-2.13	0	-3.1	0
<i>arn A</i>	Bifunctional polymyxin resistance protein ArnA	-2.35	-3.79	-2.14	-2.91	-1.57	-2.08	0	-2.55	-2.23	-2.92	-1.85	-1.97	-1.97
<i>arn B</i>	UDP-4-amino-4-deoxy-L-arabinose-oxoglutarate aminotransferase	-2.19	-2.91	-2.17	-2.95	-1.68	-2.16	-2.23	-2.25	0	0	0	2.2	0
<i>arn C</i>	Undecaprenyl-phosphate 4-deoxy-4-formamido-L-arabinose transferase	-2.35	-3.57	2.2	-2.6	-1.51	-1.69	2.84	-1.99	-1.72	-1.96	0	-1.89	0
<i>arn D</i>	putative 4-deoxy-4-formamido-L-arabinose-phosphoundecaprenol deformylase ArnD	-2.18	-2.91	-2.35	-2.44	-1.56	-1.86	-3.51	-2.09	-3.88	-2.63	-3.76	-3.19	-3.19
<i>arn F</i>	putative 4-amino-4-deoxy-L-arabinose-phosphoundecaprenol flippase subunit ArnF	-4.6	-4.57	0	0	0	0	-5.08	-4.34	-2.61	0	-4.3	-3.73	-3.73
<i>arn T</i>	Undecaprenyl phosphate-alpha-4-amino-4-deoxy-L-arabinose arabinosyl transferase	-1.47	-1.57	-1.78	-1.71	0	0	-5.02	-1.7	-5.54	-2.25	-5.05	-1.93	-1.93
<i>aro B</i>	3-dehydroquinate synthase	-4.14	-5.83	-2.13	-2.6	-2.71	-3.28	2.99	0	2.39	0	3.05	0	0
<i>aro K</i>	Shikimate kinase 1	-5.3	-5.97	-2.16	-2.74	-2.87	-3.51	3	-2.43	2.1	1.76	-4.9	0	0
<i>aro L</i>	Shikimate kinase 2	-1.65	-1.45	0	1.26	0	0	2.49	0	2.74	1.55	4.03	2.21	2.21
<i>asc D_1</i>	CDP-6-deoxy-L-threo-D-glycero-4-hexulose-3-dehydrase reductase	-5.51	-3.98	0	0	-5.63	-5.74	4.67	0	0	0	1.69	-1.66	-1.66
<i>asn A</i>	Aspartate-ammonia ligase	0	0	1.39	0	0	0	0	0	5.81	2.88	3.34	3.71	3.71
<i>asp A</i>	Aspartate ammonia-lyase	0	-3.14	-1.79	-5.17	0	-3.22	122.13	11.98	45.82	2.18	111.25	5.65	5.65
<i>asp C</i>	Aspartate aminotransferase	-1.86	-1.88	0	0	-1.69	-1.98	1.86	-1.76	3.04	1.78	3.41	0	0
<i>ast B</i>	N-succinylarginine dihydrolase	1.61	0	0	1.45	1.51	1.57	-2.68	0	-7.4	0	-2.45	0	0
<i>bio H</i>	Pimeloyl-[acyl-carrier protein] methyl ester esterase	0	0	0	0	0	0	-2.29	-1.46	-2.09	0	-4.55	-2.09	-2.09
<i>cod A</i>	Inducible lysine decarboxylase	2.2	0	1.46	-1.63	2.17	0	25.58	2.45	16.71	-2.04	14.15	0	0
<i>cod B</i>	putative cadaverine/lysine antiporter	0	0	-1.31	-1.94	1.62	-1.91	30.09	1.54	9.84	0	24.2	0	0
<i>car A</i>	Carbamoyl-phosphate synthase small chain	0	0	0	2.31	0	2.28	2.72	0	3.1	3.98	0	1.49	1.49
<i>ccm F_1</i>	Cytochrome c-type biogenesis protein CcmF	1.39	0	0	0	0	0	-5.81	0	-3.03	-1.75	-5.76	-1.42	-1.42
<i>ccm F_2</i>	Cytochrome c-type biogenesis protein CcmF	0	0	0	0	0	0	-4.59	-1.82	-2.23	-2.2	-2.55	-1.48	-1.48
<i>cds A</i>	Phosphatidate cytidylyltransferase	-3.04	-3.51	-1.9	-1.88	-2.01	-2.21	0	-1.88	0	-1.69	1.44	0	0
<i>cls B</i>	Cardiolipin synthase B	2.04	2.13	1.37	1.58	1.56	1.53	-3.56	0	-2.78	0	-2.27	-1.74	-1.74
<i>cmk</i>	Cytidylate kinase	-2.14	-2.12	-1.39	0	-1.49	-1.48	2.28	-1.55	2.08	0	3.16	0	0
<i>cpd A</i>	3',5'-cyclic adenosine monophosphate phosphodiesterase CpdA	-1.87	-2.14	-2.49	-2.3	-1.76	-1.86	1.54	0	1.64	1.55	2.91	1.74	1.74
<i>cya A</i>	Adenylyate cyclase	-2.4	-2.3	-1.51	-1.38	-1.5	-1.37	-1.6	-1.94	0	0	0	0	0
<i>dap A</i>	4-hydroxy-tetrahydrodipicolinate synthase	-3.06	-3.62	-1.82	-2.39	-1.66	-2.64	3.94	-1.84	3.28	1.69	8.08	0	0
<i>dcu A_2</i>	Anaerobic C4-dicarboxylate transporter DcuA	0	0	0	-1.27	1.62	0	13.31	1.85	8.73	0	7.08	0	0
<i>deg Q</i>	Periplasmic pH-dependent serine endoprotease DegQ	-1.43	-1.55	0	0	0	0	3.37	0	4.99	2.73	5.17	0	0
<i>deo A</i>	Thymidine phosphorylase	-1.57	-1.65	1.39	1.45	0	0	8.38	2.85	1.28	1.38	1.93	0	0
<i>deo B</i>	Phosphopentomutase	-3.18	-4.11	1.5	0	0	-1.84	25.4	15.8	3.58	0	1.61	0	0
<i>deo C</i>	Deoxyribose-phosphate aldolase	-1.5	-1.5	0	0	0	0	11.62	2.11	3.72	1.51	2.49	0	0
<i>deo D</i>	Purine nucleoside phosphorylase DeoD-type	-2.86	-3.89	0	0	0	-2.02	2.4	-2.21	2.15	0	3.53	0	0
<i>dga F_1</i>	2-dehydro-3-deoxy-phosphogluconate aldolase	0	0	0	-1.28	0	0	-4.99	-1.66	-2.19	-2.31	-3.43	-2.12	-2.12
<i>dga F_2</i>	2-dehydro-3-deoxy-phosphogluconate aldolase	0	0	0	0	0	-1.32	-4.24	0	-2.97	0	-2.25	-1.62	-1.62
<i>dut</i>	Deoxyuridine 5'-triphosphate nucleotidohydrolase	-2.41	-2.47	-1.55	-1.73	-1.69	-2.1	5.58	0	1.93	-2.93	3.97	1.5	1.5
<i>dxs_1</i>	1-deoxy-D-xylulose-5-phosphate synthase	0	0	3.8	-3.89	-2.56	-2.35	2.24	0	0	-3.81	-3.13	0	0
<i>epd</i>	D-erythrose-4-phosphate dehydrogenase	-2.5	-2.7	-1.37	-1.48	-1.59	-1.66	5.67	0	3.98	2.15	6.16	1.74	1.74
<i>eut B</i>	Ethanolamine ammonia-lyase heavy chain	2.21	1.79	6.22	0	2.98	0	-2.98	0	-2.33	0	-1.71	-1.65	-1.65
<i>eut C</i>	Ethanolamine ammonia-lyase light chain	2.1	1.45	6.82	0	8.18	1.35	3.05	0	-2.03	0	0	0	0
<i>eut D</i>	Ethanolamine utilization protein EutD	2.35	1.91	0	0	3.41	0	5	-1.79	0	0	1.79	0	0
<i>fab A</i>	3-hydroxydecanoyl-[acyl-carrier-protein] dehydratase	-2.84	-2.83	0	0	-2.11	-2.08	0	-2.12	-2.73	-3.64	0	0	0
<i>fab B</i>	3-oxoacyl-[acyl-carrier-protein] synthase 1	-5.95	-7.18	-1.76	-1.64	-3.37	-2.97	0	-7.18	1.4	0	3.19	0	0
<i>fab H</i>	3-oxoacyl-[acyl-carrier-protein] synthase 3	-6.9	-8.47	-2.65	-3.23	-5.48	-5.29	3.88	-3.11	7.03	-1.67	1.72	-1.67	-1.67
<i>fab I</i>	Enoyl-[acyl-carrier-protein] reductase [NADH] FabI	-2.51	-2.44	0	0	-1.7	-1.74	1.95	-2.85	2	-1.74	2.63	-1.84	-1.84
<i>fab Z</i>	3-hydroxyacyl-[acyl-carrier-protein] dehydratase FabZ	-6.02	-8.18	-2.57	-3.41	-3.47	-3.84	2.72	-5	0	0	0	-1.84	-1.84
<i>fad L</i>	Long-chain fatty acid transport protein	0	0	1.31	0	0	0	4.21	0	2.81	0	2.51	0	0
<i>fad H_1</i>	Formate dehydrogenase H	-1.58	-2.79	-4.4	-5.31	-3.38	-4.16	18.11	2.01	2.06	0	2.91	0	0
<i>fdo G_1</i>	Formate dehydrogenase-O major subunit	-3.7	-5.79	-1.54	-1.98	-2.03	-2.79	0	-1.67	7.51	3.33	8.56	2.25	2.25
<i>fdo G_2</i>	Formate dehydrogenase-O major subunit	-4.45	-6.96	-1.82	-2.7	-2.17	-3.15	0	-1.72	9.43	2.71	8.99	0	0
<i>fdo I</i>	Formate dehydrogenase, cytochrome b555(fdo) subunit	-4.71	-6.43	-1.81	-2.93	-2.13	-3.08	-1.94	-3.12	1.91	0	1.54	0	0
<i>feo B</i>	Fe(2+) transporter FeoB	-1.51	-1.55	0	0	0	0	-3.17	-1.82	-2.56	-1.54	-2.54	0	0
<i>fre</i>	NAD(P)H-flavin reductase	-1.85	-2.19	0	0	-1.44	-1.74	1.69	-2.27	4.26	2.52	4.11	1.73	1.73
<i>fuc O</i>	Lactaldehyde reductase	1.33	0	1.36	1.45	1.31	1.33	3.01	0	5.21	0	-3.96	-1.69	-1.69
<i>gal K</i>	Galactokinase	0	0	-1.83	-1.96	0	0	4.24	2.47	4.19	1.8	3.83	1.52	1.52
<i>gal T</i>	Galactose-1-phosphate uridylyltransferase	0	0	-1.38	-1.46	0	0	3.69	2.7	3.6	1.72	2.52	0	0
<i>gal D</i>	Galactitol-1-phosphate 5-dehydrogenase	0	0	0	0	0	0	2.35	0	-3.32	-2.8	-2.41	-1.56	-1.56
<i>gcv H</i>	Glycine cleavage system H protein	0	-1.52	-1.34	-2.83	0	-2.19	2.84	0	4.69	0	4.18	0	0
<i>gcv P</i>	Glycine dehydrogenase (decarboxylating)	0	0	0	-2.68	0	2.37	7.87	0	2.51	0	2.39	-1.7	-1.7
<i>gcv T</i>	Aminomethyltransferase (glycine cleavage system aminomethyltransferase)	0	0	0	-2.13	0	-1.83	6.38	0	8.86	2.14	9.53	0	0
<i>glg B</i>	1,4-alpha-glucan branching enzyme GlgB	0	-1.38	0	0	1.5	0	2.14	0	5.93	1.64	6.18	0	0
<i>glm S_2</i>	Glutamine-fructose-6-phosphate aminotransferase [isomerizing]	-6.29	-7.84	-2.8	-3.49	-4.56	-5.1	4.28	-1.53	1.39	-1.77	2.77	0	0
<i>glp A</i>	Anaerobic glycerol-3-phosphate dehydrogenase subunit A	0	-1.78	-3.45	-4.41	-2.9	-3.47	16.64	3.48	0	-3.43	0	-2.51	-2.51
<i>glp B</i>	Anaerobic glycerol-3-phosphate dehydrogenase subunit B	0	0	-3.07	-3.91	-2.7	-2.92	7.28	2.21	0	-3.01	0	-2.19	-2.19
<i>glp D</i>	Aerobic glycerol-3-phosphate dehydrogenase	-1.94	-3.46	-3.49	-4.02	-4.37	-5.09	1.79	-1.59	0	-3.05	-2.06	-1.99	-1.99
<i>glp F</i>	Glycerol uptake facilitator protein	-1.51	-1.76	-2.28	-2.69	-3.26	-3.71	1.81	0	7.49	0	5.95	0	0

<i>glp Q</i>	Glycerophosphodiester phosphodiesterase, periplasmic	0	-1.83	-8.44	-9.55	-9.56	-8.94	-14.58	0	3.61	-25.76	3.46	-3.08
<i>glt D_1</i>	Glutamate synthase [NADPH] small chain	-1.46	-1.56	0	0	0	0	-3.09	0	-3.11	0	-2.33	0
<i>glt P</i>	Proton/glutamate-aspartate symporter	-2.05	-2.07	0	0	-1.37	-1.45	-3.06	-3.35	5.73	-3.27	2.08	0
<i>glt X</i>	Glutamate-tRNA ligase	-2.19	-2.38	0	-1.41	-1.66	-1.93	8.13	-1.8	3.28	2.23	1	1.43
<i>gmh A</i>	Phosphoheptose isomerase	-1.6	-1.4	0	0	0	0	1.04	0	1.79	1.91	4.93	1.8
<i>gmk</i>	Guanylate kinase	-3.2	-4.14	-1.6	-1.82	-1.77	-2	2.39	-3.19	1.91	1.79	2.14	0
<i>gor</i>	Glutathione reductase	-1.57	-2.17	0	-1.26	0	-1.46	6.61	-1.43	2.68	1.91	2.8	0
<i>gph</i>	Phosphoglycolate phosphatase	-2.87	-3.73	-2.07	-2.53	-2.43	-3	1.68	-1.67	0	-1.57	0	-1.79
<i>gps A</i>	Glycerol-3-phosphate dehydrogenase [NAD(P)-]	-3.22	-3.96	-2.65	-3.48	-2.89	-3.2	4.14	-1.5	1.48	0	2.23	0
<i>gsh B</i>	Glutathione synthetase	0	0	0	0	0	0	2.64	0	2.4	0	2.99	0
<i>gua C</i>	GMP reductase	0	0	0	0	0	0	13.53	0	6.6	2.45	8.6	0
<i>hcp</i>	Hydroxylamine reductase	1.85	1.74	1.44	1.48	1.47	1.62	-3.18	0	-2.37	0	-3.04	0
<i>hem B</i>	Delta-aminolevulinic acid dehydratase	0	0	0	0	0	0	9.91	7.4	2.96	1.29	1.71	0
<i>hem C</i>	Porphobilinogen deaminase	-3.38	-3.52	-2.06	-1.96	-2.08	-2	1.64	-2.52	0	0	0	0
<i>hem D</i>	Uroporphyrinogen-III synthase	-3.19	-3.3	-1.9	-2.13	-1.97	-2.17	0	-2.17	0	0	0	0
<i>hem E</i>	Uroporphyrinogen decarboxylase	-1.78	-2.13	0	0	0	-1.5	3.31	0	3.23	1.52	2.99	0
<i>hsl V</i>	ATP-dependent protease subunit HslV	-2.34	-2.16	0	0	0	0	2.55	0	4.25	2.15	2.86	1.71
<i>hya A</i>	Hydrogenase-1 small chain	0	0	1.39	1.63	1.29	1.44	-3.85	0	-2.31	0	-2.47	-1.52
<i>hyb C</i>	Hydrogenase-2 large chain	-1.51	-2.79	-2.6	-4.11	-2.63	-3.33	13.87	3.23	3.05	0	1.4	0
<i>hyb D</i>	Hydrogenase-2 maturation protease	-1.56	-2.46	-2.74	-4.7	-2.91	-4.43	9.17	4.96	2.08	0	2.23	-1.8
<i>hyb O</i>	Hydrogenase-2 small chain	-2.32	-3.13	-4.8	-4.78	-2.76	-3.31	25.39	9.14	3.43	1.88	8.93	2.79
<i>hyc I</i>	Hydrogenase 3 maturation protease	0	-1.6	-3.31	-3.75	-2.53	-3.02	7.33	0	0	-3.58	0	-3.02
<i>ifcA</i>	Fumarate reductase flavoprotein subunit	1.59	1.55	0	1.23	0	0	-2.43	1.79	-5.93	-1.99	-3.99	0
<i>ilv A</i>	L-threonine dehydratase biosynthetic IlvA	-1.37	-1.53	0	-1.34	0	-1.35	-3.8	-1.46	-4.33	0	-2.08	0
<i>ilv C</i>	Ketol-acid reductoisomerase (NADP(+))	8.47	-9.61	0	0	-1.71	-1.88	-1.97	-3.5	-7.81	-2.04	-2.45	-1.63
<i>ilv G</i>	Acetolactate synthase isozyme 2 large subunit	0	0	0	0	0	0	-2.32	-1.52	-4.5	0	-2.07	0
<i>isp D</i>	2-C-methyl-D-erythritol 4-phosphate cytidyltransferase	-4.01	-9.13	-2.36	-2.23	-2.55	-2.56	2.3	-1.78	1.98	0	2.44	-1.4
<i>isp F</i>	2-C-methyl-D-erythritol 2,4-cyclodiphosphate synthase	-3.06	-3.63	-2.16	-2.48	-2.27	2.45	2.08	0	2.47	0	2.19	-1.53
<i>isp G</i>	4-hydroxy-3-methylbut-2-en-1-yl diphosphate synthase (flavodoxin)	-3.14	-3.31	-1.69	-1.64	-1.78	-1.87	1.46	-1.75	0	0	1.48	0
<i>isp H</i>	4-hydroxy-3-methylbut-2-enyl diphosphate reductase	-2.84	-2.95	-2.01	-2.03	-1.8	-1.87	7.04	0	0	0	0	0
<i>lex A_1</i>	LexA repressor	0	1.55	-1.58	-1.64	0	-1.5	2.94	8.06	5.23	3.45	8.75	3.48
<i>lex A_2</i>	LexA repressor	-2.9	-3.32	0	0	0	1.63	10.37	0	0	0	3.14	2.3
<i>lpt E</i>	LPS-assembly lipoprotein LptE	-2.42	-2.86	-1.53	-1.85	-2.12	-2.01	4.01	-1.55	1.73	0	0	0
<i>mae B</i>	NADP-dependent malic enzyme	0	0	0	-1.36	0	-1.64	3.18	0	1.73	0	3.24	0
<i>mal P_1</i>	Maltodextrin phosphorylase	-6.44	-7.33	0	0	-10.18	-11.19	1.83	0	4.98	1.71	-4.61	-6.08
<i>mal P_2</i>	Maltodextrin phosphorylase	1.42	0	-6.11	-6.92	0	0	2.62	0	-11.39	-19.59	-4.29	1.54
<i>mal Q</i>	4-alpha-glucanotransferase	-4.82	-4.89	-5.12	-5.55	-7.99	8.74	2.61	0	-9.55	-12.65	-5.57	-4.35
<i>man Y</i>	PTS system mannose-specific EIIC component	0	-2.26	-2.56	-2.79	-1.77	-2.4	7.66	0	2.28	-4.77	4.39	0
<i>men B</i>	1,4-dihydroxy-2-naphthoyl-CoA synthase	-1.7	-2.2	-1.32	-1.79	-1.55	-2.07	18.85	1.43	1.02	0	4.12	0
<i>men C</i>	o-succinylbenzoate synthase	-1.56	-2.15	-1.6	-1.81	-1.49	-1.91	8.13	0	2.06	0	2.44	-2.27
<i>mip A</i>	MTA-interacting protein	-3.12	-3.38	0	-1.33	-1.56	-1.81	5.45	-2.22	2.35	0	6.09	1.67
<i>nag E</i>	PTS system N-acetylglucosamine-specific EIICBA component	1.37	1.38	0	1.27	1.36	0	5.34	1.51	2.34	0	6.88	0
<i>nan A</i>	N-acetylneuraminatase lyase	0	0	0	0	0	0	5.91	-1.73	11.08	0	17.1	0
<i>nar H</i>	Respiratory nitrate reductase 1 beta chain	1.9	2.05	1.38	1.5	1.54	1.72	-2.2	0	-4.77	0	-2.66	0
<i>nem A</i>	N-ethylmaleimide reductase	0	0	0	0	0	0	7.17	2.84	7.36	1.75	2.35	0
<i>nha A</i>	Na(+)/H(+) antiporter NhaA	-1.62	0	0	0	0	0	24.08	16.93	4.89	5.67	4.48	3.83
<i>nrp A</i>	Ribonucleoside-diphosphate reductase 1 subunit alpha	7.36	-8.87	-4.85	-5.11	-9.2	-10.17	15.79	0	8.11	4.13	9.75	2.1
<i>nrp B</i>	Ribonucleoside-diphosphate reductase 1 subunit beta	-7.62	-8	-4.19	-5.06	-8.39	-8.92	11.29	0	5.23	1.97	7.62	0
<i>nud B</i>	Dihydropyrimidin triphosphate diphosphatase	-3.15	-3.94	-1.56	-2.07	-2.46	-2.88	2.05	-2.58	0	-5.64	0	-3.71
<i>ots A</i>	Trehalose-6-phosphate synthase	3.88	3.2	3.04	2.98	3.24	3.24	0	0	2.97	2.3	1.89	0
<i>ots B</i>	Trehalose-6-phosphate phosphatase	-3.6	-4.18	2.21	3.6	2.77	3.31	0	0	2.26	1.96	1.54	0
<i>pal</i>	Peptidoglycan-associated lipoprotein	-6.41	-8.45	-2.11	-3.48	-3.66	-5.14	2.35	-4.18	0	-3.3	7.4	-2.28
<i>pep B</i>	Peptidase B	-1.98	-2.45	-1.84	-2.22	-1.68	-1.79	3.64	2.87	2.12	-1.48	3.44	-1.48
<i>pep E</i>	Peptidase E	0	0	0	0	0	0	2.69	1.5	2.5	0	3.52	0
<i>pfi B</i>	Formate acetyltransferase I	-5.19	-16.48	-5.32	-12.17	-3.73	-8.81	69.62	3.05	1.69	-18.46	6.05	-10.13
<i>pho R</i>	Phosphate regulon sensor protein PhoR	0	0	0	1.3	0	0	2.87	0	3.17	3.06	1.8	2.58
<i>pls X</i>	Phosphate acyltransferase	-3.77	-6.03	-2.13	-2.26	-5.18	-4.53	0	-4.95	0	-2.18	-2.67	-1.79
<i>prc</i>	Tail-specific protease	-3.42	-3.9	-2.35	-2.28	-2.61	-2.64	1.21	-1.46	0	0	1.73	0
<i>prs</i>	Ribose-phosphate pyrophosphokinase	-2.74	-2.91	2.1	2.15	2.25	2.19	14.51	0	2.08	0	4.38	0
<i>ptr B</i>	Protease 2	0	1.57	1.55	1.75	1.48	1.74	71.72	131.22	2.08	8.49	3.66	3.58
<i>pts H</i>	Phosphocarrier protein HPr	-3.78	-6.47	-2.17	-2.89	0	0	1.01	-2.15	1.52	-2.1	0	0
<i>pts N</i>	Nitrogen regulatory protein	-2.26	-2.85	0	-1.55	-1.65	-2.02	4.44	0	2.78	0	2.57	0
<i>pur A</i>	Adenylosuccinate synthetase	-2.66	-3.17	0	-1.36	0	-1.76	8.39	-1.97	2.89	2.02	3.75	0
<i>pur C</i>	Phosphoribosylaminoimidazole-succinocarboxamide synthase	1.33	0	-1.39	-1.45	0	0	2	0	2.51	0	2.98	0
<i>pur L</i>	Phosphoribosylformylglycinamide synthase	0	0	0	0	0	0	-2.09	-1.65	-2.18	-1.74	-4.47	0
<i>puu B</i>	Gamma-glutamylputrescine oxidoreductase	0	0	1.25	0	0	0	-2.46	0	-3.74	0	-2.31	0
<i>pyr G</i>	CTP synthase	-3.91	-4.58	-1.79	-1.88	-2.14	-2.25	5.89	0	3.24	1.42	2.27	0
<i>pyr H</i>	Uridylate kinase	-4.71	-6.08	-1.95	-2.56	-3.12	-3.29	0	-2.86	0	-1.79	0	-1.85

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<i>rsc C_1</i>	Sensor histidine kinase RcsC	1.39	1.76	1.62	1.72	1.48	1.85	-2.31	-1.57	-3.27	-1.75	-4	-1.56
<i>rel A</i>	GTP pyrophosphokinase	-1.77	-2.23	-1.3	0	0	0	2.54	0	2.91	2.34	3.4	2.14
<i>rff G</i>	dTDP-glucose 4,6-dehydratase 2	-2.77	-3.95	-1.72	-2.13	-2.04	-2.45	-1.67	1.48	-2.38	-3.48	-1.82	-2.14
<i>rha A</i>	L-rhamnose isomerase	0	0	0	0	0	0	-2.16	0	-3.7	0	-2.35	0
<i>rib B</i>	3,4-dihydroxy-2-butanone 4-phosphate synthase	-2.21	-2.32	-1.39	-1.51	-2.62	2.49	0	-1.68	-3.28	1.75	3.08	1.98
<i>rib F</i>	Riboflavin biosynthesis protein RibF	-2.82	-3.46	-1.61	-1.83	-2.04	2.26	2.07	0	1.63	0	2.2	0
<i>rib H</i>	6,7-dimethyl-8-ribitylumazine synthase	-3.65	-5.45	-1.62	-2.14	-1.97	-2.85	5.93	0	2.78	1.5	5.25	1.74
<i>rpe_2</i>	Ribulose-phosphate 3-epimerase	-2.97	-3.93	-1.59	2.5	-2.35	2.97	1.76	-1.41	-1.56	-2.15	0	-2.1
<i>sda B</i>	L-serine dehydratase 2	-2.42	-2.43	-3.76	-3.88	5	5.36	7.13	0	-1.37	-3.9	0	-2.92
<i>sda C</i>	Serine transporter	-3.86	-3.88	-5.84	-5.37	-7.13	-6.73	13.84	0	0	-3.72	0	-2.51
<i>ser A</i>	D-3-phosphoglycerate dehydrogenase	-1.43	-1.48	0	0	0	0	-2.01	0	-2.44	0	-3.51	0
<i>ser C</i>	Phosphoserine aminotransferase	0	-1.52	0	0	0	0	2.79	-1.89	2.18	1.81	2.13	0
<i>spe G</i>	Spermidine N(1)-acetyltransferase	-1.52	-1.69	0	0	0	0	7.84	0	4.84	2.5	13.4	4.78
<i>spo T</i>	Bifunctional (p)ppGpp synthase/hydrolase SpoT	-3.76	-4.66	-1.75	-1.93	-2.48	2.57	-2.25	-2.67	-1.58	-1.64	0	-1.57
<i>tal B</i>	Transaldolase B	-2.13	-3.36	-1.76	-2.15	-1.51	-1.93	6.83	0	2.7	1.49	2.98	1.69
<i>tam B</i>	Translocation and assembly module TamB	-1.64	-1.78	-1.44	-1.4	0	0	-2.01	0	-2.23	0	-2.32	0
<i>tdc B</i>	L-threonine dehydratase catabolic TdcB	1.38	0	0	-1.73	0	0	5.67	0	28.28	0	30.89	0
<i>tdc C</i>	Threonine/serine transporter TdcC	0	0	1.69	-1.41	0	0	3.56	0	36.43	0	36.78	0
<i>tdh</i>	L-threonine 3-dehydrogenase	-1.97	-2.17	-2.16	-2.3	-2.14	-2.63	4.75	-1.52	1.81	-1.91	2.35	-1.47
<i>tkt A_1</i>	Transketolase 1	0	0	-2.25	-2.81	-3.24	-3.3	2.78	0	3.08	0	-2.06	-3
<i>tkt A_2</i>	Transketolase 1	-2.92	-4.23	-5.36	-4.63	-3.08	-3.77	6.27	-1.57	0	-4.01	-2.66	-1.84
<i>tor A</i>	Trimethylamine-N-oxide reductase I	0	0	0	0	0	0	-2.4	-1.39	-2.3	-1.7	-2.11	-1.42
<i>tor C</i>	Cytochrome c-type protein TorC	0	0	0	0	0	0	-2.82	-1.6	-2.44	-2.48	-2.73	-1.43
<i>tre B</i>	PTS system trehalose-specific EIIBC component	-1.99	-2.4	-4.17	-4.33	-3.69	-4.93	3.3	1.84	-6.95	-3.9	-2.38	-2.46
<i>tre C</i>	Trehalose-6-phosphate hydrolase	-2.27	-2.91	5.2	-5.49	-5.28	-5.86	12.13	1.77	-4.24	-3.08	-3.15	-2.75
<i>trx B</i>	Thioredoxin reductase	-3.1	-3.38	-1.53	-1.52	-1.77	-1.81	4	-3.88	3.83	3.34	6.83	2.26
<i>tsx</i>	Nucleoside-specific channel-forming protein tsx	-3.08	-3.15	0	0	-6.03	-6.55	11.6	0	1.48	-4.14	2.17	-2.43
<i>ubi F</i>	2-octaprenyl-3-methyl-6-methoxy-1,4-benzoquinol hydroxylase	0	0	0	0	0	0	7.84	2.57	2.52	2.39	4.3	3.04
<i>wec C</i>	UDP-N-acetyl-D-mannosamine dehydrogenase	-2.98	-3.75	-2.2	-2.43	-1.97	-2.22	-1.45	1.76	-1.43	-1.67	-1.47	-1.85
<i>wec E</i>	dTDP-4-amino-4,6-dideoxygalactose transaminase	-2.17	-2.76	-1.49	-1.66	-1.68	-1.9	-2.21	0	-2.09	-3.08	-2.23	-2.47
<i>wec F</i>	TDP-N-acetylglucosamine:lipid II N-acetylglucosaminyltransferase	-2.04	-2.32	-1.44	-1.52	-1.53	-1.79	-2.13	0	-4.66	-2.79	-4.38	-1.82
<i>wec G</i>	UDP-N-acetyl-D-mannosaminuronic acid transferase	0	-1.55	-1.38	-1.25	-1.27	-1.3	-2.75	0	-4.31	0	-2.68	-1.73
<i>wzx E</i>	Lipid III flippase	-1.94	-2.17	-1.49	-1.61	-1.57	-1.8	-2.01	0	-4.52	-6.57	-3.45	-1.77
<i>yna_1_2</i>	Low conductance mechanosensitive channel YnaI	1.7	2.09	1.71	2.1	1.73	2.14	0	0	2.18	3	0	0
<i>ace C</i>	GDP-mannose:cellobiosyl-diphosphopolyprenol alpha-mannosyltransferase	-4.31	-6.37	0	0	-2.16	-2.08	3.04	0	0	0	4.71	2.28
<i>ahp F</i>	Alkyl hydroperoxide reductase subunit F	1.66	-2.27	2.37	0	-1.67	-1.82	2.29	0	36.93	38.99	6.75	6.18
<i>apa G</i>	Protein ApaG	-4.47	-4.4	-2.28	-2.86	-3.17	-2.98	1.69	0	0	0	0	0
<i>app B_1</i>	Cytochrome bd-II ubiquinol oxidase subunit 2	1.43	1.66	1.53	1.62	1.43	1.46	-2.65	0	-2.27	0	-4.23	0
<i>ari R</i>	putative two-component-system connector protein AriR	2.1	2.88	2.06	2.82	1.86	2.1	0	0	0	0	0	0
<i>asc D_2</i>	CDP-6-deoxy-L-threo-D-glycero-4-hexulose-3- dehydrase reductase	-3.74	-5.28	-2.59	-2.4	-2.95	-2.93	3.78	0	2.78	0	2.13	0
<i>ccm E</i>	Cytochrome c-type biogenesis protein CcmE	0	0	0	0	0	0	-3.94	-1.98	-3.85	-3.66	-2.8	0
<i>chI P</i>	Chitoporin	1.63	1.79	0	1.26	1.4	1.46	-1.97	0	-3.1	-4.4	-2.41	0
<i>cit F_1</i>	Citrate lyase alpha chain	1.36	0	0	0	-2.1	-2.21	-2.08	0	-2.02	0	-1.71	-2.13
<i>cit F_2</i>	Citrate lyase alpha chain	0	0	0	1.25	-1.27	-1.3	5.08	1.67	-2.38	-7.58	-2.82	0
<i>clp B</i>	Chaperone protein ClpB	0	-1.57	0	0	0	0	3.9	1.44	4.78	2.33	5.06	3.11
<i>coa BC</i>	Coenzyme A biosynthesis bifunctional protein CoaBC	-1.6	-1.84	-1.56	-1.54	-1.42	-1.42	2.11	0	2.96	2.57	2.32	1.5
<i>col D</i>	GDP-4-keto-6-deoxy-D-mannose-3-dehydratase / pyridoxamine-phosphate transaminase	-6.34	-9.18	0	0	-3.77	-5.94	3.11	-1.64	2.04	0	8.06	-3.69
<i>com EC</i>	ComE operon protein 3	1.61	1.75	1.34	1.53	1.54	1.61	-2.4	-1.39	-5.16	0	-2.22	0
<i>com M</i>	Competence protein ComM	0	-1.4	0	0	0	0	-4.65	-1.62	-2.69	0	-2.51	0
<i>csp C</i>	Cold shock-like protein CspC	2.84	-3.29	-2.73	-3.38	-3.08	3.97	0	0	-7.52	-2.95	0	-2.76
<i>csp E</i>	Cold shock-like protein CspE	-12.13	-9.41	-6.46	-8.63	-4.95	-4.49	1.54	-18.33	-2.49	-3.3	0	3.08
<i>cue O</i>	Blue copper oxidase CueO	1.51	0	0	1.39	1.37	0	12.77	0	9.02	19.71	6.01	2.4
<i>def</i>	Peptide deformylase	-2.24	-2.3	-1.58	-1.67	-1.49	-1.72	1.25	0	2.86	6.39	2.17	2.05
<i>din F</i>	DNA damage-inducible protein F	-1.55	-1.66	-1.31	-1.27	0	0	8.63	3.83	0	1.95	1.66	0
<i>din G_1</i>	putative ATP-dependent helicase DinG	-1.48	-1.32	0	0	0	0	1.65	1.66	1.8	2.21	1.96	0
<i>din G_2</i>	putative ATP-dependent helicase DinG	2.41	-2.28	-1.67	-1.6	-2.02	-1.87	5.74	1.76	0	1.39	2.13	2.24
<i>dps</i>	DNA protection during starvation protein	-2.13	3.67	4.88	2.11	4.53	3.23	5.49	0	28.74	11.21	36.53	24.62
<i>dtp B</i>	Dipeptide and tripeptide permease B	0	0	-1.48	-1.55	0	-1.41	4.29	0	3.68	0	6.52	1.49
<i>dus</i>	putative tRNA-dihydrouridine synthase	-3.09	-7.46	0	0	-1.7	-1.42	-4.22	-12.32	-2.48	-2.76	0	-1.53
<i>eeo</i>	Intimin	1.51	1.56	0	1.32	1.34	1.49	-3.94	0	-2.59	-1.72	-2.76	0
<i>eda</i>	KHG/KDPG aldolase	0	0	0	0	0	0	9.57	4.08	1.84	0	1.1	0
<i>eps J</i>	putative glycosyltransferase EpsJ	0	0	0	0	0	0	2.54	0	-2.55	0	-2.39	0
<i>eut K</i>	Ethanolamine utilization protein EutK	1.91	1.68	3.08	0	4.05	1.4	-2.45	0	0	0	0	0
<i>eut L</i>	Ethanolamine utilization protein EutL	2.31	1.75	5.06	0	7.19	1.42	-2.04	0	0	0	0	0
<i>eut M</i>	Ethanolamine utilization protein EutM	2.91	1.62	6.62	0	8.35	0	0	0	0	0	0	0
<i>eut N</i>	Ethanolamine utilization protein EutN	2.43	0	4.39	0	6.66	0	0	0	0	0	0	0
<i>fab D</i>	Malonyl CoA-acyl carrier protein transacylase	-7.14	-10.04	-2.53	-3.64	-4.6	-5.82	1.43	-3.22	2.08	-1.88	1.31	-1.68
<i>fab F</i>	3-oxoacyl-[acyl-carrier-protein] synthase 2	-6.53	-8.11	-2.49	-2.8	-4.21	-5.65	9.86	-2.29	1.5	0	2.67	0

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<i>fab G_1</i>	3-oxoacyl-[acyl-carrier-protein] reductase FabG	-9.13	-14.04	1.36	1.41	-5.63	-7.59	-4.1	-7.8	0	0	3.72	0
<i>fab G2</i>	putative oxidoreductase	0	0	-1.95	-2.11	0	-1.77	1.91	0	4.28	3.65	-9.64	-4.35
<i>fab H</i>	Protein FdhE	-2.18	-2.55	4.4	5.51	-3.38	-1.82	0	-1.93	1.57	0	0	0
<i>fhu E</i>	FhuE receptor	1.88	2.26	1.54	1.71	1.56	1.71	-3.69	0	-4.1	0	-2.69	0
<i>fkp A</i>	FKBP-type peptidyl-prolyl cis-trans isomerase FkpA	-2.05	-4.09	-2.04	-2.57	-1.74	-2.06	9.4	0	2.06	0	4.08	1.72
<i>fkp B</i>	FKBP-type 16 kDa peptidyl-prolyl cis-trans isomerase	2.8	-3.02	-2.08	-2.16	-2.27	-2.16	2.01	0	1.52	0	0	-1.56
<i>fld A</i>	Flavodoxin 1	-2.03	-1.71	-1.33	-1.3	-1.51	-1.5	1.93	0	5.08	3.74	7.96	3.8
<i>fld B</i>	Flavodoxin 2	0	-1.4	0	0	0	0	2.47	1.61	2.06	0	3.55	0
<i>fnr</i>	Fumarate and nitrate reduction regulatory protein	-2.53	-2.52	0	0	-1.45	0	3.12	0	2.79	3.06	4.92	3.71
<i>foc A</i>	putative formate transporter 1	-4.36	-4.72	-4.43	-4.19	-2.08	-2.06	19.73	-2.08	-2.9	-5.55	2.55	-2.14
<i>glm U</i>	Bifunctional protein GImU	-4.87	-5.67	-2.53	-2.66	-3.09	-3.26	3.3	-2.26	3.34	2.45	3.34	1.8
<i>gly A1</i>	Serine hydroxymethyltransferase 1	0	-1.73	0	-1.56	0	0	38.28	1.45	18.4	3.95	11.53	1.78
<i>grc A</i>	Autonomous glycol radical cofactor	-3.67	-5.08	-3.03	-4	-2.58	-3.61	8.22	0	2.38	-3.96	5.57	1.86
<i>gro S</i>	10 kDa chaperonin	-2.24	-4.01	-1.59	-1.87	0	-1.56	10.69	0	11.74	3.16	17.53	1.7
<i>grx C</i>	Glutaredoxin 3	-4.65	-7.5	-2.43	-3.54	-2.87	-3.61	12.95	-1.68	2.58	0	5.38	0
<i>gsp A_1</i>	General stress protein A	-2.85	-3	-1.75	-1.69	-1.6	0	7.04	0	2.53	0	1.64	0
<i>gsp A_2</i>	General stress protein A	-3.11	-2.37	0	-1.69	-1.53	0	2.6	0	0	0	2.95	1.6
<i>hjl C</i>	Modulator of FtsH protease HfIC	-2.91	-5.12	-1.58	-2.06	-2	-2.87	2.72	-1.42	0	-1.66	0	0
<i>hjl K_1</i>	Modulator of FtsH protease HfIK	-3.3	-6.72	0	0	-2.26	-3.44	2.88	0	0	0	1.85	0
<i>hmu U</i>	Hemin transport system permease protein HmuU	1.75	1.86	1.32	1.39	1.4	0	3.01	0	-4.03	0	-3.34	0
<i>hsc A</i>	Chaperone protein HscA	-2.24	-2.48	-2.25	-2.35	-2.1	-2.19	4.61	8.79	3.26	3.06	2.86	1.89
<i>hsc B</i>	Co-chaperone protein HscB	-2.21	-2.38	-1.99	-2.2	-2.25	-2.19	6.13	19.4	4.17	4.32	4.05	3.9
<i>hsl R</i>	Heat shock protein 15	-2.05	-1.9	0	0	0	0	2.51	0	3.48	2.46	2.24	1.57
<i>hsl U</i>	ATP-dependent protease ATPase subunit HslU	-2.34	-3.04	0	0	0	-1.35	2.93	-1.69	2.81	1.51	3.08	0
<i>htp G</i>	Chaperone protein HtpG	0	-1.32	0	0	0	0	5.54	0	3.38	1.71	3.54	0
<i>hyb B</i>	putative Ni/Fe-hydrogenase 2 b-type cytochrome subunit	-1.52	-2.16	-3.38	-4.06	-2.52	-3.04	11.58	3.62	2.21	0	4.57	0
<i>hyb E</i>	Hydrogenase-2 operon protein HybE	-1.6	-2.05	-3.12	-3.9	-3.06	-3.52	3.43	3.28	0	0	0	-1.92
<i>hyc D</i>	Formate hydrogenlyase subunit 4	0	-1.53	-2.95	-3.52	-2.26	-2.6	4.48	1.44	0	-4.71	0	-2.06
<i>hyc E</i>	Formate hydrogenlyase subunit 5	0	-1.94	-3.06	-4.37	-3.06	-3.61	3.88	1.55	-1.53	-3.57	0	-2.37
<i>hyc G</i>	Formate hydrogenlyase subunit 7	0	-1.72	-2.68	-4.18	-2.82	-3.61	3.36	1.43	0	-2.56	0	-2.23
<i>hyf A_2</i>	Hydrogenase-4 component A	-1.44	-1.82	-3.08	-3.08	-2.28	-2.62	7.54	0	0	-4.2	0	0
<i>hyf A_3</i>	Hydrogenase-4 component A	0	-1.58	-2.43	-2.57	0	0	15.79	1.65	0	-2.3	0	0
<i>hyf B</i>	Hydrogenase-4 component B	0	-1.41	-2.32	-2.49	-2.3	-2.5	10.06	0	0	-2.08	0	-1.89
<i>hyp B</i>	Hydrogenase isoenzymes nickel incorporation protein HypB	-1.51	-1.81	-1.56	-1.75	0	-1.4	16.7	3.28	3.4	2.19	7.33	3.97
<i>hyp C</i>	Hydrogenase isoenzymes formation protein HypC	0	-1.57	0	-1.46	0	-1.57	15.45	3.92	2.84	2.01	5.07	4.08
<i>hyp D</i>	Hydrogenase isoenzymes formation protein HypD	0	-1.49	-1.56	-1.58	0	-1.43	11.89	7.66	3.12	2.93	6.14	3.44
<i>hyp E</i>	Hydrogenase isoenzymes formation protein HypE	0	-1.4	-1.59	-1.77	0	-1.39	9.66	3.27	2.02	1.68	5.51	2.79
<i>ibp A_2</i>	Small heat shock protein ibpA	-1.4	0	0	0	0	0	0	0	3.05	2.68	2.14	2.26
<i>ica B</i>	Poly-beta-1,6-N-acetyl-D-glucosamine N-deacetylase	0	0	0	0	0	0	-2.09	0	-2.83	0	-2.73	0
<i>ira M</i>	Anti-adaptor protein IraM	0	1.54	2.31	2.33	1.61	1.91	4.78	0	0	0	6.92	10.55
<i>kbl</i>	2-amino-3-ketobutyrate coenzyme A ligase	-2.23	-2.38	-2.61	-3.42	-3.04	-3.46	4.71	-1.34	2.94	-1.48	2.17	-1.43
<i>lim B</i>	Limonene 1,2-monooxygenase	0	0	0	0	0	0	-2.17	0	-2.85	-1.87	-3.74	-1.45
<i>mdo D</i>	Glucans biosynthesis protein D	0	0	1.51	1.48	0	1.37	6.08	0	3.7	2.38	3.54	1.54
<i>mit G</i>	Endolytic murein transglycosylase	-1.65	-1.45	0	0	0	1.27	-2.25	0	-2.36	-2.66	-2.34	0
<i>nfd A</i>	N-substituted formamide deformylase	1.8	2.06	1.33	1.45	1.64	1.77	3.25	0	-2.92	0	-3.53	0
<i>nlp I</i>	Lipoprotein NlpI	-8.65	-10.77	-1.85	-2.08	-3.56	-3.05	5.18	0	1.46	2.73	2.61	3.07
<i>nrd A</i>	Anaerobic ribonucleoside-triphosphate reductase	-3.24	-3.3	-1.94	-1.99	-2.22	-2.39	9.37	2.26	7.35	3.38	4.72	1.65
<i>nrf B</i>	Cytochrome c-type protein NrfB	0	0	0	0	0	0	-2.75	0	-3.16	-2.14	-2.08	0
<i>osm B</i>	Osmotically-inducible lipoprotein B	2.34	2.6	2.97	3.46	2.85	3.62	0	0	0	0	0	0
<i>osm C</i>	Peroxisredoxin OsmC	3	3	2.64	2.69	2.05	1.91	0	0	0	0	0	0
<i>osm E</i>	Osmotically-inducible putative lipoprotein OsmE	6.08	2.52	3.68	2.45	2.92	2.84	2.83	0	2.45	0	3.36	0
<i>osm Y_1</i>	Osmoprotectant import permease protein OsmY	2.14	2.46	1.61	2.19	1.8	2.12	0	1.96	0	2.2	-6.91	0
<i>osm Y_2</i>	Osmotically-inducible protein Y	-9.77	-6.68	-1.43	-1.47	0	-1.32	3.25	0	0	0	0	0
<i>osm Y_3</i>	Osmotically-inducible protein Y	-2.16	-2.96	9.79	3.9	16.38	9.12	1.8	2.8	4.3	1.86	3.33	0
<i>pap D</i>	Chaperone protein PapD	0	1.33	0	0	0	0	-3.14	0	-6.99	-2.23	-2.24	0
<i>pcm</i>	Protein-L-isoaspartate O-methyltransferase	-2.56	-2.83	-1.78	-2.02	-2.09	-2.06	1.36	-1.59	1.44	0	2.03	0
<i>pdx A1</i>	4-hydroxythreonine-4-phosphate dehydrogenase 1	-3.82	-4.24	-2.65	-2.94	-2.82	-3.23	-1.74	-1.77	-3.38	-2.45	-2.11	-1.95
<i>pep A</i>	Cytosol aminopeptidase	-1.44	-1.71	0	-1.34	0	-1.43	1.93	0	3.73	1.43	1.96	0
<i>pep D_2</i>	Cytosol non-specific dipeptidase	0	-1.63	0	0	0	0	3.97	0	3.37	1.65	3.74	0
<i>pep P</i>	Xaa-Pro aminopeptidase	-1.98	-2.49	-1.69	-1.87	-1.5	-1.9	6.39	1.86	3.29	2.19	3.04	0
<i>pep Q</i>	Xaa-Pro dipeptidase	-1.9	-2.8	-1.55	-1.87	-1.39	-1.87	9.85	-1.78	3.23	0	1.11	0
<i>pot D</i>	Spermidine/putrescine-binding periplasmic protein	-1.61	-1.73	0	0	0	0	18.34	0	4.06	0	4.88	0
<i>pps A</i>	Phosphoenolpyruvate synthase	0	0	1.68	0	0	0	5.9	0	7.66	1.76	2.38	0
<i>pqi A</i>	Paraquat-inducible protein A	-1.63	-1.56	-1.37	-1.24	-1.43	-1.49	-1.94	-1.47	-3.09	-2.14	-2.73	-1.73
<i>pqi B_1</i>	Paraquat-inducible protein B	-1.78	-1.72	-1.32	0	-1.48	-1.42	-3.29	-1.4	-3.05	-2.42	-1.81	-1.87
<i>pqi B_2</i>	Paraquat-inducible protein B	-1.53	-1.57	0	1.21	0	1.27	-1.88	-2.17	-2.27	-1.87	-1.71	0
<i>pro P_1</i>	Proline/betaine transporter	-1.93	-2.18	1.79	1.89	0	0	-4.97	-2.73	-2.66	0	0	0
<i>pro Q</i>	RNA chaperone ProQ	-3.34	-5.76	-2.85	-2.96	-3.53	-3.68	3.54	-2.25	0	0	2.3	0

<i>psp A</i>	Phage shock protein A	-2	-4.27	3.78	5.31	6.96	9.2	2.83	7.78	6.98	8.46	2.11	6.33
<i>psp B</i>	Phage shock protein B	1.82	1.76	1.76	4.21	6.69	8.8	4.16	8.97	5.29	3.29	0	5.16
<i>psp D</i>	Phage shock protein D	2.64	3.72	2.68	3.22	4.38	3.2	0	3.27	6.18	5.11	0	4.46
<i>ram A</i>	(R)-stereoselective amidase	0	1.74	1.83	1.61	0	1.42	0	0	-3.28	0	0	0
<i>rib Z</i>	Riboflavin transporter RibZ	1.67	1.92	1.33	1.69	1.5	1.68	-3.7	0	-2.63	-3.41	-3.88	0
<i>rid A</i>	2-Iminobutanoate/2-Iminopropanoate deaminase	-1.46	-1.66	0	-1.27	0	-1.35	2.64	0	-6.3	0	3.13	0
<i>rsx D</i>	Electron transport complex subunit RxD	-2.22	-1.95	0	0	-1.44	-1.4	-2.14	0	-3	-2.35	-3.61	-1.65
<i>rsx E</i>	Electron transport complex subunit RxE	-2.52	-1.99	0	0	-1.59	-1.57	-5.97	0	-2.4	0	-3.29	0
<i>rsx G</i>	Electron transport complex subunit RxG	-2.39	-2.26	0	0	-1.36	0	-2.29	0	-2.35	0	-3.13	0
<i>rtc A</i>	RNA 3'-terminal phosphate cyclase	0	0	1.33	0	0	0	-2.38	-1.57	-2.06	-1.95	0	0
<i>rtc B_1</i>	RNA-splicing ligase RtcB	1.36	0	1.47	0	0	0	-3.28	0	-4.64	-7.31	0	0
<i>sdc S_2</i>	Sodium-dependent dicarboxylate transporter SdcS	1.34	1.28	0	0	0	0	-3.08	0	-10.77	0	-3.54	-1.46
<i>skp</i>	Chaperone protein Skp	-8.95	-17.9	-3.99	-9.84	-4.67	-10.67	1.24	-5.95	1.44	-3.43	2.57	-3.67
<i>sly D</i>	FKBP-type peptidyl-prolyl cis-trans isomerase SlyD	-2.23	-2.9	-1.46	-1.6	-1.43	-1.65	14.07	-1.73	4.74	1.47	8.66	1.69
<i>smc</i>	Chromosome partition protein Smc	0	0	0	0	0	0	113.34	389.08	0	0	0	0
<i>smg</i>	Protein Smg	-7.13	-2.04	-1.47	-1.48	0	0	2.16	0	2.4	1.67	1.52	3.26
<i>spy</i>	Periplasmic chaperone Spy	-5.88	2.05	2.08	2.45	6.55	2.28	2.45	1.77	2.94	0	5.2	0
<i>sth A</i>	Soluble pyridine nucleotide transhydrogenase	0	0	0	0	0	0	2.71	1.36	10.12	10.11	6.38	3.3
<i>sur A</i>	Chaperone SurA	-4.06	-4.88	-2.78	-3.64	-2.85	-3.39	1.5	-1.89	0	0	0	-2.18
<i>tig</i>	Trigger factor	-7.96	10.64	-2.55	-3.31	4	5.34	6.38	6.43	1.91	0	0	0
<i>tqs A_1</i>	AI-2 transport protein TqsA	1.63	1.79	1.66	1.83	1.6	1.76	-2.79	0	-3.36	0	-2.78	0
<i>ubi E_2</i>	Ubiquinone/menaquinone biosynthesis C-methyltransferase UbiE	-2.71	-3.3	-1.7	-2.03	-1.86	-2.14	2.84	-1.95	2.18	1.7	1.53	0
<i>ubi G_2</i>	Ubiquinone biosynthesis O-methyltransferase	0	0	0	0	0	1.3	2.23	0	3.33	1.41	2.94	0
<i>usp A</i>	Universal stress protein A	0	-1.53	0	0	1.61	0	14.85	0	10.71	5.28	10.83	2.66
<i>usp E</i>	Universal stress protein E	0	0	1.45	1.47	1.7	1.62	2.97	-2.05	5.48	2.89	6.64	2.36
<i>usp G</i>	Universal stress protein UP12	0	0	0	0	0	0	2.52	0	4.52	0	14.1	6.12
<i>wec D</i>	dTDP-fucosamine acetyltransferase	-2.28	-2.83	-2.03	-2.06	-1.73	-2.09	-2.83	0	-2.79	-2.35	-2.01	-1.87
<i>yba B</i>	Nucleoid-associated protein YbaB	-5.22	-5.52	-2.74	-3.11	-2.96	-3.07	1.64	-1.92	1.56	0	0	0
<i>yce D</i>	Large ribosomal RNA subunit accumulation protein YceD	-6.81	-11.05	-2.19	-2.85	-3.55	-3.62	2.76	-25.63	2.3	0	5.61	0
<i>yci V</i>	5'-3' exoribonuclease	-1.33	0	0	1.29	0	0	4.34	0	2.82	2.3	2.58	3.31
<i>ycj G</i>	L-Ala-D/L-Glu epimerase	0	0	0	1.41	1.71	1.66	2.03	1.42	2.55	0	2.48	1.5
<i>ydg I</i>	Putative arginine/ornithine antiporter	0	0	1.68	1.84	1.47	1.66	-3.39	-1.46	-4.04	-2.55	-3.61	-2.24
<i>yeb F</i>	Protein YebF	0	0	0	0	0	0	464.99	274.85	12.7	24.12	17.22	12.55
<i>yec D</i>	Isochorismatase family protein YecD	0	0	0	0	0	1.35	2.48	0	6.72	0	2.41	0
<i>yic I</i>	Alpha-xylosidase	0	0	0	0	0	0	-2.91	0	-2.12	-1.44	-2.57	-1.49
<i>yjf K</i>	putative transport protein YjfK	0	-1.79	0	0	0	0	-3.88	-1.74	-2.77	-2.14	-2.17	0
<i>yjf C</i>	Putative acid-amine ligase YjfC	0	0	0	0	0	0	-2.75	0	-3	0	-3.03	-1.45
<i>yod B</i>	Cytochrome b561	-3.11	-2.98	-3.29	-3.07	-3.1	-2.73	0	-1.83	-4.05	-2.77	0	0
<i>ywl C</i>	Threonylcarbamoyl-AMP synthase	-1.7	-1.56	0	0	0	0	13.73	3.6	4.62	2.6	4.09	3.72

Capítulo 5

Considerações Finais

5.1 Considerações Finais

Neste trabalho, além de ser observada uma heterogeneidade em relação aos níveis de *persisters* formados por diferentes isolados, cada isolado respondeu em níveis diferentes aos distintos antibióticos aos quais foi exposto, incluindo até mesmo a ausência de formação de *persisters*. Esta variação de resposta frente à exposição a fármacos distintos também foi evidenciada pelos distintos padrões de expressão diferencial encontrados na análise transcritômica. Dentro da mesma linha, foi constatado que as condições de cultivo, planctônico ou biofilme, podem influenciar nos níveis de *persisters*, assim como as *persisters* mostraram ser capazes de formar colônias regulares e *small* frente a um mesmo estressor e, até mesmo, retomaram o crescimento na presença de concentrações letais do antimicrobiano ao qual se manteve suscetível. Também foi possível observar que tanto células oriundas de colônias regulares como *small* apresentaram septo de divisão e filamentação quando observadas microscopicamente. Esses dados nos sugerem que um mesmo isolado pode dar origem a populações de *persisters* fenotipicamente distintas que as capacitam a sobreviver a variados desafios. Também pode ser destacado que a exposição prévia a concentrações subinibitórias de estressores iguais ou diferentes ao subsequentemente empregado não induziu níveis mais elevados de *persisters*, o que, além de indicar a não seleção de mutantes altamente persistentes, aponta para um importante papel da formação estocástica de *persisters*.

A análise preliminar dos transcritos diferencialmente expressos indicou que o padrão apresentado por alguns genes, como por exemplo aqueles relacionados ao estresse oxidativo e à resposta ao estresse, seja adicionalmente investigado empregando qRT-PCR, talvez delimitando tempos intermediários de exposição, o que poderia incrementar a elucidação dos mecanismos envolvidos na regulação das células tolerantes.

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